FINAL DRAFT
ECAO-CIN-D002
March, 1994

DRINKING WATER CRITERIA DOCUMENT FOR CHLORAMINES

HEALTH AND ECOLOGICAL CRITERIA DIVISION
OFFICE OF SCIENCE AND TECHNOLOGY
OFFICE OF WATER

DISCLAIMER

This report is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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 1986; however, more recent data have been added during the review process, and final revisions updating this document were made.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, 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.

Tudor Davies, Director
Office of Science and
Technology

James Elder, Director
Office of Ground Water
and Drinking Water

DOCUMENT DEVELOPMENT

Carolyn L. Smallwood, A.B., Document Manager
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Authors

Carolyn L. Smallwood
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Patricia A. Murphy
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Annette M. Gatchett
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Rita Schoeny
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Frank E. Scully, Jr.
Dept. of Chemical Sciences
Old Dominion University
Norfolk, VA 23508

Scientific Reviewers

Randall F. Bruins
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

W.B. Peirano
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

David J. Reisman
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Cynthia Sonich-Mullin
Environmental Criteria and Assessment Office, Cincinnati
U.S. Environmental Protection Agency

Joseph R. Bianchine
Medical Research and Development
American Critical Care
McGaw Park, IL

Joseph P. Gould
Environmental Chemistry
Georgia Institute of Technology
Atlanta, GA

Richard J. Bull
College of Pharmacy
Washington State University
Pullman, WA

Herbert H. Cornish
Professor Emeritus
University of Michigan
Ypsilanti, MI

Curtis D. Klaassen
University of Kansas Medical Center
Dept. of Pharmacology and Toxicology
Kansas City, KS

Loren K. Koller
College of Veterinary Medicine
Oregan State University
Corvallis, Oregan

David Jollow
Medical University of South Carolina
Charleston, SC

Editorial Reviewers

Erma Durden, B.S.
Environmental Criteria and Assessment
Office, Cincinnati
U.S. Environmental Protection Agency

Judith Olsen, B.A.
Environmental Criteria and Assessment
Office, Cincinnati
U.S. Environmental Protection Agency

Document Preparation

Technical Support Services: Bette Zwayer, Environmental Criteria and Assessment Office, Cincinnati

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Alk-P	Alkaline phosphatase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BUN	Blood urea nitrogen
bw	Body weight
CHO	Chinese hamster ovary
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
DPD	Diethyl-p-phenylenediamine
DWEL	Drinking water equivalent level
EAA	Essential amino acid
EKG	Electrocardiogram
FEV	Forced expiratory volume
FVC	Forced vital capacity
GGTP	q-Glutamyltranspeptidase
GI	Gastrointestinal
GSH	Glutathione
HA	Health advisory
HCT	Hematocrit
HGB	Hemoglobin percent

LIST OF ABBREVIATIONS (cont.)

i.p.	Intraperitoneal
i.v.	Intravenous
LOAEL	Lowest-observed-adverse-effect level
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
NADH	Nicotinamide-adenine dinucleotide dehydrogenase
NCP	N-chloropiperidine
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
PHA	Phytohemagglutinin
RBC	Red blood cell
RfD	Reference dose
s.c.	Subcutaneous
SCE	Sister chromatid exchange
T_4	Plasma thyroxine
TCA	Trichloroacetic acid
TPA	12-o-Tetradecanoylphorbol-13-acetate
TrpC	Tryptophan C
TWA	Time-weighted average

I. SUMMARY

Inorganic chloramines are alternate disinfectants that are rapidly formed when free chlorine is added to water containing ammonia. To achieve the desired chloramine concentration, chlorine may also be intentionally added to the already naturally occurring ammonia in the water source. Under the usual conditions of water and wastewater chlorination, monochloramine is the principal chloramine encountered. In the event of excess hypochlorite or at lower pH, ammonia can be di- and trichlorinated and organic amines can be dichlorinated. Comparatively little is known about the physical properties of pure dichloramine because it is not stable and is difficult to synthesize. Trichloramine is formed in acidic solutions where chlorine concentrations are much greater than those of ammonia. This document will deal primarily with inorganic chloramines; however, where data are limited or not available various chlorinated amino compounds (including organic chloramines) will be provided as supplemental information.

Inorganic dichloramine is unstable and decomposes to nitrogen, hypochlorous acid and other products. This reaction accounts to a large extent for the breakpoint reaction seen in water chlorination. Inorganic monochloramine is a much poorer disinfectant than hypochlorous acid, and it reacts slowly with organic amino nitrogen compounds to produce organic N-chloramines, which are even poorer disinfectants.

Conventional methods of monochloramine analysis have led to confusion in the use of the term "chloramines." The term has been used to describe many compounds in

complex mixtures that oxidize iodide to iodine, the amount of which is subsequently determined by colorimetric or amperiometric analysis. Organic N-chloramines and N-chloramides respond similarly to monochloramine; however, they are poor disinfectants compared with inorganic chloramine. From a public health viewpoint, these ambiguities can present serious problems since the disinfecting capabilities of waters that contain organic N-chloramines could be overestimated. Improved analytical methods are needed for the determination of organic and inorganic N-chloramines in chlorinated waters. For the purpose of this document, the term "chloramines" will refer to a combination of inorganic mono-, di- or trichloramines unless otherwise stated.

Human exposure to chloramines is through ingestion of chlorinated water containing ammonia or chloraminated water. Chloramination is a technique that is being adopted by many communities to avoid formation of trihalomethanes on water disinfection. Organic chloramines have been shown to form upon chlorination of stomach fluid *in vitro*. However, the significance of their formation on ingestion of chlorinated water is not clear.

Information on the absorption of inorganic chloramines is extremely limited. In one study an absorption rate constant was calculated for monochloramine at 0.278 mg/hour (after 8 hours) with an absorption half-life of 2.5 hours after a single oral dose of ~4.6 mg/kg/day was administered to Sprague-Dawley rats. After 48 hours, the rate constant was 0.018 mg/hour. Absorption rates with respect to various dosage media and different routes of exposure were not available.

The distribution of radiolabeled chlorine in the subfractions of rat liver homogenates in organs, tissues, and fluids was similar (120 hours) after oral administration of either $^{36}Cl^-$ (200 mg/L as $Na^{36}Cl$) or $NH_2^{36}Cl$ (370 mg/L $NH_2^{36}Cl$). Plasma contained the highest concentrations of 36Cl radioactivity for $NH_2^{36}Cl$ followed by whole blood, skin, testes, packed cells, bone marrow, kidney, lung, stomach, thyroid, thymus, duodenum, spleen, carcass, liver, ileum and fat. The major metabolite of $NH_2^{36}Cl$ was $^{36}Cl^-$.

Information on the metabolism of chloramines is also extremely limited. One experiment indicated that chloramines are transformed to the chloride moiety and eliminated primarily in this form.

Chloramines are eliminated primarily through the urine. During the first 24 hours after a single dose of $NH_2^{36}Cl$ (1.1 mg/animal) to Sprague-Dawley rats, only 0.40 and 0.08% of the total dose was eliminated in the urine and feces, respectively. At the end of 120 hours, 25.15 and 1.98% of the dose was eliminated through the urine and feces, respectively. By comparison 16.1 and 0.92% of $^{36}Cl^-$ radiolabel (200 mg/L as $Na^{36}Cl$) was eliminated in the urine and feces, respectively, in the first 24 hours. Over twice as much of the $^{36}Cl^-$ radiolabel was eliminated over a 120-hour period. The major difference between the $NH_2^{36}Cl$ and $^{36}Cl^-$ studies was in the total amount of label excreted over the test period.

Several short-term studies showed no observed adverse hematologic effects in mice, rats and monkeys. In A/J mice administered chloramine solutions between 2.5 and 200 mg/L (pH 8.9) for 30 days, the only observable effect was a slight increase in hematocrit. In another study of similar duration (45 days) rats treated with 10, 50 or 100 mg/L monochloramine experienced a decrease in the amount of methemoglobin present in the blood, the opposite of what was expected. Monochloramine in drinking water for 6 weeks at 100 mg/L had no detectable effects on 18 hematologic tests of 12 African Green monkeys.

In a 12-month study using Sprague-Dawley rats administered 1, 10 and 100 mg/L monochloramine, glutathione levels, red blood cell count and hematocrit were found to be decreased at sporadic intervals. However, there was a lack of dose- and time-dependent response in the results. Plasma thyroxine levels were significantly decreased and cholesterol elevated in pigeons administered 15 mg/L monochloramine for 3 months.

Rats and mice were administered monochloramine in drinking water at concentrations of 0, 25, 50, 100, 200 or 400 ppm (0, 25, 50, 100, 200 or 400 mg/L) for 91 days. Decreased body weight gain and liver damage were observed at 200 and 400 mg/L in rats and 100, 200 and 400 mg/L in mice. Histopathologic observation revealed mild to moderate cytologic alteration in the liver of male mice administered 200 and 400 mg/L chloramines. Chronic liver inflammatory changes occurred at 100, 200 and 400 mg/L in female mice and to a lesser extent in male mice at the 100 ppm level. Microscopic

examination of rat tissues at the 400 mg/L level did not reveal any treatment-related lesions. The investigators suggested a NOEL of 50 mg/L or ~8.3 mg/kg/day monochloramine based on chronic liver inflammatory changes in mice.

In a 90-day study, male and female Sprague-Dawley rats were administered monochloramine in drinking water at concentrations of 0, 25, 50, 100 and 200 mg/L. At 200 mg/L the average weight gain was 51% of controls. There were also reductions in organ weights (absolute, relative or both) at the high dose level. The authors identified the 100 mg/L dose as the NOAEL.

B6C3F1 mice were administered monochloramine in their drinking water for 90 days at 0, 12.5, 25, 50, 100 and 200 mg/L. There were weight gain reductions, and reductions in absolute and relative organ weights at the 100 and 200 mg/L dose levels. Based on these reductions the authors identified a NOAEL of 50 mg/L.

In a 2-year study, F344/N rats and B6C3F1 mice were administered 0, 50, 100 and 200 ppm monochloramine in their drinking water. There was a decrease in mean body weight in high-dose rats. The high-dose group had decreases in organ weights and increases in organ-to-body weight ratios at 14- or 66-week evaluations. There was also a dose-related decrease in mean body weights of dosed male and female mice throughout the study. There were decreases in organ weights and increases in organ-to-body weight ratios observed in high-dose mice at 15- or 66-week evaluations.

Monochloramine was not teratogenic in mature female Sprague-Dawley rats exposed to 1, 10 or 100 mg/L in drinking water, nor did 40, 100 and 200 mg/L solutions induce sperm-head anomalies in B6C3F1 mice. In addition, no significant differences in fertility, viability, litter size, day of eye opening or day of vaginal patency were observed between control and exposed Long-Evans rats given $\geq$10 mg/kg chloramines. There were no alterations in sperm count, direct progressive sperm movement, percent mobility or sperm morphologic characteristics.

Results on the mutagenicity of chloramines are inconclusive. Monochloramine has been found to be marginally mutagenic in *Bacillus subtilis* and in *Vicia faba* plant seeds. In *Salmonella typhimurium* (TA97, TA100 and TA102), chloramines (40 um) marginally increased the number of revertant colonies over untreated controls. It was responsible for cellular hypertrophy, increased mitotic figures and bizarre chromatin patterns in B6C3F1 mice exposed to 200 and 400 mg/L in drinking water. In another study, monochloramine at 40, 100 and 200 mg/L did not induce chromosomal aberrations or micronuclei in bone marrow of CD-1 mice.

The organic chloramine, N-chloropiperidine, was found to be marginally mutagenic in the reverse mutation plate incorporation assay (Ames test). It was cytotoxic and cytostatic in CHO cells and produced chromosomal aberrations, the frequency of which was proportional to the concentration of the compound. It produced SCEs in CHO cells, but not in baby hamster kidney cells. The analogous chloramine, N-chlorodiethylamine, was more toxic but nonmutagenic. When the synthetic N-Cl compound chloramine T

(sodium p-toluene-sulfonyl chloramide) was tested, SCEs were significantly increased in a dose-dependent manner in CHO cells.

Organic concentrates of water treated with monochloramine produced papillomas, squamous cell carcinomas and lung adenomas in SENCAR mice. These data are inadequate, however, to assess the carcinogenic potential of monochloramine. In a 2-year study using male and female F344 rats and B6C3F1 mice, monochloramine was administered in drinking water at 0, 50, 100 and 200 ppm. Equivocal evidence of carcinogenic activity was found in female rats because of the slightly increased incidence of mononuclear cell leukemia. There was no evidence of carcinogenic activity in male rats or female or male mice, which was attributed to chloraminated drinking water.

Information concerning human exposure to chloramines is extremely limited. In humans, acute exposure by inhalation of chloramine fumes has been observed after mixing 4-5% solutions of ammonia and sodium hypochlorite in a small room. Pneumonitis resulted, but no permanent pulmonary damage occurred. Very few experimental studies have been conducted. Individuals ingesting levels of chloramines between 0.01 and 24.0 mg/L for 1 day or 5 mg/L for 12 weeks showed no hematologic or detrimental physiologic responses resulting from chloramine ingestion.

There are no epidemiologic studies that have been designed to address specifically the potential adverse effects of exposure to chloramines on human health. One study was conducted to see if there was a difference in cancer mortality among

communities using chlorine compared with communities using chloramine for disinfection. This study was not designed to assess adverse effects from exposure to chloramine but rather to consider the chloramine-exposed participants as controls.

Chloramines appear to be produced by normal human neutrophils as part of their bactericidal action. Chloramines are believed to exert their effects by interfering with enzymatic reactions. Monochloramine oxidizes and denatures hemoglobin and inhibits the hexose monophosphate shunt. It also causes strand-breaks in DNA.

Lack of sufficient data preclude the derivation of a 1-day HA for chloramines. It is recommended that the 10-day HA of 1 mg/L be adopted as the 1-day HA. The 10-day HA was derived from a drinking water study using African Green monkeys. The 10-day HA for a 10 kg child is 1 mg/L based on the absence of hematologic effects. The longer-term HAs for a 10 kg child and 70 kg adult are 1 and 4 mg/L, respectively. These HAs are based on a NOAEL for body and organ weight changes in rats exposed to chloramines in drinking water. The DWEL of 4 mg/L is derived from a proposed RfD of 0.1 mg/kg/day from a NOAEL in a chronic drinking water study using rats, based on absence of decreased organ weight changes.

There was one 2-year bioassay with equivocal evidence of carcinogenic activity in female rats. The CRAVE Work Group verified (12/02/92) a classification for monochloramine of group D, not classifiable as to human carcinogenicity, meaning that there is inadequate human and animal evidence of carcinogenicity.

II. PHYSICAL AND CHEMICAL PROPERTIES

Introduction

Inorganic chloramines are alternate disinfectants that are rapidly formed when free chlorine is added to water containing ammonia. This reaction is represented by the following equation with its respective reaction rate constant (at 25°C = 298°K) (Morris, 1967):

$$NH_3 + HOCl = NH_2Cl + H_2O$$

$$(k_{298} = 6.1x10^6\ M^{-1}\ sec^{-1})$$

Since ammonia has more than one hydrogen that can be replaced by a chlorine atom, it reacts with an excess of hypochlorous acid to form dichloramine (Morris, 1967; Gray et al., 1979):

$$NH_2Cl + HOCl = NHCl_2 + H_2O$$

$$(k_{298} = 3.4x10^2\ M^{-1}\ sec^{-1})$$

or trichloramine (Morris and Isaac, 1983):

$$NHCl_2 + HOCl = NCl_3 + H_2O$$

$$(k_{298} = 2.1\ M^{-1}\ sec^{-1})$$

The distribution of mono-, di- and trichloramine is dependent on pH, temperature and relative concentrations of ammonia and hypochlorite as described in the following section on physical and chemical properties.

Physical and Chemical Properties of Chloramines

Under the usual conditions of water and wastewater chlorination, monochloramine is the principal chloramine encountered. Anhydrous monochloramine is a colorless, water-soluble liquid (Kirk-Othmer, 1979) that freezes at -66°C. The pure liquid decomposes above -50°C with formation of nitrogen, chlorine and nitrogen trichloride (Colton and Jones, 1955; Kovacic et al., 1970). The known chemical and physical properties of pure monochloramine are summarized in Table II-1. The environmental significance of monochloramine, however, is generally restricted to its aqueous solutions, where it is useful in water treatment for destruction of pathogenic bacteria (Butterfield, 1948; Wolfe et al., 1984). The rate of its formation is so rapid that determination of rates between pH 6.5 and 10 were impossible because of the rapidity of the reaction in this range (Weil and Morris, 1949). At pH 8.5 the rate of its formation reaction reaches a maximum (Weil and Morris, 1949). Monochloramine is the only chloramine formed when the pH of ammonia containing water is >8 and the molar ratio of hypochlorite to ammonia is <1 (Gray et al., 1979).

At hypochlorite to ammonia ratios >1 or at lower pH values, dichloramine and trichloramine are formed. At pH values <5.5 monochloramine slowly converts to form dichloramine (Gray et al., 1979):

$$2\ NH_2Cl + H^+ = NHCl_2 + NH_4^+$$

The relative proportions of monochloramine and dichloramine formed as a function of pH and temperature are listed in Table II-2.

Comparatively little is known about the physical properties of pure dichloramine because of its instability and difficulty of preparation. Its odor, volatility from aqueous solution, and relative solubility in various solvents are intermediate between those of monochloramine and trichloramine (NRC, 1980). Under equilibrium conditions at pH 4 it is the only product of the reaction of equimolar concentrations of chlorine and ammonia. Under normal conditions, however, dichloramine solutions are unstable (Corbett et al., 1953) and decompose by several mechanisms, not all of which have been elucidated (Chapin, 1931; Wei and Morris, 1974; Hand and Margerum, 1983).

Trichloramine (nitrogen trichloride) is formed in acid solutions where chlorine concentrations are much greater than those of ammonia. At these high chlorine concentrations and at pH values <3, trichloramine is the only chloramine present. Nitrogen

trichloride occurs in diminishing proportions at chlorine-to-ammonia mole ratios >2 and pH values of ≤7.5. At pH >7.5, no trichloramine is found, regardless of the ratio of chlorine to ammonia. Pure trichloramine is a bright yellow liquid that, because of its limited solubility, can be isolated from aqueous solution by solvent extraction (Dowell and Bray, 1917). Its chemical and physical properties are summarized in Table II-1. It can be explosive in concentrated solutions and is an effective chlorinating agent, particularly in nonaqueous media (Dowell and Bray, 1917; Jander, 1955; Kovacic et al., 1970). In aqueous solutions at neutral pH it decomposes slowly to ammonia and hypochlorous acid (Ryan et al., 1980) by an autocatalytic pathway (Hand and Margerum, 1983). Aqueous solutions of trichloramine are stabilized by small amounts of acid (Corbett et al., 1953).

When chlorine is added to waters containing ammonia, the breakpoint phenomenon becomes significant in the pH range of 6-9. The breakpoint is that dosage of chlorine that produces the first detectable amount of free chlorine residual. At chlorine-to-ammonia weight ratios of <5:1 ~pH 7, monochloramine is formed and the combined residual increases to a maximum. This is the case up to the hump in the curve as displayed in Figure II-1. At chlorine-to-ammonia weight ratios 5:1, dichloramine is formed. The residuals formed in this reaction occur between the top and the dip of the curve. With the addition of chlorine the previously formed dichloramine is oxidized to nitrous oxide (N_2O), nitrogen trichloride (NCl_3) and nitrogen (N_2). The formation of these nitrogen compounds oxidize chlorine which in turn results in a decrease of ammonia nitrogen. As the chlorine-to-ammonia nitrogen weight ratio reaches 10:1 at ~pH 7, the breakpoint

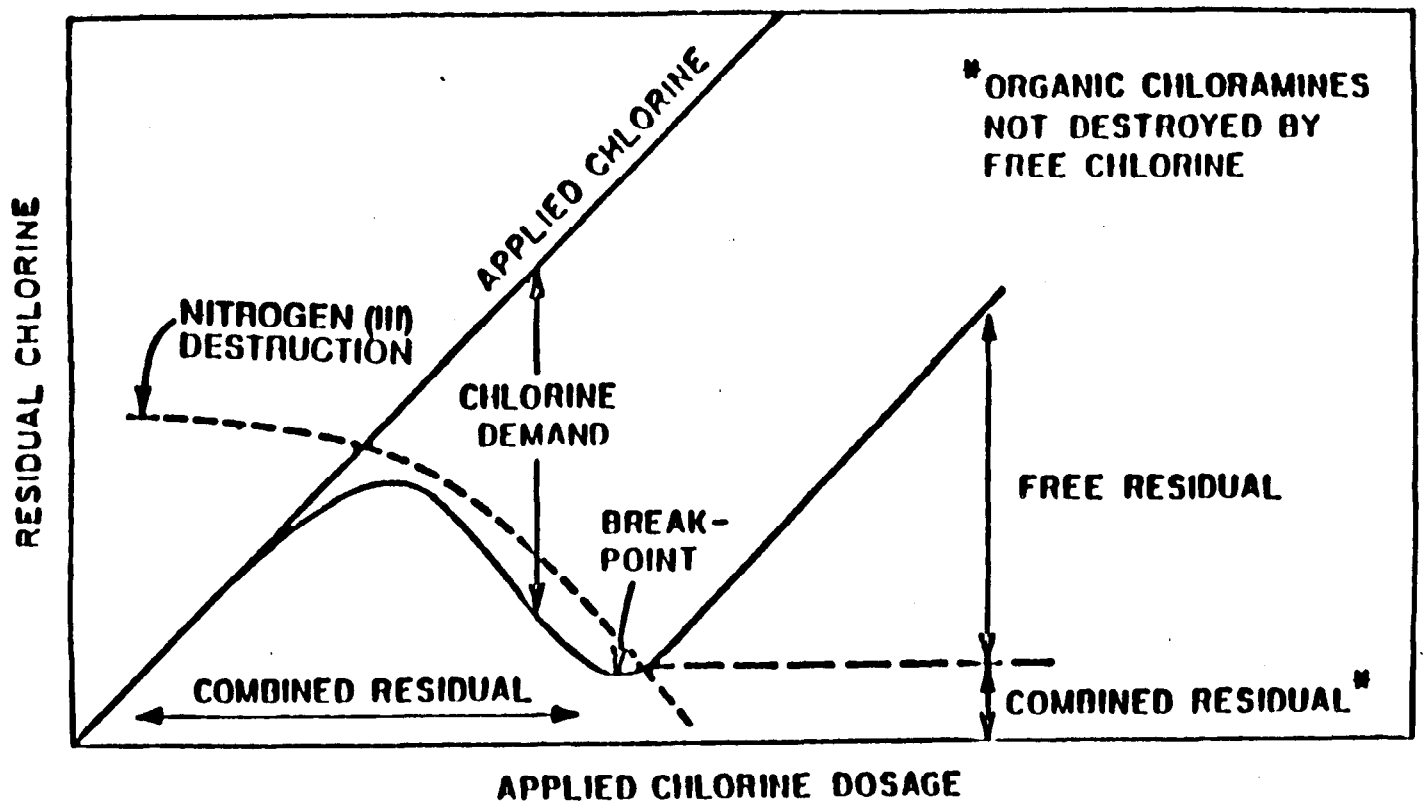

FIGURE II-1

Graphic Description of Breakdown Phenomenon

phenomenon occurs (NRC, 1980; White, 1972). At the breakpoint dosage, some resistant chloramines are present (primarily di- and trichloramine); however, they are not of any importance (NRC, 1980).

