SUMMARY REPORT

OF THE EXTERNAL PEER REVIEW OF THE DRAFT

TOXICOLOGICAL PROFILE FOR

CHLORINE

Submitted to:

The Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, MS F-32
Atlanta, GA 30333

Submitted by:

Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

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QUALITY NARRATIVE STATEMENT

ERG selected reviewers according to selection criteria provided by ATSDR. ATSDR confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled ATSDR's selection criteria. Reviewers conducted the review according to a charge prepared by ATSDR and instructions prepared by ERG. ERG checked the reviewers' written comments to ensure that each reviewer had provided a substantial response to each charge question (or that the reviewer had indicated that any question[s] not responded to was outside the reviewer's area of expertise). Since this is an independent external review, ERG did not edit the reviewers' comments in any way, but rather transmitted them unaltered to ATSDR

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SECTION I

PEER REVIEWERS' SUMMARY COMMENTS

SUMMARY COMMENTS RECEIVED FROM

John Balmes, M.D.
Professor in Residence
Department of Medicine
University of California, San Francisco
San Francisco, CA 94110
415-206-8953
Email: john.balmes@ucsf.edu

John R. Balmes

REVIEW OF CHLORINE TOXICOLOGICAL PROFILE

Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?

No

Are there any general issues relevant to child health that have not been discussed in the profile and should be?

No

CHAPTER 1. PUBLIC HEALTH STATEMENT

Does the chapter present the important information in a non-technical style suitable for the average citizen?

Yes

Do the answers to the questions of the major headings adequately address the concerns of the lay public? Are these summary statements consistent, and are they supported by the technical discussion in the remainder of the text?

Yes

Are scientific terms used that are too technical or require additional explanation?

p. 4, "Short-term exposure to chlorine in air" box The fourth bullet uses the term, "respiratory rhythm." This term may not be understood by the lay reader. I would substitute "breathing rate." The fifth bullet uses the term, "toxic pneumonitis." This term is too technical without additional explanation. I would revise the bullet as follows: "lung injury (toxic pneumonitis) ..."

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

Do you agree with those effects known to occur in humans as reported in the text?

Yes

Are the effects only observed in animals likely to be of concern to humans?

Yes.

Have exposure conditions been adequately described?

Yes

p. 10, lines 21-24 With the exception of emphysema, the description of the further complications of chlorine inhalation fits the histological condition known as diffuse alveolar damage that is associated with the clinical condition known as the adult respiratory distress syndrome. To reduce confusion, this should be noted in the text. In addition, while emphysema may have been described in war victims of chlorine gassing, it is not part of the early response to inhalation of chlorine or any other irritant gas. I would delete emphysema from line 23 to reduce confusion.

p. 11, lines 20-21 Studies do not conduct tests, investigators do. I would revise this sentence as follows: "No other study of chlorine-exposed subjects has included neurobehavioral testing,..."

p. 18, lines 7-8 Suggested revision as follows: "...possible exposures included sulfur dioxide, hydrogen sulfide, and methyl mercapatan, in addition to various particulates) found that, relative to a control group of rail workers, the pulp mill workers complained more frequently of usual phlegm, wheeze without cold, and chest illness (Enarson et al. 1984)."

CHAPTER 3. HEALTH EFFECTS

Section 3.1 INTRODUCTION

Section 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

Toxicity – Quality of Human Studies

Were adequately designed human studies identified in the text? If not, were the major limitations of the studies sufficiently described in the text?

Adequately designed human studies were identified for the acute effects of low-level exposures. This was not the case for long-term exposure to low-levels of chlorine, but the limitations of the studies of occupationally exposed humans were sufficiently explained in the text.

Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the profile?

Yes

Were all appropriate NOAELs and/or LOAELs identified for each study?

Yes

Were statistical test results of study data evaluated properly?

Yes

Are you aware of other studies which may be important in evaluating the toxicity of cresols.

No

Toxicity – Quality of Animal Studies

Were adequately designed animal studies identified in the text?

Yes

Were the animal species appropriate for the most significant toxicological endpoint of the study?

Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the profile?

Yes

Were all appropriate NOAELs and/or LOAELs identified for each study? Were all appropriate toxicological effects identified for the studies?

Yes

Were statistical test results of study data evaluated properly?

Yes

Are you aware of other studies which may be important in evaluating the toxicity of cresols.

Levels of Significant Exposure (LSE) Tables and Figures

Are the LSE tables and figures complete and self-explanatory? Does the "users Guide" explain clearly how to use them? Are exposure levels accurately presented for the route of exposure?

Yes

Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

Yes

If MRLs have been derived, are the values justifiable?

Yes

Evaluation of Text

Have the major limitations of the studies been adequately and accurately discussed?

Yes

Has the effect, or key endpoint, been critically evaluated for its relevance in both humans and animals?

Yes

Have "bottom-line" statements been made regarding the relevance of the endpoint for human health?

Yes

Are the conclusions appropriate given the overall database?

Yes

Has adequate attention been paid to dose-response relationships for both human and animal data?

Yes

Has the animal data been used to draw support for any known human effects?

Yes

p. 34, lines 30-32 This sentence about mediastinal air is confusing and should be deleted. Pneumomediastinum is not specific to chlorine and likely resulted from severe coughing.

pp. 36, last para. continued on p. 37 For consistency and increased clarity, I would use airway hyperresponsiveness instead of airway hyperreactivity. The usual abbreviation used in the literature is AHR rather than HR. I would not use the term, "bronchial hyperresponsiveness," even when used by the

primary authors (pp. 40-42), both because it is imprecise (methacholine induces constriction of small, non-bronchial airways) and unnecessarily different from airway hyperresponsiveness.

p. 40, line 10 Should be as follows: "...symptoms and chest x-rays and..."

p. 40, line 23 Suggest "airway" instead of bronchial responsiveness.

p. 40, line 31 Should be as follows: "Pulmonary function testing...

p. 40, line 33 Should be as follows: "...with an alveolar-capillary injury."

p. 41, line 31 Suggest "methacholine challenge tests" instead of bronchial responsiveness tests.

p. 41, lines 32 and 34 Suggest "airway" instead of bronchial responsiveness and bronchial hyperresponsiveness.

p. 42, line 17 Suggest "airway" instead of bronchial responsiveness.

p. 44, lines 27-28 I would revise this sentence as follows: "...it appeared that nitric oxide (NO) production may have contributed to the airway response to inhaled chlorine."

p. 46, line 22 The statement that reduced airflow at 25% vital capacity indicates some degree of small airway involvement may confuse clinicians because this point on the maximal expiratory flow-volume (MEFV) curve in human pulmonary function testing is referred to as FEF75 (forced expiratory flow at 75% of the vital capacity). I reviewed the Kutzman 1983 report and its use of EFR25 refers to the same point on the MEFV curve as the FEF75. Therefore, I would revise this sentence as follows: "...reduction in airflow at 75% of exhaled vital capacity in all exposed groups,..."

p. 47, line 21 Goblet cell should not be capitalized.

p. 47, line 30 Should be as follows: "Pulmonary diffusing capacity for CO..."

p. 47, lines 32-34 This sentence provides unnecessary detail that may cause confusion. I would delete it and revise the next sentence as follows: "There was no evidence of treatment-related effects on pulmonary function at any interval during the study."

p. 49, line 34 Should be as follows: "...due to a decrease in intravascular fluid..."

p. 54, line 18 Should be as follows: "...high concentrations of chlorine gas..."

p. 57, line 13 Suggest "respiratory protective gear" instead of protective garments (mouthpieces).

Section 3.3 GENOTOXICITY

Section 3.4 TOXICOKINETICS

Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance?

Yes

Have the major organs, tissues, etc. in which the substance is stored been identified?

Yes

Have all applicable metabolic parameters, pharmacokinetic/pharmacodynamic models and supporting data been presented?

Yes

Is there adequate discussion of the differences in toxicokinetics between humans and animals?

Yes, given the limited database available for such comparisons.

Is there an adequate discussion of the relevance of animal toxicokinetic information for humans?

Yes, given the limited database available for such a discussion.

If applicable, is there a discussion of the toxicokinetics of different forms of the substance?

Yes

Section 3.5 MECHANISMS OF ACTION

Have all possible mechanisms of action been discussed?

Yes

p. 77, line 35 For increased clarity, I suggest the following: "...in mice, a) an aerosol of sodium hypochlorite and b) chlorine gas, at equivalent concentrations,..."

Section 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Section 3.7 CHILDREN'S SUSCEPTIBILITY

Section 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Are the biomarkers of exposure specific for the substance or are they for a class of substances? Are there valid tests to measure the biomarker of exposure?

There are no adequate biomarkers of exposure for chlorine available at the current time and the text makes this clear.

Are the biomarkers of effect specific for the substance or are they for a class of substances? Are there valid tests to measure the biomarker of effect?

There are no adequate biomarkers of effect for chlorine available at the current time and the text makes this clear.

Section 3.9 INTERACTIONS WITH OTHER CHEMICALS

Is there adequate discussion of the interactive effects with other substances?

Yes

If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions?

Yes

p. 85, lines 23-24 Should be as follows: "...a recovery period..."

Section 3.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Is there a discussion of populations at higher risk because of biological differences which make them more susceptible? Do you agree with the choices of populations?

Yes

p. 86, line 16 Suggest "hyperresponsiveness" instead of hyperreactivity.

Section 3.11 METHODS FOR REDUCING TOXIC EFFECTS

Is the management and treatment specific for the substance, or is it general for a class of substances?

There is no specific treatment for chlorine-induced health effects. Only general therapeutic approaches for acute chlorine toxicity can be recommended. On p.89, lines 5-8, the text describes treatment for pulmonary edema in a manner that is both naïve and too specific. I would delete this sentence and substitute the following: "If pulmonary edema occurs, emergent treatment and monitoring in an intensive care unit is often necessary."

Is there any controversy associated with the treatment?

The use of 5% nebulized bicarbonate to neutralize the hydrochloric acid that forms in the airways after inhalation of chlorine is not accepted as standard therapy. On p. 89, line 3 specific beta-agonist bronchodilators are mentioned. I would revise this sentence as follows: "Nebulized bronchodilators should be used to treat bronchospasm."

Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance?

No

Are there treatments available to prevent the specific substance from reaching the target organ(s), or are the actions general for a class of substance?

There is no specific treatment to prevent chlorine from reaching target organs. Only general approaches can be recommended.

Is there any controversy associated with the treatment?

No

Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance?

No

Are there treatments to prevent adverse effects as the substance is being eliminated from the major organs/tissues where it has been stored?

No

Section 3.12 ADEQUACY OF THE DATABASE

Existing Information on Health Effects of Chlorine

Do you know of other studies that may fill a data gap?

No

Identification of Data Needs

Are the data needs presented in a neutral, non-judgmental fashion?

Yes

Do you agree with the identified data needs?

Yes

Does the text indicate whether any information on the data needs exists?

Yes

Does the text adequately justify why further development of the data need would be desirable; or conversely, justify the "inappropriateness" of developing the data need at present?

Yes

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

Are you aware of any information or values that are wrong or missing in the chemical and physical properties tables?

No

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

Are you aware of any information or values that are wrong or missing in the chemical and physical properties tables?

No

p. 104, line 35 There appears to be something missing from this sentence. It's not clear to what "hypo" is referring.

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites?

Yes

Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of cresols in all media?

Yes

Does the text cover pertinent information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there adequate discussion of the quality of the information?

Yes

Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of cresols, as well as populations with potentially high exposures? Do you agree with the selection of these populations?

Yes

Existing Information on Potential for Human Exposure to Chlorine

Do you know of other studies that may fill a data gap?

No

Identification of Data Needs

Are the data needs presented in a neutral, non-judgmental fashion?

Yes

Do you agree with the identified data needs?

Yes

Does the text indicate whether any information on the data needs exists?

Yes

Does the text adequately justify why further development of the data need would be desirable; or conversely, justify the "inappropriateness" of developing the data need at present?

Yes

CHAPTER 7. ANALYTICAL METHODS

Are you aware of additional methods that can be added to the tables?

No

Have methods been included for measuring key metabolites mentioned previously in the text?

Yes

If unique issues related to sampling for the substance exist, have they been adequately addressed in the text?

Yes

Existing Information on Analytical Methods for Chlorine

Do you know of other studies that may fill a data gap?

No

Identification of Data Needs

Are the data needs presented in a neutral, non-judgmental fashion?

Yes

Do you agree with the identified data needs?

Yes

Does the text indicate whether any information on the data needs exists?

Yes

Does the text adequately justify why further development of the data need would be desirable; or conversely, justify the "inappropriateness" of developing the data need at present?

Yes

CHAPTER 8. REGULATIONS AND ADVISORIES

Are you aware of other regulations or guidelines that may be appropriate for the table?

No

CHAPTER 9. REFERENCES

Are there additional references that provide new data or are there better studies than those already in the text?

Not of which I am aware.

UNPUBLISHED STUDY

Kutzman et al. 1983

This study was adequately designed. The methods used were appropriate. The reporting of results and their interpretation by the author are reasonable. I agree with the interpretation of the author.

SUMMARY COMMENTS RECEIVED FROM

Meryl Karol, Ph.D.
Professor Emeritus
Associate Dean for Academic Affairs and Research
University of Pittsburgh
Pittsburgh, PA 15261
412-431-6688
Email: mhk@pitt.edu

ATSDR Toxicological Profile on Chlorine

Summary Report

by

Meryl Karol

Overview

The profile has identified and presented the important sources of information relative to chlorine. Children's health and development have been addressed appropriately.

Chapter 1. Public Health Statement

This Chapter presents important information in a non-technical style. The use of questions as major headings is effective and the answers are appropriate for a lay public. The Section on Animal Testing (p. 3) is inappropriate and should be deleted.
Occasionally, alternative wording is suggested (by an underline) for clarification and/or tone.
The frequent misplacement of adjectives (see *) throughout this Chapter (and many others in the Report) often creates misinterpretations and always detracts from an otherwise excellent Report.

1. Page 1, line 14 Change *"so"* to highly
2. Page 1, line 19 Needs examples of how you contact chlorine. Insert at end of line 19, through inhalation, via skin contact, or by ingestion.
3. Page 2, line 4 Needs information regarding what chlorine reacts with when released into the atmosphere. Insert with a variety of chemicals, including water, to form strong acids, after *"and reacts"*.
4. Page 2. Line 8, "elemental chlorine" is a scientific term that needs further explanation for the intended readership.
5. *Page 3 line 3 (and throughout the review) misplaced modifiers cause misinterpretations. Change statement to "Chlorine gas ~~only~~ enters your body only when you breathe it in.
6. Page 3 line 3 Insert here - Chlorine is also absorbed following skin contact.
7. Page 3, lines 8-19. This section is inappropriate here. It is judgmental and out of place in a Public Health overview. I suggest it be deleted.

8. Page 4, section titled *Long-term exposure to chlorine in air.* Clarification needed of what is meant by the phrase, "relatively low concentrations". Suggest adding an example of such a concentration.
9. Page 4, insert after "(less than a cup)" typically of chlorine-treated drinking water
10. Page 5, line 5, Examples are needed of " Short-term exposures" (ie, 1 hr) and "longer-term" (ie, > 1 week). Most importantly, as worded, this section appears to contradict statements made elsewhere in the report that children are not small adults. This section **must be reworded** to indicate that the effect from chlorine depends on both the concentration of chlorine, the route of exposure, and, because of developmental considerations, on the age of the infant/child.
11. Page 5, **Birth defects** insert after "pregnant women" or pregnant animals
12. Page 5, **Birth defects** after "hypochlorite solution" insert the concentration used in the study described.
13. Page 5, line 15 last sentence, insert after "ingestion of" large amounts of hypochlorite
14. Page 6, lines 14-16. Change to: levels that affect animals; they are then adjusted to levels that will ~~help protect~~ not harm humans. ~~Sometimes~~ These not-to-exceed levels ~~differ among federal organizations because they used different~~ specify exposure times (an 8-hour workday or a 24-hour day). ~~, different animal studies, or other factors.~~ Sometimes they are based on animal studies.
15. Page 6, line 23 **Levels in air set by EPA,** reword as follows:
    EPA established an environmental air limit or 0.5 ppm. Exposure to higher levels could result in discomfort and irritation~~.~~ Dependent upon the concentration, these effects ~~are~~ may be reversible when exposure ends.

Chapter 2. Relevance to Public Health

This Chapter presents the important effects known to occur in humans exposed to chlorine. Appropriate extrapolation of animal data to human effects is addressed by specific comments below as are appropriate descriptions of exposure conditions.

1. Page 8, line 19. Usually reference is made to a chemical's half-life, not lifetime. Should the half-life should be used here? Graedel 1978 reference contained neither value.
2. Page 10, line 17. Description of "alveolar capillary *congestion*" unclear.

Should state that edema is due to damage to the lung tissue.

