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Aqueous Chlorine-Based Antimicrobial/Disinfectant Products:
Final Report

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Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ATTACHMENT

EXCEL Workbook: SERA EXWS 52-15-04a: Custom Worksheets for Aqueous Chlorine Risk Assessment

ACRONYMS AND ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AEL	adverse-effect level
a.i.	active ingredient
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
bw	body weight
calc	calculated value
CBI	confidential business information
CI	confidence interval
cm	centimeter
DAA	days after application
DAT	days after treatment
DER	data evaluation record
d.f.	degrees of freedom
EC_x	concentration causing X% inhibition of a process
EC_{25}	concentration causing 25% inhibition of a process
EC_{50}	concentration causing 50% inhibition of a process
EFED	Environmental Fate and Effects Division (U.S. EPA/OPP)
ExToxNet	Extension Toxicology Network
F	female
FH	Forest Health
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FQPA	Food Quality Protection Act
g	gram
GLP	Good Laboratory Practices
ha	hectare
HED	Health Effects Division (U.S. EPA/OPP)
HQ	hazard quotient
IARC	International Agency for Research on Cancer
IRED	Interim Reregistration Eligibility Decision
IRIS	Integrated Risk Information System
k_a	absorption coefficient
k_e	elimination coefficient
kg	kilogram
$K_{o/c}$	organic carbon partition coefficient
$K_{o/w}$	octanol-water partition coefficient
K_p	skin permeability coefficient
L	liter
lb	pound
LC_{50}	lethal concentration, 50% kill
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LOC	level of concern
m	meter
M	male
mg	milligram

mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
mM	millimole
mPa	millipascal, (0.001 Pa)
MOS	margin of safety
MRID	Master Record Identification Number
MSDS	material safety data sheet
MW	molecular weight
NAWQA	USGS National Water Quality Assessment
NCI	National Cancer Institute
NCOD	National Drinking Water Contaminant Occurrence Database
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	not otherwise specified
NRC	National Research Council
NTP	National Toxicology Program
OM	organic matter
OPP	Office of Pesticide Programs
OPPTS	Office of Pesticide Planning and Toxic Substances
OSHA	Occupational Safety and Health Administration
Pa	Pascal
PBPK	physiologically-based kinetic
POC	Port Orford Cedar
ppm	parts per million
RED	re-registration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
SOD	Sudden Oak Death
TEP	typical end-use product
T.G.I.A.	Technical grade active ingredient
TRED	Tolerance Reassessment Eligibility Decision
UF	uncertainty factor
U.S.	United States
USDA	U.S. Department of Agriculture
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WHO	World Health Organization

COMMON UNIT CONVERSIONS AND ABBREVIATIONS

To convert ...	Into ...	Multiply by ...
acres	hectares (ha)	0.4047
acres	square meters (m^2)	4,047
atmospheres	millimeters of mercury	760
centigrade	Fahrenheit	1.8℃+32
centimeters	inches	0.3937
cubic meters (m^3)	liters (L)	1,000
Fahrenheit	centigrade	0.556℉-17.8
feet per second (ft/sec)	miles/hour (mi/hr)	0.6818
gallons (gal)	liters (L)	3.785
gallons per acre (gal/acre)	liters per hectare (L/ha)	9.34
grams (g)	ounces, (oz)	0.03527
grams (g)	pounds, (oz)	0.002205
hectares (ha)	acres	2.471
inches (in)	centimeters (cm)	2.540
kilograms (kg)	ounces, (oz)	35.274
kilograms (kg)	pounds, (lb)	2.2046
kilograms per hectare (hg/ha)	pounds per acre (lb/acre)	0.892
kilometers (km)	miles (mi)	0.6214
liters (L)	cubic centimeters (cm^3)	1,000
liters (L)	gallons (gal)	0.2642
liters (L)	ounces, fluid (oz)	33.814
miles (mi)	kilometers (km)	1.609
miles per hour (mi/hr)	cm/sec	44.70
milligrams (mg)	ounces (oz)	0.000035
meters (m)	feet	3.281
ounces (oz)	grams (g)	28.3495
ounces per acre (oz/acre)	grams per hectare (g/ha)	70.1
ounces per acre (oz/acre)	kilograms per hectare (kg/ha)	0.0701
ounces fluid	cubic centimeters (cm^3)	29.5735
pounds (lb)	grams (g)	453.6
pounds (lb)	kilograms (kg)	0.4536
pounds per acre (lb/acre)	kilograms per hectare (kg/ha)	1.121
pounds per acre (lb/acre)	mg/square meter (mg/m^2)	112.1
pounds per acre (lb/acre)	µg/square centimeter ($\mu g/cm^2$)	11.21
pounds per gallon (lb/gal)	grams per liter (g/L)	119.8
square centimeters (cm^2)	square inches (in^2)	0.155
square centimeters (cm^2)	square meters (m^2)	0.0001
square meters (m^2)	square centimeters (cm^2)	10,000
yards	meters	0.9144

Note: All references to pounds and ounces refer to avoirdupois weights unless otherwise specified.

CONVERSION OF SCIENTIFIC NOTATION

Scientific Notation	Decimal Equivalent	Verbal Expression
$1 \cdot 10^{-10}$	0.0000000001	One in ten billion
$1 \cdot 10^{-9}$	0.000000001	One in one billion
$1 \cdot 10^{-8}$	0.00000001	One in one hundred million
$1 \cdot 10^{-7}$	0.0000001	One in ten million
$1 \cdot 10^{-6}$	0.000001	One in one million
$1 \cdot 10^{-5}$	0.00001	One in one hundred thousand
$1 \cdot 10^{-4}$	0.0001	One in ten thousand
$1 \cdot 10^{-3}$	0.001	One in one thousand
$1 \cdot 10^{-2}$	0.01	One in one hundred
$1 \cdot 10^{-1}$	0.1	One in ten
$1 \cdot 10^{0}$	1	One
$1 \cdot 10^{1}$	10	Ten
$1 \cdot 10^{2}$	100	One hundred
$1 \cdot 10^{3}$	1,000	One thousand
$1 \cdot 10^{4}$	10,000	Ten thousand
$1 \cdot 10^{5}$	100,000	One hundred thousand
$1 \cdot 10^{6}$	1,000,000	One million
$1 \cdot 10^{7}$	10,000,000	Ten million
$1 \cdot 10^{8}$	100,000,000	One hundred million
$1 \cdot 10^{9}$	1,000,000,000	One billion
$1 \cdot 10^{10}$	10,000,000,000	Ten billion

EXECUTIVE SUMMARY

The term *aqueous chlorine* literally refers to chlorine gas (Cl_2) dissolved in water. In the context of Forest Service uses, aqueous *chlorine* refers to aqueous solutions of sodium hypochlorite (NaClO), commonly referred to as *bleach*. The general uses of bleach can be classified as equipment or facilities disinfection, dust suppression, and the disinfection of water used in fire suppression.

Under normal conditions of use, there is no plausible basis for asserting that aqueous chlorine is likely to cause adverse effects in either workers or members of the general public. If standard and sensible precautions are followed, no adverse effects in workers would be anticipated. No significant exposure to members of the general public are likely and thus no adverse effects would be anticipated. Nonetheless, the misuse of aqueous chlorine by workers or accidental exposures to members of the general public are considered. If workers handle solutions of aqueous chlorine without gloves, skin irritation is likely if the skin is exposed to undiluted bleach formulations and may occur when working with some diluted solutions. The only accidental exposures for members of the general public that are quantified in the current risk assessment involve the accidental spill into a small pond of water treated with aqueous chlorine at 50 ppm and used for fire suppression. For this scenario, the levels of potential oral exposure are below the level of concern by factors of 4 to over 300 and the levels of dermal exposure are below the levels of concern by factors of 10,000 to over 300,000.

Allergic contact dermatitis may develop in some individuals who repeatedly use bleach. If individuals using aqueous chlorine develop severe skin irritation, respiratory impairment, or other signs of toxicity that might suggest an allergic response, it would be prudent to discontinue exposure and promptly seek medical attention.

Except for accidental exposure scenarios, risks to nontarget organisms appear to be unlikely for all organisms except soil microorganisms. When used for dust suppression, soil microorganisms on or within the road surface will be adversely effected. While these risks cannot be quantified, there is little doubt adverse effects in soil microorganisms will occur. The effects would be transient, localized, and are not likely to cause detectable secondary effects. The effects on microorganisms are the only risks to nontarget species that are likely to occur in the normal use of aqueous chlorine in Forest Service programs.

A number of accidental exposure scenarios are developed for nontarget species. Except for incidents involving gross mishandling of aqueous chlorine, no risks to birds and mammals are apparent. Risks to other terrestrial organisms cannot be quantified but do not appear to be substantial. While risks to aquatic organisms are quantified, there are substantial uncertainties in the quantitative risk characterization that relate both to the exposure and dose-response assessments. Nonetheless, the accidental release of relatively large amounts of aqueous chlorine into surface water could adversely affect aquatic organisms, particularly sensitive species of aquatic invertebrates and aquatic microorganisms. Under worst-case conditions – i.e., the release of a large amount of aqueous chlorine into a small body of water – adverse effects and perhaps substantial mortality in all groups of aquatic organisms are plausible.

1. INTRODUCTION

Aqueous chlorine is used in Forest Service programs to disinfect equipment or vehicles as well as to disinfect water drawn from ponds or streams used for fire suppression and the suppression of road dust. The present document includes risk assessments for human health and ecological effects associated with the use of aqueous chlorine in Forest Service programs.

This document contains four chapters, including the introduction, program description, risk assessment for human health effects, and risk assessment for ecological effects or effects on wildlife species. Each of the two risk assessment chapters has four major sections, including an identification of the hazards associated with the use of aqueous chlorine disinfectants, an assessment of potential exposure, an assessment of the dose-response relationships, and a characterization of the risks associated with plausible levels of exposure.

Although this is a technical support document and addresses some specialized technical areas, it is intended to be understood by individuals who do not have specialized training in the chemical and biological sciences. Certain technical concepts, methods, and terms common to all parts of the risk assessment are described in plain language in a separate document (SERA 2007a).

The published literature on chlorine and aqueous solutions of chlorine was identified using TOXLINE (http://toxnet.nlm.nih.gov/), reviews by ATSDR (2007), WHO (IPCS 2006; WHO/FAO 1967; WHO/FAO 1985), the U.S. EPA (U.S. EPA/OPP 1999a,b; U.S. EPA/OPP 2005a, 2006a,b; U.S. EPA/OHEA 1994) on chlorine and related compounds, and reviews published in the open literature (e.g., Bruch 2007; Racioppi et al. 1994; Vetrano 2001; Winder 2001). Additional information on aqueous chlorine solutions was identified through standard Internet search engines. The literature on aqueous solutions of chlorine is large and complex. As discussed further in Section 2 (Program Description), the primary agents of concern are the hypochlorite anion (OCl^-) and hypochlorous acid (HOCl) in formulations (i.e., dilute solutions of sodium hypochlorite) typically marketed as bleach (e.g., Clorox®). While there are many different types of bleaches, the term bleach is used in the current Forest Service risk assessment to designate aqueous solutions of chlorine.

Most Forest Service risk assessments are accompanied by an EXCEL workbook that contains a relatively standard set of exposure scenarios that are generally applicable to applications of insecticides and herbicides. These standard workbooks are generated by a computer program and follow uniform structure and organization (SERA 2008a). While the current risk assessment is also accompanied by an EXCEL workbook, the uses of aqueous chlorine in Forest Service programs differ substantially from the uses of insecticides and herbicides. Consequently, the workbook that accompanies this Forest Service risk assessment on aqueous chlorine contains only custom worksheets that are designed specifically for the current risk assessment. The design of the individual worksheets in the EXCEL workbook for aqueous chlorine is similar to the design of worksheets in standard Forest Service workbooks but the number and nature of the exposure scenarios are different, as detailed further in the exposure assessments for human health effects (Section 3.2) and ecological effects (Section 4.2).

2. PROGRAM DESCRIPTION

2.1. Overview

The term *aqueous chlorine* literally refers to chlorine gas (Cl_2) dissolved in water. In the context of Forest Service uses, aqueous *chlorine* refers to aqueous solutions of sodium hypochlorite (NaClO), commonly referred to as *bleach*. While thousands of formulations of bleach are available, only two formulations of bleach, both provided by the Clorox Company, are designated for use in Forest Service programs: Ultra Clorox Brand Regular Bleach and Clorox Commercial Solutions Ultra Clorox Germicidal Bleach. Both formulations are aqueous solutions of sodium hypochlorite (NaClO) with lesser amounts of sodium hydroxide. The primary role of sodium hydroxide in aqueous solutions of sodium hypochlorite is to adjust the pH of the solution to about 11. At this pH, the predominant forms of chlorine are hypochlorous acid (HOCl) and hypochlorite ions (OCl^-), which in combination are often referred to as available chlorine. Bleach solutions contain very low concentrations of chlorine gas. Although this risk assessment explicitly covers only the two formulations of bleach designated by the Forest Service, it encompasses other comparable formulations of aqueous sodium hypochlorite. This risk assessment does not cover formulations of aqueous sodium hypochlorite that contain additional active ingredients (e.g., sodium dichloroisocyanurate).

The use of bleach by the Forest Service generally involves attempts to control the spread of pathogens, such as those causing Port Orford cedar root disease or Sudden Oak Death, amphibian pathogens such as Chytrid fungus, or human pathogens such as Hantavirus. The general uses of bleach, which can be classified as equipment or facilities disinfection, dust suppression, and the disinfection of water used in fire suppression, are remarkably different from the application of insecticides and herbicides. Consequently, only a subset of the exposure assessments typically used in Forest Service risk assessments are included in the current risk assessment on bleach.

Relative to fire suppression and dust suppression, equipment or facilities cleaning involves the use of small amounts of bleach at concentrations ranging from about 200 to greater than 10,000 ppm available chlorine and exposures in relatively small areas. Fire suppression and dust abatement, which involve the treatment of large quantities of water, require lower concentrations of available chlorine (i.e., 50-200 ppm). Also, unlike equipment and facilities disinfection, water treated with bleach for fire suppression or dust abatement may be broadcast over a large area.

2.2. Chemical Description and Commercial Formulations

The various molecular species of chlorine are easy to confuse. The following list may be useful for understanding the chemistry of chlorine bleach, as discussed below:

Cl^-	chloride ion
Cl_2	chlorine gas
NaClO	sodium hypochlorite
HOCl	hypochlorous acid
OCl^-	hypochlorite ion

Although chlorine (Cl_2) is a gas, chlorine gas is soluble in water. All of the references to chlorine in the following sections refer specifically to aqueous chlorine.

Figure 1 provides an overview of the common reactions that occur in solutions of bleach as well as an overview of how bleach reacts in the environment. In the following subsections of the Program Description, the former set of reactions is referred to as product chemistry, and the reactions are relatively simple (Section 2.2.1.1). The later set of reactions, referred to as environmental chemistry, can be far more complex, depending on the types of environmental material with which the hypochlorite ion reacts (Section 2.2.1.2).

2.2.1. Chemistry of Aqueous Chlorine Disinfectants

2.2.1.1. Product Chemistry

Table 1 summarizes information on the chemical and physical properties of sodium hypochlorite. The Clorox Company is a major provider of aqueous solutions of sodium hypochlorite, commonly referred to as *Clorox*.

Aqueous chlorine refers generally to chlorine gas (Cl_2) dissolved in water. The aqueous chlorine disinfectants used in Forest Service programs are formulated as aqueous solutions of sodium hypochlorite (NaClO) and are commonly referred to as *bleach*.

The sodium hypochlorite solutions considered in this risk assessment are produced by the chlorination of sodium hydroxide solutions (Farr et al. 2003, p. 51, Eq. 9):

$$Cl_2 + 2\ NaOH \leftrightarrow NaClO + NaCl + H_2O \qquad \text{(Eq. 1)}$$

The name *Clorox* was originally selected as a trade name for the combination of chlorine and sodium hydroxide [http://www.thecloroxcompany.com/company/history/index.html]. Sodium hydroxide [NaOH] is the only other ingredient listed in the MSDS for the bleach formulations covered in this risk assessment.

In an aqueous solution, the hypochlorite (OCl^-) ion will react to form solutions which also contain hypochlorous acid (HOCl) and aqueous chlorine gas (Cl_2). The equilibria reactions for this mixture are illustrated in Figure 1 and summarized below:

$$Cl_2 + H_2O \leftrightarrow HOCl + H^+ + Cl^- \leftrightarrow OCl^- + H^+ \quad \text{(Eq. 2)}$$

The equilibrium kinetics of these reactions are pH dependent. As summarized in Figure 2, the predominant species of chlorine in very acidic solutions (pH 0 to 2) is chlorine gas (Cl_2). In less acidic to weakly basic solutions (pH ranging from 2 to 8), the predominant chlorine species is hypochlorous acid (HOCl). In more basic solutions (pH > 8), the predominant chlorine species is the hypochlorite ion (OCl^-).

Aqueous solutions of sodium hypochlorite (NaClO) are relatively stable but will degrade slowly to sodium chlorite ($NaClO_2$), sodium chlorate ($NaClO_3$), and sodium chloride (NaCl):

$$\begin{gathered} 2\ NaOCl \rightarrow NaCl + NaClO_2 \\ NaOCl + NaClO_2 \rightarrow NaCl\ NaClO_3 \\ NaOCl \rightarrow NaCl + \tfrac{1}{2}\ O_2 \end{gathered} \quad \text{(Eq. 3)}$$

Although the kinetics of the reactions cited above are very complex, the overall reaction rates are slow (Lister 1956a). These reactions involving the degradation of aqueous sodium hypochlorite occur in commercial bleach solutions. For example, the product label for Ultra Clorox Brand Regular Bleach indicates that the formulation contains 6% sodium hypochlorite; however, the MSDS for this formulation indicates the formulation contains 6-7.35% sodium hypochlorite. The reason for the difference is that 6.0% sodium hypochlorite is the minimum target level for the formulated product. The initial concentration may be higher in the originally formulated product to account for the slow degradation of the formulation during normal storage (Green 2008).

As discussed by Lister (1956a), the rate of degradation involves bimolecular reactions. Thus, expressions of first-order half-lives are not meaningful. Empirical degradation patterns for sodium hypochlorite solutions indicate that the degradation of available chlorine is more rapid as the concentration of hypochlorite increases—e.g., a 1% solution will degrade by about 40% in 100 weeks while a 12% solution will degrade by about 90% over the same period (Medlicott 2001, Figure 1, p. 11).

2.2.1.2. Environmental Chemistry

Sodium hypochlorite will not persist in the environment. One degradation reaction involves the catalytic decomposition of the hypochlorite ion to oxygen and the chlorine ion:

$$2\ OCl^- \rightarrow O_2 + 2\ Cl^- \quad \text{(Eq. 4)}$$

The above reaction can be catalyzed by a number of transition metals including cobalt, nickel, and copper and is much more rapid than the degradation of sodium hypochlorite in the absence of catalysts—i.e., in sodium hypochlorite formulations (Gray et al. 1977; Lister 1956b). In addition to catalytic breakdown by transition metals, the hypochlorite ion is also subject to

decomposition by UV light (Abel-Gawad and Bewtra 1988; Farr et al. 2003; Feng et al. 2007; Young and Allmand 1949).

The decomposition of hypochlorous acid is pH dependant and the kinetics of decomposition are complex – i.e., a third order process (Adam et al. 1992). The approximate half-life of free chlorine (i.e., combined concentrations of hypochlorous acid and hypochlorite) in chlorinated effluents ranges from about 1.3 to 5 hours (Jolley 1983). In municipal effluents, decay rates for active chlorine ranged from 0.116 to 0.816 day^{-1} (Abdel-Gawad and Bewtra 1988, Table 2, p. 953), corresponding to half-lives from about 0.84 to 6 days ($t_{½} = \ln(2) \div k$). In pond water treated with chlorine for the disinfection of shrimp pathogens, the chlorine levels dropped by a factor of about 100 in a 24-hour period (Bratvold et al. 2007).

Hypochlorous acid (HOCl) is formed naturally in soil via microbial chloroperoxidases (Archibald et al. 1998; Wever 1988, 2004; van Ginkel and Nobel 2004; Wannstedt et al. 1990) and will form the hypochlorite ion (OCl^-):

$$H_2O_2 + H^+ + Cl^- \rightarrow H_20 + HOCl \leftrightarrow OCl^- + H^+ \qquad \text{(Eq. 5)}$$

The quantitative significance of this reaction, relative to the deposition of bleach onto soil, is unclear.

Although the fate of active chlorine in soil is less clearly characterized, there is no evidence that active chlorine will persist or that the applying bleach to soil will have a substantial or prolonged impact on soil chemistry. One class of reactions involves the oxidation of several organic molecules (designated generically as R-H) by hypochlorous acid which results in the formation of chlorinated organics:

$$HOCl + R\text{-}H \rightarrow R\text{-}Cl + H_2O \qquad \text{(Eq. 6)}$$

The above reaction can include a number of naturally occurring organic compounds, like complex organics (e.g., Casey 2002) and very simple organic compounds, like methane (Scholer and Thiemann 2005). Although these reactions lead to the dissipation of hypochlorous acid in soil, they may, nevertheless, generate toxic organochlorine compounds. The formation of potentially hazardous organochlorine compounds is discussed further in Section 3.1.15.

Sodium hypochlorous acid will also react with amine groups. For example, after oral administration in mammals, hypochlorous acid solutions react with stomach contents to form various chloramines (U.S. EPA/ECAO 1994) as well as other low molecular weight chlorinated hydrocarbons, like chloroform and chloroacetic acids (Mink et al. 1983; Scully et al. 1986).

2.2.2. Commercial Formulations

Sodium hypochlorite is an ingredient in more than 2700 active pesticide formulations; furthermore, there are over 300 active formulations consisting solely of sodium hypochlorite as the active ingredient (PAN 2008). Therefore, it is not feasible to list all of the formulations of

sodium hypochlorite that might be used in Forest Service programs. Table 2 summarizes information on two formulations that are specifically labeled for the control of forest pathogens—i.e., Ultra Clorox Brand Regular Bleach and Clorox Commercial Solutions Ultra Clorox Germicidal Bleach. As discussed in further detail below, these formulations may be used to control the spread of the pathogenic agents in Port Orford cedar root disease (*Phytophthora ramorum*) and Sudden Oak Death (*Phytophthora lateralis*).

Some other formulations of sodium hypochlorite are listed in Table 4. While relatively few of the hundreds of available formulations of sodium hypochlorite are listed in Table 4, the current risk assessment will cover all formulations of sodium hypochlorite and sodium hydroxide in which sodium hypochlorite is the only active ingredient. These other formulations may be used in equipment cleaning and disinfection. Uses involving the control of forest pathogens, however, would be limited to the formulations of Clorox that are specifically labeled for forest pathogens. Notwithstanding this limitation, it should be noted that aqueous solutions of sodium hypochlorite are essentially identical except for the concentrations of sodium hypochlorite in the different formulations.

The concentration of sodium hypochlorite varies considerably among Clorox formulations, which has little impact on the current risk assessment, given the substantial dilution required for the various uses of aqueous solutions of sodium hypochlorite (Table 4). For example, the most dilute formulation of sodium hypochlorite summarized in Table 4 contains 0.45% sodium hypochlorite, which corresponds to 4.5 parts per thousand (ppt) or 4500 parts per million (ppm or mg/L). As discussed further in Section 2.3, this concentration is substantially higher than the concentrations required for most uses of bleach in Forest Service programs.

Sodium hydroxide is characterized as a *stabilizer* (Clorox Professional Products Division 2008). Note that the chlorination of water (Figure 1) will tend to acidify the water and acidification will favor the occurrence of molecular chlorine (Figure 2). Molecular chlorine, in turn, will rapidly volatilize from water (ATSDR 2007, p. 158). In addition, note that the pH of Ultra Clorox Brand Regular Bleach is very high—i.e., a pH of 11.4, which is a very basic, as opposed to acidic, solution (Table 1). As also illustrated in Figure 2, aqueous solutions of chlorine at a pH of about 11 will consist almost entirely of the hypochlorite ion (OCl^-) with virtually no molecular chlorine (Cl_2). In other words, sodium hydroxide is added to aqueous solutions of sodium hypochlorite to increase the pH of the aqueous solution and decrease the loss of chlorine species through the volatilization of molecular chlorine.

2.2.2. Label Specifications

With respect to the interpretation of mixing instructions and other types of information considered in this risk assessment, the term *available chlorine* is important because many of the label directions specify the desired concentration as *available chlorine*. As detailed by Farr et al. (2001), available chlorine (Cl_{Avail}) refers to the amount of chlorine (Cl_2) needed to produce the oxidant in units of moles ($Oxid_{Moles}$)—e.g., hypochlorous acid in Equation 2—which can be calculated as:

$$Cl_{Avail} = 70.9_{g/mole} \times Oxid_{Moles} \times \boldsymbol{N} \qquad \text{(Eq. 7)}$$

where 70.9 is the molecular weight of Cl_2 and ***N*** is the number of *active chlorine atoms* per molecule of oxidant. By definition, the term *active chlorine* refers to the number of Cl^- atoms that can accept two electrons. As indicated in Equation 2, this value is one in the case of both aqueous chlorine and hypochlorous acid. Thus, *active chlorine* is always half the value of *available chlorine*.

Using Ultra Clorox Brand Regular Bleach as an example, the product label indicates that the formulation contains 6% sodium hypochlorite and 5.7% *available chlorine*. Given a 6% (w/w) solution of sodium hypochlorite (i.e., 6 g/100 g), the available chlorine is:

$$Cl_{Avail} = 70.9_{g/mole} \times [(6\ g/100g) \div (74.44\ g/mole)] \times 1$$
$$Cl_{Avail} = 5.71467\ g\ /100\ g \approx 5.71\% \approx 57{,}100\ ppm$$

where 74.44 g/mole is the molecular weight of sodium hypochlorite. Thus, the label specifications for Ultra Clorox Brand Regular Bleach are consistent with the definition of available chlorine provided by Farr et al. (2001).

This is not the case with the label specification for Clorox Commercial Solutions Ultra Clorox Germicidal Bleach, also called CPPC Ultra Bleach 2. The label specifications for this product indicate that the formulation contains 6.15% sodium hypochlorite and yields 5.84% or 58,425 ppm available chlorine. Taking the 6.15% sodium hypochlorite concentration, the available chlorine is:

$$Cl_{Avail} = 70.9_{g/mole} \times [(6.15\ g/100g) \div (74.44\ g/mole)] \times 1$$
$$Cl_{Avail} = 5.85754\ g\ /100\ g \approx 58{,}575.4\ ppm \approx 5.86\%.$$

The discrepancy between 5.84% and 5.86% is not substantial but could be a source of confusion. The source of the discrepancy cannot be attributed to rounding errors; however, it is modest and does not impact the current risk assessment.

The term *free chlorine* is defined as the total amount of hypochlorous acid and hypochlorite in water (ATSDR 2007). Test strips commonly used to assay for free chlorine are designed to measure the concentration of hypochlorous acid and hypochlorite in water (Industrial Test Systems 2003). The product label for Ultra Clorox Brand Regular Bleach as well as similar products like household Clorox Bleach (EPA Reg. No. 5813-1) indicate that *available chlorine* may be assayed with standard chlorine test strips. In other words, the terms *available chlorine* and *free chlorine* are, in effect, synonymous.

2.3. Uses in Forest Service Programs

2.3.1. Labeled and General Uses

Aqueous chlorine-based antimicrobial/disinfectant products have a number of uses. Many of these uses involve household or domestic applications, including cleaning or preventing mildew,

controlling algae and other microorganisms in swimming pools, sanitizing household surfaces in kitchens and bathrooms, and as an additive with laundry detergents for cleaning clothes (Rutala 1995). Although some of these uses may pertain to applications in Forest Service programs, the focus of the current risk assessment is on applications that are directly related to forestry.

While not a labeled use, sodium hypochlorite is an approved direct food additive and indirect additive and is classified as a GRAS (Generally Recognized As Safe) compound that is used to strengthen dough, bleach flour, and fumigate foods (Clydesdale 1997; Fukayama et al. 1986).

A summary of forestry uses along with a representative summary of some more general uses of sodium hypochlorite, arranged in order of increasing concentration of available chlorine, is given in Table 4. In this table, uses that are clearly and directly related to forestry applications are shaded; the other more general uses are not.

All information given in Table 3 is taken from the product labels for Ultra Clorox Brand Regular Bleach and Clorox Commercial Solutions Ultra Clorox Germicidal Bleach from the U.S. EPA label web site (http://www.epa.gov/pesticides/pestlabels). The latter product is also referred to as *CPPC Ultra Bleach 2*.

The uses that are most directly related to forestry involve two plant pathogens, *Phytophthora lateralis* and *Phytophthora ramorum*. *Phytophthora lateralis* is the pathogenic agent in Port Orford cedar (*Chamaecyparis lawsoniana*) root disease, and *Phytophthora ramorum* is the pathogenic agent in Sudden Oak Death. Both of these organisms are water molds.

Two distinct forestry uses related to these pathogens are specified: cleaning tools and preventing pathogen spread by disinfecting water that may be broadcast in fire suppression. Both of these uses specify concentrations of 50 ppm available chlorine. Many domestic cleaning procedures for equipment and utensils recommend available chlorine concentrations of 200 ppm.

Water drafted from ponds or streams for use in fire suppression is treated at 50 ppm available chlorine for 5 minutes prior to use. While this water treatment is recommended specifically for preventing the spread of pathogens responsible for Port Orford cedar root disease and the tool cleaning precautions are recommended specifically for preventing the spread of the pathogens responsible for Sudden Oak Death, it seems likely that both types of treatments might be used for both types of pathogens. Fire suppression will typically involve helicopter applications in which loads of 75 gallons to 700 gallons of treated water may be dropped in a discrete and limited area or fixed-wing air tankers with loads of 100 to 3,000 gallons which may be spread over a larger area (California Office of Emergency Services 2004).

Disinfected water (50 ppm) has many more uses than fire suppression, including road watering for dust abatement as well as equipment washing (e.g., fire suppression equipment, dozers, engines, boots, road building equipment, or passenger vehicles). With fire suppression, bleach may be used directly in water tenders or water drops from helicopters. To reduce the likelihood

of getting bleach in streams, the bleach is added to fire trucks and road watering equipment after they leave the stream area where they are filled.

The use of treated water for dust suppression is somewhat different from other uses of bleach in that the bleach solution is intentionally applied over a relatively wide area – i.e., the road surfaces being treated. This is the only use of bleach for which application rates may be expressed in units of mass per unit surface area. While application rates for dust suppression may be variable depending on local road surface conditions, Errington (2009) suggests that a 2000 gallon water truck would typically cover about ¾ miles of a road that is 10 to 12 feet wide. This corresponds to a surface area of about 39,000 ft^2 to 47,520 ft^2 [0.75 x 5,280 ft x 10 to 12 ft]. Using a conversion factor of 0.0929 m^2/ft^2, these areas are equivalent to about 3623 m^2 to 4415 m^2. As indicated in Table 5, water used for dust suppression will consist of solutions with 50 ppm (mg/L) available chlorine. A 2000 gallon capacity corresponds to 7570 liters (1 gallon = 3.785 liters). At a concentration of 50 ppm, the truck will contain 378,500 mg of available chlorine [50 mg/L x 7570 L]. Thus, the application rate of chlorine for dust suppression ranges from about 86 mg/m^2 [378,500 mg / 4415 m^2] to 104 mg/m^2 [378,500 mg / 3623 m^2].

Bleach may also be used in *mix water* to produce solutions of fire retardants, like Phos-Chek. As noted in Table 4, the use of bleach in *mix water* for fire retardants may involve solutions of up to about 200 ppm available chlorine (Betlejewski 2008). Although the use of bleach in *mix water* is included in the current risk assessment, not all Forest Service Regions or National Forests will use bleach in *mix water* for fire suppressants.

More concentrated solutions of sodium hypochlorite are recommended for the treatment of citrus canker (seedling treatment with solutions of about 5700 ppm available chlorine) and parasitic nematodes, and other plant pathogens in nursery stock (a root dip at 8500 ppm available chlorine). These types of uses, however, are not relevant to Forest Service programs.

The highest concentration—i.e., a 20% solution of a 6% formulation—is recommended for cleaning tools, equipment, and enclosures used in amphibian care and/or collection to prevent the spread of *Batrachochytrium dendrobatidis*, a Chytrid fungus pathogen of amphibians. This use is relevant to Forest Service activities involving any field activity that could entail the transmission of the Chytrid pathogen from one location to another.

2.3.2. Special Forest Service Uses

In addition to the standard and labeled uses for bleach, the Forest Service requested that specific uses be addressed explicitly in the current risk assessment. These special Forest Service uses are summarized in Table 5.

As indicated in Table 5, these special uses designated by the Forest Service generally involve concentrations higher than those used in water treatment for fire suppression (50-200 ppm a.i.) but less than the concentration used to clean equipment to prevent the spread of Chytrid fungus (i.e., 10,432 ppm a.i.). Thus, all uses specified in Table 5 are encompassed by the product labels and are included in the current risk assessment.

Notably, all of the special uses designated in Table 5 appear to correspond to specific applications of labeled uses. The disinfectant uses for dreissenid veligers, whirling disease, and *Didymosphenia geminata* appear to be labeled for uses associated with equipment cleaning, and the use for Hantavirus appears to be a special case of facilities surface cleaning. Various formulations of Clorox, including both Ultra Clorox Brand Regular Bleach and Clorox Commercial Solutions Ultra Clorox Germicidal Bleach, are specifically labeled as disinfectants for human Influenza A virus (U.S. EPA/OPP 2009).

2.4. Use Statistics

Most Forest Service risk assessments attempt to characterize the use of the pesticide in Forest Service programs, relative to the use of the pesticide in agricultural and other applications. The information on Forest Service use is typically taken from Forest Service pesticide use reports (http://www.fs.fed.us/ foresthealth/pesticide/reports.shtml), and information on agricultural use is typically taken from use statistics compiled by the U.S. Geologic Survey (http://ca.water.usgs.gov/pnsp/ pesticide_use_maps/) and/or detailed pesticide use statistics compiled by the state of California (http://www.calepa.ca.gov/).

Over the period from 2000 to 2004 (the most recent year that Forest Service statistics are available), only three applications of sodium hypochlorite are listed, all in Forest 11 of Region 6 (the Pacific Northwest). The total use is reported as about 1750 gallons, and the applications are reported in units of acre feet (i.e., a unit of volume often used to designate a volume of water), and the total amount treated is specified as 103.07 acre-feet. One acre-foot contains about 325,900 gallons of water.

