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The organic ion hydroxymethanesulfonate (HMSA) has been measured in micro-molecular concentrations in acidic fogs in southern California. HMSA is a stable adduct in fogs with a pH range of 3-5, but it is likely to dissociate at pH 6.6, the pH of the human airways fluid lining. It was hypothesized that HMSA may have a specific bronchoconstrictor effect because HMSA may dissociate in the airway lumen generating SO_2 and CH_2O, both of which have bronchoconstrictor potential. In order to determine whether HMSA has such an effect, a total of 19 subjects with mild to moderate asthma were studied in an exposure chamber in which freely breathing and exercising subjects inhaled simulated fogs containing HMSA at a concentration (1000 uM) higher than the ambient levels, for 1 hour. The results indicated no significant bronchoconstrictor effect for acute exposures of HMSA.

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TABLE OF CONTENTS

Acknowledgement
List of Figures
Abstract
Conclusions
Recommendations
Body of Report
References
Publications/Presentations
Tables
Figures
Appendix

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LIST OF FIGURES

Table 1 Subject characteristics.

Table 2 Exposure characteristics, chamber study.

Table 3 Mean changes in symptom scores after inhalation of 3 types of fogs, chamber study.

Figure 1 Schematic of exposure chamber with fog generation and monitoring systems.

Figure 2 Mean specific airway resistance (SRaw) at baseline and after inhalation of each of 5 sequentially administered aerosols containing either hydroxymethanesulfonate (HMSA) in sulfuric acid (H_2SO_4) or H_2SO_4 alone for 9 subjects, mouthpiece study.

Figure 3 Mean SRaw before, during, and immediately after inhalation of fogs containing either HMSA in H_2SO_4 or H_2SO_4 alone for 10 subjects, chamber study.

Figs. 4-7 SRaw before, during and immediately after inhalation of fogs containing HMSA in H_2SO_4, H_2SO_4 alone, or neutral saline for subjects #2, 4, 6, and 7, respectively, chamber study.

ABSTRACT

The organic ion, hydroxymethanesulfonate (HMSA), has been measured in micromolar concentrations in acid fogs in southern California. HMSA is formed in the atmosphere by the combination of bisulfite (HSO_3^-) and formaldehyde (CH_2O). HMSA is a stable adduct in fogs with a pH range of 3-5, but it is likely to dissociate at pH 6.6, the pH of the fluid lining human airways. The dissociation of inhaled HMSA under the conditions present in the airway lumen should theoretically generate sulfur dioxide and CH_2O, both of which have bronchoconstrictor potential. Thus, we hypothesized that hydroxymethanesulfonic acid may have a specific bronchoconstrictor effect independent of its strength as an acid.

In order to determine whether HMSA has such a specific bronchoconstrictor effect, we studied a total of 19 subjects with mild to moderate asthma, following 2 separate protocols. Because of the lack of precedent for exposing human subjects to HMSA, the initial study involved inhalation during rest of sequentially increasing concentrations for a short duration (3 minutes) via a mouthpiece system. After no significant bronchoconstrictor effect of HMSA was demonstrated under the conditions of this pilot study, we then performed an experiment in an exposure chamber in which freely breathing and intermittently exercising subjects inhaled simulated fogs containing HMSA, at a concentration (1000 μM) higher than what has been measured in the atmosphere, for 1 hour. The results of the exposure chamber study again indicated no significant bronchoconstrictor effect for HMSA. Thus, we conclude that individuals with asthma are not likely to develop clinically significant bronchoconstriction when exposed to fogs containing HMSA in the ambient range.

CONCLUSIONS

The project completed under this contract permits the following conclusions:

1. Inhalation of dense (87 g/m^3) aerosols containing up to 1000 μM hydroxymethanesulfonate (HMSA) through a mouthpiece during resting breathing did not cause clinically significant bronchoconstriction in subjects with mild to moderate asthma. Administration of aerosol through a mouthpiece bypasses the scrubbing effect of the nose, and thereby increases the effective dose to the airways.

2. Clinically significant bronchoconstriction also did not occur in subjects with mild to moderate asthma exposed to HMSA-containing fogs for 1 hour in a chamber during intermittent exercise. The HMSA concentration administered in this study (1000 μM) is approximately 3 times higher than that which has been measured in southern California.

3. Clinically significant bronchoconstriction also did not occur in subjects with mild to moderate asthma exposed to fogs containing sulfuric acid (H_2SO_4) at a concentration ~ 1000 μg/m^3 for 1 hour in a chamber during intermittent exercise. This result confirms that of a previous ARB-funded project already reported to the board (Research contract final report re: A5-179-33, Project 3).

RECOMMENDATIONS

1. More information should be collected about the bronchoconstrictor effects of various acids, including hydroxymethanesulfonic acid, when administered in submicronic aerosols of low relative humidity, rather than in the dense fogs studied under this contract. We make this recommendation because recently it has been hypothesized that differences in the relative humidity of sulfuric acid aerosols administered by various investigators may explain the differences in the reported bronchoconstrictor potency of sulfuric acid.

2. Effects of acid aerosols, including those containing HMSA, on end-points other than bronchoconstriction should be studied. We make this recommendation for a number of reasons: a) bronchoconstriction is not likely to be a sensitive end-point for assessing the potential for acid aerosols at ambient concentrations to cause adverse health effects because of the high concentrations of sulfuric acid that have been required to induce significant bronchoconstriction in published reports of human exposure studies; b) there is evidence from animal studies that other end-points, such as mucociliary clearance and the ability to resist experimental infections, are affected by inhalation of acid aerosols; c) our laboratory and other investigators are working to develop new assays of respiratory tract toxicity, such as bronchoalveolar lavage (BAL) for evidence of inflammatory cellular response and mediator release, *in vivo* ciliary beat frequency and epithelial permeability, release of heat shock proteins by alveolar macrophages recovered from BAL, *in vitro* secretion of glycoproteins by cultured respiratory epithelial cells, etc.

DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

INTRODUCTION

The organic ion, hydroxymethanesulfonate (HMSA), has been measured recently in micromolar concentrations in acid fogs in southern California (1). HMSA is formed in the atmosphere by the combination of bisulfite (HSO_3^-) with formaldehyde (CH_2O). Formation of HMSA explains observed excesses of sulfur in the S(IV) state (4+ oxidation state) and formaldehyde in fogs and clouds (1). HMSA may represent an important source of acidity for water droplets and also may play a role in the transformation and long-distance transport of sulfur dioxide (SO_2).

While hydroxymethanesulfonic acid may cause bronchoconstriction in persons with asthma in the same manner as the inorganic acids previously studied in our laboratory, (i.e., bronchoconstriction induced by sulfuric, nitric, and hydrochloric acid aerosols appears to be chiefly a function of titratable acidity (2)), this organic acid deserves specific consideration because it is formed from HMSA. HMSA is a stable adduct in highly acidic solutions, but it is likely to dissociate into HSO_3^- and CH_2O at pH 6.6, the approximate pH of the airway lumen (1). Under the conditions present in the airways, the HSO_3^- generated from HMSA dissociation will be in equilibrium with SO_2 (3). SO_2 is a potent bronchoconstrictor (4). Although CH_2O is not as potent when inhaled as a gas (5), its lack of bronchoconstrictor effect may be due in part to uptake in the upper airways (6). By delivering SO_2 and CH_2O directly to the airway mucosa, an aerosol containing HMSA might bypass the normal scrubbing mechanisms of the upper airways and thereby be an effective stimulus to bronchoconstriction. If HMSA is a more potent bronchoconstricting agent than the inorganic acids we have studied

previously, then it also may cause greater potentiation of the bronchoconstrictor effect of hypoosmolarity (7).

The primary purpose of the experiments performed under this contract was to evaluate the bronchoconstrictor potency of HMSA in subjects with asthma. Because of the lack of precedent for exposing human subjects to HMSA, the initial study involved inhalation during rest of sequentially increasing concentrations for short durations via a mouthpiece system. After no significant bronchoconstrictor effect of HMSA was demonstrated under the conditions of this pilot study, we then performed an experiment in an exposure chamber in which freely breathing and intermittently exercising subjects inhaled simulated hypoosmolar fogs containing HMSA (at a concentration higher than what has been measured in the atmosphere) for 1 hour.