3. Page 10, lines 21-24 are unclear. What does "which" (line 22) refer to?
4. Page 10, line 25, misplaced modifier confuses meaning of the sentence. Change to "concentrations of chlorine may still be ~~still~~ in danger of delayed…"
5. Page 10, line 32 another misplaced adjective, change to "departments following ~~high~~ exposure to high chlorine gas"
6. Page 11, lines 1-2. Change to "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. ~~; the same can be said about anxiety~~.
7. Page 11, line 15. Change to "residual effects will be present ~~are detected~~, including"
8. Page 11, line 21. Delete "easily"
9. Page 12, line 9. Change to "though, under normal pH, the predominant species are expected to be hypochlorous acid and hypochlorite. ~~under normal pH.~~
10. Page 12, line 25. Consistency is needed in presentation of units. Providing both ppm and mg/L throughout the document is suggested.
11. Page 13, line 5. Change to "it is this property that may result in the irritant contact dermatitis."
12. Page 13, lines 16-26. For each study, please provide the number of months that the animals were exposed to the agent.
13. Page 13, line 20. Change "immune ~~on~~ or nervous system".
14. Page 13, line 21. More information is needed here regarding the nature of the immune parameters that were affected.
15. Page 13, line 32. Change to "risk of adverse (noncarcinogenic) effects ~~(noncarcinogenic)~~ over a specified"
16. Page 14, line 20 . Define "sensory irritant".
17. Page 14, lines 21-25. Reword the rambling sentence. The following is suggested: "Information that could be used for quantitative risk assessment regarding effects ~~of~~ from acute exposure of humans to chlorine ~~in humans that could be used for quantitative risk assessment~~ is available from ~~several~~ studies ~~in~~ of volunteers exposed to chlorine gas ~~under controlled conditions for periods ranging between~~ for 15 minutes ~~and~~ - 8 hours "
18. Page 15, line 19 insert word as follows: "decrease in respiratory rate has been "
19. Page 15, lines 26-28. Clarification needed as to whether the sensory irritation was measured in humans or in animals.
20. Page 15, line 29. Clarify what is meant by a "co-principal" study?

21. Page 16, lines 7-8 need clarification. The following is suggested: "suggesting that the response was related to ~~some function of concentration and~~ duration <u>in addition</u> ~~rather than~~ to concentration. ~~alone.~~
22. Page 16, line 13. It is not clearly stated here how the 0.2 ppm MRL was derived. Refer to a fuller explanation of how the NOEL of 0.5 ppm was used to derive a 0.2 ppm MRL.
23. Page 16 Justification is needed for the use of the unpublished Kutzman study (1983) to derive an MRL. Further, the Kutzman study is compromised by the use of animals with underlying lung disease.
24. Page 22 Delete sentence on lines 11-13 since there is no basis for this negative statement regarding absence of mechanistic information. There is no reason to discount the results of the Exon et al (1987) study (especially when it is considered in the deliberation on P. 23).

Chapter 3. Health Effects

This Chapter is appropriate for public health officials, physicians and concerned citizens. Specific comments regarding appropriate use of animals, interpretation of studies and identification of NOAELS and LOAELs are presented below.

1. Page 28, lines 16-28. Where possible, please provide estimates of the airborne chlorine concentrations resulting from these spills.
2. Figure 1 is too complicated for meaningful interpretation; it must be simplified. The legend should explain the numbers used in the figure; *Less* and *More Serious* should be defined both in the figure and in Table 3-1. Does *h* refer to human?
3. Table 3-1. Convert 960 min into hrs.
4. Page 31, line 1. The statement that the upper portion of the respiratory system is the target for exposure should be qualified with inclusion of statements as to the species (and its breathing pattern) and the concentration at exposure.
5. Table 3-2 should be updated using more recent data. Does it refer only to human data ?
6. Page 31, line 23. Isn't there information more recent than a 1976 NIOSH review?
7. Page 37, lines 28-34. The (in)appropriateness of the 0.5 ppm NOEL for sensitive populations should be stated.
8. Page 39, lines 16-17. Clarify what is meant by "in principle" the concentration was always below 0.5 ppm.

9. Page 40, lines 26-33. A statement should be added that without knowledge of exposure concentration or duration, this study has minimal value.
10. Page 41, lines 5-18. A statement similar to the one above should also be made about the study reported here.
11. Page 43, line 7. Insert the underlined as follows: "The concentration of the chemicals that induces a 50% decrease in respiratory rate is termed RD50."
12. Page 46, lines 28-29. An explanation is needed for why the unpublished Kurtzman 1983 report was selected to be the basis for the MRL derivation.
13. Page 67, Section 3.2.2.7. A brief overview is needed of the carcinogenicity of chlorinated organics that form as a result of chlorination of drinking water. Emphasis should be placed on the concentration of chloride in the water and the frequency, and types, of cancers noted.
14. Page 70, lines 10-13. Unpublished information should not be included in this review without careful scrutiny (self- scientific review) of the study.
15. Page 70 lines 17-23. The term "allergic contact dermatitis" should be changed to "contact dermatitis" in agreement with the author's (Osmundsen) description of the lesion. Further this information should be moved to section 3.2.3.2 **Dermal effects**
16. Page 77 line 1, EPA has reported development of a PBPK model for chlorine. I suggest you contact them for a possible preprint of the article (see Abstract below)

Modular Application of Computational Models of Inhaled Reactive Gas Dosimetry for Risk Assessment of Respiratory Tract Toxicity: Chlorine

Authors: Annie Jarabek[1], Jeffry Schroeter[2], Melvin Andersen[2], Julia Kimbell[2]
[1]U.S. EPA/Office of Research and Development (ORD)/National Center for Environmental Assessment (NCEA)/Immediate Office (IO) and National Health and Environmental Effects Research Laboratory (NHEERL)/Experimental Toxicology Division (ETD)/Pulmonary Toxicology Branch (PTB)
[2]The Hamner Institutes for Health Sciences, United States

Keywords: respiratory tract, reactive gases, epithelial perturbation, oxidative stress, chlorine

Inhaled reactive gases typically cause respiratory tract toxicity with a prominent proximal to distal lesion pattern. This pattern is largely driven by airflow, and interspecies differences between rodents and humans result from factors such as airway architecture, ventilation rate, breathing mode, and the metabolic capacity of different tissue types. Accurate extrapolation of the dose-response for respiratory toxicity observed in rodents to predict human health risk requires description of these factors at a level of detail commensurate with the experimental data and understanding of the mode of action (MOA) for the inhaled gas. A suite of models can be employed in a modular fashion to address the need for different descriptions that depend on the species and level of detail in the data. Hybrid computational fluid dynamics- physiologically-based pharmacokinetic (CFD-PBPK) models afford the flexibility to predict different dose metrics in the upper respiratory tract (URT) that range from average flux in the entire region to localized estimates. The dose description can be extended into the tissues with PBPK compartments for metabolism and other reactions. A modular application is provided by a CFD-PBPK model for inhaled chlorine. The hypothesized MOA for chlorine is that its irritant effects are due to oxidative stress mediated by hypochlorous acid (HOCl). HOCl forms in epithelial tissues by hydrolysis and downstream biological responses. A CFD-PBPK model was developed using experimental data on chlorine uptake delivered in situ to the isolated URT of F344 rats. Tissue chlorotyrosine (3-chloro- and 3,5-dichlorotyrosine), measured in samples from four different regions representing respiratory and olfactory tissues in both septal and lateral airstreams, was used as an internal dosimeter. The CFD mesh was segmented to provide estimates of chlorine flux in each region. The PBPK model of the tissue describes rates for chlorine hydrolysis, reaction of HOCl with proteins, and scavenging of reactive species by soluble anti-oxidants. Human dose estimates, which require consideration of delivery to the lower respiratory tract (LRT) due to mouth breathing, are calculated by calibration of the CFD-PBPK model structures of the human URT to typical-path descriptions of the entire respiratory tract.

This abstract does not reflect U.S. Environmental Protection Agency policy.

Point of Contact:
Annie Jarabek
Special Assistant/Senior Toxicologist
U.S. EPA/ORD/NCEA/IO and NHEERL/ETD/PTB
B-143-01

17. Page 79, line 1. Shouldn't this line read "and no single animal species has emerged as a preferred animal model for human gastric toxicity"?
18. Page 80, line 11, change "no" to "not".
19. Page 80, lines 15-17. Specify the chlorine species to which the rats were exposed.
20. Page 89, Section 3.12.1, figs 3-4 and 3-5 are clear and provide some good information. However, they could be made even more informative. Include target organs (information given on p. 90 lines 8-16 re lung, eye, etc) and (LOAEL for each organ). It would be very helpful to designate the quality of each the studies (employing, perhaps, a scale of 1-5).

21. Page 91, Section 3.12.2 The studies should be *evaluated* before concluding that more research is, or is not, needed. Studies may disagree with regard to persistent effects, but the quality of the study may suggest whether its conclusions are valid.
22. Page 92, lines 26-27. It is inappropriate to select an unpublished study as the principal work on which to base a MRL, especially when peer-reviewed, published studies are available.
23. Page 93, line 8. The meaning of this sentence is unclear.
24. Page 94, line 12-14. I see no need to conduct this study if epidemiologic data do not suggest such an effect in humans.
25. Page 96, lines 19-29. A mechanism utilizing oxidative reactions could be hypothesized for immunotoxicity from chlorine in water.
26. Page 96, lines 31-33. Clarification is needed as to whether the reports concluded "contact dermatitis' or 'allergic contact dermatitis".
27. Page 97, lines 27-28. Considering the size and expense of such a study, it does not seem prudent to suggest undertaking a long-term, low-dose inhalation study in monkeys to evaluate what is expected to be minimal nasal changes.
28. Page 100, Section 3.12.3. There appear to be ongoing studies at the EPA. See Abstract of Jabarek et al 2007 (p. 5 of this Report).

Chapter 4. Chemical and Physical Information

The information presented in this Chapter is appropriate and complete.

Chapter 5. Production, Import/Export, Use and Disposal

The information is appropriate. Specific suggestions for clarification are below.

1. Page 104, lines 1-4. The numbers in Table 5-2 should be checked; there seems to be a problem with columns 3 and 4.
2. Page 104, Section 5.3. Reference should be made to Table 5-2 that contains information on uses.

Chapter 6. Potential for Human Exposure

The text is appropriate and appears to be complete. Specific comments are below.

1. Page 106, line 21, and P. 110, line 23, Can half-life be calculated?
2. Page 108, lines 2-13. Please indicate why, from all the known accidental spills that have occurred, these particular reports were selected for presentation here.
3. Page 114, lines 20-28. If this is a direct quote from the NRC monograph, there should be quotation marks.
4. Page 116, Section 6.8.1. *Environmental Fate.* It would be very helpful to include the half-life of chlorine released into the air (as a function of altitude) and to specify the factors that accelerate and retard its decomposition.
5. Page 117, lines 26 and 33-34. Specify how humans would be monitored. What species/characteristics/activities would be monitored?
6. Page 119, Section 7.1 Information should be added concerning the *in vivo* formation of organochlorine products such as di- and trichloroacetic acids. Chloroform has been identified in the stomach contents of the animals dosed with NaOCl but was not detected in control animals.
7. Page 124, line18. Insert the chlorine concentration used in the exposure.

Chapter 7. Analytical Methods

The Chapter appears to be complete.

Chapter 8. Regulations and Advisories

The Chapter appears to be complete.

Chapter 9. References

The Chapter is complete.

Unpublished Study

See Review of Kurtzman 1983 BNL report

Review of Kurtzman 1983, "*A Study of Fischer -344 Rats Subchronically Exposed to 0,0.5, 1.5, or 5.0 ppm Chlorine*". BNL report 32710

The objective of the study was to relate chlorine-induced compositional, structural and functional changes in Fischer 344 rats. Exposure, via inhalation, was for 62 days (6h/day, 5 d/wk) at 0, 0.5, 1.5 or 5 ppm chlorine. They were then held for 6 days before examination.

Major findings

1. Upper respiratory tract and ocular irritation
2. at all concentrations, female rats fared worse than male rats (reduced weight gain).
3. Lung physiology and lesions not remarkable; subtle tracheal changes in high exposure group.
4. Collagen increase noted in lungs of 1.5and 5 ppm groups.
5. Can discriminate between exposed and control groups by collagen, elastin, and functional reserve capacity (although not statistically significant).

Critique

1. The effects from possible by-products of the chlorine exposure, and from its reactions with animal waste must be considered. This is a serious drawback of the study. The authors note that chloramines were formed from the reaction of chlorine with urine in the chamber. In addition, endogenously produced ammonia was measured in the chamber and was dependent on the animal loading, airflow through the chamber, and the animal waste. The reaction of chlorine with stainless steel was assessed by measurement of several metals, but reaction of chlorine with Lucite was not considered. Being an acrylic, lucite is likely susceptible to oxidizing agents such as chlorine. Chloramine is an irritant and damages mucous membranes, and has been associated with asthma. The mean concentrations in the chambers were reported to be o.42, 0.49 and 0.63 ppm.

2. P.11 look up recent studies of immune effects from chlorine.
3. The female rats were severely affected by the chlorine as indicated by their lack of weight gain at all chlorine concentrations.
4. Adaptation of animals to chlorine was noted making difficult extrapolation of findings to acute exposure situations. In addition, the effect of the 6 day delay following chlorine exposure before assessing the health effects should be considered. The rationale given for the delay (avoidance of acute effects) is not reasonable since acute effects are not expected after 62 days of exposure unless there is complete recovery overnight, each night.
5. What is serious and less serious (p.2)
6. Barrow (ref 6) reported more severe effects in rats exposed to 1,3,9 ppm Chlorine for 30 days. Evaluated rats within 1 day following exposure, 10 females and 10 males. Weight gain similar as here.
7. Animal to human factor of 3 or 10?
8. Figure 3-1 is incomprehensible. The information it contains should be in Table format.
9. The lungs of the control animals were compromised in that there was evidence of low grade pneumonia and focal acute alveolitis. This compromises pulmonary LOAEL and NOAEL calculations. Moreover, the authors cite refs 30 and 44 as indicating that the "pathology observed in the rodent respiratory system by exposure to chlorine gas seems to be dependent upon the initial health of the lung." Thus the pathology observed in this study is of questionable relationship to chlorine exposure.
10. The absence of a correlation between structural and functional changes in the lungs is troubling, as is the negative correlation of pathology rank with elastin and hydroxyproline, and the negative relationship between protein and lung function parameters in the controls.

SUMMARY COMMENTS RECEIVED FROM

Dennis Shusterman, M.D., MPH
Faculty Physician
Occupational and Environmental Medicine Program
University of Washington
Seattle, WA 98104
206-744-9398
Email: dennis3@u.washington.edu

Review of
DRAFT Toxicological Profile for Chlorine

Reviewer: Dennis Shusterman, MD, MPH
Seattle, WA

In completion of Consulting Agreement 0133.06.004/46 with Eastern Research Group, Inc.

The above document, marked DRAFT 2, was reviewed in accordance with the Guidelines for Peer Review supplied to the reviewer. Special attention is focused on child health and development, specifically, in answering the following questions: * Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? * Are there any general issues relevant to child health that have not been discussed in the profile and should be? Annotation was made directly on the draft, and in addition, the following editorial comments are offered:

Section 1 – Public Health Statement

The listing of health effects due to "short-term exposure to chlorine in air" should include concentration ranges for all health endpoints for which an exposure duration is not offered (e.g., it currently states "eye irritation at 5 ppm.") This is not only because of uncertainty and biological variability, but also because exposure time is a co-factor in the occurrence of health effects. (p. 4) More specifically, in Anglen (1981), dose- and time-related trends in subjective eye irritation were apparent at and above 1.0 ppm.

The tabular listing stating "Short-term exposures to high concentration of chlorine affect children in the same manner they affect adults." First, these effects should be briefly synopsized, as indicated in the attached annotations. Equally important, however, aside from the acute effects involving rhinitis, conjunctivitis, tracheo-bronchitis, bronchospasm, RADS and chemical pneumonitis, we actually do not know whether the *sequelae* of acute, high-level exposures in children differ from those in adults. (p. 5)

Under protective recommendations, the authors indicate that "OSHA has set a legal limit of 1 ppm chlorine in air averaged over an 8-hour working day." In fact, this 8-hour limit is a CEILING concentration (i.e., not to be exceeded at any time). Both NIOSH and ACGME recommend a 0.5 ppm 8-hour time-weighted average (as well as a 1.0 ppm 15-min. STEL), but neither of these have been adopted by OSHA. (p. 6).

Section 2 – Relevance to Public Health

After paraphrasing a series of studies by Kilburn purporting to document long-term neurobehavioral effects of chlorine exposure, the report states: "...this could be *easily* examined in animal models." [italics mine] In fact, while standardized methods for study neurobehavioral toxicity in animals do exist, *extrapolation* to humans can be quite problematic. Suggested alternative language: "...could *potentially* be examined in animal models." (p. 11)

At the bottom of page 15, the report catalogs acute-duration human inhalation studies, including those with pulmonary, sensory, and rhinologic endpoints. On the next page the discussion goes on to focus on those studies incorporating pulmonary function endpoints, then to justify an acute-duration MRL of chlorine of 0.2 ppm based on these alone. There are two problems with this argument: 1) The discussion cones down on pulmonary endpoints (and excludes rhinologic) without explicitly stating that it is doing so; 2) the mathematical derivation of the MRL is not provided.

Section 3 – Health Effects

In the introduction to Section 3.2 (p. 26), the report lists potential health effects ("death, systemic...") without listing respiratory.

On page 33, line 14, the abbreviation "FEF" is taken to mean "fixed expiratory flow," when in fact it is "forced expiratory flow."

On page 35, line 14, the term "chlorine fumes" is used, when the correct terminology is, in fact, "chlorine gas." The term "fume" is reserved to refer to combustion products (specifically, nascent oxides of metals or polymers).

On page 36, line 7, the report refers to Rotman, 1983 as a "follow-up study" to Anglen, 1981. However, it is unclear whether this was, indeed, a separate study or a substudy (phase II) of Anglen. This ambiguity is reinforced by the following statement in Rotman (pp. 1122): "...the subjects found that the 1-ppm exposure days were distinguishable from the control or sham days by the occurrence of itchy eyes (etc..)..." Rotman then cites Anglen for this statement. Further, the number of subjects in Anglen's phase II (9) closely approximates the number in Rotman (8). I'm not sure if it is possible to clear up this ambiguity other than by contacting the authors for clarification.

