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Health Effects of Atmospheric Salts and Gases of
Sulfur and Nitrogen in Association with Photochemical Oxidant

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Health Effects of Atmospheric Salts and Gases of
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OF AEROSOLS AND GASES. ACID MISTS, SALTS OF S AND N,

SYNERGISM BETWEEN SALTS AND NO_2 AND OTHER GASES

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Presented at the Conference on Health Effects of

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in Association with Photochemical Oxidant

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CHAPTER I.

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Introduction

In assessing the biological effects of air pollutants we are confronted with basically two types of situations. The first one is to assess the possible effects of long-term exposure to low levels of pollutants in order to establish at what levels individuals can be exposed without significant increase in respiratory diseases, pulmonary impairment, possible shortening of expected lifespan, etc. The second one is to assess the possible effects of short-term exposures, air pollution episode types, when the concentration of pollutants may rise several folds above normal background levels due to a variety of reasons. In this case possible effects such as sensory irritation of the eye-nose-throat and effects on the bronchial tree as well as on deeper pulmonary structures are important to evaluate. In this case not only must the acute effects be evaluated per se but also considerations must be given to the possible effects from repeated exposures to these levels.

We must also be concerned about the effects on some sensitive sub-groups as well as on healthy individuals within the exposed population. There will obviously be some cut-off point as far as evaluating the effects on sensitive sub-groups since there is an infinite number of them. Quite frankly an arbitrary decision will be made as to the level of protection to be reached.

In this article an attempt is made to evaluate the methodology used to investigate the effects of pollutants which primarily affect the respiratory tract. The article is divided into two parts: short-term and long-term effects and concerned with methodology used in laboratory animals.

I. METHODOLOGY FOR SHORT-TERM EXPOSURES

1. Introduction

This section is based on the following articles (1,2,3,4,5 and 6) and on three review articles (7,8,9). For discussion purposes the following is proposed:

i)   Sensory nerve endings are found in the membranes lining the respiratory tract, from the tip of the nose to the pulmonary alveoli.

ii) These sensory nerve endings are stimulated when irritants impinge upon them.

iii) Stimulation of the endings will increase the frequency of impulses on the afferent nerve.

iv) Information will be integrated at the C.N.S. and various reflex reactions will occur through efferent nerve(s).

v)   The increase in impulse frequency and the intensity of the reflex reaction(s) will be proportional to the concentration of the irritant.

vi) By measuring either of the above a dose-response relationship can be established and threshold values determined.

vii) These nerve endings are cholinergic in nature, belonging to either the trigeminal, laryngeal or vagal nerves depending upon the level of the respiratory tract and are stimulated by specific mechanisms.

viii) Methods which will measure increases in impulse frequency on the afferent nerves or reflex reactions occuring are likely to be more sensitive in detecting the effects of airborne chemicals on the respiratory tract than any other biological evaluation.

If we examine at which level of the respiratory tract airborne chemicals can impinge and act we can determine the following:

i) nasal
ii) laryngeal
}   upper respiratory tract

iii) central conducting airways (large airways)
iv) peripheral conducting airways (small airways)
v) alveoli
}   lower respiratory tract

and therefore adequate methodology must be devised to recognize an effect at each level. In this article, the methodology will be restricted to measurement of reflex reactions occuring from the action of irritants at each level described above since measurement of impulses from afferent nerves is still a difficult procedure and necessitates anesthesia, surgery, etc. while measurement of reflex reaction can be done in unanesthetized animals and is better suited for the investigation of large numbers of pollutants and mixtures. Depending on the nature of the airborne chemicals the net dose received by each area of the respiratory tract will vary. For example, sulfur dioxide because of its high solubility is retained primarily by the upper respiratory tract while nitrogen dioxide can penetrate deeper. A useful classification is presented in Table 1. This classification is arrived at by determining which level of the respiratory tract is affected first and by the lowest concentration of the chemical.

2. Methodology Used

A. Sensory irritation of the upper respiratory tract

i) Method

A method to measure sensory irritation of the upper respiratory tract, nasal and laryngeal area but mainly nasal passages, is based on the fact that when nasal trigeminal nerve endings are stimulated (and to a lesser extent laryngeal) a reflex reaction occuring is a decrease

in respiratory rate as described in Table 2. This decrease in respiratory rate is characterized by a lengthening of the expiratory phase as shown in Figure 1 and is specific for stimulation of trigeminal nerve endings as presented in Table 3.

With this method, dose-response curves have been obtained in mice with a very wide variety of sensory irritants. Since it is quite simple to measure respiratory rate in animals the method can be proposed to investigate numerous pollutants and mixtures of pollutants. Currently we are using this method to evaluate the effects of combustion products of various plastics, a quite complex mixture and the results look promising. Similarly we have used this technique in the past to evaluate irritancy of cigarette smoke with excellent results.

ii) Animal species

In the test described above mice have been used extensively although other species such as rats, guinea pigs, rabbits, dogs, etc. will react in a similar way as shown in Table 4. Mice are selected basically because they are inexpensive and easy to keep and they breathe exclusively via the nose. Some attempts have been made to determine which animal species is more sensitive and would be more useful to predict the reaction in humans. The results indicate some variation depending on the type of irritants. For sulfur dioxide it would appear a comparison is difficult because the pattern of response is not the same in both species.

iii) Pattern of response

With sensory irritation of the upper respiratory tract the most frequent time-response pattern observed in mice is that a maximum response

is established for each concentration tested and remains at this
level for the duration of exposure. This is demonstrated in Figure
3. However, this pattern of response can vary. For sulfur dioxide
the time-response pattern observed in mice is that a maximum res-
ponse is reached rapidly and is followed by a recovery although the
exposure continues as shown in Figure 4. We have called this phe-
nomenon "desensitization". From the review of the literature on
subjective response of humans to sulfur dioxide (7) and also from
a very recent article on this subject (10) it does appear that humans
exposed to a concentration of 10-25 ppm experienced immediate dis-
comfort which gradually fades with time thus resembling the response
we observed in mice.

iv)   Effect of repeated exposure

With repeated exposure to sensory irritants the same level of
response is usually obtained. One notable exception is with sulfur
dioxide. With this gas, the maximum response observed in a series
of exposures gradually faded until the 10th exposure when no response
was observed. This is illustrated in Figure 5.

v)   Prediction to humans

We feel that the test described will correctly predict which
airborne chemicals are likely to induce sensory irritation in humans.
As far as quantitative prediction is concerned a concentration capable
of decreasing respiratory rate by 50% in mice would be predicted to
be absolutely intolerable for humans and approximately 1/10 of this
concentration would evoke subjective responses of sensory irritation
of eye-nose-throat in humans within 1 or 2 minutes of exposure. For

sulfur dioxide these concentrations would be 120   ppm and 12   ppm

respectively. From an evaluation of the literature on humans exposed

to sulfur dioxide (7) and also from a very recent article on this sub-

ject (10) these values seem appropriate.

vi) Conclusions

Measurement of decrease in respiratory rate in mice appears to

evaluate reliably the action of airborne chemicals and can be of use in

predicting at what concentration sensory irritation will occur in hu-

mans as well as predicting the time-response pattern of the reaction

during continuous or repeated exposures.

B. Action on Lower Respiratory Tract

The receptors located in the lower respiratory tract are basically of

three types (8)

· lung irritant receptors

· stretch receptors

· type J receptors

Stimulation of any of them always results in an increase in respiratory

rate as shown in Tables 5 and 6. With stimulation of lung irritant recep-

tors, which are the most centrally located, coughing and bronchoconstriction

is also observed but not with stimulation of deflation or type J receptors.

1. Determination of an effect on central airways

i) Method

The work of Amdur, as reviewed (7) has been an attempt to establish

that several airborne chemicals are capable of inducing the broncho-

on
constriction by reacting the central airways and acting upon them.

To determine their action at this level airflow resistance was measured in intact as well as in tracheostomized animals. The results obtained by Amdur and other workers are summarized in Table 7 and Figure 6. If we compare concentrations necessary to produce 50 or 100% increase in airflow resistance in intact guinea pigs, sulfur dioxide would be the least potent while zinc ammonium sulfate would be the most potent of all the chemicals tested. The use of this technique in tracheostomized animals certainly reveals that constriction of the central and perhaps peripheral airways does occur when an increase in resistance occur. However, with present knowledge, when intact guinea pigs are exposed and an increase in airflow resistance is recorded one cannot reach a direct conclusion that this increase in resistance is due to bronchoconstriction. Indeed several mechanisms exist by which an increase in resistance can be obtained as presented in Table 8.

ii) Animal species

In the test described above the guinea pig has been used most extensively with also experiments done in cats and dogs as reviewed (7). Although we are constantly told that the guinea pig is the most sensitive species, I cannot find evidence such as dose-response curves supporting this statement. Since guinea pigs can be easily handled and are quite adaptable for this measurement they will probably be used in future studies. Such measurements can however, be very easily performed in monkeys(11).

iii)   Pattern of Response

The pattern of response of the action of sulfur dioxide in
increasing resistance to airflow is quite different in guinea
pigs than for dogs, cats or humans as reviewed (7). In guinea
pigs the increase is sustained over a period of 1 hour or more
while this is not so in humans or dogs. However, the pattern
of response in humans as recently measured by Andersen et al
(10) seems to   more closely approximate   the pattern
seen in guinea pigs. Further evaluation will be necessary on
this point.

iv)   Effect of repeated exposure

As reviewed (7) in humans the action of sulfur dioxide decreased
in sequential exposures. Few animal experiments have been per-
formed to study this aspect/but this is a most important topic.
                                     (7)

v)   Predictions to humans

Although several airborne   contaminants have been tested in
guinea pigs for their effect in increasing airway resistance
only one, sulfur dioxide, has been extensively tested in both
guinea pigs and humans (7). From the data obtained (Table 7
and Figure 6) we can reach two different predictions. First
if we choose a level of response such as 50 or 100% increase
in resistance and the corresponding exposure concentrations
necessary to obtain such responses in guinea pigs and in humans
                                                    of sulfur dioxide
we find that it takes much higher concentrations/in guinea pigs
than in humans. Second, if we choose the minimum concentration
at which any level of response can be detected we find that

guinea pigs reacted at a lower concentration than humans. Which

selection should be made? It is particularly treacherous to

predict from the lower portion of the dose-response in guinea

pigs since a no-response concentration has not been established

and that for a 2 fold increase in concentration there is hardly

any noticeable increase in response. Also in a recent report

by   McJilton / et al (13) there was no detectable response at 1.0 ppm

contrary to the data presented by Amdur in Figure 6. However,

even if quantitative predictions are difficult to make, it would

appear that qualitative predictions can be made from this test

in guinea pigs.

vi) Conclusions

Measurements of airflow resistance in guinea pigs appears to evaluate

reliably the action of airborne chemicals, particularly on the

larger conducting airways. Some reservations are in order when

considering the contribution of each portion of the respiratory

tract (upper and lower) to the total increase in resistance and

to the various mechanisms involved in this reaction to inhaled

chemicals.

2. Determination of an effect on peripheral airways

i) Method

Several methods have been established to detect changes in the

status of peripheral airways in humans as reviewed by Macklem

(14) but unfortunately few are applicable in unanesthetized

laboratory animals. One method which can be used in unanes-

thetized monkeys is the nitrogen wash-out technique (15).

It would be difficult to adapt this technique to small rodents
although it has been used in squirrel monkeys with good success
(C.E. Ulrich, personal communication).

At this time it is impossible to assess how such techniques
will reveal the action of pollutants such as sulfur dioxide
nitrogen dioxide, etc. in short-term acute exposures because
of limited data. There is, however, a definite need to explore
this area since an action at this level is probably most impor-
tant.

3. Determination of an effect on peripheral airways and pulmonary struc-
ture.

   i) Method

      As stated above, when receptors in the lower respiratory tract
      are stimulated by inhaled chemicals there is a reflex increase
      in respiratory rate. If there is an action on the lung irritant
      receptors, there is normally also constriction of the airways in
      which they are located but this does not occur with stimulation
      of stretch receptors or type J receptors which are more peripherally
      located. There is indeed a long list of chemicals which have
      been shown to induce reflex tachypnea by their action on pulmonary
      receptors as shown in Tables 5 and 6. Unfortunately, there has
      been few quantitative reports in this area but for two of the
      pollutants of interest at this conference, ozone and nitrogen
      dioxide, a dose-response relationship has been established in
      guinea pigs as shown in Figure 7. For both ozone and nitrogen

dioxide there was no increase in airflow resistance while respiratory rate increased as shown in Figure 7. This would indicate that their action occurs on the deepest portion of the pulmonary system.

ii) Animal species

As shown in Tables 5 and 6 this reflex tachypnea occurs in a wide variety of laboratory animals exposed to airborne chemicals but there is not sufficient data to assess which animal species is more sensitive or best suited for investigation of pollutants.

iii) Prediction to humans

Unfortunately, little can be said about this. In Table 6, one pollutant, sulfuric acid mist, has been reported to increase respiratory rate in man. Sulfuric acid mist can probably be classified as a pulmonary irritant rather than a sensory irritant (also see section on long-term exposure) and indeed its action may have been on pulmonary receptors as discussed (7) resulting in an increase in respiratory rate. However, this will need further investigation.

iv) Conclusions

Measurements of increase in respiratory rate in laboratory animals appears to be reliable to evaluate the action of airborne chemicals on the deeper pulmonary structures.

3. Mechanisms by which sensory receptors in the respiratory tract are stimulated.

It has been frequently stated that the action of irritants in nonspecific, due to change in pH in the tissues, formation of acids or

bases, etc. Quite the contrary is true. Sensory receptors are stimu-
lated because of specific interactions   with airborne chemicals
although the mechanisms involved are only beginning to be defined (7).
There are good reasons to believe that interactions with SH groups
and S-S bonds in proteins of sensory receptors are two basic mechanisms
by which these receptors can be stimulated by airborne chemicals (7).

4. General Conclusion

Nerve endings lining the respiratory tract are easily accessible
to inhaled airborne chemicals. Stimulation of these nerve endings re-
sults in reflex reactions which can be evaluated in unanesthetized
laboratory animals with adequate methodology and used to predict, at
least qualitatively, the type of reaction to be expected in humans.

II. METHODOLOGY FOR LONG-TERM EXPOSURES

1. Introduction

This section is based on the following articles (18,19,20,21,22,23) and on one brief review article (24)*. It mainly discusses the studies in which I was involved and certainly does not include all possible tests that can or should be conducted in animals exposed to low levels of pollutants for a long period of time. Quite simply an evaluation of the protocol we used is given in an attempt to point out which kinds of tests are likely to be of value. Since these studies were designed in 1963-64 it is obvious that some deficiencies can be pointed-out on the basis of present knowledge. This, of course, is a frustration that scientists doing long-term chronic studies must learn to accept. The second one is that usually, when such studies are designed and reviewed, everyone will suggest the addition of a variety of tests to be performed and we usually end up with a testing schedule so overloaded that animals would be tested 6 hours/day instead of being in exposure chambers. Indeed the temptation is very great to design a study which will answer all questions. I do not think this can be done.

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*Other articles are being prepared on results of long-term chronic exposures to various pollutant systems.

1. Experimental Design: number of animals, statistical analysis

Obviously the design of long-term studies is crucial because of the economics involved. I would add that in testing for concentrations of pollutants or mixtures in order to establish a deleterious effect level we should design our experiments in such a way that an experiment could be <u>terminated</u> as soon as possible and <u>not</u> carried on to its original completion date just for the sake of completion. Indeed in our studies on sulfuric acid mist and nitrogen dioxide we could have terminated the studies much earlier than the planned termination date since early in the exposure we had obtained sufficient assurance that at some levels deleterious effects had definitely been observed. The studies, however, were continued to answer some questions about possible tolerance development. Obviously, if studies involving these pollutants would be started with other mixtures, they could now be much shorter and more economically feasible but yet provide probably as much information. We now come to some improtant decisions about the number of animals and statistical analysis.

a. Number of animals

In inhalation exposures, because of restriction in space available in exposure chambers, the number of animals will usually be much smaller than in other toxicological studies. Therefore, one must make a decision about what kind of animal selection will take place since if the number is small and the variation in the biological parameters being measured is large we automatically deny ourselves any chance of detecting small changes at a statiscally significant level. In our studies on monkeys, the space

available in exposure chamber was limited to 9 animals. We

therefore did not take animals on a random basis. Even after

a long conditioning period in our laboratory, we "screened"

animals and selected them on the basis of previously determined

"standards" thus obtaining what we called "healthy animals".

This screening procedure resulted in a 50% rejection rate of

animals received after quarantine and rejection was for a

variety of reasons from "abnormal" values for hematocrit, E.K.G.,

arterial blood pH, airway resistance, etc. With such screening

procedure we probably eliminated some "possible sensitive animals"

but since these were the first studies, we believed that establishing

the effect in "normal, healthy animals" was probably more important

since we could not define (and still today cannot define) what

type(s) of abnormality(ies) in animals would be most suitable to

represent some sensitive sub-groups.

b. Statistical Analysis

In our studies in monkeys and guinea pigs we decided from

the very beginning to measure all parameters under normal control

conditions not only once but several times in order to determine

normal variation when the pollutants were not present. Also we

decided to measure each variable as frequently as possible early

in the exposure. Furthermore, sequential analysis of the data

was undertaken in an attempt to determine if an effect would

occur and at what time it would occur. This was quite successful.

It seems to me that to measure a variable once prior to exposure

and then only after termination of exposure, some one or two years

later, and to attempt to analyze the data by analysis of variance

is an absolute waste of time, particularly when pulmonary function

tests, which are known to change with age, are performed. Some

investigators have done this and we have done this in studies in

rats (in order to save money) and the results are a worthless mass

of tables including a lot of F or t values. There must be new

ideas and concepts in designing long-term studies so that such

mistakes are avoided. The sequential analysis attempts we have

made have certainly shown to us that a lot more can be obtained

in these studies if frequent testing is made and a recent article

by Riffenberg showing how sequential analysis can be done in such

studies is certainly worth considering. Furthermore with our

present knowledge it should be possible for scientists in statis-

tics and operations research to optimize experimental design in

order that long-term studies be made at less cost.

2. Biological variables and frequency of testing

An attempt was made to evaluate pulmonary function by measuring:

·mechanical properties of the lung

·distribution of pulmonary ventilation

·diffusing capacity of the lung

·arterial blood gas measurements

The various measurements made and the frequency of testing under

each categories are presented in Tables 9, 10, 11 and 12. Also

various hematological and serum biochemical determinations were

performed as shown in Table 13. The methodology used for measurements

of these biological parameters has been described fully (11, 12, 15,

19, 23). In view of the high retention of sulfur dioxide in the upper respiratory tract (10, 25) it would have been advisable to study the effect on these membranes but this was not done. Also a possible reduction in lung clearance which may be indicated from recent studies (26) (although I have some reservation about this article) has not been investigated.

3. Results

The results of the long-term chronic studies published so far are summarized in Table 14. From this table it is easy to determine that sulfuric acid mist and nitrogen dioxide act in a very similar way as pulmonary irritants. Sulfur dioxide and sulfur dioxide with fly ash mixtures did not, with the testing methods used, induce detectable deleterious effects. It may be added that when calcium sulfate (10 mg/m^3) was added to 7.5 ppm of nitrogen dioxide in conditions similar to exposure to nitrogen dioxide alone there was no increase in the effect seen with nitrogen dioxide alone at 7.5 ppm, in fact perhaps a slight decrease (Alarie, unpublished).

4. Discussion

i) Changes observed

It is interesting to note that both pollutants which are con-
sidered as deep lung irritants as opposed to sulfur dioxide which
is primarily an upper respiratory tract irritant induced similar
type of effects. The distribution of pulmonary ventilation was
impaired, the respiratory rate was increased and decreases in
arterial blood oxygen tension were observed. Modifications of
the distribution of ventilation indicates an effect primarily on
the peripheral conducting airways. The increase in respiratory
rate may be attributed to stimulation of pulmonary receptors al-
though other factors may have contributed (7,21). With sulfur
dioxide at low concentrations or with sulfur dioxide and fly ash
mixtures no deleterious effects could be observed. However, with
an accidental exposure to a very high concentration the effects
observed were similar to those obtained with nitrogen dioxide
and sulfuric mist thus indicating that such an exposure resulted
in irritation of the peripheral pulmonary airways. What is
interesting in this case is that the abnormal pulmonary function
detected immediately after the accidental exposure persisted for
a period of 48 weeks during which the animals were maintained in
filtered air and no apparent recovery occurred during this period
Histopathological examination at the end of this 48 week period
revealed pulmonary lesions almost similar in nature as the ob-
tained following acute of subacute exposure to very high concen-
tration of this gas, apparently no healing process had taken place.

In all three instances where deleterious effects were
detected, involvement of the peripheral airways can be com-
pared with the action of other pulmonary irritants such as
phosgene and ozone (21).

ii) Animal species

The use of monkeys seems to be appropriate for evaluation
of the effects of various respiratory irritants commonly found
in either urban atmospheres or industrial situations. Following
a period of adaptation to the restraining chair and face mask
pulmonary function tests can be done in these animals in the
same manner as in humans. Monkeys either in a sitting or upright
position will remain quiet and breathe normally during the various
tests without need for anesthetics. This is an obvious advantage
since anesthesia has a profound effect on respiratory function and
will introduce confusing artifacts in studies on the effects of
low concentrations of pulmonary irritants. However, to obtain
arterial blood samples for determination of oxygen and carbon dio-
xide tranquilization should be used.