METHODS

The subjects were 19 non-smoking volunteers who were informed of the risks of the experimental protocol and signed written consent forms approved by the Committee on Human Research of the the University of California, San Francisco. All subjects had asthma as defined by a history of recurrent episodes of wheezing, chest tightness and reversible airway obstruction previously documented by a physician. All of the subjects completed the protocol. All subjects received financial compensation for their participation. No subject took theophylline preparations or inhaled beta adrenergic agonists within 24 hours or consumed caffeine-containing beverages or food within 4 hours of the experiment. No subject took oral corticosteroids within the study period. All subjects denied a history of an upper respiratory infection within 6 weeks prior to the study. Subject characteristics are listed in Table 1. Predicted values for the spirometric parameters described are those of Knudson and co-workers (8).

The subjects were divided into 2 groups. The first group, consisting of 9 subjects, was enrolled in the pilot study. On the initial study day, baseline spirometry (No. 822, Ohio Medical Products, Madison, WI) was performed and methacholine responsiveness was tested by measuring specific airway resistance (SRaw) before and after inhalation of 10 FRC (functional residual capacity)-to-TLC (total lung capacity) breaths of and doubling concentrations of methacholine (0.063, 0.125, 0.25, 0.50, 1.0, and 2.0) in phosphate buffered saline delivered by a Devilbiss nebulizer (No. 646, Devilbiss Co., Somerset, PA) with a dose-metering device calibrated to deliver 0.01 ml/breath. The concentration of methacholine that produced a 100% increase in SRaw from the post-saline SRaw baseline

was calculated by log_2-linear interpolation. Only subjects who developed ≥ 100% increase in SRaw (n=9) continued in the study. On 2 subsequent days, subjects were exposed repetitively to 5 aerosols of either 50 μM sulfuric acid (H_2SO_4) alone or 50 μM H_2SO_4 to which 1 of 5 sequentially increasing concentrations of HMSA (0, 30, 100, 300, and 1000 μM) had been added. Subjects inhaled each aerosol for 3 minutes through a mouthpiece during tidal breathing at rest. The 2 exposure days were randomly ordered and the aerosol challenges were performed in a single-blind fashion at the same time of day. Subjects were not exposed to aerosol on days when their baseline SRaws were < 50% or > 150% of their usual baseline values. To assess airway responses of the subjects to the inhaled aerosols, airway resistance (Raw) and thoracic gas volume (Vtg) were measured in a constant volume body plethysmograph (No. 09103, Warren E. Collins, Braintree, MA) and expressed as the product of Raw and Vtg, SRaw. Five measurements of SRaw, 1 every 30 seconds, were made before and starting 1 minute after each aerosol challenge. Coughs were counted throughout the experiment by an observer and recorded on a small tape recorder. Throat, respiratory, and nonrespiratory symptoms were assessed by means of a post-exposure questionnaire with an 11-point rating scale (0=least, 10=most) for each of 9 symptoms (throat irritation, chest pain, chest tightness, dyspnea, cough, sputum production, wheezing, back pain, and headache).

Aerosols for the mouthpiece study were generated by an ultrasonic nebulizer (Mistogen EN 145, Timeter Instrument Corp., Lancaster, PA). The solutions used to generate aerosols were adjusted to pH 4.0 by the addition of small amounts of 0.01M H_2SO_4 or 0.01M sodium hydroxide. The osmolarity of these solutions was adjusted to 300 mOsm, the osmolarity

of body fluids. Liquid water content (LWC) was measured by collecting droplets on a 47 mm membrafil filter (Nuclepore Inc., Pleasanton, CA) by sampling air at 1 L/minute utilizing a vacuum pump. Mass median aerodynamic diameter (MMAD) was measured with a cascade impactor (In-tox Products, Albuquerque, NM). Temperatures were ambient and were measured every minute at the mouthpiece. The pH was measured with a pH meter (Model 43, Beckman Instruments, Inc., Irvine, CA).

The second group, consisting of 10 subjects, was enrolled in the chamber study. On the initial study day, baseline spirometry was performed and methacholine responsiveness was tested as described above for the mouthpiece study. Only subjects who developed ≥ 100% increased in SRaw (n=10) continued in the study. On 2 subsequent days, subjects were exposed to simulated fogs containing either 1mM HMSA in 5mM H_2SO_4 or 5mM H_2SO_4 alone. Only subjects (n=3) who developed a substantial (i.e., ≥ 50%) increase in SRaw after exposure to either acid fog were exposed to a neutral fog as an added control measure. The fog challenges were randomly ordered and were performed in a single-blind fashion at the same time of the day. The subjects were exposed to the fogs in an 8' x 8' x 8' stainless steel and glass exposure chamber (Vista Scientific, Ivyland PA). The exposure period lasted 1 hour, with alternate 15-minute periods of rest and exercise, in that order. Exercise was performed on a constant-load cycle ergometer (No. 18070, Gould Godart, Bilthoven, the Netherlands) at a workload of 100 watts. Subjects were not exposed to fog on days when their baseline SRaws were < 50% or > 150% of their usual baseline values. In order to reduce neutralization of inhaled aerosol by oral ammonia, the subjects brushed their teeth and gargled with antiseptic mouthwash prior to each challenge. To assess

airway responses of the subjects to the inhaled fogs, SRaw was measured as described above for the mouthpiece study. Five measurements of SRaw, one every 30 seconds, were made before each challenge, after the initial 15-minute resting exposure, after the initial 15-minute exercise exposure, and after the completion of the 1-hour exposure. The subjects left the inhalation challenge chamber during the 1-hour exposure period only for the time required to measure their SRaws (approximately 3 minutes) at 15 minutes and 30 minutes after the onset of exposure. Throat, respiratory, and nonrespiratory symptoms were assessed by pre- and post-exposure administration of the same questionnaire described above for the mouthpiece study.

Fogs were generated by forcing stock solution (either 5mM H_2SO_4 alone or 1mM HMSA in 5mM H_2SO_4) under high pressure through a series of atomizers was adjusted to keep the LWC ~ 2 g/m^3. The osmolarity of the stock solutions was 30 mOsm, hypoosmolar relative to body fluids. The droplets were blown (via a 400 CFM capacity central blower) through a series of mesh screens (designed to scavenge larger droplets) and Teflon ducts into the exposure chamber. Central ceiling manifolds provided even distribution of the fog. Excurrent chamber air was drawn via perimeter floor ducts through a series of filters to remove droplets and provide 100% humidified air to the aforementioned blower to, in a continuous fashion, propel newly created fog droplets into the chamber. In this way, 90% of the chamber air was re-circulated, 10% was exhausted via a fog water collector and 10% fresh air was introduced after purification and humidification. Incurrent air temperature (24° ± 2°C) was maintained at ~ 1°C above excurrent air temperature to minimize evaporation from fog droplets. Figure 1 is a schematic of the fog generation system.

The fog droplets in the chamber were monitored in terms of both physical and chemical characteristics. A phase/Doppler particle analyzer (Model 1100, Aerometrics, Mountain View, CA) linked to a microcomputer (Model AT, International Business Machines, Armonk, NY) was used to measure the fog droplet size distribution. LWC was measured by collecting droplets on a 47 mm glass fiber filter, (type A/E, Gelman Sciences, Ann Arbor, MI; collection efficiency 99.9% at 0.3 microns) by sampling chamber air for 3.5 minutes at 14 L/minute utilizing a vacuum pump. LWC was also continuously tracked and displayed in real time using the phase/Doppler particle analyzer system.

Fog droplets were collected for chemical analysis using 2 different methods; a) by drawing chamber air at 70 CFM across a modified California Institute of Technology string fogwater collector (collection efficiency 85% at 4 microns) ; and b) by the glass fiber filter technique described above for the gravimetric measurement of LWC. Fogwater samples were obtained from the string collector before each challenge, after the initial 15-minute resting exposure, after the initial 15-minute exercise exposure, and after the completion of the 1-hour exposure. Filter samples were obtained at the beginning and end of each challenge. The filters were eluted with 5 ml of deionized water.

