2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO CHLORINE IN THE UNITED STATES

Chlorine is a greenish-yellow gas with a pungent, irritating odor. It is stored and transported as a liquid under pressure. When chlorine is released into the environment, it reacts with both organic and inorganic substances that it comes into contact with. When chlorine gas is released into water, such as during water chlorination, it quickly dissolves in the water and then disproportionates within seconds to form chloride and hypochlorous acid. Chlorine may be released into the environment from facilities where it is produced or used, or during accidents, such as a chlorine tank rupture or a liquid chlorine spill. Most of the chlorine released during these types of incidents is expected to volatilize into the air forming a greenish-yellow chlorine gas cloud. Because chlorine is approximately 2.5 times heavier than air, the chlorine cloud remains near the ground. Within a few hours, this cloud can be carried several miles from the site of release by the wind while maintaining very dangerous concentrations of chlorine. Chlorine undergoes direct photolysis in the air and its half-life in the troposphere is on the order of several minutes.

Because it is so reactive, chlorine gas is normally not detected in the environment except at low levels in seawater aerosols. Therefore, background exposure of the general population to chlorine is not expected to represent a health concern. Human biomonitoring data are not available for chlorine. Analyzing human biological tissue and fluids for chlorine is not relevant because >95% of the chlorine that is inhaled (over a 1–5 ppm range) is removed in the upper respiratory tract and eventually joins the chloride pool in the body. The amount of chlorine that would need to be inhaled to induce a significant increase in extracellular chloride in the body is probably a lethal amount.

There are two primary means by which the general population may be exposed to chlorine. Individuals located near an accidental release of chlorine, either from a manufacturing facility or the transportation of liquefied chlorine may be exposed to high levels of this gas through inhalation and dermal contact if the cloud travels in their direction. In addition, people who mix acidic solutions with hypochlorite solutions, such as bleach or certain types of swimming pool chemicals, may accidentally be exposed to chlorine gas. Children may be exposed to chlorine through the same routes that affect adults, except for occupational exposures. Occupational exposure to low levels of chlorine gas in air may occur for individuals who work at facilities that produce or use chlorine. These individuals may also be exposed to high chlorine concentrations if an accidental release occurs inside the facility.

Exposure to chlorine through drinking water is expected to be very low. Free chlorine in drinking water is defined as the sum of dissolved chlorine gas, hypochlorous acid, and hypochlorite anion. As discussed in Chapters 4 and 6, the level of dissolved chlorine in water is extremely low, except under acidic conditions; therefore, the term free chlorine in public water systems typically refers to the concentration of hypochlorous acid and hypochlorite anion. The term total chlorine as it pertains to water sanitation practices usually refers to the amount of free chlorine plus chloroamines (sometimes called combined chlorines) produced during the sanitation process. It is important to recognize that these compounds are different from molecular chlorine even though the terminology is often used interchangeably.

2.2 SUMMARY OF HEALTH EFFECTS

Chlorine Gas. The principal targets of exposure to chlorine gas are the respiratory airways and the eyes. Exposure can occur only by direct contact of inhaled chlorine gas with the respiratory epithelium or via direct contact of the eyes with the gas. The skin seems to be a less sensitive target to direct contact with chlorine gas possibly because it lacks the moisture of mucous membranes. The effects of acute-duration exposures to high concentrations of chlorine have been known for almost a century, starting with its use as a chemical weapon at Ypres, Belgium, during World War I. Additional information regarding the effects of brief high-level exposures to chlorine has been collected from accidental exposures following leaks during transport of tanks containing liquid chlorine, leaks from storage tanks, domestic accidents involving bleach solutions, mishandling of chemicals used at swimming pools, and even accidents in high school science experiments. These and many additional studies, including studies in volunteers exposed to controlled concentrations of chlorine, indicate that exposures to 1–3 ppm produce mild irritation of the nose that can be tolerated for about 1 hour; 5 ppm may produce eye irritation; headache and throat irritation may occur at concentrations of 5–15 ppm; 30 ppm produces immediate chest pain, nausea and vomiting, dyspnea, and cough; 40–60 ppm produces toxic pneumonitis and pulmonary edema; 430 ppm usually causes death in 30 minutes, and 1,000 ppm is fatal within a few minutes. In most cases, death is the result of pulmonary edema. Accidental releases of chlorine have affected adults and children, and a few reports suggested that children might be more susceptible than adults to the effects of chlorine. This may be due to the smaller diameter of the airways of children compared to adults.