The combined applications in Forest 11 correspond to a dilution of about 0.000052 [1750 gallons / (325,900 gallons/acre foot x 103.07 acre-feet)]. Assuming that the gallons reported apply to a formulation with 5.71% available chlorine (i.e., Ultra Clorox Brand Regular Bleach), the available chlorine concentration would be about 30 ppm [0.0571 x 0.000052 x 1,000,000 = 2.97 ppm].

Based on a review of more recent Forest Service documents (e.g., Appendix 4 in the Final Supplemental Environmental Impact Statement, Management of Port-Orford-Cedar in Southwest Oregon), it appears that water treatment with Clorox will be the major use in Forest Service programs. In 2002, this EIS reports the use of a total of 26,700 gallons of Ultra Clorox for water used in fire suppression, dust abatement, equipment cleaning, and *fire area rehabilitation* (not otherwise specified). This use is much higher than the use of 1713 gallons in 2002 (R6, Forest 11) given in the Forest Service use report. This difference probably reflects the fact that bleach is not generally regarded as a pesticide in most Forest Service activities.

While the Forest Service use of Clorox and possibly other formulations of sodium hypochlorite may be higher and perhaps much higher than the amounts given in the Forest Service use reports, it does not seem plausible that the Forest Service use of sodium hypochlorite will be substantial, relative to other uses. As summarized in (U.S. EPA/OPP 2006c), sodium hypochlorite is used

extensively in the treatment of drinking water. The most recent survey by the American Water Works Association (AWWA) was completed in 1998 and estimates that about 20% of community water systems that serve populations greater than 10,000 use sodium hypochlorite for water disinfection. For community water systems serving a population of less than 10,000, sodium hypochlorite is used by about 34% of systems that process groundwater and about 17% of the systems that process surface water (U.S. EPA/OPP 2006c). In addition to this use, as noted in Section 2.3, aqueous solutions of sodium hypochlorite have extensive use in household applications.

3. HUMAN HEALTH RISK ASSESSMENT

3.1. HAZARD IDENTIFICATION

3.1.1. Overview

Aqueous chlorine is a strong oxidizing agent that will react directly with cellular components such as DNA, proteins, lipids, and carbohydrates. At sufficiently high doses, these reactions will result in gross damage to tissue and this damage can lead to serious signs of toxicity including death. Because aqueous chlorine is very reactive, the tissues that are damaged by aqueous chlorine will depend on the route of exposure. Oral exposure may result in damage to gastrointestinal tissue and dermal exposure may result in damage to skin tissue. While chlorine gas is highly toxic by inhalation, the concentration of chlorine gas in bleach, the form of aqueous chlorine used in Forest Service programs, is very low and inhalation is not a significant route of exposure. Damage to respiratory tissue by aqueous chlorine is only likely to occur if aqueous chlorine is ingested in large amounts. In such cases, vomiting may occur and aqueous chlorine may be aspirated into lung tissue.

Because aqueous chlorine is a common household product – i.e., bleach – there is substantial human experience with the ingestion of aqueous chlorine either by mischance or attempted suicide. The approximate acute lethal dose in humans is in the range of about 300 mg/kg bw to 900 mg/kg bw. This rather wide range of doses may reflect either differences in sensitivity among individuals, the extent of aspiration of bleach into the lungs, uncertainties in exposure estimates from accidental or suicidal incidents, and/or the nature of medical attention received by different individuals after ingestion. The ranges of approximate lethal doses in humans is similar to the range of reported lethal doses in experimental mammals – i.e., about 225 to 675 mg/kg bw. While the ingestion of aqueous chlorine can be lethal, most accidental cases of ingestion of household bleach occur in children and these incidents generally do not result in serious adverse effects. Many of these non-serious incidents involving the ingestion of aqueous chlorine by children appear to involve doses in the range of about 24 to 30 mg/kg bw. As with acute lethal doses, the non-fatal and often asymptomatic doses in humans are similar to the rat NOAEL of 25 mg/kg bw.

In the normal use and handling of bleach, dermal contact is the most likely exposure pathway. As with oral toxicity, the dermal effects of exposures to aqueous chlorine in humans are well-documented. The concentrations of aqueous chlorine in normal household bleach – i.e., 5.25% to 6% - can cause dermal irritation over periods of exposure as low as 20 minutes. Dilute solutions of 1% or less do not appear to cause overt signs of dermal irritation. The effects of intermediate concentrations of greater than 1% but less than 5% appear to be variable depending on the duration of exposure. No irritant effects were noted in 15 to 90 minute exposures to 4% solutions but moderate irritation has been noted in some individuals in 48 hours exposures to 2% solutions.

Some individuals may become sensitized to aqueous chlorine. In other words, individuals who are exposed to bleach repeatedly over a prolonged period of time may develop an allergic

reaction. In cases of dermal exposure, this can lead to very severe and painful damage to the skin. Signs of systemic effects – e.g., respiratory impairment – associated with dermal sensitization to bleach have not been reported. Systemic allergic responses, however, have been documented in individuals exposed to aqueous chlorine solutions during root canals. Other than the rare reports of systemic allergic responses, solutions of aqueous chlorine do not appear to be associated with any signs of systemic toxicity.

3.1.2. Mechanism of Action

The mechanism of action of bleach – i.e., an aqueous solution of sodium hypochlorite – is based on the oxidation of molecules in biological tissue. As illustrated in Figure 1, hypochlorous acid may be involved in a large number of non-specific oxidation reactions resulting in the formation of chlorinated organic compounds as well as chloramines. Depending on the number of such reactions that occur – i.e., depending on the dose – these chemical reactions may interfere with or degrade tissue integrity and/or result in the formation of toxic chlorination byproducts.

The relative importance or predominance of these two types of reactions – i.e., direct tissue damage versus the formation of toxic chlorinated byproducts – has not been well characterized. As reviewed by ATSDR (2007), the mechanism of action of aqueous chlorine is essentially identical to that of chlorine gas, which converts to aqueous chlorine when in contact with biological tissue. Aqueous chlorine will interact with tissue at the site of administration. Thus, oral exposure will damage tissue in the upper gastrointestinal tract (Section 3.1.4), dermal exposure will result in damage to the skin (Section 3.1.11), and inhalation exposure will result in damage to lung tissue (Section 3.1.13).

The formation of chlorinated byproducts has been reviewed by Fukayama et al. (1986), Mink et al. (1983), and Scully et al. (1989). Aqueous chlorine may interact with endogenous carbohydrates, lipids, or protein and a very wide spectrum of chlorinated organic compounds may be formed. While the chemistry of these reactions is at least partially characterized, the toxicological importance of the endogenous formation of chlorinated byproducts is less clear and it is difficult to separate from the impact of the structural degradation tissue. The bactericidal efficacy of hypochlorous acid is based on direct damage to normal molecular structures in bacteria (Section 4.1.2.6) and it seems reasonable to suggest that this is also be the case in higher organisms.

While many pesticides are classified as xenobiotics – i.e., compounds that are not formed naturally in an organism – this is not the case for hypochlorous acid. As detailed by Wang et al. (2007), hypochlorous acid is produced by phagocytes such as neutrophils, a specialized type of immune cell, through the reaction of hydrogen peroxide, formed during respiratory bursts, with endogenous chloride ions and hydrogen ions:

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_20. \qquad \text{(Eq. 8)}$$

The hypochlorous acid formed during these reactions is the primary oxidant used by neutrophils to destroy microorganisms and this reaction is catalyzed by myeloperoxidases (McKenna and

Davies 1988). This mechanism essentially mimics the standard uses of bleach covered in the current Forest Service risk assessment – i.e., mammals produce and use hypochlorous acid to kill bacteria.

3.1.3. Pharmacokinetics and Metabolism

For most pesticides covered in Forest Service risk assessments, the discussion of pharmacokinetics and metabolism focuses on how rapidly the pesticide is absorbed and excreted as well as how the pesticide is altered metabolically by the organism. Aqueous chlorine, however, is inorganic and highly reactive. Rather than the organism metabolically altering the pesticide, aqueous chlorine will react with molecules in the organism through mechanisms discussed in the previous subsection.

Abdel-Rahman et al. (1983) have studied the basic pharmacokinetics of ^{36}Cl-labelled hypochlorous acid after oral dosing in rats. It should be noted that this study does not involve the kinetics of hypochlorous acid itself. Rather, the study provides information on how rapidly the labeled chlorine is absorbed, eliminated, and distributed by the organism. In this study, the labeled chlorine was rapidly absorbed (half-life of 2.2 hours) and rapidly cleared from plasma (half-life of about 44 to 88 hours). After 96-hours, the labeled chlorine was widely distributed – i.e., readily incorporated into the chlorine pool – with the highest concentration in the plasma and the lowest concentration in the fat. Approximately 50% of the labeled chlorine was excreted in the urine (≈36%) and feces (≈15%) by 96 hours after dosing with most of the label (≈81%) excreted as the chloride ion. These kinetics are similar to the kinetics of ^{36}Cl-labelled NaCl (common salt), with a plasma clearance half-time of about 52 hours (Suh and Abdel-Rahman 1983).

On oral exposure, aqueous chlorine will react with the stomach contents of the animal and a large number of different chlorinated organic compounds may be formed (Mink et al. 1983; Scully et al. 1986; Vogt et al. 1979). Mink et al. (1983) dosed rats with 7 mL of an 8,000 mg/L solution of sodium hypochlorite. The dose to the rats is not specified and the average body weight of the rats is not specified. Assuming the young rats were used (an approximate body weight of 0.25 kg), the approximate dose would be 224 mg/kg bw [0.007 L x 8,000 mg/L / 0.25 kg]. The animals were sacrificed one hour after dosing and the stomach contents were assayed by GC/MS analyses. Chlorinated reaction products included chloroform, trichloroacetic and dichloroacetic acid, dichloroacetonitrile, and trichloroacetonitrile. Mink et al. (1983) do not provide detailed estimates of the concentrations of these reaction products but indicate that the concentrations in plasma ranged from about 0.06 mg/L to 1.3 mg/L. In the study by Scully et al. (1986) rats weighing 200 to 350 g were administered 3 mL of hypochlorite at concentrations of 220 mg/L or 1016 mg/L. Taking an average body at 0.275 kg, the doses of hypochlorite to the rats were approximately 2.4 mg/kg bw or 11 mg/kg bw [0.003 L x 220 mg/L or 1016 mg/L / 0.275 kg]. The stomach contents of the rats were removed and analyzed 10 minutes after dosing. Several N-chloramines detected including N-chloroalanine, N-chloroglycine, and N-chlorophenylalanine – i.e., the chlorination of amino acids. In the study by Vogt et al. (1979), concentrations of aqueous chlorine from 4000 ppm to 16,000 ppm were associated with the formation of chloroform in the stomach of rats 1.5 hours after dosing.

The nature of the reaction products of aqueous chlorine with stomach contents may also be inferred based on studies of the reaction of hypochlorous acid with food stuffs. As reviewed by Fukayama et al. (1986), chlorine will oxidize a number of different carbohydrates resulting in the formation of carboxylic acid groups (-COOH) and will depolymerize starches. Some hydrocarbons may be halogenated. In an accidental fatal exposure to a young child, chloroform was identified in the stomach (Jakobsson et al. 1991). Chlorination is a common reaction with lipids, proteins, and amino acids. Based on studies with model lipid compounds, the extent of chlorination increases with the number of double bonds in lipids – i.e., unsaturated fats are chlorinated more readily than saturated fats (Ghanbari et al. 1982).

In most Forest Service risk assessments, the assessment of dermal absorption and quantification of dermal absorption rates is central to the risk assessment because many of the most common pathways for workers and the general public involve dermal exposures. While dermal exposures to solutions of sodium hypochlorite are likely, dermal absorption resulting in systemic toxicity is not likely to occur because of the reactivity of hypochlorous acid and the hypochlorite ion. As detailed further in Section 3.1.11.1 (Skin Irritation), aqueous chlorine can be highly irritating to the skin through the reactions of hypochlorous acid and the hypochlorite ion with components of the skin, most likely proteins and lipids. In this process, however, hypochlorous acid and the hypochlorite ion are consumed and dermal absorption of these components in aqueous chlorine is precluded. As noted by Brunch (2007), the lack of dermal absorption is also indicated by the available dermal toxicity studies on sodium hypochlorite and hypochlorous acid in which localized effects on the skin are prominent (Section 3.1.11.1) but no signs of systemic toxicity are apparent (Section 3.1.12).

3.1.4. Acute Oral Toxicity

The acute oral toxicity of bleach/aqueous chlorine is central to the current risk assessment. As discussed further in Section 3.2, the only plausible exposures for members of the general public involve acute ingestion associated with the consumption of contaminated water after the inadvertent spill of treated water into a stream or lake.

One type of acute toxicity information involves time-specific LD_{50} or LC_{50} values (i.e., doses or concentrations of a toxicant that result in or are estimated to result in 50% mortality of the test species during a specified exposure or observation period). These values can be viewed as an index of acute lethal potency. The acute lethal potency of aqueous chlorine can be estimated from relatively standard LD_{50} studies in experimental mammals. In addition, because bleach is a common household product, there is substantial human experience with accidental or suicidal ingestion of bleach and, in some instances estimates of lethal human doses can be made.

The available data on the acute lethal potency of aqueous chlorine are somewhat inconsistent. As reviewed by both ATSDR (2007) and Racioppi et al. (1994), the reported LD_{50} values in rats for sodium hypochlorite range from about 5,000 mg/kg bw to 13,000 mg/kg bw. As might be expected for a corrosive agent, the reported LD_{50} values for relatively concentrated solutions (i.e., 5.25%) are somewhat lower (i.e., 5,000 to 8,200 mg/kg bw) than the reported LD_{50} values

for less concentrated solutions (i.e., 13,000 mg/kg bw for a 5.25% solution). Much lower doses, however, have been associated with mortality in rats. Jakobsson et al. (1991) note mortality in groups of 2 to 4 rats within 0.5 to 5 hours after single gavage doses of 225 to 675 mg/kg bw (using a 4.5% formulation) with a general inverse relationship between dose and time to death.

Racioppi et al. (1994) has reviewed a large number of accidental or suicidal incidents involving humans. In most instances, the amount of bleach consumed is not known or can only be approximated crudely. Lethal exposures have been noted in adults after the consumption of 200 mL to 500 mL of 12.5 % solution of bleach. Assuming a 70 kg body weight, the estimated dose is 357 to 893 mg/kg bw [125,000 mg/L x 0.2 L to 0.5 L ÷ 70 kg bw]. In some fatal human exposures to bleach, death may be associated with aspiration of the bleach solution into the lungs. In these instances, the apparent lethal dose for aqueous chlorine may be somewhat lower than 357 mg/kg bw. For example, the U.S. EPA/OPP (1991a) cites a study by Done (1961) in which an 18-month-old girl died after ingesting … *a few tablespoons of bleach* (U.S. EPA/OPP 1999a, p. 9). Assuming a bleach solution of 6% aqueous chlorine (i.e., 60,000 mg/L) and using a volume of about 15 mL per tablespoon, the consumption of 3 tablespoons of bleach would be equivalent to about 2700 mg of aqueous chlorine [0.015 L/tablespoon x 3 tablespoons x 60,000 mg/L]. Assuming a body weight of 9.2 kg for an 18-month-old child (U.S. EPA/NCEA 2008, Table 8-1, p. 8-2), the estimated dose would be about 293 mg/kg bw. Overall, the lethal human doses appear to be in the general range of about 300 mg/kg bw to 900 mg/kg bw.

The estimates of acute lethal doses in humans and experimental animals are somewhat difficult to compare. In animal studies, the doses are administered to the test animals and observations are made at differing times. In cases of human exposure, bleach is consumed by mischance or intent and, in most of the cases of bleach ingestion, the individuals consuming the bleach are given medical care. For example, Ward and Routledge (1988) report an incident in which a 66-year-old woman survived the consumption of 500 mL of a 10% (100,000 mg/L) solution of sodium hypochlorite. The body weight of the woman is not specified in the publication. Taking 64.8 kg as the average body weight for females 65 to 74 years old (U.S. EPA/ORD 1997, Table 7 5, p. 7-6), the estimated dose is about 772 mg/kg bw [0.5 L x 100,000 mg/L ÷ 64.8 kg], which is substantially above the lowest estimated lethal dose of 357 mg/kg bw. As detailed by Ward and Routledge (1988), however, this woman received prompt medical attention – i.e., gastric lavage within 2 hours of ingesting the bleach. While somewhat speculative, the dose of 772 mg/kg bw might have been lethal to the woman if she had not received prompt medical attention. In a very similar incident reported by Ross and Spiller (1999), another 66-year old woman consumed an unknown quantity of a 5.25% solution of sodium hypochlorite and died, even though she appears to have received medical attention within 1-hour after consuming the bleach solution. This individual died about 4.5 hours after the consumption of the bleach, within the range of the times to death in rats reported by Jakobsson et al. (1991). This report, however, as well as other similar reports of gross over-exposure from the consumption of bleach cannot be used to assess the toxicity of bleach to humans relative to experimental mammals because the amount of bleach that is consumed is not known.

Within the limitations and uncertainties in the available human case reports, the available data do not suggest a remarkable difference in the approximate lethal doses in humans and experimental mammals – i.e., about 300 to 900 mg/kg bw in humans and 225 to 675 mg/kg bw in experimental mammals.

While the ingestion of bleach may be fatal, most incidents of bleach consumption are nonfatal. The common signs of toxicity in the consumption of nonfatal doses of sodium hypochlorite involve irritation to the mouth and throat as well as vomiting and these signs of toxicity do not generally require aggressive medical intervention (Mack 1983; Pike et al. 1963). As reviewed further by ATSDR (2007), serious adverse effects are not typically seen after the consumption of bleach solutions so long as the amount is less than about one cup. This is consistent with a number of surveys of case reports in which the consumption of sodium hypochlorite generally results in gastrointestinal irritation and less commonly injury to the esophagus but not in fatalities (e.g., Jakobsson et al. 1991; Pike et al. 1963; Landau and Saunders 1964; Hook and Lowry 1974).

Most accidental/non-suicidal ingestions of bleach involve young children. In these types of incidents, dose estimates are highly uncertain. Mack (1983) estimates that pre-school children will generally consume about 4 to 5 mL of bleach. Assuming that the bleach consists of a 6% solution of sodium hypochlorite (60,000 ppm or mg/L) and taking 10 kg as the body weight of a toddler, the dose of sodium hypochlorite consumed would be about 24 mg/kg bw to 30 mg/kg bw [0.004 L to 0.005 L x 60,000 mg/L ÷ 10 kg]. This dose estimate is below the range of lethal doses in humans and animals and very similar to the 25 mg/kg bw/day NOAEL in mice exposed to chlorine in drinking water at a concentration of 200 mg/L for a 30 day period (Blabaum and Nichols 1956). As discussed further in Section 3.3.2 (Acute RfD), the mouse NOAEL of 25 mg/kg bw is the basis for the 10-day drinking water health advisory for aqueous chlorine.

Only one study, Lubbers et al. (1982), is available on controlled human exposures to sodium hypochlorite. In this study, 10 volunteers were administered aqueous chlorine, in two doses at a volume of 500 mL per dose at a 4 hour interval, on 6 days over a 16 day period at progressively increasing concentrations: 0.1 mg/L on Day 1, 1 mg/L on Day 4, 5 mg/L on Day 7, 10 mg/L on Day 10, 18 mg/L on Day 13, and 24 mg/L on Day 16. No adverse effects were noted based on subjective reports, serum chemistry, blood counts, urinalysis, and a general physical exam. Lubbers et al. (1982) do not specify the body weight of the individuals but do indicate that the subjects were 21 to 35 year old males whose body weights were within 10% of normal values. Taking 70 kg as a standard male body weight, the highest and final dose was about 0.34 mg/kg bw [1 L x 24 mg/L ÷ 70 kg], substantially below the lower bound dose of 24 mg/kg bw associated with nonfatal exposures that do not require extraordinary medical intervention as well as the acute NOAEL of 25 mg/kg bw/day in experimental mammals.

3.1.5. Subchronic or Chronic Systemic Toxic Effects

The long-term toxicity of aqueous chlorine has been studied primarily because chlorine is used in the disinfection of public drinking water. As discussed in Section 3.2 (Exposure Assessment), however, longer-term exposures to aqueous chlorine are not likely to occur in programs related

to Forest Service uses. Consequently, the summary of most chronic studies in experimental mammals is relatively cursory.

In addition to the acute study in human volunteers summarized in the previous section, Lubbers et al. (1982) also exposed groups of 10 human volunteers to 0.5 liters/day of aqueous chlorine at a concentration of 5 mg/L for 12 weeks. No effects were noted based on subject reports (i.e., symptoms), hematology or urinalysis. Assuming a body weight of 70 kg, these exposures corresponded to estimated daily doses of about 0.0375 mg/kg bw [0.5 L x 5 mg/L ÷ 70 kg]. As noted by ATSDR (2007), the subjects in the Lubbers et al. (1982) study may have consumed aqueous chlorine from sources other than the liquid administered in the study and actual daily doses of aqueous chlorine may have been higher than 0.0375 mg/kg bw. In another controlled study involving human volunteers, Wones et al. (1991) exposed groups of 30 men and 30 women to 1.5 L/day of aqueous chlorine at a concentration of 20 mg/L for 4 weeks. No effects were noted on standard clinical parameters (i.e., body weight, blood pressure, pulse rate, and temperature) as well as serum lipid profile and serum levels of thyroid hormones. Based on reported average body weights, 66.7 kg for women and 73.7 kg for men, the aqueous chlorine exposures corresponded to 0.44 mg/kg bw/day for women [1.5 L/day x 20 mg/L ÷ 66.7 kg] and 0.4 mg/kg bw/day for men [1.5 L/day x 20 mg/L ÷ 73.7 kg]. These longer-term NOAELs are modestly higher than the acute NOAEL of 0.34 mg/kg bw in the acute study by Lubbers et al. (1982), discussed in the previous section.

Most longer-term toxicity studies in experimental mammals report no adverse effects at doses up to 133 mg/kg bw/ day in mice and rats (Cunningham 1980; Daniel et al. 1990, 1991; Hasegawa et al. 1986; NTP 1992). As discussed further in Section 3.3.3 (Chronic RfD), the U.S. EPA's Agency-wide chronic RfD on IRIS (U.S. EPA/ORD 1994) in based on a NOAEL of 14.4 mg/kg/day from carcinogenicity study in rats (NTP 1992). This dose estimate is based on a 2-year exposure to aqueous chlorine at a concentration of 250 mg/L, the highest dose tested. This NOEAL and corresponding chronic RfD is also adopted by the Office of Pesticides (U.S. EPA/OPP 1999a).

As detailed in Section 3.3.2 (Acute RfD), the subchronic study by Blabaum and Nicholas (1956) is used by the U.S. EPA (U.S. EPA/OHEA 1994; U.S. EPA/OPP 1999a) to derive a short-term health advisory for aqueous chlorine. In this study, groups of 20 mice, 10 males and 10 females, were exposed to aqueous chlorine at a concentration of 100 mg/L for 50 days or 200 mg/L for 33 days. No effects were noted based on gross or histopathological changes, signs of toxicity, or body weight. Based on an average body weight of 0.02 kg and water consumption rate of 0.0025 L/day, U.S. EPA/OHEA (1994) estimated the dose to the mice at 25 mg/kg bw/day [200 mg/L x 0.0025 L/day ÷ 0.02 kg]. All of these longer-term toxicity values are free-standing NOAELs – i.e., the NOAELs are not associated with any higher dose levels that cause toxic effects.

3.1.6. Effects on Nervous System

While exposures to high concentrations of chlorine gas have been associated with neurological effects in humans (ATSDR 2007), there is little indication that aqueous chlorine will lead to neurological effects. The most detailed study involving histopathological examinations of

nervous system tissue is the life-time bioassay in mice and rats conducted by NTP (1992) in which no remarkable gross or histopathological changes were noted in the brains of either rats (at doses of up to 85 mg/kg bw/day) or mice (at doses of up to 39.2 mg/kg bw/day). Similarly, the chronic study by Hasegawa et al. (1986) reported no effects on brain pathology in rats at doses of up to 133 mg/kg bw/day. Shorter term studies in experimental mammals also do not report any adverse effects or histopathology that would be associated with neurotoxicity (Cunningham 1980; Daniel et al. 1990; Daniel et al. 1991; Furukawa et al. 1980).

3.1.7. Effects on Immune System

There are various methods for assessing the effects of chemical exposure on immune responses, including assays of antibody-antigen reactions, changes in the activity of specific types of lymphoid cells, and assessments of changes in the susceptibility of exposed animals to resist infection from pathogens or proliferation of tumor cells. Sensitization may also be classified as an effect on immune function – i.e., the induction of an allergic reaction – and the studies on sensitization are covered in Section 3.1.11.2 (Skin Sensitization).

Three studies specifically focused on the effects on immune function – i.e., Fidler (1977), Hermann et al. (1982), and Exon et al. (1987). The study by Fidler (1977) exposed female mice to sodium hypochlorite at total chlorine concentrations of 25 to 30 mg/L for four weeks. The control group consisted of mice given tap water with chlorine levels of 0.5 to 1 mg/L. After the first week, a significant decrease was noted in the number of macrophages isolated from peritoneal exudate. By week 2, a decrease was also noted in the cytotoxicity of the macrophages to mouse melanoma and fibrosarcoma cells. During week 3 to 4 of the study, the peritoneal macrophages displayed no cytotoxicity to tumor cells. The Fidler (1977) study is a very brief report that does not provide sufficient information on the mice to reasonably estimate the doses to the mice in units of mg/kg bw.

In a very similar study (Hermann et al. 1982), no effects on either humoral or cell-mediated immune function were noted. In this study, mice were exposed to aqueous chlorine at concentrations of 15 or 30 mg/L for 120 days and immune function was assayed based on delayed hypersensitivity to sheep red blood cells, serum antibody responses to the sheep red blood cells, and clearance of colloidal carbon by phagocytosis. No effects were noted for any of these endpoints.

In the study by Exon et al. (1987), rats were administered sodium hypochlorite in drinking water at concentrations of 5, 15, and 30 mg/L (chlorine) for 9 weeks – i.e., the rats were 3 weeks old at the start of the study and the exposures were continued until the rats were 12 weeks old. In the high dose group, immunotoxicity was evidenced by a significant ($p<0.05$) decrease in spleen weight, a delayed hypersensitivity reaction to bovine serum albumin, and increased prostaglandin E_2 synthesis by macrophages. No effects were observed at lower concentrations. The average body weight of the rats was about 0.355 kg (Exon et al. 1987, p. 263, Table 1). The amount of water consumed by the rats is not specified in the publication. U.S. EPA/ORD (1993, Eq. 3-17, p. 3-10) suggests the following allometric relationship for estimating water requirements in mammals:

$$WC_{(L/day)} = 0.099\ Wgt^{0.9}_{kg}. \quad \text{(Eq. 9)}$$

Assuming that the rodent chow used in the study did not provide a substantial amount of water, the estimated water consumption for a 0.355 kg mammal would be about 0.039 L and the 30 mg/L exposure would correspond to a daily dose of 3.3 mg/kg bw [0.039 L x 30 mg/L ÷ 0.355 kg].

The toxicological significance of the effects of aqueous chlorine on the immune system is not entirely clear. The recent review by ATSDR (2007) suggests that the effects may not be viewed as significant adverse effects:

> *Studies in animals have provided no evidence that exposure to aqueous chlorine adversely affects the immune or nervous system, although an 8-week study in rats reported alterations in some immune parameters of unknown toxicological significance (reduced delayed type hypersensitivity reaction, increased prostaglandin E2 synthesis by macrophages, and reduced oxidative metabolism by macrophages following stimulation with phorbol myristate acetate).*
>
> ATSDR (2007, p. 14)

The ATSDR review, however, does not cite the study by Fidler (1977) and the above assessment by ATSDR is based only on the study by Exon et al. (1987). Based on the results of the Exon et al. (1987) alone, however, ATSDR (2007) clearly indicates that the effects observed by Exon et al. (1987) are not considered adverse. Similarly, the review by U.S. EPA/OHEA (1994, p. 1-4), which considers the study by Fidler (1977) but not the study by Exon et al. (1987) states that: *Mice exposed to chlorinated drinking water showed no evidence of humoral or cell-mediated immune responses*.

3.1.8. Effects on Endocrine System

Assessment of the direct effects of chemicals on endocrine function are most often based on mechanistic studies on estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone availability, hormone receptor binding, or post-receptor processing). In addition, changes in structure of major endocrine glands (i.e., the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis) may also be indicative of effects on the endocrine system. Disruption of the endocrine system during development may give rise to effects on the reproductive system which may be expressed only after maturation. Consequently, multigeneration exposures are recommended for toxicological assessment of suspected endocrine disruptors. The endocrine system is also important in normal growth and development, and changes in growth can be an indicator of effects on the endocrine system.

Few studies have assayed for the effects of aqueous chlorine on hormone levels in humans or experimental mammals. As discussed in Section 3.1.5 (Subchronic or Chronic Systemic Toxic Effects), Wones et al. (1991) noted a slight reduction in serum levels of thyroid hormones (T3 and T4) in groups of male but not female volunteers exposed to aqueous chlorine at a dose of

approximately 0.4 mg/kg bw/day for 4 weeks. No changes were noted in levels of thyroid-stimulating hormones in either sex. The small changes in thyroid hormones were …*judged not to be meaningful because of borderline statistical significance and because thyroid-stimulating hormone levels did not change* (Wones et al. 1991, p. 379). As discussed further in Section 3.1.9, no effects on thyroid hormone levels were noted in male or female rats in a reproduction study at gavage doses of 5 mg/kg bw/day. In standard subchronic and chronic toxicity studies, no effects on organs associated with endocrine function have been noted (Daniel et al. 1990, 1991; Furukawa et al. 1980; Hasegawa et al. 1986; NTP 1992).

3.1.9. Reproductive and Developmental Effects

3.1.9.1. Developmental Studies

Developmental studies are used to assess whether a compound has the potential to cause birth defects—also referred to as teratogenic effects—as well as other effects during development or immediately after birth. Very specific protocols for developmental studies are established by U.S. EPA/OPPTS and are available at http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized. No such studies, however, have been required for aqueous chlorine.

Several teratology studies, however, are available in the published literature. An early report by McKinney et al. (1976) suggested that seasonal variations in chlorine concentrations in tap water might be associated with decreases in the number of viable offspring in a population of experimental mice. Two controlled studies in which groups of mice were exposed to either chlorinated or non-chlorinated tap water failed to demonstrate any association between chlorine exposures and fetotoxicity or developmental effects (Chernoff et al. 1979; Staples et al. 1979).

3.1.9.2. Reproduction Studies

Reproduction studies involve exposing one or more generations of the test animal to a chemical compound. Generally, the experimental method involves dosing the parental (P or F0) generation (i.e., the male and female animals used at the start of the study) to the test substance prior to mating, during mating, after mating, and through weaning of the offspring (F1). In a 2-generation reproduction study, this procedure is repeated with male and female offspring from the F_1 generation to produce another set of offspring (F_2). During these types of studies, standard observations for gross signs of toxicity are made. Additional observations often include the length of the estrous cycle, assays on sperm and other reproductive tissue, and number, viability, and growth of offspring.

Carlton et al. (1986) conducted a single generation study in which male and female rats were dosed with aqueous chlorine by gavage at 5 mg/kg bw/day for 66 days. No effects were noted on fertility or fetotoxicity. In drinking water exposures to aqueous chlorine at concentrations equivalent to doses of 10 mg/kg bw/day, Abdel-Rahman et al. (1982) report soft tissue anomalies in rats. However, as reviewed by ATSDR (2007, p. 97), this observation does not appear to be consistent with the data presented in the Abdel-Rahman et al. (1982). The report by Abdel-Rahman et al. (1982) is also inconsistent with the seven generation reproduction study by

Druckery (1968) in which the rats were exposed to aqueous chlorine in drinking water at a concentration of 100 mg/L, equivalent to a dose of 10 mg/kg bw/day. Druckery (1968) observed no adverse effects on any reproductive parameters and mortality in neonates in the dosed group was about 20% less than mortality in the control group.

3.1.9.3. Target Organ Toxicity

Meier et al. (1985) administered gavage doses of sodium hypochlorite or hypochlorous acid to male mice at doses of 1.6 to 8 mg/kg bw/day for 5 days and assayed for sperm head anomalies at weeks 1, 3, and 5 after the last dose. No effects on sperm morphology were noted at week 1 or week 5 after dosing. At week 3, a dose-related increase in sperm head anomalies were noted at 1.6 and 4 mg/kg bw/day for sodium hypochlorite but not hypochlorous acid. In addition, no dose-response relationship between the 4 mg/kg bw/day and 8 mg/kg bw/day dose groups were noted for sodium hypochlorite. As reviewed by ATSDR (2007), … *In the absence of corroborating information from other studies and lack of internal consistency of the results, the toxicological significance of these results is difficult to ascertain*. No effects on sperm morphology or reproductive organs were noted in the subchronic gavage study in rats at a dose of 3.4 mg/kg bw/day (Carlton et al. 1986).

3.1.10. Carcinogenicity and Mutagenicity

As with chronic toxicity, the carcinogenicity of aqueous chlorine has been studied extensively because of the use of aqueous chlorine in the disinfection of public water. The available studies have been reviewed extensively and in detail (i.e., ATSDR 2007; IARC 1997; U.S. EPA 1994, 1999a). Consequently, the review of carcinogenicity in the current risk assessment is brief and relies on the reviews cited above.

Both the U.S. EPA (U.S. EPA/OPP 1999a) as well as the WHO (IARC 1997) have reviewed the available information on the carcinogenicity of aqueous chlorine and have determined that the carcinogenicity of aqueous chlorine cannot be determined with any certainty. i.e., aqueous chlorine is classified as a Group D carcinogen by the U.S. EPA and Group 3 by IARC. Both of these rankings indicate that the potential carcinogenicity of aqueous chlorine to humans cannot be classified. As with chronic toxicity, however, these assessments of potential carcinogenicity have a limited impact on the current risk assessment because the uses of aqueous chlorine in Forest Service programs will not involve chronic exposures.

3.1.11. Irritation and Sensitization (Effects on the Skin and Eyes)

The U.S. EPA typically requires a standard set of toxicity studies to assess dermal and ocular irritation as well as skin sensitization. Because of the well-known corrosive and irritant properties of aqueous chlorine, the U.S. EPA/OPP (1999a) has waived standard testing of aqueous chlorine and has classified aqueous chlorine a Toxicity Category I for dermal and ocular irritation as well as skin sensitization.