On page 37, lines 12-13, in reviewing the report by Schins et al. (2000) the report states "...subjective complaints by the subjects were judged to be not treatment-related." This point was critiqued as methodologically flawed in correspondence by **Shusterman et al. (2002)** (ATTACHED).

On pages 38-39, several studies of chlorine-exposed pulp mill workers are reviewed. It is not until the last review (p. 38; lines 33-34) that the complexity of potential pulp mill exposures is reviewed. It might serve well to move up a generic discussion of exposures to an introductory paragraph so that this information is in mind for all of the studies. In addition, more specific information as to the type of pulp mill (kraft, sulfite) would help establish competing exposures.

On page 40, in the summary of a case series by Moulick et al., the paragraph concludes with the phrase "...and pulmonary function tests were normal" (lines 11-12). Ideally, summaries of all case reports and case series should include a statement whether or not a test of nonspecific bronchial responsiveness (e.g., methacholine challenge) has been performed.

On page 43, line 11, the report references "...rats pre-exposed to chlorine at 1, 5, or 10 ppm..." It would be useful in understanding this experiment to know, not only the duration of this pre-exposure, but also the interval between pre-exposure and measurement of the RD_{50}.

On page 44, line 16, the report states "...showed no specific airway pathology" in referring to rabbits allowed to recover from chlorine-induced "chronic pneumonitis and anatomic emphysema." Since complete recovery from these conditions seems unusual, perhaps the reviewers meant to distinguish between "airway pathology" and "pulmonary pathology" (the latter also including alveolar pathology)?

On page 46, line 16, the report refers to "diffusing capacity for CO_2." Is the intended reference to "diffusion capacity for CO?"

On page 54, lines 3-5, the report states: "...headache, dizziness, anxiety, and syncope are commonly reported following acute high exposures to chlorine and are thought to be due, at least in part to anoxic anoxia induced by chlorine." If by "anoxic anoxia" the authors are referring to simple asphyxia, it is important to note that, in order to achieve an "oxygen-deficient atmosphere (FIO2 < 19% at sea level, or a 10% relative reduction of FIO2), it would be necessary to have chlorine levels of at least 10% (or 100,000 ppm), concentrations that would be rapidly fatal.

On page 60, line 26, there is an apparent missing word "...caused the death 8 dogs..."

On page 67, line 22, there is an awkward dependent clause: "... did not occur in males..."

On page 70, lines 7-13, reference is made to "irritation" of the cornea. Irritation is generally gauged by a vascular inflammatory response, and in the absence of neovascularization, the cornea is normally avascular. Thus, the word "irritation" is more often applied to the conjunctiva, whereas "erosion" is applied to the cornea.

On page 72, line 23 et seq., some discussion is line for the use of the term "absorption." The word implies that a substance (xenobiotic) passes an epithelial or mucosal barrier and is either taken up into the circulation or is at least locally deposited in tissue. In the case of chlorine gas, the agent's surface reactivity is so great that this criterion is not met. Thus, Nodelman and Ultman's experiments could best be described as measurements of chlorine "clearance," rather than absorption. This point is key to the lack of data in the subsequent discussions of distribution, metabolism, and elimination, since there is no tissue burden of chlorine to distribute, metabolize, or eliminate.

Also on page 72, line 35, the statement is made "...absorption appeared to be concentration-related." A more precise expression might be "...absorption appeared to be non-saturable."

On page 86, line 13, the report indicates that people with hay fever are more susceptible to the effects of chlorine, but does not cite the work on this (**Shusterman et al., 1998** [ATTACHED]; 2003b).

On page 89, lines 3-4, the report states: "Therapy with corticosteroids has not been proved to produce improvement in chlorine gas poisoning." This statement, which presumably refers to systemic / oral steroids, is unreferenced. In fact, individuals experiencing bronchospasm and/or persistent bronchial hyperreactivity (i.e., incipient or fully developed RADS) should be treated with inhaled steroids and other anti-asthma measures per NHLBI guidelines.

On page 92, the report references "eye and skin irritation" in volunteers exposed to 1 ppm in the Anglen & Rotman studies. "Skin irritation" was not included in Anglen's questionnaire (Figure 4, page 22), and, as noted above, the Rotman analysis appears to be based on the same experiment.

On page 97 the report advocates "...neurobehavioral evalutions in subjects known to have been exposed to high concentrations of chlorine..." I would add: "with suitable comparison populations matched for the prior occurrence of a non-chemically related traumatic event."

Section 8 – Regulations and Advisories

On page 125, line 18, the report refers to an "8-hour time-weighted average" OSHA exposure limit for chlorine. As noted above, this should read "ceiling."

Auxiliary review: NTP Bioassay

No comments

Additional References supplied as .pdf files:

Shusterman D, Murphy M, Balmes J. Seasonal allergic rhinitic and non-rhinitic subjects react differentially to provocation with chlorine gas. *J Allergy Clin Immunol* 1998; 101:732-740.

Shusterman D, Solomon C, Balmes J, Blanc P. Chlorine exposure and the upper respiratory tract. *Eur Respir J* 2002;19:381-383.

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SECTION II

ADDITIONAL REFERENCES AND DATA SUBMITTED BY THE PEER REVIEWERS

ADDITIONAL REFERENCES AND DATA SUBMITTED BY

Dennis Shusterman, M.D., MPH
Faculty Physician
Occupational and Environmental Medicine Program
University of Washington
Seattle, WA 98104
206-744-9398
Email: dennis3@u.washington.edu

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CORRESPONDENCE

Chlorine exposure and the upper respiratory tract

To the Editor:

We read with great interest the report of SCHINS *et al.* [1] on their controlled human-exposure study of chlorine inhalation. The air pollutant studied, gaseous chlorine, is one of substantial relevance in terms of total industrial usage and involvement in emergency release scenarios.

The authors referred to "...a paucity of human data on the effect of chlorine on the upper respiratory tract". Their literature review, however, overlooked two recent and pertinent studies from our institution pertaining to the effects of Cl_2 on both the upper and lower respiratory tracts. D'ALESSANDRO *et al.* [2] documented a significantly greater acute bronchial (obstructive) response in asthmatic *versus* normal volunteers exposed to 1.0, but not 0.4 parts per million (ppm) Cl_2 for 15 min [2]. SHUSTERMAN *et al.* [3] demonstrated significantly higher nasal irritation ratings and nasal congestion (assessed by rhinomanometry) among seasonal allergic rhinitic volunteers (as compared to normal controls) exposed to chlorine at 0.5 ppm×15 min. A common denominator of these studies is the need to identify potentially susceptible subpopulations in order to provide the most sensitive assay for potential population-based health effects.

The inability of SCHINS *et al.* [1] to document significant subjective complaints in response to Cl_2 exposures as high as 0.5 ppm×6 h, may relate to the manner in which symptoms were recorded, which did not include baseline (pre-exposure) measures and was tempered by a physician's subjective estimation of the likelihood of relatedness exposure. Moreover, the study did not employ objective physiological measures of nasal irritant response (*e.g.* rhinomanometry, acoustic rhinometry, nasal peak flow measurement, or rhinostereometry). Given these limitations, the negative findings of the study should be viewed with caution, especially in light of other positive studies with comparable exposure levels that were not discussed.

D. Shusterman*, C. Solomon#, J. Balmes*, P. Blanc*
*Division of Occupational and Environmental Medicine, and #Lung Biology Centre, University of California, San Francisco, CA, USA.

References

1. Schins RPF, Emmen H, Hoogendijk L, Borm PJA. Nasal inflammatory and respiratory parameters in human volunteers during and after repeated exposure to chlorine. *Eur Respir J* 2000; 16: 626–632.
2. D'Alessandro A, Kuschner W, Wong H, Boushey HA, Blanc PD. Exaggerated responses to chlorine inhalation among persons with nonspecific airway hyperreactivity. *Chest* 1996; 109: 331–337.
3. Shusterman D, Murphy M, Balmes J. Seasonal allergic rhinitis and non-rhinitic subjects react differentially to provocation with chlorine gas. *J Allergy Clin Immunol* 1998; 101: 732–740.

From the authors:

We read with interest the comments of D. Shusterman and colleagues to our human exposure study with gaseous chlorine. Although it may seem like we have "overlooked" the two studies referred to in their letter, there are several reasons why these controlled human exposure studies were not discussed in our paper.

The major reason is that we set out to study potential adverse effects of chlorine in a healthy population, specifically excluding those with rhinitis or nonspecific bronchial hyperreactivity in our extensive screening efforts. We don't see why our data should be "viewed with caution", when we aimed to study the nasal and pulmonary effects in healthy individuals, instead of subjects that are known to be more sensitive (at lower concentrations) showing exaggerated responses to inhaled irritants in general. In addition a nonsignificant congestive and obstructive response in normal subjects exposed to 0.4 parts per million (ppm, 60 min) or 0.5 ppm (15 min) of chlorine were reported in their own studies.

The authors, however, do have a point when they suggest objective physiological measures of nasal irritant responses. Although such measurements, which also included eye-irritation, were suggested in our initial study proposal, they were not included in the final protocol due to technical and financial reasons. However, the utmost precision was taken to score "subjective" symptoms in all four exposure conditions, where consistency, driven by an exposure-response relationship was needed to establish a symptom as an adverse effect related to chlorine exposure. In addition, a detailed medical investigation was performed at prestudy intake, and a daily short check-up was conducted before each exposure session. This information was not provided in the paper. With regard to subjective symptoms, in our study most subjects indicated they could smell the presence of chlorine already at the lowest concentration (0.1 ppm) but they were not able to discriminate between the

three different exposure levels. Considering our experience, we find it surprising that none of the subjects tested in their studies were aware of chlorine exposure, whereas half of them were hyperreactive and exposed well over the mean odour threshold of chlorine [1].

Taken together these data suggest that normal subjects do not show adverse effects <0.5 ppm chlorine up to several hours (repeated) exposure, whereas sensitive subjects (with rhinitis or hyperreactivity) show objective effects at such levels. It is up to regulatory committees to decide whether occupational exposure levels should be set to a no-effect level in highly sensitive groups.

P.J.A. Borm*, R.P.F. Schins*, H. Emmen#
*Institut für Umweltmedizinische Forschung (IUF), Düsseldorf, Germany. #TNO Voeding, Zeist, the Netherlands.

References

1. D'Allesandro A, Kuschner W, Wong H, Boushey HA, Blanc PD. Exaggerated responses to chlorine inhalation among persons with non-specific airway hyperreactivity. *Chest* 1996; 109: 331–337.

Subjects with seasonal allergic rhinitis and nonrhinitic subjects react differentially to nasal provocation with chlorine gas

Dennis J. Shusterman, MD, MPH,[a] Mary Alice Murphy, MD, MPH,[b] and John R. Balmes, MD[a] *San Francisco, Calif.*

Background: Nasal irritation and associated symptoms (nasal congestion, rhinorrhea, and sinus headache) are important elements of the response to indoor and outdoor air pollution. Marked interindividual variability in such symptoms has been suggested clinically and epidemiologically, but little experimental data exist on this issue.

Objective: We sought to test the hypothesis that subjects with seasonal allergic rhinitis (SAR) exhibit a more marked physiologic response (congestion) after nasal irritant provocation than do nonrhinitic subjects.

Methods: We studied eight subjects with SAR and eight nonrhinitic subjects; subjects with SAR were studied out of season. In a single-blind crossover study, subjects had their nasal airway resistance (NAR) measured in triplicate before, immediately after, and 15 minutes after a 15-minute exposure to either filtered air or 0.5 ppm chlorine in filtered air, administered through a nasal mask in a climate-controlled chamber. Log-transformed NAR values were analyzed in a repeated-measures analysis of variance model, with confirmatory testing using paired t tests.

Results: The net (chlorine minus air day) percent change in NAR from baseline (before exposure) to immediately after exposure was +24% in the SAR group and +3% in the nonrhinitic group ($p < 0.05$). The corresponding net changes from baseline to 15 minutes after exposure were +21% in the SAR group and −1% in the nonrhinitic group ($p < 0.05$).

Conclusions: The observed augmented nasal congestive response of subjects with SAR versus nonrhinitic subjects to a controlled low-level chemical irritant provocation is consistent with epidemiologic surveys showing a higher prevalence of nasal symptoms among subjects with SAR than nonrhinitic subjects in environments involving irritant air pollutants. (J Allergy Clin Immunol 1998;101:732-40.)

Key words: Seasonal allergic rhinitis, nasal irritation, rhinomanometry

Epidemiologically, eye, nose, and throat irritation (trigeminally mediated sensations) are among the acute symptoms most frequently reported by individuals exposed to environmental tobacco smoke,[1,2] workers in problem buildings,[3-6] and residents living near selected industrial emission sources.[7,8] In addition, irritant-associated symptoms of the upper respiratory tract (e.g., nasal congestion, rhinorrhea, and sinus headache) may mimic an allergic response, posing a potential problem of differential diagnosis for the clinician.[9] In light of these facts, any systematic differences in nasal-irritant sensitivity within the population would be of interest to clinicians, public health practitioners, and chemical risk assessors.

Abbreviations used

ETS:	Environmental tobacco smoke
NAR:	Nasal airway resistance
RANOVA:	Repeated-measures analysis of variance
SAR:	Seasonal allergic rhinitis

Several observers have linked nasal reactivity to environmental irritants (including environmental tobacco smoke and volatile organic compounds) with preexisting allergic rhinitis. This link has appeared in epidemiologic surveys,[2-4] as well as in limited experimental studies.[9,10] If this link is real, it could have important implications because up to 20% of the United States population has allergic rhinitis and could therefore constitute a susceptible subgroup with respect to the effects of irritant air pollutants.[11] The current experiment seeks to examine this issue directly, comparing the physiologic reactivity to irritant provocation of two groups: subjects with seasonal allergic rhinitis (SAR) and nonrhinitic subjects.

METHODS

The study consisted of a randomized cross-over experiment in which each subject, serving as his or her own control, breathed either an irritant atmosphere (chlorine gas at 0.5 ppm) or clean air during 15-minute exposure periods 1 week apart (Fig. 1). The physiologic endpoint of interest was nasal airway resistance (NAR), as documented by active posterior rhinomanometry performed before, immediately after, and 15 minutes after the exposure sessions. Equal numbers of subjects with SAR and nonrhinitic subjects were tested, and subjects with SAR were tested out of season. The study design was counterbalanced with respect to subject gender and order of exposure (i.e., chlorine or air first). The aim of the experiment was to test the hypothesis that subjects with SAR will exhibit a more marked physiologic response (congestion) to a given nasal irritant provocation than will nonrhinitic subjects.

From [a]the Division of Occupational and Environmental Medicine and [b]the Department of Pediatrics, University of California, San Francisco.

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Reprint requests: Dennis Shusterman, MD, MPH, Upper Airway Biology Laboratory, University of California, Berkeley/Richmond Field Station, 1301 So. 46th St., Bldg. 112, Richmond, CA 94804.

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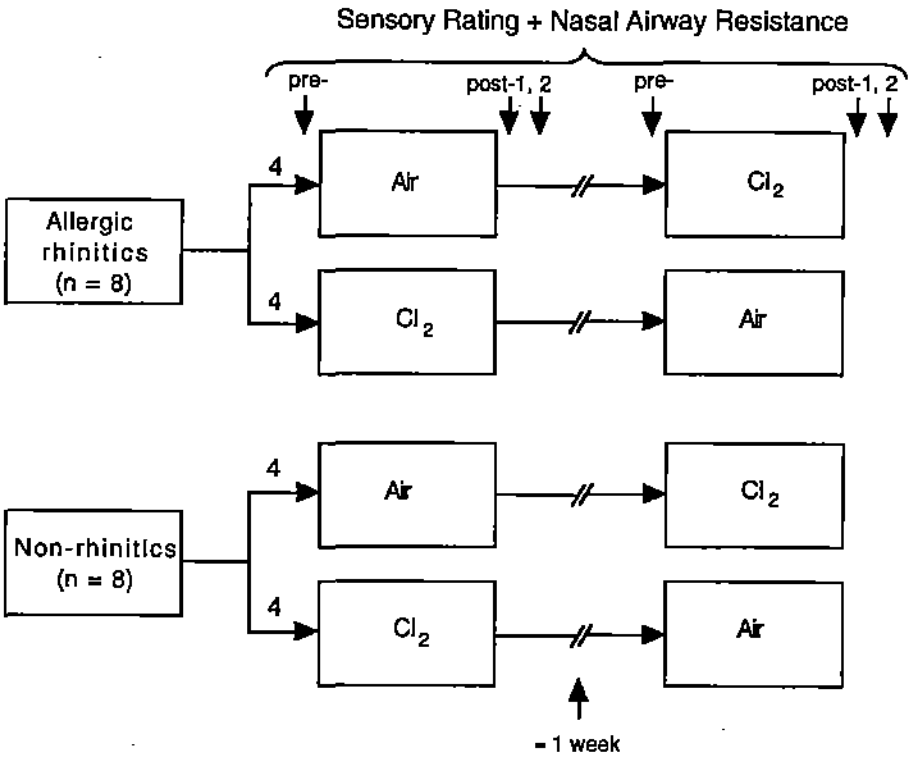

FIG. 1. Overall study design for chlorine provocation. Study was counterbalanced with respect to both gender and order of exposure (i.e., equal numbers of male and female rhinitic and nonrhinitic subjects were exposed to air or chlorine first).