The effects of exposure to chlorine seem to depend, at least above a certain minimal exposure concentration, on the duration of exposure and exposure concentration, and the moisture content of the surface contacted by the gas (i.e., the respiratory epithelium). Exposures to relatively low concentrations

of chlorine (<5 ppm) are not expected to affect deep lung structures since most of the inhaled chlorine (>95%) is scrubbed in the upper portion of the respiratory tract, whether breathing is through the nose or through the mouth. With the exception of cough, substernal pain, and respiratory distress, the symptoms occurring after exposure to moderate concentrations of chlorine generally subside within 24 hours. Edema, observed following high exposures, is caused by marked alveolar capillary congestion followed immediately by focal and confluent area of fluid with a high content of fibrinogen. Pulmonary edema peaks in 12–24 hours and the resulting hypoxia further increases capillary permeability, which creates a vicious cycle. Initially, the pulmonary fluid is interstitial, but if it overwhelms the capacity of the lymphatic system to drain it, the alveoli become filled. A further complication is the formation of hyaline membranes from the alveolar fluid with high-fibrinogen content, which along with developing areas of atelectasis (collapse), and right to left shunting of blood, explains the poor oxygen diffusion with resultant hypoxemia and later hypercapnea. Subjects surviving the acute phase of exposure to high concentrations of chlorine may still be in danger of delayed death due to bronchial pneumonia or pneumonia. The complications of chlorine inhalation fit the histological condition known as diffuse alveolar damage that is associated with the clinical condition known as the adult respiratory distress syndrome.

Not all of the signs and symptoms exhibited by subjects exposed to moderate to high concentrations of chlorine gas are caused directly by chlorine. In general, it is believed that effects such as nausea and vomiting are reflex in origin, and headache and loss of consciousness are probably due to the hypoxia caused by pulmonary edema. Leukocytosis is almost always found in subjects admitted to emergency departments following exposure to high chlorine gas and is most likely a general response to inflammation. Anxiety and changes in blood pressure and heart rate also are commonly mentioned in case reports. While cardiovascular alterations can be due in part to a ventilation perfusion mismatch, they may also represent a general response to the stress and anxiety of having been involved in a chemical accident and being admitted to a health facility.

Prolonged exposures to relatively low concentrations of chlorine in occupational settings have not given indications of respiratory or other health problems among the workers, but additional better-controlled studies are necessary to add confidence to these early findings. Workers occasionally experience brief episodes of high exposure (“gassing” incidents), in some cases to concentrations high enough to warrant a visit to the emergency room. In some of these cases and also in some cases of exposure of the general population, long-term followup has shown persistent respiratory alterations that included airway obstruction and reactive airway dysfunction syndrome (RADS). RADS is defined as an asthma-like

illness after a single acute exposure to a respiratory irritant in otherwise healthy individuals, characterized by increased responsiveness to methacholine challenge. There are many factors that can play a role in whether residual effects will be present, including exposure level and duration of exposure, medical treatment following exposure, length of the follow-up, underlying respiratory disease, and smoking status.

A series of reports suggested that acute exposure to high concentrations of chlorine produced long-term neurobehavioral effects (i.e., memory loss, slow reaction time, impaired balance, hearing loss, visual alterations). No other study of chlorine-exposed subjects has included neurobehavioral testing, but this could potentially be examined in animal models. It is not known whether exposure to chlorine gas can affect reproduction or development in humans. Only one early study reported that pregnancy outcome was not affected among female workers at a chlorine plant. There is also no relevant information regarding effects of chlorine exposure on the immune system. A few studies of workers in the chemical industry did not find any evidence that chlorine gas is carcinogenic. The EPA, the International Agency for Research on Cancer (IARC), and the Department of Health and Human Services (DHHS) have not classified chlorine gas as to its carcinogenicity.

The respiratory system is also the target of chlorine toxicity in animals. Animals exposed briefly to high concentrations of chlorine gas have shown respiratory effects similar to those observed in humans, with the added observations of severe gross and microscopic changes in the respiratory airways. Chlorine, in relatively low concentrations (1–3 ppm), also induced histological alterations in the respiratory tract, particularly the upper portion, in intermediate- and chronic-duration inhalation studies in animals. In these studies, there was no indication that chlorine exposure affects reproductive parameters. No studies are available that evaluated whether chlorine affects immunocompetence or the development of young organisms. Chlorine gas was not carcinogenic in rats and mice exposed to up to 2.5 ppm for 2 years.

Aqueous Chlorine (Hypochlorite Solutions). At very low pH (<2), it is theoretically possible that chlorine gas can be formed; therefore, exposures to hypochlorite solutions are briefly discussed even though, under normal pH, the predominant species are expected to be hypochlorous acid and hypochlorite. The principal targets of exposure to aqueous chlorine are the upper gastrointestinal tract and the skin. Exposure to aqueous chlorine can occur via accidental or intentional ingestion of chlorine bleach or via direct contact of the skin with aqueous chlorine. In most cases, ingestion of small amounts (less than a cup) of sodium hypochlorite bleach (approximately 5.25% sodium hypochlorite, 52,250 ppm, or 52,250 mg/L) (ppm in water = mg/L) can produce esophageal irritation without permanent