3.1.11.1. Skin Irritation

The effects of aqueous chlorine on human skin are well-documented and the most relevant studies are summarized in Table 6. Solutions of aqueous chlorine at concentrations in the range

of 5.25% to 6% - i.e., concentrations comparable to those found in commercial formulations used in Forest Service programs (Table 2) – are highly irritating to the skin over exposure periods of 20 minutes (Hostynek et al. 1989) to 4 hours (Nixon et al. 1975). The symptoms of exposure to concentrated solutions of aqueous chlorine include redness, itching, burning, and sometimes severe pain (Cathcart et al. 2008). As discussed further in Section 3.1.11.2, repeated exposures to aqueous chlorine may also lead to skin sensitization.

While dose-duration relationships are not well-defined, it seems reasonable to assert that increasing durations of exposure are likely to result in increasing levels of irritation. This pattern is suggested in the studies by Goffin et al. (1997) – in which no signs of skin irritation were noted at an aqueous chlorine concentration of 4% over 15 to 90 minutes – and Habets et al. (1986) – in which weak to moderate irritation was noted in about 20% of the individuals exposed to a 2% solution for 48 hours.

In addition to the concentration of aqueous chlorine and the duration of exposure, the volume of the solution may be a factor in the severity of the response. This assertion is based on the study by Hostynek et al. (1989) in which a 24 hour exposure to a 1% solution of aqueous chlorine caused no adverse effects at an application volume of 0.02 mL but slight reddening of the skin when applied at a volume of 0.1 mL.

While Hostynek et al. (1989) noted some skin reddening with the 0.1 mL application of a 1% solution of aqueous chlorine, the study by Habets et al. (1986) noted no grossly apparent signs of irritation in individuals after 48 hour exposures to concentrations of 0.5% or 1%. As noted above, Habets et al. (1986) did note moderate skin irritation at an aqueous chlorine concentration of 2%. The study by Habets et al. (1986) does not provide a detailed description of the study conditions and the basis for the inconsistency between the reports by Habets et al. (1986) and Hostynek et al. (1989) cannot be determined.

Concentrations of aqueous chlorine of about 0.5% may be viewed as an approximate threshold for readily observable skin irritation in humans. A similar assessment has been made by Bruch (2007) in a review of studies in which aqueous chlorine has been used for wound treatment. Nonetheless, Goffin et al. (1997) noted no threshold for subclinical signs of toxicity at aqueous chlorine concentrations in the range of 0.04% (400 ppm) to 0.2% (2000 ppm) over exposure periods of two hours and an application volume of 0.15 mL. The subclinical signs of skin damaged were assayed based on color changes of in vitro sheets of stratum corneum taken from exposed subjects. Given the mechanism of aqueous chlorine (Section 3.1.2), the lack of a clear threshold over the 400 ppm to 2000 ppm range tested by Goffin et al. (1997) may not be surprising.

Additional studies are available on experimental mammals, primarily rabbits and guinea pigs, and these studies have been reviewed in the open literature (e.g., ATSDR 2007; Vetrano 2001). Given the amount and quality of the human studies, however, the dermal irritancy data on experimental mammals is not required to assess the potential effects on humans who might be exposed to aqueous chlorine in Forest Service programs. In addition, as detailed in the study by

Nixon (1975), humans appear to be more sensitive to aqueous chlorine than either rabbits or guinea pigs. The skin of pigs may be a better animal model for human skin but the only available data on pigs (as opposed to guinea pigs) is from a study on the corrosive effects of bleach to a human cadaver and a pig cadaver (Adair et al. 2007).

3.1.11.2. Skin Sensitization

Skin sensitization and skin irritation are related effects in that dermal exposures may cause reddening of the skin and the development of hives. Skin sensitization, also referred to allergic contact dermatitis, is mechanistically different from skin irritation in that the underlying cause of the effect involves an immunologic mechanism, in which the chemical or other agent acts as an antigen and elicits an allergic reaction. The U.S. EPA has a relatively standard test protocol for skin sensitization involving an induction period – i.e., repeated exposure to the skin of the test organism, typically a guinea pig – followed by a challenge exposure to determine if the response is hypersensitive, which is indicative of an allergic response (U.S. EPA/OPPTS 1998). A common clinical test for skin sensitization in humans is the *patch test*. This test involves applying a presumed antigen to the skin and observing the response, typically reddening, after 24, 48, or sometimes 72 hours. The assessment of the reactions to patch tests are typically made as semiquantitative scores using plus (+) symbols: "+" for a low grade response, "++" for a moderate response, and "+++" for a severe response. Unlike the standard animal bioassays for skin sensitization, patch testing does not entail an induction period. The induction of the allergy is presumed to have occurred through prior exposure to the allergen.

As discussed in the previous section, much of the literature on the dermal irritancy of aqueous chlorine solutions involves the development of conditions for patch tests – i.e., concentration, volume, and duration of exposure – that would permit a differentiation of skin irritation from skin sensitization. Also, as noted at the start of Section 3.1.11, the U.S. EPA has not required a standard test for skin sensitization and has classified aqueous chlorine as a Category I skin sensitizer – i.e., the highest or most severe of the designations used by EPA.

Bruch (2007) has reviewed the open literature as well as several unpublished studies on the allergenic potential of various medicinal formulations of sodium hypochlorite. As noted in the Bruch (2007) review, 1.1% formulations of sodium hypochlorite are used as antiseptic in wound treatment and this type of use has been common since the 1920s. Unpublished studies on the allergic potential of these medical formulations suggest that these dilute solutions are not skin sensitizers. As noted by ATSDR (2007), however, "*Although sodium hypochlorite generally is not considered a contact sensitizer, several cases of allergic contact dermatitis have been reported"* (ATSDR 2007, p. 103). Specifically, ATSDR (2007) cites that studies by Eun et al. (1984, Habets et al. (1986), Osmundsen (1978), and Van Joost et al. (1987). In each of these studies, standard patch tests indicated moderate to strong responses to 0.1% to 0.5% solutions of sodium hypochlorite. In addition, the history of individuals with positive responses indicated prior and prolonged exposures to sodium hypochlorite – i.e., the induction period. Lower concentrations – 0.01% or 100 ppm – do not elicit a positive response even in individuals who respond positively to higher concentrations (Habets et al. 1986).

In addition to the use of sodium hypochlorite as a medicinal antimicrobial agent, sodium hypochlorite is commonly used during root canals to disinfect the root of the tooth being treated. In general, concentrations of less than 1% are recommended for use during the conduct of root canals (Farren et al. 2008) but concentrations of up to 5.25% have been used in some instances (Mehdipour et al. 2007). While the use of sodium hypochlorite during root canals does not generally lead to adverse effects, a number of cases of severe response to sodium hypochlorite have been reported (e.g., Farren et al. 2008; Mehdipour et al. 2007; Spencer et al. 2007). The adverse reactions are typically characterized as severe pain, edema, and hemorrhage. In some cases, sodium hypochlorite, rather than an anesthetic, has been accidentally injected into the mucosa of the mouth (Motta et al. 2009). In these cases, the tissue damage is severe and most probably attributable to the direct corrosive effect of sodium hypochlorite.

While many of the cases of adverse reactions to sodium hypochlorite during root canals may involve irritant rather than allergic mechanisms (Spencer et al. 2007), Caliskan et al. (1994) report one case involving a young woman that resulted in what appeared to be a very severe allergic reaction involving not only localized pain but also difficulty in breathing and a drop in blood pressure. After the patient had recovered, the allergy to sodium hypochlorite was confirmed with a standard patch test. On further questioning, the patient indicated that she had previously experienced skin rashes as well as difficulty in breathing after using …*a household cleaning agent*. The report by Caliskan et al. (1994) does not clearly identify the cleaning agent that caused both the skin rashes and difficulty in breathing prior to the dental incident as sodium hypochlorite but this is implied in the publication.

Despite the assertion by Bruch (2007) that sodium hypochlorite … *has not shown any potential as a skin sensitizer* (Bruch 2007, p. 36), the weight-of-evidence suggests that sodium hypochlorite may be allergenic in some individuals. The mechanism of sensitization is not clear and has not been addressed in the literature. Given the highly reactive nature of sodium chlorite and hypochlorous acid, it does not seem plausible that these compounds are direct allergens. While somewhat speculative, it seems likely that hypochlorous acid or the hypochlorite ion will react with proteins, fats, or other endogenous molecules to form a modified compound that will eventually generate the allergic reaction. This type of mechanism is typical of many low molecular weight compounds that appear to induce allergic responses (Rice and Cohen 1996).

3.1.11.3. Ocular Effects

As reviewed by Racioppi et al. (1994) and also noted by ATSDR (2007), splashing of commercial bleach solutions into the eye is a relatively common occurrence based on reports to poison control centers and these incidents do not typically result in severe or permanent eye damage.

As with dermal irritation, relatively standard tests for eye irritation are typically conducted in experimental mammals but these tests were waived by the U.S. EPA for aqueous chlorine. Many standard tests use the Draize scoring system which ranks eye damage with numeric scores: 0-15 for practically nonirritating, 15-25 for slightly irritating, 25-50 for moderately irritating, and 50-110 for severely irritating or corrosive. In one such assay, Griffith et al. (1980) instilled 0.01 to

0.1 mL of a solution 5% sodium hypochlorite into the eyes of rabbits and observed the condition of the eyes over a 21 day period. Only slight irritation was noted at an application volume of 0.01 mL (i.e., scores of 0 to 11). Applications of 0.03 mL or 0.1 mL resulted in moderate irritation on Day 1 (scores of 28 and 31) which persisted over the 21 day observation period (i.e., scores of about 40).

A 0.5% solution of sodium hypochlorite has been assessed as a cytotoxic agent against ocular melanoma cells (Missotten et al. 2008). While this study did not directly address the ocular toxicity of sodium hypochlorite, Missotten et al. (2008) do indicate that sodium hypochlorite at concentration of 0.5% may be an effective therapeutic agent with only a small likelihood of causing adverse effects to eye tissue.

3.1.12. Systemic Toxic Effects from Dermal Exposure

As with irritant and allergenic effects discussed in the previous subsection, the U.S. EPA/OPP (1999a) has not required any standard subchronic toxicity studies on aqueous chlorine. Instead, the U.S. EPA/OPP (1999a) classified aqueous chlorine as Category I, suggesting that aqueous chlorine is toxic on dermal exposure. This classification is appropriate in that it reflects the irritant properties of aqueous chlorine. Nonetheless, there are no data indicating that aqueous chlorine will cause systemic toxic effects after dermal exposures. Even in cases of oral exposure, relatively concentrated solutions of bleach will cause damage to the tissue that is directly effected. For such oral exposures, secondary systemic effects appear to be due not to sodium hypochlorite itself but to the direct tissue damage done to the gastrointestinal tract (Jakobsson et al. 1991) or aspiration of sodium hypochlorite into the lungs (Racioppi et al. 1964).

3.1.13. Inhalation Exposure

While exposure to chlorine gas is highly toxic and can cause extensive damage to lung tissue, inhalation is not a significant route of exposure for sodium hypochlorite under normal circumstances (ATSDR 2007). As illustrated in Figure 2, the concentration of chlorine gas in bleach solutions is negligible in moderately acidic (pH >5), neutral, and basic solutions of aqueous chlorine. However, if aqueous chlorine solutions are mixed with strong acids such as phosphoric acid or hydrochloric acid, the pH of commercial bleach solutions may change from basic to acidic. In an acidic solution of sodium hypochlorite, chlorine gas may be released in amounts that can lead to toxic effects (Mrvos et al. 1993). As discussed in Section 3.1.4 and noted above, oral exposures to aqueous chlorine may result in damage to the lungs but this damage occurs as the result of aspiration of aqueous chlorine into the lungs (Racioppi et al. 1964) rather than through an inhalation pathway.

3.1.14. Inerts and Adjuvants

As discussed in Section 2, the formulations of aqueous chlorine that are used as disinfectants in Forest Service programs contain only sodium hypochlorite and sodium hydroxide. Some formulations of aqueous chlorine may contain other ingredients such as surfactants or other antimicrobial agents. These other types of formulations, however, are not used in Forest Service programs and are not encompassed by the current risk assessment.

3.1.15. Chlorination Byproducts

Most Forest Service risk assessments contain sections addressing environmental metabolites as well as toxicological interactions. The section on environmental metabolites addresses changes to the pesticide based on common environmental processes – e.g., biodegradation, hydrolysis, and photolysis – and the section on toxicological interactions addresses the impact of exposure to the pesticide as well as other toxic agents. For aqueous chlorine, these two sections are combined because the primary toxicological interactions of aqueous chlorine all involve the reactions of aqueous chlorine with other compounds that are found in the environment.

As noted in Section 2.2.1, one very significant degradation pathway involves the chlorination of organic molecules (R-H) by hypochlorous acid. This general reaction is sufficiently important to repeat:

$$HOCl + \boldsymbol{R}\text{-}H \rightarrow \boldsymbol{R}\text{-}Cl + H_2O \qquad \text{(Eq. 10)}$$

The formation of chlorinated byproducts from the use of bleach or chlorine gas in water-treatment facilities is well documented (Lebedev 2007; Michaowicz et al. 2007; Nakamura et al. 2007; Odabasi 2008; Patton et al. 1972; Pereira et al. 1973; Vetrano 2001; Zhang and Minear 2002; Zwiener et al. 2007). Chlorinated aliphatics as well as chlorinated phenols are also formed naturally in both aquatic and terrestrial environments by the action of chloroperoxidases (Scholer and Thiemann 2005; Wannstedt et al. 1990; Wever 2004; Wever and Hemrika 2001).

The problem in assessing the risks associated with the formation of chlorinated byproducts involves the nature of ***R*** in the above equation. The type of byproducts that are formed and the amount of byproducts that will be formed will vary with the nature of the organic molecules (***R***) that are present in water when bleach is added to the water. One of the simpler hazards to assess in terms of water treatment is the formation of low molecular weight chloromethanes that occur during the chlorination of drinking water. This single and relatively simple issue has been a focus of the U.S. EPA's Office of Research and Development for more than a decade (e.g., Teuschler et al. 2004; U.S. EPA/ORD 2004,2006). While not attempting to over-simplify the resolution, the continued practice of chlorinating drinking water, primarily with chlorine gas treatment, reflects a general consensus that the benefits of disease prevention outweigh the potential risks associated with the chlorination of drinking water.

As detailed in Section 3.1.3 (Pharmacokinetics and Metabolism), the general types of reactions of hypochlorous acid to naturally occurring organic molecules will also occur *in vivo* when animals drink solutions of aqueous chlorine – e.g., the formation of chloroform, trichloroacetic, dichloroacetic acid, dichloroacetonitrile, and trichloroacetonitrile as noted by Mink et al (1983). To some extent, the exposures that occur during *in vivo* toxicity studies in which animals are exposed through drinking water may mimic and in some ways be more severe than the reaction of lower concentrations of aqueous chlorine that will occur in the environment.

3.2. EXPOSURE ASSESSMENT

3.2.1. Overview

An overview of the exposure assessments for workers and members of the general public is given in Table 7. The exposure assessments for aqueous chlorine are very different from exposure assessments included in most Forest Service risk assessments. Most Forest Service risk assessments focus on estimates of absorbed doses from routine and accidental worker exposure as well as absorbed doses in members of the general public associated with exposures to contaminated water, contaminated fish, or contaminated vegetation. As detailed in the hazard identification, however, exposures to aqueous chlorine will result in portal of entry effects – i.e., gastrointestinal damage after ingestion and skin irritation after dermal contact. Systemic effects are not likely to occur.

For workers, the only plausible exposures of concern involve contact with the skin and eyes. Ocular effects are not addressed quantitatively in either the exposure assessment or dose-response assessment but are considered qualitatively in the risk characterization. Thus, for workers, the exposure assessments are based simply on the range of concentrations that are likely to be used in Forest Service programs – i.e., 50 ppm to somewhat over 10,000 ppm – as well as contact with undiluted beach.

For members of the general public, no significant exposures to aqueous chlorine are anticipated under normal conditions. While Forest Service risk assessments attempt to present very conservative exposure assessments, most of the uses of aqueous chlorine – i.e., equipment and facilities cleaning – do not suggest that any exposure to concentrated solutions of aqueous chlorine are likely. While less plausible than the case with workers, it is conceivable that accidental exposures to solutions of aqueous chlorine over the range of 50 ppm to 10,000 ppm could occur. As discussed in the Risk Characterization (Section 3.4), the consequences of these exposures would be essentially identical to those for workers. Thus, for members of the general public, only two types of exposures are considered quantitatively: the consumption of water and swimming in water accidentally contaminated with aqueous chlorine during firefighting operations. These exposure scenarios are presented because the use of aqueous chlorine during firefighting is essentially the only time that any substantial accidental exposures might reasonably occur.

3.2.2. Workers

As described in SERA (2007a), worker exposure rates in typical Forest Service risk assessments for pesticides and herbicides are expressed in units of mg of absorbed dose per kilogram of body weight per pound of chemical handled. These types of typical occupational exposures involve multiple routes of exposure (i.e., oral, dermal, and inhalation).

This approach, however, is not appropriate for aqueous chlorine. As detailed in Section 3 (see especially Section 3.1.12), aqueous chlorine is highly reactive and the major effects of chlorine will involve portal of entry effects – i.e., gastrointestinal effects after ingestion and skin irritation

after dermal exposures. Except for allergic reactions, which are discussed qualitatively in Section 3.4.4, there is very little indication that aqueous chlorine will cause any systemic toxic effects. Given the types of uses of aqueous chlorine, as detailed in Section 2.2, the predominant route of exposure will involve contact with the skin. The endpoint of concern, however, will be dermal irritation rather than systemic toxicity.

While dermal irritation may be dependent on the duration of exposure as well as the volume of liquid coming in contact with the skin, the predominant factor appears to be the concentration of active chlorine in the solution. Thus, the exposure assessment for workers is very simple. The concentrations of aqueous chlorine in the various uses of aqueous chlorine in Forest Service programs are given in Table 4 and Table 5. While standard and prudent handling practices suggest that workers will not intentionally expose their skin to aqueous chlorine, inadvertent dermal exposures to concentrations over the range of 50 ppm to somewhat over 10,000 ppm are plausible. Exposures to undiluted formulations, in the range of about 5% to 6%, are considered accidental exposures – i.e., the assumption is made that workers would always attempt to avoid direct contact with undiluted formulations of bleach.

The range of concentrations of aqueous chlorine from Tables 4 and 5 as well as the concentration of aqueous chlorine in the most concentrated formulation covered in this risk assessments – i.e., 5.84% from Table 2 – are listed in Worksheet C01 in the EXCEL workbook that accompanies this risk assessment. Risks of dermal irritation are based on these concentrations and the approximate human NOEC for dermal irritation, which is derived in Section 3.3.4. As discussed further in Section 3.4, the risks of dermal irritation are based on the ratio of the concentration for each use to the human NOEC. This approach is analogous to the standard hazard quotient (HQ) method that is used on Forest Service risk assessments.

3.2.3. General Public

3.2.3.1. General Considerations

Bleach is a common household product. Individuals who use bleach for cleaning or as an additive to laundry are likely to routinely come into contact with aqueous chlorine. Aqueous chlorine is also used for the disinfection of drinking water but the concentrations of aqueous chlorine are very low – i.e., residual concentrations of 0.1 to 0.2 ppm (Table 4). In natural surface water, chlorine concentrations are likely to be even lower because of the reactions with organic and inorganic compounds commonly found in surface water (e.g., Abel-Gawad and Bewtra 1998). Clydesdale (1997) provides an estimate of the individual consumption of chlorine of 2.9661 mg/kg bw/day. The basis of this estimate is not clear and this estimate may include exposure to the chloride ion from the consumption of food and water.

Members of the general public are not likely to be exposed to aqueous chlorine as used in Forest Service programs. Most of the uses of aqueous chlorine in Forest Service programs involve the cleaning of tools and other items. These activities will typically be conducted in a manner that is not likely to expose members of the general public. If any exposures were to occur, they would most likely involve dermal exposure and the exposure assessments for these accidental dermal exposures would be identical to those for workers (Section 3.2.2).

For the current risk assessment, only two exposure scenarios are explicitly developed for members of the general public: the accidental consumption of water and swimming in water that is contaminated with chlorine (Table 7). Both of these exposure scenarios should be viewed as accidental. As discussed further below, the likelihood of either scenario occurring appears to be remote.

3.2.3.2. Water Consumption

As summarized in Table 4 and Table 5, the concentrations of aqueous chlorine used in Forest Service programs ranges from 50 ppm (0.005%) to somewhat over 10,000 ppm (1%). For most uses, the likelihood of an individual consuming contaminated water is remote. For example, the highest concentration, 10,342 ppm, is used for cleaning tools in order to prevent the spread of amphibian chytrid fungus. This use is a very controlled and a plausible scenario involving the consumption of contaminated water is not apparent. This is also true for most other Forest Service uses.

A possible exception involves the use of aqueous chlorine for water disinfection during fire suppression. As discussed in Section 2.3.1, water used for fire suppression is treated with 50 ppm (equivalent to 50 mg/L) aqueous chlorine. Helicopters or fixed wing aircraft will then drop this water into burning regions of a forest.

Individuals in close proximity to a forest fire are not considered at significant risk from aqueous chlorine relative to the risks associated with fire. However, it is possible that a load of water treated with aqueous chlorine could be accidently dropped during transit into an area that is not involved in a fire and has not been evacuated. This type of exposure scenario is detailed in Worksheet D01, which involves the consumption of contaminated water by a small child. The variability in the exposure assessment is based on the range of water consumption rates for a young child as well as the volume of the solution of aqueous chlorine that is dumped into the pond. This scenario uses the standard small pond from Forest Service risk assessments – i.e., a pond that is 1000 m^2 in surface area (about a quarter of an acre) and one meter deep. The amount of water that is dropped into the pond is taken as 700 (75 to 3000) gallons. As noted in Section 2.3.1, the lower bound (75 gallons) and central estimate (700 gallons) are the range of capacities for helicopters used in firefighting. The upper bound (3000 gallons) is the highest fixed-wing aircraft capacity from the California Office of Emergency Services (2004). All of these aircraft load capacities are much smaller than the volume of water in the pond – i.e., 1 million liters. Thus, concentration in the treated water, 50 ppm, is diluted substantially – i.e., about 0.12 (0.014 to 0.57) ppm. The doses associated with this scenario are about 0.01 (0.0007 to 0.06) mg/kg bw.

3.2.3.3. Dermal Exposure from Swimming in Contaminated Water

The dermal exposure scenario for an individual swimming in contaminated water is detailed in Worksheet D02. In terms of the concentration of aqueous chlorine in pond water, this scenario is identical to exposures scenario given in Worksheet D01 for the consumption of contaminated water – i.e., 700 (75 to 3000) gallons of treated water with an aqueous chlorine concentration of

50 ppm is dropped into a small pond. The only difference between these scenarios involves the endpoint that is assessed. For the swimming scenario, the assessment of a biological response is based on dermal irritation.

Some incidental consumption of water is likely to occur during swimming. As discussed in Section 3.4.3, however, the drinking water exposures discussed in the previous subsection result in hazard quotients that are below the level of concern. Consequently, the incidental consumption of aqueous chlorine during swimming is not explicitly considered.

3.3. DOSE-RESPONSE ASSESSMENT

3.3.1. Overview

Just as the exposure assessment for aqueous chlorine is different from exposure assessments in most Forest Service pesticide risk assessments, the dose-response assessment is also atypical. Two types of exposures, both acute, are assessed quantitatively: the consumption of contaminated water by members of the general public and dermal exposures of workers to concentrated or dilute solutions of aqueous chlorine.

The consequences of acute oral exposures are typically assessed using acute reference doses (RfDs) derived by the U.S. EPA or acute oral minimum risk levels (MRLs) derived by ATSDR. No such values are available, however, for aqueous chlorine. A surrogate acute RfD of 0.25 mg/kg bw/day can be derived from the 1-day health advisory recommended by U.S. EPA.

Dermal toxicity values are not typically derived in Forest Service risk assessments because such values are not typically supported by the available animal data. For aqueous chlorine, however, human data are available indicating that acute dermal exposures to aqueous chlorine at a concentration of 0.5% (5,000 ppm) is not likely to lead to overt signs of skin irritation. This concentration is used as a surrogate human no-observable-adverse-effect concentration. Higher concentrations will increase the likelihood of observing skin irritation. Concentrations of 2% to 6% may lead to clear signs of skin irritation.

Allergic reactions to aqueous chlorine appear to be plausible. These effects are not assessed quantitatively but are qualitatively considered in the risk characterization.

3.3.2. Acute Oral RfD

As detailed in Section 3.2.3 (Exposure Assessment for the General Public), acute oral exposures are the most plausible exposure scenario for members of the general public. In terms of quantifying risks – i.e., the derivation of hazard quotients as detailed further in Section 3.4 – the acute oral RfD is the only toxicity value that is used in this current Forest Service risk assessment. Typically, Forest Service risk assessments will adopt RfDs directly from the RfDs derived by the U.S. EPA or MRLs (a risk value comparable to the RfD) derived by ATSDR. Aqueous chlorine is atypical in that neither the U.S. EPA nor ATSDR have derived acute oral risk values for this agent and a de novo surrogate RfD must be proposed. Thus, this section contains a somewhat more elaborate discussion than is typical in Forest Service risk assessments.

Acute RfDs for pesticides are intended to represent levels of oral exposure over the course of a single day that are not likely to cause any adverse effects in any member of the general public. Typically, acute RfDs are based on developmental studies in which an observed adverse effect can be associated with a single dose and a NOAEL for the adverse effect can be defined.

Aqueous chlorine is somewhat atypical in that the U.S. EPA has not developed an acute RfD. Instead, the U.S. EPA/OPP (1999a) has adopted a 1-Day Health Advisory from the Drinking

Water Criteria document on aqueous chlorine (U.S. EPA/OHEA 1992). Health advisories are concentrations of a compound in drinking water that are considered safe for all members of the population. As detailed in U.S. EPA/OHEA (1992), a health advisory (*HA*) is derived using the following algorithm:

$$HA = \frac{NOAEL_{mg/kg\ bw/day} \times BW_{Kg}}{UF \times WC_{L/day}}$$

where *NOAEL* is typically an animal NOAEL in units of mg/kg bw/day, BW is the body weight of the individual in kilograms, UF is an uncertainty factor, and WC is the water consumption for the individual in units of liters per day.

The U.S. EPA/OHEA (1992) did not derive a 1-day health advisory for aqueous chlorine because an appropriate study could not be identified. U.S. EPA/OHEA (1992) did consider the controlled human study by Lubbers et al. (1982) but dismissed this study because … *the dosages appear to be well below the threshold level of effect* (U.S. EPA/OHEA 1992, p. VIII-15). As discussed in 3.1.4, this assessment appears to be correct. The highest dose used in the study by Lubbers et al. (1982) appears to be about 0.34 mg/kg bw. The dose of 0.34 mg/kg bw is substantially below the lower bound of the estimated human dose of 24 mg/kg that does not appear to cause substantial or even overt signs of toxicity or medical intervention (Section 3.1.4).

The U.S. EPA/OHEA (1992), however, does derive a 10-day health advisory of 2.5 mg/L and recommends the 10-day health advisory as a conservative/protective surrogate for the 1-day health advisory. This health advisory is based on the subchronic NOAEL in mice of 25 mg/kg bw/day from the study by Blabaum and Nicholas (1956). As discussed in Section 3.1.5, the NOAEL is based on a drinking water exposure to aqueous chlorine at a concentration of 200 mg/L over a 33 day period that resulted in no overt signs of toxicity or histopathological changes. The health advisory is derived for a 10 kg child consuming 1 liter of water per day and using an uncertainty factor of 100 – i.e., a factor of 10 for species extrapolation and a factor of 10 for sensitive subgroups. This acute health advisory could be used to derive a surrogate acute RfD of 0.25 mg/kg bw/day – i.e., 25 mg/kg bw/day ÷ 100. Note, however, that this surrogate RfD would actually be lower than the dose of 0.34 mg/kg bw based on the study by Lubbers et al. (1982), which was dismissed by the U.S. EPA/OHEA (1992) as being far below the threshold level for effects.

For aqueous chlorine, ATSDR (2007) declined to derive an acute oral MRL (i.e., a Minimum Risk Level, which is the ATSDR equivalent of an RfD). The rationale for not deriving an MRL is given by ATSDR as:

> *Oral MRLs were not derived for aqueous chlorine for the following reasons. MRLs are derived when reliable and sufficient data exist to identify a target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. Scientifically, as part of having sufficient and reliable data, it is important to be able to see the full, or at least a significant*

> *range, of the dose-response curve. In the case of the oral database for aqueous chlorine, no reliable LOAEL could be identified at levels of aqueous chlorine that could reasonably be encountered in the environment. It is a matter of policy of ATSDR not to derive free-standing MRLs.*
>
> ATSDR (2007, p. 21)

In other words, ATSDR declined to derive an MRL because it is not clear that aqueous chlorine would be associated with any adverse effects under plausible conditions of exposure.

The ATSDR position that hazardous concentrations of aqueous chlorine are not likely to occur in the environment appears to be correct, as discussed further in Section 3.4 (Risk Characterization). The assertion that target organs and adverse effects cannot be defined, however, is questionable. As discussed in Section 3.1.4, there is substantial human experience with the accidental or suicidal consumption of bleach. Lethal doses in humans can be estimated at 357 to 893 mg/kg bw and these estimates are reasonably consistent with estimated lethal doses in experimental mammals of 225 to 675 mg/kg bw. The target organ is clearly the gastrointestinal tract. Other systemic effects appear to be secondary to damage to the gastrointestinal tract or, in cases of aspiration, damage to the lungs.

In addition, doses that are not associated with serious adverse effects in children, specifically incidents in which no remarkable medical care is warranted, can be estimated at about 24 to 30 mg/kg bw (Mack 1983). This range of doses is very similar to the 25 mg/kg bw NOAEL in mice from the study by Blabaum and Nicholas (1956).

For the current Forest Service risk assessment, the surrogate acute RfD of 0.25 mg/kg bw/day is adopted from the U.S. EPA//OHEA (1992) 10-day health advisory of 2.5 mg/L. As discussed above, this is a very conservative and perhaps overly conservative approach. This has no practical impact on this risk assessment because, consistent with the position taken by ATSDR, plausible oral exposures of humans to aqueous chlorine are substantially below the surrogate acute RfD, as detailed further in Section 3.4.3.

3.3.3. Chronic Oral RfD

Unlike the case with the acute RfD, the U.S. EPA/OPP (1999a) has derived a chronic RfD for aqueous chlorine of 0.1 mg/kg bw/day. This is also the chronic RfD that is derived on IRIS (Integrated Risk Information System), the Agency-wide database of risk values that is maintained by the U.S. EPA's Office of Research and Development (U.S. EPA/ORD 1994).

The chronic RfD is based on the rat NOAEL of 14.4 mg/kg bw/day from the National Toxicology Program's cancer bioassay on aqueous chlorine. In this study, rats were exposed to aqueous chlorine at concentrations of 0, 70, 140, and 275 ppm aqueous chlorine for two years. No adverse effects were seen at any exposure level. For female rats, the 275 ppm concentration corresponded to an average dose of 14.4 mg/kg bw/day based on measured body weights and water consumption rates. In the 10-day health advisory, discussed in Section 3.3.2., an uncertainty factor of 100 was used – i.e., a factor of 10 for species extrapolation and a factor of

10 for sensitive subgroups. As discussed in Section 3.1.5, this RfD is based on a free-standing NOAEL. Thus, the RfD may underestimate the dose that could be tolerated with no adverse effects.

This chronic RfD is cited only for the sake of completeness. This RfD is important in that it is used to assess risks associated with the chlorination of drinking water. As discussed in Section 3.2 (Exposure Assessment), however, chronic exposures to aqueous chlorine in Forest Service programs are not plausible. Consequently, this chronic RfD is not used in the current Forest Service risk assessment.

3.3.4. Dermal Irritation

Pesticide risk assessments conducted by both the U.S. EPA as well as the Forest Service do not typically quantify risks associated with dermal irritation. Instead, risks are typically discussed qualitatively based on relatively standard studies on dermal irritation and dermal sensitization in laboratory mammals. While the standard studies in experimental mammals are appropriate for categorizing the potential for dermal irritation, these studies are not generally used quantitatively in a risk assessment to derive HQ values for dermal irritation.

A different approach is taken in the current Forest Service risk assessment and HQ values for dermal irritation are derived. This approach is taken for three reasons. First, as discussed in Section 3.2.2, dermal exposures are the most likely exposure scenario for workers. Secondly, the available data on the dermal effects of aqueous chlorine are reasonably well-defined in humans (Section 3.1.11.1). Lastly, the concentrations of aqueous chlorine used in Forest Service programs spans a very wide range from about 50 ppm to over 10,000 ppm. Based on the available data on the effects of aqueous chlorine in humans, very different effects in workers might be associated with this range of concentrations and the use of HQ values may provide reasonable guidance for individuals involved in the many different types of applications for aqueous chlorine in Forest Service programs.

As discussed in Section 3.1.11, the standard studies on dermal irritation and sensitization have been waived by the U.S. EPA because of the ample data on the corrosive effects of aqueous chlorine. As an alternative, the U.S. EPA/OPP (1999a) has classified aqueous chlorine as Toxicity Category I for dermal toxicity, dermal irritation, and skin sensitization. As discussed in SERA (2007a, Table 3-2), Toxicity Category I is the classification for the most toxic compounds.

As detailed in Section 3.1.12, the data on aqueous chlorine do not suggest that any systemic toxic effects associated with dermal exposures are plausible. Dermal exposures may lead to dermal irritation. As discussed in Section 3.1.11, dermal irritation may occur over relatively brief dermal exposures (i.e., 20 minutes) to concentrations of aqueous chlorine that are typically used in bleach formulations (i.e., 5.25% to 6%) (Hostynek et al. 1989). While concentration-duration relationships are not well-defined, increasing either the concentration of aqueous chlorine or the period of exposure will increase the likelihood of dermal irritation. While Goffin et al. (1997)

has noted subclinical signs of skin damage at concentrations in the range of 0.4% to 0.2%, a practical threshold for observable signs of skin irritation appears to be about 0.5%.

For the current Forest Service risk assessment, aqueous chlorine concentrations of 0.5%, equivalent to 5,000 ppm is taken as a functional threshold for signs of dermal irritation. In the risk characterization (Section 3.4), this concentration is used only to provide guidance in the likelihood of observing skin irritation in workers involved in handling solutions of aqueous chlorine over the range of concentrations used in Forest Service programs (Table 4 and Table 5).

Note that no dose-response relationship is proposed for individuals who might develop a sensitization to aqueous chlorine. Risks to this group of individuals are discussed qualitatively in the risk characterization (Section 3.4.4).

3.3.5. Dose-Severity Relationships

Forest Service risk assessments will often develop explicit dose-severity relationships to assist in the interpretation of hazard quotients (HQs) that exceed the level of concern. For aqueous chlorine, no formal dose-severity relationships are developed.