Samples were analyzed for sulfate concentration by high performance ion chromatography utilizing Dionex columns (AS4A, P/N 037041, S/N 6037), a 2.5 ml/minute flow rate, and an eluant composed of 3.6×10^{-3} M sodium bicarbonate and 3.1×10^{-3} M sodium carbonate. Samples were analyzed for HMSA concentration by mobile phase ion

chromatography using Dionex columns (E/N 035321, P/N 30956, S/N 0777), a flow rate of 1 ml/minute, and an eluant composed of 2×10^{-5} M HCl, 2×10^{-3} M tetrabutyl ammonium chloride, and 20% methanol by volume. Technical limitations made analysis of small concentrations of HMSA (i.e., < 0.1mg/ml, as on glass fiber filters) exceedingly difficult. Tank solution pH was measured with a pH meter (Model 43, Beckman Instruments, Inc., Irvine, CA) and fogwater pH was determined as the -log [H+].

To determine whether there were significant differences in the subjects' airway response to inhalation of the aerosols in the mouthpiece study, we compared the mean change in SRaw after administration of each of the 5 aerosols inhaled sequentially on the HMSA-in-H_2SO_4 study day with the mean change in SRaw after administration of each of the corresponding 5 aerosols inhaled sequentially on the H_2SO_4-only study day. To analyze the symptoms experienced after the inhalation of the 5 aerosols administered on each study day, we grouped the 9 symptom scores into 3 categories: a) lower respiratory symptoms (chest pain, chest tightness, wheezing, shortness of breath, cough, and sputum production); b) throat irritation; and c) non-respiratory symptoms (back pain and headache). To determine whether there was a significant difference between the reported symptoms following inhalation of the aerosols on the 2 study days, we compared the mean symptom category scores. We also compared the mean baseline SRaw values prior to the inhalation of the aerosols on the 2 study days.

To determine whether there were significant differences in the subjects' airway response to inhalation of the fogs in the chamber study,

we compared the mean baseline SRaw, mean change in SRaw after 1-hour fog exposure, and mean maximum change in SRaw (i.e., baseline to highest SRaw) between the HMSA-containing and H_2SO_4-only fog exposures. The pre- and post-exposure symptom scores in the chamber study were categorized as described above for the mouthpiece study. To determine whether there was a significant difference between the reported symptoms following inhalation of the 2 fogs, we compared the mean changes in score for the 3 symptom categories.

We used the Wilcoxon Rank Sum test for the comparisons described above. A p value of < 0.05 was considered statistically significant.

RESULTS

Mouthpiece Study

The mean ± SE changes in SRaw (in L x cm H_2O/L/S) from pre-exposure values after inhalation of each of the 5 repeatedly administered aerosols on the HMSA-in-H_2SO_4 exposure day were as follows: + 1.2 ± 0.5 after 50 μM H_2SO_4 alone; + 0.4 ± 0.7 after 30 μM HMSA in 50 μM H_2SO_4; - 0.8 ± 0.7 after 100 μM HMSA in 50 μM H_2SO_4; + 0.5 ± 0.3 after 300 μM HMSA in 50 μM H_2SO_4; and - 0.9 ± 0.5 after 1000 μM HMSA in 50 μM H_2SO_4. There were no significant differences in mean change in SRaw among these 5 aerosols (by 2-way analysis of variance (ANOVA)). The mean ± SE changes in SRaw from pre-exposure values after inhalation of each of the 5 repeatedly administered aerosols containing 50 μM H_2SO_4 on the H_2SO_4-only exposure day were as follows: + 1.6 ± 1.2, - 0.3 ± 1.0, + 0.1 ± 0.3, - 0.1 ± 0.4, and + 0.4 ± 0.3. There were no significant differences in mean change in SRaw among these 5 aerosols (by 2-way ANOVA), nor were there any significant differences between these values and the corresponding values obtained on the HMSA-in-H_2SO_4 exposure day. Figure 2 displays the mean SRaw values for the 9 subjects after each inhaled aerosol. Three of the 9 subjects developed increases in SRaw ≥ 50 % from pre-exposure baseline values; 2 subjects (#2,4) after inhalation of aerosols containing 30 μM HMSA in 50 μM H_2SO_4 and 2 subjects (#2,6) after inhalation of aerosol containing only 50 μM H_2SO_4.

No subject in the mouthpiece study experienced as much as "moderate" (i.e., symptom score ≥ 4) throat irritation and only 1 subject (#2) experienced moderate (symptom score 4) wheezing, sputum

production, and shortness of breath. There were no significant differences in the mean scores for throat irritation, respiratory symptoms, and nonrespiratory symptoms between the HMSA-in-H_2SO_4 and H_2SO_4-only exposure days. One subject (#2) coughed frequently during inhalation of aerosols on both exposure days, but there was no significant difference in cough frequency between the 2 days.

The MMAD (geometric standard deviation (GSD)) of the aerosols generated in the mouthpiece study was 6.1 (1.5) microns. The LWC was 87.1 g/m^3. The pre-exposure pH was 4 and there was no significant post-exposure change in pH. There were no significant differences in mean temperature (range, 21.7-22.6°C) among the aerosols.

Chamber Study

The mean ± SE post-exposure SRaw value for the HMSA-containing fog and for the H_2SO_4-only fog were 8.8 ± 1.9 and 8.7 ± 2.1, respectively. There was no significant difference in mean post-exposure SRaw between the 2 fogs. Figure 3 displays the mean SRaw values for the 10 subjects after each fog exposure. Two of the 10 subjects developed increases in SRaw ≥ 45% from pre-exposure baseline values; 1 subject (#6) after exposure to both acid fogs (Figure 6) and 1 subject (#2) after exposure to the H_2SO_4-only fogs (Figure 4). Both of these subjects failed to develop substantial increases in SRaw after exposure to neutral saline fog. Analysis of the maximum change in SRaw, rather than post-exposure SRaw, demonstrated that 2 additional subjects (#4,7) developed substantial (77% and 57%, respectively) increases in SRaw during the H_2SO_4-only fog exposure, but not during the HMSA-containing fog exposure

(Figures 5 and 7). The mean ± SE% maximum change in SRaw was significantly less for the HMSA-containing fog, 15 ± 45%, than for the H_2SO_4-only fog, 37 ± 60 % ($p < 0.02$). There was no significant difference between the pre-exposure baseline SRaw values between the 2 fog exposures.

The mean pre-exposure, post-exposure, and change in symptom scores for the HMSA-containing fog were 9.0, 11.7, and 2.7, respectively. The corresponding values for the H_2SO_4-only fog were 7.9, 13.0, and 5.1. When these scores for total symptoms were divided into throat, respiratory, and nonrespiratory categories (Table 3), there was no significant difference in the mean change in score for both throat and nonrespiratory symptoms between the HMSA-containing and H_2SO_4-only fogs. The mean ± SE change in score for respiratory symptoms was significantly different between the 2 fogs, 1.5 ± 3.1 for the HMSA containing fog compared to 3.5 ± 5.1 for the H_2SO_4-only fog ($p < 0.05$). For each fog exposure, only 2 subjects (#6,7 for the HMSA-containing fog and #2,7 for the H_2SO_4-only fog) reported a ≥ 9-point change in total symptom scores.

The exposure characteristics for the chamber study are listed in Table 2. The volume median diameter of the simulated fogs was ~ 7 microns. The LWC was ~ 2 g/m^3. The mean was 2.0 for the HMSA-in-H_2SO_4 fogs and 2.1 for the H_2S0_4-only fogs. The mean temperature range was 24.2 °C for the HMSA-in-H_2SO_4 fogs and 24.4°C for the H_2SO_4-only fogs. There were no significant differences between the HMSA-containing and H_2SO_4-only fogs for any of these exposure characteristics.

DISCUSSION

We hypothesized that hydroxymethanesulfonic acid would have a specific bronchoconstrictor effect independent of its strength as an acid. We anticipated such a bronchoconstrictor effect because hydroxymethanesulfonate, the bisulfite adduct of formaldehyde, is theoretically capable of dissociating to sulfur dioxide and formaldehyde at the pH of fluid lining human airways. However, the results of both the pilot, mouthpiece study and the exposure chamber study indicate that HMSA is not a potent stimulus to bronchoconstriction in subjects with asthma. HMSA, even when administered at a concentration (1000 μM) more than 3 times greater than what has been measured in the atmosphere, did not cause significant bronchoconstriction in either of our studies.