Chlorine was chosen as the provocation agent of choice because (1) it is highly water soluble and hence likely to produce predominantly upper respiratory tract symptoms when administered nasally at an appropriate concentration; (2) it is considered neither a carcinogen or teratogen by the US Environmental Protection Agency; (3) as a gas its concentration is relatively easily controlled; and (4) it is environmentally relevant in terms of its role in accidental releases and household chemical mishaps.[12, 13] In keeping with the goal of achieving a predominant upper airway effect, the concentration and duration of exposure was chosen on the basis of a review of prior controlled human exposure studies with this agent.[14-16] Finally, as a further safeguard against potential adverse testing events, subjects who were identified as having asthma were excluded (see below).

Subjects were recruited by using an advertisement in a student newspaper and postings at a college campus and university medical center. The single inclusion criterion was age between 18 and 40 years. Exclusion criteria included current cigarette smoking (or within the previous 6 months), a previous diagnosis of asthma, pregnancy (current or contemplated within 6 months), active lactation, a history of severe allergic reactions (anaphylaxis or angioedema), and continuous therapy with medications having antihistaminic side effects (e.g., tricyclic antidepressants). After completion of a screening questionnaire, subjects read and signed an informed consent document approved by both the Committee on Human Research of the University of California, San Francisco and the Committee for the Protection of Human Subjects of the University of California, Berkeley. A detailed questionnaire was then administered that solicited information on prior smoking history, prior otolaryngologic diagnoses, symptoms consistent with upper respiratory tract allergies, prior allergy testing, prior allergen desensitization therapy, current medications, potential workplace exposures to irritants, and self-reported upper respiratory tract reactivity to physical and chemical agents. These latter measures included an *environmental tobacco smoke (ETS) Score* of 0 to 15 (upper respiratory tract symptoms related to ETS exposure) and a *vasomotor rhinitis score* of 0 to 5 (rhinorrhea or congestion in response to changes in temperature or humidity, exposure to household cleaning products, bright lights, perfumes or colognes, and consumption of hot or spicy foods).[17]

After questionnaire administration, potential subjects underwent skin prick testing. This involved a standardized panel consisting of 13 regionally common aeroallergens (or mixes) plus histamine and saline controls. For purposes of this study, subjects with SAR were defined as subjects with (1) a history of seasonally occurring sneezing, nasal pruritis, rhinorrhea, postnasal drip, and/or nasal congestion, with or without known precipitants; and (2) skin test reactivity to at least one seasonally occurring agent from the panel that corroborated the history. Skin test reactivity is defined as a wheal reaction to skin prick testing with a diameter greater than or equal to the histamine control. Subjects with SAR who also had skin test reactivity to perennial allergens were retained in the study if allergen control measures in their home, place of work, or both rendered them essentially symptom-free outside of their pollen season. Nonrhinitic subjects were defined as subjects who

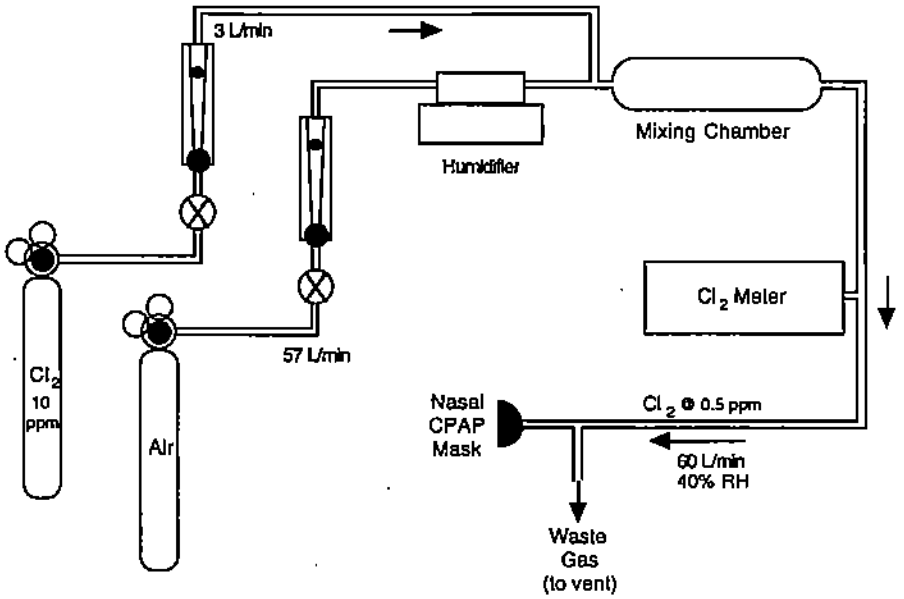

FIG 2. Schematic of chlorine dilution apparatus. Note that exposure was by nasal mask with scavenger hose attached to T-piece, allowing subject to transition from exposure to physiologic testing without leaving climate-controlled chamber. *CPAP*, Continuous positive airway pressure.

report, at most, infrequent nasal symptoms without identified seasonal variation or precipitants and with significant skin test reactivity to no more than one agent in the panel of 13 aeroallergens. Before skin testing, subjects were asked to refrain from taking antihistamines for 72 hours (terfenadine or hydroxyzine for 3 weeks, astemizole for 12 weeks).

Once subsequent testing was scheduled, subjects were asked to contact study personnel and reschedule testing if they experienced symptoms consistent with an acute respiratory tract infection or an acute exacerbation of their allergic rhinitis. Testing was delayed until subjects were asymptomatic for a period of at least 7 days (presumed allergies) or 3 weeks (suspected infection) if such symptoms were reported. Subjects were asked to refrain from wearing perfumes, colognes, or aftershaves on days in which provocation testing was scheduled. In addition, the following medication preclusions applied: no antihistamines for at least 72 hours (terfenadine and hydroxyzine, 2 weeks; astemizole, 12 weeks), no nasal or oral steroids for at least 2 weeks, no nasal cromolyn sodium for at least 48 hours, no oral or nasal decongestants for at least 48 hours, and no miscellaneous nasal sprays (e.g., saline) for at least 24 hours.

A week before provocation testing, subjects visited the laboratory to learn the technique of active posterior rhinomanometry.[18] The rhinomanometer used for this purpose was a model NR6-2 (GM Instruments, Kilwinnig, U.K.) modified to allow the use of a microbiologic filter (Model MQ306; Vacumetrics, Inc., Ventura, Calif.) between the mask and pneumotachometer. On the occasion of this visit, a variety of coaching techniques were used as needed during rhinomanometry; however, consistent with the experience of other investigators, two subjects were unable to produce meaningful pressure-volume tracings and were subsequently discontinued from the study (see below).

Provocation testing took place in a 950 cubic foot custom-built climate-controlled chamber with a charcoal- and HEPA-filtered air supply regulated at 22° ± 1° C and 40% ± 3% relative humidity. On provocation testing days, subjects entered the climate-controlled chamber and rested quietly for 15 minutes before any provocation testing occurred. During this time, the day's procedures were explained, and pulmonary peak flow was measured in triplicate with a peak flowmeter (Wright Peak Flow Mini-Meter; Clement Clarke International, Ltd.). After the acclimation period, subjects rated any preexisting nasal irritation (burning, stinging, or tingling on the inside of the nose) by adjusting the dial of a rotary potentiometer calibrated with the descriptors *none*, *slight*, *moderate*, *strong*, *very strong*, and *overpowering*.[19] The output of the potentiometer (ranging from 0.00 to 5.00 units) was recorded by the one of the investigators from the display of a digital voltmeter. A paper-and-pencil checklist labeled with the same descriptors noted above was given to the subject to rate the following additional symptoms/sensations: nasal congestion, runny nose, postnasal drip, headache, and odor. Symptom rating was followed by a triplicate measure of NAR by using the rhinomanometry technique outlined above. Each of the three NAR measurements consisted of the average, over 2 to 4 consecutive breaths, of the inspiratory and expiratory resistance calculated by using the pressure-cutoff method (75 Pa).[20] If a given recording contained a hysteresis loop that crossed the 75 Pa cutoff line, or if the automatic triggering of the rhinomanometer's software recorded fewer than two full breaths, the recording was repeated. The rhinomanometer was calibrated on a daily basis; the pressure channel to a tolerance of ±3% by using a Model 405 incline manometer (Airflow Developments, Inc., High Wycombe, G.B.) and flow to a tolerance of ±5% with a Model 235 flowmeter (Cole-Parmer/Gilmont Instruments, Vernon Hills, Ill.).

After eliciting baseline symptoms and NAR, the investigator stepped behind a translucent screen and adjusted the breathing

mixture for the nasal mask assembly. The chlorine dilution apparatus (Fig. 2) blended compressed medical-grade air (Nellcor Puritan-Bennett, San Ramon, Calif.) and compressed chlorine (diluted to 10 ppm in medical-grade air; AGA Gas, Inc., Maumee, Ohio) in a stainless steel mixing chamber (Model FMX7311; Omega Engineering, Stamford, Conn.). Diluent air, which comprised either 95% or 100% of the total flow depending on the exposure condition, was preconditioned to 22° C and 40% RH by using a Model 009700 humidifier-heater (Intertech Corporation, Bannockburn, Ill.). Immediately downstream from the mixing chamber was the sampling port for an electrochemical chlorine monitor (Model 1340; Interscan Corp., Chatsworth, Calif.), which continuously sampled the gas mixture and fed its output to a strip-chart recorder (Model 1200; Linear Instruments, Inc., Irvine, Calif.). The gas mixture was conveyed to the subject with 2.5 cm diameter corrugated respiratory tubing connected by T-piece to a nasal continuous positive airway pressure mask (Series 3121; Respironics, Inc., Murraysville, Pa.), which was sized according to the individual subject. The second limb of the T-piece connected to a low-pressure scavenger system, which led to an exhaust outside of the chamber and building. The combination of a high flow rate (60 L/min) and the scavenger system allowed subjects to breathe with negligible superimposed pressure or resistance. The chlorine meter was recalibrated on a daily basis by using the certified contents of the chlorine cylinder as the standard.

The 15-minute exposure period through a nasal mask took place on a single-blind basis, and the order of presentation was subject to limited randomization (within the constraints of the counterbalanced study design). Immediately after cessation of exposure (and then again 15 minutes later) the investigator asked subjects to rerate any sensation of nasal irritation by using the sensory potentiometer, as well as to score additional symptoms/sensations by using the paper-and-pencil checklist. The second odor rating referred to the subject's impression at the end of exposure, immediately before removing the nasal mask. NAR was remeasured times three after each symptom-rating session, and finally, pulmonary peak flow was reassessed times three. At the conclusion of the last testing session, the investigator asked each subject, "Between last week and this week, were you aware of your exposure condition?"

The statistical hypothesis tested was that subjects with SAR would show a significantly greater increase in NAR (comparing chlorine- vs air-exposure days), as well as significantly greater symptom rating increases, than would nonrhinitic subjects. For each metric, the Shapiro-Wilk test was applied for normality. Given the skewed distribution of both crude NAR and pre- to postexposure changes in NAR (as well as the wide range of baseline NAR values) proportional changes in NAR were studied throughout.[16] This metric took the form of percent change in NAR (from daily baseline) for purposes of analysis of variance (ANOVA) and graphical representation, and log-transformed NAR for repeated-measures ANOVA (RANOVA). The latter consisted of a 5-factor RANOVA model with three grouping variables (rhinitis status, gender, and order of exposure) and two trial variables (exposure condition and time). For symptom rating, the pre- to postexposure difference was examined. For each statistical hypothesis, the above RANOVA model was applied, and, if significant for the main or interactive effect of interest, confirmatory testing was performed using a subject-matched two-tailed t test comparing outcomes for the risk/exposure stratum in question. Finally, net percent change in NAR (chlorine minus air) was compared for rhinitic subjects versus nonrhinitic subjects by using an unmatched t test. It was also examined in linear regressions against vasomotor score, ETS score, and against the change in subjective congestion rating (before exposure to 15 minutes after exposure).

TABLE I. Pooled NAR data: Mean crude values (Pa/L/sec [SEM])

		Before exposure (baseline)	End of exposure	15 minutes after exposure
Rhinitic subjects	Cl_2	274 (29)	347 (59)	331 (56)
	Air	248 (18)	246 (31)	239 (26)
Nonrhinitic subjects	Cl_2	264 (19)	275 (19)	278 (15)
	Air	240 (27)	243 (27)	258 (26)

RESULTS

Subject recruitment and screening

A total of 68 subjects responded to various postings and advertisements and were provided with a screening questionnaire. Of 51 initial respondents, three were eliminated because of the presence of a contraindicating condition (asthma, pregnancy, or lactation), and 11 were held in reserve because of an excess of nonrhinitic respondents. Informed consent forms were conveyed to the remaining 37 prospective subjects, 25 of whom returned them. Detailed questionnaires were then distributed, and all were returned completed. Two subjects withdrew from the study at this stage because of time limitations, and an additional two nonrhinitic subjects were placed on reserve. Twenty-one subjects were referred for allergy skin tests. Of these, three were eliminated because of discrepancies between their questionnaire responses and skin test results. Of the 18 qualified subjects, two were unable to reproducibly perform the rhinomanometry technique. The sixteen remaining study participants were evenly divided by gender, with mean ages of 25.8 years for the SAR group and 29.4 years for the nonrhinitic group.

NAR

Table I presents the mean of crude (untransformed) NAR values for rhinitic and nonrhinitic subjects before, immediately after, and 15 minutes after air and chlorine exposures. Table II presents the corresponding values for mean percent changes in NAR from baseline for chlorine, air, and chlorine minus air (net percent change in NAR). Fig. 3 shows the mean (± SEM) net percent change in NAR from baseline for postexposure conditions 1 and 2. The mean net percent change in NAR from baseline to immediately after exposure was +24% in the SAR group and +3% in the nonrhinitic group. The corresponding net changes from baseline to 15 minutes after exposure were +21% in the SAR group and −1% in the nonrhinitic group. In the RANOVA model the interaction term for rhinitis*time*condition was significant ($p < 0.05$). In a paired t tests among rhinitic subjects (two-tailed), the distribution of NAR values (percent change from baseline) was significantly different when comparing chlorine and air days ($p < 0.05$

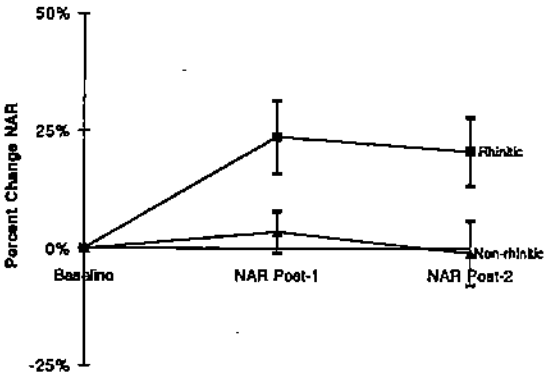

FIG. 3. Net percent change in nasal airway resistance (from baseline) ± SEM (chlorine minus air condition) for postexposure times 1 and 2 by rhinitis status ($p < 0.05$ by ANOVA at both testing times).

TABLE II. Individual NAR data: Mean percent change from baseline

	Mean % Change in NAR (SEM)	
	End of exposure	15 minutes after exposure
Rhinitic subjects		
Cl_2	+22.3% (9.4)	+16.8% (9.4)
Air	−1.4% (7.0)	−3.9% (6.2)
Net difference (Cl_2 − Air)	+23.7%	+20.7%
Nonrhinitic subjects		
Cl_2	+4.7% (4.6)	+7.4% (6.5)
Air	+1.3% (2.4)	+8.4% (4.2)
Net difference (Cl_2 − Air)	+3.4%	−1.0%
Rhinitis Effect (Cl_2 − Air, Rhinitic subjects − Nonrhinitic subjects)	+20.3%*	+21.7%*

*$p < 0.05$ for (rhinitis*time*condition) effect in repeated-measures model (chlorine vs air; post- vs preexposure; rhinitic subjects vs nonrhinitic subjects).

for both postexposure times 1 and 2); no such differences were apparent for nonrhinitic subjects (Fig. 4). Finally, examining net percent change in NAR from baseline (chlorine minus air values) in separate one-way ANOVAs (for postexposure times 1 and 2), the distribution of values for subjects with SAR and nonrhinitic subjects was significantly different ($p < 0.05$). In sum, subjects with SAR experienced congestion to a significantly greater degree than did nonrhinitic subjects when chlorine and air exposure conditions were compared immediately after, as well as 15 minutes after, provocation exposure.

In terms of self-reported nasal reactivity to irritants and physical stimuli, vasomotor scores ranged from 0 to 3, with a mean of 1.25, and ETS scores ranged from 0 to 3, with a mean of 0.31. Separate linear regressions were performed for net percent change in NAR versus each of these scores at postexposure times 1 and 2. For both testing times, the vasomotor score yielded small positive regression coefficients (+3% per score unit); however, neither was significantly different from zero. The regression coefficients for ETS score were somewhat more substantial (+13% to 14% per score unit), and for postexposure time 1, the slope was significantly different from zero. However, given the fact that only three of 16 subjects had nonzero ETS scores, and that the highest ETS score reported here was only one-fifth of the maximum possible (15), the generalizability of these findings is probably limited.

Symptoms

In general, symptom intensities were modest, with odor ratings averaging 1.25 (and irritation ratings averaging 0.61) at the end of chlorine exposure (1.00 being slight and 2.00 being moderate). Some subjects did not detect the odor of chlorine at 0.5 ppm, and a quarter of the subjects were unable to distinguish between the exposure conditions on the two testing days. On a pooled basis (subjects with SAR plus nonrhinitic subjects), significant chlorine-related increases were apparent for mean ratings of odor (end of exposure; $p < 0.001$), nasal irritation (immediately after exposure; $p < 0.01$), and nasal congestion (15 minutes after exposure; $p < 0.05$). Odor, nasal irritation, and nasal congestion were subsequently analyzed separately by rhinitis status. As noted in Fig. 5, subjects with SAR showed greater time-related increases in these three symptoms as a group than did nonrhinitic subjects. No significant exposure-related changes were observed for rhinorrhea, postnasal drip, or headache, either on a pooled or stratified basis.