Chlorine that is added after the breakpoint exists as free chlorine [that is, elemental chlorine, hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-)] (Pressley et al., 1973; Wei and Morris, 1974). When natural waters and wastewater are chlorinated, there is a residual oxidant formed, which remains stable at the breakpoint and in the presence of hypochlorite beyond the breakpoint. This residual oxidant responds to conventional methods of analysis in the same way monochloramine does. It can be shown that some organic amino nitrogen compounds form very stable organic N-chloramines that, unlike inorganic chloramines, do not decompose in the presence of excess hypochlorite. This residual oxidant is, therefore, believed to be due to organic N-chloramines.

Monochloramine is less effective as a chlorinating agent than hypochlorous acid by a factor of $\sim 10^4$ (Morris, 1967). However, when chloramines (mostly monochloramine) were used to treat raw water, Stevens et al. (1978) determined that trihalomethane (THM) formation was minimized. Thus, during the chlorination of water, when the ammonia breakpoint is not achieved, THM production may be significantly reduced. Rickabaugh and Kinman (1978) determined that chloramination of Ohio River water with monochloramine at 10 mg/L, pH 7-9 and 25°C resulted in 90.7-99.9% less THM formation, as compared with THM production from chlorination with 10 mg/L chlorine as hypochlorous

acid or hypochlorite. Presumably many of the reaction products of water treated with free chlorine (Cl, OCl^- and HOCl) are produced by the reaction of combined chlorine residual (chlorinated waters that contain chloramines) with free chlorine. This is a result of the slow hydrolysis of chloramines to hypochlorous acid. The products should occur in low concentrations because of the low equilibrium concentration of the hypochlorous acid formed (Margerum et al., 1979).

Margerum et al. (1979) indicated that the formation of hydroxylamine (NH_2OH)

$$NH_2Cl + OH^- = NH_2OH + Cl^-$$

at pH 8 has a reaction half-time of 350 years. Therefore, it probably does not occur in water treatment.

When low concentrations (mg/L range) of monochloramine and phenol are mixed, chlorophenols appear after a reaction time of several days (Burttschell et al., 1959). This probably results from the hydrolysis of NH_2Cl and subsequent reaction of HOCl with phenols.

Organic amines and amino acids in natural waters also react rapidly with hypochlorite to form organic N-chloramines (Calvert, 1940; Wright, 1926, 1936; Taras, 1950; Crane et al., 1946; Ellis and Soper, 1954; Mauger and Soper, 1946; Sandford et al., 1971; Edmond

and Soper, 1949; Ingols et al., 1953; Wajon and Morris, 1980). Morris (1967) concluded that the reaction rates of free chlorine with amino nitrogen compounds increases with the basicity of the compound. As with ammonia, organic amines can also form dichloramines, but the reaction rates are considerably slower for the addition of the second chlorine atom than the addition of the first chlorine atom. Formation of N,N-dichloromethylamine from N-chloromethylamine occurs with a second order reaction rate constant at 25°C of 1.1×10^3 M^{-1} sec^{-1} which is faster for organic than inorganic dichloramine (Morris, 1967):

$$CH_3NHCl + HOCl = CH_3NCl_2 + H_2O$$

Both inorganic chloramines and organic N-chloramines are formed when wastewater effluents are chlorinated. The relative amounts depend on the concentration ratios of ammonia to organic amino-N, the temperature, pH and the relative reaction rates (Isaac and Morris, 1980).

Organic N-chloramines can also form slowly by the reaction of inorganic chloramine with organic amines (Snyder and Margerum, 1982; Isaac and Morris, 1983, 1985):

$$NH_2Cl + RNH_2 = NH_3 + RNHCl$$

The transfer of chlorine from monochloramine to organic amines has been shown to involve two mechanisms: hydrolysis of the monochloramine to ammonia and hypochlorous

acid and direct chlorine transfer from a protonated monochloramine NH_3Cl^+ to the organic amine (Snyder and Margerum, 1982; Isaac and Morris, 1983, 1985). Because the chlorine transfer reaction is slow, its significance may be limited to large water distribution systems using inorganic chloramine as the disinfectant where retention time in the system becomes considerable.

From a water treatment standpoint organic N-chloramines are undesirable, since they are not effective disinfectants (Feng, 1966; Marks and Styandskov, 1950; Wolfe et al., 1984; Wolfe and Olson, 1985).

Uses of Chloramines

The combining of ammonia with chlorine for the purpose of forming chloramines to treat drinking water has been called combined residual chlorination, chloramination or the chloramine process (NRC, 1979). This process has been employed to provide a more persistent disinfecting residual than free chlorine. It also generally reduces the unpleasant taste and odors resulting from the formation of chlorophenolic compounds (Symons et al., 1978).

Inorganic chloramines have been considered poorer disinfectants than hypochlorous acid, since nearly 25 times as much chloramine as free chlorine was required to obtain a 100% kill with equivalent contact times (Butterfield, 1948). Brodtmann and Russo (1979) refuted the idea that chloramine was a poor biocide for use in the treatment of drinking

water. They found that chloramine treatment of drinking water was effective in destroying ~60% of the total bacterial population remaining after clarification, with a contact time of <10 minutes. Furthermore, the chloramine treatment was effective in destroying ~88% of the remaining coliform bacteria before sand filtration. Thus, the authors concluded that chloramine properly applied at effective dosages (1.5-1.8 mg/L) produced 100% kills of pathogenic bacterial species and reduced the total population of bacteria to an acceptable range.

Analytical Methods

Methods deemed acceptable for the determination of chlorine residuals in natural and treated waters include the following: colorometric methods, amperometric titration, stabilized neutral orthotolidine (SNORT) method, ferrous diethyl-p-phenylenediamine (DPD) method, and the leucocrystal violet (LCV) method.

The most common methods of analysis of monochloramine and most monochlorinated organic N-chloramines use the ability of these compounds to oxidize iodide to iodine followed by determination of the amount of iodine formed (APHA, 1980). The iodine formed can be titrated with a standardized solution of sodium thiosulfate and the endpoint detected visually with the aid of a starch solution. It can also be detected colorimetrically by addition of DPD (diethyl-p-phenylenediamine), which is converted to a red oxidized form. The intensity of the red color is measured with a spectrophotometer at 515 nm or with a filter photometer equipped with a filter having maximum transmission in the

wavelength range 490-530 nm. The oxidized DPD can also be titrated with standardized ferrous ammonium sulfate. This method is known as the FAS-DPD titrimetric method. Another method of measuring the iodine formed involves amperometric titration using a standardized solution of phenylarsine oxide. Leucocrystal violet is a colorless form of a dye that is converted by iodine to its colored form and measured with a spectrophotometer (592 nm), using a filter photometer or Nessler tubes.

Organic amines and albumenoid nitrogen compounds interfere with the analysis of monochloramine in chlorinated natural waters because they respond in a similar manner. In addition, as described below, the use of iodometric methods for analysis of chloramines has contributed a considerable amount of confusion to the meaning of the term "chloramines" (Johnson, 1978; Jolley and Carpenter, 1983; Wajon and Morris, 1980; Cooper et al., 1982). There is a need for new methods for distinguishing the various chemical compounds that respond to conventional analyses as "free residual chlorine" and "combined residual chlorine."

Most kinetic measurements of the formation, reactions and decomposition of inorganic mono-, di- and trichloramine have employed a direct spectrophotometric determination of their concentrations. Each has characteristic absorption spectra (Hand and Margerum, 1983). However, because of their low molar extinction coefficients the method is only good for measurements of solutions with concentrations $>10^{-4}$ M.

Evans (1982) reported a voltammetric method for analysis of inorganic chloramines in aqueous solution from pH 4-12. However, the method has approximately the same sensitivity as the spectrophotometric method.

Scully et al. (1984a) studied solutions of organic and inorganic N-chloramines in acid solution (pH 2) by cyclic voltammetry. Monochloramines can be distinguished from dichloramines and hypochlorous acid, but the method also lacks the sensitivity needed to measure concentrations $<10^{-4}$ M.

Scully et al. (1984b) developed a method for the analysis of organic and inorganic chloramines in dilute aqueous solution. The method involves derivatization of the chloramines with the sodium salt of 5-dimethylaminonaphthylene-1-sulfinic acid in bicarbonate buffer to form highly fluorescent sulfonamide derivatives. These derivatives can be separated by high pressure liquid chromatography so that organic chloramines can be measured in the presence of inorganic chloramine. The method has not yet been used successfully to identify organic N-chloramines in chlorinated natural waters. This will be necessary before the importance of organic N-chloramines in drinking water can be determined.

From a strictly chemical standpoint only ammonia and organic amines (R = alkyl or aryl) can form chloramines. Considerable confusion, however, has been introduced into the nomenclature of chloramines because of the methods used in the analysis of these

compounds in chlorinated natural waters and wastewaters (Johnson, 1978; Jolley and Carpenter, 1983; Wajon and Morris, 1980; Cooper et al., 1982). The term "combined residual chlorine" has been used to describe compounds in chlorinated waters that can be analyzed by amperometric and colorimetric methods only after addition of iodide, and the term "free residual chlorine" has been used to describe compounds that are generally analyzed by the same methods before the addition of iodide (APHA, 1980). Because solutions of organic and inorganic chloramines respond to these analyses as "combined residual chlorine" and because natural waters that contain high concentrations of ammonia form correspondingly high concentrations of combined residual chlorine, the terms combined residual chlorine and chloramines have become almost synonymous. Consequently, chlorinated waters that contain combined residual chlorine are said to contain chloramines. While the major fraction of combined residual chlorine is probably inorganic chloramines, other compounds including organic amines that are likely to contaminate natural waters and wastewaters react with chlorine to form compounds that respond to analyses as combined residual chlorine. For instance, proteinaceous or albumenoid nitrogen compounds contain amide linkages that react slowly with hypochlorite to form chloramides, R(C=O)NClR, which respond to analyses as combined residual chlorine.

In addition, because solutions of hypochlorite are most commonly associated with free residual chlorine, hypochlorite and free residual chlorine also have become almost synonymous. Nevertheless, N-chlorosuccinimide, which is a chlorimide, and

trichloroisocyanuric acid respond at least partially to conventional analyses as free residual chlorine (Morris et al., 1980). The term free residual chlorine most accurately refers to elemental chlorine, hypochlorus acid (HOCl) and hypochlorite ion (OCl^-). From a public health viewpoint these ambiguities can present serious problems; for instance, organic chloramines are poor disinfectants compared with inorganic chloramine (NH_2Cl) (Johnson, 1978). Jolley and Carpenter (1983) suggested the terms "combined oxidant" and "free oxidant" to avoid the ambiguities that have become associated with the residual chlorine term.

Further ambiguity exists in the term chloramine because it implies simply that a compound, organic or inorganic, contains both a chlorine atom and an amino nitrogen functional group. This would then include the highly carcinogenic nitrogen mustards that are not formed when water containing ammonia or organic amines is chlorinated. For the purposes of this document, only the toxicology and health effects data of organic and inorganic N-chlorinated amino nitrogen compounds will be reviewed.

Environmental Fate and Transport

The major source of chloramines in waters is the reaction of ammonia compounds with added chlorine. This occurs as a by-product of chlorination (i.e., in drinking water processing and sewage effluents) and from use of the chlorine-ammonia process (chloramination). An inventory of municipal water supplies undertaken in 1963 indicated that 308/11,590 surveyed used an ammonia-chlorine process (chloramination) (Moore and

Calabrese, 1980). For a discussion of rates of formation and breakdown of chloramines see the Physical and Chemical Properties Section and the Uses of Chloramines Section of this chapter. A 1984 survey of some U.S. utilities using chloramines (Trussell and Kreft, 1984) showed a possible distribution of chloramine residuals. A typical range of chloramine concentrations in drinking water supplies where it is used as a primary disinfectant or to provide a residual in the distribution system is 1.5-2.5 mg/L.

Summary

The alternate disinfectant inorganic chloramines are formed when water containing ammonia is chlorinated. The type and extent of each is dependent on pH, temperature and relative concentrations of ammonia and hypochlorite. Under the usual concentrations and conditions of water and wastewater treatment, monochloramine is the principal chloramine formed. Ammonia reacts with an excess of hypochlorous acid to form dichloramine or trichloramine. Dichloramine solutions, however, are unstable and readily decompose. When chlorine concentrations are much greater than ammonia and the solutions are acidic (pH<3), trichloramine is formed. Monochloramine at standard temperature and pressure is a liquid, whereas trichloramine at standard temperature and pressure is a solid.

Organic amines or amino acids react rapidly with hypochlorite in natural waters to form organic N-chloramines. Organic N-chloramines are also formed by the slow reaction of

inorganic chloramines with organic amines. Information on organic chloramines and their reactions is very limited.

Chloramines are used primarily as disinfectants since they are a more persistent disinfecting residual than free chlorine. They also reduce the unpleasant taste and odors in drinking water resulting from chlorophenolic compound formation.

There are several acceptable methods for determining chlorine residuals in natural and treated waters. They are as follows: colorimetric methods, amperometric titration, stabilized neutral orthotolidine (SNORT) method, ferrous diethyl-p-phenylenediamine (DPD) method, and the leucocrystal violet (LCV) method. New methods with greater sensitivity and the ability to distinguish various chemical compounds are needed.

The reaction of ammonia compounds with added chlorine is the major source of chloramine release to the environment. This is due to processes and by-products of chlorination.

TABLE II-1

Summary of Chemical and Physical Properties of Mono- and Trichloramines

Properties	Monochloramine[a]	Trichloromine[b,c]
Chemical structure	NH_2Cl	NCl_3
Molecular weight	51.48	120.38
Chemical Abstracts Registry Number	10599-90-3	10025-85-1
Registry of Toxic Effects of Chemical Substances (RTECS) Number	FN0275000	-
Synonyms	-	Nitrogen trichloride Nitrogen chloride Chlorine nitride Trichlorine nitride
Color	Yellow (liquid)	Yellow oil (liquid)
	Colorless crystals (solid)	Rhombic crystals (solid)
Physical state (25°C, 1 atm)	Liquid	Thick oil or rhombic crystals
Melting point, °C	-66°	<-40°
Boiling point, °C	-	<71° Explodes at 93°
Density	-	1.653
Solubility in water	Soluble	Insoluble in cold, decomposes in hot
Other solubilities	ETOH, ether, CCl_4, benzene	CS_2, PCl_3, benzene, CCl_4, $CHCl_3$

[a]RTECS, 1984
[b]Merck Index, 1983
[c]Weast, 1983

TABLE II-2

Proportions of Monochloramine (NH_2Cl) and Dichloramine ($NHCl_2$) Formed in Water Chlorination with Equimolar Concentrations of Ammonia and Chlorine[a,b]

pH	Proportion (%) at 0°C		Proportion (%) at 10°C		Proportion (%) at 25°C	
	NH_2Cl	$NHCl_2$	NH_2Cl	$NHCl_2$	NH_2Cl	$NHCl_2$
4	0	100	0	100	0	100
5	34	66	20	80	13	87
6	77	23	67	33	57	43
7	94	6	81	9	88	12
8	99	1	98	2	97	3
9	100	0	100	0	100	0

[a]Source: NRC, 1980

[b]Consideration of kinetic effects leads to calculated values with lower proportions of $NHCl_2$

III. TOXICOKINETICS

Introduction

Studies relevant to the toxicokinetics of inorganic chloramines are severely limited. However, studies done with various chlorinated amino compounds (including organic chloramines) give information on the pharmacokinetics of chloramines.

Absorption

Scully et al. (1985) have shown that solutions of hypochlorite can react with amines and amino acids in stomach fluid to form the corresponding N-chloramines. In separate experiments piperidine (0.2 M solution) and glycine (0.5 M solution) were administered to Sprague-Dawley rats followed by a solution (either ~200 or 1000 mg/L) of aqueous hypochlorite. Stomach fluid was recovered from these animals and shown to contain the corresponding N-chloramino derivatives. The "chlorine demand" of stomach fluid was determined by chlorinating the stomach fluid to various levels, incubating the chlorinated fluid in the dark for 1 hour and measuring the residual chlorine - in essence developing a breakpoint curve for the fluid. The chlorine dosage at which a breakpoint was found was taken as the chlorine demand. The shape of the breakpoint curve was very similar to that of wastewater, exhibiting a significant irreducible minimum at the breakpoint and a high chlorine demand (400-800 mg/L) (Scully et al., 1986). Scully et al. (1985) also found that when N-chloropiperidine (1.3 mL of 1700 mg/L as chlorine containing 100 μCi tritium labeled N-chloropiperidine) was administered to

Sprague-Dawley rats, small amounts (1-3 μCi) of the added chloramine appeared in the plasma at 30, 60 and 120 minutes after the exposure.

Abdel-Rahman et al. (1983) administered single oral doses of 3 mL $NH_2{}^{36}Cl$ (370 mg/L) to four male Sprague-Dawley rats that had been fasted overnight. Each rat received 1.1 mg of test material (~4.6 mg/kg/day). A peak ^{36}Cl plasma level (10.3 μg/L) was reached 8 hours after administration, and the absorption rate constant was 0.278 mg/hour with an absorption half-life of 2.5 hours. The ^{36}Cl plasma level remained at a plateau from 8 to 48 hours after administration. After 48 hours the radiolabel was eliminated from the plasma with a half-life of 38.8 hours and a rate constant of 0.018 mg/hour.

Toxicokinetics data on other absorption rates with respect to various dosage media and different routes of exposure were not available.

Distribution

At 24 hours following the single oral treatment to rats of $NH_2{}^{36}Cl$ as described above, Abdel-Rahman et al. (1983) found the level of ^{36}Cl radioactivity in the plasma to be 0.87%/mL of the administered dose. During the first 24 hours the portion of the administered $NH_2{}^{36}Cl$ dose eliminated through the urine was 0.40% and 0.08% by the feces. After the addition of TCA, 0.14%/mL was precipitated from 1 mL of plasma, possibly indicating binding to the protein fraction. Packed cells had an activity of 0.20%/mL that decreased to 0.06%/mL after washing twice with saline, indicating that

most of the ^{36}Cl radioactivity in the packed cells was loosely bound to the erythrocyte membrane or was exchangeable with the chloride in saline.

Abdel-Rahman et al. (1983) also characterized the subcellular distribution of ^{36}Cl radioactivity in rat liver preparations 24 hours following $NH_2{}^{36}Cl$ administration. The major portion of the activity (75%) in the whole liver homogenate was recovered in the cytosol, 2.5% was recovered in the microsomal, 1.5% in the nuclear and <0.1% in the mitochondrial fractions. Only 4.0% of the total ^{36}Cl radioactivity of the whole homogenate was precipitated by TCA. In the control experiment using $^{36}Cl^-$ Suh and Abdel-Rahman (1983) found a similar liver subcellular distribution of ^{36}Cl radioactivity: 0.82 μg/g in the whole homogenate, 0.66 μg/g in the cytosol, 0.03 μg/g in the microsomes, 0.01 μg/g in nuclei, and 0.001 μg/g in mitochondria. ^{36}Cl was administered as 200 mg/L $Na^{36}Cl$ (0.6 mg/animal) solution orally.