Despite the lack of any mean increases in SRaw after HMSA exposures in the mouthpiece study, 2 of the subjects did develop increases in SRaw following inhalation of aerosols containing 30 μM HMSA. Although these relatively mild increases in SRaw (< 80 %) were not accompanied by substantial increases in respiratory symptoms, their occurrence provides some evidence of potential adverse health effect. However, there was no dose-response effect demonstrated for these subjects, since they did not develop any further increases in SRaw with the inhalation of higher doses of HMSA. In the exposure chamber study, the only subject to develop a substantial increase in SRaw during or after exposure to HMSA-containing fog did not report a substantial increase in respiratory symptoms. Again, this 1 subject's SRaw response is suggestive of a potential adverse health effect of HMSA.

Although the pilot, mouthpiece study was not designed to simulate natural exposure to acid fogs, the range of HMSA concentrations administered did encompass the highest reported ambient level (300 μM) (1). However, the LWC of the aerosols administered by mouthpiece was approximately 87 g/m^3. Since this value is many times higher than the LWC that has been measured during even "worst-case" natural fog conditions, it is not possible to extrapolate directly from the results of the mouthpiece study to predict the effects of naturally occurring fog containing HMSA.

The characteristics of the simulated fogs administered in the exposure chamber study were closer to those of a worst-case ambient fog. The LWC of the simulated fogs was approximately 2 g/m^3, the upper limit of the ambient range (9). Whereas the aerosols administered in the mouthpiece study were isoosmolar (300 mOsm), i.e., the same ionic strength as body fluids, the osmolarity of the simulated fogs was low (~ 30 mOsm). This relatively low osmolarity was selected because it is within the range of osmolarities reported for naturally occurring fogs, which primarily consist of water (7). Since hypoosmolarity is a well-described stimulus to bronchoconstriction (10-12), matching the osmolarity of the simulated fogs to that of ambient fogs was an important consideration in terms of our study design. While the concentration of HMSA in the simulated fogs (1000 μM) was again higher than that which has been measured in southern California, the LWC and osmolarity of the fogs were more representative of ambient conditions. Thus, the results of the chamber study suggest that clinically significant bronchoconstriction is unlikely to occur in people exposed to naturally occurring fog containing HMSA.

To our knowledge, this is the first report of human exposure to HMSA-containing aerosols. The only published study of the potential health effects of HMSA, by Last and coworkers (13), involved rats exposed to 5 mg/m^3 sodium hydroxymethanesulfonate; the compound was apparently not delivered in an acid aerosol. Tracheal explants and lung homogenates were assayed for the rate of secretion of mucous glycoproteins and for DNA, RNA and protein contents, respectively. No significant differences in these endpoints were demonstrated between exposed and control rats. Although comparison of our study to that of Last and coworkers is obviously limited by the differing species and endpoints used, both studies failed to document a toxic effect of inhalational exposure to HMSA.

The exposure chamber study was not designed specifically to examine the bronchoconstrictor effects of H_2SO_4. However, the absence in our subjects of a mean increase in SRaw after 1-hour exposure to a hypoosmolar fog containing approximately 1000 $\mu g/m^3$ H_2SO_4 confirms the results of a previous study from our laboratory in which we found no bronchoconstrictor effect of H_2SO_4 fogs at this concentration (14). This finding is also in agreement with that of the only published report of H_2SO_4 fog exposures in human subjects by Avol and coworkers (15). These investigators demonstrated no substantial change in forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), peak expiratory flow rate (PEFR), and SRaw in subjects with asthma after exposure to 2000 $\mu g/m^3$. However, the lack of significant bronchoconstriction after inhalation of H_2SO_4-containing fog reported by both laboratories does not preclude significant effects on end-points other than bronchoconstriction, e.g., mucociliary clearance, mediator release in bronchoalveolar lavage

fluid, etc.

Despite its theoretical capacity to generate SO_2 and CH_2O in the airways, inhaled HMSA failed to provoke bronchoconstriction in subjects with mild to moderate asthma. The lack of bronchoconstrictor effect of HMSA is probably explicable on the basis of its dissociation kinetics and the near-zero order accumulation kinetics of the products. HMSA is formed from HSO_3^- and CH_2O at neutral to alkaline pH, is most stable in the pH range 3-5, and dissociates with increasing rapidity as pH rises (1). At 6.6, the pH of the airway lining fluid, the dissociation half-life of HMSA is 1.2 hours (16). After dissociation, the reactive products, HSO_3^- and CH_2O, are probably rapidly consumed, primarily through binding by glycoproteins in the mucus layer of the airway lining fluid. Thus, it is likely that little, if any, HSO_3^- or CH_2O penetrates the mucus layer to reach the respiratory epithelium, let alone the subepithelial nerve receptors and smooth muscle.

Before concluding that inhalation of HMSA-containing aerosols is free of risk for individuals with asthma, it will be necessary to expose such individuals to aerosols that are of much lower relative humidity than the dense fogs administered in the studies reported here. Recently, it has been hypothesized that differences in the relative humidity of H_2SO_4-containing aerosols administered by various investigators may explain the differences in reported bronchoconstrictor potency of H_2SO_4. Furthermore, bronchoconstriction is probably not an especially sensitive end-point by which to assess the potential for adverse health effects of acid aerosols at ambient concentrations, given the high concentrations of H_2SO_4 required to induce significant bronchoconstriction in published reports of human exposure studies. Other end-points, such as mucociliary clearance and

release of inflammatory mediators into bronchoalveolar lavage fluid need to be studied before final judgement can be rendered on the toxicity of HMSA

In summary, this study is the first to assess the effect of HMSA on airway function in humans. While the results we report suggest that HMSA-containing acid fogs in the natural environment are not likely to produce clinically significant bronchoconstriction in people with asthma, other experiments should be performed to further evaluate the relative toxicity of HMSA for the respiratory tract.

REFERENCES

1. Munger J.W., Christine T., Hoffmann M.R. Identification of hydroxymethane-sulfonate in fog. water. Science 1986; 231: 247-49.

2. Fine J.M., Gordon T., Thompson J.E., Sheppard D. The role of titratable acidity in acid aerosol-induced bronchoconstriction. Am. Rev. Respir. Dis. 1987; 135: 826-30.

3. Fine J.M., Gordon T., Sheppard D., The roles of pH and ionic species in sulfur dioxide- and sulfite-induced bronchoconstriction. Am Rev. Resp. Dis. 1987; 136: 1122-1126.

4. Sheppard D., Wong W.S., Vehara C.F., Nadel J.A., Boushey H.A. Lower threshold and greater bronchomotor responsiveness of asthmatic subjects to sulfur dioxide. Am Rev. Resp. Dis. 1980; 122: 873-8.

5. Sheppard D., Eschenbacher W.L., Epstein J., Lack of bronchomotor responses up to 3 ppm formaldehyde in subjects with asthma. Environ. Res. 1984; 35: 133-9.

6. Egle J.L., Retention of inhaled formaldehyde, propionaldehyde, and acrolein in the dog. Arch. Environ. Health. 1972; 25: 119-24.

7. Balmes J.R., Fine J.M., Christian D.C., Gordon T., Sheppard D. Acidity potentiates bronchoconstriction induced by hypoosmolar aerosols. Am. Rev. Resp. Dis. 1988; 138: 35-39.

8. Knudson R.J., Lebowitz M.D., Holberg C.J., Burrows B. Changes in the maximum expiratory flow-volume curve with growth and aging. Am. Rev. Respir. Dis. 1983; 127: 725-34.

9. Dickson D.H., Loveland R.B., Hatch W.H., Atmospheric waterdrop size distribution at Capistrano test site from April 16th through May 11th, 1974. White Sands Missile Range, NJ. Atomspheric Sciences Laboratory 1975. (Report No. ECOM-DR 75-3).

10. Anderson S.D., Schoeffel R.E., Finney M. Evaluation of ultrasonically nebulized solutions as a provocation in patients with asthma. Thorax 1983; 38: 284-91.

11. Sheppard D., Rizk N.W., Boushey H.A., Bethel R.A. Mechanism of cough and bronchoconstriction induced by distilled water aerosol. Am. Rev. Respir. Dis. 1983; 127: 691-4.