Cynomolgus monkeys have been used in these studies instead of
rhesus monkeys because they are less susceptible to tuberculosis.
Also infestations with lung mites is much lower or non-existant.
This is advantageous for histopathological evaluation. Whether or
not monkeys have some advantages over other species in terms of
predicting the effects to be observed in man from exposure to the
various pollutants listed in Table 1 remains unanswerable at this
time. Upper respiratory tract retention and alveolar deposition

of aerosols seems similar in monkey and man (27) and their tidal volume and respiratory frequency as well as other mechanical properties of the lung are very close to those found in young infants. However, other aspects are less well known.

As with other animal species, pulmonary function tests such as determination of maximum ventilatory capacity which requires cooperation of human subjects cannot be made in monkeys. A serious drawback in the use of monkeys is their increasing scarcity and cost. Also a period of at least three months should be observed after a quarantine period for tuberculosis detection so that the animals will be well adapted to their new laboratory environment and be in good health.

Other Considerations

Since we are getting together to discuss various aspects of air pollution I would like to place forward some statements for discussion.

1. Sulfur dioxide, possible synergism, sensitive individuals. It seems rather difficult for me to admit that sulfur dioxide is responsible for all the bad effects that we attribute to air pollution. Also of all the conditions presented so far in which potentiation was observed I remain very skeptical that any would be sufficient to cause death or severe discomfort in normal or sensitive individuals when the concentration of sulfur dioxide is lower than 2 ppm. In reports of controlled human exposure to sulfur dioxide it is sometimes reported that "sensitive" individuals reacted. I don't think the data presented is sufficient, particularly, since in a recent report (10) is was discussed that "an individual experienced intense discomfort even during clean air exposure". This is a rather remarkable observation since no subject reported such levels of discomfort when exposed to 25 ppm SO_2 (concentration increasing from 0 to 25 ppm in about one hour) and points out some of the difficulties to be expected in non-controlled experiments.

2. Tolerance - Desensitization with SO_2.

In mice, cats, or dogs sensory receptors in the respiratory tract become desensitized to the action of SO_2 and this probably also occurs in man (7) although the report by Andersen et al ([10]) pointing out that if humans are suddenly exposed to 25 ppm of sulfur dioxide they found this situation intolerable while the subjects exposed to 25 ppm but reaching this concentration slowly in one hour never reported this. I interpret this situation as desensitization or tolerance development. This raises some

interesting questions as far as evaluation of sensory irritation in

day to day air pollution effects in humans.

3. Formation of irritant sulfates

In Figure 6, the dose response obtained with SO_2 and zinc ammonium

sulfate indicate that this salt is much more potent than sulfur dioxide

in increasing airflow resistance in guinea pigs. I cannot asses the

possible formation of this salt under urban atmospheric conditions and

it seems rather difficult to admit that formation of "irritant sulfates"

occurs when we do not yet know the exact nature of the sulfates. Ob-

viously, if calcium sulfate is formed this is quite different than zinc

ammonium sulfate. In long-term chronic studies exposure of monkeys

and rats to calcium sulfate no deleterious effects could be detected

(Alarie, unpublished). Therefore, I strongly object to the use of the

term "irritant sulfates" until further definition of their nature can

be obtained.

4. Current work

Presently we are engaged in research work on sensory irritation and

although not directly engaged in the effects of sulfur dioxide or ni-

trogen dioxide our work may be of some relevance to urban atmospheric

pollution. Our present hypothesis is that specific organic chemicals

which may be formed as a result of interactions between hydrocarbons,

sulfur dioxide and nitrogen dioxide are much more important to consider

than sulfur dioxide or nitrogen dioxide in terms of evoking sensory

irritation of the eye-nose-throat. For example, nitroolefins are much

more potent sensory irritants than either sulfur dioxide or nitrogen

dioxide (7). Similarly some simple sulfonyl halides are about 50 to

100 times more potent sensory irritants than sulfur dioxide and some carbodiimides and diimines are tremendously potent sensory irritants (Alarie, unpublished). Similarly we have identified some other organic classes of chemicals which are potent sensory irritants. To what extent are these formed in photochemical smog or in some air pollution episodes is still undefined and therefore we are not suggesting these products as the only possible important ones. A recent article by Yeung and Phillips (16) based on data obtained by Heuss and Glasson (17) is encouraging us to pursue our work on chemical reactivity and sensory irritation activity for organic chemicals (7).

TABLE 1

Classification of Airborne Chemicals Capable of Stimulating Nerve Endings in the Respiratory Tract *

A — Sensory Irritant

1. Definition: Chemical which when inhaled via the nose will stimulate trigeminal nerve endings, evoke a burning sensation of the nasal passages, and inhibit respiration. Also, most will induce coughing from laryngeal stimulation.
2. Other characteristics: These chemicals are also capable of stimulating trigeminal nerve endings of the cornea and induce tearing. At high concentration, particularly on moist facial skin, they are capable of inducing a burning sensation. Some have odorant and/or gustatory qualities. Most will induce bronchoconstriction, usually at concentrations in the air higher than required for stimulation of nerve endings in the nasal passages.
3. Equivalent terms to describe their action: Upper respiratory tract irritant, nasal or corneal trigeminal stimulant, common chemical sense stimulant, chemogenic pain stimulant, suffocant, lachrymator, and sternutator.
4. Typical examples: Chloracetophenone, o-chlorobenzylidene malononitrile, β-nitrostyrene, diphenylaminochloro-arsine, sulfur dioxide, ammonia, acrolein, and inert dust. (See Tables 5 to 13.)†

B — Pulmonary Irritant

1. Definition: Chemical which when inhaled will stimulate sensory receptors within the lung and increase respiratory rate with a decrease in tidal volume resulting in rapid shallow breathing. Their action, as opposed to that of sensory irritants or bronchoconstrictors, is to evoke a sensation of dyspnea and breathlessness rather than a conscious painful sensation.
2. Other characteristics: These chemicals are capable of inducing pulmonary edema which is then accompanied by painful breathing. They have little or no action as sensory irritants of the eye or nasal passages at concentration sufficient for pulmonary irritation, and, therefore, they provide little warning of their presence. Some have odorant or gustatory qualities and some exert a bronchoconstricting action.
3. Equivalent terms to describe their action: Lower respiratory tract irritant, lung irritant, and deep lung irritant.
4. Typical examples: Phosgene, nitrogen dioxide, sulfuric acid mist, ozone, sulfur and nitrogen mustard, and sulfur pentafluoride. (See Tables 15 and 16.)†

C — Bronchoconstrictor

1. Definition: Chemical which when inhaled will induce an increase in resistance to airflow within the conducting airways of the lung. The action can be via direct effect on smooth muscles of the conducting airways by axonal reflex, vago-vagal, or trigeminal-vagal reflexes following stimulation of nerve endings belonging to these systems or by liberation of histamine.
2. Other characteristics: Most of these chemicals are also sensory irritants. Their action on the bronchial mucosa produces a painful sensation.
3. Equivalent terms to describe their action: None
4. Typical examples: Sulfur dioxide, ammonia, inert particles, sensitization by allergens such as foreign proteins or chemicals acting as hapten such as toluene diisocyanate, and aerosols of histamine or cholinergic agonists. (See Tables 15, 16, and 17.)†

D — Respiratory Irritant

1. Definition: Chemical which when inhaled can act as sensory irritant, bronchoconstrictor, and pulmonary irritant. These chemicals are capable of all three actions and there is little difference between the concentration at which they are sensory irritant and pulmonary irritant.
2. Other characteristics: Similar to sensory irritants and lung irritants.
3. Equivalent term to describe their action: Any of the terms mentioned in this table, depending on the exposure conditions involved.
4. Typical examples: Chlorine, ketene, chloropicrin, dichloromethyl ether, and chlorine pentafluoride.

*Adapted from Henderson and Haggard.[159] (ref. 7)
† see Ref. 7, this paper

TABLE 2 [*]

Characteristic Actions from Stimulation of Nasal Trigeminal Nerve Endings by Chemical or Physical Stimuli in Unanesthetized or Lightly Anesthetized Laboratory Animals and Man

Adequate Stimuli	Receptor	Afferent nerve	Processor	Efferent nerves	Reflex reactions observed
a. Physical: tempera- ture pressure b. Chemical: specific receptor agonists	Free nerve endings of trigeminal nerve at surface of nasal respiratory epithelium	Increase impulse activity in afferent trigeminal nerve	Integration at CNS level: medulla and other centers and inter- connections with efferent nerves	a. Phrenic: inhibition during expiration phase of respiration b. Vagus: increase activity c. Splanchnic: increase activity	a. Decrease in respiratory rate; lengthened expiratory phase b. Decrease in heart rate c. Peripheral vasocon- striction and rise in systolic arterial blood pressure d. Bronchoconstriction? -- bronchodilation? e. Decreased renal clea- rance and blood flow f. Decreased coronary blood flow g. Decrease in pulmonary blood flow h. Closure of the glottis i. Closure of the nares and increase in nasal flow resistance
References see Tables 5 to 13	References 91, 145, 204, 251, 262, 328	References see Table 6	References 94, 151, 198 266, 303	References see Table 4	References see text, Tables 4 and 5

[*]from Alarie, Ref. 7

TABLE 3[*]

Demonstration of Reflex Pathways Involved in Reflex Reactions from Sensory Irritation of the Upper Respiratory Tract by Airborne Chemicals

Reactions observed under various conditions
Conditions

Systems Affected	(a)	same as (a)+	same as (a)+	same as (a)+	same as (a)+	same as (a)+	same as (a)+	
	Normal conditions unanesthetized breathing via nose φφ	Section of superior laryngeal nerves*	Section of trigeminal nerves or local anesthesia with cocaine	Section of olfactory nerves or removal of olfactory bulb**	Section of the splanchnic nerves or sympathetic blockade	Section of vagus nerves or parasympathetic blockade	Section of vagus nerves with section of splanchnic nerves or sympathetic blockade	Breathing via tracheal cannula φ
Respiratory Rate	↓	↓	—	↓	↓	↓	↓	↑
Systolic Arterial Blood Pressure	↑	↑	—	↑	—	↑	—	− ↓
Heart Rate	↓	↓	—	↓	↓	—	—	− ↓
References***	see Table 5	197, 209	15, 17, 123, 197, 209, 270, 306	15, 197 209, 270	6, 16, 114, 209	6, 16, 17, 123, 133, 193, 209, 210	6	

↓   :   decrease
↑   :   increase
—   :   no change.
*   :   see text for secondary role
** :   see text for secondary role
*** :   other secondary references are noted in text
φ   :   this condition is added to demonstrate the opposite effect of irritants when acting on lower airways; see section 4.0 for details and references
φφ :   or chemical restricted to the upper respiratory tract only

[*]from Alarie, Ref. 7

TABLE 4[†]

Sensory Irritants[*]

A. Chemical	Animal species	Exposure conditions[**]	References
Acetic acid	cat, rabbit, mouse, man	A, B	4, 18, 197
Acrolein	rabbit, guinea pig	A, B	107, 209, 227
Ammonia	rabbit, cat, man	A, B	15, 18, 50, 109, 197, 270
Automobile exhaust	guinea pig	A	229
Benzene	rabbit, dog	A, B	15, 105
Bromacetone	rabbit, dog	B	209, 210
Bromine	rabbit	A, B	270
Capsaicin	mouse	A	8
Chloracetophenone (CAP)	mouse	A	8
Chlorine	rabbit, dog	B	209, 210
o-chlorobenzylidene malononitrile (CBMN)	mouse, rabbit, cat, man, rat	A, B	4, 5, 6, 7, 9, 254, 306
Chloroform	rabbit, dog	A, B	114, 123, 147, 197, 209, 210
Chloromethyl-chloroformate	rabbit, dog	B	209, 210
Chloropicrine	rabbit, dog	B	209, 210
Cigarette smoke	rabbit, mouse	A, B	4, 88, 107, 133, 142, 192, 193, 197, 270
Cinnamylidene-malononitrile	mouse	A	7
Diphenylamino-chloroarsine	mouse	A	6
Ethanol	rabbit, mouse	A, B	6, 197
Ether	rabbit, duck, rat, guinea pig, hamster, mouse	A, B	50, 142, 192, 193, 197
Formaldehyde	mouse, rabbit, guinea pig	A, B	4, 29, 107, 133
Hydrochloric acid fumes	rabbit	B	197
Nitroolefins	guinea pig	A	228
β-nitrostyrene	mouse	A	7
Phosgene	rabbit	B	209
Sodium metabisulfite	mouse	A	10
Sulfur dioxide	mouse, guinea pig, rat, man	A, B	9, 39, 107, 205, 284
Sulfuric acid fumes	rabbit	B	197
Toluene	rabbit	A	50
Trichloromethyl-chloroformate	rabbit, dog	B	209, 210
Xylene	rabbit	B	15
B. Physical			
Cooling with carbonic acid	rabbit	B	197
Cooling with cold water or cold air	dog, rat, cat, man	B	123, 147, 214, 215
Tickling or pinching	rabbit, man	B	50, 188, 197, 212

*Chemical and Physical stimuli capable of inducing a decrease in respiratory rate and other reflex reactions listed in Table 3 because of their action on nasal trigeminal nerve endings.
**Conditions: A: while breathing via the nose. B: chemical or physical stimuli restricted to upper respiratory tract.

†from Alarie, Ref. 7

TABLE 5 [†]

Inhaled Chemicals Reported to Evoke Reflex Reactions* from their Action on
Pulmonary Nerve Endings

Chemicals	Species tested	Exposure Conditions**	References
Acrolein	guinea pig	B	107
Ammonia	rabbit, dog, cat	B,C	16,65,98, 281,325
Benzene	rabbit	B,C	16
Bromacetone	rabbit, dog	B	209,210
Chlorine	rabbit, dog	B	209,210
o-chlorobenzylidene malononitrile (high concentration)	cat	B	83
Chloroform	rabbit, dog	B	209,210
Chloropicrin	rabbit, dog	B	209,210
Cigarette smoke	rabbit, man, guinea pig	A,B	102,107, 219,325
Ether	rabbit, dog	B	98,219,325
Formaldehyde	guinea pig	B	107
Histamine aerosol	rabbit, guinea pig	A,B	72,219
Sensitization with inhalation of foreign proteins	dog, monkey, rabbit, guinea pig	A,B	80,148,149, 218,219,221, 281,290
Nitrogen dioxide	guinea pig	A	230
Ozone	guinea pig, rat, rabbit	A,B	23,167, 230,236
Phosgene	dog, cat	A,B	65,146,200, 313
Sulfur dioxide	guinea pig, cat	B	107,316,317
Sulfuric acid mist	man	A	45
Toluene	rabbit	B,C	16

*Reflex reactions include an increase in respiratory rate usually with a decrease in
tidal volume preceeded or not with coughing and a very brief period of apnea and
accompanied or not with bronchoconstriction.

**A: normal breathing; B: breathing via tracheal cannula; C: breathing via nose
following trigeminal section.

TABLE 6[†]

Inhaled Chemicals Reported to Increase Afferent Vagal Fibers Activity by Stimulation of Various Pulmonary Nerve Endings or by Sensitization of Nerve Endings[****]

Chemicals	Species	Action[*]	Receptors stimulated[◊◊◊]	References
Ammonia[**]	rabbit, cat	A,B	lung irritant, stretch, tracheobronchial	219,317, 318,324
Carbachol aerosol	rabbit	A	stretch	178,179
Carbon dioxide	cat	A	type J	241
Carbon tetrachloride	cat	B	stretch	314
Charcoal dust	cat, rabbit	A	lung irritant, cough	282,325 327
Chlorine	rabbit, cat	A	type J	240,241
Chloroform	cat	B	stretch	314,315
Cigarette smoke	rabbit	A	lung irritant	219,282
Cyclopropane	cat	B	stretch	314,315
Ethyl chloride	cat	B	stretch	314
Fluothane (halothane)	cat	A,B	stretch type J	240,241, 314
Histamine, serotonin aerosol or i.v.	rabbit	A,B	lung irritant, stretch	156,219, 282,320
Nitrous oxide	cat	B	stretch	314,315
Ozone	rabbit, cat	A	stretch	167
Sensitization by foreign proteins[**]	rabbit, guinea pig	A	lung irritant, deflation, stretch	148,178, 195,196, 218,219
Starch (powdered)	cat	A,B	tracheobronchial	316,317
Sulfur dioxide[***]	cat	A,B	tracheobronchial	316,317
Talc (powdered)	cat	A,B	tracheobronchial	316,317
Trichloroethylene	cat	A,B	stretch, deflation	314,315

Other conditions

Chemicals	Species	Action[*]	Receptors stimulated[◊◊◊]	References
Atelectasis	rabbit, cat	B	stretch	320
Hyperpnea	rabbit	A	lung irritant	219,281,325
Large deflation	rabbit	A	lung irritant	219,325
Large inflation	rabbit	A	lung irritant	219,325
Mechanical	rabbit	A	lung irritant	218
Microembolism	rabbit	A	lung irritant	182,218, 219,325
Pneumothorax	rabbit, guinea pig	A	lung irritant deflation, stretch	182,195,196, 219,281,325
Pulmonary congestion	rabbit	A	lung irritant type J	219,240,241, 281,325
Pulmonary edema	rabbit, cat	B	stretch	320

[*] A: stimulation
 B: sensitization
[**] Also efferent vagal fibers (148,318)
[***] Stimulation followed by refractoriness
[◊◊◊] Type J receptors were originally called deflation receptors and tracheobronchial are probably similar to lung irritant receptors (242). However, the name of the receptor is as given in the original references.
[****] Usually an increase in respiratory rate and bronchoconstriction were also reported, preceeded or not by coughing and a brief period of apnea.

[†]from Alarie, Ref. 7

TABLE 7[†]

Airborne Chemicals Reported to Increase Resistance to Airflow in the Respiratory System and Concentrations Necessary for Increasing Resistance by 50 or 100% Above Control Values Estimated from Curves in Figure 16

Chemicals Conditions and References*	Exposure concentrations mg/m³	% increase in resistance above control value	Concentration for 50% increase in resistance		Concentration for 100% increase in resistance	
			mg/m³	umol/l	mg/m³	umol/l
Acetic acid	13.2	30	70	1.33	220	3.66
guinea pigs	99.5	54				
nose breathing	303	120				
Reference 30	1448	383				
Curve A in Figure 6						
Acrolein	0.276	3	0.8	0.016	1.1	0.022
guinea pigs	0.414	18				
nose breathing	0.805	52				
Reference 228	1.380	132				
Curve B in Figure 6						
Formaldehyde	0.06	7	4.8	0.16	23	0.77
guinea pigs	0.372	14				
nose breathing	0.696	22				
Reference 29	1.46	29				
Curve C in Figure 6						
Formaldehyde	1.08	65	0.75	0.025	2.2	0.073
guinea pigs	6.24	132				
breathing via tracheal cannula	24	209				
Reference 29	60	264				
Curve D in Figure 6						
Formic acid	0.646	30	2.2	0.048	30	0.65
guinea pigs	1.9	43				
breathing via nose	5.32	66				
Reference 29	12.54	82				
Curve E in Figure 6	25.65	93				
	80.75	142				
Histamine aerosol	3	50	3.0	0.017	4	0.023
guinea pigs	6	150				
breathing via nose	9	264				
Reference 29						
Curve F in Figure 6						
2–Nitro–2–butene	1.739	46	2.0	0.017	12	0.11
guinea pigs	3.384	66				
breathing via nose	5.358	76				
Reference 228**						
Curve G in Figure 6						
Sulfur dioxide	0.42	10	110	1.7	370	5.7
guinea pigs	1.3	12				
breathing via nose	6.8	21				
Reference 32	49.4	26				
Curve H in Figure 6	293	79				
	426	112				
	879	178				
	2171	265				
Sulfur dioxide	0.96	38	2	0.031	9	0.14

†from Alarie, Ref. 7

TABLE 7 (Cont.)

Airborne Chemicals Reported to Increase Resistance to Airflow in the Respiratory System and Concentrations Necessary for Increasing Resistance by 50 to 100% Above Control Values Estimated from Curves in Figure 16

Chemicals Conditions and References*	Exposure concentrations mg/m³	% increase in resistance above control value	Concentration for 50% increase in resistance		Concentration for 100% increase in resistance	
			mg/m³	umol/l	mg/m³	umol/l
guinea pigs	2.6	61				
breathing via tracheal cannula	5.5	64				
Reference 32	58.8	164				
Curve I in Figure 6	283	230				
Sulfur dioxide	2.6	0	17	0.26	100	1.5
humans	13	40[ΦΦ]				
breathing via mouth	33.8	70[ΦΦ]				
Reference 140						
Curve J in Figure 6						
Sulfuric acid mist (0.8uMMD)	1.9	50	5	0.051	28	0.286
guinea pigs	5.3	54				
breathing via nose	15.4	70				
Reference 27***	26.1	88				
Curve K in Figure 6	42.0	120				
Sulfuric acid mist (2.5 uMMD)	2.3	38	4.8	0.049	17	0.173
guinea pigs	8.9	60				
breathing via nose	15.4	96				
Reference 27	43.6	310				
Curve L in Figure 6						
m-Terphenyl (0.3 uMMd)	5	77	2.7	0.012	8.0	0.035
guinea pigs	9	110				
breathing via nose	22	150				
Reference 38****						
Curve M in Figure 6						
Zinc ammonium sulfate (0.3 uMMMD)						
guinea pigs	0.25	22	0.5	0.0012	1.2	0.003
breathing via nose	0.5	40				
Reference 37Φ	1.1	81				
Curve N in Figure 6	1.8	130				

*Data given in references were transformed to % increase in resistance above control value and mgm/m³ when other units were given. The following conversion factors were used for ppm to mgm/m³:

  acetic acid: 1 ppm = 2.5 mgm/m³
  acrolein: 1 ppm = 2.3 mgm/m³
  formaldehyde: 1 ppm = 1.2 mgm/m³
  formic acid: 1 ppm = 1.9 mgm/m³
  2-nitro-2-butene: 1 ppm = 4.7 mgm/m³
  sulfur dioxide: 1 ppm = 2.6 mgm/m³

**Data for 3-nitro-3-hexene and 4-nitro-4-nonene also given in this reference

***Data for 7 μ particles also given in this reference

****Data for larger particles also given in this reference

ΦData for larger particles also given in this reference

ΦΦHigher and lower increase in resistance have also been reported at this concentration[138,139,288]

ΦΦΦSimilar increase in resistance has also been reported at this concentration[234]

TABLE 8 [†]

Suggested Mechanisms Contributing to an Increase in Resistance to Airflow in the Respiratory Tract When Animals Breathing via the Nose Are Exposed to Airborne Chemicals*

A. Upper Respiratory Tract
  - Nasal and laryngeal constriction
  - Swelling, congestion of membranes
  - Increased secretions
  - Nasobronchial and laryngobronchial reflexes

B. Lower Respiratory Tract
  - Constriction of central and peripheral conducting airways by:
    1. direct action on smooth muscles
    2. axonal reflexes
    3. vago-vagal reflexes
    4. release of histamine, serotonin, etc.
  - Accumulation of secretions
  - Inflammatory changes of the walls
  - Collapsing airways

*Adapted from 111 and references given in sections 3 and 4.