The distribution of ^{36}Cl in various tissues was determined 120 hours following $NH_2{}^{36}Cl$ administration (Abdel-Rahman et al., 1983). Plasma contained the highest concentration of ^{36}Cl radioactivity (3.15 μg/g), followed by whole blood (2.66 μg/g), skin (2.13 μg/g), testes (2.09 μg/g), packed cells (1.90 μg/g), bone marrow (1.82 μg/g), kidney (1.62 μg/g), lung (1.58 μg/g), stomach (1.53 μg/g), thyroid (1.36 μg/g), thymus (1.36 μg/g), duodenum (1.20 μg/g), spleen (1.11 μg/g), carcass (0.77 μg/g), liver (0.74 μg/g), ileum (0.59 μg/g) and fat (0.18 μg/g) (Figure III-1). In the control experiment with ^{36}Cl-labeled chloride Suh and Abdel-Rahman (1983) using 47% as much labeled

material as in the $NH_2{}^{36}Cl$ study found an approximately proportional amount of ^{36}Cl distributed similarly

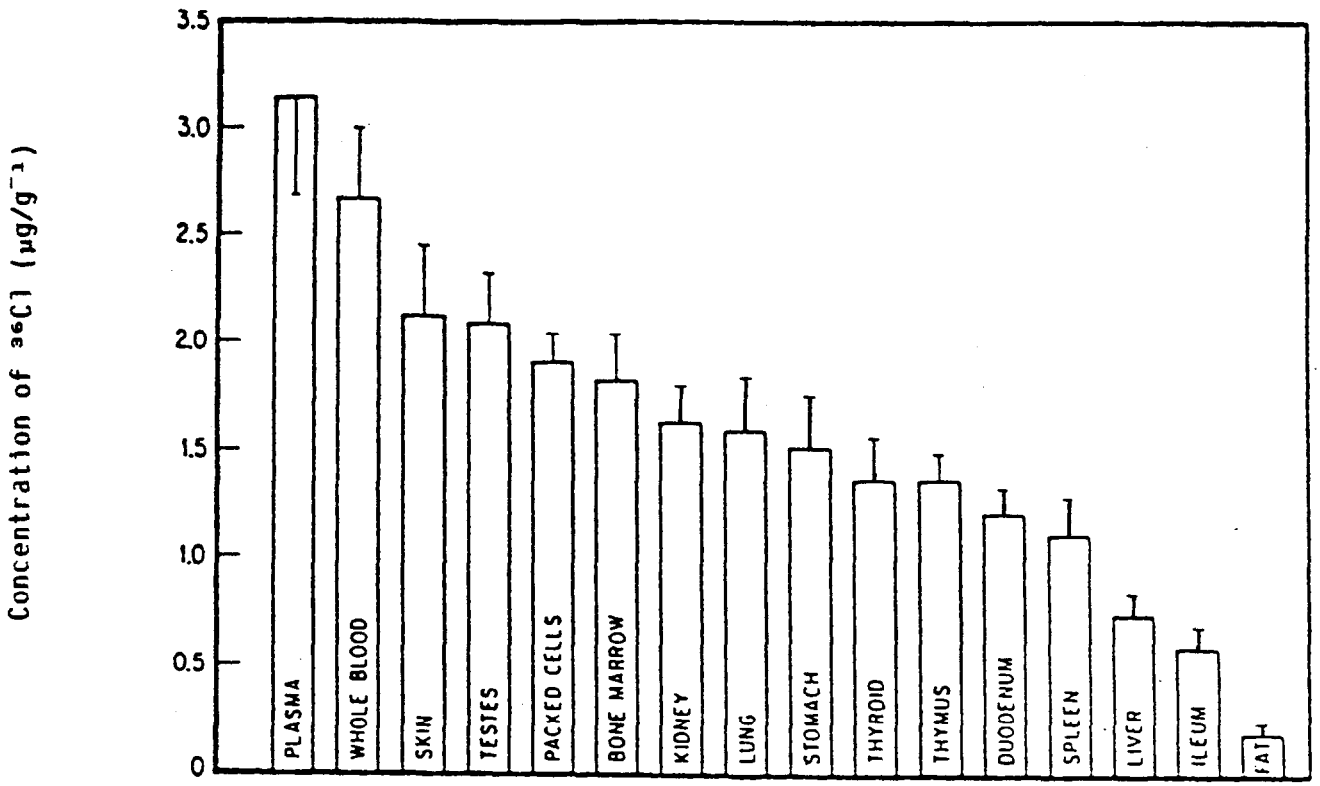

FIGURE III-1

$NH_2{}^{36}Cl$ Radioactivity Distribution in the Rat 120 Hours After the Administration of $NH_2{}^{36}Cl$ (1.1 mg) Orally

Source: Abdel-Rahman et al., 1983

throughout the same organs, fluid and tissues (Figure III-2). The ^{36}Cl radioactivity was highest in plasma (1.4 μg/g) followed by whole blood (1.3 μg/g), testes (1.2 μg/g), packed cells (1.1 μg/g), skin (0.9 μg/g), kidney (0.8 μg/g), lung (0.8 μg), bone marrow (0.8 μg/g), stomach (0.7 μg/g), thymus (0.7 μg/g), spleen (0.6 μg/g), duodenum (0.5 μg/g), carcass (0.3 μg/g), liver (0.3 μg/g), ileum (0.4 μg/g) and fat (0.2 μg/g).

Metabolism

Abdel-Rahman et al. (1983) measured chloride, chlorite and chlorate in plasma 120 hours after administration of $NH_2^{36}Cl$ in rats for determination of its metabolites. Neither ^{36}Cl-labeled chlorite nor chlorate was detected in rat plasma. Most of the total ^{36}Cl was identified as ^{36}Cl-chloride, which (according to the investigators) indicated that the chlorine moiety was eliminated primarily in this form (Table III-1).

Scully et al. (1986) identified three organic N-chloramines (N-chloroglycine, N-chloroalanine and N-chlorophenylalanine) formed *in vitro* when stomach fluid from Sprague-Dawley rats was chlorinated with a concentration of 400 mg/L hypochlorite. After *in vitro* chlorination of stomach fluid from rats fasted for 8, 24 and 48 hours, Scully et al. (1990) identified and quantified N-chloroleucine or N-chloroisoleucine, N-chloroglycine and N-chlorophenylalanine by GC/MS. Scully et al. (1986) also found that because stomach fluid already contains high concentrations of amino nitrogen, the percent conversion of a single exogenous amine to its chloramino compound is low. They determined the actual yield of chloramine by administration of tritium-labeled piperidine followed by aqueous hypochlorite. The quantity of N-chloropiperidine formed

FIGURE III-2

$^{36}Cl^-$ distribution in the rat. Four fasted rats were each administered 200 mg/L $Na^{36}Cl$ in a 3 mL solution orally. The rats were sacrificed 120 hours after administration, and $^{36}Cl^-$ was determined in different organs. Values represent the mean ± SE as μg $^{36}Cl^-$/mL (or per gram tissue) from four rats.

Source: Suh and Abdel-Rahman, 1983

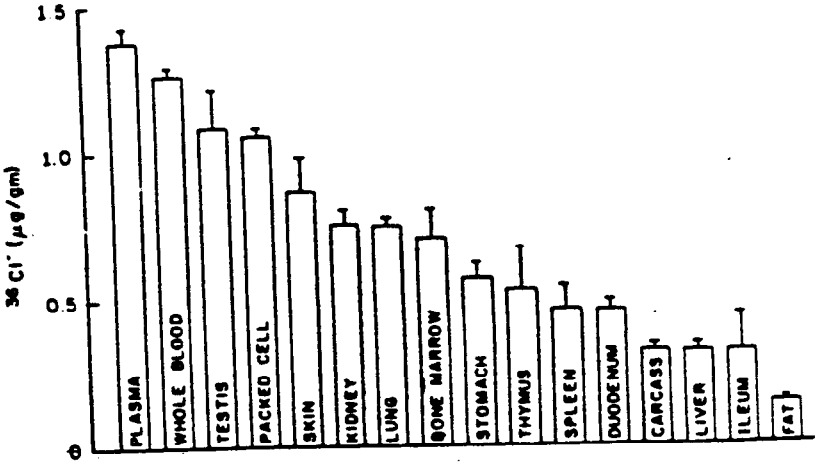

FIGURE III-2

$^{36}Cl^-$ distribution in the rat. Four fasted rats were each administered 200 mg/ℓ $Na^{36}Cl$ in a 3 mℓ solution orally. The rats were sacrificed 120 hours after administration, and $^{36}Cl^-$ was determined in different organs. Values represent the mean ± SE as μg $^{36}Cl^-$/mℓ (or per gram tissue) from four rats.

Source: Suh and Abdel-Rahman, 1983

was determined by direct liquid chromatography of the compound. This quantity combined with the average chlorine demand determined for stomach fluid was used to calculate the yield of chloramine formed. For concentrations of hypochlorite ranging from 200-1000 mg/L, yields of organic chloramines varied from 50-75% of the theoretical amount expected for these compounds. However, Scully et al. (1986) showed that administration of hypochlorite to animals at low (<40 mg/L), medium (40-800 mg/L) and high (>800 mg/L) dosages can produce very different results. This may be due to different chemical reactions taking place at varying hypochlorite concentrations below the minimum chlorine demand. They cautioned that toxicologic studies conducted at high oxidant concentrations may not reflect chloramine reactions that would account for any toxicologic effects observed at lower oxidant concentrations or at actual drinking water concentrations.

Under conditions designed to simulate the GI tract, Bercz and Bawa (1986) found that monochloramine caused covalent binding of radioiodide to nutrient biochemicals. Saliva and gastric juice were obtained from rhesus monkeys under mild anesthesia. Depending on their solubilities, nutrient substrates were dissolved in solvents and added to a mixture of 600 ppm monochloramine, 0.02 N HCl and 0.1 M KI. Monochloramine was believed to oxidize iodide to iodine, which subsequently reacted with nutrient chemicals to form iodinated organic compounds. Tyrosine, 4-aminobenzoic acid, arachidonic acid, and folic acid were among the compounds that became iodinated under the conditions of the experiment. Some of the reactions occurred under basic pH conditions. The observed percent binding was generally lower for reactions occurring

at lower pHs. While complex mixtures of nutrients such as gastric juice and saliva appeared to bind iodine in dilute aqueous solution, it is important that these results be correctly extrapolated to physiologic pH before their significance is fully understood.

The biologic effects of chloramine, its persistence in biologic fluids and its possible conversion to more toxic products were examined by the U.S. EPA (1990). The persistence of monochloramine in saliva and gastric fluid was examined to determine the extent of formation of the products dichloramine, trichloramine and molecular chlorine. Pooled human saliva and gastric fluid samples were treated with monochloramine to produce samples with initial concentrations of monochloramine between 1.0 and 20 ppm. These samples were continuously analyzed by membrane introduction mass spectrometry and tandem mass spectrometry for monochloramine, dichloramine, trichloramine and chlorine. The continuous monitoring experiments for the saliva monochloramine mixtures were done using the technique of selected ion monitoring. Because of constituents present in the gastric fluid samples, selected ion monitoring experiments were shown not to give reliable results and the multiple reaction monitoring procedure was used.

Monochloramine was completely depleted in saliva in ~5 minutes at the 1 ppm level, the 5 ppm solution was incompletely depleted in 2 hours and the monochloramine in the higher concentration solutions was largely unaffected. Dichloramine, trichloramine and molecular chlorine were not produced in detectable levels from the chloramine-treated saliva samples.

Gastric fluid monochloramine disappeared completely in <30 seconds at all concentrations, and dichloramine, trichloramine and molecular chlorine were not observed.

Excretion

Abdel-Rahman et al. (1983) collected the urine, feces and expired air of Sprague-Dawley rats over a 5-day period after the administration of 370 mg/L (1.1 mg/animal or ~4.6 mg/kg/day) $NH_2{}^{36}Cl$ to four Sprague-Dawley rats. The amount of ^{36}Cl-radiolabeled material excreted by urinary and intestinal routes are summarized in Table III-2. During the first 24 hours after administration of $NH_2{}^{36}Cl$, only 0.40 and 0.08% of the total dose administered was eliminated in the urine and feces, respectively. The proportion of the dose eliminated through the urine and feces at the end of the 120-hour study period was 25.15 and 1.98%, respectively. By comparison, Suh and Abdel-Rahman (1983) found that over twice as much of the ^{36}Cl-radiolabel was eliminated over the 120-hour study period when ^{36}Cl-radiolabeled chloride ion (200 mg/L as $Na^{36}Cl$) was administered (Table III-3). They found that 57.2% of the administered ^{36}Cl-radiolabel was eliminated in urine and 3.0% was eliminated in feces. Unlike $NH_2{}^{36}Cl$, more of the ^{36}Cl-label was eliminated in the first 24 hours, 16.1% in urine and 0.92% in feces. After 48 hours ^{36}Cl was eliminated with a half-life similar to that found after 24 hours in the $NH_2{}^{36}Cl$ study ($t_{1/2}$ = 24 hours).

Discussion

In order to interpret the results of the toxicokinetics correctly, it is necessary to understand the complications of the synthesis of $NH_2{}^{36}Cl$ and possible interferences.

^{36}Cl-radiolabeled chloride ion is available commercially. $HO^{36}Cl$ is synthesized by acidic permanganate oxidation of the $^{36}Cl^-$ to $^{36}Cl_2$:

$$2KMnO_4 + 16H^{36}Cl = 2KCl + 2MnCl_2 + 5^{36}Cl_2 + 8H_2O.$$

The ^{36}Cl-labeled Cl_2 gas is passed from the generation flask to a receiving flask where it is dissolved in deionized water:

$$^{36}Cl_2 + H_2O = HO^{36}Cl + H^{36}Cl$$

The resulting hydrolysis converts half the labeled chlorine to $HO^{36}Cl$, the desired compound, and half to $^{36}Cl^-$.

Chlorine-36 radiolabeled inorganic chloramine is synthesized by reaction of the solution of $HO^{36}Cl$ (containing an equivalent amount of $^{36}Cl^-$) with aqueous ammonia in bicarbonate buffer:

$$NH_3 + {}^{36}ClO^- = NH_2{}^{36}Cl + HO^-$$

However, the observed kinetics of $NH_2{}^{36}Cl$ absorption and elimination are necessarily affected by the presence of an equivalent amount of $^{36}Cl^-$ formed during the preparation of the $HO^{36}Cl$.

In addition, the stomach contains high concentrations of Cl^-. There is a lack of information on the rate of chloramine-chloride exchange

$$NH_2{}^{36}Cl + Cl^- = NH_2Cl + {}^{36}Cl^-$$

in solutions of high chloride concentration. If this exchange rate is significant, the toxicokinetics of $NH_2{}^{36}Cl$ could appear to resemble chloride when, in fact, the compound has simply lost its radiolabel through exchange. This is the case for $HO^{36}Cl$ (Anbar et al., 1959) where the exchange reaction is so fast that at 25°C in the presence of 0.1 M chloride the exchange is 99% complete in <0.1 sec at any pH <10.8. In the absence of specific information on the isotopic exchange rates for $NH_2{}^{36}Cl$, the rate of hydrolysis of NH_2Cl as reported by Margerum et al. (1979) ($k = 1.9 \times 10^{-5}$ sec^{-1}) and by Anbar and Yagil (1962) ($k = 6.3 \times 10^{-5}$ M^{-1} sec^{-1}) would suggest that the rate of exchange is slow and that it would require between 4 and 10 hours for half the radiolabel to be lost at 25°C. Nevertheless, monochloramine exchanges its chlorine with organic amino nitrogen compounds at a rate faster than hydrolysis (Snyder and Margerum, 1982; Isaac and Morris, 1983, 1985). Since the stomach contains high concentrations of organic amino nitrogen, the chlorine from the $NH_2{}^{36}Cl$ can be transferred to these organic amino nitrogen compounds. How this affects the rate of isotope exchange is unknown.

The control reagent used in the above toxicokinetic study (Anbar et al., 1959) of $NH_2{}^{36}Cl$ was $^{36}Cl^-$. It is not likely that an oxidant as strong as NH_2Cl will survive intact absorption, distribution, and excretion from an animal. If the chloramine is rapidly detoxified in the stomach to ^{36}Cl-radiolabeled chloride and ammonia, the observed toxicokinetics will be identical to the control kinetics. However, if the chloramine acts as a chlorinating agent, other more stable chlorinated organic compounds may form. The observed kinetics may then be due to exposure to these chlorinated compounds.

Summary

Information on the absorption of inorganic chloramines is extremely limited. In one study Abdel-Rahman et al. (1983) calculated an absorption rate constant for ^{36}Cl at 0.278 mg/hour (after 8 hours) with an absorption half-life of 2.5 hours after male Sprague-Dawley rats were administered a single oral dose of ~4.6 mg/kg/day. After 48 hours the radiolabel was eliminated from the plasma with a half-life of 38.8 hours and a rate constant of 0.018 mg/hour. Absorption rates with respect to various dosage media and different routes of exposure were not available. However, studies done with various chlorinated amino compounds (including organic chloramines) give information on the pharmacokinetics of chloramines. Scully et al. (1985) found that when the organic chloramine, N-chloropiperidine, was administered to Sprague-Dawley rats, 1-3 μCi of the chloramine appeared in the plasma at 30, 60 and 120 minutes after the exposure.

The distribution of radiolabeled chlorine in the subfractions of rat liver homogenates, in organs, tissues and fluids was similar (120 hours) after oral administration of either

^{36}Cl (200 mg/L as $Na^{36}Cl$) or $NH_2^{36}Cl$ (370 mg/L $NH_2^{36}Cl$) (Abdel-Rahman et al. 1983; Suh and Abdel-Rahman (1983). Plasma contained the highest concentrations of ^{36}Cl radioactivity for $NH^{36}Cl$ followed by whole blood skin, testes, packed cells, bone marrow, kidney, lung, stomach, thyroid, thymus, duodenum, spleen, carcass, liver, ileum and fat (Abdel-Rahman et al., 1983).

Information on the metabolism of chloramines is also extremely limited. Abdel-Rahman et al. (1983) indicated that chloramine ($NH_2^{36}Cl$) administered to rats was metabolized primarily to ^{36}Cl-chloride, which indicated that the chlorine moiety was eliminated in this form.

When pooled saliva and gastric fluid were treated with monochloramine (U.S. EPA, 1990), saliva monochloramine was depleted in 5 minutes at 1 ppm, and in higher concentrations was not affected, while gastric monochloramine disappeared in <30 seconds at all concentrations tested. Dichloramine, trichloramine and molecular chlorine were not produced in detectable levels from the chloramine-treated saliva or gastric samples.

Chloramines or their metabolites (principally chloride) are eliminated primarily through the urine. Abdel-Rahman et al. (1983) found that during the first 24 hours after a single administration of $NH_2^{36}Cl$ (1.1 mg/animal) to Sprague-Dawley rats, only 0.40-0.08% of the total dose was eliminated in the urine and feces, respectively. At the end of 120 hours 25.15 and 1.98% of the dose was eliminated in the urine and feces,

respectively. By comparison, Suh and Abdel-Rahman (1983) found that over twice as much of the ^{36}Cl-radiolabel was eliminated over a 120-hour period when 200 mg/L of $Na^{36}Cl$ was administered orally to rats. Unlike $NH_2{}^{36}Cl$, more of the ^{36}Cl-label was eliminated in the first 24 hours, 16.1% in urine and 0.92% in feces. The major difference between the $NH_2{}^{36}Cl$ and $Na^{36}Cl$ studies (Abdel-Rahman et al., 1983; Suh and Abdel-Rahman 1983) was in the total amount of label excreted over the test period. Only half as much label was eliminated in the chloramine study as in the chloride study.

Interpretation of the toxicokinetics of inorganic chloramines is difficult since synthesis of $NH_2{}^{36}Cl$ is complicated by possible interferences. There is a lack of information on the rate of chloramine/chloride exchange in solutions of high chloride concentrations.

$$NH_2{}^{36}Cl + Cl^- = NH_2Cl + {}^{36}Cl^-$$

If this exchange rate is significant the toxicokinetics of $NH_2{}^{36}Cl^-$ could appear to resemble chloride when, in fact, the compound has simply lost its radiolabel through exchange. Since the stomach contains high dose concentrations of organic amino nitrogen, the chlorine from the $NH_2{}^{36}Cl$ can be transferred to these organic amino nitrogen compounds (Snyder and Margerum, 1982; Issac and Morris, 1983, 1985). How this affects the rate of exchange is unknown.

TABLE III-1

Metabolism of $NH_2{}^{36}Cl$ in Rat Plasma[a]

Treatment	Analyte (%/mL)[b,c]	
	Total ^{36}Cl	^{36}Cl
$NH_2{}^{36}Cl$ (1.1 mg)	0.41 ± 0.08	0.35 ± 0.08

[a]Source: Abdel-Rahman et al., 1983

[b]Neither $^{36}ClO_2$ nor $^{36}ClO_3$ was detected in rat plasma at the time period studied.

[c]Values represent mean ± SE from seven (fasted) rats after 120 hours following $NH_2{}^{36}Cl$ treatment expressed as percentage of total administered dose per mL of plasma.

TABLE III-2

Excretion of ^{36}Cl in Rat 120 Hours After Single Oral Administration of $NH_2{}^{36}Cl$[a]

Collection Period (hours)	Proportion of $NH_2{}^{36}Cl$ Excreted (%)[b]		
	Urine	Feces	Total
0-8	0.07 ± 0.07		
8-16	0.13 ± 0.02		
16-24	0.21 ± 0.08		
0-24	0.40 ± 0.02	0.08 ± 0.05	0.48 ± 0.02
24-48	6.28 ± 2.87	0.48 ± 0.24	6.75 ± 3.10
48-72	6.30 ± 5.52	0.31 ± 0.29	6.60 ± 5.80
72-96	5.03 ± 3.03	0.26 ± 0.21	5.29 ± 3.24
96-120	7.16 ± 1.93	0.87 ± 0.49	8.02 ± 1.44
0-120	25.15 ± 13.32	1.98 ± 0.29	27.13 ± 13.61

[a]Source: Abdel-Rahman et al., 1983

[b]Values represent the mean ± SE from four treated (fasted) rats expressed as the proportion of administered dose. ^{36}Cl was not detected in expired air throughout the 120 hours studied.

TABLE III-3

Excretion of $^{36}Cl^-$ in Rat 120 Hours After Single Oral Administration of $Na^{36}Cl$[a]

Collection Period (hours)	Proportion of $Na^{36}Cl$ Excreted (%)[b]		
	Urine	Feces	Total
0-12	8.2 ± 1.9		
12-24	9.7 ± 1.9		
0-24	16.1 ± 3.8	0.92 ± 0.43	17.0 ± 3.4
24-48	8.2 ± 1.8	0.43 ± 0.12	8.6 ± 1.8
48-72	14.8 ± 3.2	0.55 ± 0.50	15.4 ± 2.8
72-96	9.5 ± 1.9	0.93 ± 0.17	10.4 ± 2.0
96-120	8.6 ± 1.6	0.17 ± 0.07	8.8 ± 1.6
0-120	57.2 ± 10.6	3.0 ± 0.91	60.2 ± 9.7

[a]Source: Suh and Abdel-Rahman, 1983

[b]Values represent the mean ± SE from four treated rats, expressed as percentage of administered dose. $^{36}Cl^-$ was not detected in expired air through the 120-hour study.

V. HEALTH EFFECTS IN ANIMALS

Introduction

Animal studies on chloramines are limited. Few health-related studies have been conducted on chloramines in drinking water. Since chloramines may be used as an alternate means of disinfection, more studies are being conducted. Summaries of the acute, subchronic and chronic oral studies are presented in Tables V-1 and V-2.

Short-Term Exposure

Because individuals undergoing long-term hemodialysis have been shown to develop hemolytic anemia when water containing monochloramine (NH_2Cl) has been inadvertently used in the dialysis treatment, hematologic parameters have been examined in several studies. Moore et al. (1980) exposed 12 male A/J mice per group for 30 days to a bicarbonate buffered solution (pH 8.9) of monochloramine in drinking water at concentrations of 2.5-200 mg/L. They found no statistically significant (two-way analysis and Kruskal-Wallis one-way analysis of variance) changes in nine measured blood parameters; however, hematocrit did rise slightly with increasing levels of monochloramine. The authors concluded that monochloramine ingested by A/J strain mice did not appear to produce hemolysis even at very high dose levels.