12. Eschenbacher W.L., Boushey H.A., Sheppard D. Alteration in osmolarity of inhaled aerosols cause bronchoconstriction and cough, but absence of a permeant anion causes cough alone. Am. Rev. Respir. Dis. 1984; 129: 211-5.

13. Last J.A., Dasgupta P.K., Etchison J.R. Inhalation toxicology of sodium sulfite aerosols in rats. Toxicol. Appl. Pharacol. 1980; 55: 229-234.

14. Sheppard D., Balmes J.R., Christian D. Effects of acid fog on airway function in people with asthma. Research Contract Final Report to State of California Air Resources Board, re: A5-179-33, 1988.

15. Avol E.L., Linn W.S., Wrightman L.H., et al. Short term respiratory effects of sulfuric acid in fog: a laboratory study of healthy and asthmatic volunteers. J. Air Pollut. Contr. Assoc. 1988; 38: 258-63.

16. Hoffman MR. Personal communication.

PUBLICATIONS/PRESENTATIONS

Balmes JR, Fine JM, Christian D, Sheppard D. Bronchoconstrictor potency of hydroxymethanesulfonic acid. Am Rev Respir Dis 1988; 137, no. 4 (part 2): 167.

Aris RM, Christian D, Sheppard D, Balmes JR. Hydroxymethanesulfonate in acid fogs does not contribute to bronchoconstriction. Am Rev Respir Dis 1989; 138, no. 4 (part 2): A281.

Aris R, Christian D, Sheppard D, Balmes JR. Acid fog-induced bronchoconstriction: the role of hydroxymethanesulfonic acid. Am Rev Respir Dis (in press).

Table 1

SUBJECT CHARACTERISTICS

Subject	Sex	Age (yrs)	Ht (cm)	FEV_1 (L)	FEV_1 (% pred)	FVC (L)	FVC (% pred)	Baseline SRaw * (L x cm H_2O/L/s)	Methacholine Responsiveness † (mg/ml)	Medications ††
						MOUTHPIECE STUDY				
1	M	27	178	3.84	105	5.52	122	8.3	0.15	A,B
2	F	30	173	2.51	77	4.12	104	10.9	0.24	A
3	F	31	165	2.91	97	4.07	112	5.1	1.64	A,B
4	F	28	168	1.84	60	2.87	77	13.5	<0.03	A,T
5	F	21	165	3.16	98	4.07	105	6.2	0.35	A
6	F	32	157	2.51	90	3.58	107	5.3	0.09	A
7	M	24	183	2.49	53	4.40	80	5.7	0.16	A,B,T
8	F	27	163	2.79	92	3.53	97	4.6	0.44	A
9	M	32	185	4.65	102	5.75	101	4.1	0.55	A
						CHAMBER STUDY				
1	M	33	177	3.75	90	5.10	99	4.4	0.40	A
2	M	35	177	2.06	50	4.74	93	8.9	0.19	A
3	M	29	182	2.72	60	5.93	106	20.6	0.32	B
4	M	29	166	3.69	99	5.38	117	6.3	0.29	A
5	M	24	172	3.32	79	4.74	95	7.5	0.15	- -
6	M	20	170	3.38	79	3.62	70	5.0	0.01	A,C
7	M	40	172	2.21	60	4.11	89	5.3	0.26	A
8	F	23	155	3.68	127	4.42	128	4.0	0.64	A
9	F	30	167	2.35	76	2.98	79	9.8	0.21	A,T
10	M	23	177	3.12	71	5.19	100	15.4	0.38	A,T

* mean of baseline values of 3-4 separafe days

† concentration of methacholine required to produce a 100% increase in SRaw above baseline calculated by linear log interpolation

†† A: ß-adrenergic agonist; B: beclomethasone; C: cromolyn; T: theophylline

TABLE 2

EXPOSURE CHAMBER DATA *

	HMSA (n=10)	H_2SO_4 (n=10)	NaCl (n=3)
LWC (g/m^3)	2.0 ± 0.05	2.0 ± 0.05	2.2 ± 0.11
VMD (μm)	6.97 ± 0.05	7.00 ± 0.05	7.09 ± 0.01
temp (°C)	24.2 ± 0.35	24.4 ± 0.25	25.7 ± 0.08
HMSA (mM)	1.15 ± 0.04	- - -	- - -
(mg/m^3)	0.26 ± 0.03		
H_2SO_4 (mM)	5.59 ± 0.19	5.04 ± 0.25	- - -
(mg/m^3)	1.09 ± 0.11	1.10 ± 0.15	

* mean data ± SE

Key to abbreviations: LWC = liquid water content, VMD = volume median diameter, HMSA = hydroxymethanesulfonic acid, H_2SO_4 = sulfuric acid, NaCl = sodium chloride

Table 3

CHANGES IN SYMPTOM SCORES

SYMPTOMS	HMSA	H_2SO_4	NaCl
Throat	0.4	1.1	0.3
Respiratory	1.5	3.5	4.3
Non-respiratory	0.5	0.5	0.7
Total	3.2	5.1	5.3

Mean changes in symptom scores after inhalation of 3 fogs, chamber study.

Key to abbreviations: HMSA= hydroxymethanesulfonate, H_2SO_4 = sulfuric acid, NaCl = sodium chloride