[★]

TABLE 9

MECHANICAL PROPERTIES OF THE LUNG AND RESPIRATORY SYSTEM MEASURED IN CYNAMOLGUS MONKEYS DURING EXPOSURE TO FLY ASH FOR A PERIOD OF 78 WEEKS.

Parameter	Abbreviation	Frequency of Measurement
Total respiratory system flow resistance during inspiration*	Rrs(i)	A
Total respiratory system flow resistance during expiration*	Rrs(e)	A
Tidal Volume	V_T	B
Respiratory rate	RR	B
Minute Volume	MV	B
Dynamic compliance of the lung†	Cdyn(l)	B
Pulmonary flow resistance†	Rl	B
Pulmonary flow resistance during inspiration‡	Rl(i)	B
Pulmonary flow resistance during expiration‡	Rl(e)	B
Work of breathing during inspiration per ml tidal volume	$W(i)/ml\ V_T$	B
Work of breathing during expiration per ml tidal volume	$W(e)/ml\ V_T$	B

* Method similar to that of SWANN et al. (1965) and KING (1966).
† Method similar to that of MEAD (1960).
‡ Method similar to that of MURPHY & ULRICH (1964).
A Measured every week during the pre-exposure control period, every week during the first nine weeks of the exposure period, and every four weeks thereafter.
B Measured on five occasions during the pre-exposure control period, every two weeks during the first ten weeks of the exposure period, and every four weeks thereafter.

‡from Alarie, Ref. 7
*from MacFarland, Ref. 23

TABLE 10[†]

VENTILATION AND DISTRIBUTION OF INSPIRED AIR (NITROGEN WASHOUT)
MEASURED IN CYNAMOLGUS MONKEYS DURING EXPOSURE TO FLY ASH FOR A
PERIOD OF 78 WEEKS

Parameter	Abbreviation	Frequency of Measurement
Tidal volume	V_T	A
Respiratory rate	RR	A
Minute volume	MV	A
Number of breaths to 1% nitrogen	$N(1\% N_2)$	A
Time to 1% nitrogen	$t(1\% N_2)$	A
Cumulative tidal volume to 1% nitrogen	$CV_T(1\% N_2)$	A
Graph displaying the % and expiratory nitrogen concentration vs. number of breaths, time, or cumulative tidal volume		A

A. Measured on six occasions during the pre-exposure control period, every two
weeks during the first four weeks of the exposure period, and every four weeks
thereafter.

TABLE 11[†]

. GAS DIFFUSION MEASUREMENTS IN CYNAMOLGUS MONKEYS DURING
EXPOSURE TO FLY ASH FOR A PERIOD OF 78 WEEKS

Parameter	Abbreviation	Frequency of Measurement
Diffusing capacity of the lung for carbon monoxide	DL_{CO}	A

A. Measured on six occasions during the pre-exposure control period, every two
weeks during the first four weeks of the exposure period, and every four weeks
thereafter.

TABLE 12[†]

. ARTERIAL BLOOD GAS MEASUREMENTS IN CYNAMOLGUS MONKEYS DURING
EXPOSURE TO FLY ASH FOR A PERIOD OF 78 WEEKS

Parameter	Abbreviation	Frequency of Measurement
Arterial blood oxygen tension	P_aO_2	A
Arterial blood carbon dioxide tension	P_aCO_2	A
Arterial blood acidity	pH	A

A. Measured once during the pre-exposure control period, every eight weeks for
the first 24 weeks during the exposure period, and every 12 weeks thereafter.

[†] from MacFarland, Ref. 23

TABLE 13   †

HAEMATOLOGICAL AND SERUM BIOCHEMICAL DETERMINATIONS IN CYNA-
MOLGUS MONKEYS PERFORMED DURING EXPOSURE TO FLY ASH FOR A PERIOD OF
78 WEEKS

Parameter	Abbreviation	Frequency of Measurement
Haematocrit	Hct.	A
Haemoglobin	Hgb.	A
Erthyrocytes	RBC	A
Leucocytes	WBC	A
Lymphocytes	Lymph.	A
Segmented neutrophils	Seg.	A
Blood urea nitrogen	BUN	A
Total bilirubin	—	A
Serum total protein	—	A
Serum albumin	—	A
Sodium	Na	A
Potassium	K	A
Chlorides	Cl	A
Calcium	Ca	A
Serum glutamic-oxaloacetic transaminase	SGOT	A
Serum glutamic-pyruvic transaminase	SGPT	A
Serum lactic acid dehydrogenase	LDH	A
Serum alkaline phosphatase	Alk. PO_4	A

A Measured once during the pre-exposure control period and at 11, 16, 25, 49,
and 77 weeks.

†from Mac Farland, Ref. 23

SUMMARY OF MAIN FINDINGS IN CYNOMOLGUS MONKEYS EXPOSED TO VARIOUS POLLUTANTS

| Pollutants	Duration	Concentration		Particle Size	Pulmonary Microscopic Alterations*						Pulmonary Function		
	months	ppm	mg/m³	MMD,μ	A	B	C	D	E	F	Distribution of Ventilation	Respiratory Rate	Arterial Oxygen Tension
Control	18				No Significant Alteration Present								
Sulfur Dioxide	18	0.11			No Significant Alteration Present						No Significant Alteration		
Sulfur Dioxide	18	0.64			No Significant Alteration Present						No Significant Alteration		
Sulfur Dioxide	18	1.28			No Significant Alteration Present						No Significant Alteration		
Sulfur Dioxide	7	4.69									No Significant Alteration		
Sulfur Dioxide**		4.69+			9/9 (++)	9/9 (++)	9/9 (++)	8/9 (++)			Deterioration (+++)	Increase	Decrease
Fly Ash	18		0.16	2.73	Presence of Fly Ash Detected No Significant Reaction Detected						No Significant Alteration		
Fly Ash	18		0.46	2.63	Presence of Fly Ash Detected No Significant Reaction Detected						No Significant Alteration		
Nitrogen Dioxide	21	0.5			No Significant Alteration Present						No Significant Alteration		
Nitrogen Dioxide	24	6.8			7/9 (++)	7/9 (++)			6/9 (+)	2/9 (+)	Deterioration (++)	Increase	Decrease at Beginning and Recovery
Sulfuric Acid Mist	18		0.38	2.15	5/8 (+)	3/8 (+)					No Change	Increase	No Change
Sulfuric Acid Mist	18		2.43	3.60	8/8 (++)	8/8 (++)	8/8 (++)				Deterioration (++)	Increase	Decrease
Sulfuric Acid Mist	18		0.48	0.54	No Significant Alteration Present						Deterioration (+) at Some Time Intervals	No Change	No Change
Sulfuric Acid Mist	18		4.79	0.73	8/8 (++)	8/8 (+++)					Deterioration (++)	Increase	No Change
Sulfur Dioxide & Fly Ash	18	0.11	0.62	2.62	Presence of Fly Ash Detected No Significant Reaction Detected						No Significant Alteration		
Sulfur Dioxide & Fly Ash	18	1.02	0.52	3.38	Presence of Fly Ash Detected No Significant Reaction Detected						No Significant Alteration		
Sulfur Dioxide & Fly Ash	18	5.11	0.54	2.76	Presence of Fly Ash Detected No Significant Reaction Detected						No Significant Alteration		

*A: Bronchiolar epithelial hyperplasia
B: Thickening of walls of respiratory bronchioles
C: Thickening of alveolar walls
D: Bronchiectasis, bronchiolectasis
E: Bronchitis
F: Focal emphysema
Number under each column indicate animals affected in each group (+) slight, (++) moderate, (+++) moderate to severe.

**This group was exposed to 4.69 ppm SO2 for 7 months prior to an overexposure accident (1 hour ≈ 200-1,000ppm). Animals were then kept under control conditions for eleven months prior to sacrifice and histopathological examination. Results on pulmonary function are those obtained during these eleven months.

FIGURE 1[†]
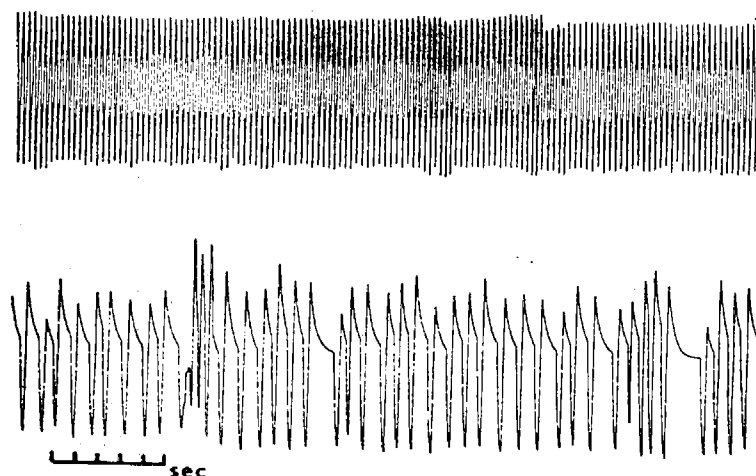
Top tracing: normal breathing rate in mice. Bottom tracing: breathing rate during exposure to o-chlorobenzylidene malononitrile as reported in Reference 6. This shows the characteristic decrease in respiratory rate during exposure of animals to sensory irritants as reported in Table 4. Note that the duration of expiration is increased and that tidal volume is relatively unchanged.

FIGURE 2[†]
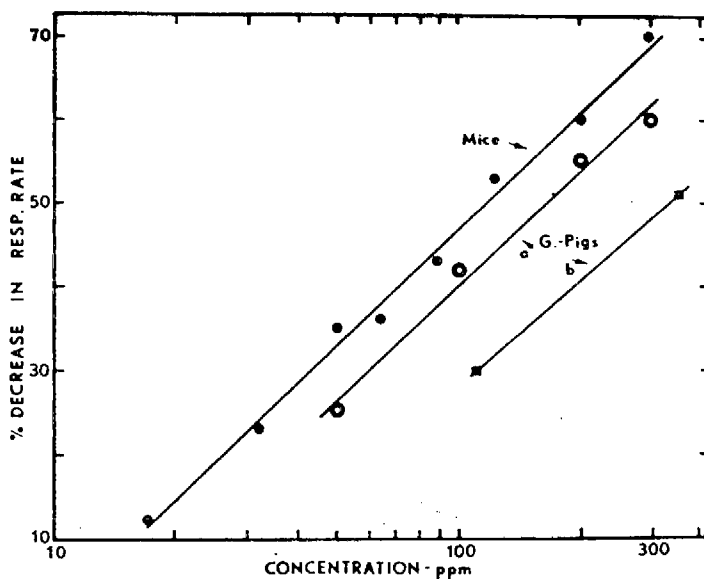
Dose-response curves obtained with sulfur dioxide in mice (from Reference 9) and in guinea pigs (Curve a, open circles, from Alarie, DeRosa, and Wakisaka, unpublished, and Curve b, black squares from Reference 39). Data points for mice and curve a for guinea pigs represent the maximum decrease in respiratory rate obtained at each exposure concentration. In Curve b for guinea pigs data points represent the average decrease in respiratory rate during a one hour exposure. The maximum response does not occur at the same time in mice and guinea pigs

[†]from Alarie, Ref. 7

FIGURE 3[†]
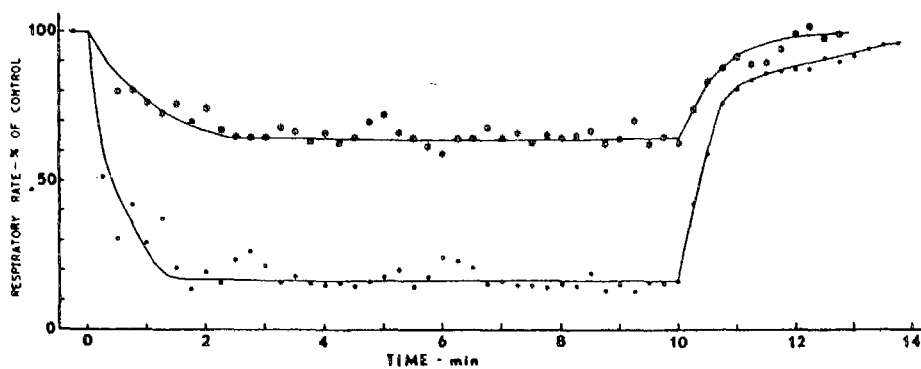
. Time-response curves obtained in mice with o-chlorobenzilidene malononitrile at 2.4 and 75 µg/l air (top and bottom of line respectively) during 10 min of exposure. This demonstrates that the response occurs rapidly, reaches a stable level, and that recovery occurs rapidly following termination of exposure. (Reproduced from Alarie, Y., Wakisaka, I., and Oka, S., *Environ. Physiol. Biochem.*, 3, 1973

FIGURE 4 [*]
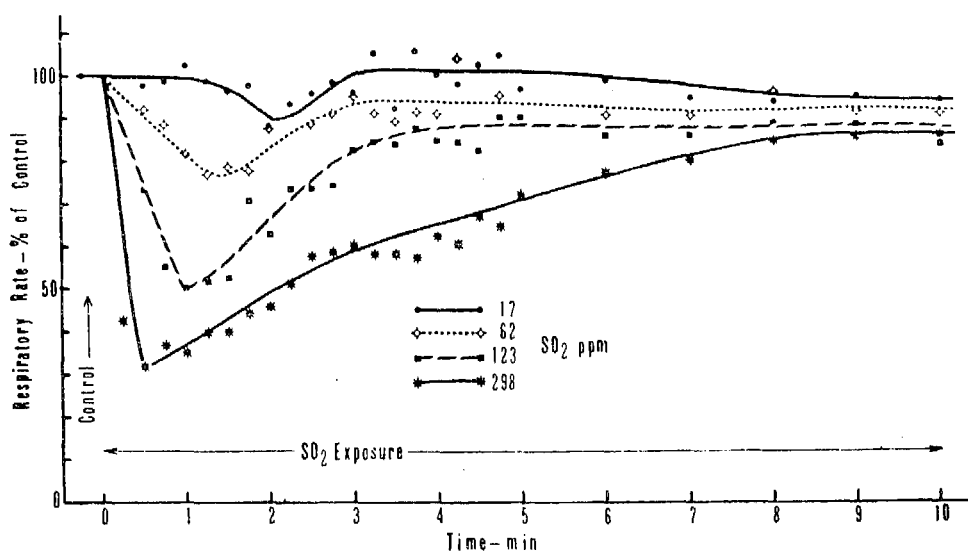
Time-response curves, fitted by visual inspection, during exposure to SO_2. Each data point represents the mean obtained with four mice exposed simultaneously. The coefficient of variation—(s.D./$\bar{X}$) × 100—for all data points was below 22% and usually below 12%.

†from Alarie, Ref. 7
*from Alarie, Ref. 3

FIGURE 5[†]
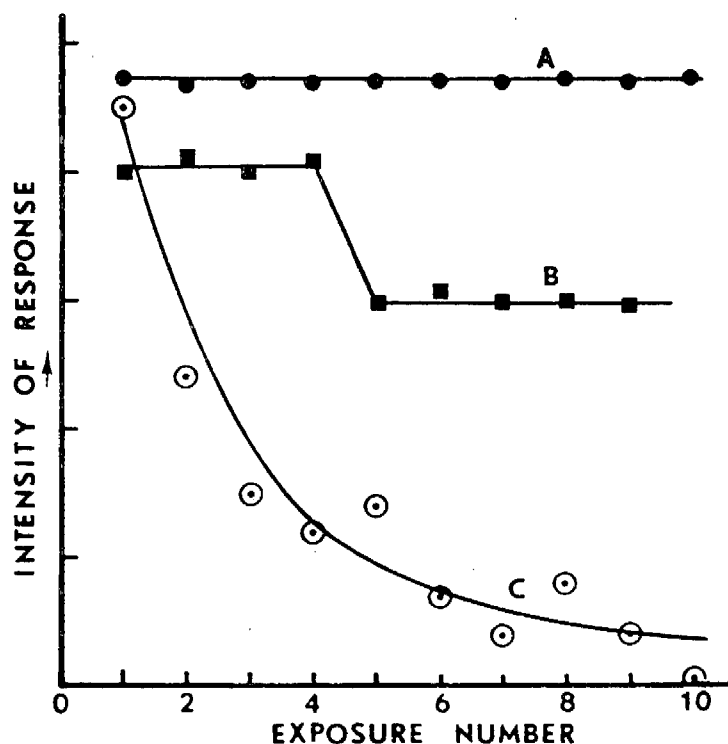
Intensity of response with repeated exposures. Curve A represents: 1. data with o-chlorobenzylidene malononitrile in mice: the maximum response, decrease in respiratory rate reflecting sensory irritation of the upper respiratory tract remained the same with repeated exposures. From References 8, 9, and Alarie and Wakisaka, unpublished. 2. data with o-chlorobenzilidene malononitrile in humans: the intensity of the response, subjective reports of sensory irritation of the upper respiratory tract and eyes remained the same with repeated exposures. From Reference 254. Curve B represents: 1. data with capsaicin (low concentration) in mice: the maximum response, decrease in respiratory rate reflecting sensory irritation of the upper respiratory tract remained the same for the first 4 exposures and was then less intense during the following 5 exposures. From Reference 8. Curve C represents: 1. data with sulfur dioxide in mice: the maximum response, decrease in respiratory rate reflecting sensory irritation of the upper respiratory tract decreased with each successive exposure until no response is observed by the 10th exposure. From Reference 9. 2. data with sulfur dioxide in man: the maximum response, increase in airflow resistance reflecting mainly bronchoconstriction decreased from the first to the second exposure. From References 135, 138, and 140.
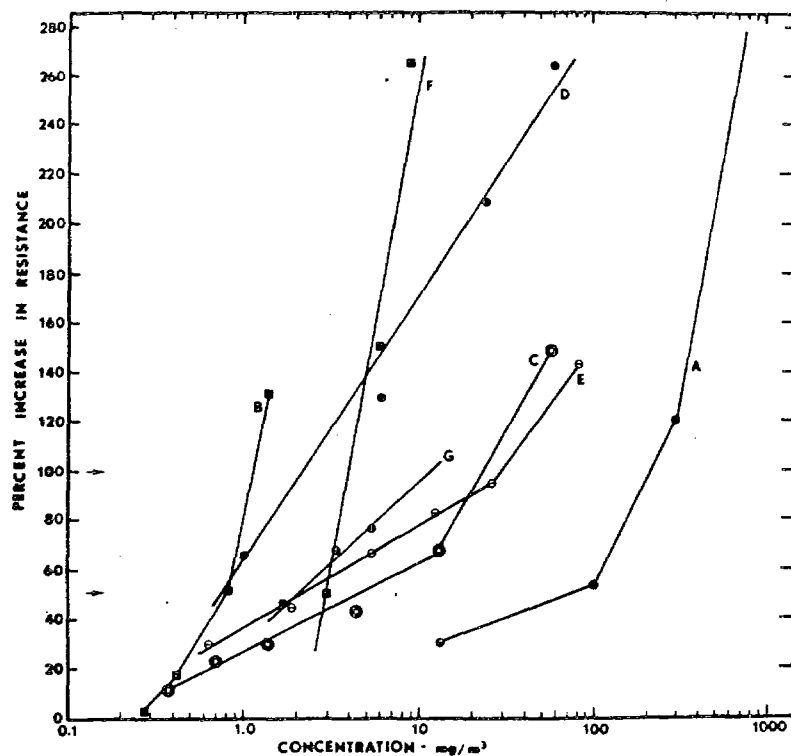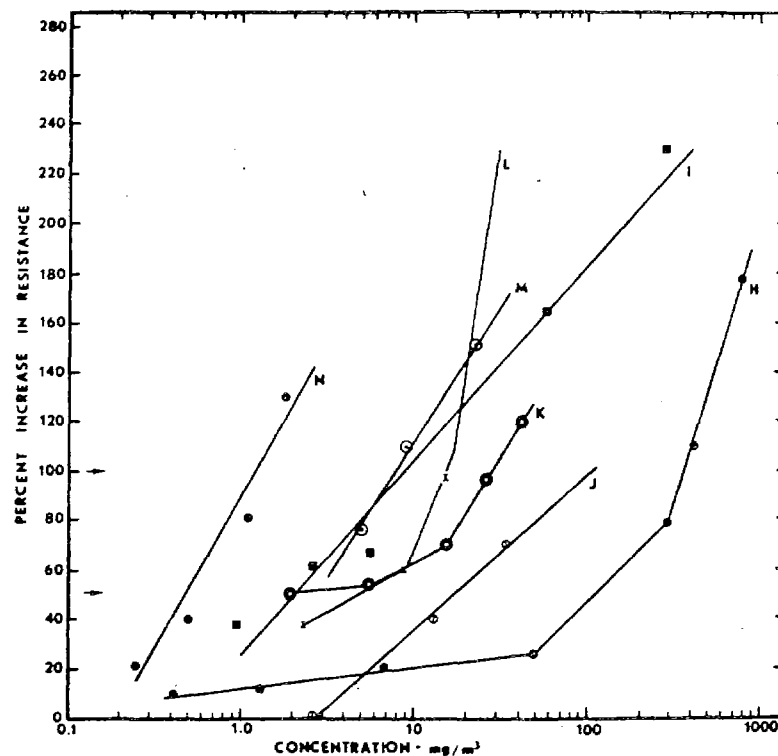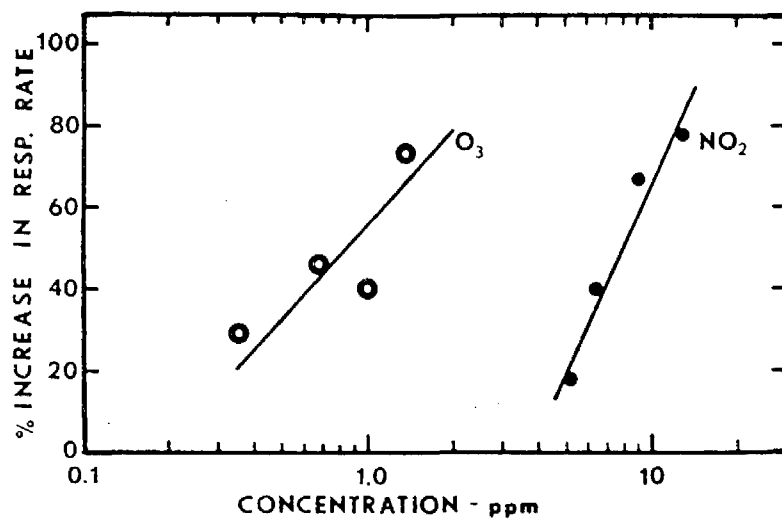
[†]from Alarie, Ref. 7

FIGURE 6 A and B.   Dose-response curves obtained with exposure of guinea pigs to various airborne chemicals. The increase in resistance is plotted against the exposure concentration for each chemical from the data in Table 7. Description of conditions and chemicals for curves A to N as given in Table 7. Concentrations required to increase resistance by 50 or 100% (arrows on graph) listed in Table 7 were obtained from these curves. Highest exposure concentration data point for curves A, H, and L not included but curves drawn toward it. See Table 7 for these values.