While a crude dose-severity relationship could be developed for oral exposure, none of the hazard quotients associated with acute oral exposures exceed the level of concern (Section 3.4 Risk Characterization). Consequently, there is no need for the development of oral dose-severity relationships.

For dermal exposures, the highest hazard quotients are associated with accidental direct dermal contact with undiluted bleach formulations. As detailed in Section 3.3.4, human data are available for assessing the likely consequences of such contact. Thus, as with oral exposures, formal concentration-severity relationships do not need to be elaborated.

3.4. RISK CHARACTERIZATION

3.4.1. Overview

Under normal conditions of use, there is no plausible basis for asserting that aqueous chlorine is likely to cause adverse effects in either workers or members of the general public. All product labels also indicate that gloves and protective eyewear should be used to prevent splashing or direct contact of either the undiluted formulation or aqueous dilutions of aqueous chlorine on to the skin or into the eyes. If these standard and sensible precautions are followed, no adverse effects in workers would be anticipated. The uses of aqueous chlorine in Forest Service programs would not lead to any significant exposure to members of the general public and thus no adverse effects would be anticipated.

Notwithstanding the above assessment, the misuse of aqueous chlorine by workers or accidental exposures to members of the general public are considered. If workers handle solutions of aqueous chlorine without gloves, skin irritation is likely if the skin is exposed to undiluted bleach formulations and may occur when working with some diluted solutions, particularly in some types of applications – i.e., treatments for citrus canker, the use of cleaning solutions for the prevention of hantavirus, treatment of plant roots for parasitic nematodes and fungi, cleaning tools to prevent the spread of amphibian chytrid fungus. Similarly, splashing either undiluted formulations or some dilute solutions could result in eye irritation.

While members of the general public will not generally be exposed to aqueous chlorine in most Forest Service uses – i.e., cleaning equipment – adverse effects comparable of those in workers might occur if exposures are not prevented. The only accidental exposure for members of the general public that is quantified in the current risk assessment involves the accidental spill of water treated with aqueous chlorine at 50 ppm and used for fire suppression. In this instance, however, the levels of potential oral exposure are below the level of concern by factors of 4 to over 300 and the levels of dermal exposure are below the levels of concern by factors of 10,000 to over 300,000.

The above risk characterization, however, does not apply to individuals who may develop a sensitization to aqueous chlorine. Allergic contact dermatitis may develop in some individuals who repeatedly use bleach. Skin irritation in these individuals may occur at concentrations that are much lower than those required to cause skin irritation in individuals who are not sensitized to aqueous chlorine. In individuals with a sensitivity to aqueous chlorine, dermal irritation would be the most likely effect. While the irritation could be painful, more severe adverse effects would not be anticipated. One report in the literature, however, suggests that some individuals with a severe allergy to aqueous chlorine could develop respiratory symptoms – i.e., difficulty breathing – after dermal exposure to aqueous chlorine. While these severe respiratory effects are not well-documented, they are noted as a concern. If individuals using aqueous chlorine in Forest Service programs develop skin irritation as well as respiratory impairment, it would be prudent to ensure that the individual receives prompt medical attention.

3.4.2. Workers

The risk characterization for workers is based on dermal exposure scenarios involving the concentrations of aqueous chlorine used in Forest Service programs (concentrations of 50 ppm to about 10,000 ppm as discussed in Section 3.2.2) and the human NOAEL for dermal irritation (5000 ppm as discussed in Section 3.3.2). The numeric expression of the risk characterization is given simply as the ratio of the exposure concentration to the NOAEL. These ratios are summarized in Worksheet C01 of the EXCEL workbook that accompanies this risk assessment. As a convenience to the reader, this worksheet is reproduced as Table 8 in this risk assessment document.

The dermal HQ values given in Worksheet C01 and Table 8 have the same form as HQ values given most Forest Service risk assessments – i.e., the ratio of exposure to a NOAEL. The interpretation of the HQ values for dermal exposure differs, however, from HQ values given for most pesticides. In a typical pesticide risk assessment, the toxicity value is based on systemic toxicity and an HQ value of greater than one (1.0) indicates the potential for a systemic toxic effects. This is not the case with aqueous chlorine. As detailed in the hazard identification, dermal exposures to aqueous chlorine may cause dermal irritation but these exposures are not likely to result in any systemic toxic effects. Thus, the HQ values of greater than one simply indicate a potential for dermal irritation if the skin comes into contact with the solution of aqueous chlorine.

Based on the HQ values, the uses of aqueous chlorine that are most likely to result in signs of dermal irritation include treatments for citrus canker or the use of cleaning solutions for the prevention of hantavirus (HQ=1.1), treatment of plant roots for parasitic nematodes and fungi (HQ=1.7), cleaning tools to prevent the spread of amphibian chytrid fungus (HQ = 2.1). The highest hazard quotient – i.e., 11.7 – is associated with direct contact with undiluted bleach formulations.

The severity of these effects would likely vary with the individual as well as the duration of exposure. As discussed in Section 3.1.11.1, the most likely effects would be redness or itching. More serious adverse effects would only be expected in individuals who develop a sensitization to aqueous chlorine. This is discussed further in Section 3.4.4.

All of the hazard quotients should be regarded as the result of accidental rather than routine exposures. All of the product labels specify that direct and prolonged contact with skin should be avoided and that the skin should be washed thoroughly if direct contact occurs. For any uses involving prolonged exposures, the product labels also recommend the use of protective gloves and other protective clothing such as gowns or long-sleeved shirts and long pants. Normal Forest Service practice requires workers to employ these protective measures. Thus, the likelihood of skin irritation in workers involved in Forest Service projects is low.

All product labels also indicate that protective eyewear should be used to prevent splashing either the undiluted formulation or aqueous dilutions of aqueous chlorine into the eyes. As discussed in Section 3.1.11.3, splashing solutions of bleach into the eyes is a relatively common

occurrence when handling solutions of aqueous chlorine but these incidences do not typically result in severe or permanent eye damage. Nonetheless, prudence dictates that protective eyewear should be used when handling aqueous chlorine and that mitigating measures should be taken if aqueous chlorine is splashed into the eyes.

The greatest concern with handling bleach may be complacency. As noted by Smolinske and Kaufman (2007), bleach is a common household product that most people use and most members of the general public as well as workers do not regard bleach as a hazardous substance. While the current risk assessment does not suggest that bleach should be treated as a highly toxic substance, bleach can cause adverse effects and should be handled sensibly.

3.4.3. General Public

The above cautionary language on handling bleach is applicable to members of the general public as well as workers. Individuals who handle bleach should be careful to avoid contact of bleach with the skin or eyes. Notwithstanding this cautionary language, however, there is no basis for asserting that members of the general public will be adversely affected by the use of bleach in Forest Service programs.

Under normal circumstances, no significant oral exposures are likely. Based on rather extreme accidental exposure scenarios involving oral exposure from the contamination of a small pond, the HQ values are about 0.04 (0.003 to 0.255), below the level of concern by factors of about 4 to over 300. As discussed in Section 3.3.2, these HQ values are based on a very conservative surrogate acute RfD, 0.25 mg/kg bw. This RfD is below the human NOAEL of 0.34 mg/kg bw from the study by Lubbers et al. (1982) and substantially below the estimated dose of 24 to 30 mg/kg bw that is not associated with effects that require substantial medical intervention (Mack 1983). In terms of a simple risk characterization, the statement by ATSDR bears repeating:

> *In the case of the oral database for aqueous chlorine, no reliable LOAEL could be identified at levels of aqueous chlorine that could reasonably be encountered in the environment.*
>
> ATSDR (2007, p. 21)

Significant dermal exposures to members of the general public are also not plausible. During the use of concentrated bleach solutions or even the relatively dilute solutions used in equipment cleaning, it seems reasonable to assert that workers in involved in Forest Service programs would exercise reasonable oversight to prevent exposing members of the general public to bleach or more dilute solutions of aqueous chlorine. If effective steps were not taken to prevent dermal exposures to members of the general public, the risk characterization for dermal exposure would be essentially identical to that for workers.

The only uncontrolled dermal exposure that seems remotely plausible involves the accidental release of a 50 ppm solution of aqueous chlorine into surface water where an individual might be swimming. As detailed in Worksheet D02, this exposure scenario leads to HQ values of 0.00003 (0.000003 to 0.0001), below the level of concern by factors of 10,000 to over 300,000.

3.4.4. Sensitive Subgroups

Given the very general mechanism of action of aqueous chlorine (Section 3.1.2) – i.e., direct reactions with biological tissue – there is little basis for suggesting that certain subgroups will be unusually sensitive to aqueous chlorine. As noted by ATSDR (2007, p. 122), … *No information was located regarding populations unusually susceptible to exposure to hypochlorite bleach.*

One exception, however, appears to be individuals who may develop an allergic sensitization to aqueous chlorine. As discussed in Section 3.1.11.2, aqueous chlorine is not generally considered to be a skin sensitizer (ATSDR 2007; Brunch 2007). The U.S. EPA/OPP (1999a), however, has classified aqueous chlorine as a dermal sensitizing agent and this classification is supported by patch test assays for allergic contact dermatitis as well as some very well documented cases of allergic response in individuals exposed to aqueous chlorine during the conduct of root canals.

The extent to which allergic contact dermatitis may be a practical concern for the use of aqueous chlorine in Forest Service programs is uncertain. Nonetheless, it seems plausible that some individuals may display dermal irritation when exposed to concentrations of aqueous chlorine that are below the approximate dermal NOAEL of 5,000 ppm for members of the general population (Section 3.3.4). Based on the study by Habets et al. (1986), some individuals with an apparent allergy to aqueous chlorine may not display adverse dermal reactions to concentration below 100 ppm. If this pattern is generally applicable, individuals involved in the use of aqueous chlorine for fire suppression or dust abatement (i.e., 50 ppm solutions) would not be expected to display any atypical adverse dermal reactions. On the other hand, several Forest Service uses of aqueous chlorine involve the use of solutions in the range of 200 ppm to about 2000 ppm. These solutions would not be expected to cause marked dermal irritation in members of the general population but might be associated with dermal irritation in individuals who are sensitized to aqueous chlorine.

The potential severity of sensitization to aqueous chlorine is also not clear. In most instances, individuals with a sensitivity to bleach will exhibit only dermal irritation, although the irritation may be severe. As discussed in Section 3.1.11.2, however, Caliskan et al. (1994) document a severe allergic response to sodium hypochlorite during root canal therapy. While other such incidents have been reported, Caliskan et al. (1994) also report that the individual involved in this incident stated that prior dermal exposures to a *household cleaning agent,* presumably sodium hypochlorite, had lead to difficulty in breathing. Respiratory impairment is a common symptom in severe allergic reactions but the paper by Caliskan et al. (1994) is the only published report indicating that an allergic reaction to a dermal exposure to bleach might cause breathing difficulty. While this type of severe allergic reaction to aqueous chlorine may be uncommon, individuals involved in the use of the aqueous chlorine solutions in Forest Service programs should be aware that this type of reaction may occur. In this event, the affected individual should receive prompt medical care.

3.4.5. Connected Actions

Under the National Environmental Policy Act (NEPA), connected actions are those actions that must occur if a prior action occurs. Implicit in this definition is a consideration of risk. If a connected action results or might result in a risk, that risk needs to be considered.

Within the context of the use of bleach in Forest Service programs, connected actions that might impact human health involve unintended exposures of members of the general public to bleach as well as chemical reactions of bleach with other compounds. To the extent possible, these connected actions are considered in this section of the risk assessment – i.e., risks to the general public are addressed in Section 3.4.3 and risks associated with chlorination byproducts are discussed in Section 3.1.15. Other connected actions are associated with unintended but unavoidable exposures to wildlife and these are considered in Section 4 (Ecological Risk Assessment).

3.4.6. Cumulative Effects

Cumulative effects refers generally to either repeated exposures to an agent or to combined exposures to the agent of concern as well as other agents that may cause the same effects – i.e., agents which have the same mechanism of action. The U.S. EPA is required to address cumulative effects under the Food Quality Protection Act of 1996. In the re-registration eligibility decision (RED) for chlorine, which also covers aqueous chlorine, the U.S. EPA/OPP (1999a) has not addressed cumulative effects. In the more recent RED on inorganic chlorates, another class of disinfectants, the U.S. EPA/OPP (2006a) addresses cumulative risks but indicates that the Agency … *has not made a common mechanism of toxicity finding* (U.S. EPA/OPP 2006a, p. 42).

As discussed in Section 3.1.2, bleach is a strong oxidizing agent. There are many other strong oxidizing agents, such as inorganic chlorates, and it is likely that co-exposure to bleach and other oxidizing agents would lead to effects that are similar to but greater than an equivalent exposure to bleach alone. A more important class of co-exposures, however, may involve the formation of chlorinated byproducts. As detailed in Section 3.1.15, aqueous chlorine may interact with and chlorinate various organic compounds and some of the chlorinated compounds that are formed may be hazardous. As in the risk assessments of chlorine conducted by ATSDR (2007) and the U.S. EPA/OPP (1999a), the potential risks associated with the chlorination of organic matter are acknowledged but cannot be addressed quantitatively in the current risk assessment of aqueous chlorine.

The current risk assessment does address the issue of repeated exposures to aqueous chlorine. For the most part, however, longer term exposures to aqueous chlorine are not likely to occur as a result of Forest Service programs. A major exception may involve workers who might use aqueous chlorine repeatedly and over a prolonged period of time. Risks to these individuals are addressed in Sections 3.4.2 and 3.4.4 of this risk assessment.

4. ECOLOGICAL RISK ASSESSMENT

4.1. HAZARD IDENTIFICATION

4.1.1. Overview

For birds and mammals, the only significant route of exposure to aqueous chlorine will involve oral exposures from contaminated water. The oral toxicity of aqueous chlorine to mammals is relatively well-defined. The range of lethal doses in mammals is about 225 to 675 mg/kg bw and the short-term NOAEL from drinking water exposures is about 25 mg/kg bw. Compared to mammals, much less information on the oral toxicity of aqueous chlorine to birds is available. Based on an acute LD_{50} and two dietary studies on lithium hypochlorite , the U.S. EPA has classified chlorine as only slightly toxic to birds. While these classifications are consistent with the general approach used by U.S. EPA, dietary exposures are not relevant to the current Forest Service risk assessment. A single 28-day study is available on the toxicity of aqueous chlorine, generated from sodium hypochlorite, to broiler chickens and this study indicates an NOEC of 300 ppm with a corresponding LOEC for changes in organ weight of 600 ppm.

Information on the toxicity of aqueous chlorine to other terrestrial species is scant. No data have been encountered on the toxicity of aqueous chlorine to reptiles, soil invertebrates, or terrestrial plants. Aqueous chlorine is an effective microbicide and bacterial culture studies are available indicating adverse effects on microorganisms at concentrations as low as about 0.1 mg/L. No studies, however, are available on the toxicity of aqueous chlorine to microorganisms in a soil matrix. This is unfortunate because soil microorganisms will be exposed to aqueous chlorine in some Forest Service applications, such as dust suppression. While it is likely that concentrations of aqueous chlorine recommended for the control of microbial pathogens will be toxic to soil microorganisms, the extent and duration of adverse effects on soil microorganism cannot be well-defined.

Aqueous chlorine is very toxic to fish, aquatic invertebrates, and aquatic plants. Relatively standard flow-through LC_{50} values range from 0.005 mg/L to about 1 mg/L for fish and 0.017 mg/L to about 1 mg/L for aquatic invertebrates. For both fish and invertebrates, the NOEC values for mortality are generally below the LC50 values by factors of about 2 to 3. While the mechanism of action of aqueous chlorine to fish and aquatic invertebrates is not well-characterized, toxicity is likely due to the oxidizing potential of sodium hypochlorite. While aqueous chlorine is used extensively as an algicide, algae do not appear to be remarkably more sensitive to aqueous chlorine than fish or aquatic invertebrates. Most short-term studies indicate that effective algicidal concentrations are in the range of about 0.05 mg/L to 1 mg/L. Longer-term studies evidence LOEC values for algae as low as 0.002 mg/L. These toxicity values, however, are not relevant to Forest Service uses of aqueous chlorine because longer-term exposures will not occur in Forest Service programs. Studies on the toxicity of aqueous chlorine to aquatic microorganisms are relatively scant compared to the number of studies on other groups of aquatic organisms. Based on relatively short-term exposures, the NOEC values for aquatic microorganisms are in the range of about 0.006 mg/L to 0.01 mg/L.

4.1.2. Toxicity to Terrestrial Organisms

4.1.2.1. Mammals

The hazard identification for mammalian wildlife is based on a subset of the information used for the hazard identification for the human health risk assessment (Section 3.1). As with humans, the potential adverse effects of aqueous chlorine to mammalian wildlife are likely to be attributable to the oxidizing action of aqueous chlorine. Also as in the human health risk assessment, concerns for aqueous chlorine are based on short-term exposures because the uses of aqueous chlorine in Forest Service programs will not lead to longer-term exposures.

The human health risk assessment focuses on two routes of exposure: dermal exposures for workers and oral exposures for members of the general public. For mammalian wildlife, however, dermal exposures are not a primary concern. As discussed in Section 3.1.11.1, humans appear to be more sensitive than experimental mammals to dermal irritation following exposures to aqueous chlorine. In addition and as detailed further in Section 4.2.2, mammalian wildlife are not likely to come into direct dermal contract with concentrated solutions of aqueous chlorine – e.g., undiluted bleach formulations or relatively high concentrations of aqueous chlorine used in equipment cleaning. Mammalian wildlife also have fur covered skin, which will further reduce the likelihood of significant dermal exposures.

Short-term oral exposures of aqueous chlorine to mammalian wildlife will be plausible. As noted in Section 3.1.4, the acute oral toxicity of aqueous chlorine to experimental mammals is similar to that in humans. Acute oral LD_{50} values in rats for sodium hypochlorite range from about 5,000 mg/kg bw to 13,000 mg/kg bw (ATSDR 2007; Racioppi et al. 1994) and the approximate lethal dose for rats is about 225 to 675 mg/kg bw (Jakobsson et al. 1991). While the U.S. EPA/OPP (1999a) does not specifically discuss mammalian wildlife in the RED coving aqueous chlorine, the rat LD_{50} values of 5,000 mg/kg bw to 13,000 mg/kg bw (Section 4.1.2.1) would classify aqueous chlorine as practically nontoxic to mammals.

An acute/single-dose NOAEL in experimental mammals is not available. The subchronic study by Blabaum and Nicholas (1956) defines a NOAEL of 25 mg/kg bw/day and this study is used for deriving the acute drinking water health advisory for aqueous chlorine (Section 3.3.2). This NOAEL is also used for deriving the acute toxicity value for mammalian wildlife (Section 4.3.2.1). This NOAEL is far above any plausible levels of exposure and thus dose-severity relationships are not a concern in the current risk assessment.

4.1.2.2. Birds

Relatively little information is available on the toxicity of aqueous chlorine to birds. As summarized in U.S. EPA/OPP (1999a), the U.S. EPA accepted data on lithium hypochlorite for assessing the effects of aqueous chlorine in birds. The acute oral LD_{50} for lithium hypochlorite to mallard ducks is 567 mg/kg bw (Piccavillo 1977a). Based on this toxicity value, the U.S. EPA/OPP (1999a) classifies lithium hypochlorite as only slightly toxic to birds. As noted in the previous subsection on mammals, the LD_{50} values for rats range from 5,000 mg/kg bw to 13,000

mg/kg bw. Based on these values for mammals and the single reported LD_{50} in birds, aqueous chlorine would be considered somewhat more toxic to birds than mammals.

The U.S. EPA/OPP (1999a) also provides a very brief summary of two acute dietary studies in birds which report acute dietary LC_{50} values of greater than 5000 ppm (Piccavillo 1977b,c). As discussed further in Section 4.2 (Exposure Assessment), dietary exposures are not anticipated. The effect to birds of drinking water containing aqueous chlorine has been assayed by Hulan and Proudfoot (1982). In this study, groups of 20 male and 20 female broiler chickens were exposed to dilutions of a commercial bleach formulation (Javex) of sodium hypochlorite in drinking water at available chlorine concentrations of 0 (control), 37.5, 75, 150, 300, 600, and 1200 ppm for up to 28 days. At the highest concentration tested in the study by Hulan and Proudfoot (1982) nearly 50% of the birds died over the 28 day exposure period. Hulan and Proudfoot (1982) report changes in body weight as well as weights of the heart, liver, kidney, and testes. At 600 ppm, decreases were noted in heart and testes weight. At the next lower concentration, 300 ppm, no effects were noted on mortality, body weights, or organ weights. This study is considered further in the dose-response assessment for birds (Section 4.3.2.2).

4.1.2.3. Reptiles

The database maintained by Pauli et al. (2000) on reptiles and amphibians does not include toxicity data for aqueous chlorine. Furthermore, no other sources of such data were identified in the published literature on aqueous chlorine. Generally, in the absence of toxicity data concerning reptile exposure to pesticides, the EPA recommends the use of birds as suitable surrogates (e.g., U.S. EPA/EFED 2001).

4.1.2.4. Terrestrial Invertebrates

No information has been encountered on the effects of aqueous chlorine on terrestrial invertebrates. Given the non-specific nature of the oxidative damage caused by aqueous chlorine (Section 3.1.2) as well as the effects of aqueous chlorine on human skin (Section 3.1.11), it seems intuitive that organisms such as earthworms will be adversely affected if directly exposed to concentrated solutions of bleach and perhaps to some of the more highly concentrated bleach solutions that are used in Forest Service programs. The most prevalent exposures, however, will be to more dilute solutions of 50 ppm used for dust abatement and fire suppression. The effects of these more dilute solutions of aqueous chlorine cannot be assessed directly. As discussed further in Section 4.1.3.3, freshwater oligochaetes appear to be very sensitive to aqueous chlorine (Ewell et al. 1986). Whether this pattern would hold for terrestrial invertebrates is not known. More importantly, however, may be the effect of the soil matrix on toxicity. While somewhat speculative, it is likely that aqueous chlorine would react quickly with soil components and this could reduce the potential for adverse effects on soil invertebrates. This supposition is supported by the aquatic study by Raikow et al. (2007) which noted that the daphnid ephippia were tolerant to exposures to aqueous chlorine in a sediment matrix that were lethal to ephippia in the absence of sediment (Section 4.1.3.3).

As discussed further in the Section 4.1.2.6, soil applications of aqueous chlorine may adversely affect soil microorganisms but recovery from any adverse effects may be anticipated. Any

adverse effects on terrestrial invertebrates would also likely be transient. No information, however, is available on the duration that might be required for recovery. Methyl bromide is used to treat soils for nematodes and complete recovery of soil nematodes may be in excess of 166 days (Yeates et al. 1991). It does not seem likely, however, that Forest Service uses of aqueous chlorine would correspond in severity to soil sterilization with methyl bromide.

4.1.2.5. Terrestrial Plants (Macrophytes)

No toxicity studies have been encountered on the effects of aqueous chlorine on terrestrial plants. As discussed in the following subsection, aqueous chlorine is likely to have adverse effects on soil microorganisms and this could impact terrestrial plants, as discussed further in Section 4.4.2.4, the risk characterization for terrestrial plants.

4.1.2.6. Terrestrial Microorganisms

As discussed in Section 3.1.2 (Mechanism of Action), aqueous chlorine is a strong oxidizing agent and this mechanism is the primary mode of action in the toxicity of aqueous chlorine to microorganisms (ATSDR 2007, p. 180). Aqueous chlorine is highly reactive but is non-specific. As reviewed by U.S. EPA/OHEA (1994), the specific mechanisms of toxicity to microorganisms have not been characterized in detail but the general mechanisms of toxicity to microorganisms are likely to include interactions with proteins in the cell wall (resulting in the disruption of mechanisms for maintaining cellular homeostasis) and reactions with cellular enzymes (resulting in various modifications to normal cellular metabolism). In other words, aqueous chlorine is likely to interfere with many different biological processes resulting in general damage due to oxidative stress (Small et al. 2007; Tandon et al. 2007).

Interactions of aqueous chlorine with RNA and/or DNA may play a role in the toxicity of aqueous chlorine to some microorganisms, resulting in the disruption of a large number of different cellular processes, mutagenicity, or cell death (U.S. EPA/OHEA 1994; Rosenkranz 1973). In addition, damage to the structural components of viruses or bacterial spores may be sufficiently severe to cause indirect damage to RNA or DNA through structural damage resulting in the release of genetic material to the surrounding media (Maillard et al. 1998; Young and Setlow 2003). The study by McKenna and Davies (1988) in *Escherichia coli* suggests that damage to DNA may be an early sign of sublethal toxicity in bacteria resulting in growth inhibition and that DNA damage occurs at lower concentrations than those required for gross structural damage associated with bacterial death.

As with mechanisms of toxicity in mammals, the toxicity of aqueous chlorine may be enhanced by the formation of chlorination byproducts (Maruyama et al. 1988) but the relative significance of direct oxidative damage for microbial sub-cellular structures compared to the toxicity of chlorination by-products has not been characterized.

While no field studies have been directly conducted on the toxicity of aqueous chlorine to soil microorganisms, the efficacy information and labeling directions, summarized in Table 4, suggest that sodium hypochlorite will be toxic to some soil organisms at available chlorine concentrations ranging from as low as 0.1 ppm to over 10,000 ppm. As would be expected from

the range of labeled treatment rates, substantial variability in sensitivity is apparent among different species of microorganisms. Laboratory bioassays using cultures of single fungal species conducted with a 1 hour period of exposure indicates minimum fungicidal concentrations ranging from 0.17 mg/L (*Candida albicans*) to 86.6 mg/L (*Aspergillus niger*) (Wang et al. 2007). A series of single bacterial species bioassays involving a 1 day period of exposure resulted in growth inhibition at concentrations of 250 mg/L (*Streptococcus faecalis*) to 5000 mg/L (*Pseudomonas aeruginosa*) (Hidalgo et al. 2002). The higher effective concentrations noted by Hidalgo et al. (2002) relative to the lower effective concentrations over a shorter period of exposure appear to reflect differences in the test species and perhaps test design between the two studies. The toxicity of aqueous chlorine to microorganisms is generally directly related to both the concentration and duration of exposure (e.g., Dequeiroz and Day 2007; Koponen et al. 1992; McKenna and Davies 1988; Mezzanotte et al. 2007).

As noted in the discussion of labeled uses (Section 2.3.1), disinfected water (50 ppm solutions of sodium hypochlorite) may be used for dust abatement – i.e., the treated water is intentionally sprayed on dirt roads in forests to reduce the formation of dust due to road traffic. No studies have been encountered on impact of sodium hypochlorite solutions on soil microorganisms. Nonetheless, based on the effectiveness of sodium hypochlorite as an antimicrobial agent, there is little doubt that the application of bleach solutions to a dirt road surface will have adverse effects on microbial populations in the treated areas. As discussed further in Section 4.4.2.5, however, the exposures will be very short-term and the effects are likely to be transient.

4.1.3. Aquatic Organisms

A large number of studies have been conducted on the effects of aqueous chlorine on aquatic organisms. As in the human health risk assessment (Section 3), many of the studies have focused on the use of aqueous chlorine in drinking water disinfection as well as sewage treatment. Chlorine is also used in the production of paper products and the toxicity of Kraft pulp mill effluents to aquatic organisms has been extensively studied (e.g., Soivio et al. 1988). This literature, however, is not relevant to the uses of aqueous chlorine in Forest Service programs and is not further addressed in this risk assessment.

Much of the early literature has been summarized in the Ambient Water Quality Criteria Document for Chlorine (U.S. EPA/OW 1984) and some of the more recent literature has been reviewed by Vetrano (2001). Key studies from these reviews as well as the more recent literature and studies focusing on sublethal toxicity are given in Appendix 1 (fish), Appendix 2 (aquatic invertebrates), Appendix 3 (aquatic plants), and Appendix 4 (aquatic microorganisms). Most of the published reviews focus heavily on LC_{50} values to both describe the toxicity of aqueous chlorine and recommend exposure limits. While LC_{50} values are important in terms of characterizing the variability in sensitivities among different species, Forest Service risk assessments prefer to focus on sublethal effects as well as no-effect levels. These types of studies, however, are far fewer than the more standard LC_{50} determinations.

4.1.3.1. Fish

Based on the mechanism of action of aqueous chlorine in mammals (Section 3.1.2) and microorganisms (Section 4.1.2.6), it is reasonable to suppose that aqueous chlorine will react with and cause oxidative damage to fish tissue. Several studies report that damage to gill tissue appears to be the primary tissue damaged in fish (Cohen and Valenzuela 1977; Middaugh et al. 1977a; Soivio et al. 1988). Zeitoun (1977, p. 189) has suggested that aqueous chlorine will *…diffuse readily through the gills, oxidizing the hemoglobin to methemoglobin*. The rationale for asserting that aqueous chlorine will easily diffuse through gill tissue is not clear. The histopathology studies conducted by Cohen and Valenzuela (1977) do appear to clearly demonstrate that aqueous chlorine will cause extensive damage to gill tissue and that this damage is associated with the lethal effects of aqueous chlorine in fish. While it is possible that species differences may exist in the susceptibility of gill tissue to aqueous chlorine, the weight of evidence suggests that gill tissue is likely to be a target tissue. This effect is similar to and consistent with effects in mammals in which aqueous chlorine primarily affects the portal of entry. As noted in Zeitoun (1978), gill damage is consistent with the effects of aqueous chlorine on the blood.

A recent study by de Paiva Magalhas et al. (2007) has focused on both lethal and sublethal effects of aqueous chlorine in zebrafish. The lethality study is discussed further below with other LC_{50} determinations. At low sublethal concentrations – i.e., 10% of the LC_{50} – the primary sign of toxicity was hyperactivity. At somewhat higher sublethal concentrations – i.e., 20% of the LC_{50} – the primary sign of toxicity was hypoactivity. While somewhat speculative, it seems reasonable to suggest that the hyperactivity could reflect gill irritation and that the hypoactivity are higher concentrations could reflect hypoxia, either secondary to gill damage and/or as a direct result of the effects aqueous chlorine on blood.

The irritant effects of aqueous chlorine are also evident in a large number of studies indicating that fish will exhibit avoidance behavior when exposed to sublethal concentrations of aqueous chlorine (Cherry et al. 1977; Middaugh et al. 1977a; Schumacher and Ney 1980). Because of the variability in LC_{50} values, as discussed further below, and the nature of the avoidance studies in fish, it is difficult to suggest meaningful threshold concentrations for avoidance behavior. Rainbow trout, however, appear to be the most sensitive species with evidence of avoidance behavior at concentrations as low as 0.04 mg/L (Schumacher and Ney 1980) to 0.05 mg/L (Cherry et al. 1982).

The most common type of toxicity data available on fish consists of 96-hour LC_{50} values. Consequently, these toxicity values are used by U.S. EPA/OPP (1999a) to classify the toxicity of aqueous chlorine to fish and are probably the best data set for assessing the variability in toxicity studies. Aqueous chlorine is classified by the U.S. EPA/OPP (1999a, p.25) as highly toxic to fish based on registrant submitted studies using lithium hypochlorite which report LC_{50} values of 0.28 mg/L for bluegills (Buccafusco 1978a) and 0.2 mg/L for trout (Buccafusco 1978b).

The U.S. EPA/OPP (1999a) also references LC_{50} values reported in the more extensive review on aqueous chlorine by U.S. EPA/OW (1984) citing LC_{50} values that range from 0.045 mg/L for

a shiner and 0.71 mg/L for a stickleback. All of these bioassays involve flow-through exposures in which the concentrations of aqueous chlorine are maintained at a constant concentration. The lower range LC_{50} of 0.045 mg/L from U.S. EPA/OPP (1999a, Table 1, p. 25) is referenced to the LC_{50} in the pugnose shiner (*Notropis anogenus*) from the study by Ward and DeGraeve (1980). Ward and DeGraeve (1980, Table 2, p. 43) report a somewhat lower LC_{50} of 0.029 mg/L for the pugnose shiner. More importantly, however, the Ward and DeGraeve (1980) study involved bioassays of chlorinated industrial waste water which was itself toxic to the fish. As noted by these investigators, *…the Pugnose shiner LC_{50} concentration of 0.029 mg/l Cl_2 is probably unrealistically low, since more than 50 percent of the pugnose shiners exposed to 100 percent non-disinfected effluent died* (Ward and DeGraeve 1980, pp. 42-43). Thus, the study by Ward and DeGraeve (1980) as well as other bioassays on chlorinated effluent (e.g., Zillich 1972) are not used quantitatively in the current Forest Service risk assessment. U.S. EPA/OW (1984), however, also summarizes a toxicity study in trout fry by Wolf et al. (1975) in which a similar LC_{50} of 0.040 mg/L is reported. The upper bound LC_{50} of 0.71 mg/L cited in U.S. EPA/OPP (1999a) appears to be from the bioassay in the threespine stickleback (*Gasterosteus aculeatus*) in the unpublished study by Esvelt et al. (1971). Taking the 96-hour LC_{50} of 0.71 mg/L as the highest valid value, the reported 96-hour LC_{50} values which involve 96-hour exposure periods to aqueous chlorine appear to vary over a factor of about 18 [0.71 mg/L ÷ 0.04 mg/L ≈ 17.75].

The focus by the U.S. EPA on 96-hour exposures and 96-hour LC_{50} values appears to reflect concern with longer-term releases of aqueous chlorine from industrial processes or drinking water treatment. As noted in the exposure assessment for aquatic organisms (Section 4.2.5), no significant releases of aqueous chlorine to surface water are likely to occur in the normal uses of bleach in Forest Service programs. The most plausible accidental exposure scenario involves the release of 50 ppm solution of aqueous chlorine into a small pond. This could result in initial very high concentrations in the affected section of the pond that would rapidly diminish over time as a result of mixing, the reaction of aqueous chlorine with components in pond water, and the degradation of aqueous chlorine.

As also summarized in Appendix 1, some static bioassays report LC_{50} values substantially greater than the upper bound of the LC_{50} values from flow-through bioassays. For example, static 96-hour LC_{50} values in fathead minnows range from 5.9 mg/L (Curtis and Ward 1981) to 10 mg/L (Ewell et al. 1986). The highest reported static LC_{50} is 48 mg/L (De Paiva Megalhas et al. 2007), which is approximately the concentration of aqueous chlorine used in fire suppression. In these studies, however, the actual exposures over the course of the 96-hour study probably involved much lower concentrations than are reflected in the reported LC_{50} values.