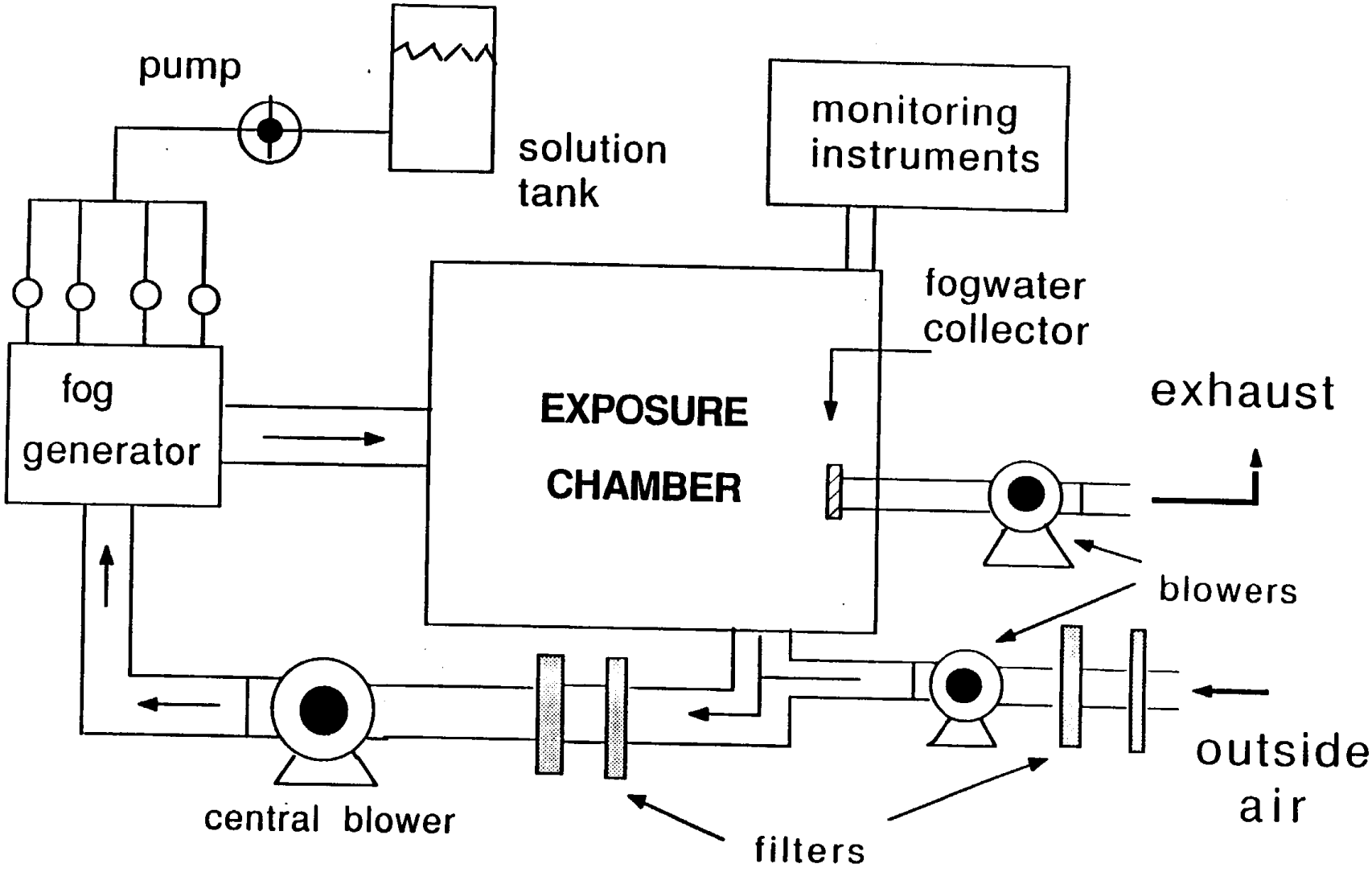
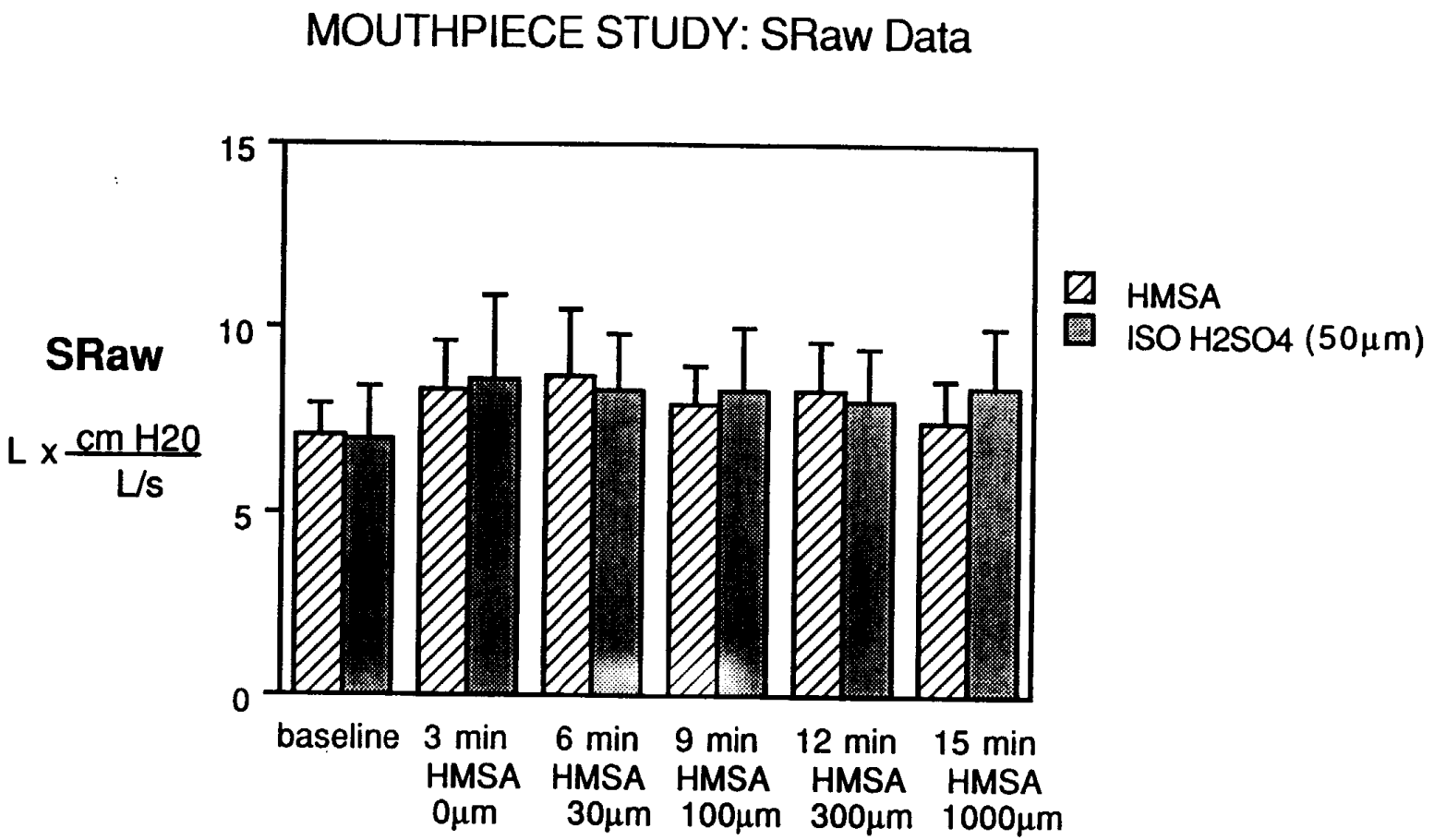


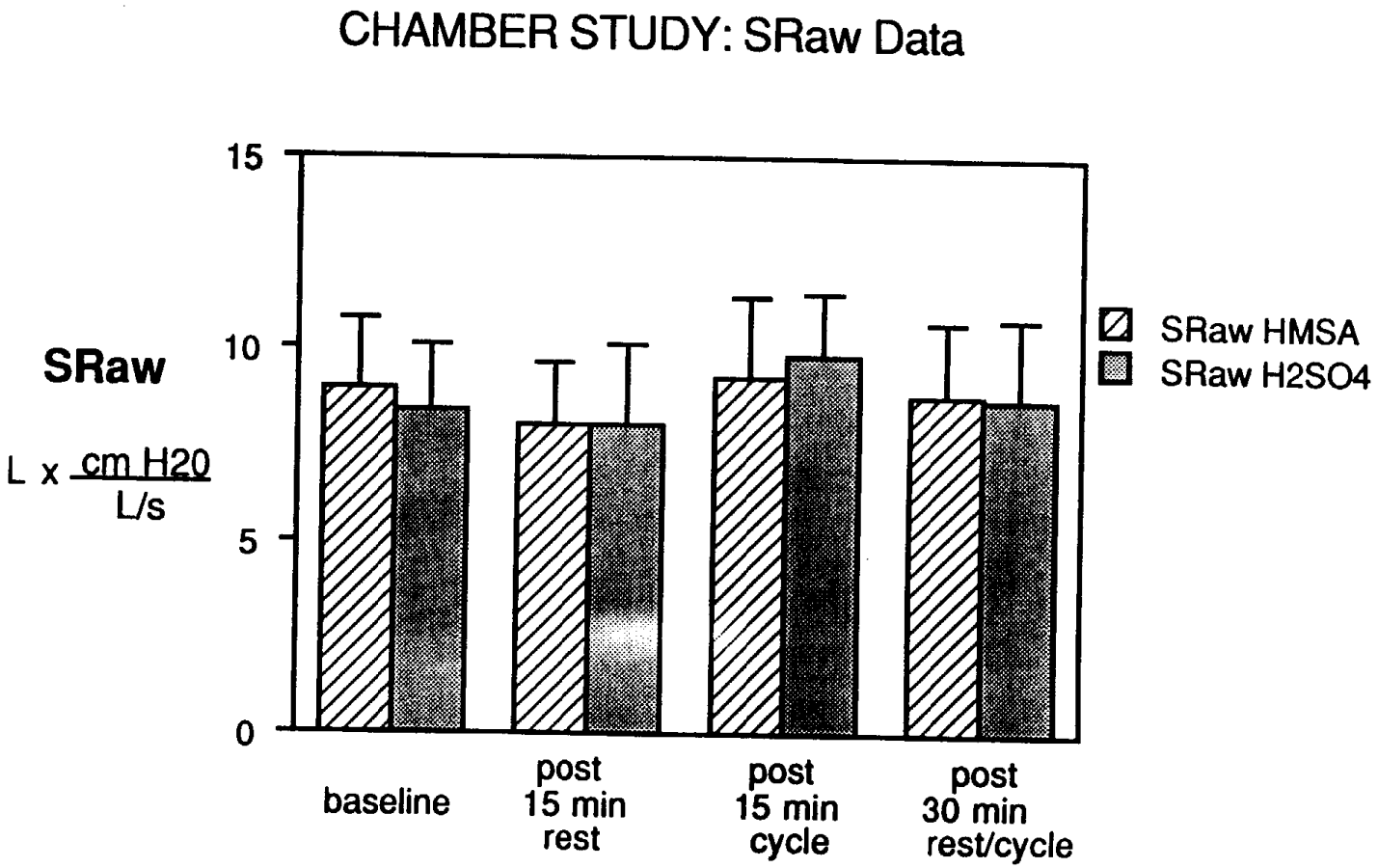
Figure 1 Schematic of exposure chamber with fog generation monitoring systems.