FIGURE 7[†]

Dose-response curves obtained with exposure of guinea pigs to ozone and nitrogen dioxide. The maximum increase in respiratory rate (% increase) occurring during exposure is plotted for each exposure concentration. From data in Reference 230.

[†]from Alarie, Ref. 7

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SULFUR OXIDES AND PARTICLES: EFFECTS ON

PULMONARY PHYSIOLOGY IN MAN AND ANIMALS

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Presented at the Conference on Health Effects of

Atmospheric Salts and Gases of Sulfur and Nitrogen

in Association with Photochemical Oxidant

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CHAPTER II.

Table of Contents

When we were invited to speak about the functional changes caused by sulfur oxides and suspended particles in man, we accepted with great pleasure, but made one minor stipulation: that additionally, we be allowed to discuss a recent study in our laboratory involving guinea pigs. The results of that study have implications for the effects of air pollution on health. The Academy most graciously accepted the stipulation.

$$\underline{SO_2}$$

First, however, let us consider SO_2 alone. There is a large body of experience gained from studies employing just SO_2 which will serve as a useful preamble to our discussion of the consequences of mixing the gas with an aerosol. Acute exposure to SO_2 in concentrations of about 1-2 parts per million by volume (ppm) may cause slight changes in the maximal ventilatory performance of healthy individuals (Snell and Luchsinger, 1969; Anderson, et al., in press). These concentrations are encountered industrially but are probably rare in urban environments. In the most recent of these studies (Anderson, et al., in press), evidence was adduced that the upper airways, specifically the nasopharynx, could in response to irritation by SO_2 initiate reflex broncho-constriction. We will return to this study later. Nonetheless, it is reasonable to state that low ambient concentrations of SO_2 alone are not <u>directly</u> irritating to the lungs. The reason is that the gas is highly soluble in tissue fluids, so that little penetrates beyond the upper airways.[1] An example of the efficiency of the nasopharyngeal

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[1] SO_2 is over 50 times more soluble than carbon dioxide in water at body temperature

passageway in removing SO_2 from inspired air is shown in Figure 1. In these subjects only about 1-2% of the 16 ppm of SO_2 entering the nose reached the posterior pharynx during quiet breathing (Speizer and Frank, 1966). The findings have since been confirmed (Anderson, et al., in press).

It is well to note that the subjects in both studies were at rest and breathing nasally. We do not know what percentage of the inspired SO_2 might have reached the lower airways (tracheo-bronchial tree) had they been exercising and were obliged to breathe by mouth. A study in anesthetized dogs in which $^{35}SO_2$ was administered to either the nose or mouth at two widely different rates of flow suggests an answer (Frank, et al., 1969). The results are shown in Figure 2. Note that the scrubbing efficiency of the oro-pharynx was only slightly less than that of the naso-pharynx at a low flow, but fell rapidly at the high flow. We can infer from these results that the amount of SO_2, or of any soluble pollutant gas, that reaches the lower airways per unit of time will increase during exercise for two reasons: its fractional up-take by the upper airways falls, while the ventilatory rate rises. For these reasons, it is likely that children who play a lot out-of-doors are being unduly exposed to air pollution. Some of the clear-cut associations between urban air pollution and both respiratory functional impairment and disease in children may reflect their greater physical activity.

Analysis of the uptake of SO_2 by the respiratory system can be carried a step further to consider what happens to the gas once it reaches the trachea. What are the subsequent sites of transferral from the air stream to the tissue fluids? The level(s) at which transfer occurs

should have bearing on the nature of the functional, biochemical, and structural responses. Unfortunately, there is no direct information on the subject. Nonetheless, we would like to present briefly the results of a computer analysis of the problem (McJilton, et al., 1972). The results must be considered preliminary; they have not yet been tested experimentally. We relied on a modified version of Weibel's (1963) model of the human lung, i.e., for computational convenience, we used segments that differed slightly from his convention of numbering branches or generations of airways. Three independent variables were treated: tidal volume, duration of the breathing cycle, and the absorption characteristics or mass transfer coefficient of the gas. The results are indicated in Figures 3 and 4.

. Figure 3 compares the sites of uptake in the lungs beginning at the trachea of two gases of interest to this Conference, SO_2 and ozone (O_3). In this analysis the respiratory bronchioles comprise segments 23-24. Note that for a tidal volume of one-half liter and a respiratory frequency of about 15 breaths/minute, SO_2 is taken up (virtually) entirely in the pre-bronchiolar airways. The uptake of O_3 is more peripheral.

Figure 4 relates mass transfer of the gas to tissue surface area in each segment; i.e., it provides an index of local tissue dosage. On the basis of this analysis, one would expect the effects of SO_2 to be confined to the airways, while those of O_3 would be distributed between the airways and alveoli.

SO_2-Aerosol Mixtures

Our experiment on guinea pigs referred to earlier was the first in a projected series designed to test factors that might influence the interaction between SO_2 and suspended particles and thereby modify the

biological response. We chose the guinea pigs and a sodium chloride (NaCl) aerosol for historical reasons.[2] Virtually the only convincing evidence that a gas-aerosol mixture may have synergistic effects on the lungs was obtained by Amdur (1961) using this animal and aerosol.

NaCl aerosol is deliquescent. At a low relative humidity (RH), the particles are solid. At an RH of about 68-72%, the particles form droplets. Thereafter, as the RH continues to rise, the droplets increase in size. Figure 5 illustrates the deliquescent behavior of a NaCl aerosol. The light scattering ratio of the aerosol (Bscat), measured with a nephelometer (Charlson, et al., 1969) is shown on the ordinate. The ratio is an approximate index of the mass concentration of submicronic aerosols. RH is shown on the abscissa. There is a sharp increase in mass concentration starting at an RH of about 68%.

We reasoned that SO_2 and NaCl aerosol would not interact extensively if mixed in air at an RH below deliquescence.[3] Once RH was sufficiently high for droplets to form, the SO_2 would enter solution. Less than two to three minutes would be required for equilibration between the gas and liquid, while the average residence-time of the mixture in our reaction chamber would be eight minutes. Admittedly, if a dry NaCl particle were inhaled, it also would rapidly become a droplet in the warm, humid upper airways. The process might take only 20-30 msec (Keith and Arons, 1954) and approach completion in the vicinity of the naso-pharynx. But

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[2] NaCl aerosols are present in maritime cities and certainly in the area around Salt Lake City, Utah. More importantly, NaCl serves as a model for any deliquescent aerosols. The presence of which has recently been demonstrated in the St. Louis area (i.e., $(NH_4)_2SO_4$).

Dry aerosols adsorb small amounts of SO_2 on their surfaces. From Pilat's calculations (1968) it can be predicted that 1.5 mg of SO_2 will be adsorbed in each m^3 of gas when the SO_2 concentration is 1 ppm and the aerosol concentration 1 mg/m^3. We assume this occurred in our experiment. Once the aerosol becomes a droplet, the amount of SO_2 entering solution will substantially exceed the amount adhering to the solid surface, and will of course dissociate into H^+, HSO_3^- and $SO_3^=$ ion.

while the droplet was forming, the surrounding mucosa would have removed most of the SO_2. Thus, the more favorable circumstance for synergism would be that in which the aerosol, before entering the airways, was in droplet form and equilibrated with the gas.

Figure 6: The animals were lightly sedated with sodium pentobarbital to reduce their sense of discomfort and struggling within the plethysmograph. We used 6 experimental modes: 1 ppm of SO_2 alone at low RH (< 40%) and high RH (>80%); $1mg/m^3$ of NaCl aerosol alone at the two RHs. The aerosol was generated by flowing filtered air through a fritted glass sparger submerged in a 5% NaCl solution. The size distribution was essentially that of urban air as described by Junge (1963) and Butcher and Charlson (1972).

Following a control period, each animal was exposed to one mode for one hour, allowed to recover on filtered, ambient air for one hour, and re-exposed to another mode for one more hour. The sequence of modes was randomized. In this way, 36 animals were exposed 72 times; there were 12 exposures per mode, half being initial and half being final exposures.

The average changes in pulmonary flow resistance (R_L) for the entire hour are shown in Figure 7. The only significant increase in R_L occurred with the mixture at a high RH.

To assess the changes in R_L as a function of time, we divided the one-hour exposure into four 15 minute periods, and obtained an average value of R_L for each period. To simplify the presentation, the results of only two modes are shown in Figure 8: SO_2 alone and the mixture at the high RH. The increase in R_L in response to the mixture was at least

as great during the last half-hour of exposure as during the first half-hour.

One other finding is worthy of note: the pH of the aerosol droplet (high RH) in the presence of SO_2 collected in a midget impinger averaged 3.5; i.e., it was an acid aerosol.[4] The pH of the aerosol droplet in the absence of SO_2 was 6.8. Sulfurous acid, formed when SO_2 dissolves in an aqueous solution, is highly dissociated into hydrogen (H^+) and bi-sulfite (HSO_3^-) ions. The presence of bisulfite ion and absence of sulfate ion were confirmed quantitatively by mass spectrometry. Sulfuric acid mist was not detectable with a method described by Covert, et al. (1972).

There are differences worth noting between Amdur's experimental results and our own. Whether her NaCl aerosol was in liquid or solid state just prior to inhalation is unknown since RH was unknown. She did employ higher concentrations of aerosol, i.e., 4 and 10 mg/m^3, and in general, higher concentrations of SO_2. We estimate from one of her reports that the increase in R_L in response to a mixture of 1 ppm of SO_2 and 10 mg/m^3 of NaCl aerosol averaged about 30% (by extrapolation), that the increase in R_L in response to a mixture of 1 ppm of SO_2 and 4 mg/m^3 of aerosol averaged slightly under 20%, and that the change evoked by 1 ppm of the gas alone was about 15% (Amdur, 1961). Conceivably, had she used only 1 mg/m^3 of NaCl in combination with SO_2 the response might have been no different from that of the gas alone. It will be recalled that the increase in R_L in our animals (gas-aerosol mixture, high RH) averaged 47%. On this basis, we suspect that her aerosol was dry.

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[4]The presence of an acid aerosol (either or both H_2SO_4 and NH_4HSO_4) in the ambient air in the St. Louis area was recently confirmed by real-time measurements, by graduate students working under the direction of Dr. Robert Charlson of the University of Washington.

Perhaps one way of reconciling the differences between the two studies is to suggest that if enough soluble dry aerosol and gas were inhaled together, the former in the process of becoming a liquid in the upper airways may present an absorptive surface area large enough to compete effectively for the gas with the nasal mucosal surfaces. Also, since 4-10 times as much aerosol was used in Amdur's experiments, there would have been a correspondingly greater amount of SO_2 adsorbed on the dry surface of the aerosol prior to inhalation.

It is debatable whether synergism arising from a mixture of SO_2 and a soluble aerosol has been shown experimentally in man. This, despite a number of attempts here and in Japan (Toyama, 1962; Toyama and Nakamura, 1964; Frank, et al., 1964; Burton, et al., 1969; Snell and Luchsinger, 1969). Generally the aerosol has been NaCl, but plain water and hydrogen peroxide have also been used as substrates. In the study by Toyoma purporting to show synergism, the technique for measuring pulmonary flow resistance (Ainsworth and Eveleigh, 1952; 1953; Clements, et al., 1959) appears to have been used improperly, judging from an illustration (Figure 2) in the text. In one of the studies in which synergism could not be demonstrated (Frank, et al., 1964), there was a deliberate effort to replicate the concentration and size distribution of the NaCl aerosol previously shown to be effective in guinea pigs (Amdur, 1961). Unfortunately, none of these studies provided information about the ambient RH, so that it is difficult to interpret the results or to resolve ostensible conflicts. The experiments ought

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[5]The nasal surface area lying between the bony opening of the nose and the posterior ends of the turbinates is estimated to be about 160 cm^2 (Proctor, 1964), compared with a surface area for the aerosol (1 mg/m^3) of about 3 x 10^{-5} cm^2.

to be redone with proper attention given not only to RH but also to other factors such as changes in ambient temperature and the addition of metallic catalysts to the mixture. All of these circumstances might be expected to modify the interaction between SO_2 and soluble aerosols and thereby modify the toxicity of urban air pollution.

Effects of SO_2 on Upper Airways

Thus far we have considered just two functional parameters: the mechanical behavior of the lungs and maximal ventilatory performance. Until recently, they were the basis for virtually all the information we had about the effects of SO_2 on man. The scope of information is now increasing. A year ago, Andersen, et al. (in press) exposed a group of young male subjects on three successive days to the following sequence of SO_2: 1 ppm on Day 1, 5 ppm on Day 2, 25 ppm on Day 3, each exposure lasting six hours. Among the functional changes that occurred during the first day of exposure to 1 ppm of SO_2 were: slowing of the rate of nasal mucus flow; a decrease in nasal patency as measured by the resistance to airflow; and confirmatory evidence, mentioned earlier, that the uptake of SO_2 by the nose was virtually complete. Notwithstanding this capacity of the nose to remove SO_2 from the inspired air, there was a reduction in forced expiratory flow rate over the middle range of the vital capacity (FEF 25-75%). The authors interpreted their findings as evidence for a naso-bronchial reflex, i.e., irritation

of the nose by SO_2 had caused reflex tracheo-bronchial constriction.[6]
The accessibility of the upper airways and the fact that they do parti-
cipate in the response to inhaled pollutants, all commend them for in-
tensified stury in this field.

Conclusion

SO_2 alone in concentrations typical of urban communities presents
only slight hazard to the lungs. When the gas combines with a submicron-
ic wet aerosol, the hazard may increase significantly. Among the factors
influencing this interaction are the ambient temperature and RH, and
the presence of catalytic agents. Thus, the amount of SO_2 emitted or
its concentration in any locale at a particular time, may be expected
to have different implications for health depending on these additional
factors. These considerations should carry weight when emission controls
and air quality standards are attempted.

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[6]At a symposium on the Nose and Adjoining Cavities at the Armed Forces
Institute of Pathology in Washington, D.C. on December 1-3, 1969, S.
Ingelstedt of Sweden presented data during an open discussion on several
patients with asthma who manifested the naso-bronchial reflex while breath-
ing cold air. The reflex could be abolished by applying a topical anes-
thetic to the nose. Since there is evidence that ^{35}S is excreted by the
lungs during exposure of the isolated upper airways to $^{35}SO_2$ (Frank, et
al., 1967), it would appear that the tracheo-bronchial tree can also be
exposed to SO_2 "from below". One wonders whether an anesthetic applied
topically to the nose in conjunction with exposure to SO_2 might be use-
ful in distinguishing between the effects of the naso-bronchial reflex
and this form of internal exposure.
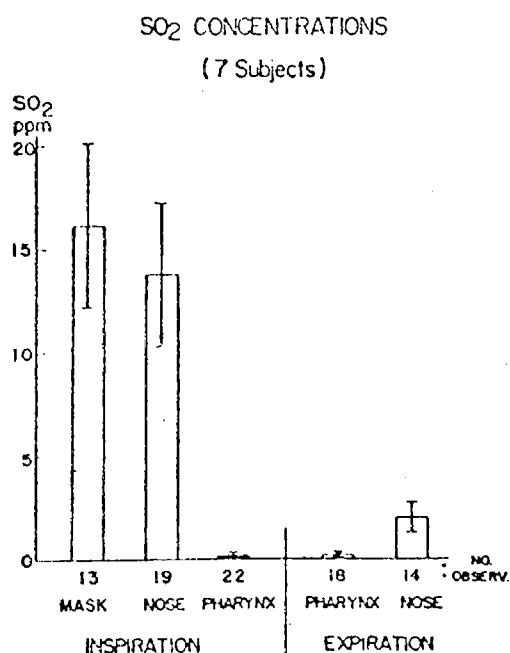
SO$_2$ CONCENTRATIONS

(7 Subjects)

—The mean concentrations and standard deviations of SO$_2$ during inspiration are shown for the following sites: within the mask, just within the nose, and the pharynx. Nasal and pharyngeal values are also given for expiration. The inspiratory samples at the nose and pharynx were collected simultaneously, as were the expiratory samples at the same sites.

Figure 1. Reprinted from Speizer, Frank E., and Frank, N.R.: The uptake and release of SO$_2$ by the human nose. Arch. Environ. Health 12:726, 1966.

Penetration of upper airways by $^{35}SO_2$ (1 ppm)

| Dog No. | NOSE | | | | MOUTH | | | |
| | 3.5 LPM | | 35 LPM | | 3.5 LPM | | 35 LPM | |
	Expos. min.	Penetra-tion, %	Expos. min.	Penetra-tion, %	Expos. min.	Penetra-tion, %	Expos. min.	Penetra-tion, %
4	5	<0.01	2	10.7	5	0.1	1	72
	5	<0.01	2.5	6.4				
5	5	<0.01	1.5	<0.01	5	0.1	2.7	48
	5	<0.01			4.5	<0.01		
	5	0.1			5	3.4		
					5	3.1		

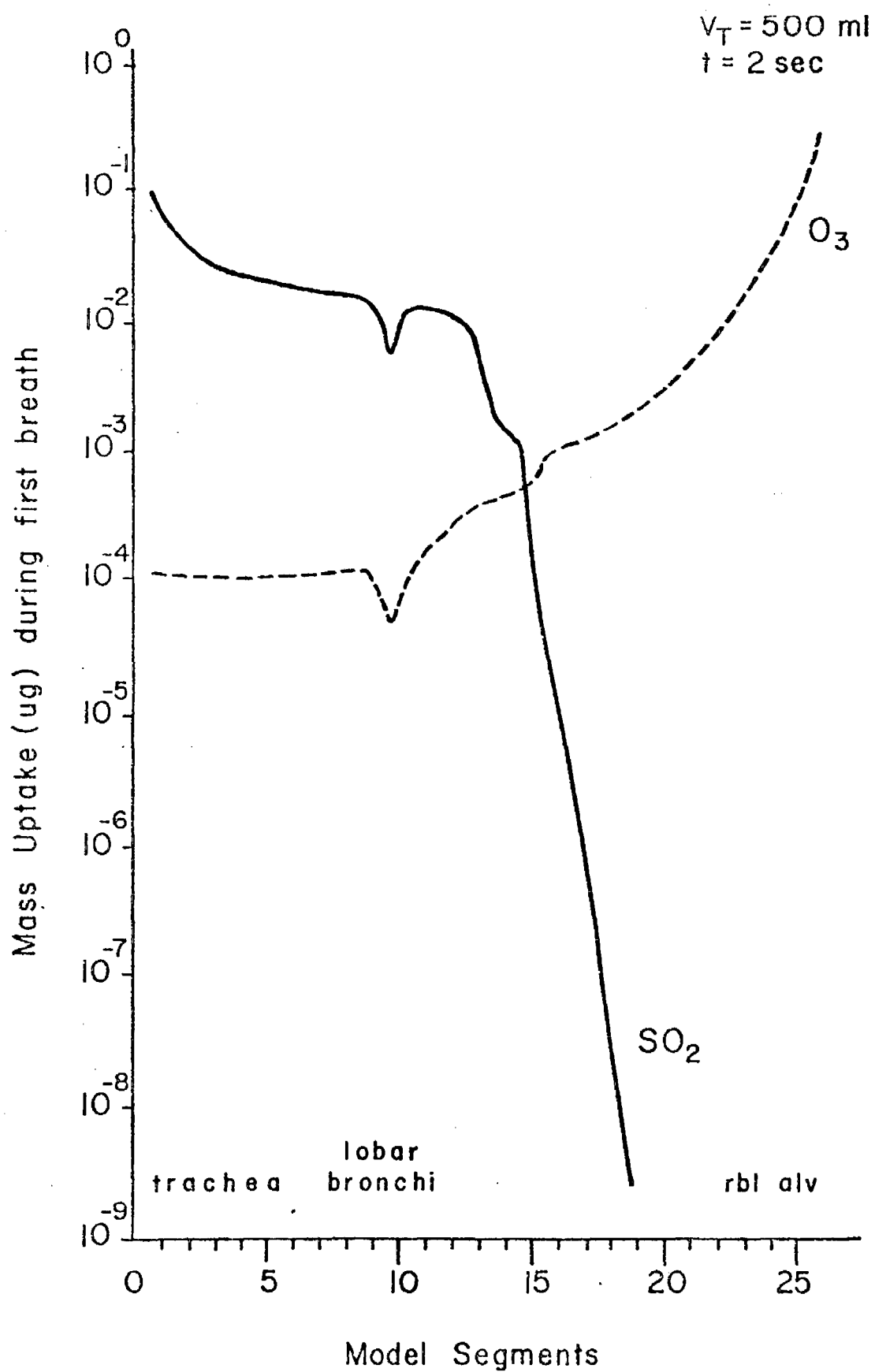Figure 2. Reprinted from Frank, Yoder, Brain and Yokoyama, 1969.