consequences. However, fatalities due to ingestion of sodium hypochlorite have been reported. In a reported case, an autopsy of a woman who drank an unknown amount of bleach revealed esophageal and gastric mucosal erosions, perforations at the gastroesophageal junction, and extensive necrosis of adjacent soft tissue. Aspiration of hypochlorite bleach into the lungs following ingestion of bleach also has been reported as a cause of death. The lethal dose of sodium hypochlorite in adults has been reported to be approximately 200 mL of a solution containing 3–6% chlorine. No significant additional toxicities have been reported in humans following oral exposure to aqueous chlorine. Two intermediate-duration studies in which volunteers were exposed to known amounts of aqueous chlorine provided no evidence of adverse effects. In one of them, consumption of water containing 5 mg/L chlorine (approximately 0.036 mg Cl/kg/day) had no significant effect on hematology, serum chemistry, urinalysis, or additional physiological parameters. The other study, although of limited scope, showed that consumption of water containing 20 ppm chlorine (approximately 0.4 mg Cl/kg/day) had no significant effect on serum lipids or serum levels of thyroid hormones. It is not known whether oral exposure to chlorine can affect the immune and nervous systems, or reproduction or development in humans. There are no studies of cancer in humans exposed to chlorine itself. Based on inadequate evidence for carcinogenicity of hypochlorite salts in animals and no data from studies in humans, IARC determined that hypochlorite salts are not classifiable as to their carcinogenicity in humans.

Direct contact of the skin with household chlorine bleach can cause skin irritation in humans. Although sodium hypochlorite generally is not considered a contact sensitizer, several cases of allergic contact dermatitis have been reported. Commercial household bleaches are prepared with sodium hydroxide and are typically very alkaline; it is this property that may result in the irritant contact dermatitis. The limited information regarding ocular effects of direct contact of the eye with hypochlorite solutions suggest that splashes in the eye with house solutions of sodium hypochlorite rarely result in serious consequences.

For the most part, the results of oral and dermal studies of chlorine in animals support the observations in humans. Studies in which hypochlorite bleach was placed in the esophagus of animals reproduced the observations following high exposure in humans. Additional intermediate- and chronic-duration studies that examined hematology and clinical chemistry parameters and conducted gross and microscopic examination of tissues from rats and mice following exposure to chlorine in the drinking water provided little evidence of chlorine-related toxicity. In the intermediate-duration studies, Sprague-Dawley rats, Fischer-344 rats, and B6C3F_1 mice were dosed for 90 days with up to 24.9, 85, and 39.2 mg Cl/kg/day, respectively. In the chronic-duration studies, rats were exposed for 2 years to up to 14.4 mg Cl/kg/day

and mice to up 24.2 mg Cl/kg/day. 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). Exposure of male and female rats to aqueous chlorine before and during breeding and of the females during gestation and lactation did not cause reproductive effects in either sex or adverse developmental effects in the offspring. Cancer bioassays in rats and mice have been negative except for equivocal evidence of increased incidence of leukemia in female Fischer-344 rats in the NTP bioassay.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for chlorine. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

- An MRL of 0.07 ppm has been derived for acute-duration inhalation exposure (14 days or less) to chlorine gas.

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The effects of acute-exposure of humans and animals to chlorine have been well characterized (see Sections 2.2 and 3.2.1.2). Chlorine is a sensory irritant (substance capable of eliciting sensory irritation) and the most sensitive target for chlorine toxicity in humans and in animals is the respiratory system. Information that could be used for quantitative risk assessment regarding effects from acute exposure of humans to chlorine is available from studies of volunteers exposed to chlorine gas for 15 minutes to 8 hours (Anglen 1981; D'Alessandro et al. 1996; Rotman et al. 1983; Schins et al. 2000; Shusterman et al. 1998, 2003b). Some of these studies, as detailed below, also included sensitive individuals. Collectively, the results of these studies suggest that brief exposures to concentrations of chlorine ≤0.5 ppm do not cause sensory irritation or significant alterations in pulmonary function tests, but exposure to ≥1 ppm chlorine can induce transient respiratory and eye irritation and slight alterations in pulmonary function tests. Evaluations of soldiers gassed during World War I provide information on the effects of acute exposure to very high concentrations of chlorine and also on potentially persistent effects of acute exposure (Berghoff 1919; DOA 1933; Meakins and Priestley 1919). Similar information is available in many reports of accidental exposures to chlorine gas of workers and members of the general population (i.e., Agabiti et al. 2001; Agency for Toxic Substances and Disease Registry 1998; Bonetto et al. 2006; CDC 1991, 2005; Chasis et al. 1947; Chester et al. 1977; Hasan et al. 1983; Schönhofer et al. 1996; Sexton and Pronchik 1998; Weill et al. 1969). In both the war cases and the accidental exposures to chlorine gas, the concentrations of chlorine were generally not known. These high exposure cases have provided data on respiratory effects and on additional signs and symptoms of intoxication with chlorine that are not due to a direct action of chlorine, but that probably represent reflex responses and/or general responses to inflammation and stress. Some of these responses include nausea, vomiting, headache, anxiety, alterations in blood pressure, and leukocytosis.