As summarized in Appendix 1, other toxicity studies with aqueous chlorine focus on relatively brief periods of exposure. Some of these LC_{50} values are reported as 96-hour LC_{50} values because a 96-hour observation period was used to detect latent toxicity (e.g., Fandrei and Collins 1979). While very short-term exposures may not be directly applicable to the scenario involving spills of aqueous chlorine into a pond, short-term exposures may mimic the spill of aqueous chlorine into a rapidly flowing stream. The shortest duration of exposure reported in the literature is 30 minutes from the studies by Fandrei and Collins (1979) and Seegert et al. (1977).

As summarized in Appendix 1, these studies report LC_{50} values ranging from 0.23 mg/L in the emerald shiner at 25°C (Fandrei and Collins 1979) to 7.7 mg/L in adult yellow perch at 10°C (Seegert et al. 1977). The bounds of this range of toxicity values for 30-minute exposures are only factors of about 6 to 11 greater than the 96-hour flow-through toxicity values – i.e., 0.04 mg/L to 0.71 mg/L – even though the 30 minute period of exposure is almost 200 times shorter than 96-hours. This is similar to the pattern noted by U.S. EPA/OW (1984) in the comparison of 24-hour to 96-hour LC values in which the average ratio of 24- to 96-hour LC_{50}s was only 1.4.

Another pattern noted by U.S. EPA/OW (1984) involves the relatively steep concentration-response curve for aqueous chlorine. Based on the early studies by Mattice and Zittel (1976) and Brooks and Seegert (1977a), U.S. EPA/OW (1984) notes that concentrations of about one-half to one-third of the LC_{50} values for different species are often associated with no mortality. Similar results have been reported by Brooks and Bartos (1984) – i.e., ratios of the LC_{50} to the LC_1 in three species of fish are less than a factor of 2 – as well as Seegert and Brooks (1978) – i.e., ratios of 100% lethality to 0% lethality of about 1.9 to 2.6. Steep concentration-response curves are also noted in the concentration-response curves for aquatic invertebrates, as discussed further in Section 4.1.3.3. As noted above, however, most of the bioassay data do not provide information on sublethal effects and the lack of mortality is not equivalent to a no-observable-effect concentration (NOEC) for sublethal effects. This is illustrated in the recent study by De Paiva Magalhas et al. (2007) in which the sublethal NOEC for hypoactivity is a factor of 5 below the LC_{50} for zebrafish.

The toxicity of many pesticides to fish and other aquatic organisms tend to increase with increasing temperatures. This pattern has been noted in some studies (e.g., Fandrei and Collins 1979; Seegert and Brooks 1978) but other studies (e.g., Bass and Heath 1977; Thatcher et al. 1976) do not suggest a consistent or pronounced pattern among species. U.S. EPA/OW (1984) notes that water alkalinity does not appear to have a substantial impact the toxicity of aqueous chlorine and this is supported by the more recent study by Kitamura (1990).

4.1.3.2. Amphibians

Very little information is available on the effects of aqueous chlorine on amphibians. Gauthier et al. (1989) conducted an assay on the ability of sodium hypochlorite to cause chromosomal damage in newt larvae (*Pleurodeles waltl*). The bioassay involved 12-day exposures to 0.06, 0.125, and 0.25 ppm available chlorine. The exposures appear to have been static renewal, with chlorine added to the water each day when the organisms were fed. At the two higher concentrations, an increase was noted in the number of micronuclei – i.e., an index of chromosomal damage. The paper does not report any signs of toxicity. This paper, however, is focused on newt larvae as an assay for DNA damage and it is not clear that the authors would have reported sublethal signs of toxicity but it does seem more likely that mortality or gross signs of toxicity would have been reported.

4.1.3.3. Aquatic Invertebrates

As with fish, the predominant type of information available on the toxicity of aqueous chlorine to aquatic invertebrates consists of LC_{50} estimates. By convention, most acute toxicity bioassays

for aquatic invertebrates are conducted over a 48-hour exposure period but several 96-hour exposure studies have been conducted with aqueous chlorine in both small invertebrates – e.g., the copepod study by Beeton et al. 1976 – and larger invertebrates – e.g., the isopod study by Bosnak and Morgan 1981. Also as in the bioassays on fish, very large differences in LC_{50} estimates are apparent between flow-through studies and static bioassays. As reviewed by U.S. EPA/OW (1984), the reported LC_{50} values for flow-through studies range from 0.017 mg/L in *Daphnia magna* to 0.960 mg/L in a species of crayfish.

While several more studies on the toxicity of aqueous chlorine are summarized in Appendix 2, most of the newer studies involve static exposures, as discussed further below. Of the more recent flow-through exposure studies, most are reasonably consistent with the data reviewed by U.S. EPA/OW (1984). Fisher et al. (1999) reports a 48-hour LC_{50} values in crustaceans of 0.032 mg/L (daphnids) and 0.078 mg/L (amphipods) which are consistent with the range of toxicity values identified by the U.S. EPA. Klerks and Fraleigh (1991) conducted relatively long-term toxicity studies in zebra mussels but report LT_{50} values (time to 50% lethality) of 4.5 days at a concentration of 1.0 mg/L and 3.2 days at concentration of 2.5 mg/L. Similarly, Cameron et al. (1989) reported LT_{50} values of 8.7 days at 0.5 mg/L and 5.9 days at 5 mg/L for the Asiatic calm. These studies suggest that adult bivalves may be somewhat more tolerant than other aquatic invertebrates.

In terms of the current Forest Service risk assessment, the main focus is on the identification of the most sensitive species. One more recent study, Taylor (1993), suggests that *Ceriodaphnia dubia* may be somewhat more sensitive than would be suggested from the toxicity values taken from earlier studies reviewed by U.S. EPA/OW (1984). As summarized in Appendix 2, Taylor (1993) conducted a series of static and flow-through bioassays at pH 7 and pH 8. The two pH values were selected in an attempt to differentiate the toxicity of hypochlorous acid (which will predominate at pH 7) and the hypochlorite ion (which will predominate at pH 8). As illustrated in Figure 2 and discussed in Taylor (1993), the resulting solutions of aqueous chlorine will differ in relative concentrations of hypochlorite and hypochlorous acid but the differences are not substantial. These relatively minor differences in the species of aqueous chlorine are reflected in the toxicity values reported by Taylor (1993), flow-through 48-hour LC_{50} values of 0.005 mg/L at pH 7 and 0.006 mg/L at pH 8. Taylor (1993) also reports static LC_{50} values for *Ceriodaphnia dubia* that are in the range of flow-through toxicity values in *Daphnia magna* – i.e., 0.035 mg/L at pH 7 and 0.048 mg/L at pH 8. Manning et al. (1996) reports a somewhat higher static 24-hour LC_{50} value for *Ceriodaphnia dubia* of 0.12 mg/L. As discussed by Manning et al. (1996), however, the *Ceriodaphnia dubia* used in this bioassay was an Australian cladoceran that is taxonomically distinct from the North American *Ceriodaphnia dubia* used in the study by Taylor (1993). U.S. EPA/OW (1984) does not report any bioassay data on *Ceriodaphnia dubia* or other species of *Ceriodaphnia*. *Ceriodaphnia dubia* is similar to but much smaller than *Daphnia magna.* The greater sensitivity of *Ceriodaphnia dubia* relative to *Daphnia magna* may simply reflect the greater surface area per unit body weight of the small organism.

Another variation in the study by Taylor (1993) involved separate static bioassays in which the *Ceriodaphnia dubia* were fed a mixture of trout chow, yeast, and a commercial mix at a

concentration of 2 mg/mL solids. In each of these bioassays, the LC_{50}s for aqueous chlorine were higher by factor of about 2 to 4 and that this decrease in toxicity was associated with a more rapid dissipation of aqueous chlorine which reacted with the daphnid food.

As with fish, there are very large differences between static studies which report only nominal concentrations and flow-through studies which report measured concentrations of aqueous chlorine. Some static studies are included in Appendix 2 because they involve bioassays in several different species that can be used to assess differences in sensitivity in different groups of invertebrates. For example, Ewell et al. (1986) assayed six different types of aquatic invertebrates and noted that daphnids, amphipods, and segmented worms were much more sensitive to aqueous chlorine (96-hour LC_{50} values of 2.1 to 3 mg/L) than flatworms, isopods, or snails (LC_{50} values of 32 to 59 mg/L). Consistent with the observations by Ewell et al. (1986), most studies indicate that daphnids and other relatively small invertebrates such as copepods and even amphipods are much more sensitive to aqueous chlorine (Brown et al. 1994; Gregg 1974; Mattice et al. 1981) than larger aquatic invertebrates such as snails, crayfish, and bivalves (Cameron et al. 1989; Doherty et al. 1986; Klerks and Fraleigh 1991; Martin et al. 1993).

As discussed in Section 4.1.3.1, the U.S. EPA/OW (1984) has noted relatively steep concentration-response curves for fish in which the NOEC for lethality is typically a factor of about 2 to 3 below the LC_{50}. Most studies in aquatic invertebrates that provide concentration-response data also note a relatively steep concentration-response relationship. As summarized in Appendix 2, Brown et al. (1994) conducted bioassays on five species of small aquatic invertebrates. Ratios of the LC_{50} to the NOEC for mortality ranged from about 1.33 for a copepod (*Mysocyclops longisetus*) to about 3.3 for a mosquito (*Anopheles farauti*).

The only exception to the general pattern of very steep slopes in the concentration-response curve is from the study by Latimer et al. (1975) in which a series of bioassays were conducted at various temperatures on two species of copepods, *Cyclops bicuspidatus* and *Limnocalanus macrurus*. Like many of the studies in fish, Latimer et al. (1975) used a 30-minute exposure period and assayed for mortality at 24 hours after exposure. *Limnocalanus macrurus* evidenced a very steep concentration-response curve with log-concentration probit-response slopes of about 7.9 to 10.4. *Cyclops bicuspidatus*, however, displayed much shallower slopes, ranging from 1.14 at 10°C to about 2.0 at 15°C.

In addition to the magnitude of the differences, the slopes from the study by Latimer et al. (1975) are noteworthy for two reasons. First, each bioassay involved the use of a very large number of organisms – i.e., 459 to 1411 per bioassay – and each bioassay involved a large number of concentrations – i.e., ranging from about 13 to 29 in the different bioassays. Thus, the concentration-response curves for both species are statistically well-defined. Secondly, Latimer et al. (1975) indicate that the organisms used in the bioassays were all wild-caught. Thus, the differences in sensitivity between these two species of copepods may reflect differences in field populations. From a practical perspective, the substantial differences in slopes between these two species of copepods impacts the current risk assessment in the use of empirical observations

of ratios of LC_{50} to NOEC values, as discussed further in the dose-response assessment for aquatic organisms (Section 4.3.3).

Very little information is available on the sublethal effects of aqueous chlorine to invertebrates. As noted in Section 4.1.3.1, aqueous chlorine appears to cause gill irritation and fish will display avoidance behavior to chlorine concentrations as low as 0.04 mg/L (Schumacher and Ney 1980). The only information on avoidance behavior in aquatic invertebrates is the study by Leynen et al. (1999) indicating that tubifex worms (small invertebrates that inhabit sediment) will withdraw into the soil column at aqueous chlorine concentrations of 0.5 ppm. Because of the very small size of most aquatic invertebrates, avoidance behavior may be displayed but, except for sediment dwelling organisms, the ability to move away from localized areas of aqueous chlorine contamination may be limited. In a stream simulation study by Newman et al. (1987), a concentration of 0.25 mg/L was associated with a decrease in the colonization of stream vegetation by amphipods. This effect was not noted at a lower concentration of 0.075 mg/L. It is not clear, however, if the decrease in colonization was associated with avoidance behavior or toxicity.

While daphnids are very sensitive to aqueous chlorine, Raikow et al. (2007) has noted that ephippia from *Daphnia mendotae* are very tolerant to aqueous chlorine with an LC_{50} value – assayed as eggs that did not produce viable daphnids – of 55 mg/L, a factor of over 3000 above the LC_{50} of 0.017 mg/L in *Daphnia magna*. This tolerance would be expected. Ephippia, also known are resting eggs or winter eggs, are formed by sexual reproduction by daphnids under adverse conditions. They are eggs surrounded by a hard chitinous case which is less sensitive to the oxidant effect of aqueous chlorine than viable daphnid tissue. A more interesting observation in the Raikow et al. (2007) study is that ephippia within sediment were much more tolerant to aqueous chlorine at concentrations of up to 2000 mg/L. This suggests that aqueous chlorine will react rapidly with abiotic components of sediment and it is possible that benthic organisms that burrow or can withdraw into sediment may be much more tolerant to aqueous chlorine as measured in the water column.

4.1.3.4. Aquatic Plants

One of the more common domestic uses of sodium hypochlorite is as an algicide in swimming pools. As indicated in Table 4, the recommended concentration for the control of algae is 1 ppm (1 mg/L) available chlorine with residual concentrations of at least 0.6 ppm. This range of concentrations is clearly toxic to most species of algae. As summarized in Appendix 3, various signs of algicidal activity such as a decreases in chlorophyll-a, decreases in primary productivity, microscopic signs of cell damage are apparent at concentrations in the range of 0.05 mg/L to about 0.4 mg/L in most studies involving either single cell or mixed cultures (Ahamed et al. 1993; Brooks and Liptak 1979; Kott and Edlis 1969; Peterson et al. 1995; Pratt et al. 1988; Steinman et al. 1992). A major exception is the study by Betzer and Kott (1969) which notes an NOEC of 1 mg/L in *Cladophora*, a genus of filamentous algae. NOEC values for non-filamentous algae appear to be much lower – i.e., in the range of 0.035 mg/L (Peterson et al. 1995).

The study by Pratt et al. (1988), however, suggests that reported NOEC and LOEC values for algae may be highly dependant on experimental conditions. Under conditions of continuous exposure – i.e., a flow-through assay – Pratt et al. (1988) noted a 28-day LOEC of 0.0021 mg/L. In the same study, a static renewal assay over a 24 day period noted a NOEC of 0.079 mg/L with an LOEC of 0.261 mg/L. Thus, the flow-through exposure with constant levels of chlorine was about 200 times more toxic to the algae than the static renewal. As discussed by Pratt et al. (1988), other differences between the two assays could have contributed to the very large differences in the results but differences in the exposures – i.e., relatively constant flow-through versus fluctuating static – were probably the most important factor.

The study by Watkins and Hammerschlag (1984) is the only study available on the toxicity of aqueous chlorine to macrophytes. As summarized in Appendix 3, this study also noted very substantial differences in response based on the exposure schedule. Under flow-through conditions over a 96-hour period of exposure, concentrations of 0.05 mg/L and greater were associated with reduced growth and visible discoloration of Eurasian watermilfoil. The 96-hour NOEC for continuous exposure was 0.02 mg/L. At a concentration of 1 mg/L, visible damage was apparent within 6 hours. In a separate series of intermittent exposures – i.e., 2 hours/day for 3 days – only the 1 mg/L concentration was associated with adverse effects and the intermittent NOEC was 0.5 mg/L.

4.1.3.5. Aquatic Microorganisms (other than algae)

As summarized in Appendix 4, several studies have been published on the effects of aqueous chlorine on aquatic microorganisms. Only one study, Newman et al. (1987), focused on microbial decomposition and the remaining studies focus on the impact of aqueous chlorine protozoan communities (Cairns et al. 1990; Dickson et al. 1977; Pratt et al. 1988).

These studies are very diverse in terms of experimental design and are difficult to compare. In terms of the current Forest Service risk assessment, Newmann et al. (1987) is the most relevant in terms of the duration of exposure, defining a 4-day NOEC 0.01 mg/L and a corresponding LOEC of 0.075 mg/L for a reduction in microbial populations. A functional impact on the degradation of vegetation, however, was noted only at 0.25 mg/L over a 35 day period of exposure. Over a 7-day period of exposure, Cairns et al. (1990) noted a dose-related decrease in the species richness of periphyton communities at concentrations of 0.0063 mg/L and 0.0566 mg/L. At the lower concentration, the decrease was substantial (about 20% lower than controls) but not statistically significant.

As discussed in the previous subsection on aquatic plants, Pratt et al. (1988) conducted two bioassays, an indoor study involving continuous exposure and an outdoor study involving static renewal exposures. As with the results in algae, the continuous study yielded a much lower NOEC (0.0061 mg/L) relative to the static renewal study (a NOEC of 0.079 mg/L).

The study by Dickson et al. (1977) reports NOEC values ranging from 0.66 mg/L to 1.45 mg/L, much higher than NOEC values reported in the other studies. As noted in Appendix 4, the

specific exposure regime used in this study is not well-described and it is not clear when specific observations were made.

4.2. EXPOSURE ASSESSMENT

4.2.1. Overview

An overview of the exposure assessments used in the ecological risk assessment is given in Table 9. As in the human health risk assessment, only a small subset of the scenarios used in standard Forest Service pesticide risk assessments are used for aqueous chlorine. The reasons for the limited number of scenarios are essentially identical to those in the human health risk assessment. Most uses of aqueous chlorine in Forest Service programs are highly localized and the potential for wide-spread exposures are limited. The major exceptions are the use of relatively low concentrations of aqueous chlorine (i.e., 50 ppm) for dust and fire suppression. The use of 50 ppm solutions of aqueous chlorine for fire suppression would not generally be viewed as hazardous to wildlife relative to the hazards posed by the fire.

As in the human health risk assessment, exposure scenarios for birds and mammals are developed for the consumption of contaminated water after an accidental spill of aqueous chlorine from an aircraft into a small pond in an area not impacted by fire. The resulting concentrations in water are estimated at 0.13 (0.014 to 0.56) ppm. A more extreme accidental scenario is also developed involving the consumption of unattended solutions of aqueous chlorine over the range of concentrations used in Forest Service programs – i.e., 50 ppm to 10,342 ppm. The lower bound of this range may be plausible in terms of transient pools of water that might exist after the application of aqueous chlorine for dust suppression. Higher concentrations would essentially reflect small amounts of more highly concentrated solutions that would be left unattended and accessed by birds or mammals. This is a highly extreme and probably highly implausible event that is included in the risk assessment only to illustrate the consequences of such extreme exposures.

A similar approach is taken for the exposure assessment of aquatic species. The concentration of aqueous chlorine in surface water is modeled as the accidental deposition of an aircraft load of chlorine treated (50 ppm) water used for fire suppression into a small pond. This scenario is identical to that used for birds and mammals – i.e., a concentration of 0.13 (0.014 to 0.56) ppm. The toxicity data on some aquatic organisms, however, supports the assessment of very short-term exposures that could exist in water prior to complete mixing. For this scenario, the maximum concentration in water is taken as the concentration used to treat the water – i.e., 50 ppm.

No quantitative exposure assessments are conducted for terrestrial plants, invertebrates, or microorganism. While exposures to soil invertebrates and soil microorganisms will occur in the use of aqueous chlorine for dust suppression, the available toxicity data do not support a dose-response assessment. Thus, risks to these organisms are considered qualitatively.

4.2.2. Mammals and Birds

4.2.2.1. Extreme Accidental Exposures

Only two exposure scenarios are considered quantitatively and both of these scenarios involve the consumption of contaminated water. The accidental exposure scenario involves the consumption of field solutions of aqueous chlorine that reflect the range of concentrations that may be used in Forest Service programs. As summarized in Table 8, these concentrations range from 50 ppm (fire and dust suppression) to 10,342 ppm (chytrid fungus control) and these concentrations are used as the lower and upper bounds. The central estimate is taken as 200 ppm, the concentration of aqueous chlorine used for general equipment cleaning. In this scenario, the assumption is made that the solution of aqueous chlorine is left unattended and that a small mammal (Worksheet F01a) or small bird (Worksheet F01b) uses this solution for drinking water over the course of a day.

This exposure scenario is atypical and is not used in other Forest Service risk assessments. While accidental exposure scenarios in Forest Service risk assessments are intentionally extreme, this exposure scenario for aqueous chlorine is probably unrealistic with the possible exception of the lower bound of the concentration – i.e., 50 ppm. Forest Service personnel will not leave concentrated solutions of aqueous chlorine unattended. Even if this were done, it does not seem plausible that mammals or birds would intentionally consume water with high concentrations of aqueous chlorine.

The lower bound of the concentration, however, is associated with fire or dust suppression – i.e., broadcast application. In these cases, this accidental exposure scenario could occur if water applied for dust suppression or water for fire suppression were accidentally dropped into an area far from a forest fire. In these cases, contaminated water could form a temporary pool and might be consumed by a small mammal or small bird. Even in this scenario, however, the exposures in Worksheets F01a (small mammal) and F01b (small bird) are likely to be overestimates because of the rapid dissipation of aqueous chlorine – i.e., 1.3 to 5 hours from the study by Jolley (1983). While this degradation could be considered quantitatively, this is not done in this exposure scenario because the lower bound concentration of 50 ppm does not exceed the level of concern for either birds or mammals (Section 4.4.2).

Most Forest Service risk assessments provide drinking water scenarios only for a small mammal (20 g) or a small bird (10 g). This approach is taken because allometric relationships for food and water consumption indicate that smaller mammals and birds will consume more food and water per unit body weight than larger mammals and birds (e.g., U.S. EPA/ORD 1993). For aqueous chlorine, a series of body weights rather than a single body weight is used. For mammals, the body weights are taken as 0.02 kg (typical of a very small mammal such as a mouse), 4 kg (typical of a moderate size mammals such as a raccoon), and 70 kg (typical of a moderate sized deer). For birds, the body weights are taken as 0.01 kg (typical of a very small bird), 1 kg (typical of a small game bird), and 4 kg (typical of a larger bird such as a Canada goose). This elaboration is made only for this extreme accidental exposure because the risk characterization varies with the size of the bird or mammal, as detailed further in Section 4.4.2.1.

4.2.2.2. Plausible Accidental Exposures

While the accidental exposures of mammals or birds to field solutions of aqueous chlorine are highly unlikely, other accidental exposures are plausible. As in the human health risk assessment (Section 3.2.3.2), a more plausible accidental exposure scenario involves the accidental spill of water used for fire suppression – i.e., 50 ppm which is equivalent to 50 mg/L) into a small pond. These exposure scenarios are given in Worksheet F02a for a small mammal and F02b for a small bird.

As in the human health risk assessment, the amount of water that is dropped into the pond is taken as 700 (75 to 3000) gallons and this scenario uses the standard small pond from Forest Service risk assessments – i.e., a pond that is 1000 m^2 in surface area (about a quarter of an acre) and one meter deep. The amount of water consumed by a small mammal and a small bird are estimated from allometric relationships defined by U.S. EPA/ORD (1993). As with the more extreme accidental exposure scenario discussed in the previous subsection, aqueous chlorine is likely to degrade rapidly in surface water. This degradation is not considered because the levels of exposure are far below the level of concern.

4.2.3. Other Terrestrial Organisms

No quantitative exposure assessments are conducted for terrestrial invertebrates, plants, or microorganisms. Incidental exposures to these organisms are plausible but, as discussed in the hazard identification, no meaningful information is available for proposing dose-response assessments. When aqueous chlorine is used for dust abatement, soil invertebrates and soil microorganisms will be exposed to aqueous chlorine concentrations of 50 ppm, at least on and immediately below the surface of treated dirt. While adverse effects to soil invertebrates are plausible and adverse effects to soil microorganisms are virtually certain, the risks cannot be meaningfully expressed quantitatively. These risk, however, are addressed qualitatively in the risk characterization (Section 4.4).

4.2.5. Aquatic Organisms

The exposure assessment for aquatic organisms is similar to that for drinking water exposures for birds and mammals. The general exposure scenario involves the accidental release of a 50 ppm solution of aqueous chlorine into a pond or lake from different types of aircraft. As in the exposure assessment for birds and mammals (Section 4.2.2.2) as well as the human health risk assessment (Section 3.2.3.2), the amount of water that is dropped into the water body is taken as 700 (75 to 3000) gallons.

For birds and mammals, the only exposure scenario considered is the release of a plane load of 50 ppm aqueous chlorine into a small pond with a surface area of 1000 m^2 (about 0.25 acres) and a depth of 1 meter (Section 4.2.2.2). This small pond is identical to that used in accidental spill scenarios in all Forest Service risk assessments. This is a very conservative exposure scenario because the low volume of the pond reduces the extent to which the pesticide is diluted. As discussed further in Section 4.4.2 (Risk Characterization for Terrestrial Organisms), this exposure scenario leads to hazard quotients that are far below the level of concern for both mammals and birds and the exposure scenario is not further elaborated.

This is not the case for aquatic organisms. As discussed in the hazard identification for aquatic organisms (Section 4.1.3) and detailed further in the dose-response assessment for aquatic organisms (Section 4.3.3), many aquatic organisms are very sensitive to aqueous chlorine and will be adversely affected by concentrations in surface water that are likely to be harmless to birds and mammals. Consequently, the exposure assessment for aquatic organisms is elaborated to include not only the small pond but also a larger pond and a small lake. The large pond is characterized as a pond that is about 1 acre in surface area (4,000 m^2) and two meters deep. This pond is about the same the size as the pond used in most Forest Service risk assessments for Gleams-Driver modeling as well as the size of the standard farm pond used in many EPA risk assessments (SERA 2007b). No standard convention for a small lake is used regularly in Forest Service or EPA risk assessments. For this risk assessment on aqueous chlorine, a small lake is defined as a lake with a surface area of about 10 acres (40,000 m^2) and an average depth of 5 meters. The calculations of the concentrations of aqueous chlorine in each of these three water bodies are given in Worksheet G03. This worksheet also gives the resulting hazard quotients for different groups of aquatic organisms, as discussed further in Section 4.4.3.

As discussed in Section 4.1.3 (Hazard Identification for Aquatic Organisms) and detailed further in Section 4.3.3 (Dose-Response Assessments for Aquatic Organisms), some aquatic organisms are highly sensitive to aqueous chlorine and data are available indicating that even very short-term exposures can lead to lethality. In the case of an accidental spill into a small pond, some time will be necessary before complete mixing occurs. The concentrations of aqueous chlorine will initially be very high in some parts of the pond – i.e., the area where the spill occurred – and much lower in other parts of the pond. The kinetics of this process will be highly variable and cannot be well-characterized. This uncertainty is addressed qualitatively in the risk characterization (Section 4.4.3).

4.3. DOSE-RESPONSE ASSESSMENT

4.3.1. Overview

A summary of the dose-response assessment for nontarget species is given in Table 10. Because no long-term exposures to aqueous chlorine are plausible given the uses of bleach in Forest Service programs, only acute toxicity values are proposed. The toxicity values for mammals and birds are similar: 25 mg/kg bw for mammals and 19 mg/kg bw for birds. Both of these values are NOECs from subacute studies in which aqueous chlorine was administered in drinking water. Thus, these NOEC values are likely to over-estimate risks to both mammals and birds from the very short-term exposures that are plausible in Forest Service programs. No quantitative dose-response assessments are proposed for soil invertebrates, other terrestrial invertebrates, or terrestrial plants. The U.S. EPA does not require data for these groups of organisms and appropriate studies on which to base a dose-response assessment for these groups of organisms has not been located in the open literature. Thus, risks to terrestrial invertebrates and terrestrial plants are considered qualitatively in the risk characterization.

Aqueous chlorine is very toxic to aquatic organisms. Separate toxicity values are derived for sensitive and tolerant fish, aquatic invertebrates, aquatic microorganisms, and algae. For aquatic macrophytes, data are available on only a single species. For all groups of aquatic organisms, the types of available toxicity data do not correspond well to the anticipated types of exposure – i.e., accidental spills in which concentrations of aqueous chlorine may be very high initially and then rapidly diminish with both dilution and dissipation. Following general practices in Forest Service risk assessments, a generally conservative approach is taken in which preference is given to flow-through toxicity studies conducted over period of 1 to 4 days. As with the toxicity studies in birds and mammals, the toxicity values selected for groups of aquatic organisms are likely to overestimate risks. The most sensitive groups of aquatic organisms appear to be small invertebrates (lowest NOEC of 0.0015 mg/L) and microorganisms (lowest NOEC of 0.006 mg/L). Sensitive species of fish and algae have NOEC values 0.02 mg/L and 0.035 mg/L, respectively. The NOEC for macrophytes is 0.02 mg/L. While it is not clear that this NOEC would apply to sensitive or tolerant species, the macrophyte NOEC is identical to the NOEC for sensitive species of fish and very close to the NOEC for sensitive species of algae.

4.3.2. Terrestrial Organisms

4.3.2.1. Mammals

As discussed in Section 3.3.2, the U.S. EPA bases the 1-day and 10-day health advisory for aqueous chlorine on the subchronic study by Blabaum and Nicholas (1956) in which no adverse effects were noted in mice over a 33 day exposure period to aqueous chlorine at a concentration of 200 mg/L. The estimated NOAEL from this study is 25 mg/kg bw/day. In the absence of a suitable shorter-term study, the NOAEL of 25 mg/kg bw/day is used in the current Forest Service risk assessment to characterize risks associated with short-term exposures in mammalian wildlife.

As discussed further in Section 4.4.2.1, all of the plausible exposure scenarios for mammalian wildlife are far below the NOAEL. The scenarios for the consumption of unattended solutions of relatively concentrated aqueous chlorine – i.e., concentrations of up to10,000 ppm – do lead to substantial excursions above the NOAEL. The consequences of these exposures are characterized using the approximate lethal doses in experimental mammals. As discussed in Section 3.1.4, these lethal doses are in the range of about 225 to 625 mg/kg bw.

4.3.2.2. Birds

As discussed in Section 4.1.2.2, the toxicity of aqueous chlorine to birds is not characterized as well as in mammals. The U.S. EPA/OPP (1999a) uses an acute oral LD_{50} of 567 mg/kg bw for lithium hypochlorite to characterize acute toxicity to birds (Piccavillo 1977a). The Forest Service, however, prefers to use NOEC values rather than LD_{50} values for risk characterization whenever possible.

The study by Hulan and Proudfoot (1982) involves the administration of sodium hypochlorite as an aqueous solution to birds. This study is similar to the study by Blabaum and Nicholas (1956), used in the dose-response assessment for mammalian wildlife, in that it defines a subchronic NOAEL for exposure to an aqueous solution of sodium hypochlorite. As detailed in Section 4.1.2.2, the study consisted of exposing broiler chickens to dilutions of a commercial bleach formulation at available chlorine concentrations of 0 (control), 37.5, 75, 150, 300, 600, and 1200 ppm for up to 28 days. The concentration of 300 ppm is a NOAEL. The next higher concentration, 600 ppm is a LOAEL based on decreases in heart and testes weight. The highest concentration, 1200 ppm, is a frank effect level based on a significant increase in mortality.

Hulan and Proudfoot (1982, Table 2, p. 1805) provide data on both body weight and water consumption. These data are summarized in a custom worksheet, Worksheet H01, in the EXCEL workbook that accompanies this risk assessment. This worksheet is provided solely to document the conversion of aqueous chlorine concentrations in water to dose estimates in units of mg/kg bw/day. The body weight data given in Table 2 of Hulan and Proudfoot (1982) are relatively simple. The body weights are given for days 7, 14, 21, and 28 of the study in units of grams. In Worksheet H01, these data are converted to units of kilograms. The nature of the water consumption is not clearly specified in the Hulan and Proudfoot (1982) paper. They indicate that … *each treatment was offered to 3 replicate units (pens) of 20 male and 20 female chicks*. Thus, each of the doses involved a total of 120 animals – i.e., 60 males and 60 females. The water consumption is expressed as weekly consumption in liters over the four week duration of the study. Based on the reported body weights and water consumption, the 300 ppm NOEC corresponds to a daily dose of about 19 mg/kg/day bw, very close to the mammalian NOEC of 25 mg/kg bw/day from the subchronic study in mice by Blabaum and Nicholas (1956). The LOEC of 600 ppm from the study by Hulan and Proudfoot (1982) corresponds to a daily dose of about 34 mg/kg bw. The frank effect level (i.e., mortality) of 1200 ppm corresponds to a daily dose of about 94 mg/kg bw/day.

The NOEC of 19 mg/kg bw is used to derive HQ values in the risk characterization for birds. The LOEC of 34 mg/kg bw and the frank effect level of 94 mg/kg bw/day are used to assess the consequences of exposures that exceed the LOC, as discussed further in Section 4.4.2.2.

4.3.2.3. Other Terrestrial Organisms

As discussed in the hazard identification, no data are available on the toxicity of aqueous chlorine to soil invertebrates (Section 4.1.2.4) or terrestrial plants (Section 4.1.2.4) and dose-response assessments for these groups of organisms cannot be proposed.

Data are available on microorganisms and these data indicate that adverse effects on microorganisms may be expected in laboratory cultures of microorganisms at chlorine concentrations as low as 0.17 mg/L – i.e., *Candida albicans*, a yeast, from the study by Wang et al. (2007). As noted in Section 4.2.3, concentrations of 50 ppm aqueous chlorine are used for dust suppression and exposures to terrestrial microorganisms associated with this use are virtually certain. It is far less certain, however, that the available laboratory studies on the toxicity of aqueous chlorine to microorganisms can be used to derive risk quotients. When aqueous chlorine is applied to a dirt surface, it is likely that hypochlorous acid and the hypochlorite ion will react rapidly with soil constituents, including microorganisms. No toxicity studies on the impact of aqueous chlorine on microorganisms in a soil matrix have been encountered. Thus, no formal dose-response relationship for soil microorganisms is proposed and risks to soil microorganisms are considered qualitatively (Section 4.4.2.5).

4.3.3. Aquatic Organisms

4.3.3.1. Fish

The selection of toxicity values for fish is somewhat atypical in that the types of toxicity studies that are available do not reflect the types of exposures that are likely to occur. As discussed in Section 4.1.3.1 (hazard identification for fish), many different types of toxicity studies are available in fish and the differences generally reflect the various types of exposures of concern to specific investigators – e.g., waste water or drinking water treatment or antifouling applications in power plants.

Studies addressing accidental spills of chlorine into a small pond or lake – i.e., the exposure scenario of concern in the current Forest Service risk assessment – have not been conducted. Static toxicity studies are similar to the type of accidental spill scenarios that are considered in the current Forest Service risk assessment in that the concentrations immediately after a spill will be relatively high but then will diminish rapidly due to dissipation and degradation of aqueous chlorine. As discussed in Section 4.1.3.1 and summarized in Appendix 1, several static toxicity studies report LC_{50} values in excess of 0.5 mg/L and up to about 50 mg/L (e.g., Curtis et al. 1979; de Paiva Magalhas et al. 2007; Ewell et al. 1986). In contrast, more standard flow-through bioassays, in which the concentrations of aqueous chlorine are maintained at reasonably constant levels over the course of the study, lead to LC_{50} values in the range of about 0.04 mg/L to 0.7 mg/L.