Abdel-Rahman et al. (1984) investigated the toxicity of NH_2Cl in male Sprague-Dawley rats. Acute exposure to a single dose at 10, 20 or 40 mg/L NH_2Cl induced a statistically significant ($p<0.05$) increase in blood glutathione levels within 30

TABLE V-1

Summary of Studies on Acute and Subchronic Oral Exposure to Monochloramine

Mode of Administration	Species	Sex, Weight	Duration	Dose	Effect	Reference
Gavage	Sprague-Dawley rats	4 males/group, 150-170 g	1 dose	10 mg/L x 3 mL (0.18-0.2 mg/kg bw)	Significant increase in blood glutathione at 30 and 60 minutes (7.9 and 26%, respectively), but not at 15 or 120 minutes. No significant effect on blood osmotic fragility.	Abdel-Rahman et al., 1984
				20 mg/L x 3 mL (0.35-0.4 mg/kg bw)	Significant increase in blood glutathione at 15, 30 and 60 minutes (17, 15 and 25%, respectively) but not at 120 minutes. No significant effect on blood osmotic fragility.	
				40 mg/L x 3 mL (0.71-0.8 mg/kg bw)	Significant increase in blood glutathione at 15, 30 and 60 minutes (13, 17 and 33%, respectively) but not at 120 minutes. No significant effect on blood osmotic fragility.	
Oral/drinking water	African Green monkeys	5 males, 7 females, 3.0-5.7 kg	6 weeks	100 mg/L	No detectable effect in 18 hematologic tests.	Bercz et al., 1982
Oral/drinking water	CD-1 mice	10 males, 10 females	30 days	100x*	No significant differences in body weight; significant increase in liver weights in females; significant decrease in lung weights of both sexes; significant decrease in brain and kidney weights in males.	Miller et al., 1986
				400x*	No significant difference in body weight; significantly decreased kidney and liver weights in males and significantly increased ovary weights in females.	
Oral/drinking water	rat	NS	45 days	10, 50 or 100 mg/L	No effect on weight gain or hematologic parameters	Bull, 1980

TABLE V-1 (cont.)

Mode of Administration	Species	Sex, Weight	Duration	Dose	Effect	Reference
Oral/drinking water	B6C3F1 mice	10/sex/group	91 days	25 and 50 mg/L	No adverse effects	GSRI, 1981
				100, 200 and 400 mg/L	Chronic liver inflammatory changes; increased frequency of mitotic figures, hypertrophy and bizarre chromatin patterns; decreased liver weights at 400 mg/L in males and >100 mg/L in females.	
	Fischer 344 rats			25, 50 and 100 mg/L	No adverse effects	
				200 and 400 mg/L	Decreased body weights; trend toward reduced liver size but not significant.	
Oral/drinking water	Sprague-Dawley rats	10/sex/group	90 days	25 mg/L	No adverse effects	Daniel et al., 1990
				50 mg/L	Significant reduction in body weight gain in males. Males had decreased absolute lung weights.	
				100 mg/L	Significant reduction in body weight gain in males. Significant decreased absolute liver weights.	
				200 mg/L	Reduction in body weight gain; average weight gain was 51% of controls; decreased organ weights (absolute and relative); liver and spleen weights decreased in males and females.	

TABLE V-1 (cont.)

Mode of Administration	Species	Sex, Weight	Duration	Dose	Effect	Reference
Oral/drinking water	B6C3F1 mice	10/sex/group	90 days	12.5 and 25 mg/L	Decrease in MCV in females, decrease in Alk-P (25 mg/L) (neither effect considered treatment related).	Daniel et al., 1991
				50 mg/L	Decrease in MCV in females, significant decreases in Alk-P in males and significant increases in AST in females (none considered treatment related).	
				100 and 200 mg/L	Significant differences in weight gain and water consumption. Increase in lymphocytes in males and decrease in kidney and lung weights in both sexes. In males decreased absolute testes and spleen weights (200 mg/L). Also at 200 mg/L signficant increases in relative brain, kidney, lung and testes in males and brain and kidney in females.	
Oral/drinking water	Sprague-Dawley rats	12 males/dose, 100 g	9 weeks	9, 19 and 38 mg/L	Significant reduction in spleen weight (38 mg/L); decreased antibody synthesis (9 and 19 mg/L); augmented PGE2 production (19 and 38 mg/L)	Exon et al., 1987

*Samples were concentrated equal to the original concentrate (400x) or one-fourth of the original concentrate (100x). The original concentrate or monochloramine residual was 2.1 mg/L. The original concentrate of chloramines was not specified.

NS = Not specified

TABLE V-2

Summary of Studies on Chronic Oral Exposure to Monochloramine in Drinking Water

Species	Sex, Weight	Duration	Dose	Effect	Reference
Sprague-Dawley rats	4 males/group, 150-570 g	12 months	1 mg/L (~0.1 mg/kg bw/day)	Significant decrease in blood glutathione at 4, 6 and 12 months (27, 24 and 25%, respectively). No effect on blood osmotic fragility. No effect on blood cell compartment. Increase in [^{3}H]-thymidine incorporation in nuclei, significant in kidney (190%) and spleen (230%) but not liver, testes or intestinal mucosa, at 3 months. No effect on body weight.	Abel-Rahman et al., 1984
			10 mg/L (~1.0 mg/kg bw/day)	Significant decrease in blood glutathione at 6 and 12 months (20 and 24%, respectively); significant increase at 10 months (27%). Significant increase in blood osmotic fragility at 2 and 10 months (44 and 39%, respectively). Significant decrease in RBC count (8.8%) and HCT (11%) at 3 months, no effect on HGB, MCV, MCH or MCHC. Significant increase in [^{3}H]-thymidine incorporation in nuclei of kidney (290%) and spleen, (160%) but not other organs, at 3 months. No effect on body weight.	
			100 mg/L (~10 mg/kg bw/day)	Significant decrease in blood glutathione at 4, 6 and 12 months (20, 23 and 22%, respectively). Significant increase in blood osmotic fragility at 2 and 6 months (27 and 67%, respectively). Significant decrease in RBC count (8.8%) and HCT (12%) at 3 months, and decrease in HGB (22%) and MCH (23%) at 10 months. Significant increase in [^{3}H]-thymidine incorporation in nuclei of testes (200%) at 3 months. Body weight significantly reduced at 3-12 months (8.4-17%).	
F344/N rats	10/sex/group	2 years	50 ppm (2.1-2.8 mg/kg/day)	No treatment-related effects	NTP, 1990
			100 ppm (4.8-5.3 mg/kg/day)		
			200 ppm (8.7-9.5 mg/kg/day)	Lower mean body weights and decreases in liver and kidney weights in males and increases in brain and kidney-to-body weight ratios at 14- or 66-week evaluations.	

TABLE V-2 (cont.)

Species	Sex, Weight	Duration	Dose	Effect	Reference
B6C3F1 mice	10/sex/group	2 years	50 ppm (5.0-44.9 mg/kg/day)	No treatment-related effects	NTP, 1990
			100 ppm (8.9-9.0 mg/kg/day)		
			200 ppm (15.9-17.2 mg/kg/day)	Mean body weights of male mice were 10-22% lower after week 37 and body weights of female mice were 10-35% lower after week 8. At 15 or 66 weeks decreases in liver weights and increases in brain or kidney-to-body-weight ratios occurred.	

minutes after administration of a 3 mL aqueous solution by gavage. On the other hand, chronic administration at 1, 10 or 100 mg/L doses in drinking water induced a statistically significant ($p<0.05$) decrease in glutathione after 4 months of exposure at the 1 and 100 mg/L dose levels. The difference in glutathione blood levels following subchronic versus chronic exposures may be due to different physiologic responses such as 1) an increase in reduced glutathione levels resulting in increased RBC glutathione reductase activity in subchronically exposed animals, and 2) a decrease in reduced glutathione levels in chronically exposed animals, which is due to depletion of glutathione body stores.

Bercz et al. (1982) studied the toxicity of monochloramine administered in drinking water to 5 adult males and 7 adult female African Green monkeys with body weights ranging from 3.0-5.7 kg. Monochloramine was administered for 6 weeks at 100 mg/L. The authors estimated the mean daily dose to be ~10 mg/kg/day. Such ingestion of monochloramine had no detectable effect in 18 hematologic tests on the 12 monkeys, including red cell GSH levels. No evidence of thyroid suppression was detected in serum. Maziarka et al. (1976) also found that rats exposed for 9 months to 9.0 mg/L chloramines showed no observable hematologic effects. However, the study was not a dose-response type and many of the blood parameters reflecting oxidant stress were not measured.

In a review of alternate disinfectants Bull (1980) reported the results of a 45-day study in which 10, 50 or 100 mg/L (1.4, 7.0 and 14.0 mg/kg assuming rats consume 0.14 L/day) monochloramine in drinking water was administered to laboratory rats. Body

weight gain and hematologic parameters in exposed animals did not differ significantly from control animals. The only significant finding was a decrease in the amount of methemoglobin present in the blood at 100 mg/L monochloramine. This is the opposite of what was expected. There were no signs of overt toxicity (Bull, 1980). Details of this study were not reported.

A subchronic 30-day toxicity test was performed on groups of 10 male and 10 female CD-1 mice using concentrated drinking water samples collected at a pilot scale drinking water treatment plant using monochloramine for disinfection purposes (Miller et al., 1986). The residual monochloramine level was 2.1 mg/L before concentration of organics by reverse osmosis. Samples were concentrated (100x or 400x) before toxicologic testing. Post exposure, the mice were examined for gross pathologic changes. Body weights were not significantly different from controls. There was a statistically significant increase ($p<0.05$) in liver-to-body weight ratios of females and a statistically significant decrease ($p<0.05$) in the lung weights of males and females at 100x. The brain and kidney weights were significantly decreased ($p<0.05$) in males at 100x. At 400x the kidney and liver weights of males were significantly decreased ($p<0.05$) and the ovary weights of females were significantly increased ($p<0.05$). The authors conclude that these data did not reveal any overt toxicity; therefore, histopathologic examinations of major organs was not performed.

A draft report by GSRI (1981) investigated the effects following exposure to monochloramine in Fischer 344 rats and B6C3F1 mice. Rats (10/sex/group) were

administered concentrations of 0, 25, 50, 100, 200 and 400 ppm (25, 50, 100, 200 and 400 mg/L) monochloramine in drinking water ad libitum for 91 days. Using water and food consumption data provided in the report, corresponding doses were calculated to be 2.5, 4.9, 10.2, 18.8 and 40.7 mg/kg/day for males and 3.8, 6.5, 13.8, 26.6 and 53.9 mg/kg/day for females. No animals died during the course of the study. After 25 days the buffer system for monochloramines was changed because of a palatability problem at the high-dose levels. Terminal body weight gains in male rats decreased at the 200 and 400 ppm levels by 13 and 24%, respectively, when compared with their controls. Female rats gained 8 and 14% less weight than their controls at the 200 and 400 ppm levels, respectively. The decrease in the percentage of body weight gain was most marked at 0-4 weeks and appeared to be related to the palatability of the 200 and 400 ppm solutions. Absolute liver weights showed a trend toward reduced liver size; however, when liver weights were expressed as a percentage of total body weight no significant differences were observed. Protein excretion increased in male rats when 200 and 400 ppm monochloramine was administered. Microscopic examination of tissues at the 400 ppm level did not reveal any treatment-related lesions. This study identified a NOAEL for rats of 100 ppm (10.2 mg/kg/day). (Confidence in this study is low because of questions regarding the conduct of this study, the histopathologic evaluations and lack of corroboration of its findings.)

Similarly, mice (10/sex/group) were administered concentrations of 0, 25, 50, 100, 200 and 400 ppm (25, 50, 100, 200 and 400 mg/L) monochloramines in drinking water ad libitum for 91 days. Using water and food consumption data provided in the report

corresponding doses were 4.9, 8.3, 14.5, 31.3 and 50.7 mg/kg/day for males and 7.7, 12.1, 21.9, 34.6 and 88.5 mg/kg/day for females. During the course of the study no animals died. The buffer system for monochloramines was changed after 25 days since palatability was a problem at the high dose levels. Male and female mice gained less weight than the controls at the 200 and 400 ppm levels. There was a reduction in absolute liver weights and liver-to-body weight ratios in male mice at the 400 ppm levels and in female mice at $\geq$100 ppm. Histopathologic observations revealed necrotic changes at the low doses and mild to moderate cytologic alteration in the livers of male mice administered 200 and 400 ppm. Chronic liver inflammatory changes occurred at 100, 200 and 400 ppm in female mice and to a lesser extent in male mice at the 100 ppm level. At concentrations of 100, 200 and 400 ppm increased frequency of mitotic figures, hypertrophy and bizarre chromatin patterns occurred in males and in one female at 200 ppm. This study identified a NOAEL for mice of 50 ppm (8.4 mg/kg/day).

In a more recent study, Daniel et al. (1990) administered 0, 25, 50, 100 and 200 mg/L monochloramine to male and female Sprague-Dawley rats (10/sex/dose) in their drinking water for 90 consecutive days. Using food and water consumption data provided in the report, corresponding doses were calculated to be 0, 1.8, 3.4, 5.8 and 9.0 mg/kg/day for males and 0, 2.6, 4.3, 7.7 and 12.1 mg/kg/day for females. A control group received distilled water buffered with sodium bicarbonate to a pH of 8.0-8.5. Mortality, clinical signs, body weight, organ weights, food consumption, hematology, clinical chemistry, gross pathology, and histopathology were examined. Water consumption was significantly decreased at all monochloramine doses. At 200 mg in males water

consumption was ~31% of controls and in females at 200 mg water consumption was ~34% of controls.

Food consumption was significantly reduced in males at the highest dose tested. In males there was a significant reduction in body weight gain at doses of 50 mg/L and higher. The reduced body weight gain in females was significant only at 200 mg/L. For males and females the average weight gain at 200 mg/L was ~51% of controls. At the 200 mg/L dose, reductions in the absolute weight of liver and spleen were observed in both sexes. In females, the absolute weight of the thymus was also decreased as was the weight of the lung and heart of the males. Also at 200 mg/L, females had increased relative kidney and decreased relative liver weights while males had increased relative brain, testes and kidney weights.

At 100 mg/L males had significantly decreased absolute liver weights and at 50 mg/L males had decreased absolute lung weights. At the 25 mg/L dose, there was no significant effect on absolute organ weights in either sex. The authors concluded that the 100 mg/L dose is considered the NOAEL. Although reductions in organ weights appeared to be dose-related in males, subsequent histopathologic examination did not reveal any target organ or treatment-related changes. Although reduced RBC count (200 mg/L) and decreased calcium levels in males were significant, the authors did not consider them biologically significant, dosage-related or within the normal range for rats of this age and strain.

Daniel et al. (1991) administered monochloramine in drinking water for 90 days to male and female B6C3F1 mice (10/sex/dose) at 0, 12.5, 25, 50, 100 and 200 mg/L. Using food and water consumption data provided in the report, corresponding doses were calculated to be 0, 2.5, 5.0, 8.6, 11.1 and 15.6 mg/kg/day for males and 0, 2.8, 5.3, 9.2, 12.9 and 15.8 mg/kg/day for females. Mortality, clinical signs, body weight, organ weights, food consumption, hematology, clinical chemistry, gross pathology and histopathology were examined. Food consumption was decreased in males and females at the two highest dose levels with a statistically significant decrease in females. Water consumption was decreased in all treated groups with statistically significant decreases in female mice at all treatment levels and male mice at the two highest levels.

In males there was an increase in lymphocytes at 100 and 200 mg/L and in females there was a decrease in neutrophils at 100 mg/L and a decrease in MCV in all dose levels. None of the hematology results were considered to be dose related. Alk-P was decreased in males at all concentrations, statistically significant at 25, 50 and 100 mg/L, while AST was increased in females at all dose levels, and statistically significant at 50 and 200 mg/L.

There were significant reductions in body weight gain in males at the three highest dose levels, and at the two highest dose levels in females. Significant decreases in water consumption occurred in all doses in female mice and at the two highest dose levels in males. Significant reductions in absolute organ weights were evident in males and females drinking 200 mg/L monochloramine. In male and female mice, liver and heart

weights were decreased at the two highest dose levels, and relative and absolute spleen weights were decresed in the two highest doses for females. At the highest dose, the kidney and lung weights were decreased in males and females; also at the highest dose, absolute testes and spleen weights were decreased in males. When analyzed relative to body weight, brain, kidney, lung and testes in males and brain and kidney in females were significantly increased at the highest dose compared with the control value. Based on relatively minor changes at 100 mg/L of monochloramine, including <10% depression of body weight and the greater changes at 200 mg/L (19-25% decreased body weight), the 100 mg/L dose (12.9 mg/kg/day) can be identified as a NOAEL in this study.

No compound-related gross or microscopic lesions were observed, and no target tissues were identified in the monochloramine-treated animals.

Exon et al. (1987) administered monochloramine at 0, 9, 19 and 38 ppm (mg/L) in the drinking water of male Sprague-Dawley rats (12/sex/dose) from weaning to 12 weeks of age (9 weeks of exposure). Based on reference body weight and water consumption values for subchronic exposure (U.S. EPA, 1986), the corresponding intake of monochloramine was 0, 1.3, 2.6 and 5.3 mg/kg/day. Following treatment, the test animals were assessed for immune competence. Parameters of immunity measured were spleen and thymus weights, antibody production, delayed-type hypersensitivity (DTH) reactions, natural killer cell (NKC) cytotoxicity, oxidative metabolism response and phagocytosis by macrophages and production of two immunoregulatory cytokines, interleukin 2 (I12) and prostaglandin E2 (PGE2). Significant ($p<0.05$) reduction of spleen weights (38 ppm),

decreased antibody synthesis (9 and 19 ppm) and augmented PGE2 production (19 and 38 ppm) were reported. The authors state that these results indicate that B lymphocyte, as well as macrophage function may be adversely affected. While monochloramine appears to exert immunotoxic effects, the biologic importance of effects on the immune system as a factor in the toxicity of monochloramines is not clear. In addition, there is a lack of correlation among various endpoints examined, as well as deficiencies in some of the methodologies (i.e., the use of the ELISA test for antibody analysis). Based on these considerations, a NOAEL or LOAEL cannot be determined from this study.

Since pigeons may be susceptible to cardiovascular disease, Revis et al. (1986) investigated the relationship of monochloramine to plasma cholesterol and thyroid levels. 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 monochloramine *ad libitum* for 3 months. The treatment diets consisted of either (A) a diet reduced to 0.35% calcium (80% of the minimal daily requirement for a pigeon) i.e., a normal diet, or (B) a diet reduced to 0.35% calcium with the addition of 10% lard and 0.5% cholesterol, i.e., a high cholesterol diet. The controls were given either diet and deionized water *ad libitum*. 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. The study reported that plasma T_4 levels were significantly decreased in pigeons fed a normal diet (A) or high cholesterol diet (B) and drinking water containing 15 ppm monochloramine. Following a 3-month exposure to diet B and receiving either deionized water or water containing 15 ppm monochloramine, plasma cholesterol levels were

1266±172 and 2049±212 mg/dL, respectively, a difference of 783 mg/dL. Significant increases in plasma cholesterol were also observed in pigeons at the 2 ppm level compared with deionized water and when both groups were fed only the calcium-deficient diet (A). No significant changes were observed in the comparative 15 ppm diet (A) group. T_4 levels were not significantly altered when 2 ppm monochloramine and diet (A) were administered. There was no clear dose-response effect for plasma cholesterol observed in any of the treatment groups. Thus, factors associated with the effects of monochloramine on plasma cholesterol are not known. The authors suggested that the changes in plasma cholesterol may be mediated by products formed when monochloramine reacts with organic matter in the upper GI tract. Pigeons may or may not mimic humans in thyroid function, so the significance of effects in the pigeons relationship to humans is unknown. Also, pigeons were maintained on a calcium deficient diet in order to depict an average human diet. It is not known if pigeons metabolize calcium in the same manner as humans.

Other Short-term Effects

Robinson et al. (1986) treated female Sencar mice (5/dose) with aqueous solutions of monochloramine by whole-body exposure (except head) for a 10-minute period for 4 days to assess hyperplastic effects. The backs of the animals were shaved 3 days before treatment of 1, 10, 100 and 1000 mg/L of monochloramine. Animals were sacrificed the day following the last treatment and skin thickness measured. TPA (12-o-tetradecanoylphorbol13-acetate) was applied at a dose of 1.0 μg in 0.2 mL acetone/mouse to the positive control mice. Epidermal thickness on the fifth experimental

day measured ~32.8 μm in the positive controls. The epidermal layer in control animals given water measured ~15.4 μm. Exposure to 1, 10, 100 and 1000 mg/L monochloramine decreased epidermal thickness (~14.0, 14.4, 13.1 and 13.6, respectively); however, this was not significant when compared with controls. In addition, cell counts were not increased at any of the doses when compared with controls.

Flour bleached with trichloramine administered in the diet has been shown to produce "canine hysteria" or "running fits" in dogs. However, one study suggests that this is a species-specific phenomenon for dogs (Mellanby, 1946; Silver et al., 1947a,b,c; Newell et al., 1947) and does not affect humans (Pollock, 1949). Trichloramine is formed in waters at high chlorine-to-ammonia ratio concentrations and at lower pHs than normally found in drinking water.

Long-Term Exposure

Abdel-Rahman et al. (1984) conducted a study to investigate the toxicity of NH_2Cl in drinking water (see Table V-2). Groups of four male Sprague-Dawley rats drank either 0, 1, 10 or 100 mg/L NH_2Cl in deionized water daily for $\leq$12 months. The levels of chlorine, dichloramine and trichloramine in the NH_2Cl solution were <1%, <1% and 0%, respectively, of the total NH_2Cl added. Food was available ad libitum and body weight was measured during the treatment. Cardiac puncture and collection of heparinized blood was performed at 2, 4, 6, 8, 10 and 12 months after treatment. Hematologic parameters, blood glutathione (GSH) levels and osmotic fragility were monitored throughout the treatment period. ^{3}H-Thymidine incorporation was studied in liver, kidney,

testes, intestinal mucosa and spleen after 3 months of treatment. Increased incorporation of ^{3}H-thymidine was observed in the kidney and spleen at 1 and 10 mg/L and in testes at 100 mg/L at 3 months; other time periods were not examined. Body weights were significatnly reduced after 3 months of treatment at 100 mg/L and remained lower than controls throughout the experiment. Blood levels of chloroform monitored during the study were no different than in untreated rats. Results varied over the 12-month study period, but at 6 and 12 months after initiation of the study statistically lower GSH levels were observed at all doses. After 3 months of treatment significant decreases in RBC count and hematocrit were observed at the higher dose levels. A reduction in hemoglobin concentration and mean corpuscular hemoglobin occurred at 100 mg/L after 10 months of treatment. The decrease in blood GSH could be due to a protective role by GSH against damage caused by oxidants. The health significance of these types of changes is uncertain. Furthermore, results were analyzed by inappropriate statistical methods and a number of "significant" changes in hematologic parameters relative to control values were identified that had no consistent relationship with dose and were not observed consistently throughout the period of exposure.