The acute-duration database in animals is extensive, and includes a great number of studies conducted after the use of chlorine as a chemical weapon during World War I (for review, see DOA [1933] and Withers and Lees [1985b]). Most of the early studies provide information regarding lethal concentrations of chlorine as well as descriptions of the pathology of the respiratory tract caused by exposure to relatively high concentrations of chlorine. Although qualitatively informative, the early data do not meet current guidelines for use in quantitative risk assessment. More recent studies in animals, mainly rodents, have confirmed the earlier findings regarding sensory irritation and pathological changes in the respiratory tract (Barrow and Smith 1975; Buckley et al. 1984; Demnati et al. 1995; Jiang et al. 1983; Yildirim et al. 2004). In general, morphological alterations in the nasal mucosa of rats and mice occurred with chlorine concentrations >5 ppm. Specific lowest-observed-adverse-effect levels (LOAELs) for

sensory irritation in rodents are not available. However, in response to exposure to irritant substances, a reflex mechanism allows rodents to decrease the respiratory rate as a protective response (Alarie 1973). The concentration of the irritant that induces a 50% decrease in respiratory rate has been termed RD_{50} and is commonly used to compare the irritant potencies of chemicals. This reflex reaction has also been demonstrated in humans, dogs, and cats (Alarie 1973). Acute-duration inhalation studies provided very little information regarding end points other than those involving the respiratory system. Body weight loss, which is due to reduced food consumption, was reported in some studies (Dodd et al. 1980; Jiang et al. 1983).

Evaluation of the acute-duration inhalation database summarized above indicates that sensory irritation and pulmonary function in humans are the most sensitive end points for exposure to chlorine and will serve as the basis for derivation of an acute-duration inhalation MRL for chlorine. These findings were reported in a group of studies that can serve as co-principal studies (Anglen 1981; D'Alessandro et al. 1996; Rotman et al. 1983; Schins et al. 2000; Shusterman et al. 1998, 2003b). A detailed description of these studies is provided in Appendix A.

Collectively, this group of studies provides evidence of sensory irritation and transient pulmonary changes occurring in humans exposed to 1 ppm chlorine for up to 8 hours/day. The pulmonary changes indicated increased airway resistance and reduced air flow. No such changes were reported in volunteers exposed to 0.5 ppm chlorine (0.4 ppm in the D'Alessandro et al. [1996]) study. The longest exposure duration was 8 hours (Anglen 1981; Rotman et al. 1983). These studies also included sensitive individuals: an atopic subject in the study by Rotman et al. (1983), subjects showing methacholine hyperresponsiveness in the study by D'Alessandro et al. (1996), and subjects exhibiting seasonal allergic rhinitis (Shusterman et al. 1998). Also of significance is the fact that Rotman et al. (1983) reported that exposure to 1 ppm for 8 hours induced greater changes in pulmonary function tests than exposure to the same concentration for 4 hours, suggesting that the response was related to duration in addition to concentration. Given this information, an acute-duration inhalation MRL for chlorine can be derived by duration adjustment of the no-observed-adverse-effect level (NOAEL) of 0.5 ppm for continuous exposure (0.5 ppm x 8 hours/24 hours = 0.2 ppm) (8 hours was the longest period of exposure for which there is information). Although sensitive individuals were tested in some of these studies, the number of individuals tested at the region of the NOAEL (0.4–0.5 ppm) was small. Therefore, an uncertainty factor of 3 is used to account for sensitive populations. The resulting acute-duration inhalation MRL for chlorine is 0.07 ppm (0.2 ppm/3).

- An MRL of 0.002 ppm has been derived for intermediate-duration inhalation exposure (15–364 days to chlorine gas.

No human studies were available that could serve as the basis for derivation of an intermediate-duration inhalation MRL. The animal database for intermediate-duration exposure to chlorine is limited to two studies. In one study, male and female Fischer 344 rats were exposed to 0, 1, 3, or 9 ppm chlorine 6 hours/day, 5 days/week for 6 weeks (Barrow et al. 1979). In the other study, male and female Fischer 344 rats were exposed to 0, 0.5, 1.5, or 5 ppm chlorine 6 hours/day, 5 days/week for 62 days (Kutzman 1983). Aside for a reduction in final body weight of approximately 11% relative to controls in female rats exposed to 0.5 ppm chlorine (most likely due to reduced food consumption) in the Kutzman (1983) study, the most sensitive target for chlorine exposure was the respiratory tract. Barrow et al. (1979) described inflammation of the nasal turbinates in rats exposed to ≥1 ppm chlorine, whereas loss of cilia and epithelium in the trachea was seen in rats exposed to ≥0.5 ppm in the Kutzman (1983) study. No NOAELs for respiratory effects were established in either study. Since incidences of animals with respiratory lesions were presented in the Kutzman (1983) study, but not in the Barrow et al. (1979) study, the Kutzman (1983) study was selected as the principal study for derivation of an intermediate-duration inhalation MRL for chlorine (more complete descriptions of the end points evaluated and the reported results in these studies can be found in Section 3.2 and Appendix A).