Finally, the relationship between subjective and objective nasal congestion was examined. In a pooled (subjects with SAR plus nonrhinitic subjects) analysis, a one-point change in subjective nasal congestion rating was associated, on the average, with an 8% change in net percent change in NAR. However, this effect was not statistically significant ($r^2 = 0.03$, $p \cong 0.50$), and the regression line became horizontal when rhinitis status was controlled for. Thus, within either the SAR or nonrhinitic subgroup, there was essentially no relationship between subjective and objective congestion after chlorine exposure.

Pulmonary peak flow

Pulmonary peak flow was obtained before and after exposure as a safeguard to detect potential acute lower airway effects of low-level chlorine inhalation. None of the subjects exhibited clinically significant changes in peak flow (i.e., decreases ≥10% of baseline), nor did they complain of cough, wheezing, or chest tightness on chlorine exposure days.

DISCUSSION

Our study demonstrated differential upper respiratory tract physiologic reactivity to a nasal irritant challenge comparing subjects with SAR and nonrhinitic subjects,

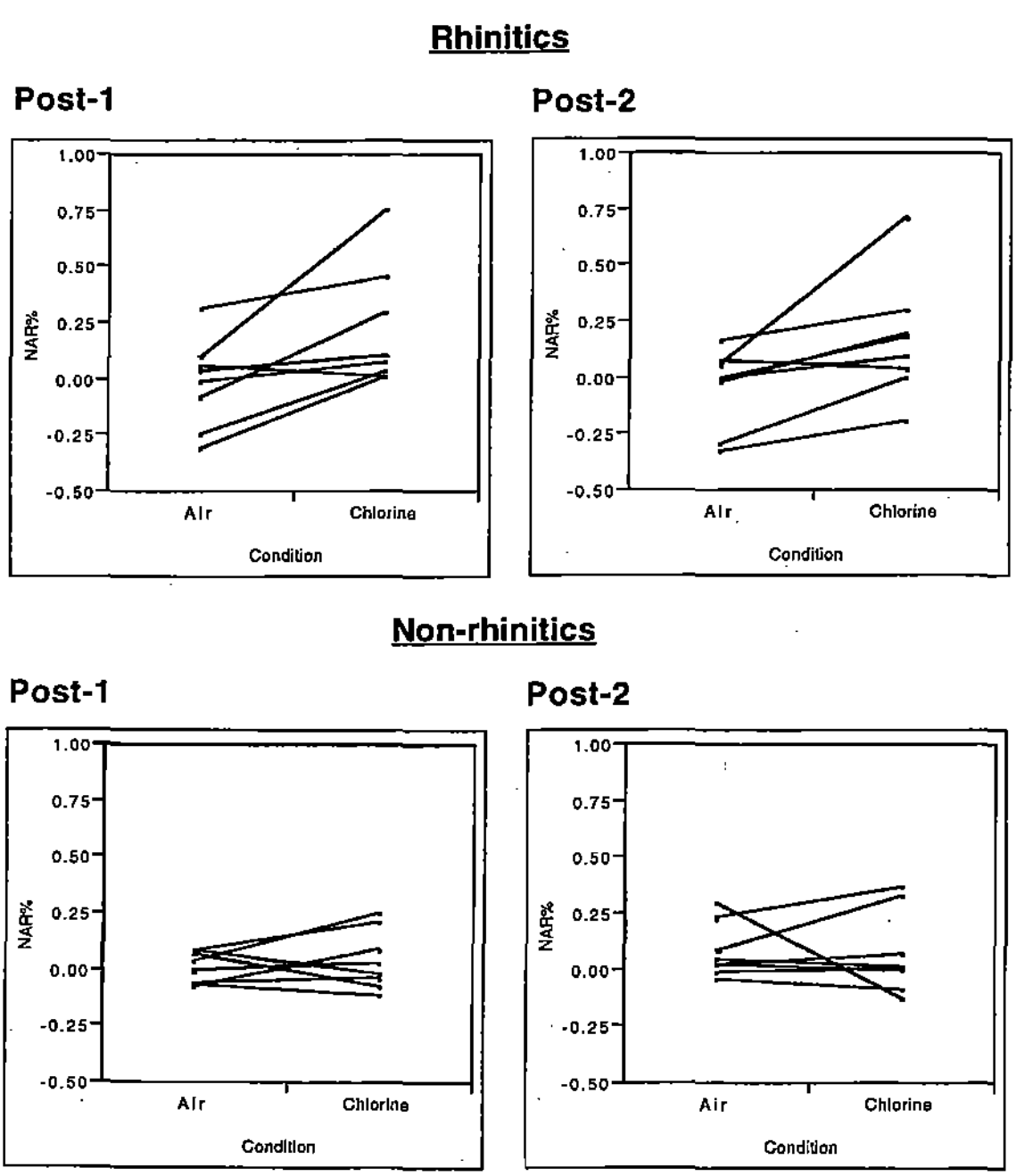

FIG. 4. Paired analyses of percent change in nasal airway resistance (from baseline) stratified by rhinitis status and time ($p < 0.05$ by paired t test for rhinitic subjects only at both testing times).

as evidenced by a greater proportional increase in NAR from baseline to after exposure when comparing the chlorine and air exposure conditions. Rhinitic subjects also reported greater exposure-related increases in perceived odor intensity, nasal irritation, and nasal congestion than did nonrhinitic subjects. The relationship between subjective and objective nasal congestion, on the other hand, was extremely weak and disappeared entirely when analyses were confined to either the rhinitic or nonrhinitic subgroup.

The results reported here are unlikely to be due to confounding because a stratified sample of rhinitic and nonrhinitic subjects was used, and the study design was counter-balanced with respect to subject gender and order of exposure. Our results agree with those of Bascom et al.[9] and Kjaergaard et al.[10] both of whom

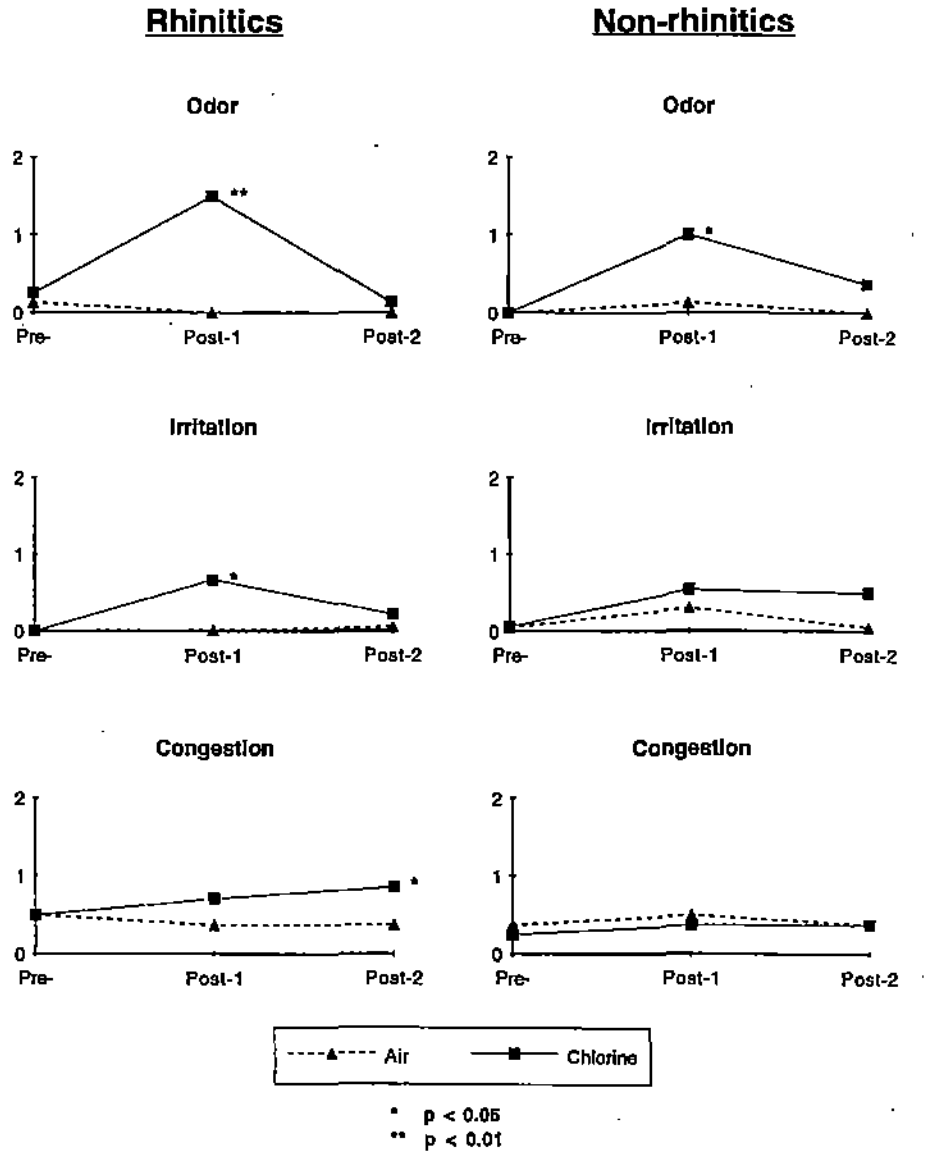

FIG. 5. Symptom ratings by time stratified by rhinitis status (1 = slight and 2 = moderate). Significant elevations were apparent, comparing chlorine and air exposures, for odor intensity ($p < 0.01$ at end of exposure for rhinitic subjects and $p < 0.05$ for nonrhinitic subjects), nasal irritation ($p < 0.05$ at end of exposure for rhinitic subjects only), and nasal congestion ($p < 0.05$ at 15 minutes after exposure for rhinitic subjects only).

showed differential nasal irritant sensitivity by allergic rhinitis status among subgroups selected either explicitly (Kjaergaard) or incidentally (Bascom) to contrast response on this trait (see below). Our failure to find a significant correlation between subjective and objective nasal congestion is also consistent with the published literature.[21]

The issue of interindividual variability in upper airway susceptibility to irritant chemicals is one of considerable clinical interest. Experimentally, the only published study directly examining atopy as a risk factor for upper respiratory tract irritant reactivity is that of Kjaergaard et al.,[10] who exposed 18 of each group of subjects (subjects with SAR and normal subjects) to either a mixture of 22 volatile organic compounds at 20 mg/m^3 or to clean air times 4 hours. In this experiment, the subjects with SAR reported greater eye, nose, and throat irritation and showed greater evidence of an inflammatory response in tear fluid than did the normal subjects. Although both

groups showed a decrease of nasal volume by acoustic rhinometry, no differential response (between rhinitic and nonrhinitic subjects) was evident in this regard. Bascom et al.[9, 22] found that subjects who are historically reactive to ETS manifest greater changes in nasal airway resistance after ETS provocation than do self-reported nonreactors. Because 60% to 70% of their historically sensitive subjects (but only 30% of their nonsensitive subjects) had positive skin test results, their studies may have indirectly addressed the issue of atopy as a risk factor. Significantly, despite the similarity of ETS-induced symptoms to those of allergic rhinitis, the usual markers of IgE-mediated allergic response (histamine, TAME-esterase, albumin, and kinins) were not elevated in nasal lavage fluid after ETS provocation.[9]

Epidemiologically, an association between preexisting atopy and nasal symptoms has been noted in investigations of so-called "problem buildings" in which no bioaerosol problem has been identified.[4] Furthermore, reports of nasal symptoms in response to ETS exposure are more common among individuals with a prior history of atopy than in nonatopic subjects.[2] Despite this empirical association with atopy, only a small proportion of ETS-sensitive subjects have positive skin test reactivity to tobacco-leaf extract or tobacco-smoke condensates.[23] The implication to be drawn from this work is that although a prior history of respiratory allergies appears to be a risk factor for upper respiratory tract reactivity to airborne irritants, the mechanism of response is probably not classical allergy. The most credible candidate for a nonallergic nasal response mechanism involves the irritant (nociceptor) receptor system of the trigeminal nerve.[24] Within this system, irritant-sensitive C and Aδ fibers innervate the nasal and oral cavities and give rise to both local (neuropeptide-mediated) and central (parasympathetic and sympathetic) reflexes.[25-32]

In the explanatory model proposed by ourselves and others, preexisting allergic inflammation primes some portion of the neurogenic reflex loop for response to chemical irritants.[33] Of note, subjects with SAR in our study were studied within 1 to 2 months of the end of their respective allergy seasons to preserve any priming effect and simultaneously avoid extraneous allergic triggering of symptoms. Depending on the actual reflex (or reflexes) involved, priming could take the form of a lowered sensory threshold and/or an augmented store of neuropeptides in afferent (trigeminal) nerve branches, a facilitated brainstem reflex, augmented acetylcholine release from the efferent (facial) nerve, or augmented responsiveness of the end organ (in this case, nasal mucosal capacitance vessels) to neuroimmune mediators. Our current data do not permit us to localize the site of modulation of irritant-induced reflexes. However, as a step to understanding this problem, future work will center on defining the relative contributions of autonomic and axon reflexes in the vasodilation/airway congestion response to irritant provocation.

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SECTION III

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ANNOTATED PAGES SUBMITTED BY

Dennis Shusterman, M.D., MPH
Faculty Physician
Occupational and Environmental Medicine Program
University of Washington
Seattle, WA 98104
206-744-9398
Email: dennis3@u.washington.edu

Short-term exposure to chlorine in air	The following effects have been observed in humans briefly exposed to chlorine: • mild nose irritation at 1–3 ppm • eye irritation at 5 ppm • throat irritation at 5–15 ppm • immediate chest pain, vomiting, changes in the respiratory rhythm, and cough at 30 ppm • toxic pneumonitis and pulmonary edema (fluid in the lungs) at 40–60 ppm • death after 30 minute exposure to 430 ppm • death after a few minute exposure to 1,000 ppm In general, people who suffer from respiratory conditions such as allergies or hay fever, or who are heavy smokers, tend to experience more severe effects than healthy subjects or nonsmokers.
Long-term exposure to chlorine in air	No significant harmful health effects were observed in workers exposed for years to relatively low concentrations of chlorine The tissues inside the nose were principally affected in animals exposed to chlorine for longer durations.
Short-term exposure to hypochlorite solution by ingestion	Drinking small amounts of hypochlorite solution (less than a cup) can produce irritation of the esophagus. Drinking concentrated hypochlorite solution can produce severe damage to the upper digestive tract and even death. These effects are most likely caused by the caustic nature of the hypochlorite solution and not from exposure to molecular chlorine.
Long-term exposure to hypochlorite solution by ingestion	There is no information on long-term ingestion of hypochlorite solution in humans. Animals that drank hypochlorite solution in water for up to 2 years did not show any significant health effects. The amount of hypochlorite solution in the water that the animals drank was much smaller than what is found in household bleach.
Skin exposure to hypochlorite solution	Spilling hypochlorite solution on the skin can produce irritation. The severity of the effects depends on the concentration of sodium hypochlorite in the bleach.

Range?

Further information on the health effects of chlorine in humans and animals can be found in Chapters 2 and 3.

How can chlorine affect children?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Children are likely to have similar effects as adults	Short-term exposures to high concentrations of chlorine affect children in the same manner they affect adults. We do not know what the effects could be in children following longer-term, low-level exposure to chlorine gas, but this type of exposure occurs only in workers and is not relevant to children. We also do not know what the effects could be in children following longer-term, low-level exposure to hypochlorite solution.
Birth defects	We do not know whether exposure to chlorine gas during pregnancy can result in damage to unborn babies because there are no studies of pregnant women exposed to chlorine. There are no studies of pregnant animals exposed to chlorine gas. One study of rats exposed to hypochlorite solution during pregnancy found no evidence of birth defects or any other developmental alteration in the baby rats.

How can families reduce the risk of exposure to chlorine?

If your doctor finds that you have been exposed to substantial amounts of chlorine, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

Do not mix bleach with household cleaners	Chlorine gas can be released to the air when bleach is mixed with other cleaning solutions that contain an acid; for example, some toilet cleaners.
Store household chemicals out of reach of young children	Always store household chemicals in their original labeled containers out of reach of young children to prevent accidental poisonings. Never store household chemicals in containers children would find attractive to eat or drink from, such as old soda bottles.
Follow instructions for swimming pool disinfection	Chlorine gas can also be released to the air when chemicals used to chlorinate swimming pools are mishandled. If you have a swimming pool at home, read the labels of the chlorination products carefully and do not let children play with these products.

Is there a medical test to determine whether I have been exposed to chlorine?

There are no medical tests available for chlorine	There are no medical tests to determine whether you have been exposed specifically to chlorine. Chlorine is transformed in the body into chloride ions, which are normal components of the body. An enormous amount of chlorine has to be inhaled or ingested in order to detect a significant increase in chloride ions in the blood. This has occurred in a few cases of ingestion of hypochlorite solution and one of them was a fatal case.

What recommendations has the federal government made to protect human health?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as "not-to-exceed" levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it.

Some regulations and recommendations for chlorine include the following:

Levels in air set by EPA	EPA established an air limit of 0.5 ppm. Exposure to higher levels could result in discomfort and irritation; these effects are reversible when exposure ends.
Levels in workplace air set by OSHA	OSHA set a legal limit of 1 ppm chlorine in air averaged over an 8-hour work day. * WRONG
Levels in drinking water set by EPA	EPA established a maximum contaminant level (MCL) and maximum residual disinfectant level (MRDL) of 4 mg/L for free chlorine in drinking water.