The NTP (1990) conducted two studies to determine the potential chronic toxicity or carcinogenic activity of chloraminated drinking water. In the first study F344/N (70/sex/dose) rats were administered chloramine for 2 years at doses of 0, 50, 100 and 200 ppm in pH 9 buffered charcoal filtered deionized drinking water. These doses were calculated on the basis of a time-weighted average to be 0, 2.1, 4.8 and 8.7 mg/kg/day for male rats and 0, 2.8, 5.3 and 9.5 mg/kg/day for female rats. There was a dose-related

decrease in the amount of water consumed by both sexes; this decrease was noted during the first week and continued throughout the study. Food consumption of treated rats was the same as the controls with males consuming more. Mean body weights of 200 ppm dosed rats were lower than their control groups. However, mean body weights of rats receiving chloraminated drinking water were within 10% of controls until week 97 for females and week 101 for males. At the end of the study all animals were given a complete histopathologic examination. The authors determined that no clinical changes were attributable to chloraminated drinking water. Survival of rats receiving chloraminated drinking water was not significantly different than controls except that, for the 50 ppm dose groups, survival was greater than that of controls.

Groups of rats (10/sex/group) were predestined for incremental sacrifice and evaluation at 14 and 66 weeks. At these times a complete hematologic examination and necropsy were performed on all animals and histopathologic examinations were performed on all control and high-dose rats. In F344/N rats at the 14-week evaluation the mean body weight at necropsy of 200 ppm dosed males was significantly lower ($p<0.01$) than that of controls while mean body weights of low- and mid-dose males was similar to that of controls. At the 66-week evaluation, there was a dose-related decrease in body weights of chloraminated treated male rats. The mean body weights of high-dose male and female rats were 94 and 92% of controls, respectively. Slight decreases ($p<0.05$) in the liver and kidney weight in high-dose males and kidney-to-body weight ratios in male and female high-dose rats were related to the lower body weights in these groups.

The second NTP (1990) study was a 2-year study to determine the potential chronic toxicity or carcinogenic activity of chloraminated drinking water. B6C3F1 mice (70/sex/dose) were administered chloramine at doses of 0, 50, 100 and 200 ppm in pH 9 buffered charcoal filtered deionized drinking water. These doses were calculated based on a time-weighted average to be 0, 5.0, 8.9 and 15.9 mg/kg/day for male mice and 0, 4.9, 9.0 and 17.2 mg/kg/day for female mice. There was a dose-related decrease in the amount of water consumed by both sexes; this decrease was noted during the first week and continued throughout the study. Male and female mice had similar food consumptions as controls except the 200 ppm dose females that were slightly lower than controls. There was a dose-related decrease in mean body weights of dosed male and female mice throughout the study. Mean body weights of high-dose male mice were 10-22% lower than their control group after week 37 and the body weights of high-dose female mice were 10-35% lower after week 8. Survival of mice receiving chloraminated drinking water was not significantly different than controls. All animals were given a complete histopathologic examination at the end of the study. The authors did not attribute any clinical findings to the consumption of chloraminated drinking water.

Groups of mice (10/sex/group) were predestined for incremental sacrifice and evaluation at 15 and 66 weeks. A complete hematologic examination and necropsy were performed on all animals and a histologic examination was performed on all controls and high-dose rats at the incremental sacrifices. At 15 weeks the group mean body weights of high-dose male and female mice were 91 and 84% of controls, respectively. At the 66-week evaluation, the differences in body weight between the high-dose mice and

controls was 87% for females but 91% of controls for mid- and high-dose males. Decreases in liver weights and increases in brain- or kidney-to-body weight ratios observed in high-dose mice at 15 or 66 weeks were attributable, according to NTP, to the lower body weights in these groups.

Because specific organic N-chloramines have not yet been identified in chlorinated water or *in vivo* on ingestion of chlorinated water, Bempong and Scully (1980a) studied N-chloropiperidine (NCP), a model for the health effects of organic chloramines as a class of compounds. They found it was toxic to C57Bl/J6 mice after i.p. injection. Groups of 10 female and 10 male mice were injected with either 50, 100, 200, 300 or 400 mg/kg bw of NCP. The animals were observed daily for toxic effects, food intake and body weight. The LD_{50} for NCP was slightly greater than 300 mg/kg as presented by the available data. However, the toxicity of NCP was increased when allowed to stand at room temperature in an aqueous solution, indicating that the degradation products are more toxic. The toxicity of the chloramine was less than that of the parent amine, but the toxicity of aqueous solutions of the chloramine increased on standing at room temperature for 24 hours or more.

Developmental Toxicity

Abdel-Rahman et al. (1982) investigated the effects of monochloramine administered in drinking water to adult female Sprague-Dawley rats. Six animals/group were administered 0, 1, 10 or 100 mg/L NH_2Cl daily in the drinking water both 2.5 months before and throughout gestation. Sacrifice of rats on the 20th day of gestation was

performed for soft-tissue and skeletal examination of the progeny. Monochloramine did not produce any significant changes in rat fetuses at any dose level; in fact, there was a slight increase in fetal weight in all NH_2Cl groups compared with controls.

Reproductive Toxicity

Eight- to 10-week-old hybrid male mice (C57Bl/J6 x DBA2) and 10-week-old inbred male golden Syrian hamsters (numbers not specified) were given daily exposures (ad libitum) to 400 mg/kg/day NCP in 0.001% ethanol for 100 days. Significant increases of abnormal sperm (either tail or head anomalies) were observed in both hamsters and mice after 5 weeks of exposure (Bempong and Scully, 1983). Another significant result among NCP-treated hamsters was reduced sperm count after 70 days of daily exposure. Greater than 80% of the hamster population was classified as sterile based on their reproductive performance. A few (number not provided) were reported as having testicular atrophy after 16 weeks of daily exposure. Gradual reductions in percent abnormal sperm were observed after the 75th day of treatment in hamsters; however, sperm counts did not increase. In mice the level of sperm abnormalities remained the same for more than 5 weeks except there was a reduction in sperm tail damage with concomitant increased anomalous sperm heads. Sperm count was also reduced in mice but did not affect percent anomalous sperm. The data support the concept that species and strain differences in sperm abnormalities are suggestive of genotoxic control of sperm development. In addition, the investigators propose that since increased levels of sperm abnormalities were observed >5 weeks after treatment, the most sensitive cells were early

spermatocytes > differentiating spermatogonia > premeiotic cells (Bempong and Scully, 1983).

Fertility studies were also undertaken wherein male mice and male hamsters were caged with two respective females after 1 week of continuous exposure as described previously (Bempong and Scully, 1983). The duration of each mating period was 7 days. Six treatment groups of 15-25 females were constituted in the following manner: 1) untreated (UT) female x UT male; 2) UT female x ethylmethane sulfonate (EMS)-treated male; 3) NCP-treated female x NCP-treated male; 4) UT female x NCP male; 5) NCP female x UT male; and 6) piperidine-treated female x piperidine-treated male. EMS served as a positive control and piperidine was included to ascertain the extent to which effects could be ascribed to the monochloramine group. Reduced fertility indices were observed on week 5 and subsequently for all matings of NCP-treated animals, except when females only were exposed (group 5). To obtain data on potential embryotoxic effects of NCP, five pregnant animals/group were allowed to reach term and the offspring were counted. Numbers of offspring/pregnancy were variable for the NCP-treated animals, but was lowest for the group (3) wherein both males and females received NCP. Treatment with NCP increased the number of uterine moles, particularly when the female was exposed. Implant frequency was reduced in all NCP treatment groups; exposure of both males and females resulted in a significantly greater reduction than treatment of either single parent (Bempong and Scully, 1983).

In a more recent study, Carlton et al. (1986) administered monochloramine by gavage at doses of 0, 2.5, 5.0 and 10 mg/kg bw/day to male (12/dose) and female (24/dose) Long-Evans rats for 66-76 days. Males were treated for 56 days and females for 14 days before mating. The administration was continued during the 10-day mating trial period, and thereafter females were gavaged with monochloramine daily throughout gestation and lactation. Males were necropsied at the end of the mating period and bled for complete blood counts and thyroid hormone levels. Their sperm was examined for normalcy and histologic examinations were performed on the reproductive tract including the testis, epididymus, prostate and seminal vesicles. Dams and some offspring were necropsied at weaning 21 days after birth of the pups. At necropsy dams were bled for complete blood counts and thyroid hormone levels. In addition, the reproductive tract was removed for histopathologic examination. Other offspring were administered chloramines after weaning until they were 28-40 days old; these pups were evaluated for vaginal patency and thyroid hormone levels. The authors reported no significant (statistical analysis not provided) differences between control and exposed rats in fertility, viability, litter size, day of eye opening, or average day of vaginal patency (day 31.8-32.6). There were no alterations in sperm count, direct progressive sperm movement, percent mobility, or sperm morphologic characteristics in adult males. Weights of male and female reproductive organs were not significantly different among test and control groups, and there were no significant morbid anatomic changes evident on tissue examination. There were no signs of toxicity, changes in hematologic parameters, or body weight suppression in adult rats of either sex at any dose level. The mean weight of the pups was unchanged from that of control litters. Based on these data, a NOAEL of 10 mg/kg/day is identified.

Mutagenicity

Lu Shih and Lederberg (1976) showed chloramine to be weakly mutagenic at the *trpC* locus of *Bacillus subtilis*. They further investigated the biologic and physical effects of chloramine on *B. subtilis* after treatment of the bacterial cells or the bacterial DNA. Both resulted in single-strand breaks and a few double-strand scissions at higher chloramine doses, with loss of DNA-transforming activity. Since some DNA repair-defective mutants seem to be more sensitive to chloramine, it would appear that chloramine's bacteriotoxic effect may be due in part to its ability to damage DNA.

Thomas et al. (1987) reported that monochloramine (40 μm) slightly increased the number of revertant colonies over untreated control levels in assays employing *Salmonella typhimurium* (TA97, TA100 and TA102). Positive controls were run concurrently. Lipophilic dichloramines were the most active mutagens in this study.

Fetner (1962) found that distilled water containing monochloramine produced chromosome breakage when used for soaking *Vicia faba* seeds. A 1-hour exposure to 10^{-4} M monochloramine produced 24% abnormal anaphases (including chromosomal bridges and fragments) in the embryonic *Vicia* roots. As there was no visible browning of the plant embryos, Fetner concluded that monochloramine produced chromosome breakage at a concentration that exhibited little evidence of tissue damage.

Meier et al. (1985) evaluated the ability of monochloramine and other oxidants to induce chromosomal aberrations (5/sex/dose) and micronuclei in the bone marrow of

CD-1 mice (4/sex/dose), and sperm-head abnormalities in male B6C3F1 mice (10/dose). Monochloramine administered by gavage at concentrations of 40, 100 and 200 mg/L showed no evidence of any significant effects in any of the tests.

Bempong and Scully (1980a) found that the organic chloramine, NCP, was weakly mutagenic in a preincubation *Salmonella* reverse mutation assay (Ames test). Mutagenic activity was detected in the absence of exogenous S9 activation in strains TA1535 and TA100. The toxicity of the chloramine appeared to overshadow its mutagenic property at doses >64 μg/plate with a concomitant reduction in the number of revertants/plate. N-Chlorodiethylamine, on the other hand, was highly toxic but nonmutagenic (Scully et al., 1983).

The effect of increasing concentrations of NCP on the toxicity, mitotic indices and chromosomal aberrations were studied in Chinese hamster cells (CHO). CHO cells exposed to NCP (0.4-2.4 μg/L) for 3 hours and analyzed during a 96-hour period proliferate less rapidly than control cells and as the concentration of NCP increased, mitotic indices were reduced and the peaks of mitotic activity shifted resulting in chromosome separation and nuclear distribution. CHO cells were exposed to increasing concentrations of NCP (0, 0.4, 0.8 and 1.2 μg/mL) for a 3-hour period, harvested 48 hours after treatment and analyzed for chromatid aberrations (breaks, exchanges, fragments, ring and centric errors). The increases in the frequency of aberrations were proportional to the increase in NCP concentration with breaks accounting for the major proportion of aberrations. Chromosomal aberrations were further investigated after a 3-hour incubation

and a 60-hour recovery period to allow for delayed mitotic activities, and increased populations of metaphases. A nonlinear dose-dependent increase in bridges, dicentrics, exchanges, fragments and ring configurations occurred (Bempong and Scully, 1980b; Bempong et al., 1981, 1986; Scully and Bempong, 1982).

Chloramine T (sodium p-toluenesulfonylchloramide) tested negative in a Salmonella reverse mutation assay (Ames test) in the presence of S-9 in strains TA98, TA100, TA1535 and TA1538 (Anderson and Styles, 1978), and negative in strains TA98, TA100, TA1535, TA1537 and TA1538 with and without S-9 fractions (Gocke et al., 1981).

Weitberg (1987) tested the ability of chloramine T (sodium p-toluene-sulfonylchloramide) to produce SCEs in cultured CHO cells. A significant dose-dependent increase was observed at 10^{-5} to 10^{-7} M and at 10^{-8} M when compared with controls. This effect was significantly diminished when cells were treated with methionine, a thioether, which reduces N-Cl back to the parent amine.

Süssmuth (1982) examined the mutagenicity of chlorinated solutions of several amino acids using several strains of bacteria including the recombination-deficient strain of *B.* M45 (*rec*), *Es. coli* P3478 (*polA1*), and three histidine auxotrophic strains of *S. himurium*. Chlorinated solutions of methionine, tyrosine, phenylalanine and glycine were mutagenic in more than one strain of bacteria. The active mutagens in Süssmuth's solutions were not defined clearly. Since the amino acids react rapidly in chlorinated water to form N-chloramino acids, the first group of compounds to form would be the

chloramine acids. These may be the active mutagens; however, because some of the chloramine acids are labile, their degradation by-products may be the active mutagens.

An Ames *Salmonella* assay was performed using concentrated drinking water samples collected at a pilot scale drinking water treatment plant disinfected with monochloramine (Miller et al., 1986). The reverse osmosis concentrates ranged from 0.025-1.0 mL. The mutagenic response was negative for strains TA98 or TA100 when tested with or without the metabolic activation system S9.

Carcinogenicity

Several initiation-promotion studies have been conducted. Although these studies are not useful for quantitative risk assessment, they may support findings in bioassay studies.

Settled, coagulated and sand-filtered Ohio River water was treated with monochloramine at 3 mg/L (Bull, 1980; Bull et al., 1982). The residual disinfectant was dissipated within 48 hours. The water was then concentrated by reverse osmosis and the concentrate subjected to a mouse skin initiation-promotion assay in SENCAR mice. A total of 1.5 mL of the reverse osmosis concentrate was applied to the backs of the mice in six doses (0.25 mL each) over a 2-week period. This was followed by thrice weekly applications of 2.5 ug of the promoting agent phorbol myristate acetate in acetone for 18 weeks. Nondisinfected water concentrates produced no tumors, while concentrated monochloramine-treated water samples induced neoplasms in 5/25 animals. Lesions

included papillomas (1/25), squamous cell carcinomas (2/25) and lung adenomas (5/25). Overall chi-square analysis comparing numbers of animals with systemic tumors indicated that the increase was not significant. Subsequent to the results published by Bull (1980), five additional experiments were undertaken using new samples of disinfected waters. Results of two of these were published by Bull et al. (1982). In initiation-promotion assays of concentrated monochloramine-treated water samples at a dose of 1.0 μg for 20 weeks, 23% and 15% of animals developed papillomas respective to the two studies. For these assays, however, papillomas were also observed in 15 and 13% of mice treated with saline and in 20% of mice exposed to nondisinfected water concentrates. The authors pointed out that the average tumor incidence of all the monochloramine concentrated water-exposed animals is almost twice that of the simultaneous controls and is more than double the historical control response. It is likely that the initiating activity of the water concentrates is not due to the monochloramine per se, but rather to organic chloramines or other materials formed as by-products of the disinfection process. The authors noted that as the reverse osmosis process used to produce the water concentrates does not concentrate low molecular weight organics, trihalomethanes were not included in these test samples.

Herren-Freund and Pereira (1986) tested chloramines for initiation-promotion activity in rat liver by using the rat liver foci bioassay. The endpoint of the assay is the occurrence of altered foci of hepatocytes. This assay uses an increased incidence of γ-glutamyltranspeptidase positive foci (GGT foci) as an indicator of carcinogenicity since initiation-promotion bioassays using GGT foci have detected both hepatic and nonhepatic

carcinogens. Male rats (authors do not clarify if Fischer 344 or Sprague-Dawley or both were used) were administered chloramine (route not specified) 14.75 mg/kg bw 24 hours following a 2/3 partial hepatectomy. Seven days after initiation, promotion by 500 ppm phenobarbital in drinking water was begun. After 10 weeks of exposure to phenobarbital, the rats were removed from the exposure to the promoter for 1 week and then sacrificed. Positive and negative controls were run concurrently. The authors reported that under conditions of this study chloramine did not initiate GGT foci.

Similar results were reported by Miller et al. (1986) when rats (10/group) were administered concentrated drinking water samples collected at a pilot scale drinking water treatment plant using monochloramine for disinfection purposes, which were concentrated (4000x or 2000x the residual concentration of 2.1 mg/L) by the macroreticular resin process. Rats were partially (2/3) hepatectomized on day 0 and treated 24 hours later with chloramines for 1 week. On day 7 the rats received 500 ppm sodium phenobarbital in their drinking water for 56 days. Chloramine-treated water did not initiate the incidence of GGT foci above that of the vehicle control group.

One of the toxicologic tests used for determining tumor-initiating potential of concentrated drinking water samples is the mouse lung adenoma assay. Concentrated drinking water samples from a pilot treatment plant using monochloramine for disinfectant purposes was administered to 6-week-old strain A mice in drinking water samples concentrated (4000x and 2000x) by the macroreticular resin process (Miller et al., 1986). The residual chloramine level before concentration was 2.1 mg/L. Both positive and

negative controls were run concurrently. The vehicle controls had 0.1 adenoma/animal and the treated animals had $\leq$0.20 adenoma/animal, indicating there was no treatment-related effect.

The National Toxicology Program (NTP, 1990) conducted a 2-year study of chloraminated drinking water in F344/N rats at doses of 0, 50, 100 and 200 ppm. This dose was calculated to be 0, 2.1, 4.8 and 8.7 mg/kg/day for males and 0, 2.8, 5.3 and 9.5 mg/kg/day for females. The purpose of this study was to determine the potential chronic toxicity or carcinogenic activity of chloraminated water. The water from all treatment groups was charcoal filtered and deionized to remove organic substances and other residues.

According to the authors there were no neoplasms or nonneoplastic lesions in rats of the 2-year study that were attributable to the consumption of chloraminated drinking water. The incidence of mononuclear cell leukemia was slightly increased relative to that of controls in the mid- and high-dose range. Although female rats receiving chloraminated water had significantly greater leukemia incidence in the high-dose group than the controls, there was no clear dose response and the incidence of leukemia in the female control group (16%) was less than the untreated historical controls (25%). There was also no evidence of a reduced latency in the occurrence of leukemia in female rats consuming chloraminated water. However, because of the marginal statistical significance the increased incidence of leukemia was considered equivocal evidence of

carcinogenic activity in female rats. There was no evidence of carcinogenic activity of chloraminated drinking water for male rats.

The National Toxicology Program (NTP, 1990) conducted another 2-year study of chloraminated drinking water in B6C3F1 mice at doses of 0, 50, 100 and 200 ppm. These doses were calculated to be 0, 5.0, 8.9 and 15.9 mg/kg/day for males and 0, 4.9, 9.0 and 17.2 mg/kg/day for females. The purpose of this study, as with the rat study, was to determine the potential chronic toxicity or carcinogenic activity of chloraminated water. The water from all treatment groups was charcoal filtered and deionized to remove organic substances and other residues.

Renal tubular cell adenomas occurred in two high-dose and one low-dose male mice; focal tubule hyperplasia was seen in one low-dose, three mid-dose and one high-dose male. Hyperplasia was also observed in male control animals. There were no other lesions in the kidneys of male or female mice receiving chloraminated water. Histopathology evaluation of the kidneys failed to identify any difference in the incidence or severity of renal tubular atrophy, dilation, regeneration, focal mineralization, or protein casts. Step sections of the kidneys of male mice did not provide supportive evidence of a chemical effect. There were no neoplasms or nonneoplastic lesions in mice in the 2-year study that were attributable to the consumption of chloraminated drinking water, according to the authors. There was no evidence of carcinogenic activity of chloraminated drinking water for male or female mice.

Summary and Discussion

Several short-term studies showed no observed adverse hematologic effects in mice, rats and monkeys (Moore et al., 1980; Bercz et al., 1982; Bull, 1980). In A/J mice administered chloramine solutions between 2.5 and 200 mg/L (pH 8.9) for 30 days, the only observable effect was a slight increase in hematocrit (Moore et al., 1980). In another study of similar duration (45 days) rats treated with 10, 50 or 100 mg/L monochloramines experienced a decrease in the amount of methemoglobin present in the blood, the opposite of what was expected (Bull, 1980). Monochloramine in drinking water for 6 weeks at 100 mg/L resulted in no detectable effects on 18 hematologic tests of 12 African Green monkeys (Bercz et al., 1982). The results from the three studies described above are somewhat conflicting in that similar dose levels and duration produced different changes in hematologic parameters (i.e., increased hematocrit, decreased methemoglobin in blood) while others produced no detectable effects. In one longer-term study in which blood glutathione levels were decreased after 12 months of exposure to monochloramine, there was a lack of dose- and time-dependency in the observed effects (Abdel-Rahman et al., 1984).

One possible reason for the varying observations between laboratories may be the conditions under which the solutions of monochloramine are generated. It is understood that, as the pH of a solution of monochloramine is lowered, some of the monochloramine is converted to dichloramine. To avoid this, solutions are frequently buffered to pH 8 to avoid dichloramine formation. However, in doing this, buffer concentrations may be exerting an unusually strong effect on the reactions of disinfectants in the stomach where

enzyme activity is usually greatest (lower pH). The pH and buffer concentrations of the monochloramine solutions used in toxicity studies may vary widely from laboratory to laboratory.