Figure 2



Mean specific airway resistance (SRaw in liters x cm H2O/L/s) at baseline and after inhalation of each of 5 sequentially administered aerosols containing either hydroxymethanesulfonate (HMSA) in sulfuric acid (H2SO4) or H2SO4 alone for 9 subjects, mouthpiece study.

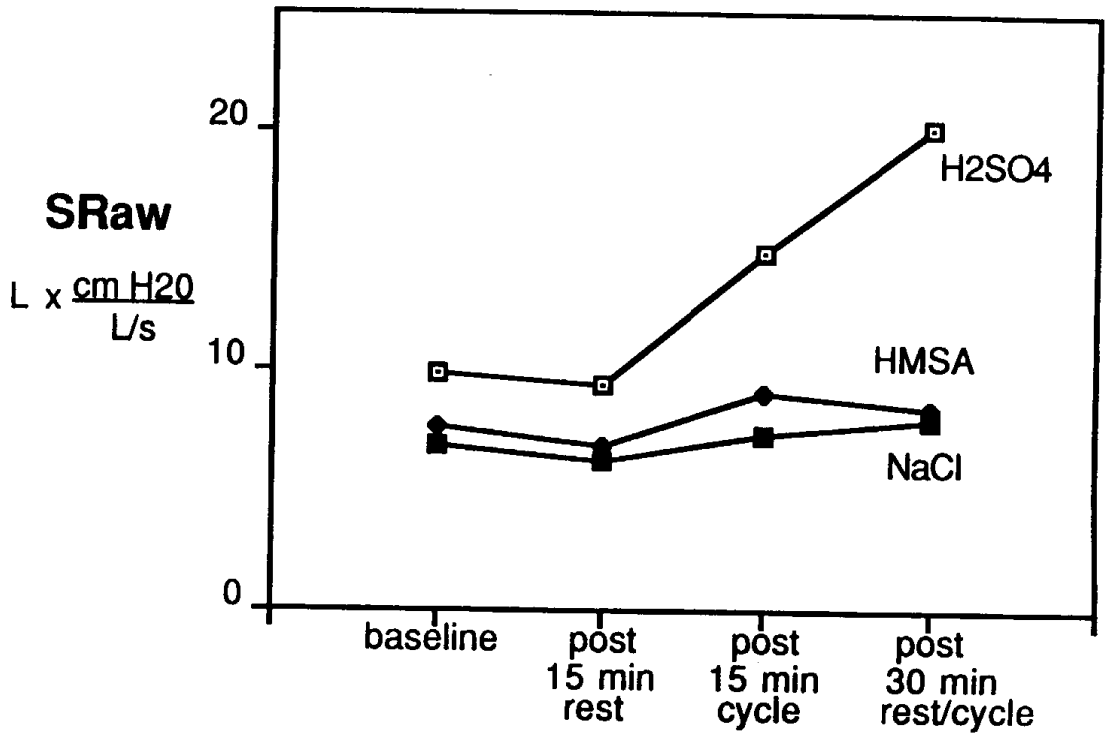
Figure 3



SRaw in liters x cm H2O/L/s, before,during and immediately after inhalation of fogs containing HMSA in H2SO4 or H2SO4 alone for 10 subjects, chamber study.

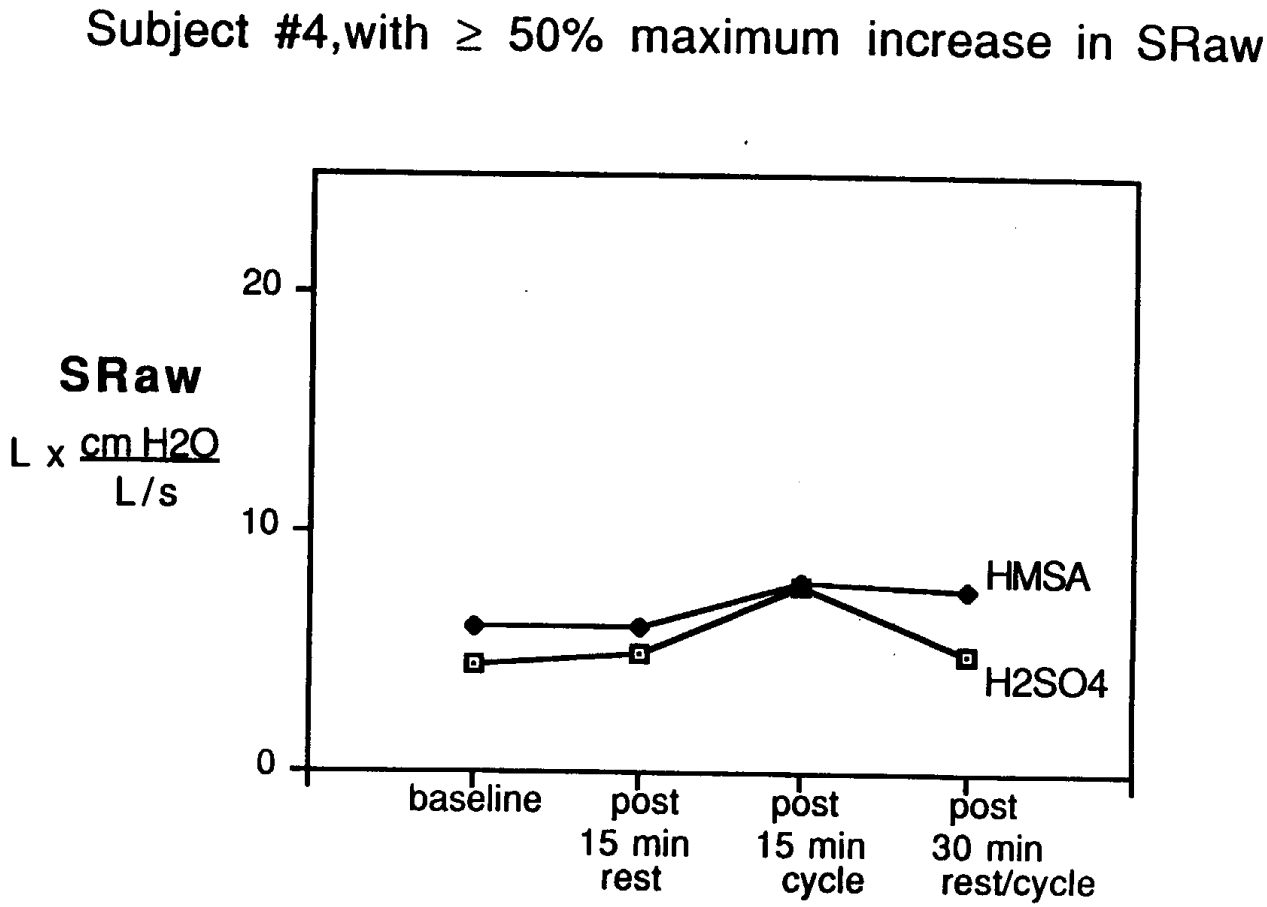
Figure 4

Subject #2, with ≥ 50% increase in post-exposure SRaw



SRaw in liters x cm H2O/L/s, before,during and immediately after inhalation of fogs containing HMSA in H2SO4 , H2SO4 alone, or neutral saline.