The current Forest Service risk assessment, however, will adopt the general approach of U.S. EPA/OW (1984) and select toxicity values based on the lower flow-through bioassays in fish. This approach is taken for two reasons. Firstly, Forest Service risk assessments will generally defer to the U.S. EPA in the selection of the most sensitive toxicity studies. Exceptions to this approach involve the identification of toxicity studies that are more conservative than those used by the U.S. EPA. In the case of aqueous chlorine, the U.S. EPA/OW (1984) considered and used the most conservative toxicity studies and this approach has been endorsed in the more recent analysis by U.S. EPA/OPP (1999a).

Another reason for using the more conservative flow-through studies involves the nature of static bioassays relative to anticipated exposures. While static bioassays are generally designed in a manner so that the fish loading of the bioassay tank will not interfere with fish survival, the density of fish in a static bioassay will be much higher than the density of fish in natural waters. For some highly toxic compounds, this may not substantially impact the results of the bioassay. For aqueous chlorine, however, the presence of the test fish in the bioassay water will likely lead to a more rapid dissipation of aqueous chlorine than would likely occur in natural waters. Thus, while a static bioassay may intuitively seem to be more relevant to a spill scenario, static bioassays may underestimate the risks to fish after an accidental spill.

The focus of U.S. EPA/OW (1984) is on deriving national water quality criteria. Thus, U.S. EPA/OW (1984) does not derive separate criteria for different groups of organisms. Instead, the Agency proposed a 4-day average criterion of 0.011 mg/L and a 1-hour average criterion of 0.019 mg/L. While not explicitly discussed in U.S. EPA/OW (1984), the criteria appear to be greatly influenced by the somewhat lower toxicity values for aquatic invertebrates relative to fish. Chronic toxicity values for invertebrates are discussed further in the following subsection (Section 4.3.3.2).

As discussed by U.S. EPA/OPP (1999a), comparable or much lower toxicity values can be derived following the standard approach used by the U.S. EPA/OPP. This approach involves multiplying the LC_{50} by various factors based on differing levels of concern – i.e., 0.5 for acute toxicity and 0.05 for threatened and endangered species. For example, U.S. EPA/OPP (1999a) derives concentrations of concern for fish based on an acute LC_{50} of 0.045 mg/L: 0.023 mg/L for acute toxicity [0.045 mg/L x 0.5] and 0.0023 mg/L for threatened and endangered species [0.045 mg/L x 0.05]. The 0.023 mg/L is very close to the 0.019 mg/L value from U.S. EPA/OW (1984) but 0.0023 mg/L value for threatened and endangered species is much lower than any value recommended by U.S. EPA/OW (1984).

Unlike the approach and intent of U.S. EPA/OW (1984), Forest Service risk assessments attempt to derive separate toxicity values for different groups of organism and also attempt to derive separate toxicity values for both sensitive and tolerant species within each group. Thus, while the current Forest Service risk assessment will defer to U.S. EPA/OW (1984) in the selection of flow-through studies rather than static bioassays, the selection of toxicity values for fish is more closely related to the approach used in U.S. EPA/OPP (1999a).

As discussed in Section 4.1.3.1, the 0.045 mg/L LC_{50} used by U.S. EPA/OPP (1999a) appears to be referenced to the bioassay of pugnose shiners by Ward and DeGraeve (1980). For the current risk assessment, however, a somewhat lower LC_{50} of 0.04 mg/L in rainbow trout fry by Wolf et al. (1975) is used for the most sensitive species. For tolerant species, the LC_{50} of 0.71 mg/L from Esvelt et al. (1971) is used. Generally, LC_{50} values are not used directly in Forest Service risk assessments. In the absence of a NOEC value, an LC_{50} value in fish is multiplied by 0.05, which is based on the approach used by U.S. EPA/OPP for threatened and endangered species. For aqueous chlorine, however, the concentration-response data clearly suggest that a value of 0.5, the factor used by U.S. EPA/OPP for acute toxicity, will be sufficient to estimate a dose that is not likely to be lethal in fish. Thus, the toxicity values used in the current Forest Service risk assessment for fish are 0.02 mg/L [0.04 mg/L x 0.5] for sensitive species and 0.035 mg/L [0.7 mg/L x 0.5] for tolerant species. The potential impacts on endangered and threatened species are discussed qualitatively in the risk characterization (Section 4.4.3.1).

4.3.3.2. Aquatic Invertebrates

The toxicity data and the types of studies that are available on aquatic invertebrates are generally similar to the data and studies on fish. Consequently, the general approach taken to the dose response assessment on aquatic invertebrates is similar to that taken for fish – i.e., the toxicity values are based on flow-through rather than static studies.

Unlike the case with fish, however, the toxicity values used in the current Forest Service risk assessment are not identical to those used by the U.S. EPA/OW (1984) or U.S. EPA/OPP (1999a). The most sensitive invertebrate toxicity value cited in U.S. EPA/OW (1984) or U.S. EPA/OPP (1999a) is the LC_{50} of 0.017 mg/L in *Daphnia magna*. As discussed in Section 4.1.3.3, Taylor (1993) has reported flow-through LC_{50} values as low as 0.005 mg/L in a smaller daphnid species, *Ceriodaphnia dubia*. Taylor (1993) also reports an NOEC for mortality in the flow-through bioassay of 0.0015 mg/L and this NOEC value is used to characterize risks in sensitive species of aquatic invertebrates. This toxicity value is substantially lower than the 4-day average criterion of 0.011 mg/L and the 1-hour average criterion of 0.019 mg/L derived by U.S. EPA/OW (1984).

For tolerant species of invertebrates, the highest toxicity value from U.S. EPA/OW (1984) is 0.96 mg/L, an LC_{50} value for *Orconectes nais*, a species of crayfish. As discussed in Section 4.1.3.3, more recent flow-through bioassays (Cameron et al. 1989; Klerks and Fraleigh 1991) suggest that bivalves may be somewhat less sensitive than crayfish. These toxicity values, however, are expressed as times to 50% mortality – i.e., concentration specific LT_{50} values – rather than LC_{50} values. Consequently, the 0.96 mg/L LC_{50} value used by U.S. EPA is also used for tolerant species in the current Forest Service risk assessment.

U.S. EPA/OW (1984) does not report the NOEC associated with the 0.96 mg/L LC_{50} value and the study to which this LC_{50} is attributed has not been published. As discussed in the dose-response assessment for fish (Section 4.3.3.1), the data on fish are consistent in indicating a very steep concentration-response relationship – i.e., a very high slope. As illustrated in the study by Taylor (1993), this pattern is reflected in the more recent literature on aquatic invertebrates.

There is, however, one notable exception. The study by Latimer et al. (1975) notes a very shallow concentration-response relationship in the freshwater copepod, *Cyclops bicuspidatus*. As discussed in greater detail in 4.1.3.3, this study involved a very large number of organisms tested at a relatively large number of concentrations. Thus, the shallow slope is very well-defined. In addition, the organisms were collect from a field population and may reflect a natural sensitivity to aqueous chlorine. Thus, for aquatic invertebrates, the more general approach used in Forest Service risk assessments will be taken and the NOEC will be estimated as a factor of 0.05 of the LC_{50}. Thus, the toxicity value used for tolerant species of aquatic invertebrates is taken as 0.048 mg/L [0.05 x 0.96 mg/L]. This is an admittedly conservative approach and may overestimate risk substantially, as discussed further in the risk characterization (Section 4.4.3.2).

4.3.3.3. Aquatic Plants

Although chlorine is registered as an algicide, the U.S. EPA does not require toxicity data on nontarget plants for microbicides. Thus, standard toxicity studies in algae and macrophytes, that are typically required for the registration of pesticides, have not been submitted to the U.S. EPA and the Agency has not designated toxicity values for aquatic plants in the RED for chlorine (U.S. EPA/OPP 1999a). As discussed in Section 4.3.3.1 (the dose-response assessment for fish), U.S. EPA/OW (1984) has derived national water quality criteria. While U.S. EPA/OW (1984) does discuss studies on the toxicity of aqueous chlorine to algae, the national water quality criteria – i.e., a 4-day average criterion of 0.011 mg/L and a 1-hour average criterion of 0.019 mg/L – appear to be based largely on toxicity data in aquatic invertebrates.

While no toxicity values for aquatic plants are derived by U.S. EPA/OPP (1999a), the Agency does note that effective algicidal concentrations of aqueous chlorine are in the range of 0.6 mg/L to 1 mg/L. As summarized in Appendix 3, these concentrations are toxic to most species of algae and are associated with adverse effects such as damage to chlorophyll-a, decreases in primary productivity, and loss of cellular integrity. While some longer-term studies indicate LOEC values as low as about 0.002 mg/L (Pratt et al. 1988), longer-term exposures are not anticipated given the uses of aqueous chlorine in Forest Service programs.

Based on short-term (<1 day) toxicity studies, the most sensitive species of algae appears to be *Aphanizomenon flos-aquae*, a blue-green algae, with an NOEC of 0.035 mg/L (Peterson et al. 1995). A much higher NOEC of 1 mg/L – i.e., the recommended algicidal concentration for aqueous chlorine – has been noted in a species of *Cladophora*, a genus of filamentous algae. No other toxicity studies in filamentous algae have been encountered and it is not clear if filamentous algae are generally more tolerant to aqueous chlorine than other types of algae. For the current Forest Service risk assessment, the NOEC values of 0.035 mg/L and 1 mg/L are used for sensitive and tolerant species of algae.

Only one study is available on an aquatic macrophyte, the bioassay by Watkins and Hammerschlag (1984) using Eurasian watermilfoil. While Eurasian watermilfoil is generally considered a target species for aquatic herbicides, it must be used as a surrogate for nontarget macrophytes because this is the only species on which data are available. As with fish and aquatic invertebrates, the study by Watkins and Hammerschlag (1984) suggests a substantial

difference in toxicity between continuous and intermittent exposures. For continuous exposures, the 96-hour NOEC is 0.02 mg/L with concentration related signs of toxicity at concentrations of 0.05 mg/L and higher. In short-term (2 hour/day) exposures, the NOEC was 0.5 mg/L. As with fish, the 96-hour toxicity is used in the current Forest Service risk assessment to characterize risks to aquatic macrophytes. Whether Eurasian watermilfoil should be regarded as a sensitive or tolerant species of aquatic macrophyte cannot be determined from the available data on macrophytes – i.e., no other species have been tested. The NOEC of 0.02 mg/L is identical to the NOEC for sensitive species of fish and relatively close to the NOEC of 0.035 mg/L for sensitive species of algae (Table 10). Consequently, the 0.02 mg/L NOEC is listed for sensitive species of aquatic macrophytes in Table 10. Uncertainties in this classification are discussed further in the risk characterization (Section 4.4.3.3).

4.3.3.4. Aquatic Microorganisms (other than algae)

As discussed in Section 4.1.3.5 and summarized further in Appendix 4, only four studies are available on the toxicity of aqueous chlorine to aquatic microorganisms. The reported NOEC values range from about 0.002 mg/L (Pratt et al. 1988) to over 1 mg/L (Dickson et al. 1977). The 0.002 mg/L NOEC, however, is from the study by Pratt et al. (1988) and involved a 28 day period of exposure. The very high NOEC values reported by Dickson et al. (1977) appear to have involved very short-term exposures that are not well-defined or documented in the publication.

For sensitive species of aquatic microorganisms, the NOEC is taken as 0.006 mg/L. This is based on the 3-day NOEC of 0.0061 mg/L for protozoan colonization from the study by Pratt et al. (1988) and is supported by the NOEC of 0.0063 for protozoan species richness from the study by Cairns et al. (1990). Although Cairns et al. (1990) noted a 20% decrease in species richness at 0.0063 mg/L, the decrease was not statistically significant. In addition, Cairns et al. (1990) employed a 7-day exposure period. Thus, using 0.006 mg/L as an estimate of the NOEC in sensitive species of aquatic microorganisms for shorter term exposures seems to be sufficiently conservative.

The selection of an NOEC for tolerant species is somewhat problematic. Newmann et al. (1987) notes an NOEC of 0.01 mg/L for reduced microbial populations in artificial streams over a 96-hour exposure period. This concentration, however, is very close to the 0.006 mg/L NOEC values from Pratt et al. (1988) and Cairns et al. (1990). As summarized in Appendix 4, the NOEC of 0.006 mg/L from Pratt et al. (1988) is from the part of the study using indoor mesocosms. Pratt et al. (1988) also conducted outdoor enclosure studies and noted a much higher NOEC of 0.261 mg/L. As noted in Section 4.1.3.5, the major difference between the indoor and outdoor study involved the type of exposures – i.e., a flow-through assay in the indoor study and daily pulse exposures (i.e., static renewal) in the outdoor study. Nonetheless, Pratt et al. (1988, p. 686) also note that the two tests involved the collection of species from somewhat different habitats and that the differences in results of the indoor and outdoor studies could have reflected differences in the species compositions in the two studies. Based on this

supposition, a concentration of 0.26 mg/L will be used as an estimate, albeit tenuous, for the NOEC of tolerant species of aquatic microorganisms.

4.4. RISK CHARACTERIZATION

4.4.1. Overview

The risk characterization for aqueous chlorine in Forest Service programs varies with the types of uses of aqueous chlorine. Most uses of aqueous chlorine involve equipment cleanings. These uses are highly controlled and localized uses and widespread exposures of nontarget organisms to aqueous chlorine are not anticipated. The uses of aqueous chlorine in fire and dust suppression both involve applications that are roughly analogous to broadcast applications. Under normal conditions of use in fire suppression, risks to nontarget organisms from exposure to aqueous chlorine would clearly be subordinate to risks associated with forest fires. The situation is somewhat different with dust suppression. In this type of application, dirt roadways are sprayed with treated water and nontarget organisms on, under, or immediately adjacent to the treated road surface will be exposed.

Under normal conditions in applications for dust suppression, the groups of nontarget organisms that would be exposed to aqueous chlorine at an initial concentration of 50 mg/L include soil invertebrates, soil microorganisms, and terrestrial vegetation on or adjacent to the roadway. Because of limitations in the available toxicity data for these groups of organisms, risks to these groups of organisms cannot be characterized quantitatively. Nonetheless, it seems plausible that spraying a dirt surface with a 50 mg/L solution of aqueous chlorine will result in adverse effects in soil microorganisms. The soil depth that would be impacted would likely depend on the permeability of the soils to water and the amount of water applied per unit area. Any adverse effects on soil microorganisms would be transient and would not be likely to cause detectable secondary effects. The potential for adverse effects on soil invertebrates and terrestrial vegetation seems more remote. While speculative, it seems likely that smaller soil invertebrates would be at greater risk than macroinvertebrates. Risks to surface vegetation cannot be excluded but there is no basis for asserting that adverse effects on surface vegetation would be substantial or even detectable.

Risks from accidental exposure scenarios for mammals, birds, and aquatic organisms are quantified using hazard quotients – i.e., the ratio of exposure to a defined toxicity value such as an NOEC. For mammals and birds, two sets of accidental exposure scenarios are developed. The more extreme scenario involves exposures to solutions of aqueous chlorine over the range of concentrations of aqueous chlorine used in Forest Service programs – i.e., 50 mg/L to over 10,000 mg/L. At the upper bound concentration, accidental exposures result in high hazard quotients that could be associated with mortality. These exposure scenarios are considered highly implausible because they involve exposures to unattended solutions of aqueous chlorine. In Forest Service programs, leaving concentrated solutions of aqueous chlorine unattended and accessible to wildlife would constitute a gross misuse. The less extreme accidental exposure scenarios for birds and mammals involves exposures to aqueous chlorine after the accidental release of treated water intended for fire suppression into a small pond. Hazard quotients for these exposure scenarios are below the level of concern by factors of 125 to 12,500.

Risks to aquatic organisms are modeled using an accidental release scenario similar to that used with mammals. Rather than modeling only a small pond, risks to aquatic organisms are elaborated using a small ¼-acre pond, a larger 1-acre pond, and a small 10-acre lake. Most groups of aquatic organisms would be at risk from accidental releases of aqueous chlorine into a small pond. The only exception appears to be tolerant species of algae. The highest hazard quotients are for sensitive species of aquatic invertebrates – i.e., HQs of 88 (9 to 374) – and sensitive species of microorganisms – i.e., HQs of 22 (2 to 94). For a large pond, accidental releases result in upper bound HQ values that exceed the level of concern for all sensitive subgroups – i.e., HQs ranging from 2 to 47. The central estimates of the HQ value for a large pond exceed the level of concern only for sensitive aquatic invertebrates (HQ=11) and sensitive microorganisms (HQ=3). The HQ values for accidental releases of aqueous chlorine into a small lake exceed the level of concern only for upper bound exposures to sensitive species of aquatic invertebrates (HQ = 1.9).

While risks to aquatic organisms are quantified, there are substantial uncertainties in the quantitative risk characterization that relate both to the exposure and dose-response assessments. A simple verbal interpretation of risk is that aqueous chlorine is toxic to aquatic organisms. If a solution of aqueous chlorine is accidentally released into surface water, adverse effects are likely, particularly in aquatic invertebrates and microorganisms. As the amount of aqueous chlorine released increases and the size of the body of water decreases, adverse effects and perhaps substantial mortality could occur in all groups of aquatic organisms.

4.4.2. Terrestrial Organisms

4.4.2.1. Mammals

Most uses of aqueous chlorine in Forest Service programs – i.e., equipment cleaning or relatively standard surface cleaning – are not likely to lead to significant exposures to terrestrial mammals. Possible exceptions involve the use of aqueous chlorine solutions for dust and fire suppression. During a forest fire, 50 ppm solutions of aqueous chlorine will be intentionally deposited onto burning vegetation. The assessment of risks to terrestrial mammals from exposure to aqueous chlorine in this situation is not necessary. Nonetheless, two exposure scenarios, both accidental, are considered.

4.4.2.1.1. Extreme Accident Scenarios

An extreme accidental scenario involves a mammal drinking field solutions of aqueous chlorine over the range of concentrations used in Forest Service programs – i.e., a central value of 200 mg/L with a range from 50 ppm to 10,342 ppm (Section 4.2.2.1). The lower bound concentration of 50 ppm involves a scenario in which a small pool of water with 50 ppm aqueous chlorine is created either from dust suppression or the accidental release of water for fire suppression into an area that is not affected by fire. Numeric expressions of risks for this scenario are the lower bound HQ values given in Worksheet F01a. The hazard quotients are 0.3, 0.2, and 0.1 for mammals weighing 0.02 kg, 4 kg, and 70 kg, respectively.

The central and upper bound HQ values are based on very different exposure scenarios in which unattended and more highly concentrated solutions of aqueous chlorine are consumed by mammals over the course of a full day. This exposure scenario is clearly extreme and as well as implausible. This type of exposure scenario is used only to illustrate the consequences of grossly mishandling solutions of aqueous chlorine. As summarized in Worksheet F01a, the HQ values for a 200 ppm solution slightly exceed the level of concern for a small mammal (HQ=1.2) but not for larger mammals. It is not likely that this modest excursion above the NOAEL of 25 mg/kg bw would result in any adverse effects.

The upper bounds of the HQ values for this exposure scenario are based on an aqueous chlorine concentration of 10,342 ppm. As indicated in Table 4, this concentration is used to prevent the spread of amphibian pathogens. The HQ values for this exposure scenario are 61 for a 0.02 kg mammal, 36 for a 4 kg mammal, and 27 for a large mammal. The exposures correspond to doses that range from about 670 mg/kg bw to 1,500 mg/kg bw. While these does are below the reported LD_{50} values for mammals – i.e., 5,000 mg/kg bw to 13,000 mg/kg bw – they are substantially higher than the approximate lethal dose for mammals – i.e., 225 to 675 mg/kg bw (Jakobsson et al. 1991). Thus, it is possible that solutions of aqueous chlorine in the upper bound of concentrations used in Forest Service programs could harm and possibly be fatal to mammals if significant exposures were to occur. Nonetheless, this risk characterization has little practical impact on the use of higher concentrated solutions of aqueous chlorine because these types of exposures would not occur unless the aqueous chlorine solutions are grossly mishandled – i.e., left in the open and accessible to mammalian wildlife.

4.4.2.1.2. Plausible Accident Scenarios

As detailed in Section 4.2.2.2, the plausible accidental exposure scenario for mammals involves the accidental release of a 50 ppm solution of aqueous chlorine by an aircraft into a small pond. This exposure scenario assumes complete mixing (which may not be a conservative assumption) but ignores degradation (which is a very conservative assumption). As summarized in Worksheet F02a, the hazard quotient for this exposure scenario is 0.0008 (0.00008 to 0.003), which are below the level of concern by factors of over 300 to 12,500.

4.4.2.2. Birds

The risk characterization for birds is essentially identical to that for mammals. The extreme accidental exposure scenario for birds is summarized in Worksheet F01b. For the 50 ppm solution of aqueous chlorine – i.e., an extreme but still plausible exposure scenario – the HQ values of 0.7 for a very small bird, 0.2 for a somewhat larger game bird, and 0.1 for a large bird such as a Canada goose. The scenarios for exposures to higher concentrations of aqueous chlorine do exceed the level of concern for a 200 ppm solution consumed by a small bird (HQ=3). Based on the study by Hulan and Proudfoot (1982), discussed in Section 4.3.2.2, an HQ of 3 might be associated with frank signs of toxicity. For the highest concentrations of aqueous chlorine used in Forest Service programs (10,342 ppm), the HQ values range from 20 to 147 and these exposures would probably results in lethality. As discussed in the risk characterization for mammals (Section 4.4.2.1.1), the exposure scenarios for the direct consumption of solutions of aqueous chlorine greater than 50 ppm are implausible. The risk

quotients for these scenarios are provided only to illustrate the consequences of grossly mishandling solutions of aqueous chlorine.

A much more plausible exposure scenario for birds involves the accidental release of a 50 ppm solution of aqueous chlorine into a small pond. The risks to a small bird for this scenario are summarized in Worksheet F02b. The HQ values for this scenario are 0.002 (0.0002 to 0.008), below the level of concern by factors of 125 to 5,000.

4.4.2.3. Terrestrial Invertebrates

No quantitative risk characterization is given for terrestrial invertebrates. While incidental exposures to flying or tree-dwelling insects might occur, no data are available for assessing the consequences of such exposures. The most plausible exposure scenario would involve 50 ppm solutions of aqueous chlorine during a forest fire. In this circumstance, the effect of treated water used to suppress the fire would be inconsequential relative to the fire itself. Other uses of aqueous chlorine would not generally lead to any significant exposures to flying or tree dwelling insects.

Exposures to soil invertebrates are much more likely to occur, particularly in the use of 50 ppm solutions of aqueous chlorine for dust suppression. Exposures to more concentrated solutions of aqueous chlorine used to clear equipment could also occur if the cleaning solutions were deposited on soil after use. As discussed in Section 4.3.2.3 (Dose-Response Assessment), no toxicity data are available to assess the consequences of such exposures. Given the irritant effects of aqueous chlorine on human skin (Section 3.1.11.1), it seems reasonable to assume that the more concentrations solutions of aqueous chlorine used in some Forest Service applications – e.g., the 10,342 ppm solution used for amphibian pathogens – would adversely impact soil organisms such as earthworms if these organisms were near the soil surface. These effects, however, would be highly localized. In addition, given the rapid degradation of hypochlorous acid and the hypochlorite ion, it is not likely that conditions damaging to soil invertebrates would persist for a prolonged period.

4.4.2.4. Terrestrial Plants

As with terrestrial invertebrates, no quantitative risk characterization is developed for terrestrial plants. For terrestrial plants, the hazard identification (Section 4.1.2.5) does not support a dose-response assessment because an adverse endpoint cannot be identified.

The U.S. EPA does not require nontarget terrestrial plant toxicity studies on microbial disinfectants and no toxicity studies on terrestrial plants have been submitted to or considered by the U.S. EPA/OPP (1999a). This limitation seems sensible, particularly for Forest Service applications such as fire suppression.

As noted in Section 4.1.2.6 (the hazard identification for terrestrial microorganisms) and discussed further in Section 4.4.2.5 (the risk characterization for terrestrial microorganisms), aqueous chlorine is likely to adversely affect some soil microorganisms. The impact of this effect, however, could be either positive (i.e., adverse effects on plant pathogens) or negative

(i.e., an adverse effect on beneficial microorganisms). These types of secondary effects on plants would be most relevant to the application of 50 ppm solutions for dust suppression. Since these applications are made primarily on roadways, it is not clear that any detrimental effects on surface vegetation would be considered adverse.

While speculative, it seems likely that spilling a concentrated solution of sodium hypochlorite on to a plant could damage plant tissue directly. These types of events would be unusual and any effects would likely be highly localized.

4.4.2.5. Soil Microorganisms

Aqueous chlorine is an effective microbicide and the microbicidal properties of aqueous chlorine are the basis for the use of this agent by the Forest Service. While a large number of efficacy studies of aqueous chlorine has been conducted (e.g., Maillard et al. 1998), no studies on the impact of soil applications of aqueous chlorine to soil microorganisms have been encountered in the literature. As discussed in Section 4.3.2.3, however, bioassays on microorganisms in cell culture indicate adverse effects at concentrations as low as 0.17 mg/L. It would not be appropriate to use cell culture bioassays to derive HQ values for soil applications of aqueous chlorine because it seems likely that aqueous chlorine would rapidly react not only with soil microorganisms but also with other soil constituents. Nonetheless, it seems self-evident that concentrations of aqueous chlorine over the range of concentrations used in Forest Service programs – i.e., 50 ppm to over 10,000 ppm – would cause mortality in at least some soil microorganisms. The soil depth that would be impacted would likely depend on the permeability of the soils to water. It is not likely that aqueous chlorine would penetrate deeply into the soil column in hard packed clay. Conversely, deeper penetration into the soil column is plausible in sandy soils.

The consequences of reducing populations of soil microorganisms in soil may include a decrease in plant productivity (e.g., Rodríguez-Echeverría and Pérez-Fernández 2005). For partially sterilized soils, however, increases in plant productivity have been noted and this increase may be associated with adverse effects of soil sterilization on microbial plant pathogens (Marschner and Rumberger 2004).

For intentional applications of sodium hypochlorite to soil in either dust abatement or fire fighting, the potential for adverse effects on plant productivity does not appear to be a serious concern. The impact of forest fires on microbial populations is in itself severe (e.g., Guerrero et al. 2005) and the benefits of reducing the spread of a forest fire will clearly outweigh any ancillary damage to soil microorganisms from sodium hypochlorite. For dust abatement on dirt roads, a decrease in soil productivity does not appear to be major concern because plant productivity on dirt roads in forests is not a desirable outcome. In addition, any effect of sodium hypochlorite on soil microorganisms is likely to be transient. In other words, microorganisms from untreated areas will recolonize treated areas and reduced but surviving populations of soil microorganisms in treated areas will recover.

The rates of repopulation or recolonization by soil microorganisms are likely to be highly variable depending on site-specific conditions. The mechanisms of microbial recolonization may involve the passive growth of microorganisms, active movement of microorganisms, or transport of microorganisms by soil water flow (Wertz et al. 2007). Additional mechanisms for the recovery of microbial populations in the field may include transport by earthworms or macroarthropods (Rantalainen et al. 2005; Yeates et al. 1991). Airborne transport of microorganisms will also occur but this mechanism is probably minor compared to other mechanisms (Rodríguez-Echeverría and Pérez-Fernández 2005).

Passive recolonization involving the movement of microorganisms due to population growth is a very slow process (Rantalainen et al. 2005), on the order of 0.14 mm/day in dry soil (Turnbull et al. 2001). In soil mesocosms consisting of 27 mm diameter disks of sterilized soil surrounded by 2 mm to 4 mm sections of untreated soil, bacterial recolonization of the sterilized soil occurred in about 2 to 8 days. Using larger soil samples – i.e., about 30 cm in diameter and 30 cm deep – sterilized with methyl bromide, much longer periods are required for recovery – i.e., several months – with bacterial recovery being more rapid than the recovery of nematodes (Yeates et al. 1991). Forest fires will also impact microbial populations in soil.

Recovery periods following forest fires may not reflect recovery periods associated with the chemical sterilization of soil because fires will alter the availability of soil nutrients differently from chemical sterilization. For example, microbial populations may increase shortly after forest fires due to an increase in soil nutrients (Vazquez et al. 1993). While soil microorganism recovery from wild fires may be different from recoveries from chemical sterilization, very long recovery periods have also been noted in forest soils after forest fires, with complete recovery to pre-fire microbial soil structure not observed even after one year (Acea and Carballas 1996; Vazquez et al. 1993). These studies, however, involve soils collected from the field and observed in the laboratory. Thus, rates of recolonization facilitated by larger organisms such as worms and macroarthropods are not encompassed by these studies and it is likely that field recovery rates of soil microorganisms would proceed more rapidly.

Although the use of 50 ppm aqueous chlorine in dust suppression will clearly have an impact on soil microorganisms, these impacts take place in a dirt road surface system. The mere practice of installing a road over a previously existing ecosystem will have altered the soil environment probably to a greater degree than the impact of the aqueous chlorine.

4.4.3. Aquatic Organisms

The risk characterization for all aquatic species is highly uncertain because of both the exposure assumptions and dose-response assessments. While these uncertainties are discussed in both the exposure assessment (Section 4.2.5) and dose-response assessment (Section 4.3.3) for aquatic species, further emphasis of these uncertainties is warranted because of the impact that these uncertainties have on the risk characterization.

As discussed in Section 4.2.5, the only plausible exposure scenario for aquatic species involves the accidental release of water treated with aqueous chlorine and used for fire suppression into

surface water. There are some obvious and substantial uncertainties associated with this scenario, specifically the amount of aqueous chlorine that is released and size of the water body into which the release occurs. These uncertainties are encompassed by assuming releases from three different types of aircraft (small, medium, and large) as well as by assuming three different sizes for the water body (a small pond, a large pond, and a small lake). As detailed in Worksheet G03, the resulting concentrations of aqueous chlorine range from about 0.00007 mg/L to 0.56 mg/L – i.e., this variability spans a factor of 8,000. All of these concentrations, however, are based on the assumption of complete and instantaneous mixing. This assumption is simply an approximation. In the event of a spill of a 50 ppm solution of aqueous chlorine into a water body, the area of the spill may have relatively high concentrations that could be substantially in excess of the concentrations based on complete mixing for some period of time. This period, however, cannot be well-defined and would likely vary with site-specific conditions. Thus, the assumption of complete mixing may underestimate risk.

Conversely, aqueous chlorine will not persist in surface water for a prolonged period of time. In this respect, the calculated values given in Worksheet G03 may overestimate risk because degradation is not considered. U.S. EPA/OPP (1999a) cites the half-lives of 1.3 to 5 hours from Jolly (1983). As discussed in Section 2.2.1.2, substantially longer half-lives of up to 6 days have been reported by other investigators (Abdel-Gawad and Bewtra 1988). Again, the rate of degradation of aqueous chlorine in natural waters is likely to vary with both temperature and organic matter. At higher temperature and higher concentrations of organic matter, aqueous chlorine is likely to degrade more quickly than in cooler waters with low levels of organic matter. This type of variability and uncertainty cannot be quantified with any precision.

In an attempt to address the uncertainties in the exposure assessment, conservative assumptions are made in the dose-response assessment (Section 4.3.3). Specifically, all toxicity values used in the dose-response assessment for aquatic species are based on flow-through toxicity values from studies that typically involve periods of exposure that range from one day to four days. In water bodies where aqueous chlorine will be rapidly diluted and/or rapidly degraded, these flow-through toxicity values may overestimate risk by an order of magnitude or more.

4.4.3.1. Fish

The hazard quotients for fish and other groups of aquatic organisms are summarized in Worksheet G03 of the EXCEL workbook that accompanies this risk assessment. For convenience, these hazard quotients are reproduced in Table 11 of this risk assessment.

Within the very substantial uncertainties in the risk characterization for all aquatic organisms, it seems reasonable to assert that the worst-case release of water treated with aqueous chlorine at 50 ppm – i.e., water used for fire suppression – could result in substantial mortality in fish. As discussed in the dose-response relationship for fish, the NOEC values used in this risk assessment are estimated by multiplying the LC_{50} by a factor of 0.5. While this may be viewed as somewhat anti-conservative relative to the more standard approach of using a factor of 0.05, this approximation has no impact on the risk characterization. If a factor of 0.05 had been used, the HQ values would simply be a factor of 10 higher. The basic interpretation, however, would

be the same. If a large aircraft were to release a large amount of treated water into a small pond, the concentration of aqueous chlorine would exceed the LC_{50} for sensitive species of fish by a factor of about 14. In other words, if the fish in the small pond were representative of fish species that are sensitive to aqueous chlorine, substantial fish mortality would be expected. Even for relatively tolerant species of fish, the upper bound HQ of 1.6 is based on a concentration of 0.56 mg/L and an LC_{50} of about 0.7 mg/L. While the concentration-response curve for fish is steep, the estimated exposure would correspond to about 80% of the LC_{50} and some fish mortality would be expected.

As the size of the water body increases, risks to fish diminish. There is no basis for asserting that the accidental release of even a large amount of aqueous chlorine into a small lake would likely lead to detectable mortality in fish. In the case of a large pond, the upper bound HQ is 4 for sensitive species of fish and mortality in sensitive species of fish would be plausible.

As discussed at the start of Section 4.4.3, concentrations of aqueous chlorine in water could be initially much higher than the calculated concentrations based on complete mixing. While this could present an additional hazard, avoidance behavior in fish is well- documented and could reduced the likelihood of adverse effects from high but transient concentrations of aqueous chlorine immediately after a spill.

4.4.3.2. Aquatic Invertebrates

Aquatic invertebrates appear to be generally more sensitive than fish to aqueous chlorine. Notwithstanding the uncertainties in the risk characterization for aquatic species, there seems to be little doubt that the release of a load of aqueous chlorine by any aircraft into a small pond would be associated with substantial mortality in sensitive and tolerant species of aquatic invertebrates. If a load of chlorine is released into a larger – e.g., 1 acre – pond, mortality in sensitive species of aquatic invertebrates is likely. In the event of a release of a large load of aqueous chlorine into a small lake, the upper bound of the hazard quotient is 1.9. This corresponds to a concentration of 0.0028 mg/L, which is about 60% of the lowest reported LC_{50} in *Ceriodaphnia dubia* – i.e., 0.005 mg/L from the study by Taylor (1996). Thus, while some mortality might be expected in very sensitive species of aquatic invertebrates in the event of a large release into a small lake, the mortality might not be substantial. The release of smaller amounts of aqueous chlorine – e.g., from helicopters rather than fixed-wing aircraft – would probably not result in substantial mortality in sensitive species of aquatic invertebrates and would be far below the level of concern for tolerant species.

Unlike fish, most invertebrates will not be able to avoid initial high concentrations of aqueous chlorine by moving rapidly to areas of lower concentration. Nonetheless, as discussed in Section 4.1.3.3, it is likely that many benthic organisms could withdraw into sediment and this behavior could significantly reduce the impact of aqueous chlorine to benthic organisms. In addition, some benthic organisms such as clams or snails may be able to alter behavior to further minimize exposure to transiently high concentrations of aqueous chlorine.