Figure 3. Reprinted from McJilton, C., Thielke, J., and Frank, R., 1972.
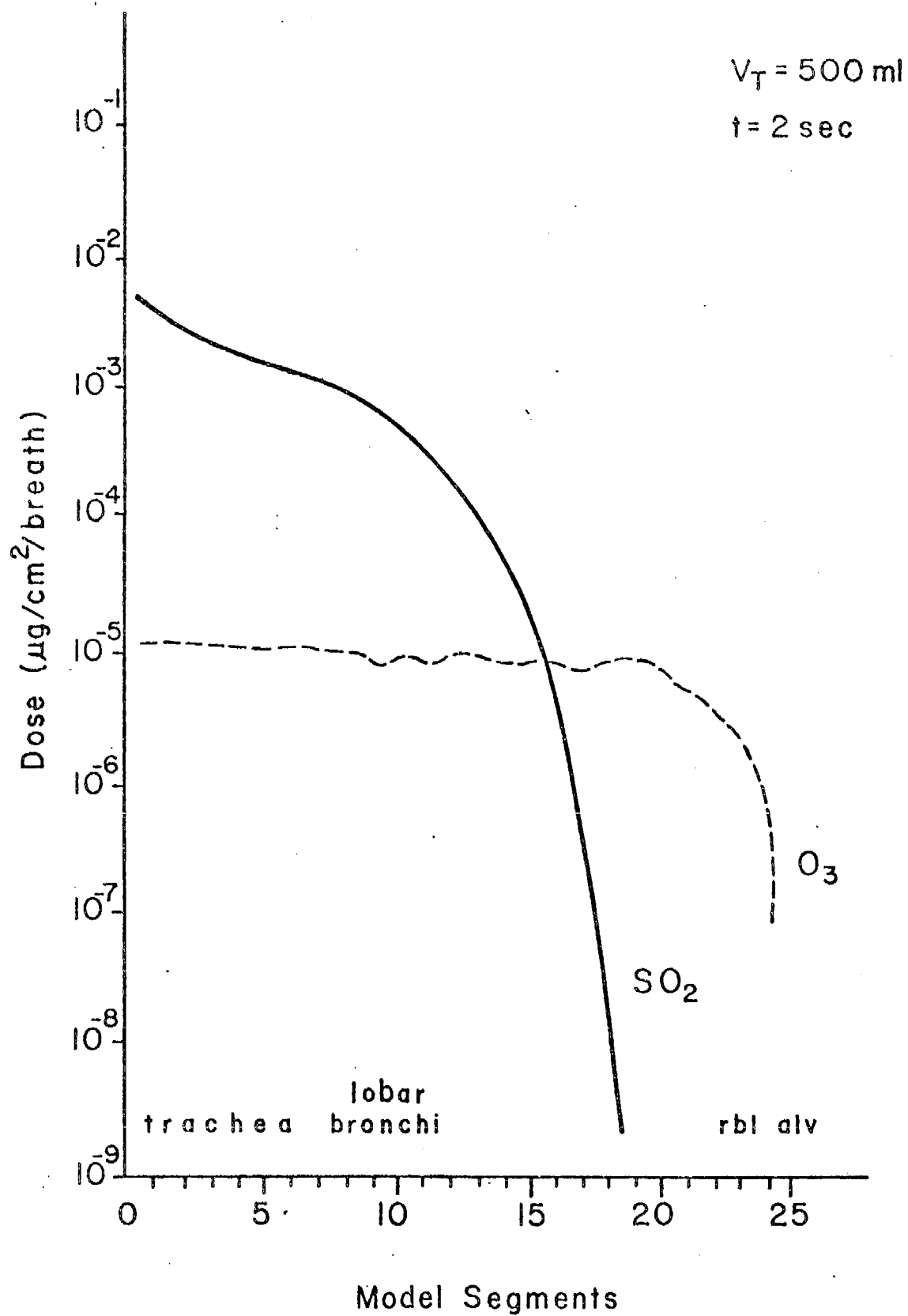
Figure 4. Reprinted from McJilton, C., Thielke, J., and Frank, R., 1972.
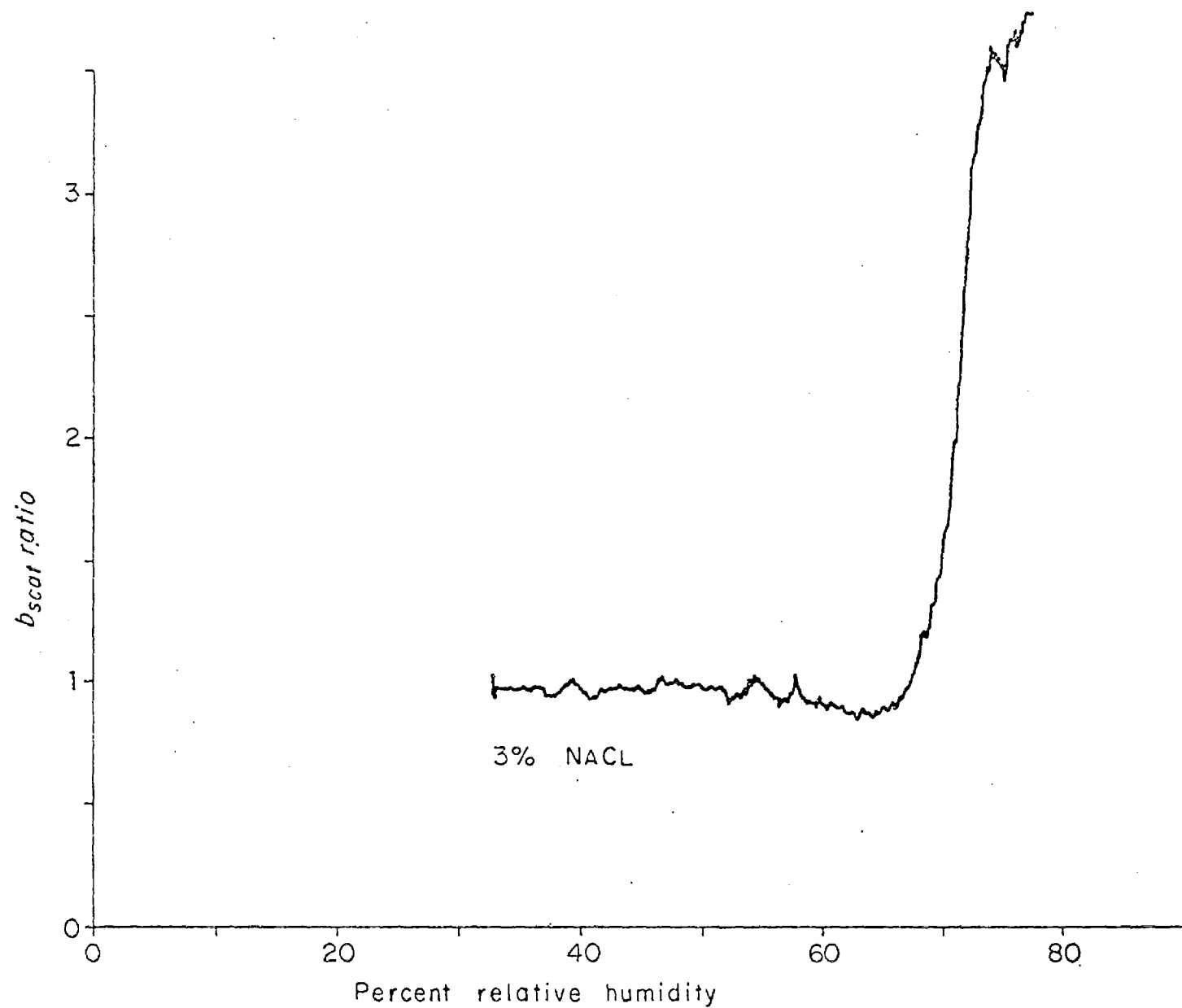
Figure 5. Reprinted from Charles E. McJilton's Doctoral Dissertation, 1973, University of Washington.

Figure 6. Reprinted from McJilton, C., Frank, R., and Charlson, R.: The role of relative humidity in the synergistic effect of SO_2-aerosol mixture on the lung. Science, in press.

A	Nephelometer
B	Sulfur dioxide monitor
C	Dew Point hygrometer
D	Differentiator
F	Sulfuric acid mist detector
P_1, P_2	Differential transducers
EC	Exposure chamber
PL	Pressure plethysmograph
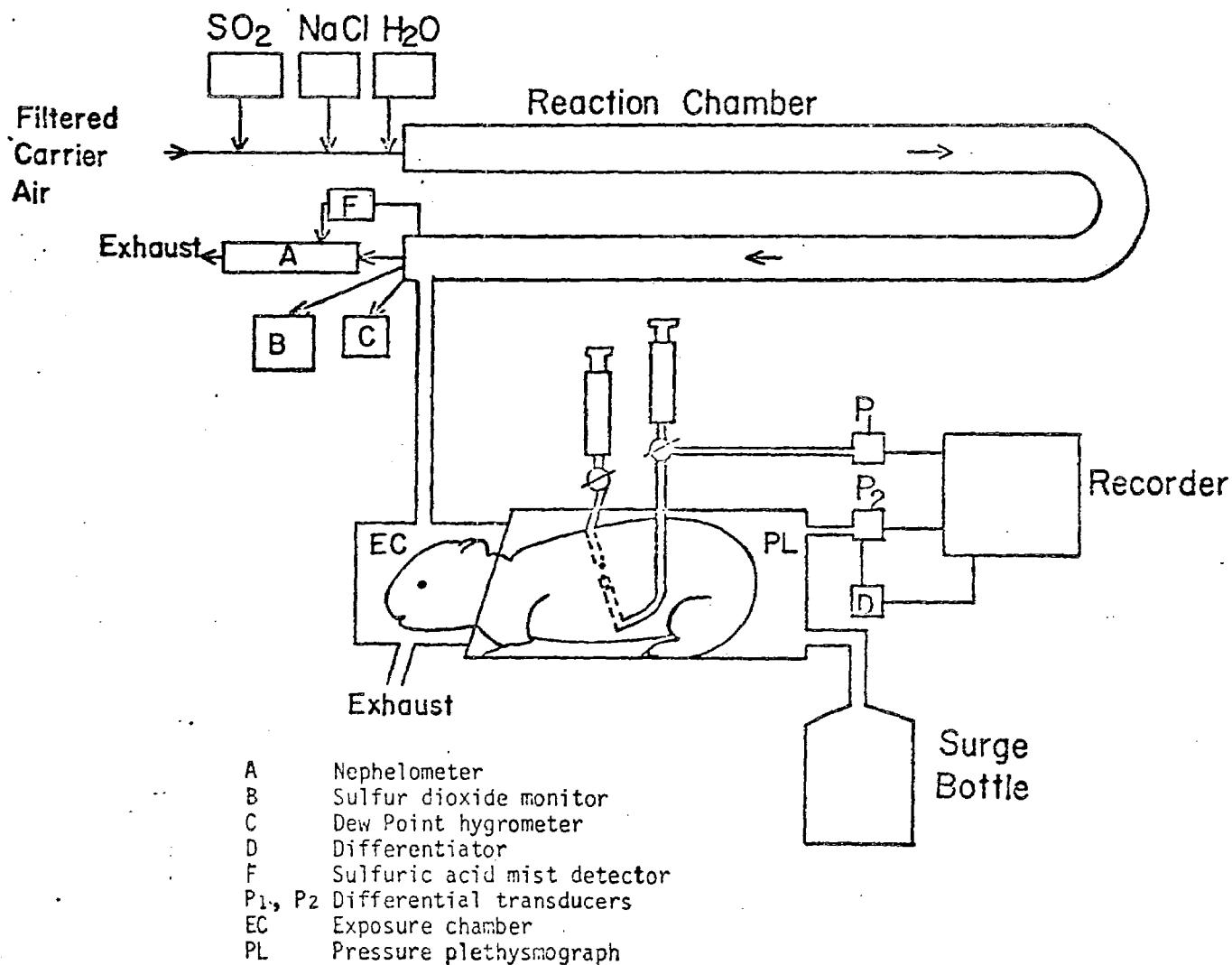
Figure 7. Reprinted from McJilton et al, in press.

Figure 8. Reprinted from McJilton et al, in press.

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Amdur, M.O.: The effect of aerosols on the response to irritant gases. Inhaled Particles and Vapours, Vol. 1. Ed. Davies, C.N., Pergamon Press, New York, 1961, pages 281-292.

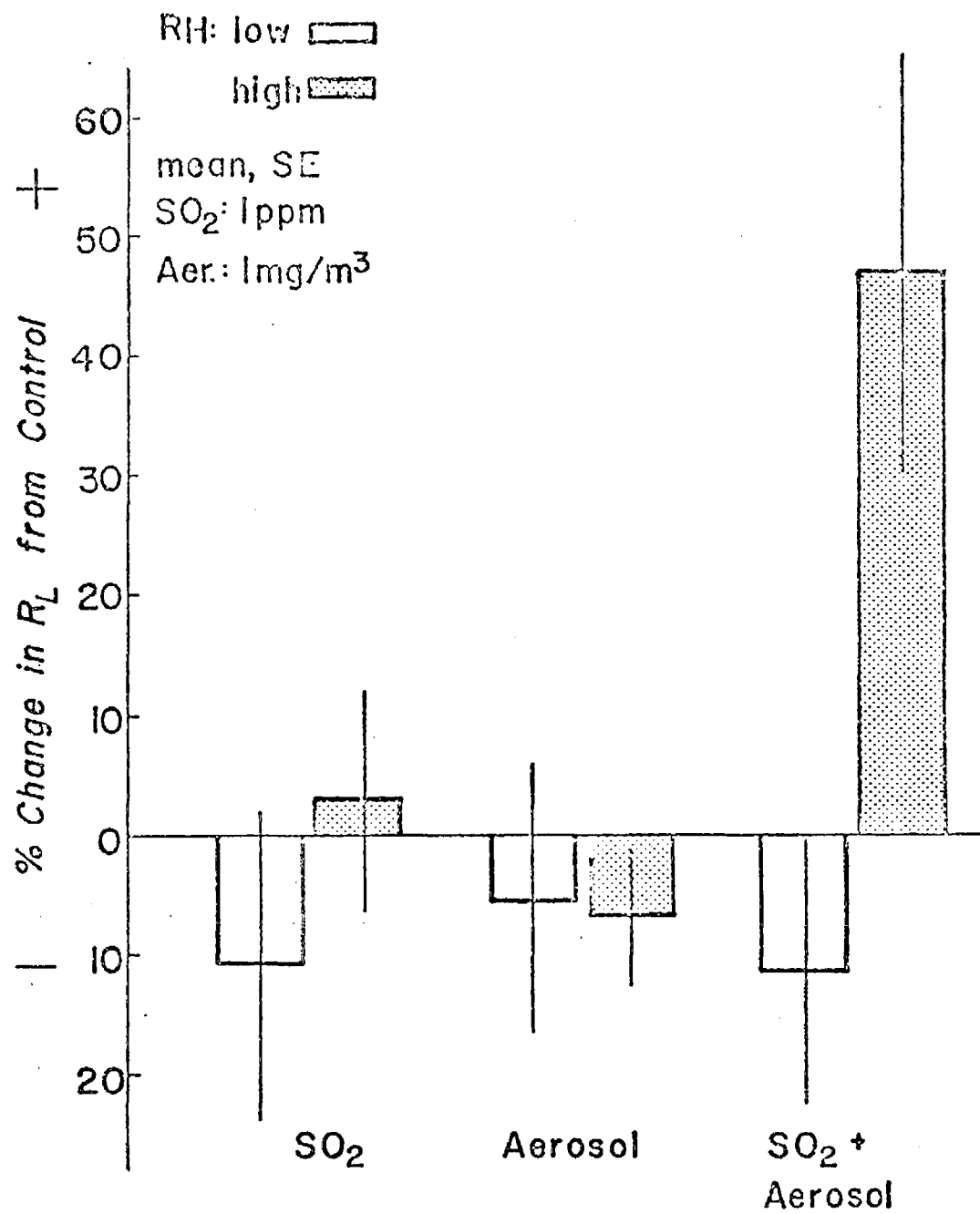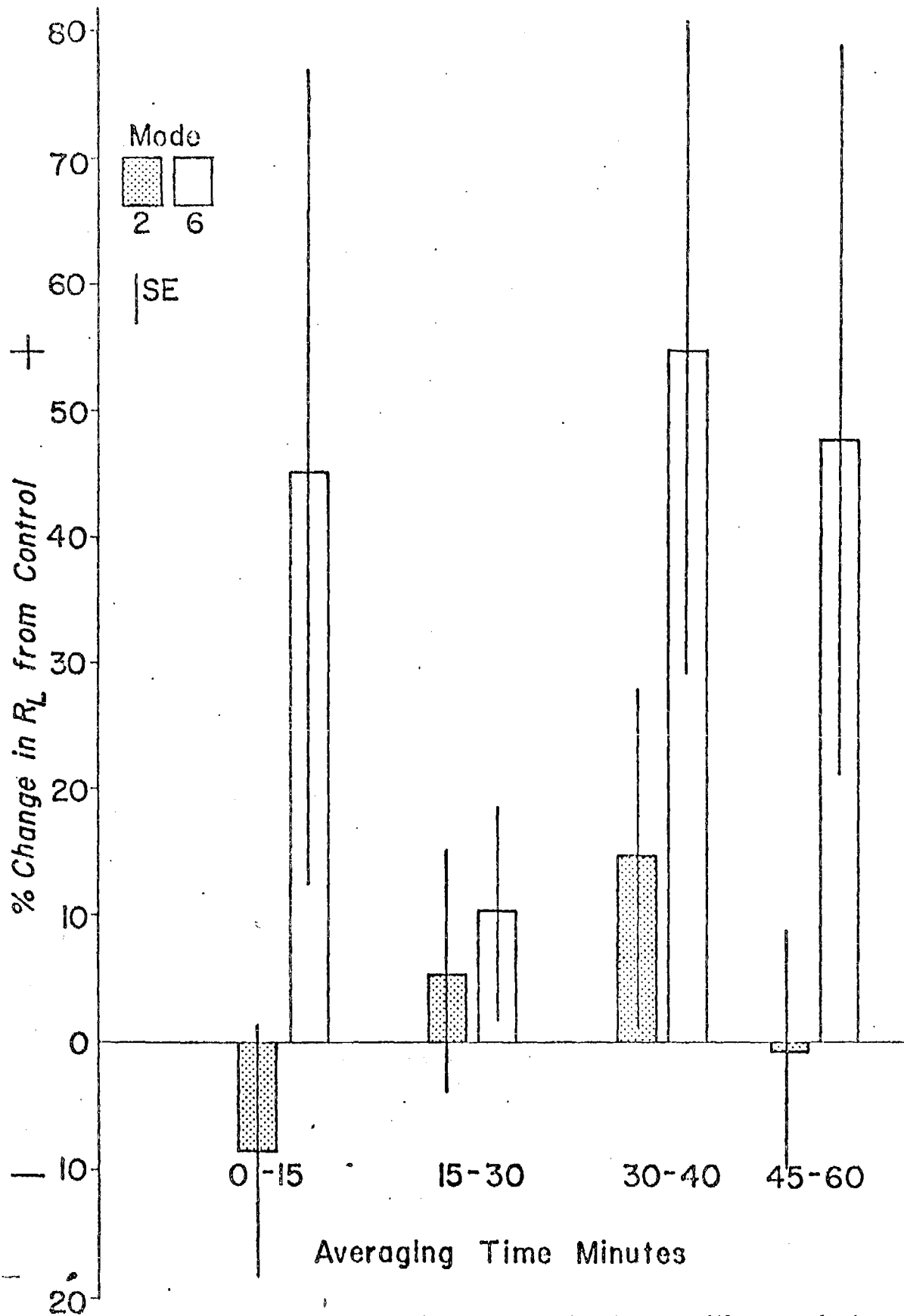Amdur, M.O., and Underhill, D.: The effect of various aerosols on the response of guinea pigs to sulfur dioxide. Arch. Environ. Health, $\underline{16}$:460, 1968.