A draft report by GSRI (1981) investigated the effects in rats and mice administered drinking water containing 0, 25, 50, 100, 200 or 400 mg/L monochloramine for 91 days. Using water and food consumption data provided in the report, corresponding respective doses were 2.5, 4.9, 10.2, 18.8 and 40.7 mg/kg/day for male rats; 3.8, 6.5, 13.8, 26.6 and 53.9 mg/kg/day for female rats; 4.9, 8.3, 14.5, 31.3 and 50.7 mg/kg/day for male mice; and 7.7, 12.1, 21.9, 34.6 and 88.5 mg/kg/day for female mice. Animals experienced decreased body weight gain and liver damage at 200 and 400 mg/L monochloramine in rats and 100, 200 and 400 mg/L monochloramine in mice. Based on these observations, the investigators suggested a NOEL of 50 mg/L or ~8.3 mg/kg/day monochloramine.

In addition monochloramine at 9, 19 and 38 ppm in drinking water may exert immunotoxic effects to male Sprague-Dawley rats. Significant ($p<0.05$) reduction of spleen weight (38 ppm), decreased antibody synthesis (9 and 19 ppm) and augmented PGE2 production (19 and 38 ppm) were reported (Exon et al., 1987). The biologic importance of effects on the immune system as a factor in the toxicity of monochloramines is not clear.

Daniel et al. (1990) investigated the effects of monochloramine administered for 90 days in the drinking water of Sprague-Dawley rats at 0, 25, 50, 100 and 200 mg/L. Based on food and water consumption data, doses were calculated to be 0, 1.8, 3.4, 5.8 and 9.0 mg/kg/day for males and 0, 2.6, 4.3, 7.7 and 12.1 mg/kg/day for females. For males and females the average weight gain at 200 mg/L dose was 51% of controls. Reductions in absolute and relative organ weights occurred at 200 mg/L. The authors concluded that 100 mg/L is considered the NOAEL.

Daniel et al. (1991) also investigated the effects of monochloramine administered for 90 days in the drinking water of B6C3F1 mice at 0, 12.5, 25, 50, 100 and 200 mg/L. Based on food and water consumption data, doses were calculated to be 0, 2.5, 5.0, 8.6, 11.1 and 15.6 mg/kg/day for males and 0, 2.8, 5.3, 9.2, 12.9 and 15.8 mg/kg/day for females. Based on decreases in liver, heart and lung weights in males and liver, heart and spleen weights in females at 100 mg/L and decreased weight gain and food consumption in both sexes and reduced water consumption in females, the 50 mg/L concentration is considered the NOAEL.

NTP (1990) conducted two studies for 2 years (F344/N rats and B6C3F1 mice) to determine the potential chronic toxicity for chloraminated drinking water. Chloramine was administered at doses of 0, 50, 100 and 200 ppm. These doses were calculated to be 0, 2.1, 4.8 and 8.7 mg/kg/day for male rats and 0, 2.8, 5.3 and 9.5 mg/kg/day for female rats, and 0, 5.0, 8.9 and 15.9 mg/kg/day for male mice and 0, 4.9, 9.0 and 17.2 mg/kg/day for female mice. Mean body weights of 200 ppm dosed rats were lower than controls. Slight

decreases in organ-to-body weight ratios in high-dose rats were related to the lower body weights. There was a dose-related decrease in mean body weights of dosed male and female mice throughout the study. No clinical changes were attributable to chloraminated drinking water in the rat or mouse study.

Studies suggest that flour treated with trichloramine may cause "canine hysteria" but one study suggests that this is a species-specific phenomenon in dogs (Mellanby, 1946; Silver et al., 1947a,b,c; Newell et al., 1947) and does not affect humans (Pollock, 1949).

Few studies have been conducted on organic N-chloramines. Studies conducted with N-chloropiperidine suggest that organic chloramines may be toxic, but determination of their environmental significance will rely on identification of specific organic N-chloramines actually formed on chlorination of natural waters (Bempong and Scully, 1980a).

Monochloramine was not teratogenic in mature female Sprague-Dawley rats exposed to 1, 10 or 100 mg/L in drinking water nor did 40, 100 and 200 mg/L solutions induce sperm-head anomalies in B6C3F1 mice (Abdel-Rahman et al., 1982).

In a more recent study there were no significant differences in fertility, viability, litter size, day of eye opening or day of vaginal potency between control and exposed Long-Evans rats given $\geq$10 mg/kg chloramines. There were no alterations in sperm

count, direct progressive sperm movement, percent mobility or sperm morphologic characteristics in adult males. Weights of male and female reproductive organs were not significantly different among test and control groups.

Data on the mutagenicity of chloramines are inconclusive. A number of studies suggest that monochloramine and some organic N-chloramines and N-chloramino acids may be weakly mutagenic. Monochloramine has been found to be marginally mutagenic in *Bacillus subtilis* bacteria (Lu Shih and Lederberg, 1976) and in *Vicia faba* plant seeds (Fetner, 1962). It was responsible for cellular hypertrophy, increased mitotic figures and bizarre chromatin patterns in B6C3F1 mice exposed to 200 and 400 mg/L in drinking water (Wolfe et al., 1984). Thomas et al. (1987) reported monochloramine (40 um) slightly increased the number of revertant colonies over untreated control levels in assays employing *Salmonella typhimurium* (TA97, TA100 and TA102). In addition, monochloramine showed no evidence of producing spermhead abnormalities in B6C3F1 mice or inducing chromosomal aberrations and micronuclei in bone marrow of CD-1 mice (Meier et al., 1985).

The organic chloramine, N-chloropiperidine, was found to be marginally mutagenic in the reverse mutation plate incorporation assay (Ames test). It was cytotoxic and cytostatic in CHO cells and produced chromosomal aberrations, the frequency of which was proportional to the concentration of the compound (Bempong and Scully, 1980b; Bempong et al., 1981, 1986; Scully and Bempong, 1982). It produced SCEs in CHO cells, but not in baby hamster kidney cells (Scully et al., 1983). The analogous

chloramine, N-chlorodiethylamine, was more toxic but nonmutagenic (Scully et al., 1983). When the synthetic N-Cl compound chloramine T (sodium p-toluenesulfonylchloramide) was tested, SCEs were significantly increased in a dose-dependent manner in CHO cells (Weitberg, 1987).

Data indicate that chloramine per se is not an initiator (Bull et al., 1982; Bull, 1980; Herren-Freund and Pereira, 1986). Two-year studies by the National Toxicology Program (NTP, 1990) have shown equivocal evidence of carcinogenic activity in female rats that is due to the slightly increased incidence of mononuclear cell leukemia compared with that of concurrent controls, although incidence in historical controls was higher. There was no evidence of carcinogenic activity that isdue to chloraminated drinking water in male rats or in male or female mice. Until the carcinogenicity of chloramines is further delineated, it is categorized in group D, not classifiable as to human carcinogenicity, meaning that there is inadequate human and animal evidence of carcinogenicity (U.S. EPA, 1986).

VI. HEALTH EFFECTS IN HUMANS

Introduction

Data on the human health effects observed following exposure to chloramines are limited to a few clinical reports and epidemiologic studies. Several cases have been reported where chloramine-T has caused allergic contact dermatitis. Clinical reports indicate that acute chloramine exposure either by inhalation or ingestion results in burning eyes and throat, dyspnea, coughing, nausea, reversible pulmonary damage and allergic responses. One epidemiologic study looked at a population exposed to chloramine in its drinking water and used the disease risk as a baseline for comparing the risk in a population exposed to chlorine in its drinking water. These findings will be addressed only briefly as they are not directly relevant to this document. Damage to red blood cells has been observed in high risk subpopulations such as hemodialyzed individuals.

Clinical Reports and Experiments

Lombardi et al. (1989) reported the work related case of a 38-year-old nurse who developed subacute eczema that appeared to be caused by the use of chloramine-T as an antiseptic disinfectant in the cleansing of burns. Only a few cases of sensitization to chloramine-T have been reported and usually from nonoccupational contact even though it has widespread use in Italy as an antiseptic, disinfectant and chemical reagent.

Laakso et al. (1982) reported the case of a 27-year-old woman who mixed ~500 mL of 4-5% household ammonia with the same amount of 5% sodium hypochlorite bleach in a small, poorly ventilated bathroom. The vapors from the mixture caused burning in the eyes and throat, dyspnea, coughing, nausea and vomiting. Inhalation of the chloramine fumes resulted in pneumonitis, which did not result in permanent pulmonary damage.

Beck (1983) reported the case of a 28-year-old nurse who suffered from rhinitis when she was in contact with a chloramine solution. Chloramine is often used as an antiseptic in the treatment of infected ulcers, and when the nurse was treated with a 2% chloramine solution for a dental abscess, a severe angioneurotic edema developed. Since this Type I reaction to chloramine was reported only once before, the authors recommended that a prick test with chloramine be used before treatment of patients with previous exposures.

A clinical study was conducted by Lubbers et al. (1981) to assess the safety of chronically administered chlorine water disinfectants in humans. This study was conducted in three parts over a 12-week period. Phase I and Phase II subjects were male college students between the ages of 21 and 35 years of age, of normal body weight and free of any history of disease or any medical or surgical condition that might interfere with the absorption, excretion or metabolism of substances by the body. Phase III subjects were glucose-6-phosphate dehydrogenase deficient, but were normal in all other respects.

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 with 10 subjects in the control group that received untreated water. Chloramine was given every 3 days for a total of 15 days, at concentrations of 0.01, 1.0, 8.0, 18.0 and 24.0 mg/L (corresponding doses of 0.14, 14, 110, 260 and 340 ug/kg/day assuming a body weight of 70 kg) in a total volume of 1000 mL.

Phase II consisted of 60 subjects randomly assigned into six treatment groups of 10 subjects/group with one group receiving untreated water. A daily concentration of 5 mg/L chloramines in a volume of 500 mL of water was administered for 12 consecutive weeks. Physicals and collection of blood and urine were conducted on a weekly basis during the treatment period and for 8 weeks following.

Phase III was conducted on male students who were deficient in glucose-6-phosphate dehydrogenase and were considered to be more susceptible to oxidative stress. These students were given 5 mg/L of sodium chlorite daily in a volume of 500 mL for 12 consecutive weeks.

Blood and urine samples were collected and physical exams were given, including blood pressure measurements and taste tests. During all three phases of this study a massive volume of raw data was acquired. No definitive finding of detrimental physiologic impact was made in any of the three phases of this human investigation of

the relative safety and tolerance of oral chlorine disinfectant ingestion. Other possible confounders such as diet and other sources of drinking water were not addressed. The fact that there were no overt adverse health effects within the limitations of this study suggests that ingestion of chloramine at these levels over a relatively short period of time produces no toxicity in healthy adult males, but does not rule out the possibility that longer treatment periods would result in any detectable adverse outcomes of biologic significance.

Epidemiology Studies

There are no epidemiologic studies that have been designed to address specifically the potential adverse effects of exposure to chloramines on human health. The study of Zierler et al. (1986, 1988) was designed in response to earlier ecologic studies that indicated that areas using chlorinated surface waters for drinking water were associated with higher cancer mortality than areas using other sources of drinking water and disinfectant practices. The addition of chlorine to surface water is known to form organic micropollutants (Murphy and Craun, 1990). Specifically, the reaction of free chlorine with naturally occurring precursor substances, primarily humic and fulvic acids, produces a group of halogen-substituted single-carbon compounds known as trihalomethanes (THMs) (Craun, 1988). The predominant THMs formed are chloroform and bromodichloromethane. The process of chloramination produces only small amounts of THMs. It was originally thought that the early findings of increased cancer mortality associated with chlorinated drinking water might be due to exposure to the THMs themselves. Zierler's study in Massachusetts was conducted to see if there was

a difference in cancer mortality among communities using chlorine compared with communities using chloramine for disinfection. In this sense, the persons who were exposed to chloraminated drinking water were used as controls with the assumption being they would be much less exposed to chlorination by-products.

The first phase of this study (Zierler et al., 1986) looked at the patterns of cancer mortality among 43 communities using either chlorine or chloramine since 1938. All resident Massachusetts deaths among those 45 years and older and occurring during 1969-1983 were eligible for the study. Deaths were selected for inclusion if the last residence listed on the death certificate was in a community using chlorine or chloramine for disinfection. Cancers of the bladder, colon, kidney, pancreas, rectum, stomach, lung and female breast were thought to be related to chlorinated by-products of disinfection and were therefore treated as cases for a mortality odds ratio (MOR) analysis. Deaths from cardiovascular and cerebravascular disease, chronic obstructive lung disease and lymphatic cancer (N=214,988), considered to be unrelated to chlorinated by-products, were used for comparison. In general, cancer mortality was not associated with type of disinfectant in the MOR analysis. There was a slight association (MOR=1.05) for chlorine use noted only with bladder cancer that increased slightly (MOR=1.15, 95% confidence interval = 1.06-1.26) when lung cancer deaths were used for controls. Standardized mortality ratio analysis of the data set were generally unremarkable. There was a small increase in mortality (SMR=118, 95% confidence interval = 116-120) from influenza and pneumonia in the chloraminated communities.

The second phase of this mortality study (Zierler et al., 1988) was designed to further pursue the bladder cancer findings in a more refined case-control analysis, which included decedent next-of-kin interviews. Information on cigarette smoking, occupation and residential history was obtained. The relationship with bladder cancer and residence in communities using chlorine for disinfection again persisted. The crude association was highest for lifetime residents of chlorinated drinking water communities relative to lifetime residents of chloraminated drinking water communities (MOR=1.5, 95% confidence interval = 1.1-2.2) when lymphatic cancers were used for controls. The MOR increased to 2.7 after controlling for the joint effects of age, gender, occupation and pack-years of cigarette smoking. Although the exposure estimates in these analyses are relatively crude, there is a consistent relationship, albeit small, with bladder cancer mortality and years of exposure to chlorinated drinking water when compared with years of exposure to chloraminated drinking water. The study was not designed to assess adverse effects from exposure to chloramine, but rather considers the chloramine-exposed participants as controls. At this time there are no epidemiologic studies that have evaluated chloraminated drinking water as the exposure of interest and not the control exposure.

High-Risk Subpopulations

Eaton et al. (1973) studied hemodialyzed patients (number of subjects not reported) from three University of Minnesota hospitals. They observed significant methemoglobinemia (>5% statistical analysis not provided) and Heinz body inclusions in the red cells of the patients in two hospitals, which used unpurified tap water and

water purified with the reverse osmosis (RO) technique during dialysis. The RO technique removes particulate matter and trace metals from the dialysis water. Charcoal-filtered RO water that did not contain chloramines was used in the third hospital, and did not result in significant methemoglobinemia. Serial observation of several patients exposed to chloramines through dialysis suggested that the RBC oxidant damage was cumulative. In addition to oxidant damage to the RBC, chloramines inhibit the metabolic pathway used by these cells to prevent and repair such damage. Thus, chloramine-containing dialysis water presented a severe threat of acute hemolytic anemia to uremic patients undergoing dialysis.

Kjellstrand et al. (1974) also observed similar effects in patients undergoing hemodialysis. The authors concluded that chloramines induced their deleterious effects through the formation of methemoglobin from the direct oxidation of hemoglobin, and through damage of the hexosemonophosphate shunt (HMPS), with which red cells defend themselves against oxidant damage. The authors suggested that chloramine-induced hemolysis may be reduced by the addition of ascorbic acid to the treatment water.

Summary and Discussion

Few acute exposures to chloramines have been reported in the literature. Laakso et al. (1982) reported pneumonitis as a result of inhalation of chloramine fumes from a mixture of household ammonia and sodium hypochlorite. Permanent pulmonary damage

was not sustained. Beck (1983) reported a Type I allergic response after treatment with a 2% chloramine solution for a dental abscess.

One experimental study on chloramines was located in the available literature. Lubbers et al. (1981) reported no hematologic or abnormal effects following routine clinical tests in individuals ingesting 0.01, 1.0, 8.0, 18.0 and 24.0 mg/L (0.14, 14, 110, 260 and 340 mg/kg/day, respectively) chloramines for 1 day in drinking water or 5 mg/L chloramines for 12 weeks in drinking water. It is unknown, however, whether exposure beyond this time would have any impact.

Although mentioned here for the sake of completeness, the work of Zierler et al. (1986, 1988) should not be used as definitive evidence that chloramine exposure is not associated with adverse health effects in humans. Although statistically stable because they are based on large numbers of deaths, the community based SMR analyses represent a relatively crude means of assessing the relationship of a specific cause of death with a specific drinking water disinfection practice. If there is a positive relationship between these variables, it may go undetected because of the attenuating effects of random misclassification of the observed deaths into exposure categories. The case-control study of bladder cancer was designed to use the chloramine exposure as the comparison group and does not address the potential adverse effects from chloramine, although often misinterpreted in this way.

Hemodialyzed patients are a high-risk subpopulation for chloramine exposure through chloraminated dialysis water. Chloramines cause oxidant damage to red blood cells and inhibition of the hexose-monophosphate shunt with which red blood cells defend themselves against oxidant damage (Eaton et al., 1973; Kjellstrand et al., 1974). Thus, this high-risk group must also be considered when using monochloramine for disinfection of water used in dialysis.

VII. MECHANISMS OF TOXICITY

General Theories

The literature on the mechanism of inactivation of microorganisms by chloramines is limited. Since low levels of inorganic chloramines are effective in inactivating bacteria, Nusbaum (1952) proposed that the mechanism of action must essentially be the same as that of hypochlorous acid on enzymes; that is, the chloramine molecules enter the cytoplasm and interfere with enzymatic reations. Ingols et al. (1953) studied bactericidal mechanisms of chlorine compounds on unspecified bacterial suspensions. They noted that monochloroamine bacteriotoxicity was not completely reversed by the addition of sulfhydryl radicals; and thus, conclude that sulfhydryl radical oxidation was not the exclusive mechanism of toxicity. They proposed that the sulfhydryl group of critical enzymes may be the point of vulnerability to a strong oxidant (for example, chlorine dioxide) but that changes in other functional groups may also be involved in cell death. Thus, a weak oxidant like monochloramine may lead to microbial inactivation by changes in groups other than sulfhydryls.

Lu Shih and Lederberg (1976) showed that chloramine applied to both intact *Bacillus subtilis* cells or to the extracted bacterial DNA resulted in double and single strand breaks. Some microbial toxicity may, therefore, be attributable to DNA damage. Monochloramine produced chromosome breaks in *Vicia faba* (Fetner, 1962), and organic chloramines have been shown to produce chromosomal abnormalities in rodent cells (NIEHS, 1982).

Through observation of patients undergoing long-term hemodialysis and concomitant analysis of the water used for this process, Eaton et al. (1973) ascertained that chloramines were probably the oxidants responsible for hemolytic anemia in the patients. *In vitro* studies using human RBCs indicated that chloramine produced denaturation of hemoglobin through direct oxidant damage to erythrocytes. It also inhibited the hexose monophosphate shunt, which generates NADPH that in turn protects RBCs from oxidant damage.

A study by Grisham et al. (1984) demonstrated that organic N-chloramines formed *in vivo* play an important role in ameliorating oxidant effects but can also contribute to monochloramine formation. These workers stimulated isolated human neutrophilic leukocytes to produce hydrogen peroxide (H_2O_2) and secrete cytoplasmic granule components such as myeloperoxidase into the medium. Myeloperoxidase catalyzed the oxidation of chloride (Cl^-) by H_2O_2 to yield hypochlorous acid (HOCl). The HOCl, in turn, reacted with endogenous nitrogen compounds to yield derivatives containing nitrogen-chlorine (N-Cl) bonds, such as hydrophilic, low molecular weight, mono-N-chloramine (RNHCl) derivatives. The RNHCl derivatives were of low toxicity, but reacted with ammonium ion (NH_4^+) to yield monochloramine (NH_2Cl). The bactericidal, cytotoxic and cytolytic activities of the organic N-chloramines (RNHCl) result from reaction of RNHCl derivatives and the ammonium ion. This indicates that neutrophil amines act as a trap for HOCl, and by competing with endogenous NH_4^+ for reaction with HOCl, protect neutrophils and other cells from oxidative attack. The RNHCl derivatives remain as a

reserve of oxidizing equivalents that convert to a toxic form when an increase in NH_4^+ concentration favors formation of monochloramine.

Nusbaum (1952) proposed that the mechanism of action of dichloramine was similar to monochloramine, but there are insufficient data to support this contention. Silver et al. (1947c) demonstrated that trichloramine reacted with cystine and cysteine residues to produce a reagent that was responsible for causing canine hysteria.

Summary

Bactericidal, cytotoxic and cytolytic activities of organic N-chloramines (RNHCl) result from the reaction of RNHCl derivatives and the ammonium ion to yield monochloramine (NH_2Cl). The RNHCl derivatives remain as a reserve of oxidizing equivalents, which convert to a toxic form when the ammonium ion concentration increases favoring monochloramine formation (Grisham et al., 1984).

It has been suggested that the chloramine molecules enter the cytoplasm and interfere with enzymatic reactions (Nusbaum, 1952). Ingols et al. (1953) found monochloramine required higher concentrations and longer contact times to destroy bacteria than hypochlorous acid, suggesting that monochloramine led to microbial inactivation through enzyme changes that may not have been involved in bacterial inactivation by hypochlorous acid. Chloramines are also capable of causing DNA damage in bacterial plant and mammalian cells, which may contribute to cytotoxicity. Eaton et al. (1973) found that chloramines produced denaturation of hemoglobin through both direct oxidant damage to RBCs and inhibition of the hexose monophosphate shunt.

VIII. QUANTIFICATION OF TOXICOLOGIC EFFECTS

Introduction

The quantification of toxicologic 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:

$$RfD = \frac{(NOAEL \text{ or } LOAEL)}{[Uncertainty \ Factor \ (s) \ x \ Modifying \ Factor]} = \_\_ \ mg/kg/day$$

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 toxicologic effects for the chemical. In order to ensure that uncertainty factors are selected and

applied in a consistent manner, the U.S. EPA (1994) 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:

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

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:

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

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 toxicologic 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 biologic 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.

Current Levels of Exposure

Monochloramine has been used as a disinfectant of drinking water. An inventory of municipal water supplies done in the early 1960s revealed that 308 of 11,590 supplies surveyed used an ammonia chlorine process (Moore and Calabrese, 1980).