* OSHA PEL = 1.0 ppm ceiling (not 8-hr average) — NIOSH REL + ACGIH TLV (8 hr) = 0.5 ppm STEL (15 min.) = 1.0 ppm

Where can I get more information?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

may also represent a general response to the stress of having been involved in a chemical accident and being admitted to a health facility; the same can be said about anxiety.

Prolonged exposures to relatively low concentrations of chlorine in occupational settings have not given indications of respiratory or other health problems among the workers (Enarson et al. 1984; Ferris et al. 1979; Patil et al. 1970), 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 (Bhérer et al. 1994; Kowitz et al. 1967; Schwartz et al. 1990) and also in some cases of exposure of the general population (Donnelly and FitzGerald 1990; Schönhofer et al. 1996), long-term follow-up 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 (Brooks et al. 1985). There are many factors that can play a role in whether residual effects are detected, 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 by Kilburn (1995, 2000, 2003b) 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 conducted neurobehavioral testing, but this could be ~~easily~~ 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 (Sklyanskaya et al. 1935). 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 (Barbone et al. 1992; Barregård et al. 1990; Bond 1983, 1985, 1986; Heldaas et al. 1989). 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 (i.e., Barrow and Smith 1975; Buckley et al. 1984; Demnati et al. 1995; Jiang et al. 1983). Chlorine, in relatively low concentrations (1–3 ppm), also induced histological alterations in the respiratory tract, particularly the

Direct contact of the skin with household chlorine bleach can cause skin irritation in humans (Hostynek et al. 1989; Nixon et al. 1975). Although sodium hypochlorite generally is not considered a contact sensitizer, several cases of allergic contact dermatitis have been reported (Eun et al. 1984; Osmundsen 1978; Van Joost et al. 1987). Commerical household bleaches are prepared with sodium hydroxide and are typically very alkaline (Racioppi et al. 1994); it is this property that may result in the 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 (Grant and Schuman 1993).

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 (Hook and Lowry 1974; Landau and Saunders 1964; Yarington 1970). More recent 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 (Daniel et al. 1990, 1991; NTP 1992). In the intermediate-duration studies, Sprague-Dawley rats and B6C3F$_1$ mice were dosed with up to 24.9 and 39.2 mg Cl/kg/day, respectively. In the chronic-duration studies, rats were exposed 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 (Exon et al. 1987). 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 (Carlton et al. 1986). Cancer bioassays in rats and mice have been negative (Hasegawa et al. 1986; Kurokawa et al. 1986; NTP 1992) except for equivocal evidence of increased incidence of leukemia in female Fischer-344 rats in the NTP (1992) 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

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) and subjects showing methacholine hyperresponsiveness in the study by D'Alessandro et al. (1996). 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 some function of concentration and duration rather than to concentration alone. 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) (8 hours was the longest period of exposure for which there is information). An uncertainty factor to account for sensitive populations is not necessary because sensitive individuals were already included in two studies. The resulting acute-duration inhalation MRL for chlorine is 0.2 ppm.

- An MRL of 0.0005 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

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of chlorine. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

respiratory?

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important

acutely ill patients in moderate to marked respiratory distress, increased respiratory rate of costal abdominal type, and both dry and moist rales. Laboratory data showed sputum with large numbers of epithelial cells showing pronounced degenerative changes. Most cultures showed microorganisms representative of the normal pharyngeal flora. Chest x-rays showed mottling of the lungs and patches of irregular density and differences in the degree of aeration between the two pulmonary fields. Spirometry was conducted on 8 patients 48 hours after exposure and showed markedly reduced vital capacity (VC) and maximal breathing capacity (1 minute); these changes showed improvement in subsequent days. The diagnosis of pulmonary changes was: pulmonary edema, tracheobronchitis, and pneumonia. In 29 patients who were followed for up to 16 months after exposure, there was no evidence of permanent pulmonary disease.

Hasan et al. (1983) reported that exposure of 28 subjects to chlorine that leaked from a storage tank caused cough, dyspnea, and nasopharyngeal irritation. Pulmonary tests conducted 18 hours after exposure showed diminished forced expiratory volume in 1 second (FEV_1), and low ~~fixed~~ forced expiratory flow rate at 50 and 25% vital capacity (FEF_{50} and FEF_{25}) and $FEF_{25-75\%}$. These abnormalities were still present 14 days after exposure in subjects whose chief initial complaint was dyspnea. Evaluation of nine subjects 5 months after chlorine exposure showed pulmonary parameters within normal limits.

In contrast to the findings of the above two studies, some studies have reported long-term effects of acute high chlorine exposure. For example, Chester et al. (1977) reported the case of a woman who was exposed following a leak in a liquid storage tank and suffered severe cough and chest pain within minutes after exposure. Chest x-rays at the time showed bilateral infiltrates in the midpulmonary zones, but 1 year after the accident x-rays were normal. However, dyspnea and chronic cough with occasional production of white to yellow sputum persisted over the next 4 years. Schönhofer et al. (1996) studied three cases that experienced nose and throat irritation, cough, shortness of breath, wheezing, chest tightness, and a feeling of suffocation minutes after exposure to chlorine gas that leaked from a tank. Chest x-rays showed no evidence of pulmonary edema. Four months after the accident, bronchoalveolar lavage showed inflammatory changes, but no such changes were seen 16 months later. However, moderate to severe bronchial hyperresponsiveness was observed up to 30 months after the accident. Schönhofer et al. (1996) noted that the condition showed the typical feature of the reactive airways dysfunction syndrome (RADS), defined as an asthma-like occupational illness after an acute exposure to concentrated respiratory irritants characterized by increased responsiveness to methacholine (Brooks et al. 1985).

Chlorine gas can be released around swimming pools when chlorinating agents are handled improperly or due to malfunction of the chlorination equipment. Sexton and Pronchik (1998) described the effects of such an exposure on 13 children who presented to the emergency department. On admission to the emergency department, most patients complained of throat irritation, chest pain, shortness of breath, wheezing, and chest tightness. Five patients who were admitted to the hospital had normal chest x-rays. At follow-up interviews 2 weeks later, the patients did not complain of residual respiratory symptoms. Ploysongsang et al. (1982) studied four patients who inhaled, for 2–5 minutes, an undetermined amount of chlorine gas that leaked from a container at a public swimming pool and experienced cough, a feeling of irritation of the upper respiratory tract, and tightness in the chest. Pulmonary function studies conducted 12–14 hours after the accident showed values within normal ranges. However, tests done 1 month later showed a significant increase in measurements of volumes, suggesting that there had been an acute reduction of lung volumes after the exposure. Ploysongsang et al. (1982) concluded that exposure to chlorine had produced an insignificant and inconsistent obstruction in large airways. Agabiti et al. (2001) reported the effects of accidental inhalation of chlorine fumes among a total of 282 subjects attending a pool. Cough and shortness of breath were common acute symptoms after the accident and 27% reported some respiratory symptoms 15–30 days after exposure. Lung function measurements at that time revealed a tendency to lower levels among those with the highest perceived exposure, but only a decrease in FEV_1 was significant. The study also found that among children (approximately half the sample), the incidences of all symptoms tended to be higher among those who had a history of chronic respiratory disease, among those who were engaged in physical exercise when the accident occurred, among those who were slow to evacuate the pool, and among those who reported higher exposure (as judged by eye irritation). Also, incidences were higher among smokers and former smokers than among never smokers. A recent study of 18 children exposed to chlorine in a swimming pool accident found that a biomarker of pulmonary inflammation, leukotriene B_4, was still significantly increased in exhaled breath condensate 2 months after exposure, long after pulmonary function parameters had returned to normal values (Bonetto et al. 2006). Immediately after exposure, the children had experienced dyspnea and burning of the throat and spirometry tests done within the first 24 hours showed reduced forced vital capacity (FVC) and FEV_1. The authors also found that hours after exposure a Clara cell-specific protein, CC16, was significantly elevated in serum compared to healthy children, suggesting that damage had occurred to the epithelial permeability barrier.

Controlled Low-level Exposure of Volunteers. Anglen (1981) exposed up to 29 male and female volunteers to 0, 0.5, 1, or 2 ppm chlorine for either 4 or 8 hours. Sensations were recorded before and during exposure and pulmonary function was monitored by measuring FVC and FEV_1 before and at

various times during exposure. Itching and burning of the throat were the highest responses and were most prevalent by the end of an 8-hour exposure to 1 ppm chlorine. Responses for sensations of itching or burning of the nose and eyes were also prevalent at 1 ppm chlorine. In general, males provided stronger irritation responses than females. Exposure to 1 or 2 ppm chlorine for 8 hours produced significant changes in pulmonary function but similar exposures to 0.5 ppm did not. Exposure to 2 ppm for up to 30 minutes produced no increase in subjective irritation and exposure to 2 ppm for 2 hours did not alter pulmonary function. A follow-up study was conducted in eight healthy male volunteers exposed to target concentrations of 0, 0.5, or 1 ppm chlorine (Rotman et al. 1983). Pulmonary tests were conducted before exposure, after a 4- and 8-hour exposure period and again 2 and 24 hours after exposure ceased. During exposure, the subjects exercised on a treadmill for 15 minutes of each hour to simulate light-to-moderate work that raised the heart rate to 100 beats per minute. Specific respiratory parameters measured included FVC, FEV_1, forced expired volume in 1 second as percent FVC (FEV_1%), peak expiratory flow rate (PEFR), FEF_{50} and FEF_{25}, TLC, expiratory reserve volume (ERV), functional residual capacity (FRC), residual volume, airway resistance (Raw), single-breath DL_{CO}, closing volume, and difference in nitrogen concentrations between 750 and 1,250 mL of inhaled vital capacity (ΔN_2). Exposure to 1 ppm chlorine caused runny nose and mild burning in the throat, but no such effects were reported at 0.5 ppm. Significant changes in pulmonary function tests were mostly restricted to the 1 ppm exposure level and were evident after 4 hours of exposure. Changes were observed in FEV_1, PEFR, FEF_{50}, FEF_{25}, TLC, Raw, and ΔN_2. Greater changes in some of these parameters were seen after 8 hours of exposure. Few changes were still evident 24 hours after exposure, but most parameters had returned to pre-exposure values by that time. It should be noted that one volunteer who was atopic experienced severe distress during exposure to 1 ppm and was forced to exit the chamber before the full 8-hour period due to shortness of breath and wheezing.

CHECK THIS

D'Alessandro et al. (1996) evaluated pulmonary function in subjects with (n=10) and without (n=5) airway hyperreactivity (HR, defined by baseline methacholine hyperresponsiveness). The HR subjects were exposed to 0.4 or 1.0 ppm chlorine, whereas the healthy subjects were exposed to 1.0 ppm chlorine. All exposures lasted 60 minutes. Airflow and airway resistance were measured immediately before and immediately after exposure. Also, lung volumes, airflow, diffusing capacity, airway resistance, and responsiveness to methacholine were measured 24 hours before and 24 hours after exposure. Exposure of the HR group to 0.4 ppm chlorine resulted in no significant change in airflow or resistance either immediately or 24 hours after exposure. Exposure to 1.0 ppm chlorine resulted in an immediate decrease in FEV_1 and $FEF_{25-75\%}$ and increase in airway resistance among normal and HR subjects, but the magnitude of the effects among HR subjects was significantly greater than in healthy subjects. Twenty-

four hours after exposure, there were no significant changes for healthy or HR subjects in airflow, lung volumes, diffusing capacity, resistance, or methacholine responsiveness. Comparing relative changes from baseline immediately after exposure between normal and HR subjects showed that HR subjects had much greater changes in pulmonary function tests.

A similar study was conducted in eight volunteers exposed to chlorine 6 hours/day on 3 consecutive days to each of the four exposure conditions, 0, 0.1, 0.3, and 0.5 ppm chlorine (Schins et al. 2000). Pulmonary function including effort-dependent parameters and effort-independent parameters were evaluated before and after exposures. In addition, nasal lavage measurements were performed before and after each exposure and 1 and 4 days after each exposure. The nasal lavage fluid was examined for total cells, epithelial cells, neutrophils, lymphocytes, eosinophils, monocytes, albumin (an indicator of epithelial permeability), and interleukin-8 (indicator of inflammatory response). Subjective complaints by the subjects were judged to be not treatment-related. Examination of the nasal lavages gave no indication of an inflammatory response or irritant effects on the nasal epithelium. The results of the pulmonary function tests showed that the only significant effect related to chlorine exposure was a difference in maximal mid expiratory flow (MMEF) between 0 and 0.5 ppm exposure; however, this was attributed to an unexplained shift in baseline values during control exposure (0 ppm).

CRITIQUE
Shusterman et al 2002

Shusterman et al. (2003b) measured nasal airway resistance in 52 healthy adults (24 males and 28 females) before and after exposure to 0 or 1 ppm chlorine for 15 minutes. Subjects were stratified on age (18–34, 35–51, 52–69 years), gender, and allergic rhinitis status (27 were positive). Nasal airway resistance was measured by active posterior rhinomanometry. Exposures to air and chlorine were a week apart. Subjects with allergic rhinitis showed a significantly greater increase in nasal airway resistance (49% increase from baseline) than healthy subjects (10% increase from baseline) 15 minutes after exposure. The increase in nasal airway resistance was most pronounced in older subjects and least pronounced in the youngest group. No significant differences were seen between males and females.

As a whole, these studies indicate that acute-duration exposures to 1 ppm chlorine can induce upper respiratory tract irritation and transient alterations in parameters of respiratory function and exposure concentrations of 0.5 ppm are generally devoid of such effects and, therefore, 0.5 ppm can be considered an acute (1–8 hours) NOAEL for sensory irritation and pulmonary function. The studies also show that individuals with compromised respiratory function constitute a susceptible group for exposure to chlorine. The NOAEL of 0.5 ppm and LOAEL of 1 ppm from these studies as a group, serve as the basis for derivation of an acute-duration inhalation MRL for chlorine.

Pulp Mills ⇒ competing exposures (GENERIC DISCUSSION) MOVE UP

Long-term, Low-level Occupational Exposures. Relatively few studies have examined the effects of long-term exposure to low levels of chlorine in humans, and the ones that have done so have not provided conclusive answers largely because of study limitations.

Patil et al. (1970) studied the health effects of chlorine in 600 workers from 25 plants producing chlorine in North America. A group of 382 workers not considered to be routinely exposed to chlorine served as controls. The average duration of exposure was 11.9 years. Each worker received one physical examination that included evaluation of medical and occupational histories, blood and urine tests, pulmonary function tests and electrocardiogram (EKG). Tobacco and alcohol use were also monitored. The concentration of chlorine was monitored in each plant every 2 months over a period of 1 year in several representative areas, but otherwise unspecified. 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). It also showed that almost all workers were exposed to <1 ppm chlorine, 94% were exposed to ≤0.5 ppm, and 70% were exposed to ≤0.2 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 there are limitations such as unclear analytical methodology, no clear definition of the case/control populations, and insufficient detail regarding the method of analysis that render the NOAEL questionable; thus, it is not included in Table 3-1.

TIMING ? / DURATION OF EXPOSURE

skewed distrib WHAT ABOUT that ... ? cutoff

Ferris et al. (1967) examined the prevalence of chronic respiratory disease among 147 workers in a pulp mill and 124 controls who worked in a paper mill and found no significant differences in respiratory symptoms or in tests for FVC and FEV_1 (tests were conducted without a nose clip) between the two groups. Duration of exposure was not provided. Chlorine levels were measured on three different occasions in 3 years; in one occasion, the mean was 7.4 ppm and only traces were reported in the other two occasions. The limit of detection of the method was 1 ppm. Examination of the same cohort 10 years later did not reveal any increased mortality or increased specific cause of death (Ferris et al. 1979). Evaluation of 200 men seen at both times did not reveal any differences in respiratory symptoms or chronic nonspecific respiratory disease.

Enarson et al. (1984) evaluated respiratory effects and pulmonary function in a group of 392 male pulp mill workers exposed to chlorine, sulfur dioxide, hydrogen sulfide, and methylmercaptan, in addition to various particulates (i.e., wood dust, ash, lime dust), for a mean duration of 101.5±86.6 months. A

control, unexposed group, consisted of 310 male rail yard workers who lived in the same community and who performed similar manual labor. End points examined included prevalence of respiratory symptoms (usual cough, usual phlegm, wheezing without a cold, dyspnea when hurrying, chest tightness, and chest illness). Pulmonary function tests conducted included FEC, FEV_1, $FEF_{25-75\%}$, and FEV_1/FVC ratio. Chlorine was the main contaminant in two areas of the pulp mill, the bleach plant and the machine room (mean 8-hour TWA 0.18 and 0.02 ppm, respectively). Overall, pulp mill workers complained more frequently of usual phlegm, wheeze without cold, and chest illness than rail workers. However, the most significant finding was that among bleach workers (n=15) and machine room workers (n=22), nonsmokers (n=4) had a significantly lower $FEF_{25-75\%}$ and FEV_1/FVC ratio than rail yard workers. Given the small number of workers involved, the possibility of exposure to multiple chemicals, and the lack of information on chlorine peak exposure levels, the validity of the 0.18 ppm as an effect level is questionable.

A study at a chlorine plant in Sweden compared the changes in vital capacity (VC) and FEV_1 that occurred between measurements separated by 10 years among 44 workers exposed to chlorine and 33 white-collar workers matched for age and smoking status (Hyback 1999). The author stated that the concentration of chlorine was measured continuously over the years and, in principle, was always below 0.5 ppm. The results of the tests showed that in fact, over the years, VC and FEV_1 declined more in white-collar workers (significantly for FEV_1) than in the workers exposed to chlorine. Hyback (1999) speculated that perhaps the low concentrations of chlorine gas may protect workers from contracting respiratory infections that over time contribute to a decline in respiratory function.