There were no significant exposure-related increases in the incidences of animals with histological lesions in any of the examined tissues with the exception of a loss of cilia in the trachea (Kutzman 1983). The incidence of slight to moderate loss of tracheal cilia were 1/23, 12/23, 4/23, and 13/23 in the 0, 0.5, 1.5, and 5 ppm, exposure groups, respectively. Although the incidence for this lesion in the mid-exposure group was not significantly different from the control incidence, a statistically significant (p=0.0055) Cochran-Armitage trend test for these data can be demonstrated. However, when attempts were made to apply dose-response models to the data, no adequate fits of EPA Benchmark Dose Software models to the data were obtained (p-values for chi-square goodness of fit statistics were <0.1). Thus, the LOAEL of 0.5 ppm was used as the point of departure for deriving an intermediate-duration inhalation MRL for chlorine, after it was converted to a human equivalent concentration (HEC) using the EPA cross-species dosimetric methodology (EPA 1994a) for a category 1 gas, as follows:

$$LOAEL_{[HEC]} = LOAEL_{[ADJ]} \times RGDR_{TB}$$

where:
$LOAEL_{[ADJ]}$ = 0.5 ppm x 6/24 hours x 5/7 days = 0.09 ppm and
$RGDR_{TB}$ = ratio of the regional gas dose in rats to that of humans for the tracheobronchial region

$$RGDR_{TB} = (VE/SA_{TB})_A / (VE/SA_{TB})_H$$

where:
VE = minute volume (0.137 L/minute for rats, 13.8 L/minute for humans [EPA 1994a]) and
SA_{TB} = surface area of the tracheobronchial region (22.5 cm^2 for rats and 3,200 cm^2 for humans [EPA 1994a])
$LOAEL_{[HEC]}$ = 0.09 ppm x (0.137 L/minute/22.5 cm^2) / (13.8 L/minute/3,200 cm^2) = 0.14 ppm

Applying an uncertainty factor of 90 (3 for extrapolation from animals to humans with dosimetric adjustment, 3 for the use of a minimal LOAEL, and 10 for human variability) to the $LOAEL_{[HEC]}$ yields an intermediate-duration inhalation MRL of 0.002 ppm for chlorine.

- An MRL of 0.00005 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to chlorine gas.

There is no information regarding chronic-duration exposure of the general population to chlorine because this type of exposure occurs only in occupational settings. There are few studies of chronically-exposed workers in which there is some documentation regarding exposure levels and in which there is no evidence, at least explicitly mentioned in the studies, of the workers having being subjected to acute episodes of high exposure or "gassing" incidents. One of these studies involved 600 workers from 25 plants producing chlorine subjected to an evaluation of medical and occupational histories, blood and urine tests, pulmonary function tests, and electrocardiogram (Patil et al. 1970). Exposure data were available for 332 workers and showed a time-weighted average (TWA) 8-hour mean of 0.15±0.29 ppm (range, 0.006–1.42 ppm). Evaluation of the 332 workers who had exposure data showed that none of the end points examined (those subjected to recall or measured) showed a dose-response relationship. The mean concentration of 0.15 ppm may be considered a NOAEL for the study, but limitations such as unclear analytical methodology, no clear definition of the case/control populations, and insufficient detail regarding the method of analysis render the NOAEL questionable. A respiratory health assessment of 392 male pulp mill workers exposed predominantly to a mean 8-hour TWA of 0.18 ppm chlorine (other possible exposures included, sulfur dioxide, hydrogen sulfide, and methylmercaptan, in addition to various particulates) found that, relative to a control group, the pulp mill workers complained more frequently of usual phlegm, wheeze without cold, and chest illness (Enarson et al. 1984). However, the most significant finding was that a subgroup of nonsmokers (n=4) had a significantly lower fixed expiratory flow rate at 25–75% vital capacity ($FEF_{25–75\%}$) and forced expiratory volume in

1 second/forced vital capacity (FEV_1/FVC) ratio than the control workers. Given the small number of workers involved and the possibility of exposure to multiple chemicals, the validity of the 0.18 ppm as an effect level is questionable. An additional issue to consider is that neither one of these studies seemed adequate to detect possible mild alterations in the nasal cavity, a sensitive target of chlorine exposure in humans and animals, as described in Sections 2.2 and 3.2.1.2. Due to the limitations mentioned above, these long-term studies are insufficient for quantitative risk assessment.