4.4.3.3. Aquatic Plants

For sensitive species, the toxicity value for algae (0.035 mg/L) is similar to the toxicity value for fish (0.02 mg/L) and thus the risk characterization for sensitive species of algae is also similar to that for sensitive species of fish. For macrophytes, only a single toxicity value is available, an NOEC of 0.02 mg/L. Thus, the HQ values for macrophytes are identical to those for fish.

In the worst-case scenario – i.e., a large spill into a small pond – the HQ value is 16. This scenario is associated with a concentration of about 0.56 mg/L. This is very close to the recommended range of algicidal concentrations – i.e., 0.6 to 1 mg/L – and adverse effects on sensitive species of algae would be expected. Based on one study in an apparently tolerant species of filamentous algae, the worst-case concentration of 0.56 mg/L is below the NOEC of 1 mg/L. Thus, even under worst-case conditions, it is possible that some species of tolerant algae would not evidence adverse effects.

Adverse effects on sensitive species of algae diminish under less extreme exposure assumptions – i.e., smaller amounts of treated water being released and/or larger bodies of surface water. For a small pond, the central estimate of the HQ is 4 and this is associated with a release of treated water by a large helicopter. The resulting concentration in water after complete mixing is 0.13 mg/L. Although this concentration is substantially below the effective algicidal concentrations of aqueous chlorine, a decrease in carbon uptake has been noted at concentrations of 0.1 mg/L (Brooks and Liptak 1979) and a decrease in nitrogen fixation has been noted at 0.07 mg/L (Peterson et al. 1995). Thus, the HQ of 4 in sensitive species of algae would likely be associated with functional impairment. Accidental releases of treated water into a larger pond are much less likely to be associated with adverse effects in sensitive species of algae. The upper bound of the estimated concentration is 0.07 mg/L, the LOAEL for decreased nitrogen fixation. Thus, the HQ of 2 for this exposure would be regarded with concern. All accidental releases of aqueous chlorine into a small lake lead to HQ values that are below the level of concern.

As discussed in Section 4.4.3, all of the HQs are based on the assumption of complete mixing. For aquatic plants and particularly for algae, it is likely that some organisms would be exposed to much higher concentrations of aqueous chlorine for at least a short period of time. A concentration of 50 mg/L – i.e., the concentration used to treat water for fire suppression – would not be maintained but concentrations in excess of 1 mg/L might occur in parts of the pond or lake for a sufficient period of time to cause death in aquatic vegetation.

While the extent of damage to aquatic vegetation cannot be well-quantified, adverse effects on aquatic vegetation are plausible in localized areas of any surface water into which aqueous chlorine is released. The duration of adverse effects is likely to be inversely related to the biomass of the aquatic vegetation. Aqueous chlorine is likely to be degraded more rapidly in bodies of water that are rich in aquatic vegetation – i.e., the aqueous chlorine will react with and be consumed by the aquatic vegetation – relative to bodies of water with little vegetation or other organic matter.

4.4.3.4. Aquatic Microorganisms

Aqueous chlorine is an effective microbicide. As is the case with terrestrial microorganisms, effective concentrations of aqueous chlorine will, by definition, kill microorganisms. Unlike the case with terrestrial microorganisms, risks to aquatic microorganisms can be expressed quantitatively. For sensitive species of microorganism, the NOEC is 0.006 mg/L and this NOEC is relatively well-documented in that it is based on two studies – the flow-through toxicity studies by Pratt et al. (1988) and Cairns et al. (1990). The accidental release of aqueous chlorine into a small pond is likely to cause adverse effects on sensitive species of aquatic microorganisms across the range of aircraft capacities considered in this risk assessment. For a larger pond, the concentrations of aqueous chlorine could exceed the level of concern only at the upper bound concentrations – i.e., those associated with the release of aqueous chlorine from a large fixed-wing aircraft. For very large bodies of water, the resulting concentrations of chlorine are below the level of concern even under worst-case conditions.

As discussed in Section 4.3.3.4, the dose-response assessment for tolerant species of microorganisms is 0.26 mg/L from the study by Pratt et al. (1988) in outdoor mesocosms. This toxicity value is atypical of toxicity values used for other aquatic organisms in that the value is based on a static renewal rather than flow-through exposure. Thus, it is not clear that the toxicity value represents a tolerant community of microorganisms or simply the less severe effects that are often noted in aquatic toxicity studies that use static exposure. Within this limitation, the quantitative risk characterization for tolerant species of microorganisms suggests that the level of concern is exceeded only at the upper bound of the exposure scenarios for a small pond (HQ=2).

As with algae, it seems likely that any sized release of aqueous chlorine into any body of surface water could result in short-term and localized concentrations of aqueous chlorine that could rapidly cause mortality in aquatic microorganisms. Also as with algae, it is likely that the duration of this effect would be greatest in surface waters with low biomasses and low concentrations of other organic matter.

5. REFERENCES

NOTE: The initial entry for each reference in braces {} simply specifies how the reference will be cited in the text. The final entry for each reference in brackets [] indicates the source for identifying the reference.

ATSDR	**References identified through the ATSDR (2007) Toxicological Profile [n=72]**
Std	**Standard references used in most Forest Service risk assessments.**
Sec	**Reference taken from secondary source.**
Internet	**Various reports on pesticide.**
ECOTOX	**References identified through a search of EPA's ECOTOX database: http://cfpub.epa.gov/ecotox/ [n=83]**
E-Docket01	**These are from the following E-Docket EPA- EPA-HQ-OPP-2006-0328 [Chlorine Dioxide and Sodium Chlorite]. To get the complete listing of items available, go to http://www.regulations.gov/search/ index.jsp and enter the docket number in the Search box.**
E-Docket02	**These are from the following E-Docket: EPA-HQ-OPP-2005-0507 [Inorganic Chlorates]. See note on E-Docket01 for details.**
SET00	**Papers from previous risk assessments.**
Set01	**Paper from initial TOXLINE search, HHRA not in ATSDR[n=33].**
Set02	**Supplemental search for ERA [n=14].**
Set03	**Initial Tree Search for additional data on fate in water and soil [n=13].**
Set04	**Supplemental search focused primarily on chlorination byproducts [n=16].**
Set05	**Supplemental literature search on soil sterilization.**
Set06	**Tree search of supplemental literature on soil sterilization.**
Set07	**Tree search of mammalian reviews.**
Set08	**Tree search of aquatic reviews. Several citations primarily from the reviews by Vetrano 2001 and U.S. EPA/OW 1984.**
Set09	**Odds and ends during internal QC.**
Supl	**Supplemental papers ordered late during preparation of risk assessment.**
Sundry	**Other sources of information including comments on Program Description.**

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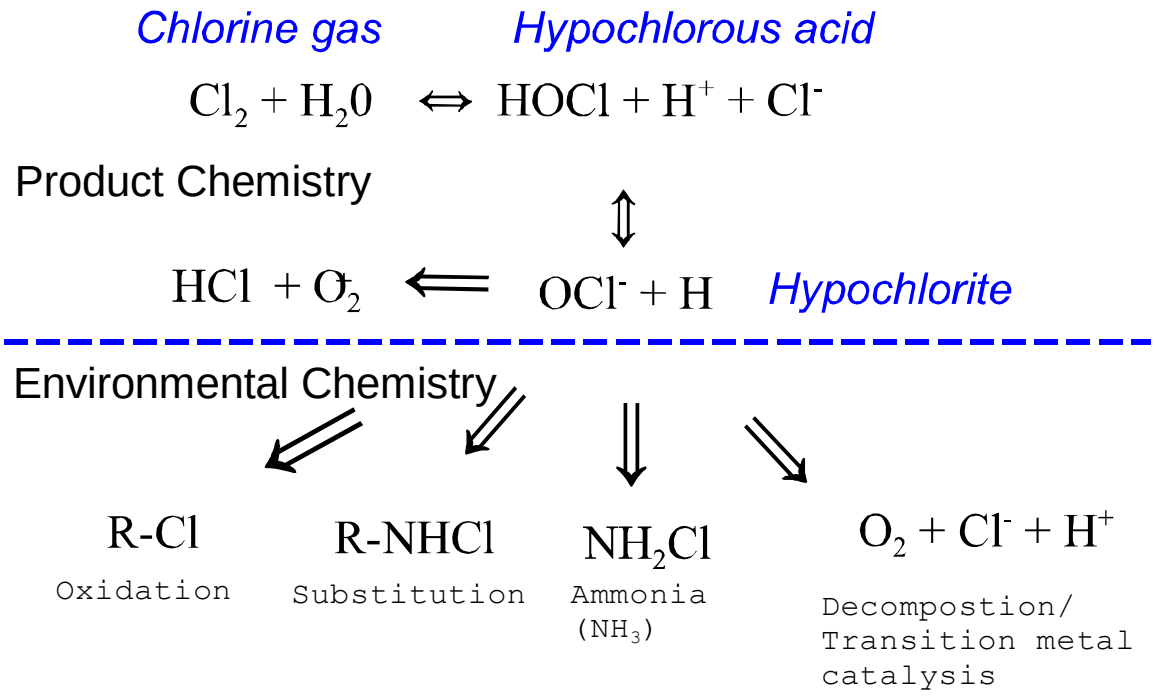

Figure 1: Common reactions of hypochlorite in aqueous solution

Modified from Farr et al. (2003) and Jolly (1987)

Note: For simplicity, the above equations are not balanced.

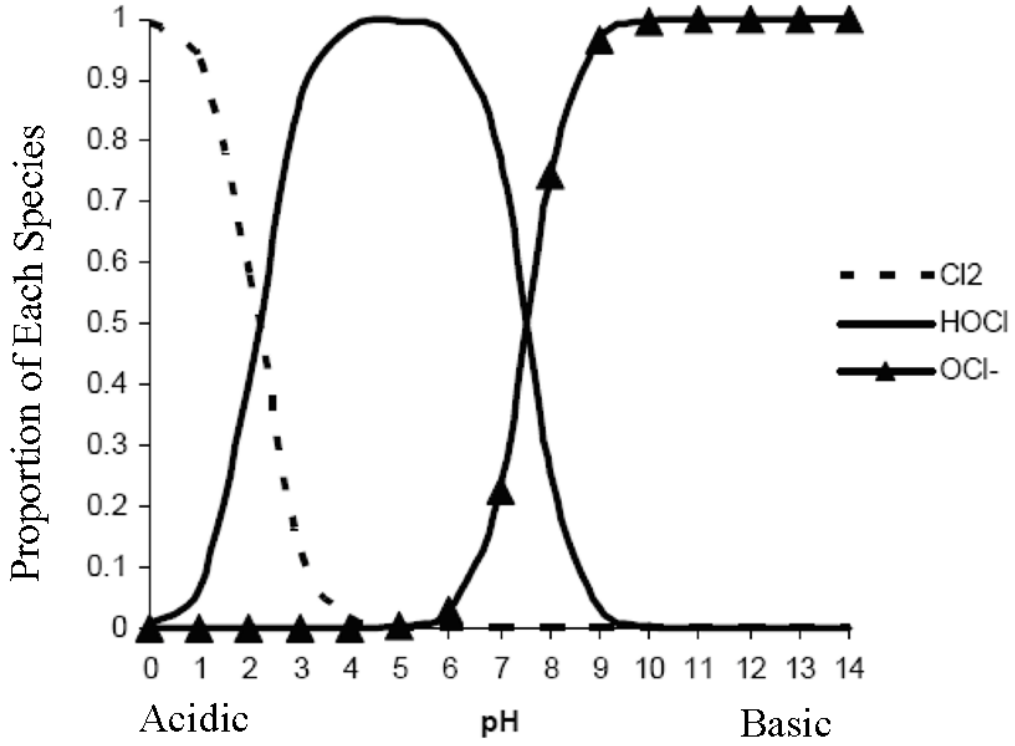

Figure 2: Speciation of Cl_2, HOCl, and OCl^- as a Function of pH

Modified from Figure 4-1 from ATSDR (2007, p. 143)

Table 1: Properties Sodium Hypochlorite and the Hypochlorite Ion

Property	Value	Reference
	Sodium Hypochlorite	
Common Name	Sodium hypochlorite Sodium Chlorate (I)	Budavari 1989
Names for aqueous solutions	Clorox; Eau de Labarraque; Dazzle	Budavari 1989
Molecular Formula	NaClO	Budavari 1989
Molecular weight (g/mole)	74.44	Budavari 1989
% Chlorine	47.62%	Budavari 1989
% Sodium	30.88%	Budavari 1989
CAS Number	7681-52-9	Budavari 1989; HSDB 1986
pK_a (hypochlorous acid)	7.5	Feng et al. 2007
U.S. EPA Docket Number	EPA-HQ-OPP-2006-0328 [Chlorine Dioxide and Sodium Chlorite] EPA-HQ-OPP-2005-0507 [Inorganic Chlorates]	http://www.regulations.gov/search/index.jsp
	Hypochlorite Ion	
Molecular Formula	ClO^-	Budavari 1989
Molecular weight (g/mole)	38.99 [74.44 – 35.45]	Budavari 1989

Sodium hypochlorite is not listed in Tomlin (2004) or USDA/ARS 2006.

Table 2: Some Typical Formulations of Sodium Hypochlorite with Labeled Forestry Uses

Property	Ultra Clorox Brand Regular Bleach	Clorox Commercial Solutions Ultra Clorox Germicidal Bleach
Synonyms		CPPC Ultra Bleach 2
EPA Registration No. [a]	5813-50	67619-8
Manufacturer	The Clorox Company	The Clorox Company
Active Ingredient	Sodium Hypochlorite	Sodium Hypochlorite
Percent Active Ingredient (w/w)	6% [MSDS indicates 6-7.35 %]	6% [MSDS indicates 6.15 %]
Percent Available Chlorine	5.71%	5.84% [58,425 ppm]
Application Rates	See Table 4 (Uses)	See Table 4 (Uses)
Application Volume	See Table 4 (Uses)	See Table 4 (Uses)
Other ingredients in formulation (Inerts)	94.75% [NOS] <1% Sodium hydroxide [CAS No. 1310-73-2]	93.55% [NOS] <1% Sodium hydroxide [CAS No. 1310-73-2]
pH	≈11.4	11 to 12
Specific gravity ($H_20 = 1$)	1.1 at 70 °F	1.1
Most Recent Label	January 15, 2009	December 22, 2008
Labeled Uses for Forest Pathogens [b]	*Phytophthora ramorum, Phytophthora lateralis*	*Phytophthora ramorum, Phytophthora lateralis*

[a] Information taken from most recent U.S. EPA/OPP labels from http://www.epa.gov/pesticides/pestlabels/ based on EPA Registration Numbers as well as the MSDS for the formulations from the Clorox Company. All alkaline formulations of sodium hypochlorite are covered in this risk assessment. See Section 2.2.2.

[b] See Section 2.3 for a discussion of the specific uses in Forest Service programs for the control of these and other pathogens of concern the Forest Service.

Table 3: Other Commercial Formulations of Sodium Hypochlorite

Product Name	Date of Most Recent EPA Label	Pesticide Registration Number	% Sodium Hypochlorite (w/w)	Other
Clorox Bleach	May, 2008	5813-1	5.25%	No phosphorous
Fresh Scent Clorox Bleach	August, 2006	5813-20	5.25%	No phosphorous
Tackle	May, 2008	5813-21	1.84%	No phosphorous
Tilex	July, 2008	5813-24	2.4%	No phosphorous
Strike Cleanser	December, 2007	5813-23	0.45%	No phosphorous

All data taken from U.S. EPA, Pesticide Product Label System at http://oaspub.epa.gov/pestlabl/ppls.home

Table 4: Labeled Uses and Mixing Instructions for Clorox Formulations

Sorted in increasing concentration of chlorine. Direct forestry uses are shaded.

All information from product labels in Table 2

Disinfectant Uses	Mixing Instructions	Available Chlorine (ppm)	Other Information
Emergency connections for drinking water systems		0.1 – 0.2	These residual levels must be maintained.
Supplementary water supplies		0.2	20 minutes
Swimming pools	1 pint per 6000 gallons	1	Maintain 0.6 to 1 ppm chlorine
Fruit and vegetable washing		25	2 minutes in wash tank. Rinse with potable water.
Fire suppression, dust suppression and other water disinfection to prevent spread of Port Orford Cedar root disease (*Phytophthora lateralis*)	1 gallon product to 1000 gallons of water	50	Let stand for 5 minutes prior to use of water.
Fire suppression, dust suppression and other water disinfection to prevent Sudden Oak Death (*Phytophthora ramorum*)	1 gallon of CPPC Ultra Bleach (5.84% available chlorine) brought to a volume of 1000 gallons with water.	50	Let stand for 5 minutes prior to use of water.
In solution with fire retardants.	Up to 4 gallons of a 5% solution per 1000 gallons of fire-retardant solution.	≈200	Information from Betlejewski (2008)
Equipment and utensils		200	Spray, soak, or scrub. At least 1 minute.
Tanks for shipping water		500	5 minutes. Maintain residual of 0.22 ppm after cleaning
Nonporous surfaces		900	Spray. 2 minutes then rinse.
Nonporous surfaces		2700	Let stand for 5 minutes then rinse. Bacteria, fungi, and viruses.
Nonporous surfaces		2700	Spray. 1 minute then rinse.
Loading and hauling equipment		2700	Pressure-spray with 1 oz powdered detergent. Let stand for 5 minutes then rinse.
Citrus canker treatment (*Xanthomonas axonopodis*)	10% dilution of 6% formulation	≈5710	Immerse seeds at 125 degrees °F or higher for 10 minutes.
Plant parasitic nematodes and plant disease-causing fungi quarantine use with tree nursery stock.	Five or six parts water with one part this product (equals approximately 0.85% active ingredient).	8500	Remove soil from roots. Dip roots for 30 to 45 seconds. Rinse with clean water.

Prevent spread of Chytrid fungus (amphibian pathogen)	20% solution (1 Clorox:4 water)	10,342	Immerse for 5 minutes. Air dry.

Table 5: Special Forest Service Uses

Disinfectant Uses	Mixing/Treatment Instructions	Available Chlorine (ppm) Calculation	Other Information
Dreissenid veligers (mussel larvae)	Gear rinsed with 0.5% bleach solution (250 ppm NaClO) Liquid oz Clorox per gallon water = 0.6 ▪Tbsp liquid Clorox per gallon water =1.1 ▪Gallons Clorox per 100 gallons water =0.5	257 51,710 × 0.5 gal ÷ (100 gal + 0.5 gal)	Tait 2008; USDA/FS/R4 2004
Whirling Disease (*Myxobolus cerebralis*, myxosporean parasite of salmonids)	0.9 gallons of 6% Bleach per 100 gallons of water for 10 minutes. 500 ppm NaClO.	465 51,710 × 0.9 ÷ 100	Tait 2008; USDA/FS/R4 2004; Hedrick et al. 2008.
Didymo (*Didymosphenia geminata*, aquatic algae)	For 1 min: 2% bleach solution (800 ppm NaClO) ▪Liquid oz Clorox per gallon water =1.8 ▪Tbsp liquid Clorox per gallon water =3.6 ▪Gallons Clorox per 100 gallons water = 1.4	713 51,710 × 1.4 gal ÷ (100 gal + 1.4 gal)	Tait 2008; USDA/FS/R4 2004
Hantavirus	1 1/2 cups to one gal of water (1:10 solution) to the insides of bunch houses and other work facilities before cleaning.	5,710 51,710 × 0.1	Valle 2008

[a] Calculation of available chlorine assumes a 6% NaClO solution with an available chlorine of 5.17% or 57,100 ppm. See calculations in Section 2.2.2.

Table 6: Dose-Severity Relationships for Skin Irritation in Humans

Exposure [a]	Response	Reference
0.04% to 0.2% x 2 hours [b]	Subclinical damage to skin based on instrumental *in vitro* measures of samples of stratum corneum.	Goffin et al. 1997
0.5% x 48 hours	No irritation in 20 individuals.	Habets et al. 1986
1% x 48 hours	No irritation in 20 individuals.	Habets et al. 1986
1% x 24 hours	No irritation (0/50) with 0.02 mL exposures. Irritation in indicated by reddening of skin with 0.1 mL exposure.	Hostynek et al. 1989
2% x 48 hours	Weak to moderate irritation in 15/69 individuals	Habets et al. 1986
4% x 15 to 90 min.	No signs of irritation after exposure.	Goffin et al. 1997
5.25% x 4 hours	Severe irritation in intact skin of 4 of 7 subjects with serum weeping in 2 of 7 subjects. Less irritation to rabbits and guinea pigs.	Nixon et al. 1975
6% x 20 min	Non-immunologic contact urticaria (hives) in 4 of 10 individuals. No systemic effects.	Hostynek et al. 1989

[a] 1% = 10,000 mg/L or ppm

[b] Dilutions of 1% to 5% of a 4% NaOCl solution.

Table 7: Summary of Exposure Scenarios for human health effects

Scenario	Person	Fire Suppression	Equipment Cleaning	Comment	Worksheet
Workers					
General Exposure	Worker	►	►	Exposure to forestry use-specific concentrations. See Tables 4 and 5.	C01
Accidental Exposures					
Direct Dermal Contact	Worker	►	►	Undiluted formulation.	C01
General Public					
Accidental Acute Exposures					
Water consumption (spill)	Child	■		Accidental release of 50 ppm aqueous chlorine into a small pond	D01
Swimming	Female	►			D02

■ Standard HQ approach for oral exposures.

► Assessment based on dermal no-effect concentration for human dermal exposure.

Table 8: Summary of Dermal Risk Characterization for Workers

Disinfectant Use	Concentration (ppm or mg/L)	Dermal HQ [4]
Labeled Uses [1]		
Fire or dust suppression	50	0.01
Solutions with fire retardants	200	0.04
General utensil cleaning	200	0.04
Tanks for shipping water	500	0.1
Nonporous surfaces, 5 min.	900	0.2
Nonporous surfaces, 2 min. or cleaning loading/hauling equipment	2,700	0.5
Citrus canker treatment	5,710	**1.1**
Parasitic nematodes and fungi on plants	8,500	**1.7**
Chytrid fungus (amphibian pathogen)	10,342	**2.1**
Special Forest Service Uses [2]		
Dreissenid veligers (mussel larvae)	257	0.05
Whirling Disease	465	0.09
Didymo (aquatic algae)	713	0.14
Hantavirus	5,710	**1.1**
Accidental Exposure [3]		
Contact with concentration formulation	58,400	**11.7**

[1] See Table 4.
[2] See Table 5.
[3] See Section 3.2.2.2.
[4] The HQ values are the ratio of the exposure concentration to the dermal NOAEL of 5,000 ppm. See Section 3.3.4 for a discussion of the dermal NOAEL.

Table 9: Exposure Scenarios for Ecological Risk Assessment

Scenario	Receptor	Assessment	Comment	Worksheet
Extreme Accidental Acute Exposures				
	Small Mammal	■	Acute exposure to range of concentrations used in Forest Service programs.	F01a
	Small Bird	■		F01b
Non-Accidental Acute Exposures				
Contaminated Water				
	Small Mammal	■	Similar to accidental spill but uses concentration in a small pond. Quantified only for fire suppression.	F02a
	Small Bird	■		F02b
	Aquatics	■	Accidental spills into a small pond, large pond and small lake. Quantified only for fire suppression.	G03
Contaminated Soil				
	Plants	►	Exposures are likely but no dose-response assessment is proposed. Risks are addressed qualitatively	N/A
	Invertebrates	►		N/A

■ Standard HQ approach

► Qualitative assessment. No worksheet.

Table 10: Summary of Toxicity Values for the Ecological Risk Assessment

Group		Endpoint	Toxicity Value	Source
Terrestrial Animals				
	Mammals	NOAEL, gross pathology	25 mg/kg bw	Section 4.3.2.1
	Birds	NOAEL, organ weights	19 mg/kg bw	Section 4.3.2.2
	Soil Invertebrates	N/A		Section 4.3.2.3
	Other Invertebrates	N/A		Section 4.3.2.3
Terrestrial Plants				
Soil exposure		N/A		Section 4.3.2.3
Foliar exposure		N/A		Section 4.3.2.3
Aquatic Animals				
Fish	Sensitive	Est. NOEC, lethality[1]	0.02 mg/L	Section 4.3.3.1
	Tolerant	Est. NOEC, lethality[1]	0.35 mg/L	Section 4.3.3.1
Invertebrates	Sensitive	NOEC, lethality	0.0015 mg/L	Section 4.3.3.2
	Tolerant	Est. NOEC, lethality[2]	0.048 mg/L	Section 4.3.3.2
Microorganisms	Sensitive	NOEC, species richness	0.006 mg/L	Section 4.3.3.4
	Tolerant	NOEC, population	0.26 mg/L	Section 4.3.3.4
Aquatic Plants				
Macrophytes	Sensitive	NOEC, growth	0.02 mg/L	Section 4.3.3.3.
	Tolerant	N/A		Section 4.3.3.3.
Algae	Sensitive	NOEC, N_2 fixation	0.035 mg/L	Section 4.3.3.3.
	Tolerant	NOEC, discoloration	1 mg/L	Section 4.3.3.3.

[1]Because of the steep concentration-response curves for aqueous chlorine in fish, the NOEC is estimated as one-half of the LC_{50}. See Sections 4.3.3.1for a more detailed discussion.

[2] The NOEC is estimated from the LC_{50} by multiplying by a factor of 0.05. While this is a standard approach in Forest Service risk assessments, this factor may substantially overestimate risk for aqueous chlorine. See Sections 4.3.3.2 and 4.4.3.2 for more detailed discussions.

Table 11: Hazard Quotients for Aquatic Organisms

Receptor	Type	Hazard Quotients			Toxicity Value	Toxicity Endpoint
		Central	Lower	Upper		
Small Pond						
Fish	Sensitive	**7**	0.7	**28**	**0.02**	Est. NOEC
	Tolerant	0.4	4E-02	**1.6**	**0.35**	Est. NOEC
Invertebrate	Sensitive	**88**	**9**	**374**	**0.0015**	NOEC
	Tolerant	**3**	0.3	**12**	**0.048**	Est. NOEC
Algae	Sensitive	**4**	0.4	**16**	**0.035**	NOEC
	Tolerant	0.1	1E-02	0.6	**1**	NOEC
Macrophyte	Sensitive	**7**	0.7	**28**	**0.02**	NOEC
	Tolerant	No toxicity data.			**N/A**	N/A
Microorganisms	Sensitive	**22**	**2**	**94**	**0.006**	NOEC
	Tolerant	0.5	5E-02	**2**	**0.26**	NOEC
Large Pond						
Fish	Sensitive	0.8	9E-02	**4**	**0.02**	Est. NOEC
	Tolerant	5E-02	5E-03	0.2	**0.35**	Est. NOEC
Invertebrate	Sensitive	**11**	**1.2**	**47**	**0.0015**	NOEC
	Tolerant	0.3	4E-02	**1.5**	**0.048**	Est. NOEC
Algae	Sensitive	0.5	5E-02	**2**	**0.035**	NOEC
	Tolerant	2E-02	2E-03	7E-02	**1**	NOEC
Macrophyte	Sensitive	0.8	9E-02	**4**	**0.02**	NOEC
	Tolerant	No toxicity data.			**N/A**	N/A
Microorganisms	Sensitive	**3**	0.3	**12**	**0.006**	NOEC
	Tolerant	6E-02	7E-03	0.3	**0.26**	NOEC
Small Lake						
Fish	Sensitive	3E-02	4E-03	0.1	**0.02**	Est. NOEC
	Tolerant	2E-03	2E-04	8E-03	**0.35**	Est. NOEC
Invertebrate	Sensitive	0.4	5E-02	**1.9**	**0.0015**	NOEC
	Tolerant	1E-02	1E-03	6E-02	**0.048**	Est. NOEC
Algae	Sensitive	2E-02	2E-03	8E-02	**0.035**	NOEC
	Tolerant	7E-04	7E-05	3E-03	**1**	NOEC
Macrophyte	Sensitive	3E-02	4E-03	0.1	**0.02**	NOEC
	Tolerant	No toxicity data.			**N/A**	N/A
Microorganisms	Sensitive	0.1	1E-02	0.5	**0.006**	NOEC
	Tolerant	3E-03	3E-04	1E-02	**0.26**	NOEC

Appendix 1: Toxicity to Freshwater Fish

Animal	Concentration/Duration	Effects	Citation
Bluegill sunfish (*Lepomis macrochirus*)	Standard flow-through bioassays. Source of chlorine not clearly specified.	96-hour LC_{50}s 0.064 mg/L at 6°C 0.063 mg/L at 15°C 0.057 mg/L at 25°C 0.065 mg/L at 32°C Based on reported confidence intervals, the 25°C exposure was more toxic than other temperatures. There is, however, no systematic relationship. See Table III of study for other duration-specific LC_{50}s.	Bass and Heath 1977
Mosquito fish (*Gambusia affinis*), males and females, 2-4 cm, 10 fish/assay	Chlorine bleach (5.25% NaOCl) in freshwater **Static** with daily renewal and aeration. Exposure to concentrations of 0.5, 1.0, or 2.0 ppm under static conditions for at least 1 hour and up to 3 hours, daily for 5 days.	Mortality: At 0.5 and 1.0 ppm fish survived daily dosing; however by day 5, survival was 60% for 0.5 ppm group and 20% for 1.0 ppm group. At 2.0 ppm, mortality rate was 90% after a single exposure on day 1. Gill damage: extensive, dose-related damage, including delamination of cell layers and separation of adjacent cells. Authors' Comment: *The cumulative gill damage of multiple exposures at 0.5 ppm was less than a single exposure of 2.0 ppm.*	Cohen and Valenzuela 1977
Fathead minnow (*Pimephales promelas*), NOS	Sodium hypochlorite (4-6% aqueous solution). Concentrations used in bioassays not specified. **Static** bioassays.	24-hour LC_{50} = 5.6 mg/L (95% CI = 4.7-7.1 mg/L) 48-hour LC_{50} = 5.9 mg/L (95% CI = 4.8-8.0 mg/L) 96-hour LC_{50} = 5.9 mg/L (95% CI = 4.8-8.0 mg/L)	Curtis et al. 1979

Appendix 1: Toxicity to Freshwater Fish *(continued)*

Animal	Concentration/Duration	Effects	Citation
Zebrafish *(Danio rerio*), >120-days-old, 0.2-0.5 g; 2.4-3.2 cm, 32/group	Sodium hypochlorite (NaOCl) 5–6% formulation (ISOFAR brand) All exposures appear to have been **static**. Exposure concentrations for LC_{50} determination: 22.8, 28.5, 34.2, 39.0, 45.6, 51.3, 57.0 mg/L for 24 hours. Sublethal concentrations: 4.8, 9.6, 14.4, or 19.2 mg/L for 5 hours to evaluate behavioral responses.	24-hour LC_{50} = 48 mg/L Behavioral response to sublethal concentrations of NaOCl included an escape response (increased swimming activity—i.e., hyperactivity) at 10% of the LC_{50} (4.8 mg/L). A decrease in swimming activity at 20% of the LC_{50} (9.6 mg/L), which was within the normal limits of variation. Significant hypoactivity at 30% (14.4 mg/L) and 40% (19.2 mg/L) of the LC_{50}. NOEC (for hypoactivity) = 9.6 mg/L (20% of LC_{50})	De Paiva Magalhas et al. 2007
Fathead minnow (*Pimephales promelas*), 0.2-0.5 g, n=10	Reagent grade sodium hypochlorite (NaClO) 5.25% under **static** conditions.	Static bioassays: 96-hour LC_{50} = 10 mg/L	Ewell et al. 1986
Emerald shiner (*Notropis atherinoides*), adults, 85.6 mm (avg.), 70-115 (range), n=500	30-minute total residual chlorine (sodium hypochlorite) exposure (slug dose) at 10 and 25°C. Flow-through	10°C: 96-hour LC_{50} = 0.87 mg/L (CI = 0.77-1.01 mg/L) 25°C: 96-hour LC_{50} = 0.28 mg/L (CI = 0.23-0.31 mg/L)	Fandrei and Collins 1979
Emerald shiner (*Notropis atherinoides*), yearlings, 56.9 mm (avg.), 40-74 (range), n=507	30-minute total residual chlorine (sodium hypochlorite) exposure (slug dose) at 10 and 25°C Flow-through	10°C: 96-hour LC_{50} = 0.71 mg/L (CI = 0.67-0.77 mg/L) 25°C: 96-hour LC_{50} = 0.23 mg/L (CI = 0.22-0.24 mg/L)	Fandrei and Collins 1979
Emerald shiner (*Notropis atherinoides*), young-of-the-year, 43.2 mm (avg.), 39-52 mm (range), n=1217	30-minute total residual chlorine (sodium hypochlorite) exposure (slug dose) at 10 and 25°C, Flow-through	10°C: 96-hour LC_{50} = 1.32 mg/L (CI = 1.27-1.36 mg/L) 25°C: 96-hour LC_{50} = 0.33 mg/L (CI = 0.32-0.34 mg/L)	Fandrei and Collins 1979

Appendix 1: Toxicity to Freshwater Fish *(continued)*

Animal	Concentration/Duration	Effects	Citation
Golden shiner (*Notemigonus crysoleucas*), young, 71.5-74.9 mm, 3180-3300 mg wet weight, 890-960 dry weight	Sodium hypochlorite (66 g chlorine/L) for 96 hours of continuous or intermittent exposure (40 minutes every 8 hours) under flow-through conditions	Continuous exposure: 96-hour LC_{50} = 304 µg chlorine/L (95% CI = 255-358 µg chlorine/L) Intermittent exposure: 96-hour LC_{50} = 572 µg chlorine/L (95% CI = 505-654 µg chlorine/L)	Fisher et al. 1999
Rainbow trout (*Oncorhynchus mykiss*), 15-days-old, 24.0 mm, 98.0 mg wet weight, 17.2 mg dry weight	Sodium hypochlorite (66 g chlorine/L) for 96 hours of continuous or intermittent exposure (40 minutes every 8 hours) under flow-through conditions	Continuous exposure: 96-hour LC_{50} = 59 µg chlorine/L (95% CI = 50-71 µg chlorine/L) Intermittent exposure: 96-hour LC_{50} = 374 µg chlorine/L (95% CI = 304-449 µg chlorine/L)	Fisher et al. 1999
White cloud mountain minnows (*Tanichthys albonubes*)	NaClO (NOS) in various degrees of hardness of the test water	48-hour LC_{50} values: 1.1 ppm (hardness = 0) 1.1 ppm (hardness = 30) 1.2 ppm (hardness = 100) 0.94 ppm (hardness = 200) 0.82 ppm (hardness = 400)	Kitamura 1990
Striped bass (*Morone saxatilis*), 24-day-old larvae	Total residual chlorine (TRC) in flowing brackish water Nominal concentrations: 1.8, 3.2, or 5.6 mg|/L as NaOCl. Measured concentrations are much lower.	Avoidance of measured concentrations as low as 0.29-0.32 mg/L. No avoidance at 0.16-0.18 mg/L	Middaugh et al. 1977a
Striped bass (*Morone saxatilis*), 2-day-old prolarvae, or 12-day-old larvae, or 30-day-old juveniles.	Total residual chlorine (TRC) in flowing water Nominal concentrations: 0.32, 0.56, 1.0, 1.4, 1.8, 3.2, 5.6, or 10.0 mg/L	Incipient ($t=\infty$) LC_{50} 0.04 mg/L for 2-day-old prolarvae 0.07 mg/L for 12-day-old larvae 0.04 for 30-day-old juveniles. Shorter-term LC_{50} values are not tabulated. Based on Figure 4 in publication, the 24-hour LC_{50} values appear to be about 0.7 mg/L. **Sublethal**: Histopathological examination indicated gill and pseudobranch damage in surviving 30-day-old juveniles exposed to TRC concentrations as low as 0.21 mg/L for 71 minutes.	Middaugh et al. 1977a