The limited information available does not suggest that long-term exposure to low levels of chlorine gas affects respiratory function, but additional, better-conducted studies are necessary to confirm this view.

High-level Occupational Exposure. Schwartz et al. (1990) studied a group of 20 workers who were briefly (minutes) exposed in a pulp mill to chlorine gas when liquid chlorine leaked from a tank and evaporated. Acute symptoms included burning of the nose and throat, and dry cough with chest tightness. Pulmonary tests were conducted within 24 hours of exposure and several times over the next 12 years. The most significant findings were a high prevalence of airflow obstruction (FEV_1/VC ratio <65%) that persisted over the observation period and a prevalence of low residual volume (RV) that increased during the follow-up period. Schwartz et al. (1990) also found that 5 of 13 subjects tested at year 12 had increased airway reactivity to inhaled methacholine. While the findings were suggestive of long-term pulmonary complication, the investigators acknowledged that without pre-exposure pulmonary function

tests and individual measures of exposure, it is difficult to determine whether the changes were due to chlorine exposure.

Moulick et al. (1992) evaluated 82 patients exposed to approximately 66 ppm chlorine that leaked from a storage tank at a chemical factory in Bombay, India. Acute symptoms of exposure included dyspnea, cough, and irritation of the throat. Pulmonary tests performed in 62 cases within 48 hours of exposure indicated obstruction in 17 cases, restriction in 2 cases, and a mixed pattern in 33 cases. Also, bronchoscopy showed tracheobronchial mucosal congestion and hemorrhagic spots. Four out of 16 patients who were followed for 1 year showed persistent cough 4–6 weeks after exposure, but after 1 year, there were no residual symptoms and x-ray and pulmonary function tests were normal. Evaluation of five nonsmoking patients 3 years after exposure did not reveal any residual symptoms and pulmonary function tests were normal.

NSBR?
(NON-SPECIFIC BRONCHIAL RESPONSIVENESS?)

Lemière et al. (1997) reported the case of a nonsmoking worker at a water-filtration plant man who was exposed to chlorine levels high enough to induce immediate burning of the nose and throat and retrosternal burning and wheezing. Five years earlier, he experienced similar symptoms after chlorine inhalation, but the symptoms had been transient. Two days after exposure, FEV_1 was significantly reduced (66% of predicted) and the response to methacholine provocation was slightly abnormal. A bronchial biopsy showed almost complete replacement of the epithelium by a fibrinohemorrhagic exudate. Subsequent biopsies taken over a 5-month period showed considerable epithelial desquamation 15 days after exposure followed by signs of regeneration 5 weeks after exposure and considerable improvement 5 months after exposure, although an inflammatory infiltrate was still present. The bronchial responsiveness to methacholine paralleled the inflammatory changes, but could be significantly improved by inhaled steroids.

Kowitz et al. (1967) described the effects of chlorine exposure that occurred when a cylinder containing chlorine that was being unloaded from a freighter leaked. Neither exposure concentration nor exposure duration was available. At least 150 men were involved and almost all experienced acute symptoms. Eleven of 17 subjects who were admitted to a hospital were evaluated over a 3-year period. All showed respiratory distress on admission; other common signs included rales, wheeze, or rhonchi, or both, and pulmonary edema. Pulmonary testing conducted over the 3-year evaluation period showed a persistent decrease in lung volume and diffusing capacity and increased airway resistance. According to Kowitz et al. (1967), these alterations were compatible with an alveolo-capillary injury.

More recently, studies in rodents have confirmed the earlier observations regarding high exposures and have provided valuable information regarding the irritant properties of chlorine.

Acute exposure to low-to-moderate concentrations of chlorine induces a reduction in the respiratory rate, a protective reflex response mediated by stimulation of trigeminal nerve endings in the nasal mucosa. The concentration of the chemical that induces a 50% in respiratory rate is termed RD_{50}. For example, RD_{50} values of 9.3 and 3.5 ppm were determined in mice exposed for 10 and 60 minutes, respectively (Barrow et al. 1977; Gagnaire et al. 1994). An RD_{50} of 25 ppm was determined in male Fischer 344 rats exposed to chlorine for 10 minutes (Barrow and Steinhagen 1982). This study also demonstrated the development of tolerance to chlorine since in rats pre-exposed to chlorine at 1, 5, or 10 ppm, the RD_{50} values were 90, 71, and 454 ppm, respectively. Barrow and Steinhagen (1982) speculated that the mechanism of tolerance may involve reactions of chlorine with sulfydryl groups in the receptors or that chlorine exposure may damage the free nerve endings in the respiratory nasal mucosa. Rats pre-exposed to chlorine also developed cross-tolerance to formaldehyde (Chang and Barrow 1984). Interestingly, rats pre-exposed to 15 ppm formaldehyde did not develop tolerance to formaldehyde, but did develop cross-tolerance to chlorine, which suggested the existence of different reactive sites for the two gases (Chang and Barrow 1984).

DURATION? INTERVAL?

A study by the same group of investigators examined the effects of chlorine on lung -SH content and on the enzymes that maintain non-protein -SH levels, glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GSSG-RED) in rats exposed to 0 or 12 ppm chlorine for up to 2 weeks and sacrificed at various times after cessation of exposure (Dodd et al. 1980). The results showed no significant alterations in lung protein -SH, non-protein -SH, G6PD, or GSSG-RED in rats sacrificed immediately after 1, 5, or 10 days of exposure. Rats sacrificed 3 or 6 days after exposure showed an increase in lung -SH, G6PD, and GSSG-RED. These parameters returned to control values after 10 days of recovery. The investigators concluded that the increase in lung -SH and enzymatic activities observed during the recovery periods may reflect reparative processes subsequent to damage induced by chlorine. A different study by the same group showed that exposures to up to 10 ppm chlorine for 12 hours did not alter the total sulfydryl content (TSH) of the olfactory mucosa but lower concentrations did reduce TSH in the respiratory mucosa, suggesting that inhaled chlorine can oxidize tissue sulfydryl groups at the point of entry, but not at deeper regions of the respiratory tract (McNulty et al. 1983). McNulty et al. (1983) also found that exposure to 5 ppm for 6 hours or 10 ppm for 3 hours (concentration times exposure=30) produced similar reductions in TSH, but exposure to 2.5 ppm for up to 12 hours did not significantly

affect TSH content. The investigators speculated that a threshold concentration may be needed to overwhelm the protective mechanism in the respiratory mucosa (perhaps the mucociliary flow) allowing chlorine to penetrate deeper into the underlying tissue.

Acute studies also have examined respiratory function in animals.

Barrow and Smith (1975) evaluated inspiratory-expiratory flow rate ratios (Vi/Ve) and volume-pressure relationships (lung compliance) in rabbits exposed to 0, 50, 100, and 200 ppm chlorine for 10 minutes. The tests were conducted 0.5 hours after exposure and after 3, 14, and 60 days without exposure. After the last test, the rabbits were killed and the lungs were removed for gross and microscopic examination. Rabbits exposed to 50 ppm showed mild pneumonitis, which was also observed in control animals; this exposure level did not induce significant changes in air flow ratios, but transiently decreased lung compliance. Exposure to 100 or 200 ppm induced transient concentration related increases in Vi/Ve and a decrease, followed by an increase, in pulmonary compliance; these changes are related to gross signs of pulmonary edema and microscopic changes characterized by chronic pneumonitis and anatomic emphysema. Rabbits allowed to recover for 14 or 60 days showed no specific airway pathology. pulmonary?

In another study, mice exposed for 15 minutes to 0.8, 2, 3.1, or 3.8 ppm chlorine showed concentration-related decreases in respiratory frequency and increases in specific airway resistance (Morris et al. 2005). Pretreatment with atropine did not alter the increase in airway resistance, suggesting that this response does not involve parasympathetic cholinergic endings. However, pretreatment with capsaicin, a sensory nerve toxin, dramatically reduced respiratory irritation and the obstructive response, suggesting the involvement of sensory nerves. Mice exposed to much higher concentrations of chlorine (100–800 ppm) for 15 minutes showed increased airways resistance and increased responsiveness to methacholine and microscopic examination of the lungs showed flattening of the epithelium and epithelial cell loss and changes associated with oxidative stress (Martin et al. 2003). Since the increased responsiveness to methacholine could be prevented by inhibition of nitric oxide synthase, it appeared that nitric oxide (NO) production may have contributed to the airway damage.

Jiang et al. (1983) studied the time course of the histopathological alterations in the respiratory tract of rats and mice exposed to the RD_{50} of 9.1 ppm chlorine 6 hours/day for 1–5 days. The animals were killed immediately after the last exposure and the nose, larynx, trachea, and lungs were processed for microscopic examination. In both species, lesions were seen in the nasal passages with less severe changes in the nasopharynx, larynx, trachea, and lungs. The lesions in the nasal passages involved both

females. The nasal turbinates showed mucopurulent inflammation with secretory material and erosions of the mucosal epithelium. Changes in the trachea and bronchi consisted mostly of hyperplasia of the epithelial lining and inflammatory reactions. The alveolar sacs contained macrophages and secretory material and epithelial cells showed necrosis, hypertrophy and hyperplasia. Alterations in rats exposed to 1 and 3 ppm were less extensive and were limited to focal mucopurulent inflammation of the nasal turbinates in females. Males exposed to 1 or 3 ppm showed deeper pulmonary changes consisting of slight to moderate inflammatory reaction around the respiratory bronchioles and alveolar ducts, increased alveolar macrophages, and isolated areas of atelectasis (incomplete expansion). A LOAEL of 1 ppm for respiratory effects can be defined in this study based on the presence of inflammatory changes in the nasal turbinates of females and in the lungs of males; no NOAEL was established.

A similar study examined clinical signs, lung function, and histopathology of the nasal turbinates and lungs from Fischer 344 rats exposed to 0, 0.5, 1.5, or 5 ppm chlorine 6 hours/day, 5 days/week for 62 days (Kutzman 1983). Pulmonary function tests (plethysmograph-based assessment of multiple end points, including lung and tidal volumes, breathing frequency, transpulmonary pressure, lung compliance, N_2 washout, diffusing capacity for CO_2, maximum expiratory flow volume, peak expiratory flow and airway resistance) were conducted in 21–24 anesthetized males 6 hours after the last exposure. Respiratory tissues from these rats were prepared for histopathology. The lungs from some of these rats were also examined for collagen, elastin, total protein, and DNA. Exposure to 5 ppm cause severe upper respiratory irritation; exposure to 1.5 ppm showed occasionally less severe signs of irritation, whereas exposure to 0.5 ppm caused no obvious signs of irritation or discomfort. The tests of pulmonary function did not reveal marked abnormalities. The most significant effect was a reduction in airflow at 25% vital capacity in all exposed groups, indicating some degree of small airway involvement. There were no histopathological alterations in the lungs and nasal turbinates, but there was a tendency in the trachea for loss of cilia and epithelium at 0.5 and 5 ppm chlorine. The lung biochemistry only showed an increased collagen concentration at 1.5 and 5 ppm. Based on upper respiratory irritation and loss of cilia and epithelium in the trachea, the exposure level of 0.5 ppm can be defined as a LOAEL for respiratory effects; no NOAEL was defined in this study. This study was used as the basis for derivation of an intermediate-duration inhalation MRL for chlorine.

Two studies have examined the effects of chronic exposure to chlorine on respiratory parameters in animals.

3.2.1.4 Neurological Effects

Symptoms effects such as headache, dizziness, anxiety, and syncope are commonly reported following acute high exposures to chlorine and are thought to be due, at least in part, to anoxic anoxia induced by chlorine.

In a case of high exposure to chlorine that resulted in the death of the patient, postmortem examination showed a swollen brain with flattening of convolutions and subarachnoid hemorrhage (Adelson and Kaufman 1971). The investigators speculated that the lesions could have been caused by hypoxia that resulted from the severe pulmonary effects. In another case report, a 60-year-old man who accidentally inhaled chlorine gas in a swimming pool accident had a magnetic resonance scan of the head conducted 2 years after the accident that showed multiple areas of decreased signal in the periventricular white matter (*Levy* et al. 1986). Other neurological tests showed no evidence of cranial nerve abnormalities or sensory deficits. This brief communication does not mention what might have prompted the subject to undergo the scan.

Kilburn (1995, 2000, 2003b) published a series of reports describing long-lasting neurological effects in subjects accidentally exposed to high concentrations chlorine gas under various scenarios. The earliest study (Kilburn 1995) reported that six subjects exposed to an undetermined concentration of chlorine for 3 minutes to 5 hours had difficulty concentrating and sleeping, dizziness, loss of balance, excessive fatigue, loss of strength, depression, and irritability during a period of 1–3 years after the accident. Neurobehavioral tests were conducted 15–50 months after exposure and the results were compared to a control group matched for sex, age, and education. It should be noted that the testers were aware of the exposure status of the subjects. The results showed impaired balance with the eyes closed and hearing loss in all of the exposed subjects. Five had decreased vibration sensitivity, color discrimination, and verbal recall; four had prolonged blink reflex latency; three had prolonged simple and choice reaction times, and three had nerve defects or constricted visual fields. In a subsequent study, 22 patients exposed briefly (the reports mentions seconds to a few minutes in one section and minutes to a few hours in another section) to chlorine gas were evaluated with a battery of tests 7–48 months after exposure. A total of 296 unexposed subjects served as controls. The results showed significant impairment among the exposed group in a number of areas including balance, reaction time, color identification, visual field performance, blink latency, cognition, verbal recall, and making trails. A similar study was conducted with subjects exposed to chlorine as a result of a train derailment (see Agency for Toxic Substances and Disease Registry [1998] under Respiratory Effects) (Kilburn 2003b). Ninety-seven subjects were tested

damage to the upper gastrointestinal tract. Pike et al. (1963) reviewed 129 cases of children who ingested Clorox® and reported that no complications of consequences were found. Sixty-five cases were examined by esophagoscopy within 96 hours of the ingestion and only 2 showed evidence of esophageal injury. The children were between 12 month and 7 years old and the amounts of bleach ingested ranged from "½ ounce to 1 cup." Landau and Sanders (1964) state that among 393 children who ingested bleach and were seen at a hospital, there were no esophageal strictures or perforations, and about 50% of the patients received no treatment. Hook and Lowry (1974) reported that among 23 definite cases of children who ingested Clorox®, severe irritation of the esophageal mucosa was observed in only 1 case. Minor transient irritation was observed in some of the patients. A report from the German literature of 23 children who accidentally ingested 3–5% sodium hypochlorite indicates that there was only 1 case with signs of superficial burns in the esophagus, which had disappeared 2 weeks later when controlled by esophagoscopy (Mühlendahl et al. 1978). Liquid bleach is a strong emetic, which helps reduce the time of residence in the stomach, but on the other hand, it increases the potential for aspiration.

Examination of fatal cases following ingestion of unknown quantities has revealed esophageal and gastric mucosal erosions, perforations at the gastroesophageal junction, and extensive necrosis of adjacent soft tissue (Ross and Spiller 1991). In a fatal case of a child who drank 4.5% sodium hypochlorite in an alkaline solution (pH 12), severe gross lesions were seen in the mouth, tongue, glottis, epiglottis, esophagus, and stomach (Jakobsson et al. 1991). Glottic and subglottic edema was described by Babl et al. (1998) in a child who drank household bleach from a cup.

In some earlier studies in animals, commercial 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). For example, commercial bleach placed in the esophagus of 151 dogs for several minutes caused the immediate death 8 dogs from perforations into their pleural cavities (Landau and Sanders 1964). Necropsy performed 3 months later on the seven dogs that survived revealed no abnormalities. Yarington (1970) reported that, in dogs, the minimum amount of bleach that caused a burn in the esophagus was 10 cm^3 applied over a 5-minute period. A volume of 30 cm^3 applied for 2 minutes caused minimal edema of the esophagus.

Few more recent studies are available. Gross and microscopic examination of multiple levels of the gastrointestinal tract of Sprague-Dawley rats that drank water that provided up to 24.9 mg Cl/kg/day for 90 days did not reveal any significant gross or microscopic alterations (Daniel et al. 1990). The same

opening and average day of observed vaginal patency were unaltered in pups evaluated at age 28 and 40 days. The developmental NOAEL of 3.4 mg Cl/kg/day is listed in Table 3-3 and plotted in Figure 3-2.

3.2.2.7 Cancer

Studies of the carcinogenicity of trihalomethanes or other organic chemicals that form in water as a result of the chlorination of drinking water are not discussed in this section since these studies were not intended to assess whether chlorine itself is responsible for cancer. For reviews on this issue, the reader is referred to IARC (1991), Koivusalo and Vartienen (1997), and EPA (1994b).