There are only two chronic-duration inhalation studies of chlorine in animals. One is a 1-year study in monkeys (Klonne et al. 1987) and the other is a 2-year bioassay in rats and mice (Wolf et al. 1995). Both studies tested similar concentrations of chlorine (up to 2.3 ppm in monkeys and 2.5 ppm in rats and mice) and evaluated multiple end points including respiratory tract histopathology, hematology, and clinical chemistry. In both studies, the upper respiratory tract was the target for chlorine toxicity. In general, lesions were less severe in the monkeys than in rats and mice, but extended more distally in the respiratory tract. In rats and mice, an increase incidence of minimal to moderate alterations occurred with the lowest exposure concentration tested, 0.4 ppm chlorine. In general, the nasal lesions were site-specific, but the severity and/or incidence were not always concentration-dependent. Lesions observed included respiratory and olfactory epithelial degeneration, septal fenestration, mucosal inflammation, respiratory epithelial hyperplasia, squamous metaplasia, and goblet cell hypertrophy and hyperplasia, and secretory metaplasia of the transitional epithelium of the lateral meatus. For the most part, monkeys exhibited only mild concentration-related respiratory epithelial hyperplasia with focal loss of cilia over the range of concentrations tested (0, 0.1, 0.5, and 2.3 ppm) and showed no evidence of the major nasal lesions seen in rats and mice. These differences are probably related to species-specific respiratory-tract airflow characteristics (Ibanes et al. 1996), which in turn, are determined by anatomical differences. Moreover, rats and mice are obligatory nose breathers with a greater surface area-to volume ratio of the upper respiratory tract than primates. Therefore, exposure of rodents and primates to equal concentrations for equal amounts of time will likely result in greater pathological changes in the nasal area of the rodent (Barrow et al. 1979). It appears, therefore, than primates are a better model to evaluate potential respiratory effects in humans than rodents. For these reasons, the study in monkeys (Klonne et al. 1987) was selected for deriving a chronic-duration inhalation MRL for chlorine.

In the principal study, male and female rhesus monkeys (4/sex/exposure level) were exposed to 0, 0.1, 0.5, or 2.3 ppm chlorine 6 hours/day, 5 days/week for 1 year (Klonne et al. 1987). The only treatment-related histopathological effects consisted of focal epithelial hyperplasia characterized by increased cell

numbers and loss of cilia and goblet cells in the respiratory epithelium of the nose and trachea. The affected areas of the nasal passages showed hypercellularity with loss of goblet cells and cilia. In some of these areas, the nuclei showed altered polarity. Lesions were more frequent on the angular margins of the turbinates and less frequent on the lateral wall or septum adjacent to these margins. In some cases, the respiratory epithelial hyperplasia was associated with mild suppurative inflammatory response. Lesions in the trachea resembled those in the nose, but were less severe and involved only a small circumferential section of the ventral and ventrolateral trachea. The combined incidences of hyperplasia in the nasal epithelium with loss of goblet cells and cilia, characterized as trace and mild in males and females, were 1/8, 3/8, 6/8, and 8/8 in the control, 0, 0.1, 0.5, and 2.3 ppm exposure groups, respectively. The lowest exposure concentration of 0.1 ppm chlorine is considered a LOAEL for nasal lesions in monkeys.

Incidence data for nasal lesions in male and female monkeys exposed to chlorine gas (Klonne et al. 1987) were analyzed using the benchmark dose (BMD) approach for MRL derivation (further details of the modeling are presented in Appendix A). Models in the EPA Benchmark Dose Software (BMDS version 1.4.1) (Gamma, Logistic, Log-logistic, Multi-stage, Probit, Log-probit, Quantal linear, Weibull models) were fit to the nasal lesion data to determine potential points of departure for the MRL. A quantal linear model provided the best fit to the data. From this model, the predicted exposure concentration associated with a 10% extra risk (BMC_{10}) for nasal lesions in monkeys was 0.04 ppm; the lower 95% confidence limit on this concentration ($BMCL_{10}$) was 0.02 ppm. The monkey $BMCL_{10}$ served as the point of departure for the chronic-duration MRL, after it was converted to a HEC ($BMCL_{10[HEC]}$) using the EPA cross-species dosimetric methodology (EPA 1994a) for a category 1 gas, as follows:

$$BMCL_{10[HEC]} = BMCL_{10[ADJ]} \times RGDR_r$$

where:

$BMCL_{10[ADJ]}$ = 0.02 ppm x 6/24 hours x 5/7 days = 0.004 ppm and

$RGDR_{ET}$ = ratio of the regional gas dose in rats to that of humans for the extrathoracic region

$$RGDR_{ET} = (VE/SA_{ET})_A / (VE/SA_{ET})_H$$

where:

VE = minute volume 2.1 m^3/day for monkeys, calculated using the allometric equation for monkeys in EPA (1988) assuming a body weight of 7 kg for Rhesus monkeys with nasal cavity surface area of 62 cm^2 (Gross and Morgan 1991); 20 m^3/day for humans (EPA 1994a) and

SA_{ET} = 62 cm^2 surface area of the nasal cavity in Rhesus monkeys weighing 7 kg (Gross and Morgan 1991); 200 cm^2 for humans (EPA 1994a)

$$RGDR_{ET} = (2.1\ m^3/day / 62\ cm^2) / (20\ m^3/day / 200\ cm^2) = 0.34$$

$$BMCL_{10[HEC]} = 0.004 \text{ ppm} \times 0.34 = 0.00136 \text{ ppm}$$

Applying an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) to the $BMCL_{10[HEC]}$ yields a chronic-duration inhalation MRL of 0.00005 ppm for chlorine.