According to Morris et al. (1980), the extent of knowledge of natural water contamination by N-containing aromatic compounds or the degree to which these compounds react with aqueous chlorine is very limited. It is known that both inorganic chloramines and organic N-chloramines are found upon chlorination of wastewater effluents (Isaac and Morris, 1980). One survey reported the presence of monochloramine ranging from 0.0321-0.9979 mg/L and dichloramine ranging from 0.0020-0.6950 mg/L in secondary sewage effluents and cooling water samples (Jolley et al., 1978). Because inorganic and organic chloramines cannot be identified separately, the levels are based on the mixture.

Noncarcinogenic Effects

The chlorination of water containing ammonia or organic amines may result in the formation of monochloramine, dichloramine, and trichloramine (nitrogen trichloride) through the reaction between ammonia and hypochlorous acid. The amount of chloramines produced depends upon the levels of chlorine and ammonia, and the pH of the water being treated. Some studies on the oral effects of chloramines were presented in Tables V-1 and V-2.

Results on the mutagenicity of chloramines are inconclusive. Chloramine is weakly mutagenic for *Bacillis subtilis* and causes DNA breaks in this bacterial species (Lu Shih and Lederberg, 1976). Thomas et al. (1987) reported that monochloramine (40 um) marginally increased the numbers of revertant colonies over untreated control levels in assays employing *Salmonella typhimurium* (TA97, TA100 and TA102). Monochloramines

produce chromosome abnormalities in both *Vicia faba* and rodent cells (Fetner, 1962; NIEHS, 1982). It was responsible for cellular hypertrophy, increased mitotic figures and bizarre chromatin patterns in B6C3F1 mice exposed to 200 and 400 mg/L in drinking water (Wolfe et al., 1984), while Meier et al. (1985) found that monochloramine at 0, 40, 100 and 200 mg/L did not induce chromosomal aberrations or micronuclei in bone marrow of CD-1 mice or spermhead abnormalities in B6C3F1 mice. Organic chloramines are mutagenic or bacteriotoxic in *S. typhimurium* and have been reported to produce SCEs and other chromosomal changes in mammalian cells (Scully et al., 1983; Bempong and Scully, 1980b; Bempong et al., 1981, 1986; Scully and Bempong, 1982). Tests for teratogenic, reproductive and carcinogenic effects have been negative or inconclusive.

Abdel-Rahman et al. (1984) investigated the toxicity of monochloramine (NH_2Cl) in groups of four male Sprague-Dawley rats/treatment weighing ~160 g. Acute exposure to a single dose at 10 (0.19 mg/kg/day), 20 (0.38 mg/kg/day) or 40 (0.75 mg/kg/day) mg/L NH_2Cl induced a significant increase in blood glutathione levels within 30 minutes after administration of 3 mL aqueous solution by gavage. As part of the same study, the long-term toxicity of NH_2Cl in drinking water was also investigated. Groups of four male Sprague-Dawley rats drank either 0, 1 (0.067 mg/kg/day), 10 (0.67 mg/kg/day) or 100 (6.7 mg/kg/day) mg/L NH_2Cl in deionized water daily for $\leq$12 months. The levels of chlorine, dichloramine and trichloramine in the NH_2Cl solution were <1%, <1% and 0% respectively of the total NH_2Cl added. Food was available *ad libitum* and body weight was measured during the treatment. Heparinized blood was collected by cardiac puncture at 2, 4, 6, 8, 10 and 12 months after treatment, and blood GSH and osmotic fragility were determined

at each of these intervals. Results varied over the 12-month study period, but at 6 and 12 months after initiation of the study, statistically lower GSH levels were observed in all treated rats. After 3 months of treatment significant decreases in RBC count and hematocrit were observed at the higher dosage levels. Hemoglobin concentration and MCH decreased significantly in the 100 mg/L group after 10 months of treatment. The health significance of these types of changes is uncertain. Furthermore, results of treatment effects were analyzed by inappropriate statistical methods (ANOVA). The multiple comparison test used for paired comparisons (Duncan's) is a nonconservative appproach. A more conservative approach would have taken into account correlations or nonindependences. In addition, the control variability was greater than variability between dose groups. A number of "significant" changes in hematologic parameters relative to control values were identified that had no consistent relationship with dose and were not observed consistently throughout the period of exposure. This could be due to the use of a multiple comparison procedure, which is liberal such as the Duncan's.

Several short-term studies showed no observed adverse hematologic effects in mice, rats and monkeys (Moore et al., 1980; Bercz et al., 1982; Bull, 1980). In A/J mice administered chloramine solutions between 2.5 and 200 mg/L (pH 8.9) for 30 days, the only observable effect was a slight increase in hematocrit (Moore et al., 1980). In another study of similar duration (45 days) rats treated with 10, 50 or 100 mg/L monochloramine experienced a decrease in the amount of methemoglobin present in the blood, the opposite of what was expected (Bull, 1980). Bercz et al. (1982) studied the toxicity of monochloramine administered in drinking water to 5 adult male and 7 adult female African

Green monkeys (3.0-5.7 kg). Monochloramine was administered for 6 weeks at 0 and 100 mg/L. The authors estimated the mean daily dose at ~10 mg/kg/day. Treatment with monochloramine had no detectable effect in 18 hematologic tests on the 12 monkeys, including red cell GSH levels. No evidence of thyroid suppression was detected from measurements of serum T_4. The results of these data are somewhat conflicting in that similar dose levels and duration produced different changes, if any, in hematologic parameters. One explanation for the varying observations between laboratories may be the conditions under which the solutions of monochloroamine were generated.

In 1981 a draft report investigated the effects of monochloramine in Fischer 344 rats and B6C3F1 mice (GSRI, 1981). Rats and mice (10 animals/sex/group) were administered concentrations of 0, 25, 50, 100, 200 and 400 ppm monochloramine in drinking water for 91 days. Using food and water consumption data provided in the report, corresponding dose levels are as follows: 2.5, 4.9, 10.2, 18.8, 40.7 mg/kg/day for male rats; 3.8, 6.5, 13.8, 26.6, 53.9 mg/kg/day for female rats; 4.9, 8.3, 14.5, 31.3, 50.7 mg/kg/day for male mice; 7.7, 12.1, 21.9, 34.6, 88.5 mg/kg/day for female mice. After 25 days the buffer system for monochloramine was changed because of a palatibility problem at the higher dose levels. Decreased body weight gain and decreased relative liver weight were observed in male and female rats at concentrations of 200 and 400 mg/L. A statistical analysis was not provided. Protein excretion increased in male rats only when 200 and 400 mg/L monochloramine was administered. Microscopic examination of rat tissues at the 400 ppm level did not reveal any treatment-related lesions.

As with rats, male and female mice gained less weight than the controls at the 200 and 400 ppm levels. There was a reduction in absolute liver weights and liver-to-body weight ratios in male mice at the 400 ppm level and in female mice at $\geq$100 ppm. Histopathologic observations revealed mild to moderate cytologic alteration in male mice administered 200 and 400 ppm levels. Chronic liver inflammatory changes occurred at 100, 200 and 400 ppm in female mice and to a lesser extent in male mice at the 100 ppm level. At concentrations of 100, 200 and 400 ppm increased frequency of mitotic figures, hypertrophy and unusual chromatin patterns occurred in males and in a female at $\geq$200 ppm. The results suggest a LOAEL for liver toxicity of 100 ppm and a NOEL of 50 ppm based on chronic liver inflammatory changes in mice.

Daniel et al. (1990) administered 0, 25, 50, 100 and 200 mg/L monochloramine to male and female Sprague-Dawley rats (10/sex/dose) in their drinking water for 90 consecutive days. These doses correspond to 0, 1.8, 3.4, 5.8 and 9.0 mg/kg/day for males and 0, 2.6, 4.3, 7.7 and 12.1 mg/kg/day for females. At $\geq$50 mg/L in males there were reductions in body weight gain with significant reductions only at the highest dose (200 mg/L). For males and females in the 200 mg/L dose group the average weight gain was 51% of that of the controls; however, water consumption for the 200 mg/L dose was 31 and 33% of controls for males and females, respectively. At the 200 mg/L dose level there were also reductions in organ weights (absolute, relative or both) and liver and spleen weight reductions in both sexes. Although authors concluded that 100 mg/L dose is considered the NOAEL, they suggest that a matched watering and feeding study would be

useful for distinguishing between systemic toxic effects and weight loss from taste aversion to more clearly identify the NOAEL.

Daniel et al. (1991) administered 0, 12.5, 25, 50, 100 and 200 mg/L monochloramine to male and female B6C3F1 mice (10/sex/dose). These doses correspond to 0, 2.5, 5.0, 8.6, 11.1 and 15.6 mg/kg/day for males and 0, 2.8, 5.3, 9.2, 12.9 and 15.8 mg/kg/day for females. Water consumption was decreased in all treated groups. There were significant water and food consumption decreases and weight gain reductions at the two highest dose groups. There were reductions in absolute and relative organ weights in male and female mice in the two highest dose groups. Based on relatively minor changes at 100 mg/L) including <10% decrease in body weight gain at 100 mg/L, and 19-25% decrease in body weight gain at 200 mg/L, a NOAEL of 100 mg/L (12.9 mg/kg/day) was identified based on a decrease in body weight gain and decreased organ weights in B6C3F1 mice consuming 200 mg/L monochloramine in the drinking water for 90 days. The authors state that the lower levels of serum enzyme and reduced organ weights were considered consistent with decreased water and nutrient consumption and altered electrolyte balance rather than disinfectant-induced toxicity. The authors conclude that the absence of histopathology or observable clinical signs of toxicity suggest that these monochloramine exposures induce a relatively mild, nonspecific toxicity by an indirect mechanism (nutritional and electrolyte deficiencies) rather than a direct toxicologic effect on specific organs or tissues.

NTP (1990) conducted 2-year studies using monochloramine administered in the drinking water. In the first study monochloramine was administered to male and female

F344/N rats at 0, 50, 100 and 200 ppm. These doses were calculated on a time-weighted average to be 0, 2.1, 4.8 and 8.7 mg/kg/day for males and 0, 2.8, 5.3 and 9.5 mg/kg/day for females. Mean body weights of high-dose rats were lower than their respective controls. However, mean body weights of rats receiving chloraminated drinking water were within 10% of controls until week 97 for females and week 101 for males. Interim evaluations made at 14 weeks revealed that body weights of high-dose males were lower than controls. At the 66-week evaluation, there was a dose-related decrease in body weight in male chloraminated-treated rats and the mean body weights of high-dose rats were 94% and 92% of controls for males and females, respectively. Decreases in liver and kidney weight in the high-dose males and increases in brain and kidney-to-body weight ratios in high-dose male and female rats were observed in the 14- and 66-week evaluations.

The NTP (1990) second study was a 2-year study of momochloramine administered in drinking water to male and female B6C3F1 mice at 0, 50, 100 and 200 ppm. These doses were calculated on a time-weighted average to be 0, 5.0, 8.9 and 15.9 mg/kg/day for males and 0, 4.9, 9.0 and 17.2 mg/kg/day for females. Mean body weights of high-dose male mice were 10-22% lower than controls after week 37 and the body weights of high-dose female mice were 10-35% lower after week 8. At the 15-week interim sacrifice the mean body weights of high-dose mice were 91% and 84% of controls for males and females, respectively. At the 66-week evaluation the differences in body weight between the high-dose and controls was 87% for females but 91% of controls for the mid- and high-dose males. Decreases in liver weights and increases in brain and

kidney-to-body weight ratios were observed in high-dose male and female mice at 15 and 66 weeks.

Quantification of Noncarcinogenic Effects

Derivation of 1-Day Health Advisory. The only report of an acute exposure to monochloramine in the literature is the study by Abdel-Rahman et al. (1984) wherein the authors observed a drop in blood GSH levels at the lowest dose administered by gavage (3 mL of 10 mg/L solution). As the health implications of these effects are uncertain, they were transitory and were not corroborated by other investigations, it would seem inappropriate to use this study as the basis for a 1-day HA. Therefore, no suitable study is available for the derivation of a 1-day HA. It is recommended that the 10-day HA of 1.0 mg/L be adopted as the 1-day HA.

Derivation of 10-Day Health Advisory. Similar NOAELs or NOELs for monochloramine of 200, 100 and 100 ug/L for hematologic parameters were identified by Bercz et al. (1982), Moore et al. (1980) and Bull (1980), respectively. Both Moore et al. (1980) and Bull (1980) did observe some changes in hematologic parameters. Moore et al. (1980) found a slight increase in hematocrit in A/J mice after 30 days of exposure whereas after 45 days of exposure to rats there was a decrease in the amount of methemoglobin present in the blood, the opposite of what was expected (Bull, 1980). Bercz et al. (1982) reported a NOEL in monkeys after treatment with 100 mg/L chloramines in drinking water. There were no detectable effects in 18 hematologic tests. The authors calculated that by the termination of the study the test animals were

consuming 10 mg chloramine/kg/day. One explanation for the varying observations between laboratories may be the conditions under which the solutions of monochloramine were generated. It is recommended that the NOEL of 100 mg/L from the Bercz et al. (1982) study be used to derive the 10-day HA for two reasons: 1) the Bull (1980) study provides an incomplete description of methods concerning study design and clinical analysis, and 2) monkeys may be a better animal model since the A/J strain of mice used in the Moore et al. (1980) study maintain an erythrocyte glucose-6-phosphate dehydrogenase activity 3 times that of human cells and may be more resistant to oxidant stress (Kiese, 1974). A NOEL is, therefore, set at 10 mg/kg/day, which the authors determined to be the rate of consumption of chloramine at the 6-week termination of the study.

A provisional 10-day HA is calculated as follows:

For a 10 kg child:

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

where:

10 mg/kg/day	=	NOAEL based on the absence of hematologic effects in monkeys (Bercz et al., 1982)
10 kg	=	assumed body weight of a child
10	=	uncertainty factor for use of an animal NOAEL and to protect sensitive members of the human population
1 L/day	=	assumed water consumption of a child

Thus, the proposed 10-day HA is 1 mg/L for a 10 kg child. The small number of animals used by Bercz et al. (1982) and the lack of other shorter- term studies for supporting data should be considered when evaluating this HA.

Derivation of Longer-Term HA. Several longer-term studies were considered for serving as the basis for the longer-term HA. The 1981 draft report by GSRI had methodology and data inconsistencies that make it unsuitable for a longer-term HA. In the Daniel et al. (1990) study, male and female rats in the 200 mg/L dose group (highest dose tested) had an average weight gain of 51% of controls and drinking water consumption was decreased 31-34%, which may suggest that the effect of weight loss may be due to a taste aversion rather than a systemic toxic effect. Although the authors identified a NOAEL of 100 mg/L (5.8 and 7.7 mg/kg/day) they also suggested that the NOAEL could be more clearly identified by a matched feeding and water study to more clearly distinguish between systemic toxic effects and weight loss from taste aversion. In the Daniel et al. (1991) study a NOAEL of 50 mg/L (8.6-9.2 mg/kg/day) was identified for B6C3F1 mice. At the two higher dose levels tested, the lower levels of serum enzymes and reduced organ weights were considered by the authors to be consistent with decreased water and nutrient consumption and altered electrolyte balance rather than chemical-induced toxicity. The studies by Daniel et al. are supportive of the NTP (1990) study, which is used as the basis for the DWEL. Using the data from either of the two studies by Daniel et al. would result in a longer-term HA of 3 mg/L, slightly less than the DWEL of 4 mg/L. The DWEL is based on a well conducted chronic NTP (1990) study with a NOAEL of 9.5 mg/kg/day. Because of the problems in interpreting the subchronic studies, it is recommended that the

DWEL of 4 mg/L be adopted for the longer-term HA for adults. For the 10 kg child drinking 1 L/day, the DWEL is modified resulting in a longer-term HA of 1 mg/L.

Assessment of Lifetime Exposure and Derivation of a DWEL. It is recommended that the study by NTP (1990) be used as a basis for a DWEL because it is a chronic 2-year study using rats and mice with chloramine in the drinking water. When monochloramine was administered to rats and mice in their drinking water there were statistically significant changes in body and several organ weights (as previously described) at the high-dose level. However, the significance of these changes is unclear because test animals consumed a reduced amount of water, which was perhaps due to palatability, and NTP does not consider these changes in body and organ weight biologically significant. It is recommended that the highest dose tested be used as the NOAEL. The NOAEL identified in the rat study was chosen over the NOAEL in the mouse study to calculate the proposed RfD because data from acute and shorter term studies indicate that the rat is the more sensitive species. The calculation of the RfD is as follows:

$$RfD = \frac{9.5\ mg/kg/day}{100} = 0.095\ mg/kg/day \quad (rounded\ to\ 0.1\ mg/kg/day)$$

where:

9.5 mg/kg/day = NOAEL based on an absence of biologically significant adverse effects in rats (NTP, 1990)

100 = uncertainty factor for protection of sensitive members of the human population and for use of an animal NOAEL

$$DWEL = \frac{0.1\ mg/kg/day\ x\ 70\ kg}{2\ L/day} = 3.5\ mg/L\ (rounded\ to\ 4\ mg/L)$$

where:

0.1 mg/kg/day	=	RfD
2 L/day	=	assumed water consumption by an adult
70 kg	=	assumed body weight of an adult

The RfD of 0.1 mg/kg/day was verified (06/23/92) by the RfD/RfC Work Group of the U.S. EPA (1994). The Work Group expressed a high degree of confidence in the critical study. Although ideally higher doses should have been tested, this was not possible due to the taste aversion. The study by NTP (1990) examined relevant endpoints in two animal species exposed to chloramine by a relevant route of exposure for a prolonged period of time. Several dosage levels were included, the number of animals per dose group was adequate, and the statistical analyses were used. The Work Group expressed a medium level of confidence in the data base. Information is available on mice, rat and monkeys for the noncarcinogenic toxicity of oral exposure to monochloramine for subchronic periods. The developmental toxicity and reproductive toxicity of monochloramine have been examined in rats but a developmental toxicity study in a second species and a 2-generation reproductive study are not available. (Information available for chlorine can be used to satisfy data gaps for monochloramine.) Confidence in the data base is limited

by the lack of information on health effects in humans. Overall confidence in the RfD is considered to be medium. A summary of the HAs and DWEL is presented in Table VIII-1.

Carcinogenic Effects

In a 2-year study by NTP (1990), there was equivocal evidence of carcinogenic activity of chloraminated drinking water in female rats, which was due to the slightly increased incidence of mononuclear cell leukemia compared with that of controls. There was no

TABLE VIII-1

Summary of HAs and DWEL for Noncarcinogenic Effects

HAs and DWEL	Dose Level (mg/kg/day)	10 kg Child (mg/L)	70 kg Adult (mg/L)	Reference
1-Day HA	10	1[a]	--	Bercz et al., 1982
10-Day HA	10	1	--	Bercz et al., 1982
Longer-term HA	9.5	1	4[b]	NTP, 1990
DWEL	9.5	--	4	NTP, 1990

[a]Adopted from the 10-day HA

[b]Adopted from the DWEL

evidence of carcinogenic activity because of chloraminated drinking water in male rats or in male or female mice. Monochloramine was verified by the CRAVE Work Group (12/02/92) and is classified in group D, not classifiable as to human carcinogenicity, meaning that there is inadequate human and animal evidence of carcinogenicity (U.S. EPA, 1986).

Existing Guidelines, Recommendations and Standards

The NAS (1987) calculated a suggested no-adverse-response level (SNARL) of 0.581 mg/L. This was based on a NOEL of 50 ppm (estimated by NAS to be 8.3 mg/kg bw/day) from a 90-day study in mice, which showed reduced body weight and liver toxicity (GSRI, 1981). It was assumed that a 70 kg human consumes 2 L of water daily, which contributes 20% of total intake. This value should be viewed cautiously since the data in this study were not verified.

Special Groups at Risk

Long-term hemodialysis patients have displayed higher risks of hemolytic anemia caused by chloramines present in dialysis baths. These chloramines denature hemoglobin through oxidation and inhibit the hexose monophosphate shunt (Eaton et al., 1973). This problem can be eliminated by using charcoal-filtered water in the dialysis bath; therefore, this group should not be given special consideration in the development of water quality criteria (U.S. EPA, 1981).

Risk Characterization

In characterizing the risk that chloramines pose in drinking water, the health effects must be considered in light of the widespread use of the chloramine-ammonia process (chloramination) for disinfection purposes. As stated earlier in this document, the range of residual in the distribution system (1.5-2.5 mg/L) is well below the DWEL of 4 mg/L. In humans, health effects do not appear to be associated with levels of residual chloramine typically found in drinking water.

IX. REFERENCES

Abdel-Rahman, M.S., M.R. Berardi and R.J. Bull. 1982. Effect of chlorine and monochloramine in drinking water on the developing rat fetus. J. Appl. Toxicol. 2(3): 156-159.

Abdel-Rahman, M.S., D.M. Waldron and R.J. Bull. 1983. A comparative kinetics study of monochloramine and hypochlorous acid in rat. J. Appl. Toxicol. 3(4): 175-179.

Abdel-Rahman, M.S., D.H. Suh and R.J. Bull. 1984. Toxicity of monochloramine in rat: An alternative drinking water disinfectant. J. Toxicol. Environ. Health. 13: 825-834.

Anbar, M. and G. Yagil. 1962. The hydrolysis of chloramine in alkaline solution. J. Am. Chem. Soc. 84: 1790-1796.

Anbar, M., S. Guttmann and R. Rein. 1959. The isotopic exchange between hypochlorite and halide ions. II. The exchange between hypochlorous acid and chloride ions. J. Am. Chem. Soc. 81: 1816-1821.

Anderson, D. and J.A. Styles. 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens. Appendix 2. The bacterial mutation test. Br. J. Cancer. 37: 924-930.

APHA (American Public Health Association). 1980. Standard Methods for the Examination of Water and Wastewater, 15th ed. p. 286-288.

Beck, H.I. 1983. Type I reaction to chloramine. Contact Dermatitis. 9(2): 155-156.

Bempong, M.A. and F.E. Scully, Jr. 1980a. Mutagenic activity of N-chloropiperidine. J. Environ. Path. Toxicol. 4(2,3): 345-354.

Bempong, M.A. and F.E. Scully, Jr. 1980b. *In vitro* cytological effect of N-chloropiperidine: Induction of mitotic anomalies in Chinese hamster ovary cells. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 3, R.L. Jolley et al., Ed. Ann Arbor Science, Ann Arbor, MI. p. 817-825.