Cancer bioassays of chlorine in drinking water have been conducted in rats and mice. In the NTP (1992) bioassay, Fischer-344 rats (70/sex/dose group) were exposed to 0, 70, 140, or 275 ppm sodium hypochlorite in the drinking water for 103–104 weeks. This provided doses of 0, 4.2, 7.3, or 13.6 mg Cl/kg/day to males and 0, 4.2, 7.8, or 14.4 mg Cl/kg/day to females. The water used in the study was deionized charcoal-filtered water. Interim sacrifices (10 rats/sex/dose) were conducted at 14 and 66 weeks. The only significant finding was an increased incidence of leukemia in female rats. The incidences were: 8/50, 7/50/, 19/51, and 16/50 in the control, low-, mid-, and high-dose females, respectively. Pair-wise comparison showed a statistically significant difference between controls and the mid-dose ($p=0.014$) and a trend test was also significant ($p=0.037$). In males, the respective incidences were 25/51, 25/51, 27/50, and 29/51. These results led NTP (1992) to conclude that there was equivocal evidence of carcinogenicity in female rats based on the fact that there was no clear dose-related response or reduced latency, did not occur in males, and the incidence in concurrent controls (16%) was significantly lower than in historical controls (25%). In a similar study, Hasegawa et al. (1986) administered sodium hypochlorite in distilled water to groups of Fischer-344 rats (50/sex/dose) in concentrations of 0, 500, or 1,000 ppm to males and 0, 1,000, or 2,000 ppm to females for 104 weeks; this was followed by a period of 8 weeks of drinking untreated water. This corresponds to doses of approximately 0, 33, or 67 mg Cl/kg/day for males and 0, 67, or 133 mg Cl/kg/day for females. The results showed no significant treatment-related increased incidence of neoplasms or alterations in latency of neoplasms.

AWK.

In the NTP (1992) study, $B6C3F_1$ mice (70/sex/dose group) drank water with 0, 70, 140, or 275 ppm sodium hypochlorite for 103–104 weeks. This corresponds to doses of approximately 0, 7.4, 14, or 24 mg Cl/kg/day for males and 0, 7.6, 14.2, or 24.2 mg Cl/kg/day for females. The water used in the study was deionized charcoal-filtered water. Interim sacrifices (10 mice/sex/dose) were conducted at 15 and

Ocular Effects. Very limited information was located regarding ocular effects of direct contact of the eye with hypochlorite solutions. In their text *Toxicology of the Eye*, Grant and Schuman (1993) state that "because most accidental splashes in the eye have been with the relatively weak 5% household solutions of sodium hypochlorite, very few human eye injuries have been reported, and recovery has been rapid and complete."

Experiments conducted in male and female New Zealand albino rabbits showed that instillation of 0.1 mL of household bleach directly to the central corneal surface and followed over a 21-day period produced moderate irritation (Griffith et al. 1980). The median day to clear was 7 days. In a review of the literature, Racioppi et al. (1994) mention unpublished data indicating that in rabbits, 0.1 mL of an 8% solution of sodium hypochlorite (without rinsing) caused moderate irritation and that the recovery time was 7 days; under similar conditions, 0.01 mL of the same solution had low irritation potential and the recovery time was 3 days.

Irritation of cornea? <OR> Irritation of conjunctiva w/ erosion of cornea?

3.2.3.3 Immunological and Lymphoreticular Effects

Although sodium hypochlorite generally is not considered a contact sensitizer, several cases of allergic contact dermatitis have been reported. Osmundsen (1978) reported that case of a woman had a strong reaction to patch testing with 0.5% sodium hypochlorite in water years after having had dermal contact with chloramine. Further tests showed positive reactions to sodium hypochlorite in 3 out of 225 patients. Habets et al. (1986) reported two cases of hand dermatitis related to sodium hypochlorite allergy, as diagnosed by patch tests. Both patients showed a positive reaction to sodium hypochlorite up to a concentration of 0.1%. Van Joost et al. (1987) reported one additional case among 40 housewives who apparently had used bleaching agents for long periods. Eun et al. (1984) also reported a case of allergic contact dermatitis in a veterinarian who occasionally washed his hands with a commercial solution containing 4–6% sodium hypochlorite.

ACD or ICD?

No information was located regarding immunological and lymphoreticular effects in animals following dermal exposure to aqueous chlorine.

No studies were located regarding the following effects in humans or animals after dermal exposure to aqueous chlorine:

There is limited information regarding the *in vivo* genotoxicity of aqueous chlorine. A study in which male B6C3F$_1$ mice were administered chlorine in the drinking water as sodium hypochlorite or hypochlorous acid for 5 days and that provided doses of up to 8 mg Cl/kg/day found no evidence of increased incidences of chromosomal aberrations or micronuclei in bone marrow (Meier et al. 1985). In another study, administration of a single intraperitoneal dose of up to 2,500 mg/kg sodium hypochlorite (1,175 mg Cl/kg/day) to male ddY mice did not increase the incidence of micronuclei in bone marrow evaluated 24 hours after dosing (Hayashi et al. 1988). Exposure of newt larvae to sodium hypochlorite in the surrounding water (0.12 or 0.25 µg/mL) for 12 days increased the frequency of micronuclei in blood erythrocytes (Le Curieux et al. 1993). However, the study did not specify in what type of water the larvae were kept. If the larvae were kept in tap water, it is possible that chlorination byproducts rather than chlorine or the hypochlorite anion were the clastogenic agents. Table 3-5 summarizes the genotoxicity of sodium hypochlorite *in vivo*. Studies of the genotoxicity of sodium hypochlorite *in vitro* are summarized in Table 3-6. As the table shows, the results have been mixed and no general statements can be made. The variability of the results may be due to differences in the experimental protocols used.

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Nodelman and Ultman (1999a) measured the fraction of an inspired chlorine bolus absorbed during a single breath as a function of the bolus penetration into the respiratory system of five nonsmoker males and females during both nasal and oral breathing at a respiratory flow of 250 mL/second using a noninvasive procedure. Measurements of the chlorine concentrations were made by means of a fast-responding thermionic chlorine analyzer. Peak concentrations of 0.5 and 3 ppm chlorine were used in nasal breathing experiments and 3 ppm in oral breathing experiments. The results indicated that almost all of the chlorine inhaled was absorbed in the upper airways whether the subjects inhaled through the nose or through the mouth. By comparing mass transfer parameters, the investigators also determined that total absorption rates for the mouth and nose were similar. When the peak concentration in the nasal breathing experiments was increased from 0.5 to 3 ppm, the mass transfer parameters remained unchanged, indicating that the dissolution, diffusion, and chemical reactions governing the absorption of the gas by the nasal mucosa are all linear processes. In other words, over the 0.5–3 ppm concentration range, absorption appeared to be concentration-related. In a separate experimental series, the investigators determined the longitudinal distribution of a bolus of 3 ppm chlorine as a function of the

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to chlorine than will most persons exposed to the same level of chlorine in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of chlorine, or compromised function of organs affected by chlorine. Populations who are at greater risk due to their unusually high exposure to chlorine are discussed in Section 6.7, Populations with Potentially High Exposures.

→ Shusterman et al 1998; 2003b

Populations unusually susceptible to chlorine gas exposure include individuals with respiratory conditions such as asthma, hay fever, and chronic bronchitis, heavy smokers, and children. Rotman et al. (1983) described the case of an atopic individual who experienced severe distress during exposure to 1 ppm chlorine, a concentration that was tolerated by healthy subjects. D'Alessandro et al. (1996) also reported that subjects with airway hyperreactivity to methacholine exhibited a much more pronounced decrease in FEV_1 and $FEF_{25-75\%}$ than healthy subjects during exposure to 1 ppm chlorine. Following an accidental leak of chlorine, individuals who had a more prevent history of smoking and asthma exhibited more hypoxemia and were more likely to have tachypnea, crackles, and wheezes during examination than subjects without asthma and/or who smoked less (Hasan et al. 1983). In the former, signs and symptoms of chlorine intoxication resolved more slowly reduced and flow rates and lung volumes were still evident 2 weeks after acute exposure to chlorine. Similar observations regarding smokers have been made in studies of workers who have experienced occasional high exposures or "gassing" episodes (Chester et al. 1969; Gautrin et al. 1999; Henneberger et al. 1996).

In a swimming pool accident involving 126 adult and 134 children, among both children and adults, the incidences of all symptoms (eye, nose, and throat irritation) and respiratory problems (shortness of breath, wheezing, cough) were higher among those who had a history of chronic respiratory disease than among healthy people (Agabiti et al. 2001). In addition, in adults, incidences were higher among smokers and former smokers than among never smokers.

Some reports in which adults and children were accidentally exposed to high concentrations of chlorine have suggested that children might be more susceptible to the effects of chlorine than adults. For example, in a case involving 106 individuals, 60 of whom were children and adolescents <18 years old, of

be avoided following ingestion of chlorine bleach. However, dilution with water or milk is recommended, but the dilution amount should be small to avoid inducing vomiting. In case of exposure of the skin to aqueous chlorine, flushing with copious amounts of plain tepid water is recommended. In case of exposure of the eyes, irrigation with saline or Ringer's lactate is recommended.

3.11.2 Reducing Body Burden

reacts with ?

There are no standard methods for reducing chlorine body burden. Studies in humans have shown that under low exposure conditions (<5 ppm), >95% of the inspired chlorine is absorbed in the upper airways and <5% is delivered to the lower airways (Nodelman and Ultman 1999a, 1999b). Chlorine that is absorbed into the mucosa of the upper respiratory airways eventually joins the pool of chloride ions in the body. Studies in animals also have shown that most of the chlorine ingested as hypochlorous acid is transformed and eliminated as chloride (Abdel-Rahman et al. 1983).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The toxic effects of chlorine gas are due to its oxidant properties and also to the added tissue damage caused by the hypochlorous and hydrochloric acids that result from the reaction of chlorine with water. There are no established methods to interfere with the oxidant properties of chlorine, but nebulized sodium bicarbonate has been used to neutralize the acid (Bosse 1994; Douidar 1997).

The treatment of exposure to chlorine gas is symptomatic, exposure to low concentrations may require only treatment for sensory irritation, but exposure to high concentrations may cause serious respiratory symptoms including pulmonary edema and respiratory failure and death. The information below has been extracted from the texts listed above and also from Baxter et al. (1989).

Before any treatment, the patient should be assessed for signs of corrosive injury to mucous membrane, eyes, and skin. The assessment should also include a check for lung sounds, peak flow, and vital signs. Patients heavily exposed who show breathing difficulties at rest should undergo baseline x-ray examination. The initial treatment consists of irrigation with water or saline and vasoconstrictive ophthalmic solutions for eye irritation, but eye damage may require referral to a health care facility. Nausea may be treated with Phenergan® and administration of clear liquids, whereas sore throat can be treated with throat lozenges or spray or a humidifier. Decongestants are recommended for rhinitis and antitussive agents for the treatment of cough. Skin burns should be treated as thermal burns. Patients exhibiting respiratory effects should receive 100% humidified oxygen, unless it is contraindicated by the

medical history. As mentioned above, 5% nebulized bicarbonate has been used in patient with respiratory effects with favorable responses in at least some patients (Bosse 1994; Douidar 1997). Nebulized salbutamol or terbutaline may be used to treat bronchospasm. Therapy with corticosteroids has not been proved to produce improvement in chlorine gas poisoning. Monitoring of respiratory function and arterial blood gases in important because pulmonary edema may occur up to 24 hours after exposure. If pulmonary edema occurs, administration of 60% humidified oxygen by face mask or mechanically is recommended and if pO_2 cannot be maintained above 50 mmHg, the patient may need to be intubated for positive end expiratory pressure ventilation. Caution should be exercised with the administration of intravenous fluids and because fluid overload is extremely dangerous in such patients. If fluid overload occurs, diuretics such as furosemide may be useful as indicated. Survivors of high chlorine exposure should be monitored periodically to determine possible persistent loss of pulmonary function.

3.12 ADEQUACY OF THE DATABASE

Inhaled steroids in RADS

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chlorine is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of chlorine.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Chlorine

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chlorine gas and aqueous chlorine are summarized in Figures 3-4 and 3-5, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of chlorine. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the

monitor the contact time between the solution and the mucosa (Hook and Lowry 1974; Landau and Saunders 1964; Strange et al. 1951; Yarington 1970). These studies are inadequate for quantitative risk assessment. Two more recent studies were of very limited scope (Cunningham 1980) or reported ambiguous results (Meier et al. 1985); therefore, could not be used for derivation of an acute-duration oral MRL for aqueous chlorine. Additional acute-duration oral studies are necessary to define dose-response relationships for aqueous chlorine.

Dermal effects have been reported in a few cases of direct acute contact of the skin with high concentrations of chlorine gas in humans (Agency for Toxic Substances and Disease Registry 1998; Joyner and Durel 1962; NIOSH 1995), and eye and skin irritation were reported in volunteers exposed to 1 ppm chlorine for up to 8 hours (Anglen 1981; Rotman et al. 1983). Information on dose-response for sensory irritation was used along with data on pulmonary effects to derive the acute-duration inhalation MRL for chlorine. Additional studies of sensory irritation with chlorine gas do not appear necessary at this time. Chlorine gas is not absorbed through the skin, so systemic effects due to contact of the skin with chlorine are not expected to occur. Dermal effects of hypochlorite bleach have been reported in humans and in animals (Habets et al. 1986; Hostynek et al. 1989, 1990; Nixon et al. 1975; Strange et al. 1951); therefore, additional dermal studies do not seem necessary at this time.

Intermediate-Duration Exposure. No studies of humans exposed specifically for intermediate duration to chlorine gas were located. However, it is likely that in many of the occupational studies available, some workers were exposed for intermediate durations. Only two intermediate-duration studies in animals are available (Barrow et al. 1979; Kutzman 1983). Both studies utilized rats and in both studies, 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. The Kutzman (1983) study was selected as the principal study for derivation of an intermediate-duration inhalation MRL for chlorine. Additional intermediate-duration inhalation studies in animals do not seem necessary at this time.

Few intermediate-duration studies in animals were located that examined a wide range of end points following exposure to hypochlorite. 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 (Abdel-Rahman et al. 1984; Cunningham 1980; Daniel et al. 1990, 1991) and also

drinking water. The highest level of chlorine allowed in drinking water is 4 ppm (EPA 2006a), which is considerably lower than the maximal concentration of chlorine used in long-term studies (275 ppm available chlorine) in rats and mice (NTP 1992), which caused no significant toxicity. Therefore, it seems unlikely that free chlorine in drinking water will represent a health concern for humans. It should be noted, however, that chlorinated water contains a variety of chlorinated byproducts whose biological effects continue to be studied.

Biomarkers of Exposure and Effect.

Exposure. There are no specific biomarkers of exposure for chlorine. Chlorine gas that enters the airways or chlorine ingested as sodium hypochlorite eventually joins the chloride pool in the body.

Effect. There are no biomarkers of effect specific for chlorine. The sensory irritation and respiratory alterations caused by exposure to chlorine gas or the esophageal irritation caused by ingestion of hypochlorite bleach can also be caused by other chemicals.

cleared by?

Absorption, Distribution, Metabolism, and Excretion. The only information regarding pharmacokinetics of chlorine gas is that from experiments in volunteers conducted by Nodelman and Ultman (1999a, 1999b) that showed that almost all (>95%) of a bolus dose of chlorine gas inhaled through the mouth or the nose is absorbed in the upper respiratory tract and none reaches the lungs. This was observed over a 0.5–3 ppm exposure range. The methodology used to generate the bolus and to monitor the concentrations of chlorine in the airways could probably be adapted to studies in animals, particularly monkeys, to test a wider range of concentrations and to correlate internal concentrations of chlorine with lesions in the respiratory tract.

There is only one study of the pharmacokinetics of aqueous chlorine, the study by Abdel-Rahman et al. (1983) that evaluated absorption, metabolism, distribution, and excretion of chlorine in rats following gavage doses or radiolabeled (^{36}Cl) hypochlorous acid. Additional studies may be useful to confirm or refute the findings of Abdel-Rahman et al. (1983). On the other hand, as Scully et al. (1988) pointed out, because aqueous chlorine is a potent oxidant, pharmacokinetic studies of radiolabeled hypochlorous acid (^{36}Cl) in animals do not reveal what happens to the parent compound, but rather to the product of the reactions of these compounds *in vivo*. Therefore, the usefulness of additional studies is questionable.

EPA (IRIS 2007) has established an oral reference dose (RfD) for chlorine of 0.1 mg/kg/day based on a NOAEL of 14.4 mg/kg/day for systemic effects in Fischer-344/N rats exposed to chlorine in the drinking water for 2 years (NTP 1992). The uncertainty factor used in this assessment was 100 (10 for interspecies extrapolation and 10 for the protection of sensitive human subpopulations).

EPA has not derived an inhalation reference concentration (RfC) for chlorine gas.

The International Agency for Research on Cancer (IARC), the National Toxicology Program (NTP), and EPA has not classified chlorine, sodium hypochlorite, or hypochlorous acid for human carcinogenicity (IARC 2006; IRIS 2007; NTP 2005). The American Conference of Governmental Industrial Hygienists (ACGIH) has classified chlorine as an A4 carcinogen (not classifiable as a human carcinogen) (ACGIH 2006).

OSHA has required employers of workers who are occupationally exposed to chlorine to institute engineering controls and work practices to reduce and maintain employee exposure at or below permissible exposure limits (PELs) (OSHA 2006c). The employer must use engineering and work practice controls to reduce exposures to or below an 8-hour time-weighted average (TWA) of 1 ppm for chlorine (OSHA 2006c).

ceiling

EPA has designated chlorine as a hazardous air pollutant (HAP) under the Clean Air Act (CAA) (EPA 2007b). Chlorine and sodium hypochlorite are on the list of chemicals appearing in "Toxic Chemicals Subject to Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986" and has been assigned a reportable quantity (RQ) limit of 10 and 1 pounds, respectively (EPA 2007e). Chlorine is also considered to be an extremely hazardous substance (EPA 2007f). The RQ represents the amount of a designated hazardous substance which, when released to the environment, must be reported to the appropriate authority.

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), chlorine gas is exempt from the requirement of a tolerance for pesticide chemicals in food when used as pre- or postharvest in solution on all raw agricultural commodities (EPA 2007h) and sodium hypochlorite is exempt from the requirement of a tolerance for residues in food (EPA 2007k).