For the purpose of comparison, using the NOAEL/LOAEL approach would yield a chronic-duration inhalation MRL of 0.0007 ppm for chlorine. This results from duration-adjusting the LOAEL of 0.1 ppm (0.1 ppm x 6/24 x 5/7 = 0.02 ppm) and then multiplying the $LOAEL_{[ADJ]}$ by the $RGDR_{ET}$ of 0.34 calculated above ($LOAEL_{[HEC]}$ = 0.02 ppm x 0.34 = 0.007 ppm). Applying an uncertainty factor of 100 (10 for extrapolation from a LOAEL to NOAEL, 3 for animal to human extrapolation using dosimetric adjustments, and 3 for human variability) to the $LOAEL_{[HEC]}$ of 0.007 ppm would result in a chronic-duration inhalation MRL of 0.00007 ppm for chlorine, which is very close to the MRL calculated by benchmark analysis. If the LOAEL is considered a minimal LOAEL, the composite uncertainty factor would be 30 and the resulting MRL would be 0.0002 ppm, which is 4 times higher than the MRL calculated by benchmark analysis.

Oral MRLs

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. A summary of the oral database is presented below.

Earlier acute-duration studies in animals tried to reproduce the lesions to the esophagus and/or stomach due to ingestion of bleach. In most studies, commercial hypochlorite bleach was administered through a tube directly into the esophagus and, in some cases, the distal end of the esophagus was artificially occluded to prolong and control the contact time between the solution and the mucosa (Hook and Lowry 1974; Landau and Saunders 1964; Strange et al. 1951; Yarington 1970). Only three acute modern studies in animals were available. Cunningham (1980) administered 0, 8, 40, or 200 ppm Cl/kg/day (as sodium

hypochlorite) to Wistar rats (10 females/dose group) by gavage in milk for 14 days and reported that this treatment had no significant effect on growth or on the weight of the brain, liver, kidney, or heart. No other end points were evaluated. In a limited scope study, Meier et al. (1985) exposed male $B6C3F_1$ mice (10/dose group) to 0, 1.6, 4, or 8 mg Cl/kg/day (from sodium hypochlorite or hypochlorous acid) for 5 days and reported that in mice treated with sodium hypochlorite and sacrificed 3 weeks after exposure, there were significant increases in sperm abnormalities at 1.6, 4, and 8 mg Cl/kg/day (not clearly dose-related), but no such increases were seen in mice sacrificed 1 or 5 weeks after exposure. In addition, no increases in sperm abnormalities were seen in mice treated with hypochlorous acid, which the investigators considered "somewhat surprising since hypochlorite should be converted to hypochlorous acid in the acid pH of the stomach." Furukawa et al. (1980) administered sodium hypochlorite in the water to male and female Fischer-344 rats for 14 days and reported weight loss at approximately ≥36 mg Cl/kg/day accompanied by marked reductions in water consumption. No histological evaluations were conducted in this study.

Two human studies were located in the available intermediate-duration oral database. A study of limited scope evaluated serum lipid profile and serum levels of thyroid hormones in human volunteers who drank 1.5 L/day of distilled water containing 0 or 20 ppm chlorine (0 or 0.4 mg Cl/kg/day based on a mean body weight of 71 kg from the study) for 4 weeks (Wones et al. 1993). No significant deviations from normality were found. In the other study, consumption of water containing 5 mg/L chlorine (approximately 0.036 mg Cl/kg/day) for 12 weeks by 10 volunteers had no significant effect on hematology, serum chemistry, urinalysis, and additional physiological parameters (Lubbers et al. 1982). Since the study did not control for non-experimental ingestion of chlorine by the volunteers, the actual dose of chlorine cannot be estimated, but is likely to have been higher than 0.036 mg Cl/kg/day.