Appendix 1: Toxicity to Freshwater Fish *(continued)*

Animal	Concentration/Duration	Effects	Citation
Striped bass (*Morone saxatilis*), developing embryos, 8-9 hours after fertilization, approximately 8500/test concentration	Total residual chlorine (TRC) in flowing water Nominal concentrations: 0.10, 0.25, 0.50, or 1.0 mg/L Measured concentrations: <0.01, 0.01, 0.07, 0.21 mg/L Exposure periods of up to 48 hours except for highest concentration, in which no embryos survived past 23.5 hours (see Fig. 3 in publication).	At highest dose tested (0.21 mg/L), no larvae emerged from embryos. Stage at which development ceased could not be determined due to rupture and escape of yolk sac. At 0.07, only 3.5% of exposed embryos hatched, many of which had a curvature of the vertebral column. Many of the embryos developed to a stage just prior to emergence, but failed to hatch. At 0.01 mg/L 23% of the treated eggs hatched; however, many larvae had difficulty detaching from the chorion as they hatched. At <0.01, the effects were similar to those observed at 0.01 mg/L; however a strong swimming response was observed in these larvae as they moved to the surface in a burst of fast swimming then sank slowly to the bottom of the aquaria with their yolk sac oriented toward the water surface.	Middaugh et al. 1977a
Rainbow trout (*Salmo gairdneri*), 20-35 cm total length	In situ exposures in discharge canal from a power plant. Sodium hypochlorite (12.5% available chlorine by weight) to achieve maximum total residual chlorine (TRC) concentrations of 0.04, 0.2, 0.6, or 1.0 mg/L. Duration: 28 to 37 minutes.	Fish demonstrated a range of avoidance beginning at concentrations of 0.04-0.10 mg/L TRC. Despite the variability of individual responses, approximately 95% avoidance behavior was observed at 0.50 mg/L, which was *well before cumulative time dose exposure approached lethal limits*.	Schumacher and Ney 1980
Alewife (*Alosa pseudoharengus*),	NaOCl used to generate TRC. 10 organisms per concentration with 5 concentrations per bioassay. For salmon, results are given	30-minute LC_{50} = 2.15 mg/L at 10°C 30-minute LC_{50} = 2.27 mg/L at 15°C 30-minute LC_{50} = 1.70 mg/L at 20°C 30-minute LC_{50} = 0.96 mg/L at 25°C 30-minute LC_{50} = 0.30 mg/L at 30°C	Seegert and Brooks 1978

Appendix 1: Toxicity to Freshwater Fish *(continued)*

Animal	Concentration/Duration	Effects	Citation
Coho salmon (*Oncorhynchus kisutch*), adult	for bioassays done in 1975 and 1976.	1975: 30-minute LC_{50} = 1.26 mg/L at 10°C 30-minute LC_{50} = 1.35 mg/L at 15°C 30-minute LC_{50} = 0.9 mg/L at 20°C 1976: 30-minute LC_{50} = 0.56 mg/L at 10°C 30-minute LC_{50} = 0.29 mg/L at 20°C	Seegert and Brooks 1978
Rainbow smelt (*Osmerus mordax*), adult		30-minute LC_{50} = 1.27 mg/L at 10°C	Seegert and Brooks 1978
Spottail shiner (*Notropis hudsonius*), adult		30-minute LC_{50} = 2.41 mg/L at 10°C 30-minute LC_{50} = 1.00 mg/L at 15°C 30-minute LC_{50} = 0.53 mg/L at 20°C	Seegert and Brooks 1978
Alewife (herring), adult (NOS)	Total residual chlorine	30-minute LC_{50} = 2.25 mg/L at 10°C	Seegert et al. 1977
Freshwater Coho salmon, adult (NOS)	Total residual chlorine	30-minute LC_{50} = 1.25 mg/L at 10°C	Seegert et al. 1977
Rainbow trout (*Salmo gairdneri*), yearling (NOS)	Total residual chlorine	30-minute LC_{50} = 2.0 mg/L at 10°C	Seegert et al. 1977
Spottail shiner, adult (NOS)	Total residual chlorine	30-minute LC_{50} = 3.2 mg/L at 10°C	Seegert et al. 1977
Yellow perch, adult (NOS)	Total residual chlorine	30-minute LC_{50} = 7.7 mg/L at 10°C 30-minute LC_{50} = 4.0 mg/L at 15°C 30-minute LC_{50} = 1.1 mg/L at 20°C 30-minute LC_{50} = 1.0 mg/L at 25°C	Seegert et al. 1977
Rainbow trout (*Salmo gairdneri*), 1-year-old, 9.4±0.5 g, 10.1±0.3 cm	Sodium hypochlorite (NaClO) in semi static dechlorinated Helsinki municipal tap water (13°C) NaClO concentrations used varied from 0.0 to 0.5 mg/L (NOS). **Static renewal.**	24-hour LC_{50} = 0.43 mg/L 48-hour LC_{50} = 0.35 mg /L At 0.2 mg/L, gills were thicker than observed in control group; at 0.4 mg Cl_2/L, epithelium of the secondary lamellae epithelium were considerably swollen, the lamellae were curled with constricted blood spaces. LC_{50} values for fish pre-exposed to Kraft effluents were lower with a 48-hours LC_{50} of 0.07 mg/L.	Soivio et al. 1988
Brook trout, adult (NOS)	Total residual chlorine	<u>96-hour LC_{50} =</u> 0.15-0.18 mg/L at 10°C 0.13-0.16 mg/L at 15°C 0.10-0.12 mg/L at 20°C	Thatcher et al. 1976

Appendix 1: Toxicity to Freshwater Fish *(continued)*

Animal	Concentration/Duration	Effects	Citation
Bluegill (*Lepomis macrochirus*), young-of-the-year	1-hour exposure/day for 4 days using proportional diluters. Chlorine was measurable in test chambers for about 2 hours each day.	96-hour LC_{50} = 0.88 mg/L (based on average concentration)	Wilde et al. 1983a
Fathead minnow, adult	1-hour exposure/day for 4 days using proportional diluters. Chlorine was measurable in test chambers for about 2 hours each day.	96-hour LC_{50} = 0.58 mg/L (based on average concentration)	Wilde et al. 1983a
Fathead minnow, juvenile	1-hour exposure/day for 4 days using proportional diluters. Chlorine was measurable in test chambers for about 2 hours each day.	96-hour LC_{50} = 0.18 mg/L (based on average concentration)	Wilde et al. 1983a
Bluegill (*Lepomis macrochirus*), young-of-the-year	1-hour exposure/day for 4 days using proportional diluters. Chlorine was measurable in test chambers for about 2 hours each day.	96-hour LC_{50} = 0.44 mg/L (based on average concentration)	Wilde et al. 1983b
Fathead minnow, adult (NOS)	1-hour exposure/day for 4 days using proportional diluters. Chlorine was measurable in test chambers for about 2 hours each day.	96-hour LC_{50} = 0.35 mg/L (based on average concentration)	Wilde et al. 1983b
Fathead minnow, juvenile (NOS	1-hour exposure/day for 4 days using proportional diluters. Chlorine was measurable in test chambers for about 2 hours each day.	96-hour LC_{50} = 0.08 mg/L (based on average concentration)	Wilde et al. 1983b
Rainbow trout (*Salmo gairdneri*), adult, 15/test group	Exposure to 3.86, 2.47, 2.75, or 1.09 mg/L for 8, 19,20,or 29 minutes, respectively. Concentrations expressed as total chlorine.	Exposed fish had blood that was darker and thicker than that of controls; chlorine was readily diffused through the gills, oxidizing the hemoglobin to methemoglobin, resulting in hemolysis by disrupting the erythrocyte membranes.	Zeitoun 1977

Appendix 1: Toxicity to Freshwater Fish *(continued)*

Animal	Concentration/Duration	Effects	Citation
Rainbow trout (*Salmo gairdneri*), 2- to 3-year-old adults, ≈30/test group	Exposure to total residual chlorine at concentrations of 1.67, 3.50, 1.10, 1.25, 1.02 or 0.0 mg/L, at water temperatures of 13.5, 14 .6,17.2, 22.6, 23.0 or 26.0° C, respectively.	<u>Cumulative mortality at 48 hours after exposure</u>: 1.67 mg/L (13.5°C) = 11.5% 3.50 mg/L(14.6°C) = 100% 1.10 mg/L (17.2°C) = 0% 1.25 mg/L = (22.6°C) 67.1% 1.02 mg/L = (23.0°C) 36.1% Increase in hematocrit, plasma protein, and methemoglobin in all surviving fish. During recovery period, the measured blood parameters returned to the control level within 24 hours, except for plasma hemoglobin (hemolysis index), which returned to the control level within 48-hours. Author notes that gill damage may have been responsible for effects on blood but no data on gill tissue are presented.	Zeitoun 1978
Rainbow trout (*Salmo gairdneri*), 3-year-old adults, ≈15/test group	Total residual chlorine concentrations of 1.67-3.86 mg/L	Chlorine toxicity appeared to disturb the mineral homeostasis in blood of exposed fish; however, the fish appear to have an active mechanism to compensate for the mineral loss.	Zeitoun et al. 1977

Appendix 2: Toxicity to Aquatic Invertebrates

Animal	Dose/Exposure	Response	Reference
Copepods (*Cyclops bicuspidatus thomasi*), 20/test concentration	96-hour exposure periods.	**Sodium hypochlorite:** 96- hour TL_{50} = 0.069 mg/L total residual chlorine **Chloramine:** 96- hour TL_{50} = 0.084 mg/L total residual chlorine	Beeton et al. 1976
Isopods, (*Caecidotea bicrenata*), a blind hypogean cave isopod	0.016-0.593 reagent-grade sodium hypochlorite (NaOCl) TRC (total residue chlorine) for 96 hours under flow-through conditions	96-hour LC_{50} = 0.110 (0.42-0.286) mg/L	Bosnak and Morgan 1981
Isopods, (*Lirceus alabamae*), an epigean isopod	0.016-0.593 reagent-grade sodium hypochlorite (NaOCl) TRC (total residue chlorine) for 96 hours under flow-through conditions	96-hour LC_{50} = 0.155 (0.082-0.295) mg/L	Bosnak and Morgan 1981
Copepods (*Mysocyclops aspericornis*), 60 per concentration	Sodium hypochlorite (NaOCl) resulting in total residual chlorine (TCR) concentrations of 0.0 to 2.0 ppm for 24 hours.	24-hour LC_{50} = 0.47 (0.40-0.53) ppm NOEC for lethality: 0.2 ppm (see Figure 1 of paper)	Brown et al. 1994
Copepods (*Mysocyclops longisetus*), 60 per concentration	Sodium hypochlorite (NaOCl) resulting in total residual chlorine (TCR) concentrations of 0.0 to 2.0 ppm for 24 hours.	LC_{50} = 1.01 (0.92-1.10) mg/L NOEC for lethality: 0.8 ppm (see Figure 1 of paper)	Brown et al. 1994
Mosquito larvae (*Aedes aegypti*),	Sodium hypochlorite (NaOCl) resulting in total residual chlorine (TCR) concentrations of 0.0 to 2.0 ppm for 24 hours.	24-hour LC_{50} = 1.07 (0.97-1.20) mg/L NOEC for lethality: 0.4 ppm (see Figure 1 of paper)	Brown et al. 1994
Mosquito larvae (*Anopheles farauti*)	Sodium hypochlorite (NaOCl) resulting in total residual chlorine (TCR) concentrations of 0.0 to 2.0 ppm for 24 hours.	24-hour LC_{50} = 1.35 (1.17-1.54) mg/L NOEC for lethality: 0.4 ppm (see Figure 1 of paper)	Brown et al. 1994
Mosquito larvae (*Culex quinquefasciatus*)	Sodium hypochlorite (NaOCl) resulting in total residual chlorine (TCR) concentrations of 0.0 to 2.0 ppm for 24 hours.	24-hour LC_{50} = 0.71 (0.69-0.73) mg/L NOEC for lethality: 0.5 ppm (see Figure 1 of paper)	Brown et al. 1994

Appendix 2: Toxicity to Aquatic Invertebrates *(continued)*

Animal	Dose/Exposure	Response	Reference
Snails (*Goniobasis livescens*), 10/container	NaOCl for 48 hours, static with 24-hour renewal	24-hour LC_{50} = 10.4 ppm 48-hour LC_{50} = 6.2 ppm	Cairns et al. 1976
Snails (*Lymnaea emarginata*) , 10/container	NaOCl for 48 hours, static with 24-hour renewal	24-hour LC_{50} = 21.8 ppm 48-hour LC_{50} = 13.6 ppm	Cairns et al. 1976
Snails (*Physa integra*), 10/container	NaOCl for 48 hours, static with 24-hour renewal	24-hour LC_{50} = 2.0 ppm 48-hour LC_{50} = 1.8 ppm	Cairns et al. 1976
Asiatic clam (*Corbicula fluminea*), juveniles and adults	Chlorine target residual concentrations: 0.5 or 5.0 mg/L. Results expressed as LT_{50}, median lethal time. Flow-through assays	At 0.5 mg/L: LT_{50} = 8.7days (*adults*) Mortality rate = 5.0%/day (± 0.18) At 5.0 mg/L: LT_{50} = 5.9 days (*adults*) Mortality rate = 8.4%/day (± 0.18) At 5.0 mg/L: LT_{50} = 4.8 days (*juveniles*) Mortality rate = 9.6%/day (± 0.48)	Cameron et al. 1989

Appendix 2: Toxicity to Aquatic Invertebrates *(continued)*

Animal	Dose/Exposure	Response	Reference
Asiatic clam (*Corbicula fluminea*), juveniles and adults; shell heights: 15-21 mm (adults) and 7-11 mm (juveniles)	0.2 to 1.0 mg/L total residual chlorine: NaOCl in 28-32-day laboratory and field (industrial water supply) bioassays	Mortality in laboratory tests with constant chlorine dosing at levels of 0.2-1.0 mg/L was <53% at <16°C (mean temp) >53% at >18°C. Adult mortality was >80% in laboratory tests involving initial low-dose contractions (0.25 mg/L TRC) for 14 days followed by 18-day high-dose concentrations (0.50-1.00 mg/L), which was comparable to mortality rates of 60-95% associated with constant high-dose (0.5-1.00 mg/L) exposure concentrations. <u>Field studies conducted during spring and fall</u>: >90% mortality at 0.25 mg/L TRC for 28 days (ambient temperatures rose from 20 to 25°C) ≤23% mortality at <0.50 mg/L TRC for 28 days (ambient temperatures fell from 20 to 12°C)	Doherty et al. 1986
Flatworm (*Dugesia tigrina*), 0.006 g, n=10	Reagent grade sodium hypochlorite (NaClO) 5.25% under **static** conditions	96-hour LC_{50} = 32 mg/L	Ewell et al. 1986
Isopod (*Asellus intermedius*), 0.012 g, n=10	Reagent grade sodium hypochlorite (NaClO) 5.25% under **static** conditions	96-hour LC_{50} = 32 mg/L	Ewell et al. 1986
Segmented worm (*Lumbriculus variegates*), 0.006 g, n=10	Reagent grade sodium hypochlorite (NaClO) 5.25% under **static** conditions	96-hour LC_{50} = 3.2 mg/L	Ewell et al. 1986
Amphipod (*Gammarus fasciatus*), 0.007 g, n=10	Reagent grade sodium hypochlorite (NaClO) 5.25% under **static** conditions	96-hour LC_{50} = 4.0 mg/L	Ewell et al. 1986
Snail (*Helisoma trivolvis*), 0.180 g, n=10	Reagent grade sodium hypochlorite (NaClO) 5.25% under **static** conditions	96-hour LC_{50} = 59 mg/L	Ewell et al. 1986

Appendix 2: Toxicity to Aquatic Invertebrates *(continued)*

Animal	Dose/Exposure	Response	Reference
Water flea (*Daphnia magna*), first and second larval instar, n=10	Reagent grade sodium hypochlorite (NaClO) 5.25% under **static** conditions	96-hour LC_{50} = 2.1 mg/L	Ewell et al. 1986
Amphipod (*Hyalella azteca*), juveniles	Sodium hypochlorite (66 g chlorine/L) for 96 hours of continuous or intermittent (40 min. every 8 hours) exposure under flow-through conditions	Continuous exposure: 96-hour LC_{50} = 78 µg chlorine/L (95% CI = 62-96 µg chlorine/L) Intermittent exposure: 96-hour LC_{50} = 301 µg chlorine/L (95% CI = 252-362 µg chlorine/L)	Fisher et al. 1999
Daphnia (*Daphnia magna*), <24-hours	Sodium hypochlorite (66 g chlorine/L) for 48 hours of continuous or intermittent (40 min. every 8 hours) exposure under flow-through conditions	Continuous exposure: 48-hour LC_{50} = 32 µg/L (95% CI = 1.0-36.0 µg/L) Intermittent exposure: 48-hour LC_{50} = 55 µg/L (95% CI = 45-68 µg/L)	Fisher et al. 1999
Amphipod (*Gammaarus pseudolimnaeus*), adult	Total residual chlorine	48-hour LC_{50} = 0.023 mg/L at 15 °C	Gregg 1974
Zebra mussels (*Dreissena polymorpha*), small (4-6 mm), large (10-15 mm)	Sodium hypochlorite dilution series: 21, 28, 35, 42, or 56 µg/L (mortality assessed every 24 hours for 9 days. All bioassays were **static.**	Large mussels (10-15 mm): 9-day LC_{50} = 1.61 mg/L (95% CI = 1.14-2.29 mg/L) Small mussels (4-6 mm): 9-day LC_{50} = 1.13 mg/L (95% CI = 0.75-1.60mg/L) **Note**: This study also investigates the effects of season, stock, and laboratory protocols on the survival of zebra mussels.	Kilgour and Baker 1994

Appendix 2: Toxicity to Aquatic Invertebrates *(continued)*

Animal	Dose/Exposure	Response	Reference
Zebra mussels (*Dreissena polymorpha*), adults, 14-16 mm, ≈25/concentration	Applied aqueous chlorine (from sodium hypochlorite) concentrations of 0, 0.5, 1.0, or 2.5 mg /L in flow-through system for maximum of 56 days OR Applied concentrations of 0, 5, or 10 mg /L in flow-through system for 28 days	56-day exposure (7-18°C): LT_{50} = 53.7 days at 0.5 mg/L *(95% CI = 52.0-55.7 days)* LT_{50} = 31.9 days at 1.0 mg/L *(95% CI =31.4-32.5 days)* LT_{50} = 16.3 days at 2.5 mg/L *(95% CI = 15.9-16.6 days)* Mortality rates: 100% at 2.5 mg Cl_2/L 94% at 1.0 mg Cl_2/L 55% at 0.5 mg Cl_2/L 28-day exposure (5-10°C): LT_{50} = 24.2 days at 5 mg/L *(95% CI = 23.8-24.9 days)* LT_{50} = 19.5 days at 10 mg/L *(95% CI = 18.8-20.3 days*) Mortality rates: 86% at 10 mg Cl_2/L 67% at 5 mg Cl_2/L	Klerks and Fraleigh 1991
Zebra mussels (*Dreissena polymorpha*), adults,4/replicate	Applied concentrations of aqueous chlorine (from sodium hypochlorite) 0, 0.5, 1.0, or 2.5 mg/L in continuous and intermittent 28-day static renewal tests. *During the 28-days of exposure, pronounced changes occurred in the room temperature….water temperatures ranged from 17.1 to 27.0°C (mean 22.1°C).*	Continuous exposure: LT_{50} = 6.9 days at 0.5 mg/L (confidence limit = 5.6-9.2 days) LT_{50} = 4.5 days at 1.0 mg/L (confidence limit = 3.9-5.0 days) LT_{50} = 3.2 days at 2.5 mg/L (confidence limit = 1.9-4.5 days) Treatment killed all mussels within 1-9 days, and mussels did not detach from substrate until 16.9 hours after death. Intermittent exposure: LT_{50} >28 days at 0.5 mg/L LT_{50} >28 days at 1.0 mg/L LT_{50} = 26.6 days at 2.5 mg/L (confidence limit = 19.9-221days) LT_{50} values could only be calculated for the highest treatment level. During intermittent exposure, filtering frequencies were much higher in clean water, compared with treated water, and mussels were almost always closed during chlorine exposures.	Klerks and Fraleigh 1991

Appendix 2: Toxicity to Aquatic Invertebrates *(continued)*

Animal	Dose/Exposure	Response	Reference
Copepods (*Cyclops bicuspidatus thomasi*),	Sodium hypochlorite (5%) added to aquaria to produce a range of residual chlorine for **30-minute static exposures** at various temperatures. Mortality assessed at 24 hours.	LC_{50}: 14.68 mg/L (at 10°C) n=1411 *(95% CI = 12.59-15.29 mg/L)* 15.61mg/L (at 15°C) n=901 *(95% CI = 13.96-18.17 mg/L)* 5.76 mg/L (at 20°C) n=920 *(95% CI = 5.10-6.99 mg/L)* 3.15 mg/L (at 20°C) n=459 *(95% CI = 2.29-3.67 mg/L)* Relatively shallow slopes: 1.13 to 2.0	Latimer et al. 1975; Seegert et al. 1977
Copepods (*Limnocalanus macrurus*)	Sodium hypochlorite (5%) added to aquaria to produce a range (NOS) of residual chlorine for **30-minute static exposures** at two different temperatures. Mortality assessed at 24 hours.	LC_{50}: 1.54 mg/L (at 5°C) n=1208 *(95% CI = 1.51-1.58 mg/L)* 1.54 mg/L (at 10°C) n=718 *(95% CI = 1.50-1.58 mg/L)* Relatively steep slopes: 7.9 to 10.4. **Note:** The identical LC_{50} values and nearly identical confidence intervals are not typographical errors. See Table 2 of study.	Latimer et al. 1975; Seegert et al. 1977
Australian cladoceran (*Ceriodaphnia dubia*), neonates (<24-hours-old)	Sodium hypochlorite in freshwater, **static renewal** for longer-term exposures.	1-hour LC_{50} 0.28 mg/L (95% CI = 0.26-0.31 mg/L) 24-hour LC_{50} 0.12 mg /L (95% CI = 0.11-0.13 mg /L) 10-day lifecycle test: All animals in two highest test concentrations (NOS) died within 4 days. Observed effect was death prior to production of offspring; no significant effects on production of offspring. LOEC = 0.066 mg /L NOEC = 0.048 mg /L	Manning et al. 1996

Appendix 2: Toxicity to Aquatic Invertebrates *(continued)*

Animal	Dose/Exposure	Response	Reference
Zebra mussels ((*Dreissena polymorpha*), small adults, 2-8 mm valve length	Sodium hypochlorite diluted from industrial grad 12% stock (12% chlorine by weight) Concentrations: 0, 0.10, 0.25, 0.50, 1.00, 2.50, or 5.00 mg/L residual chlorine (after initial demand) for up to 480 hours under **static** conditions in a darkened environment (to minimize loss chlorine loss).	!00% mortality within 360 hours at a concentration of 2.50 mg/L Mortality was less than 10% within 480 hours at concentrations <1.00 mg/L At 1.00 mg/L, 50% mortality was achieved at approximately 295 hours, and more than 20% of mussels were alive at 480 hours. At 2.50 mg/L, 50% mortality was achieved by 178 hours, with 100% mortality at 360 hours. At 5.00 mg/L, 50% mortality was achieved after approximately 157 hours, with 100% mortality after 264 hours.	Martin et al. 1993
Daphnia magna, adult	Total residual chlorine	30-minute LC_{50} = 0.097 mg/L 1-hour LC_{50} = 0.063 mg/L	Mattice et al. 1981
Microbial plant decomposers (not specifically identified)	Dosing of outdoor experimental streams with NaOCl at target TRC of 10, 75, or 250 µg/L from June 12 to October 27, 1985 – i.e., 137 days..	Nominal concentration of 250 µg/L (230 µg/L measured) resulted in reduced colonization of amphipod shredders.	Newman et al. 1987
Daphnia mendotae resting eggs extracted from sediment collected from Muskegon Lake in MI, 50 eggs/5 replicates/dose	Bioassays using **resting eggs** (i.e., ephippia) to sodium hypochlorite	LC_{50} = 55.0 ± 0.6 mg/L LC_{90} = 78.3 ± 1.6 mg/L Concentrations ≤2000 mg/L sodium hypochlorite were not toxic to resting eggs buried in sediment (i.e., burial in sediment protected the eggs from the toxicant).	Raikow et al. 2007
Rotifers (*Brachionus calyciflorus*)	Sodium hypochlorite, **static** assay over **1 hour** exposure period.	1-hour LC_{50} = 0.37 mg/L (95% CI = 0.35-0.39 mg/L)	Snell 1991; Snell et al. 1991

Appendix 2: Toxicity to Aquatic Invertebrates *(continued)*

Animal	Dose/Exposure	Response	Reference
Daphnids (*Ceriodaphnia dubia*)	Static and flow-through exposures at different pHs: pH 7 (predominantly HOCl) and pH 8 (predominantly OCl^-).	<u>pH 7 (predominantly hypochlorous acid):</u> 24- hour static LC_{50} = 0.035 mg/L (without food) 24- hour static LC_{50} = 0.14 mg/L (with food) 24- hour flow-through LC_{50} = 0.005 mg/L <u>pH 8 (predominantly hypochlorite):</u> 24- hour static LC_{50} = 0.048 mg/L (without food) 24- hour static LC_{50} = 0.08 mg/L (with food) 24- hour flow-through LC_{50} = 0.006 mg/L	Taylor 1993

Appendix 3: Toxicity to Aquatic Plants

Organism	Dose/Exposure	Response	Reference/ Classification [1]
Mixed phytoplankton, India	Power station effluent, 0.05 to 0.2 mg/L	At 30% to 70% decrease in primary productivity.	Ahamed et al. 1993
Cladophora sp. (filamentous algae)	Sodium hypochlorite at concentrations of 1 to 200 mg/L (static) with observation at 24 hours.	NOEC: 1 mg/L LOEC: 5 mg/L (discoloration) Higher concentrations cause progressively more severe effects including rupture of cell walls.	Betzer and Kott 1969
Mixed phytoplankton	0.046 to 2.7 mg/L Nominal concentrations.	Concentration-related decrease in photosynthesis and respiration. 0.046 mg/L appears to have caused over a 15% decrease in photosynthesis but there is not statistical analysis. Concentrations of 0.5 mg/L or greater caused over 50% decreases. See Figure 1 of paper.	Brooks and Baker 1972
Mixed phytoplankton	Doses of sodium hypochlorite to yield aqueous chlorine concentrations between 0.003 to about 1.5 mg/L TRC for **30 minutes**. Damage assayed over a 24 hour period. Studies conducted at temperatures from 2°C (in winter) to 12°C (in summer). Chlorophyll damage assayed as ratio of chlorophyll α to pheophytin. Carbon-14 uptake also assayed. Results are illustrated in Figures 1 and 2 of paper. No statistical analyses.	**chlorophyll α** No substantial impact on chlorophyll α at concentrations of 0.1 mg/L or less. Concentrations of 0.4 mg/L or greater decreased chlorophyll α. Different temperatures had no remarkable impact. **14C Uptake** Concentrations as low as 0.01 mg/L caused a transient decrease in carbon uptake. The only exception is a 0.029 mg/L exposure at 2°C which caused a transient stimulation in carbon uptake. All exposures >0.1 mg/L caused a decrease in carbon uptake over the 24-hour observation period.	Brooks and Liptak 1979

Appendix 3: Toxicity to Aquatic Plants *(continued)*

Organism	Dose/Exposure	Response	Reference/ Classification [1]
Single-celled fresh water alga (*Chlorella sorokiniana*),≈200 cells/mm^3	Sodium hypochlorite with an initial concentration of 0.4 mg/L. A second study with chlorine added at 5 and 10 hours. Observation period of 21 hours	A substantial decrease in algal counts by 5 hours relative to a substantial increase in algal counts in control media. The addition of chlorine at 5 and 10 hours did not have a remarkable effect.	Kott and Edlis 1969
Mixed periphyton on rocks.	Concentrations of TRC from 0.1 to 2.0 mg/L for 24 hours. **Static.**	Respiration Rates. Slight stimulation at 0.1 mg/L. Significant decreases at 0.5 mg/L and higher.	Osborne 1982
Blue-green algae (*Aphanizomenon flos-aquae*)	Sodium hypochlorite at available chlorine concentrations of 0.01, 0.035, and 0.07 mg/L. Total exposure period of 22 hours	Slight stimulation of N_2 fixation at 0.01 and 0.035 mg/L with substantial inhibition at 0.07 mg/L (Figure 1 of paper). Higher concentrations (>0.25 mg/L) over shorter periods of exposure were associated with signs of cell membrane damage (Figure 2 of paper).	Peterson et al. 1995
Mixed phytoplankton, outdoor mesocosm	Measured concentrations of 0, 0.4, 1.5, 24, 79, and 261 µg/L. 24 day observation period. Static renewal exposures.	Significant reduction in chlorophyll-*a* only at highest concentration.	Pratt et al. 1988
Mixed phytoplankton, indoor mesocosm	Nominal concentrations of 0, 3, 10, 30, 100, and 300 µg/L from a commercial bleach (NOS). Mean measured TRC of 2.1, 6.1, 25, 100, and 308. Measurements were highly variable. 28 day observation period. Chlorine added continuously.	Significant decrease in chlorophyll-*a* are all concentrations. LOEC: 2.1 µg/L.	Pratt et al. 1988

Appendix 3: Toxicity to Aquatic Plants *(continued)*

Organism	Dose/Exposure	Response	Reference/ Classification [1]
Mixed algal populations in stream microcosm	Total residual chlorine concentrations of 0.23 to 0.26 mg/L for 24 hours.	Approximately 20 to 24% reduction of chlorophyll-*a*.	Steinman et al. 1992
Eurasian watermilfoil (*Myriophyllum spicatum*), field collected	Continuous 96-hour exposure (flow-through) to 0, 0.02, 0.05, 0.1, 0.3, 0.5 or 1 mg/L TRC.	At concentrations of 0.05 mg/L and greater, dose-related signs of toxicity (reduced growth and visual damage. Rapid (within 6 hr) necrosis at 1 mg/L.	Watkins and Hammerschlag 1984
Eurasian watermilfoil (*Myriophyllum spicatum*), field collected	Intermittent (2 hr/day for 3 days) exposures to 0, 0.02, 0.05, 0.1, 0.3, 0.5 or 1 mg/L TRC.	1 mg/L: significant decrease in length, root dry weight, and chlorophyll-*a*. No adverse effects at lower concentrations. Growth stimulation was noted at lower doses.	Watkins and Hammerschlag 1984

Appendix 4: Toxicity to Aquatic Microorganisms

Organism	Dose/Exposure	Response	Reference/ Classification [1]
Periphyton communities	Periphytic communities on polyurethane foam generated from 14-day culture in an artificial pond. 7-day period of exposure to measured concentrations of 0.0063 mg/L and 0.0566 mg/L, flow-through. No description of species in culture.	A concentration-related decrease in species richness at 0.0063 mg/L (80% of control, NS) and 0.0566 mg/L (40% of control, significant at p=0.05). Specific data given in Table 1 of study. A less than additive effect with ammonia (i.e., chloramine formation)	Cairns et al. 1990
Mixed protozoan communities	2 hour exposure periods to chlorine concentrations of about 0.58, 0.66, 0.85, 1.15, 1.45, 2.15, and 2.85 ppm. Chlorine added every 20 minutes. Observations made at <24 hours up to 36 hours. The actual levels of exposure and observation times for specific assays are not clearly defined.	1.45 mg/L: NOEC for a 2-hour exposure involving 3 administrations. 0.66 mg/L: NOEC for a 2-hour exposure involving seven administrations. 0.66 mg/L: Based on Figure 8 of publication, this concentration was associate with a decrease in the number of species but the decrease does not appear to be statistically significant.	Dickson et al. 1977
Microbial plant decomposers (not specifically identified)	Dosing of outdoor experimental streams with NaOCl at target TRC of 10, 75, or 250 µg/L from June 12 to October 27, 1985 – i.e., 137 days. Different specific experiment conducted over various periods during the study.	Measured concentrations were reasonably close to target concentrations. Reduced microbial populations at two higher concentrations apparent after 4 days. NOEC: 0.01 mg/L LOEC: 0.075 mg/L The highest concentration, 0.250 mg/L, was associated with reduced breakdown of vegetation by 35 days.	Newman et al. 1987

Appendix 4: Toxicity to Aquatic Microorganisms *(continued)*

Organism	Dose/Exposure	Response	Reference/ Classification [1]
Mixed protozoan communities, outdoor mesocosm	Measured concentrations of 0, 0.4, 1.5, 24, 79, and 261 µg/L. 24 day observation period. Static renewal exposures.	A general concentration related decrease in protozoan numbers but the data were highly variable. Statistically significant decrease in colonization only at 261 µg/L.	Pratt et al. 1988
Mixed protozoan communities, indoor mesocosm	Nominal concentrations of 0, 3, 10, 30, 100, and 300 µg/L from a commercial bleach (NOS). Mean measured TRC of 2.1, 6.1, 25, 100, and 308. Measurements were highly variable. 28 day observation period. Chlorine added continuously.	Significant decrease in protozoan colonization as soon as 3 days at concentrations of 25 µg/L and higher. NOEC: 6.1 µg/L. Decrease in protozoan species numbers at 6.1 µg/L. NOEC: 2.1 µg/L.	Pratt et al. 1988