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CHAPTER III

EFFECTS OF SULFUR OXIDES IN ANIMALS AND MAN

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CHAPTER III

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Effects of Sulfur Oxides in Animals and Man

A. SO_2: acute studies

    1. Background on relevant concentrations of SO_2

        Background examples of ambient concentrations of sulfur dioxide in community air are reviewed briefly here as a guide to concentrations relevant to research. These data are more extensively summarized elsewhere in this report. The National Annual Primary Ambient Air Quality Standard is 0.08 mg/m^3 (0.03 ppm), an annual mean value. The Standard as a 24 hour mean is 0.1365 mg/m^3 (0.13 ppm). Data for Chicago in 1964 (22, Table 3-2) show maximum SO_2 concentrations at 1 hour and 8 hour averaging times of 1.12 and 1.02 ppm (3.20 and 2.92 mg/m^3) and an annual average of 0.18 ppm (0.51 mg/m^3). A sample calculation of the probable 1 hour average concentrations for SO_2 projected from an annual average is given in Reference 22, p. 40, from Larsen. Assuming an annual average 1 hour value based on a series of 1 hour average data of, for example, 0.044 ppm (0.126 mg/m^3), it was estimated from the mean and standard deviation of the 1 hour data that a concentration of 0.27 ppm (0.77 mg/m^3) would be exceeded for 1 hour every day and a maximum concentration of 1.36 ppm (3.89 mg/m^3) for 1 hour would be expected to occur once per year. Human and animal data relevant to air pollution should show effects at concentrations below the maximum ambient concentration for 1 hour per year, i.e., about 1 to 1.5 ppm (2.86 to 4.29 mg/m^3).

    2. Pulmonary function data

        Acute effects of sulfur dioxide at these concentrations have been demonstrated in several species, including experimental human subjects, by means which test increase in flow resistance of the airways. At most concentrations in the mouth- or nose-breathing animal, this response

results from bronchoconstriction, is initiated by sensory receptors in the nose and pharynx as well as in trachea and bronchi and is reversible (1, 2). In studies in dogs, Balchum and co-workers (3) and Frank and Speizer (4) demonstrated increases in flow resistance of the lungs when only an isolated segment of the trachea was exposed to sulfur dioxide at high concentrations while the lungs were ventilated with air. When the lung itself was exposed to sulfur dioxide through a tracheal cannula, bronchoconstriction was more marked than when upper airways (nose, pharynx, larynx, upper trachea) were exposed, suggesting that receptors in the lung are more sensitive.

Guinea pig data were summarized by Amdur (6) for exposures to less than 1 ppm sulfur dioxide. In observations from 71 animals at sulfur dioxide concentrations ranging from 0.03 to 0.65 ppm (mean 0.26 ppm) (0.09 to 1.86 mg/m^3, mean 0.74 mg/m^3) the mean response of the group to the 1 hour exposure was an increase in airway resistance at 12.8% above control values. The increase was statistically significant from control data in the same animals, but the increase in resistance produced by 0.36 ppm (0.74 mg/m^3) sulfur dioxide was less than the variation in resistance among the individual animals prior to exposure. Unless guinea pigs were used in controlled experiments with a large number of animals, therefore, the effects of SO_2 might not be found at 1 ppm.

Conclusion #1: The sensitivity of airway resistance as a measure of animal and human responses to SO_2 appears to reach a limit at 1 ppm in exposures of 1 hour to several hours.

Resistance to airflow due to increased bronchial smooth muscle tone may be initiated by a bronchial reflex after most, if not all, the SO_2 has been absorbed by the nose and nasopharynx according to Andersen, et al (5) as well as Speizer and Frank (7), Frank, et al (8) and Amdur (6).

The finding by Andersen, et al, that the amount of SO_2 passing to or beyond the larynx in nose-breathing human subjects was below 0.25 ppm (0.72 mg/m^3) led that group to interpret their data as supporting a nasobronchial reflex to account for the increased airway resistance. Andersen, et al (5) ask whether this reflex may be more important to bronchoconstriction than direct effects of sulfur dioxide on bronchial surfaces.

Conclusion #2: The importance of reflex bronchoconstriction is that possibly incapacitating bronchoconstriction could occur in sensitive subjects even if the irritant does not pass beyond the larynx. Pathogenesis of chronic lung disease may not occur although measureable effects can be noted in the mechanics of ventilation.

3. Nasal airway resistance

The nasopharyngeal and oropharyngeal airways were narrowed by exposure to 1, 5 or 25 ppm SO_2 as measured by reduced cross-sectional area in 15 healthy, male adult human subjects (5). Impairment of mucociliary transport and possibly increased susceptibility to infection (5) also occurred (see below). Cross-sectional areas were reduced more after 4 to 6 hours of exposure than after 1 to 3 hours. There was a greater effect with rising concentration but not a linear dose response. Nasal airway resistance increased more sharply between 1 and 5 ppm than between 5 and 25 ppm. The effective airway diameter in the nose was reduced to about 80% of normal. This was associated with slight discomfort in some but not in all subjects. There was no carryover of effect from day one to day three as the exposure sequence advanced from 1 to 5 to 25 ppm. Variable nasal resistance in man was observed also by Speizer and Frank (9) using 10 min. exposures to 15 or 28 ppm.

Conclusion #3:   It seems reasonable to assume that nasal absorption of SO_2 produces mucosal edema or vascular engorgement in the nasal mucosa (5, 10).

4.   Absorption and removal of sulfur dioxide by the upper respiratory tract

Dalhamn, et al (11) in rabbits, Frank and Speizer (5) in studies of dogs, and Speizer and Frank (8) in human subjects examined the extraction of SO_2 in upper airways at concentrations above 15 ppm.   More than 95% of the sulfur dioxide was removed in the upper respiratory tract, hence 5% or less reached the lung.   Strandberg (13) reported that the upper respiratory tract extracted proportionately less sulfur dioxide at low than at high concentrations.   At 0.1 ppm (Table 1), only 1 to 10% of the sulfur dioxide was absorbed in rabbits, hence the remainder could reach airways below the larynx.   In man, the proportion of sulfur dioxide which failed to pass beyond the moist surfaces of the nose and naso-pharynx and the oropharynx was recorded directly by Speizer and Frank (8). Only 1 to 2% of 16 ppm (5.6 mg/m^3) of SO_2 presented to the nose was de-tected below the larynx.   Findings on absorption of SO_2 depend on concentration and mode of analysis.   The problems noted by Strandberg at low concentrations may have been due to lack of sensitivity of monitor-ing methods or to real change in absorption at low concentrations.

The notion that a progressively smaller proportion of airborne SO_2 is extracted by the airways anterior to the larynx is not supported by Andersen, et al (6) who could not detect 0.25 ppm or more sampling air from the posterior pharynx during exposure of humans to 1, 5 or 25 ppm of SO_2 for up to 6 hours.   Instead of supporting the idea of a declining absorbed fraction as the concentration is reduced, the latter authors

propose that the human nasopharyngeal airways act as a sink with capacity of 25 ppm SO_2 for 6 hours. This is equivalent to a load of 65 mg SO_2/m^3 of air or 30 mg of SO_2 per hour.

Animals have been observed to require very high doses of SO_2 for significant damage and have been regarded as less sensitive to SO_2 than man. The basis for this alleged resistance of animals, particularly rabbits, mice and rats, has been the comment that the airways above the larynx of animals have a higher absorptive capacity than these air passages in man. However, absorption of SO_2 by airways above the larynx appears to be as efficient in man as in lower animals (4, 5, 7, 11, 12). Animal responses to SO_2 seem to be appropriate guides to human responses at comparable concentrations during short-term exposure in resting, nose-breathing subjects that actually receive at the nose the concentration measured in air.*

Frank, et al (5) noted that ventilatory flow rate was important to the proportion extracted in the airways anterior to the larynx (Fig.1). Exercise would increase the ventilatory flow rate and would be expected to decrease the proportion of airborne SO_2 that was removed by airways anterior to the larynx. The effective dose to the airways posterior to the larynx would be greater in physically-active persons and during mouth-breathing than in resting subjects breathing through the nose (8). Frank, et al (9) noted that labeled SO_2 could be absorbed into the circulation of the upper airways, circulate to the lung and presumably be desorbed and released into the bronchial air spaces, since significant label was found in air from the deep lung where no SO_2 had been inhaled.

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*The latter proviso means, for example, that rats huddled in a cage with noses buried in one another's fur do not receive the concentration measured in the air of the cage.

This observation suggests that airway epithelia could be exposed "from below" by SO_2.

Conclusion #4: Major portions of inhaled SO_2 are absorbed in the nose, nasopharynx and oropharynx. These structures must represent an important target for SO_2, a point of absorption of SO_2 into blood and a barrier to passage of SO_2 to deeper airways. Extraction by upper airways seems to be about equally effective in animals and man. The concept of Andersen, et al, that there is a capacity for mucosal absorption of SO_2 independent of concentration needs confirmation.

5. Mucociliary transport

Andersen, et al (5) demonstrated that 1 ppm SO_2 or more reduced the rates of posterior movement of a lightweight ^{99}Tc-labeled resin particle placed anteriorly on the superior surface of the inferior turbinate in human subjects exposed for 1 to 3 or 4 to 6 hours. The rate at which this effect occured was variable owing to variation in the subject-to-subject speed of transport in control periods. Some subjects were "fast movers" and some were slow. "Slow movers" experienced stasis of muco-ciliary transport earlier than fast movers.

Tracheobronchial mucociliary transport in rats was stopped after 2 min. exposure to 25 ppm (71.5 mg/m^3) or 12 min. exposure to 12.5 ppm (36.7 mg/m^3) sulfur dioxide (14). The rats inhaled through the nose and a sealed window in the tracheal wall was used for direct microscopic observation. By estimation from Standberg's data (13) for extraction of SO_2 by rabbit airways anterior to the larynx (Table 1), 5% of 25 ppm or 1.25 ppm (3.6 mg/m^3) of the sulfur dioxide might have actually reached the rat trachea. If the data for rabbits and rats can be manipulated in this way, an effective concentration of about 1 ppm for a very brief

period could be said to have stopped ciliary transport in rats. This manipulation is offered speculatively only because it permits an interpretation that coincides with data of Andersen, et al (5) in supporting the view that mucociliary transport in airways above or below the larynx is slowed by effective concentrations of SO_2 at or near 1 ppm.

Mucociliary transport of formalin-killed radioactive bacteria was not impaired during a 3-hour observation time in guinea pigs which had been first exposed to 6.0 or 10.4 ppm SO_2 (17.2 or 29.7 mg/m^3) for 4 weeks at 6 hours/day, 5 days per week (15). Based on Standberg's finding (13) that 20% of 5 ppm of SO_2 passes the larynx (Table 1) the effective concentration reaching the tracheobronchial system can be estimated roughly as 1.2 to 2.1 ppm (3.4 to 5.95 mg/m^3). These data are admittedly from chronic exposure but do not confirm the foregoing conclusion that about 1 ppm SO_2 for some short time suppresses mucociliary transport. However, the data from acute effects were based on direct observation of transport while the data for clearance of labeled bacteria by Rylander, et al (15) required aerosol deposition of bacteria, lung homogenization, filtration of homogenate and autoradiographic counts of labeled bacteria of the filter. Experiments of this kind have many sources of variance and are appropriate for phagocytic killing studies but less appropriate for mucociliary transport.

Incidental observations by Andersen's group (5) include the occurrence of colds in 4 of 15 subjects within a week after sequential exposure to 1, 5 and 25 ppm SO_2 on successive days. Increased frequency of respiratory disease has been recorded during or for the 3 days following episodes of increased SO_2 and particulate pollution (24) or in high vs. low pollution communities (25), hence infection resistance may be reduced during nasal absorption of SO_2, particularly to common cold

viruses which preferentially infect the nasal mucosa.

Conclusion #5: Nasal mucociliary transport is slowed or stopped during exposure to about 1 ppm (2.86 mg/m^3) sulfur dioxide. By comparison with Amdur's data (see Conclusion #1), both transport and airway resistance may be modified at thic concentration of SO_2. Parallel swelling of the nasal mucosa occurs. This level of exposure could modify local resistance to infection.

6. Macrophage killing of inhaled bacteria

Phagocytic properties of alveolar macrophages are responsible for the component of pulmonary clearance that proceeds at a lower rate than mucociliary transport. Macrophage function in clearance requires ingestion of particles and can be directly observed if the test particle is present in macrophages in tissue sections or in macrophages recovered by lavage. Phagocytosis plus intracellular killing of inhaled bacteria must both occur in test systems that depend on quantifying bacteria by lung culture or on resistance to pneumonia after inhalation of microbial pathogens.

Sulfur dioxide alone did not alter bactericidal properties of guinea pig lungs for a nonpathogenic strain of E. coli after exposure to 6.0 to 10.4 ppm (17.2 to 29.7 mg/m^3) 6 hours per day, 5 days weekly for 4 weeks (15). Macrophage function was not studied after brief exposure, and the 4-week exposure may not be appropriately compared with events that follow from short exposure.

Conclusion #6: In the effort to identify more and less sensitive targets of action, the effect of SO_2 exposure on bactericidal properties apparently required more SO_2 than was needed to increase airway resistance or slow mucociliary transport. However, bactericidal properties may be

highly relevant to production of chronic progressive lung disease; hence, this function is important even if by present methods it is not a very sensitive target of SO_2.

B. SO_2: Chronic studies

   1. Purpose and interpretation

   Repeated intermittent exposure or continuous exposure for weeks or months have been directed at evidence of chronic disease produced by SO_2. Such studies are done because levels of SO_2 and other pollutants have been higher in the workplace or community in which chronic lung disease has been prevalent than in areas of lower prevalence of disease. Studies using SO_2 alone have shown that much greater quantities of SO_2 are needed to produce chronic lung disease in animals than have been present in the human exposure settings associated with chronic lung disease.

   This observation has had two interpretations: animals are less sensitive to pathogenesis of SO_2 than man, or co-pathogens in addition to SO_2 are involved in producing human disease. The major importance of chronic animal exposure to SO_2 has been to show that major elements of the pathologic state observed in patients with chronic bronchitis can be produced in animals by SO_2 alone. While any irritant might have the same effect as SO_2, this gas has induced abnormalities upon chronic exposure. Sulfur dioxide may be, therefore, as relevant an irritant for human disease as more potent irritants but it is not a sufficient pathogen as indicated by the high concentrations required to induce pathologic changes. The current questions are whether oxides of sulfur, including SO_2, are pathogens in human chronic lung disease, what form of the oxides is most potent, what associated pathogens are acting as well, and whether the co-pathogen is so intimately associated with oxides of sulfur (as a cationic metal, for example) that "sulfates" constitute a pathogenetic unit.

2. Animal data from chronic exposure to SO_2

Reid (16) described increase in size of mucous glands and number of goblet cells in tracheobronchial epithelia of rats after daily exposures to 300-400 ppm (858-1144 mg/m^3) sulfur dioxide for three months. These lesions are also common in patients with chronic bronchitis in areas polluted by coal dust, coal smoke and SO_2. A graded response over the range 50 to 300 ppm (143-858 mg/m^3) was presumably equivalent to a lower effective (intrabronchial) concentration of 1% (13) or 3 to 4 ppm. This correction seems to apply to most species, because the high dose require- ment indicates that the rat is resistant to chronic exposure or that SO_2 is a necessary but not a sufficient pathogen for this chronic lesion.

Alarie, et al (17) exposed guinea pigs continuously to 0.13, 1.01 and 5.72 ppm (0.37, 2.9 and 16.4 mg/m^3) of sulfur dioxide for 52 weeks and monkeys (18) to 0.14 to 1.28 ppm (0.4 to 3.7 mg/m^3) for 78 weeks. Pulmonary and cardiac function measurements, blood and clinical chemistry studies, gas uptake, and histology of the respiratory tract were parallel to data for control animals before, during and after the exposure. The increase in flow-resistance observed by Amdur during and after brief exposure (1 hour) did not persist during chronic exposure. Therefore, brief exposures may not predict the concentration needed to produce the same mechanical changes upon chronic exposures. Acute studies in monkeys were not available to compare with the response to chronic exposure.

Dogs exposed for 21 hrs/day for 225 days to 5 ppm sulfur dioxide by Lewis, et al (20) developed flow resistance of 3.4 $\pm$ 0.9 cm H_2O/l/sec as compared to 1.7 $\pm$ 0.9 cm H_2O/l/sec in controls. The dog may differ from the guinea pigs referred to above (17) because the dog developed increased pulmonary resistance at 5 ppm SO_2 after chronic exposure. The data for dogs need confirmation not only by physiologic methods but by additional

techniques (clearance, morphology) to determine whether the data of Lewis, et al (20) are evidence for a pathogenetic effect of SO_2 in this species when no effect at 5 ppm was noted in guinea pigs.

In chronic exposures of guinea pigs and monkeys, the concentrations which were used without production of chronic mechanical abnormalities of breathing nor anatomic lesions were in a range approaching the levels of human industrial and community exposures. Since extraction of sulfur dioxide is highly efficient in man (19), dog (4) and rabbit (13), and presumably in guinea pigs and monkeys, air concentrations may be appropriately compared for these animals. There is no obvious reason why monkeys and guinea pigs failed to develop physiologic or anatomic lesions while dogs seem to have developed some degree of ventilatory abnormality at concentrations approaching human exposure experience.

Conclusion #7: Animal species may differ in susceptibility to SO_2, of course, but when two species (monkeys and guinea pigs) fail to respond at relevant concentrations and a third species (rat) experiences lesions only at concentrations that are two or more orders of magnitude too high, it is reasonable to re-examine the dog as a sensitive subject and to retain a serious doubt that SO_2 alone is a sufficient pathogen for chronic bronchial disease.

C. Sulfuric and sulfurous acids

1. Background on relevant concentrations

The present federal standard for occupational exposure to sulfuric acid is an 8-hour time-weighted average of 1 mg/m^3 (29). The maximum concentrations measured in London during the episode of December 2-5, 1957, were SO_2, 1.47 ppm (4.2 mg/cu^3), and H_2SO_4, 0.222 mg/cu^3, giving a weight ratio of 0.053 H_2SO_4/SO_2 (22, p. 42ff), led to the conclusion that over 0.01 mg/cu^3 of sulfate was irritant. If all of the sulfate were H_2SO_4, this would yield an unexpectedly high irritant capability for H_2SO_4 in

view of human response data underlying the occupational standard. However, the relevant range of H_2SO_4 concentrations should probably extend from 0.01 to 1.0 mg/cu^3 for experimental and epidemiologic purposes.

2. Sulfuric acid formed on catalytic salts

Data reviewed in this section suggest that the biologic effect of SO_2 depends on conversion of some fraction of the SO_2 to acid. The acid formed may be H_2SO_4 if the SO_2 is absorbed or dissolved by a particle that is catalytic, while aerosols containing water and inert solids dissolve SO_2- producing sulfurous acid as demonstrated by McJilton, et al (26).

Salt particles of Mn^{2+}, Fe^{2+} and V^5 did not produce airway resistance after 10 min. exposures of guinea pigs at 1 mg/m^3 (34). Salt particles were generated from water solutions before addition to the exposure atmosphere. Particles had Mass Median Diameters (MMD) of less than 0.1 μm in the exposure air, and were probably delivered to animals in air of moderate (say, 50%) relative humidity. When combined with SO_2, particles containing these metallic cations (which are known to catalyze SO_2 to SO_3) yielded a gas-aerosol mixture that produced three times greater effects than produced by SO_2 alone (Fig.2). That is, respiratory flow resistance in guinea pigs exposed to SO_2 alone was 10% higher than in control guinea pigs but resistance was 30% higher than in controls in the presence of SO_2 and the salts. This ratio applied over a range of SO_2 concentrations. The presumed effect of the particle-SO_2 combination was catalysis of SO_2 to SO_3 followed by hydration to H_2SO_4 upon entering the moist air of the respiratory tract or upon deposition on moist respiratory mucosa.

Amdur (6) also presented data for increase in respiratory flow resistance upon exposure of guinea pigs to SO_2 or sulfurous acid (Fig.3). The data for pulmonary flow resistance in guinea pigs upon 1 hr exposure to SO_2 at increasing concentrations are the same as for Fig.2. H_2SO_4 droplets of small size (0.3μm) were more active than large droplets (2.6μm) and the

curves for small H_2SO_4 droplets demonstrate about 3-5 times greater effect than SO_2 when both forms of sulfur are compared at the same value of mg/S.

The marked effect on guinea pigs of the catalytic salt-SO_2 combinations shown in Fig. 2 were combined with evidence of the effect of H_2SO_4 on airway resistance (Fig. 3). From these data Amdur (6) developed the concept of catalysis of SO_2 to SO_3 (or H_2SO_4) to the point of estimating the amount of H_2SO_4 formed from SO_2 in the presence of catalytic metal salts (Fig. 4). By use of Fig. 4 it can be calculated that in a salt-SO_2 mixture containing 1 mg/m^3 of catalytic salt and SO_2 at, say, 0.4 ppm (1.14 mg/m^3), 0.06 mg H_2SO_4/m^3 would be formed. This represents oxidation of about 5% of sulfur in SO_2 to H_2SO_4. Amdur concludes that the greater biologic effect of the mixture of SO_2 and a catalytic salt was caused by forming acid. The proportion of SO_2 converted to H_2SO_4 was greater at low concentrations of SO_2 (Fig. 4). Catalysis of SO_2 to SO_3 on a dry aerosol of metal salts is a slow process, i.e., several percent per hour, hence the amount of H_2SO_4 presumed to be formed in the metal salt-SO_2 systems (34) might be overestimated (59). This possibility would lead to an alternative interpretation suggested by Charlson (60) in which the particles used by Amdur and Underhill (34) may be identified as "wet" rather than "dry" owing to hygroscopicity of $MnCl_2$, $FeSO_4$ and Na_3VO_4 (see Section 4).