Few intermediate-duration studies in animals were located that examined a wide range of end points following exposure to aqueous chlorine. These studies showed that the main effect of exposure to solutions of hypochlorous acid or sodium hypochlorite, particularly at the higher concentrations levels, is a reduction of water intake that is due to taste aversion. The available intermediate-duration oral studies evaluated systemic toxicity (body weight, tissue and organ histopathology, hematology, clinical chemistry) (Abdel-Rahman et al. 1984; Cunningham 1980; Daniel et al. 1990, 1991; Furukawa et al. 1980) and also provided information, albeit limited, on immunological/lymphoreticular (organ weight and histopathology and limited immunocompetence) (Daniel et al. 1990, 1991; Exon et al. 1987), neurological (weight and histopathology of the brain and sciatic nerve) (Daniel et al. 1990, 1991), reproductive (male

and female reproductive organ weight and histopathology, fertility, and sperm parameters) (Carlton et al. 1986; Daniel et al. 1990, 1991), and developmental effects (fetal viability and fetal weight in a gestation exposure study) (Carlton et al. 1986). None of the available studies reported effects that could be attributed directly to chlorine or only reported effects that were considered of unknown toxicological significance. LOAELs were not identified in the intermediate-duration studies, but in one of these studies, rats exposed to 4.1 mg Cl/kg/day (the highest dose tested) for 8 weeks showed a statistically significant reduction in delayed-type hypersensitivity reaction (DTH) to bovine serum albumin, increased prostaglandin E2 synthesis by macrophages, and reduced oxidative metabolism (Exon et al. 1987). The toxicological significance of these findings is unknown.

The highest NOAEL identified in intermediate-duration oral studies is 76 mg Cl/kg/day for reduction in body weight gain in rats dosed with sodium hypochlorite in the drinking water for 13 weeks (Hasegawa et al. 1986). Doses of 152 and 305 mg Cl/kg/day were associated with reductions in final body weight of 19 and 47%, respectively, relative to controls. This study, however, has serious limitations including no reporting of food or water consumption and lack of presentation of data on other end points. Three other 13-week studies are available. Furakawa et al. (1980) administered up to approximately 85 mg Cl/kg/day (from sodium hypochlorite) to Fisher-344 rats for 92 days and reported significant reductions in final body weight males at 85 and 50 mg Cl/kg/day (19% and 46%, respectively) and in females at 84 mg Cl/kg/day (30%) relative to controls. This was accompanied by significant reductions of up to 66% in water consumption. For the most part, clinical chemistry hematology tests were unremarkable. Gross necropsy showed bladder abnormalities (no further description) among all groups, while microscopic examination showed endocardial hyperplasia and fibrosis of the myocardium in males and females dosed with 84 mg Cl/kg/day. Daniel et al. (1990) exposed male and female Sprague-Dawley rats to chlorine in the drinking water and evaluated a number of end points including organ histopathology, hematology, and clinical chemistry. Daniel et al. (1991) conducted a similar study in male and female $B6C3F_1$ mice. In neither study were there significant toxic effects that could be attributed to exposure to chlorine. However, in both studies, water consumption was significantly decreased, particularly in the high-dose groups. In high-dose male (16.7 mg/ Cl/kg/day) and female (24.9 mg Cl/kg/day) rats, water consumption was reduced 36 and 38%, respectively; in high-dose male (34.4 mg Cl/kg/day) and female (39.2 mg Cl/kg/day) mice, water intake was reduced 30 and 20%, respectively. The decrease in water intake, which resulted in dehydration and possibly altered electrolyte balance, could explain the reductions in weight gain and sporadic changes in hematological parameters, clinical chemistry, and organ weights

observed in both rats and mice. In the absence of effects that could be clearly attributed to chlorine toxicity, the highest doses tested represent the NOAELs for the studies; no LOAELs were defined.

No chronic-duration human study of exposure to hypochlorous acid or sodium hypochlorite was located; thus, a target for long-term exposure to chlorine in humans has not been identified. Three chronic-duration studies were available, two in rats (Hasegawa et al. 1986; NTP 1992) and one in mice (NTP 1992). In the NTP (1992) study, the only treatment-related effect was a reduction in water consumption, particularly at the higher dose levels of chlorine, which, as generally agreed, is due to taste aversion. All three studies evaluated a comprehensive number of end points including hematology and clinical chemistry and tissue and organ histopathology. The highest NOAEL was 133 mg Cl/kg/day for female rats in the Hasegawa et al. (1986) study. However, final body weight in low- and high-dose females at termination (104 weeks) was 11 and 20% lower, respectively, than in controls. Hasegawa et al. (1986) stated that in females, the daily water intake was "somewhat lower" (no data provided) than in the other groups during the first year, but that this trend was not observed during the second year. In addition, the investigators indicated that in males, water intake was comparable among groups, except during the last 20 weeks of the study, during which time water intake was consistently 10–20% higher in the experimental groups than in the controls. The latter seems to be inconsistent with findings in other studies that reported marked reductions in water consumption at lower chlorine concentrations (i.e., Daniel et al. 1990, 1991; NTP 1992). In the NTP (1992) studies, Fischer-344 rats received doses of up to 14.4 mg Cl/kg/day and B6C3F_1 mice up to 24.2 mg Cl/kg/day for up to 2 years. No significant alterations attributable to chlorine exposure were noticed in either species for a wide range of end points assessed.