Bempong, M.A. and F.E. Scully, Jr. 1983. Seminal cytology and reproductive toxicology of N-chloropiperidine. J. Am. College Toxicol. 2(2): 209-219.

Bempong, M.A., C. Montgomery and F.E. Scully, Jr. 1981. *In vitro* evaluation of N-chloropiperidine for toxic and mutagenic effects. J. Basic Appl. Sci. 39(2,3): 11-24.

Bempong, M.A., C. Montgomery and F.E. Scully, Jr. 1986. Mutagenicity and clastogenicity of N-chloropiperidine. J. Environ. Pathol. Toxicol. Oncol. 6(2): 241-252.

Bercz, J.P. and R. Bawa. 1986. Iodination of nutrients in the presence of chlorine based disinfectants used in drinking water treatment. Toxicol. Lett. 34(2 and 3): 141-147.

Bercz, J.P., L. Jones, L. Garner, D. Murray, D.A. Ludwig and J. Boston. 1982. Subchronic toxicity of chlorine dioxide and related compounds in drinking water in the nonhuman primate. Environ. Health Perspect. 46: 47-55.

Brodtmann, N.V., J.R. and P.J. Russo. 1979. The use of chloramine for reduction of trihalomethanes and disinfection of drinking water. J. Am. Water Works Assoc. 79(1): 40-42.

Bull, R.J. 1980. Health effects of alternate disinfectants and their reaction products. J. Am. Water Works Assoc. 72(5): 299-303.

Bull, R.J., M. Robinson, J.R. Meier and J. Stober. 1982. Use of biological assay systems to assess the relative carcinogenic hazards of disinfection by-products. Environ. Health Perspect. 46: 215-227.

Burttschell, R.H., A.A. Rosen, F.M. Middleton and M.B. Ettinger. 1959. Chlorine derivatives of phenol causing taste and odor. J. Am. Water Works Assoc. 51: 205-213.

Butterfield. C.T. 1948. Bactericidal properties of chloramines and free chlorine in water. Public Health Rep. 63: 934-940.

Calvert, C.K. 1940. Super chlorination. Water Works Sewerage. 87: 299-303.

Carlton, B., P. Barlett, A. Bascaron, K. Colling, I. Osis and M.K. Smith. 1986. Reproductive effects of alternative disinfectants. Environ. Health Perspect. 69: 237-241.

Chapin, R.M. 1931. The influence of pH upon the formation and decomposition of the chloro derivatives of ammonia. J. Am. Chem. Soc. 53: 912-920.

Colton, E., and M.M. Jones. 1955. Monochloramine. J. Chem. Educ. 32: 485-487.

Cooper, W.J., M.F. Mehran, R.A. Slifker, D.A. Smith, J.T. Villate and P.H. Gibbs. 1982. Comparison of several instrumental methods for determining chlorine residuals in water. J. Am. Water Works Assoc. 74: 546-552.

Corbett, R.E., W.S. Metcalf and F.G. Soper. 1953. Studies on N-halogenocompounds. IV. The reaction between ammonia and chlorine in aqueous solution, and the hydrolysis constants of chloramines. J. Chem. Soc., London. 1953: 1927-1929.

Crane, C.W., J. Forrest, O. Stephenson and W.A. Waters. 1946. The chlorination of methyl di-2-(chlorethyl)amine and related compounds. J. Chem. Soc., London. 1946: 827-830.

Craun, G.F. 1988. Surface water supplies and health. J. Am. Water Works Assoc. (Feb): 40-52.

Daniel, F.B., L.W. Condie, M. Robinson et al. 1990. Comparative subchronic toxicity studies of three disinfectants. J. Am. Water Works Assoc. 82: 61-69.

Daniel, F.B., H.P. Ringhand, M. Robinson, J.A. Stober, G.R. Olsen and N.P. Page. 1991. A comparative subchronic toxicity study of chlorine and monochloramine in drinking water in the B6C3F1 mouse. J. Am. Water Works Assoc. 83: 68-75.

Dowell, C.T. and W.C. Bray. 1917. Experiments with nitrogen trichloride. J. Am. Chem. Soc. 39: 896-905.

Eaton, J.W., C.F. Kolpin, H.S. Swofford, C.M. Kjellstrand and H.S. Jacob. 1973. Chlorinated urban water: A cause of dialysis-induced hemolytic anemia. Science. 181: 463-464.

Edmond, C.R. and F.G. Soper. 1949. The mechanism of formation of dialkyl-chloramines from hypochlorous acid. J. Chem. Soc., London. 1949: 2942-2945.

Ellis, A.J. and F.G. Soper. 1954. Studies of N-halogeno compounds. VI. The kinetics of chlorination of tertiary amines. J. Chem. Soc., London. 1954: 1750-1755.

Evans, O.M. 1982. Voltammetric determination of the decomposition rates of combined chlorine in aqueous solution. Anal. Chem. 54: 1579-1582.

Exon, J.H., L.D. Koller, C.A. O'Rielly and J.P. Bercz. 1987. Immunotoxicologic evaluation of chlorine-based drinking water disinfectants, sodium hypochlorite and monochloramine. Toxicology. 44: 257-269.

Feng, T.H. 1966. Behavior of organic chloramines in disinfection. J. Water Pollut. Control Fed. 38: 614-628.

Fetner, R.H. 1962. Chromosome breakage in *Vicia fabia* by monochloramine. Nature. 196: 1122-1123.

Gocke, E., M.-T. King, K. Eckhardt and D. Wild. 1981. Mutagenicity of cosmetics ingredients licensed by the European communities. Mutat. Res. 90: 91-109.

Gray, Jr., E.T., D.W. Margerum and R.P. Huffman. 1979. Chloramine equilibria and the kinetics of disproportionation in aqueous solution. In: Organometals and Organometalloids, Occurrence and Fate in the Environment, F.E. Brinkman and J.M. Bellama, Ed. Am. Chem. Soc., Washington, DC. p. 264-277.

Grisham, M.B., M.M. Jefferson, D.F. Melton and E.L. Thomas. 1984. Chlorination of endogenous amines by isolated neutrophils. J. Biol. Chem. 259(16): 10404-10413.

GSRI (Gulf South Research Institute). 1981. A subchronic study of chloramine generated *in situ* in the drinking water of F344 rats and B6C3F1 mice. Project No. 414-798. Draft Report prepared for Tracor-Jitco, Inc., Rockville, MD.

Hand, V.C. and D.W. Margerum. 1983. Kinetics and mechanisms of the decomposition of dichloramine in aqueous solution. Inorg. Chem. 22: 1449-1456.

Herren-Freund, S.L. and M.A. Pereira. 1986. Carcinogenicity of by-products of disinfection in mouse and rat liver. Environ. Health Perspect. 69: 59-65.

Ingols, R.S., H.A. Wyckoff, T.W. Kethley et al. 1953. Bacterial studies of chlorine. Ind. Eng. Chem. 45: 996-1000.

Isaac, R.A. and J.C. Morris. 1980. Rates of transfer of active chlorine between nitrogenous substrates. In: Water Chlorination: Environmental Impact and Health Effects, R.L. Jolley, W.A. Brungs and R.B. Cumming, Ed. Vol. 3. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. p. 183-191.

Isaac, R.A. and J.C. Morris. 1983. Transfer of active chlorine from chloramine to nitrogenous organic compounds. 1. Kinetics. Environ. Sci. Technol. 17: 738-742.

Isaac, R.A. and J.C. Morris. 1985. Transfer of active chlorine to nitrogenous organic compounds. 2. Mechanism. Environ. Sci. Technol. 19: 810-814.

Jander, J. 1955. Ein beitrage zur Kenntnis des monochloramins. Naturwissenchaften. 42: 173-179. (Ger.)

Johnson, J.D. 1978. Measurement and persistence of organic residuals in natural waters. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 1, R.L. Jolley, Ed. Ann Arbor Science, Ann Arbor, MI. p. 37-63.

Jolley, R.L. and J.H. Carpenter. 1983. A review of the chemistry and environmental fate of reactive oxidant species in chlorinated water. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 4, R.L. Jolley et al., Ed. Ann Arbor Science, Ann Arbor, MI. p. 3-47.

Jolley, R.L., G. Jones, W.W. Pitt and J.E. Thompson. 1978. Chlorination of organics in cooling waters and process effluents. Water Chlorination. 1: 105-138.

Kiese, M. 1974. Methemoglobinemia: A Comprehensive Treatise. CRC Press, Cleveland, OH.

Kirk-Othmer. 1979. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., M. Grayson, Ed. John Wiley and Sons, NY.

Kjellstrand, C.M., J.W. Eaton, Y. Yawata et al. 1974. Hemolysis in dialized patients caused by chloramines. Nephron. 13: 427-433.

Kovacic, P., M. Lowery and K.W. Field. 1970. Chemistry of N-bromamines and N-chloramines. Chem. Rev. 70: 639-665.

Laakso, M., I. Arvala, S. Tervonen and M. Sotarauta. 1982. Chloramine induced pneumonitis from mixing household cleaning agents. Br. Med. J. [Clin. Res.]. 285(6348): 1086.

Lombardi, P., M. Gola, M.C. Acciae and A. Sertoli. 1989. Unusual occupational allergic contact dermatitis in a nurse. Cont. Dermat. 20: 302-316.

Lubbers, J.F., S. Chauhan and J.R. Bianchine. 1981. Controlled clinical evaluation of chlorine dioxide, chlorite and chlorate in man. Fund. Appl. Toxicol. 1: 334-338.

Lu Shih, K.L. and J. Lederberg. 1976. Chloramine mutagenesis in *Bacillus subtilis*. Science. 192: 1141-1143.

Margerum, D.W., E.T. Gray, Jr. and R.P. Huffman. 1979. Chlorination and the formation of N-chloro compounds in water treatment. In: Organometals and Organometalloids, Occurrence and Fate in the Environment, F.E. Brinckman and J.M. Belloma, Ed. Am. Chem. Soc., Washington, DC. p. 278-291. (Cited in U.S. EPA, 1984)

Marks, H.C. and F.B. Strandskov. 1950. Halogens and their mode of action. Ann. N.Y. Acad. Sci. 53: 163-171.

Mauger, R.P. and F.G. Soper. 1946. Acid catalysis in the formation of chloramides from hypochlorous acid. J. Chem. Soc. 1946: 71-75.

Maziarka, S., I. Kongiel-Chablo, M. Rybak, S. Szulinski and J. Wojcik. 1976. Study of the effects of chlorine and chloramines in drinking water on animals. Rocj. Pastiv. Zatl. h. Heg. 27(2): 123-131.

Meier, J.R., R.J. Bull, J.A. Stober and M.C. Cimino. 1985. Evaluation of chemicals used for drinking water disinfection for production of chromosomal damage and sperm-head abnormalities in mice. Environ. Mutagen. 7: 201-211.

Mellanby, E. 1946. Diet and canine hysteria. Br. Med. J. 2: 885-887.

Merck Index. 1983. Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ. p. 74-81.

Miller, R.G., F.C. Kopfler, L.W. Condie et al. 1986. Results of toxicological testing of Jefferson Parish Pilot Plant samples. Environ. Health Perspect. 69: 129-139.

Moore, G.S. and E.J. Calabrese. 1980. The health effects of chloramines in potable water supplies. A literature review. J. Environ. Pathol. Toxicol. 4(1): 257-263.

Moore, G.S., E.J. Calabrese and M. McGee. 1980. Health effects of monochloramines in drinking water. J. Environ. Sci. Health. A15(3): 239-258.

Morris, J.C. 1967. Kinetics of reactions between aqueous chlorine and nitrogenous compounds. In: Principles and Applications of Water Chemistry, S.D. Faust and J.V. Hunter, Ed. John Wiley and Sons, Inc., New York. p. 23-53.

Morris, J.C. and R.A. Isaac. 1983. A critical review of kinetic and thermodynamic constants for the aqueous chlorine-ammonia system. In: Water Chlorination: Environmental Impact and Health Effects, Vol. 4, R.L. Jolley et al., Ed. Ann Arbor Science, Ann Arbor, MI. p. 49-62.

Morris, J.C., N. Ram, B. Baum and E. Wajon. 1980. Formation and significance of N-chloro compounds in water supplies. EPA 600/2-80-031.

Murphy, P.A. and G.F. Craun. 1990. A review of recent epidemiologic studies reporting associations between drinking water disinfection and cancer. In: Water Chlorination: Chemistry, Environmental Impact and Health Effects, Vol. 6, R.L. Jolley, L.W. Condie, J.D. Johnson et al., Ed. Lewis Publishers, Ann Arbor, MI. p. 361-372.

NAS (National Academy of Sciences). 1977. Drinking Water and Health. Vol. I. p. 19-63.

NAS (National Academy of Sciences). 1980. Drinking Water and Health. Vol. 3. p. 25-67.

NAS (National Academy of Sciences). 1987. Drinking Water and Health, Vol. 7. Safe Drinking Water Committee Board on Toxicology and Environmental Health Hazards, Assembly of Life Sciences. National Academy Press, Washington, DC.

Newell, G.W., T.C. Erickson, W.E. Gilson, S.N. Gershoff and C.A. Elvehjem. 1947. Role of "agenized" flour in the production of running fits. J. Am. Med. Assoc. 135: 760-763.

NIEHS (National Institute of Environmental Health and Safety). 1982. Subchronic study of monochloramine in the Fisher 344 rat and the B6C3F1 mouse. Draft Report, NTP, RTP, NC. (Cited in Wolfe et al., 1984)

NRC (National Research Council). 1979. Ammonia - Subcommittee for Ammonia. University Park Press, Baltimore, MD.

NRC (National Research Council). 1980. Drinking Water and Health, Vol. 2. Safe Drinking Water Committee Board on Toxicology and Environmental Health Hazards. Assembly of Life Sciences. National Academy Press.

NTP (National Toxicology Program). 1990. NTP Technical Report on the Toxicology and Carcinogenesis Studies of Chlorinated and Chloraminated Water in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR 392, National Institutes of Health.

Nusbaum, I. 1952. Sewage chlorination mechanism. A survey of fundamental factors. Water Sewage Works. 99: 295-297.

Pollock, G.H. 1949. Species specificity of agene toxicity. J. Appl. Physiol. 1: 802-806.

Pressley, T.A., D.F. Bishop, A.P. Pinto and A.F. Cassel. 1973. Ammonia-nitrogen removal by breakpoint chlorination. EPA 670/2-73-058.

Revis, N., P. McCauley, R. Bull and G. Holdsworth. 1986. Relationship of drinking water disinfectants to plasma cholesterol and thyroid hormone levels in experimental studies. Proc. Natl. Acad. Sci. USA. 83: 1485-1489.

Rickabaugh, J.F. and R.N. Kinman. 1978. Trihalomethane formation from iodine and chlorine disinfection of Ohio River water. In: Water Chlorination: Environmental Impact and Health Effects, R.L. Jolley, H. Gorchev and D.H. Hamilton, Jr., Ed., Vol. 2. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. p. 583-591.

Robinson, M., R.J. Bull, M. Schamer and R.E. Long. 1986. Epidermal hyperplasia in mouse skin following treatment with alternative drinking water disinfectants. Environ. Health Perspect. 69: 293-300.

RTECS (Registry of Toxic Effects of Chemical Substances). 1984. Ammonia and Chloramine. U.S. DHHS, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health. DHHS (NIOSH) Publ. No. 83-107-4.

Ryan, E.E., W.J. Cooper and E.P. Meier. 1980. Development of FACTS procedure for bromine, chlorine dioxide, and iodine in aqueous solutions. AD A091590. Tech. Rep. 8003, U.S. Army Med. Bioeng. Res. Develop. Lab., Ft. Detrict, Federick, MD. p. 98.

Sandford, P. A., A. J. Nafziger and A. Jeanes. 1971. Reaction of sodium hypochlorite with amines and amides: A new method for quantitating amino sugars in monomeric forms. Anal. Biochem. 42: 422-436.

Scully, F.E., Jr. and M.A. Bempong. 1982. Organic N-chloramines: Chemistry and toxicology. Environ. Health Perspect. 46: 111-116.

Scully, F.E., Jr., C.E. Bell, Jr. and M.A. Bempong. 1983. Organic N-chloramines: A chemical and toxicological assessment of environmental water contaminants. Final Report. EPA CR-807254.

Scully, F.E., Jr., D.M. Oglesby and H.J. Buck. 1984a. Cyclic voltammetry of organic and inorganic N-chloramines in aqueous solution. Anal. Chem. 56: 1449-1451.

Scully, F.E., Jr., J.P. Yang, K. Mazina and F.B. Daniel. 1984b. Derivatization of organic and inorganic N-chloramines for high-performance liquid chromatographic analysis of chlorinated water. Environ. Sci. Technol. 18(10): 787-792.

Scully, F.E., Jr., K. Mazina, D.E. Sonenshine, and F.B. Daniel. 1985. Reactions of hypochlorite and organic N-chloramines in stomach fluid. In: Water Chlorination:

Chemistry, Environmental Impact and Health Effects, Vol. 5, R.L. Jolley et al., Ed. Lewis Publishers, Inc., Chelsea, MI. p. 175-184.

Scully, F.E., Jr., K.E. Mazina, D. Sonenshine, and F. Kopfler. 1986. Quantitation and identification of organic N-chloramines formed in stomach fluid on ingestion of aqueous hypochlorite. Environ. Health Perspect. 69: 259-265.

Scully, F.E., Jr., K. Mazina, H.P. Ringhand, E.K. Chess, J.A. Campbell and J.D. Johnson. 1990. Identification of organic N-chloramines *in vitro* in stomach fluid from the rat after chlorination. Chem. Res. Toxicol. 3: 301-306.

Silver, M.L., R.E. Johnson, R.M. Kark, J.R. Klein, E.P. Monahan and S.S. Zevin. 1947a. White bread and epilepsy in animals. J. Am. Med. Assoc. 135: 757-760.

Silver, M.L., S.S. Zevin, R.M. Kark, and R.E. Johnson. 1947b. Canine epilepsy caused by flour bleached with nitrogen trichloride (agene). I. Experimental method. Proc. Soc. Exper. Biol. Med. 66: 408-409.

Silver, M.L., E.P. Monahan and J.R. Klein. 1947c. Canine epilepsy caused by flour bleached with nitrogen trichloride (agene). II. Role of amino acids. Proc. Soc. Exper. Biol. Med. 66: 410-412.

Snyder, M.P. and D.W. Margerum. 1982. Kinetics of chlorine transfer from chloramine to amines, amino acids, and peptides. Inorg. Chem. 21: 2545-2550.

Stevens, A.A., C.J. Slocum, D.R. Seeger and G.G. Robeck. 1978. Chlorination of organics in drinking water. In: Water Chlorination: Environmental Impact and Health Effects, R.L. Jolley, Ed., Vol. 1. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. p. 77-104.

Suh, D.H. and M.S. Abdel-Rahman. 1983. Kinetics study of chloride in rat. J. Toxicol. Environ. Health. 12: 467-473.

Süssmuth, R. 1982. Genetic effects of amino acids after chlorination. Mutat. Res. 105: 23-28.

Symons, J.M., J.K. Carswell, R.M. Clark, et al. 1978. Ozone, chlorine dioxide and chloramines as alternatives to chlorine for disinfection of drinking water. Water Chlorination. 2: 555-560.

Taras, M.J. 1950. Preliminary studies on the chlorine demand of specific chemical compounds. J. Am. Water Works Assoc. 42: 462-474.

Thomas, E.L., M.M. Jefferson, J.J. Bennett and D.B. Learn. 1987. Mutagenic activity of chloramines. Mutat. Res. 188: 35-43.

Trussell, R. and P. Kreft. 1984. Proceedings of AWWA Seminar on Chloramines. AWWA, Denver, CO.

U.S. EPA. 1981. Ambient Water Quality Criterion for the Protection of Human Health: Ammonia. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC.

U.S. EPA. 1986. Guidelines for Carcinogen Risk Assessment. Federal Register. 51(185): 33992-34003.

U.S. EPA. 1990. Chloramine in Saliva and Gastric Fluid. Prepared by Purdue University PO #OCO99INTEX (4/18/90) for Health Effects Research Laboratory, Cincinnati, OH.

U.S. EPA. 1994. Integrated Risk Information System (IRIS). Online. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH.

Wajon, J.E. and J.C. Morris. 1980. The analysis of free chlorine in the presence of nitrogenous organic compounds. Environ. Int. 3: 41-43.

Weast, R.C., Ed. 1983. CRC Handbook of Chemistry and Physics, 64th ed. CRC Press, Inc., Boca Raton, FL. p. 13-84, 85, 117.

Wei, I.W. and J.C. Morris. 1974. Dynamics of breakpoint chlorination. In: Chemistry of Water Supply, Treatment, and Distribution. A.J. Rubin, Ed. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. p. 297-332. (Cited in U.S. EPA, 1984)

Weil, I. and J.C. Morris. 1949. Kinetic studies on the chloramines. I. The rates of formation of monochloramine, N-choromethylamine and N-chlorodimethylamine. J. Am. Chem. Soc. 71: 1664-1671.

Weitberg, A.B. 1987. Chloramine-induced sister-chromatid exchanges. Mutat. Res. 190: 277-280.

White, G.C. 1972. Handbook of Chlorination for Potable Water, Wastewater, Cooling Water, Industrial Processes and Swimming Pools. Van Nostrand Reinhold Company, New York, NY. p. 192-201.

Wolfe, R.L. and B.H. Olson. 1985. Inability of laboratory models to accurately predict field performance of disinfectants. In: Water Chlorination: Chemistry, Environmental Impact and Health Effects, Vol. V, R.L. Jolley et al., Ed. Lewis Publishers, Inc., Chelsea, MI. p. 555-573.

Wolfe, R.L., N.R. Ward and B.H. Olson. 1984. Inorganic chloramines as drinking water disinfectants: A review. J. Am. Water Works Assoc. 76(5): 74-88.

Wright, N.C. 1926. The action of hypochlorites on amino acids and proteins. Biochem. J. 20: 524-532.

Wright, N.C. 1936. The action of hypochlorites on amino acids and proteins. The effect of acidity and alkalinity. Biochem. J. 30: 1661-1667.

Zierler, S., R.A. Danley and L. Feingold. 1986. Type of disinfectant in drinking water and pattern of mortality in Massachusetts. Environ. Health Perspect. 69: 275-279.

Zierler, S., L. Feingold, R.A. Danley and G. Craun. 1988. Bladder cancer in Massachusetts related to chlorinated and chloraminated drinking water: A case-control study. Arch. Environ. Health. 43(2): 195-200.