When a comparable degree of increase in pulmonary flow resistance, say 40% over controls, is used to estimate the equivalent biologic potency of sulfuric acid and sulfur dioxide from Fig. 3, the air concentrations appear to be: 0.2 mg H_2SO_4/m^3=100 mg SO_2/m^3. From this ratio, H_2SO_4 of particle size 0.3 um is 500 times as irritant as SO_2 on a mg-per-mg comparison of the molecules. This estimate is discussed in greater detail in section 4.

3. Humidity as a factor in irritant potential of SO_2 and H_2SO_4

   a. Animal studies

   Humidity has been studied insufficiently as a contributor
to oxidation or to solution of SO_2 in yielding products that are
health hazards. Oxidation of SO_2 dissolved in water to form sul-
furous acid proceeds rapidly to formation of H_2SO_4 in droplets
carrying metallic ions (particularly manganese, 28) and very slowly
on dry particles of metal salts (59). In addition, solution of
SO_2 in water droplets to produce H_2SO_3 accounts for the significant
increase in pulmonary flow resistance in guinea pigs exposed to
1 mg $NaCl/m^3$ (MMD=0.1 μm) plus 1 ppm SO_2 at a relative humidity
(RH) of 80% as reported by McJilton, et al. (26). The guinea
pigs were not affected by the same regimen at low RH (40%) nor
by NaCl or SO_2 alone at high RH. One irritant that could account
for the effect of H_2SO_3 is low pH; levels were estimated to be
3.2 in the moist aerosol. Sulfite ion was also present in this
aerosol. Sodium sulfite has not been found to be a respiratory
irritant in mice exposed to aerosols of unspecified particle size
at unspecified humidity at a concentration of 306 ppm, expressed
as SO_2 by the West-Gaeke method. Sodium metabisulfite (98 ppm
expressed as SO_2 by the West-Gaeke method) was about as irritant
as SO_2 (89 ppm) in a 10 min exposure of mice (50). This experiment
may not eliminate sulfite as an irritant in view of the omission
of description of the aerosol particle size and humidity of the
exposure system but it does argue against irritancy of these ions.

   About the same degree of flow resistance was produced in guinea
pigs by the humid $NaCl-SO_2$ system of McJilton, et al. (26) and
by the metal salt-SO_2 system of Amdur and Underhill (34). While
the values for percent increase in flow resistance should probably

not be compared for two laboratories, there may be some merit

in comparing the studies since both used 1 mg/m^3 of a salt at

0.1 μm-MMD (or less), both demonstrated non-toxicity of the salt alone,

and both used SO_2 at 1 ppm. Amdur (6) assumes that the irritancy

of metal salts plus SO_2 was due to H_2SO_4 formed by catalysis. The

amount of H_2SO_4 proposed by Amdur (6) as having been formed on the

metal salt-SO_2 system can be estimated from Fig. 3 to be 0.2 to 0.4

mgm/m^3 on the assumption that the postulated degree of catalysis

did occur.

This degree of catalysis is in question (59) owing to slowness

of catalysis hence the formation of H_2SO_4 will be rejected temporarily

because of an alternative which bears on the influence of humidity

on aerosol toxicity rather than on catalysis. The alternative

interpretation (60) for irritancy of the metal salt-SO_2 aerosol of

Amdur and Underhill (34) is that water droplets were already present

when the highly hygroscopic salts $MnCl_2$, $FeSO_2$ and Na_3VO_4 were aerosolized.

For this reason it would not be important that humidity was not

specified or that water vapor was not added as a part of the experimental

design. Solution of SO_2 in these presumed droplets would have been

more rapid than catalysis and could have produced the same irritant

effect as obtained by McJilton, et al. (26) and for the same reason

(60); presumably the formation of H_2SO_3.

The importance of this interpretation is that humidity need

not be very high in order to permit salt particles to serve as

droplet nuclei; humidity need only be high enough for any particular

salt to absorb water. Water absorbed by hygroscopic salts at low

RH may dissolve SO_2 as well as water absorbed by the NaCl particles

of McJilton, et al. (26) at high RH.

The possible error in the interpretation that Amdur and Underhill's (34) experiment showed irritant effects due to formation of H_2SO_3 is that catalysis in moist particles would probably have proceeded more rapidly to formation of H_2SO_4 than if the metallic salt particles had been truly "dry". Thus some amount of H_2SO_3 (with acid and sulfite ion activity), of H_2SO_4 (with acid activity), and of metallic cation (activity not evident in control animals) may all have been present, rendering the question of the major irritant moot.

The pH estimated in the sulfurous acid droplets formed in the study by McJilton, et al. (26) was 3.2. The estimated pH likely to result when Amdur's (6) 0.3 μm droplets of H_2SO_4 pass from air of unspecified (say, 50%) humidity to the very humid air of the airways is about 0.3-1.0 (59). These data suggest that if acidity is the irritant, Amdur's sulfuric acid mist should have been more irritant than the $NaCl-SO_2$-80% RH system. A possible complication in this comparison is that Amdur's mist droplets were 0.3 μm in air and would quickly have become larger in the airway while the particles of McJilton, et al., were 0.1 μm in air at 80% RH and may have grown rather little after inhalation.

Conclusion #8: At this stage in the progress of studies of irritancy of sulfuric and sulfurous acids and the related ions more work is needed to sort out particle size, relative humidity and molecular species. This is a fruitful area of research and may settle an important question regarding the source of irritant properties ascribed to sulfates.

b. Human Studies

Enhanced irritant potency of H_2SO_4 in the presence of high RH (91%) as compared to less high RH (62%) was attempted in human

trials using high concentrations of the acid at droplet sizes of 1.0 to 1.5 μm (31). Reporting of results was vague and the concentrations were higher than are regarded as relevant in this report. It is of interest, however, that 20.8 mg/m^3 (4 N acid) for 30 minutes at high RH and 1.5 μm MMD produced severe coughing and increased airway resistance by 43 to 150% above normal while 39 mg/m^3 (10 N acid) at RH of 62% and 1.0 μm MMD was tolerated well for 60 minutes. The latter exposure did increase resistance by 35 to 100% above normal despite lack of marked symptoms. The effect of high humidity may have been to promote deposition of larger particles in the upper airways but particle sizes were already different in the direction favoring deposition of the particles used with high humidity. The experiment is not fully conclusive. Better controlled studies at a wider range of RH and particle sizes are needed to determine the relevance of humidity to the irritant potency of H_2SO_4.

4. Direct short-term exposures to H_2SO_4

   a. Animal Studies

   The most satisfactory data in animals are assembled by Amdur (6) for guinea pigs (see Fig. 3). These data demonstrate that particles of 0.3 μm are more irritant than particles of 2.4 μm and that both classes of particles induce greater resistance than SO_2. In Amdur's original graph (Fig. 6 in Ref. 6) the horizontal axis was labeled "mgS/m^3". The adaptation of her Figure 6 as Figure 3 in this report has added the related values for H_2SO_4 and SO_2 in mg/m^3 or ppm for ease of comparison of concentrations.

   Amdur (6) interprets the relative toxicity of SO_2 and H_2SO_4 according to the percent increase in pulmonary flow resistance (vertical axis) per unit of sulfur in mgS/m^3 (horizontal axis).

By this reasoning, 1 mgS/m^3 would correspond with 2 mg SO$_2$/m^3 (0.7 ppm) associated with about 12% increase in resistance, and with 3.06 mg H$_2$SO$_4$/m^3, which is associated with about 90% increase in resistance. The relative toxicity of H$_2$SO$_4$ would be about 9 times the toxicity of SO$_2$, based on percentage increase in flow resistance at 1 mg/m^3 of sulfur.

An alternative method for determining relative toxicity is to select some effect level expressed as percent increase in resistance (Fig. 3) and determine the concentration of H$_2$SO$_4$ or SO$_2$ needed to produce the effect. The concentrations of SO$_2$ and H$_2$SO$_4$ in mg S/m^3 that produce a 50% increase in resistance are about 60 and 0.15. The relationship SO$_2$/H$_2$SO$_4$ of 60/0.15 = 400-fold greater toxicity for H$_2$SO$_4$, an appreciable difference from the 9-fold greater toxicity estimated by Amdur's (6) interpretation of data contained in Fig. 3. These alternative interpretations could lead to very different recommendations for action in human exposure settings.

Reviews are available (22, 29, 32) for earlier experiments in which most particle sizes were near or above 1 μm, relative humidities were not specified or were 50%, and concentrations were at or above 2 mg/m^3. Short-term exposures of guinea pigs by Amdur in 1958 (44) ranged from 2 to 40 mg/m^3 with particles of 0.8, 2.5 and 7.0 μm-MMD. The 7.0 μm-MMD particles produced increase in flow resistance at 30 mg/m^3. Few particles would be expected to pass beyond the nose so that restriction in flow was probably due to changes above the larynx. From Alarie's review (12) it would be expected that reflex bronchoconstriction and reduce respiratory rates should have occurred.

The 0.8 μm-MMD droplets produced an increase in pulmonary flow resistance accompanied by a lesser decrease in compliance, and increase in elastic and resistive work, hence increase in the total work

of breathing at the lowest concentration tested, 1.9 mg/m^3. Onset

of the effect was prompt as upon exposure to SO_2. Changes in function

were interpreted as consistent with bronchoconstriction or mucosal

swelling.

The 2.5 µm-MMD particles also increased flow resistance at 2.3

to 44 mg/m^3 but the greatest increase in effect was at the higher

concentrations. These data were interpreted that deep lung penetration

was more rapid for the smaller particles while larger particles

reached deeper airways only after large doses had been inhaled so

as to produce extensive central airway obstruction. These experi-

ments were inconclusive as to factors responsible for the physio-

logic and pathologic changes noted and should be repeated.

Slow recovery from the action of sulfuric acid mist was ob-

served. Pulmonary flow resistance persisted above pre-exposure

levels 2 hours after the 1 hour exposure had ended. Analogy was

drawn by Lewis, et al., (32) between the persistent action of sul-

furic acid mist and of aerosols of inert particles (NaCl) bearing

absorbed irritant vapor (formaldehyde) (45).

A major problem in existing data for physiologic and pathologic

effects of H_2SO_4 is inadequate control of particle size in the range

most effective for retention (0.01-0.1 µm), as well as in the size

range that is recorded for sulfates in the atmosphere (0.2-0.4 µm).

Deposition of particles is discussed in section D, below, and H_2SO_4

must reach airways as a particle, whether as a mist droplet of pure

acid or as part of the complement of chemicals absorbed on a dry

particle or a droplet nucleus. One deficit in evaluation of irritant

properties of H_2SO_4 is partly, therefore, in the description of the

physical characteristics of particles bearing H_2SO_4 as they approach

inhalation. The responses to particles described by Amdur (44) suggest that very large particles (7 μm) act like SO_2 and a deep lung irritant (e.g., O_3). These are reasonable interpretations but they are confused by Amdur's less explainable results with 2.5 μm particles. Re-evaluation of H_2SO_4 mists is thus necessary.

The additional deficit lies in ascribing irritancy to H^+ vs. other ions contained in a sulfuric acid mist or a particle carrying H_2SO_4.

Conclusion #9: Sufficient animal studies have not been done to establish the dose-response effect of H_2SO_4 in the 0.1-0.3 μm particle size range. Available evidence in guinea pigs supports the view that H_2SO_4 formed on small (less than 0.1 μm) possible "dry" particles (34), H_2SO_4 as small (0.3 μm) droplets (6) or H_2SO_3 in small (0.1 μm) droplets formed on inert salt particles (26) has an irritant effect. Additional studies are needed to establish the dose-response to H_2SO_4, H_2SO_3, and other inorganic acids with particle sizes in the range of 1.0 μm to 0.01 in order to determine whether acidity or anions are the toxic agent. The physiologic response to deposition of acid droplets in the upper and lower airways needs to be examined over this particle size range.

B. Human Studies

Studies that have been equally attentive to particle size, humidity and comprehensive measurement of physiologic function are conspicuously lacking in recorded experimental exposures of man.

Bushtueva (48) exposed 10 volunteers to H_2SO_4 over a concentration range of 0.7 to 6 mg/m^3. In the range of 0.7 to 1.1 mg/m^3 volunteers noted throat irritation. At 1.1 to 2.14 mg/m^3 all sub-

jects noticed considerable esophogeal irritation and 4 experienced eye irritation. At from 2.4 to 6.0 mg/m^3 there was pronounced cough, and irritation of eyes, mouth and nose. Respiratory rate and amplitude did not change at concentrations below 1.0 mg/m^3. Particle sizes were not recorded.

Amdur, et al. (46) noted increased respiratory rate and tidal volume in 15 volunteers within 3 minutes of starting exposure to 0.35 to 0.5 mg H_2SO_4/m^3. These changes persisted during a 15 minute exposure and returned to normal within 15 minutes after the end of the exposure. Morando (47), Bushtueva (48) and Amdur et al., (46) identified 0.5 to 0.7 mg/m^3 as the threshold for subjective detection. If Amdur, et al. (46) are correct some pulmonary physiologic response occurred below the sensory threshold but data are limited for human responses to H_2SO_4 and a large gap in concentration exists between the above reports and the report of Sim and Pattle (31).

The lowest concentrations leading to detectable effects in man were reported by Toyama and Nakamura (49) who reported 17.9% increase in airway resistance at a concentration of 0.01 to 0.1 mg H_2SO_4/m^3 at a particle size of 1.8 µm. These results were called in question (29, p. 48) because of possible interaction between H_2SO_4 and H_2O_2 in the exposure air. In addition, Lewis, et al., (32, p. 21) regard the concentrations as questionable, "since 0.01 to 0.1 mg/m^3 are concentrations below the reported thresholds for any physiological response." This conclusion is possibly challenged by Amdur's data (Fig. 3) for effects of about 0.1 mg H_2SO_4/m^3 in guinea pigs.

Conclusion #10.

a. Short-term human exposures to aerosols of H_2SO_4 at

0.35-1.0 mg/m^3 have been claimed to show responses but particle sizes have not been specified. Human exposures to sulfuric, sulfurous, and other inorganic acids should be done with particles of 0.1 μm over a range of concentrations. Aerosol pH should be measured. The hypothesis under test would be whether acidity of inorganic acids is toxic.

b) Apparently normal persons have been tested in the past. Sensitive persons should be tested, with all appropriate precautions, to determine whether H$^+$ is itself an irritant capable of disabling sensitive persons.

c) Atmospheric analyses must obtain realistic values for the pH of environmental aerosols. Experimental exposures of animals and man to acid aerosols should be done even though methodology is still being developed to measure the pH of airborne particles.

5. Long-term exposure to H_2SO_4

a. Monkeys: H_2SO_4 only

Groups of 8 cynomolgus monkeys were exposed by Alarie, et al. (54) to sulfuric acid mist at a high concentration (2.43 mg/m^3) of large particles (3.6 μm-MMD); a high concentration (4.79 mg/m^3) of small particles (0.73 μm); and at a moderate concentration (0.38 to 0.48 mg/m^3) of large (2.15 μm) or small (0.54 μm) particles. The humidity was 50%, hence particle size was probably stable while in the chamber air. Exposures were continuous for 78 weeks and during this period measurements of pulmonary function were performed. The experiment was completed by pathologic studies.

High concentrations at particle sizes of 3.6 and 0.73 μm were both effective in increasing respiratory rate and increasing retention of N_2 upon washout, indicating uneven distribution of ventilation.

This abnormality became progressive after 17 weeks in the large particle group but only after 49 weeks in the small partlicle group. Abnormal distribution of ventilation appeared late in the course of exposure to small particles even though the monkeys in this group received a total higher concentration of sulfuric acid. Pathologic changes, including thickening of respiratory bronchioles, were less marked in the monkeys exposed to a high concentration (2.43 mg/m^3) of large particles (3.6 µm) than to a high concentration (4.79 mg/m^3) of small particles (0.73 um). There were, therefore, discrepancies in the findings in the high concentration-small particle group, namely: late onset of physiobgic evidence of small airway disease (N$_2$ washout) but more extensive bronchiolar pathology at autopsy. These discrepancies would fit the interpretation that pathologic changes precede physiologic evidence of damage, and as a plausible corollary, that extensive pathologic abnormality of smaller airways induced by small particles may lead to detectable physiologic disorders only at a late stage of pathogenesis. The pathogenicity of sulfuric acid mist is shown to be significant but only at unrealistically high concentrations.

In monkeys exposed to lower concentrations (0.38-0.48 mg/m^3) at 2.15 and 0.54 µm-MMD, the larger particle size produced increased respiratory rates while the smaller particles did not. The smaller particles produced a transient abnormality of distribution of ventilation but the larger particles did not. Each of these alterations represent part of the expected effect of particles which irritate lower airways but the only pathologic abnormalities were bronchiolar wall thickening and epithelial hyperplasia in some but not all animals exposed to the larger particles. In this instance,

transient ventilatory abnormalities suggesting small airway effects were not the result of permanent pathologic changes in the low dose-small particle group.

Conclusion for chronic exposure to H_2SO_4 in monkeys

Each of these experiments fulfilled some of the generalizations made by Alarie (Chapter 1) from studies of physiologic response to pulmonary irritants upon acute exposure. For example, H_2SO_4 mist did not increase airway resistance but did induce increased respiratory rates, presumably either as a reflex response to stimulation of deep lung receptors or as a pathologic change in oxygen transport. The latter process seems unlikely since PaO_2 was normal. The delayed emergence of ventilatory abnormalities is consistent with progressive damage to small airways and pathologic findings confirm this in part. These data affirm the pathogenicity of sulfuric acid mist at concentrations of 0.48 mg/m^3 or more.

In view of the competence of the research team, this experiment might well be used as a warning against the massive investment needed to repeat such a study. Instead, these data would argue for inclusion of co-pathogens if sulfuric acid is to be studied at concentrations nearer ambient (that is, 0.1 mg/m^3 and below).

b. Monkeys: H_2SO_4 plus fly-ash

An additional study by the above group (55) did provide a particulate co-pathogen. Fly ash, 0.53 mg/m^3 (3.0 μm MMD) plus H_2SO_4, 0.99 mg/m^3 (1 μm MMD) apparently produced the same pathologic lesions observed with H_2SO_4 in the high concentrations (2.43 and 4.79 mg/m^3) of the sulfuric acid mist study. The original data were not available to this reviewer

but a co-pathogen did reduce somewhat the concentration of sulfuric acid necessary to produce pathologic changes in small and large airways.

c. Guinea Pigs

In addition to the monkey studies described above, guinea pigs were exposed by Alarie, et al (54) to sulfuric acid mist at 0.08 or 0.10 mg/m^3 (0.8 or 2.8 μm MMD) for 52 weeks. No physiologic or pathologic abnormalities developed. Since these concentrations are closer to possible realistic levels for sulfates than the concentrations used in monkeys, and since the pathogenicity of sulfates could be interpreted as resulting from their existing in an airborne state as sulfuric acid, the pathogenicity of sulfuric acid alone is not clearly established at concentrations near realistic (0.1 mg/m^3 or less).

Conclusion #11:

a) Long-term chronic exposure to H_2SO_4 at levels below 1 mg/m^3 using particles of 0.5 μm MMD or larger at RH of 50% probably does not need to be repeated since these have been done by a good enough group to suggest strongly that H_2SO_4 under these conditions is not a pathogen sufficient by itself to cause chronic lung disease at concentrations in community air. Sulfuric acid should therefore be studied in the presence of a co-pathogen (e.g., metal) or as an agent rendering the lung susceptible upon challenge with a secondary pathogen (e.g., bacteria or virus).

b) A limited possibility remains open, namely, that particle sizes near 0.1 μm may have pathogenicity that does not exist

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for particles of 0.5 μm or larger. The effect of such particles at high or low RH should be studied but the exposure should probably not continue for a year before pathologic changes are sought.

c) Pathologic changes preceded physiologic alterations. This will probably be true of any chronic, low-dose exposure study in which function is more likely impaired due to anatomic lesions than due to long-standing reflex irritation. In small animals, it may be easier to recognize pathologic changes than physiologic alterations in small airways, hence pathologic observation may represent a primary method for study.

D. Sulfates

"Not all sulfates, then, are irritant in nature, and the sulfate ion, per se, is not known to have irritant potency" (32).

1. Metallic sulfates

Amdur and Corn (51) found the irritant potency in the guinea pig of ammonium sulfate, zinc sulfate and zinc ammonium sulfate to be as 1 to 1.5 to 3, respectively. The particle size was 0.29 μm-MMD and the aerosol concentration was 1 mg/m^3. Dose-response curves correlating particle size with increase in pulmonary resistance were then obtained for zinc ammonium sulfate at a constant concentration of 1 mg/m^3. Aerosols of 1.4, 0.74, 0.51 and 0.29 μm MMD were progressively more irritant as particle size decreased. The dose-response curve relating percent increase in resistance to increasing particle concentration for zinc ammonium sulfate was steepest for the smallest particles and flattest for the largest particles.

This observation is of general value for particle deposition and retention below the larynx and corresponds to the model developed for

human lung deposition by the Task Group on Lung Dynamics (52). There is a roughly linear inverse relationship between percent deposition in a human lung model (expressed as a log-probability function) and the log of particle size over a particle MMD range of 10^{-2} to 10^2 μm. Nasal deposition occurs with a direct linear relationship to size. Nasal deposition is about 1% for 0.1 μm, about 30% for 1.0 μm and 50% for about 1.5 μm particles. These data are derived for man but are undoubtedly meaningful for animals. The data of Amdur and Corn (51) are compatible with better deposition of smaller particles deep in the lung and with a better opportunity for hydration of particles while within the humid atmosphere of the airways. The foregoing observation (51) therefore indicates that importance of particle sizing but does not establish the basis for the irritant potency of zinc ammonium sulfate.