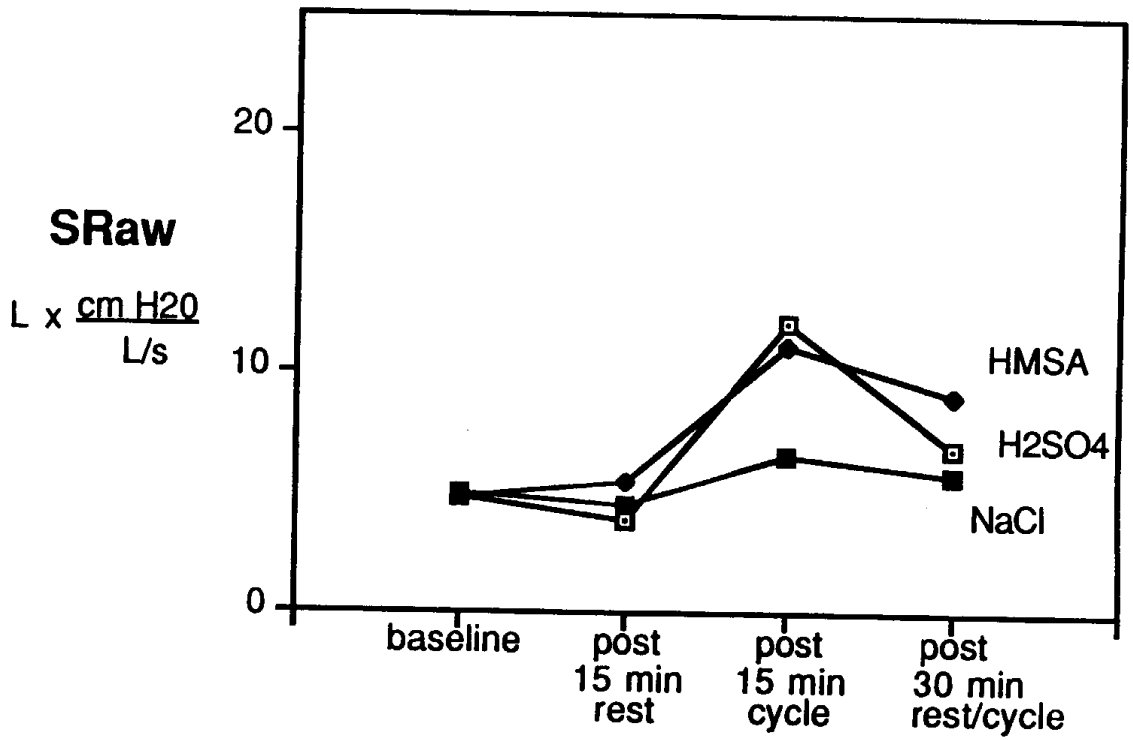
Figure 5



SRaw in liters x cm H20/L/s, before, during and immediately after inhalation of fogs containing HMSA in H2SO4, H2SO4 alone, or neutral saline.

Figure 6

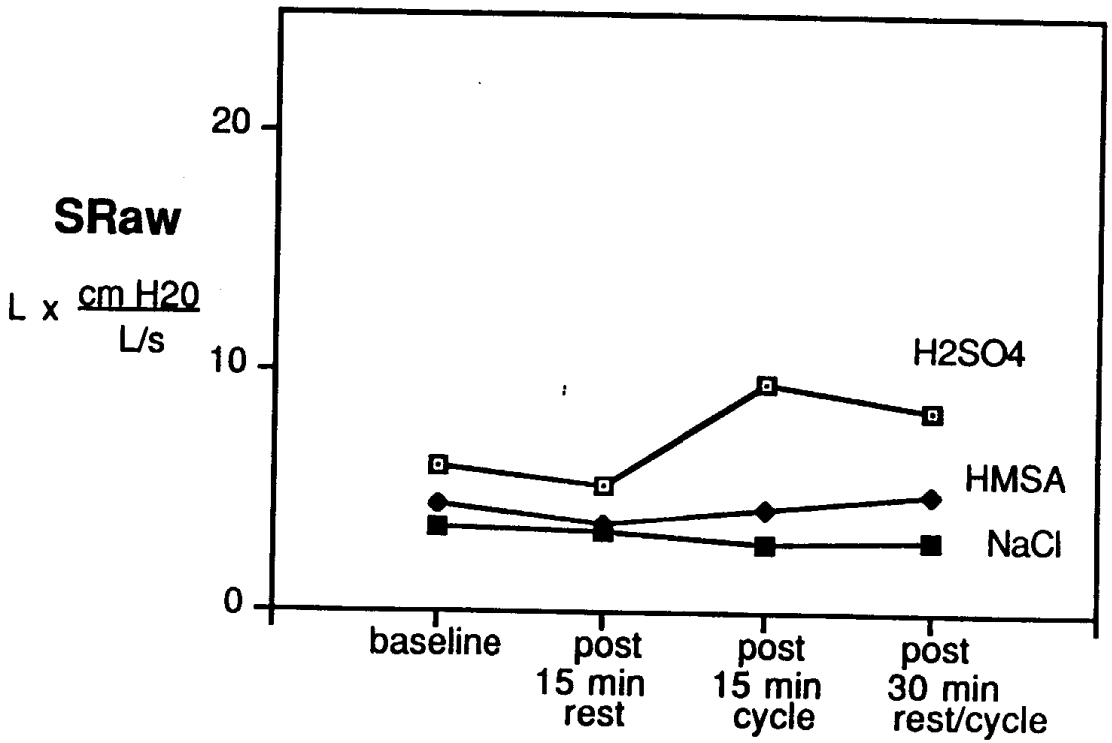
Subject #6, with ≥ 50% increase in post exposure SRaw



SRaw in liters x cm H2O/L/s, before,during and immediately after inhalation of fogs containing HMSA in H2SO4 , H2SO4 alone, or neutral saline.

Figure 7

Subject #7, with ≥ 50% maximum increase in SRaw



SRaw in liters x cm H2O/L/s, before,during and immediately after inhalation of fogs containing HMSA in H2SO4 , H2SO4 alone, or neutral saline.

APPENDIX

Our original plan was to study the effects of simulated acid fogs that were buffered with ammonium sulfate, as well as those of fogs containing hydroxymethanesulfonate (HMSA), in subjects with asthma. However, we did not expose subjects to simulated acid fogs buffered with ammonium sulfate because we realized that results from previous studies in our laboratory provided little justification for such an experiment. Our initial hypothesis concerning exposure to buffered acid fogs was that an increase in the available pool of H^+ ions through buffering would increase the bronchoconstrictor effect of a fog in a dose-dependent fashion such that a fog containing a concentration of sulfuric acid in the ambient range might induce bronchoconstriction. However, results from some of our other ARB-funded studies convinced us that this was unlikely to occur. We administered aerosols containing 3000 $\mu g/m^3$ to subjects with asthma during resting breathing for 16 minutes (Research contract final report re: A6-149-33, project 1) and simulated fogs containing over 1000 $\mu g/m^3$ to such subjects during intermittent exercise for 1 hour (Research contract final report re: A5-179-33, project 3) without demonstrating any significant bronchoconstrictor effect of these exposures. Thus, we reasoned that there was little likelihood that buffering a sulfuric acid-containing fog would increase its bronchoconstrictor potency to the point where an ambient concentration of sulfuric acid would cause significant bronchoconstriction.

A second reason that we did not study simulated acid fogs

buffered with ammonium sulfate is that, due to unexpected technical difficulties with the ion chromatographic measurement of HMSA, we were required to spend much greater amounts of time (i.e., approximately 3 months) and money (~$30,000) on the HMSA project than we had specified in the original research proposal to the ARB. We had been assured by the manufacturer of our ion chromatographic system, Dionex, that we would be able to measure HMSA using high performance ion chromatography (HPIC), which we already had on-line. We budgeted accordingly our time and monetary expenses. Unfortunately, after considerable frustrated effort, we became aware that HPIC was an inappropriate technique for the measurement of HMSA. We contacted Dr. William Munger at the Keck Laboratory of Environmental Engineering Science at the California Institute of Technology, the lead author of the report that described the measurement of HMSA in southern California acid fog, who advised us to try mobile phase ion chromatography (MPIC). Although this advice put us on the right track, it was not until we shared our experience with Karen Anderson at USC, that we began to successfully detect HMSA at the millimolar level. The successfully applied MPIC technique required us to buy different chromatography columns and suppressor columns than we had requested in the original proposal and to replace these new columns more frequently than is necessary for HPIC columns. Thus, one reason we did not perform a study involving exposure to acid fogs buffered with ammonium sulfate is that we had to exceed the planned budget for the HMSA study in order to complete it. We were left with insufficient funds to complete the proposed experiment involving ammonium sulfate-buffered acid fogs.

Dr. Sheppard discussed both the scientific rationale and the budgetary constraints behind our decision not to conduct the buffered acid fog experiment with Dane Westerdahl of the Air Resources Board Research Division by phone in June, 1988.