The irritancy of metallic sulfates could depend on acid formation upon dissociation in liquid droplets after humidification in the airways or after settling on mucosal surfaces. Analysis of this possibility was not made by Amdur and Corn (51) for zinc ammonium sulfate but Amdur (53) prepared a graph setting forth the data for irritant potency of SO_2, H_2SO_4 and the zinc, ammonium, and zinc ammonium sulfates using the equivalent content of sulfur as the basis for correlation of concentration with irritant potency. Particle sizes were not identical in the exposures used to generate these data (6, 51) hence the slopes of the curves describing increasing irritant potency with increasing concentration cannot be compared. Lewis, et al (32) review these data and appear to conclude that zinc ammonium sulfate has about 20-fold greater irritant potency than

H_2SO_4 when each aerosol contains the same amount of sulfur. This would argue against the notion that these sulfates are irritant on the basis of acidity and favor the possible toxic effect of the metallic ion.

The data from the graph of Amdur and Corn (51) were, therefore, superimposed on Amdur's graph (6), reproduced in this report as Fig. 3. The result is shown in Fig. 5. The close grouping of the points for the sulfates near the curve for H_2SO_4 suggest that there is little basis for assigning a difference in toxicity to the sulfates and H_2SO_4. Significant irritancy of ferric sulfate was reported by Amdur and Underhill (4) and this compound is included in Fig. 5.

There is clearly a problem in comparing irritancy due to the metal ions associated with sulfate and the acidity associated with H_2SO_4. On the basis of Fig. 5, it could be reasoned that acidity was the principal irritant in all these compounds or at least that there was not much greater irritant potency in ferric sulfate and in zinc ammonium sulfate than in H_2SO_4. The importance of these data lies in demonstrating that zinc and ferric ions are at least as irritant as H_2SO_4.

Conclusion #12: Lewis, et al (32) conclude that "The properties of these and other sulfur compounds have been studied thoroughly by many researchers, who have found that toxicity of the compounds is directly related to the metal ion itself rather than to the attached sulfur radical."

The conclusion of Lewis, et al (32) above, is appropriate in directing research policy toward evaluation of aerosols of

metallic ions in sulfates. Though data adequate to define irritancy and possible toxicity of metallic ions in sulfates as contrasted with acidity of sulfates are limited, there is no doubt that some metallic sulfates are as irritant (and possibly more irritant) than H_2SO_4. A list of references of possible value in assessing toxicity of metals was appended by Lewis, et al (32). The assumption guiding research on health effects of metallic cations found in association with sulfates is that the aerosol reaching human populations has already been converted to sulfate and that toxic effects may result from the metal ion itself. Acidity related to incomplete neutralization of SO_3 or to dissociation of sulfates is adequately covered in the discussion of H_2SO_4.

The contribution of metallic ions to the catalytic conversion of SO_2 to SO_3 was discussed earlier.

2. Ammonium sulfate

Sulfates formed by neutralization of H_2SO_4 may be partially neutralized, producing acid sulfates. The cations associated with the acid sulfate may be metallic and require combined analysis of the effect of acidity and a metal ion in the same aerosol. In addition, cations of weak bases, as ammonia, may have some toxicity that is identifiable with the cation or with the dissociation characteristics of the sulfate salts of such compounds.

Neutralization of H_2SO_4 by ammonia during exposure to a sulfuric acid mist relieved symptoms in adult male volunteers toward the end of a period of exposure to H_2SO_4 (31) suggesting that neutralization of sulfuric acid by producing ammonium sulfate was blocking the irritant effect of the acid without producing a separate toxic effect.

Ammonium ion induces pulmonary edema by neurotoxic effects
mediated through adrenergic fibers and abolished by adrenergic blocking
agents (57) but this effect has been observed only upon intravenous
or intraperitoneal injection. The lesions of this pathologic event
are observed within 2.5 minutes, become greater for up to 90 minutes
after intraperitoneal injection, and consist of edema of epithelial
cells, edema within the interstitium of the lung and blebbing of
endothelial cells (58). The ultrastructural changes were regarded
by Hayes and Shiga as similar to those of pulmonary edema induced
by methods other than altered hemodynamics and different from vascular
leakage occuring in acute inflammation. These effects were produced
in rats by an amount of ammonium sulfate (6g/k) far greater than could
be inhaled from city air but the lesion occured in all animals,
indicating that the effects of smaller concentrations are worthy
of test.

Summary for Oxides of Sulphur

The limited toxicity of SO_2 is based on extraction by moist mucosal surfaces where the gas is presumably dissolved to form sulfurous acid. Surfaces nearest the nose and mouth are affected directly and sensory reflexes produce bronchoconstriction. The local effect is suppression of mucociliary transport and mucosal swelling with possible impairment of defense against infection. Remote bronchoconstriction does not apparently require that SO_2 reach beyond the larynx. Bronchoconstriction may not be relevant to progressive chronic lung disease if it is initiated only by SO_2 or by aerosol particles too large to reach deeper airways. Bronchoconstriction could be highly incapacitating in persons with high reflex reactivity of bronchi, as asthmatics, or in persons with limited lung reserve due to prior cardiopulmonary disease.

Sulfur dioxide absorbs on particles and dissolves in watery aerosols. Sulfurous acid so formed is more irritant than SO_2. Oxidation of SO_2 to SO_3 (hence to H_2SO_4) occurs in the atmosphere. Acidic aerosols so formed are a toxic hazard during whatever time H_2SO_4 or HSO_4 can be inhaled. At such times, the irritant potency of sulfur oxides rise by about 3 to 9-fold (or possibly by 400-fold) above the potency of SO_2. The potential for damage to small airways is increased as particle size of acid aerosols is reduced from 3 to about 0.5 μm amd may be still greater near 0.1 μm.

Chronic lung disease associated with long-term exposure to SO_2 or H_2SO_4 can be produced in experimental animals but requires 10 to 100-fold higher than ambient quantities. The pathogenetic effect of oxides of sulfur alone is probably not sufficient to produce chronic human airway disease but oxides of sulfur in the form of sulfate particles may be candidates as pathogens if associated with metallic cations some of which have direct irritant potency. An example is ferric but not ferrous ion. Chronic effects of aerosols of such

metals have not been reported. The irritant potency of metallic sulfates

and H_2SO_4 together may be great enough to warrant study as pathogens in chronic

lung disease.

TABLE 1

Absorption of SO_2 by the Upper Respiratory Tract of Rabbits

SO_2 PPM	Absorption %	Range %
100	95	90-98
20	95	75-95
5	80	50-90
0.5	50	10-60
0.1	5	1-10

Data from Strandberg (13)

FIGURE 1[*]

SO2 CONCENTRATIONS

(7 Subjects)
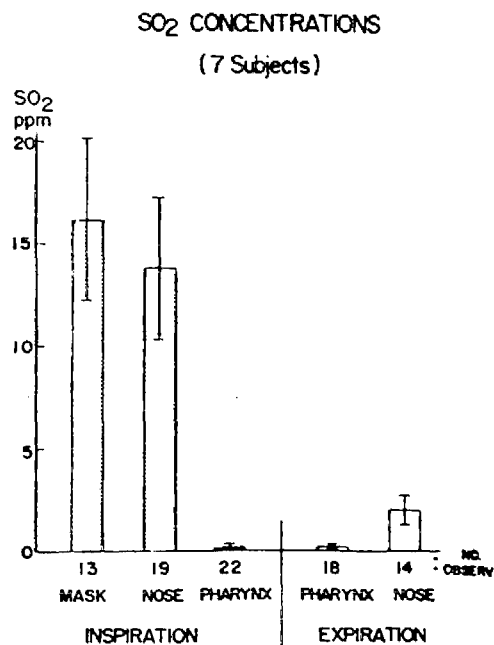
Fig 2.—The mean concentrations and standard deviations of SO_2 during inspiration are shown for the following sites: within the mask, just within the nose, and the pharynx. Nasal and pharyngeal values are also given for expiration. The inspiratory samples at the nose and pharynx were collected simultaneously, as were the expiratory samples at the same sites.

FIGURE 2

The Effect of SO_2 Alone and of SO_2 Plus Catalytic Metallic Salts on Pulmonary Flow Resistance in Guinea Pigs.   Aerosol Particles were 0.1 um MMD.[*]

● SO_2　　　　　　　▲ $SO_2 + V^{+3}$

✕ $SO_2 + Mn^{++}$　　　△ $SO_2 + Fe^{++}$

% INCREASE IN RESISTANCE

SO_2 ppm	0.1	0.2	0.5	1	2	5	10	20	50
SO_2 mg/m³	0.29	.57	1.43	2.86	5.72	14.30	28.6	57.2	143.0
S mg/m³	.05	0.1	0.25	0.5	1.0	2.5	5	10	25
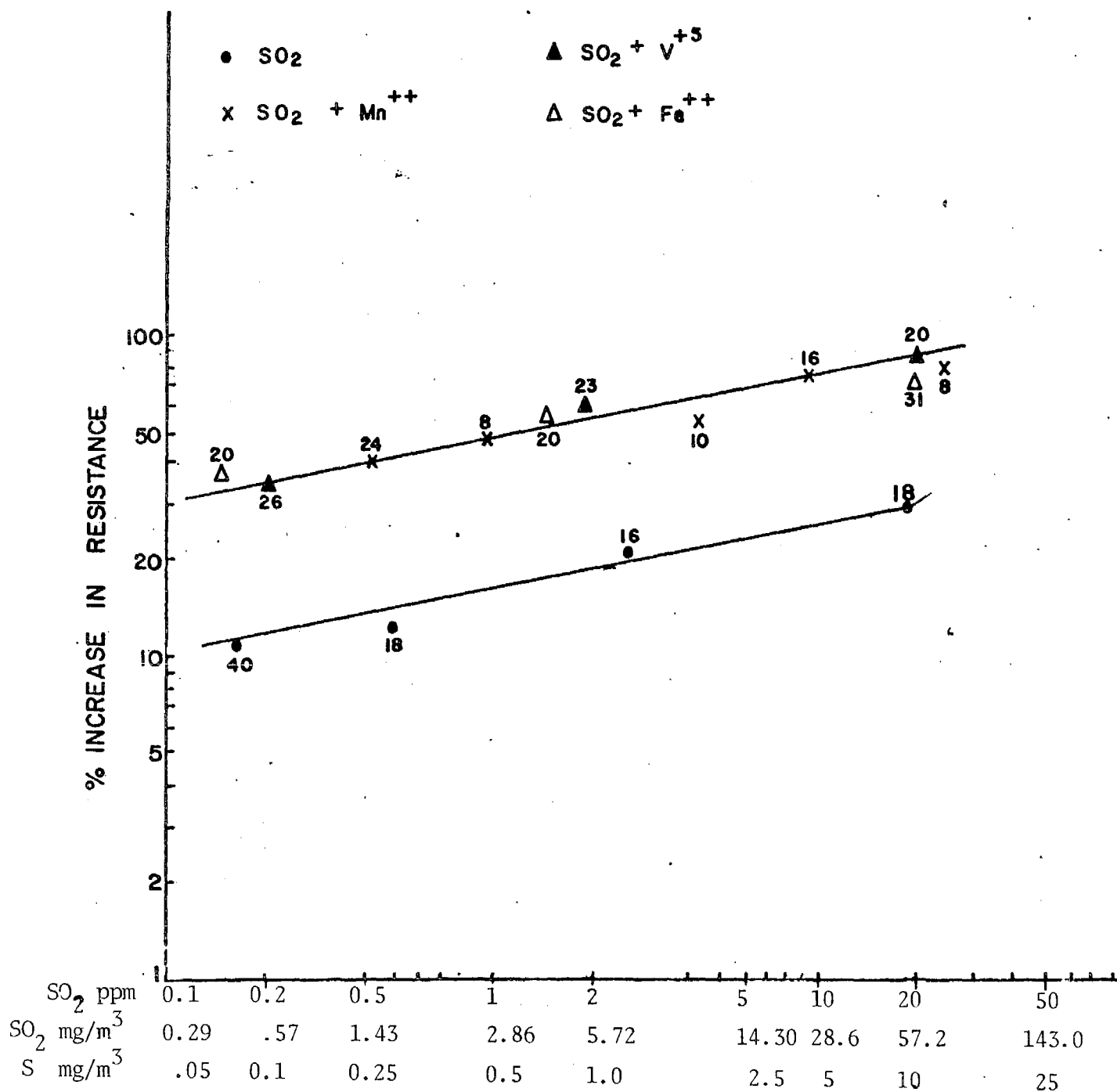
[*]from Amdur 6. See also 34

PULMONARY FLOW RESISTANCE IN GUINEA PIGS EXPOSED FOR ONE HOUR TO SULFUR DIOXIDE OR SULFURIC ACID
MIST PARTICLES OF DIFFERENT MASS MEDIAN DIAMETERS*

mg S/m^3	0.01	0.02	0.05	0.1	0.2	0.5	1	2	5	10	20	50	100	200	500
mg H$_2$SO$_4$/m^3	0.31	0.06	0.15	0.306	0.612	1.53	3.06	6.12	15.3	30.6	61.2	153	306	612	1530
mg SO$_2$/m^3	0.02	0.04	0.1	0.2	0.4	1.0	2	4	10	20	40	100	200	400	1000
ppm SO$_2$	0.007	0.014	0.035	0.07	0.14	0.35	0.7	1.4	3.5	7	14	35	70	140	350

*from Amdur, 6.

FIGURE 3

PERCENT OF SO_2 CONVERTED TO H_2SO_4 BY MIXING WITH 1 MG/M^3 OF CATALYTIC METALLIC SALTS. ESTIMATED FROM RESPONSE OF GUINEA PIGS TO H_2SO_4 AEROSOL (0.3 μm-MMD) AND TO SO_2 AND AEROSOL (0.1 μm-MMD)[*]

FIGURE 4

[*]from Amdur, 6.

PULMONARY FLOW RESISTANCE IN GUINEA PIGS EXPOSED FOR ONE HOUR TO SULFUR DIOXIDE OR SULFURIC ACID MIST PARTICLES OF DIFFERENT MASS MEDIAN DIAMETERS*
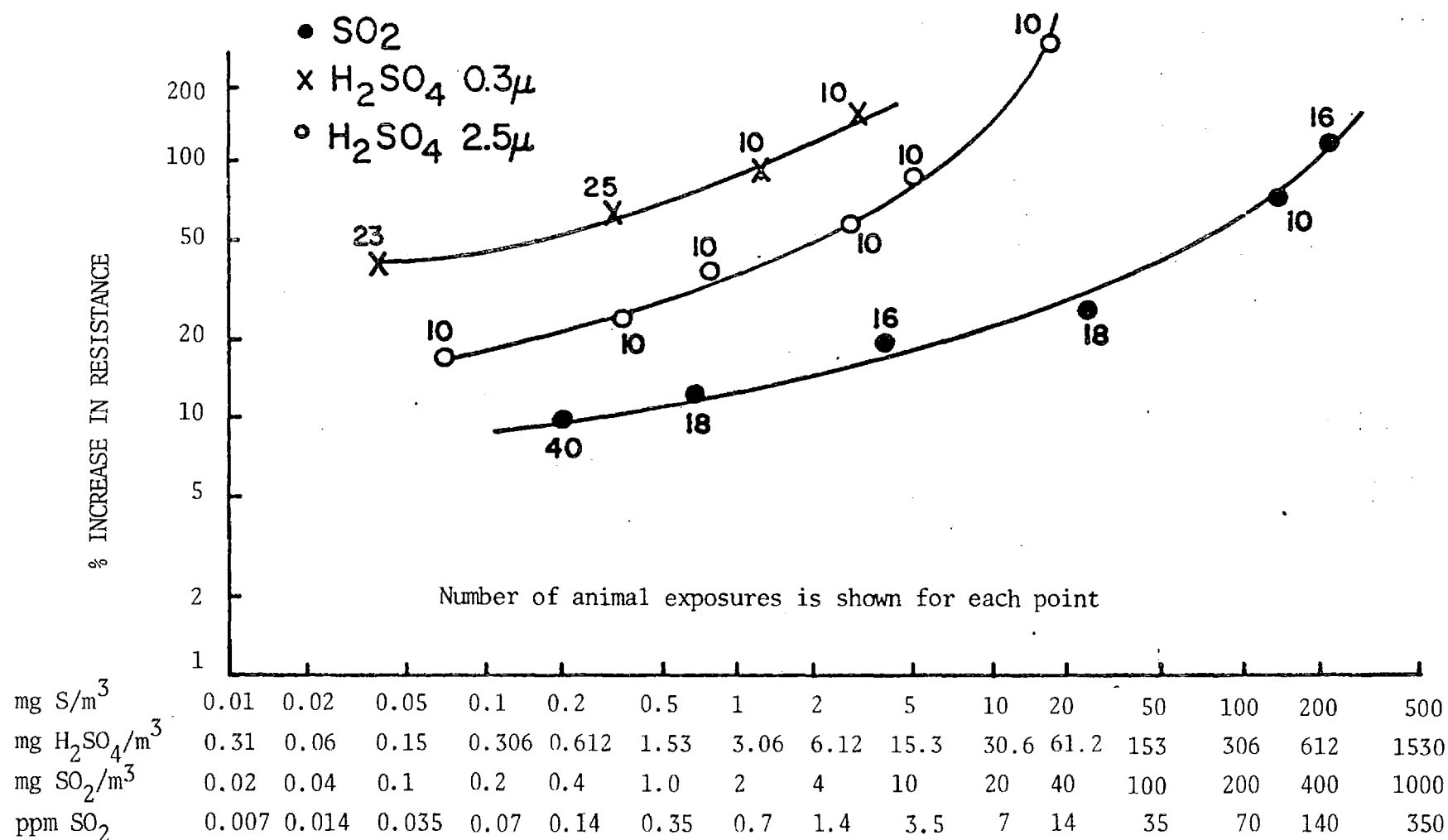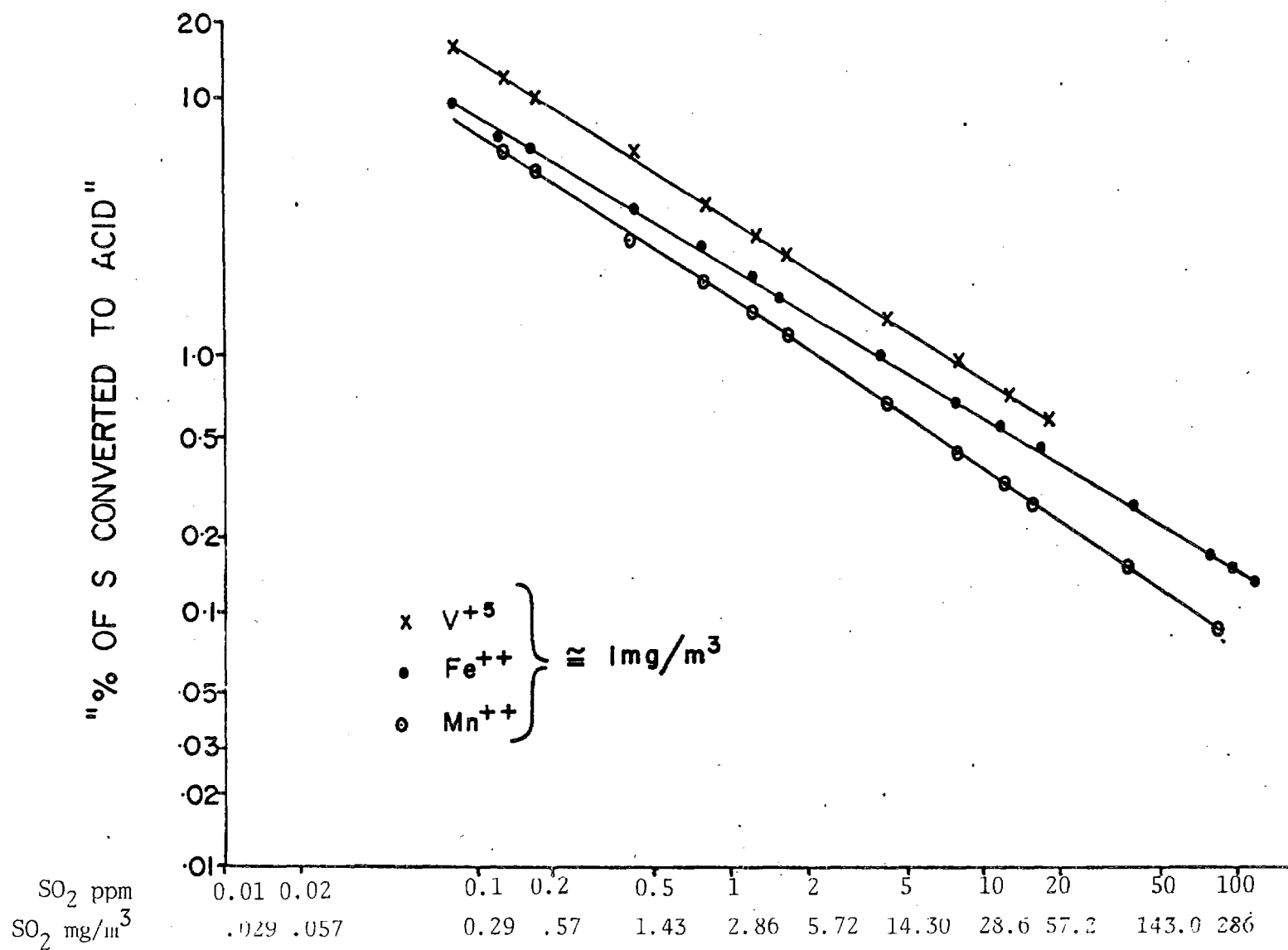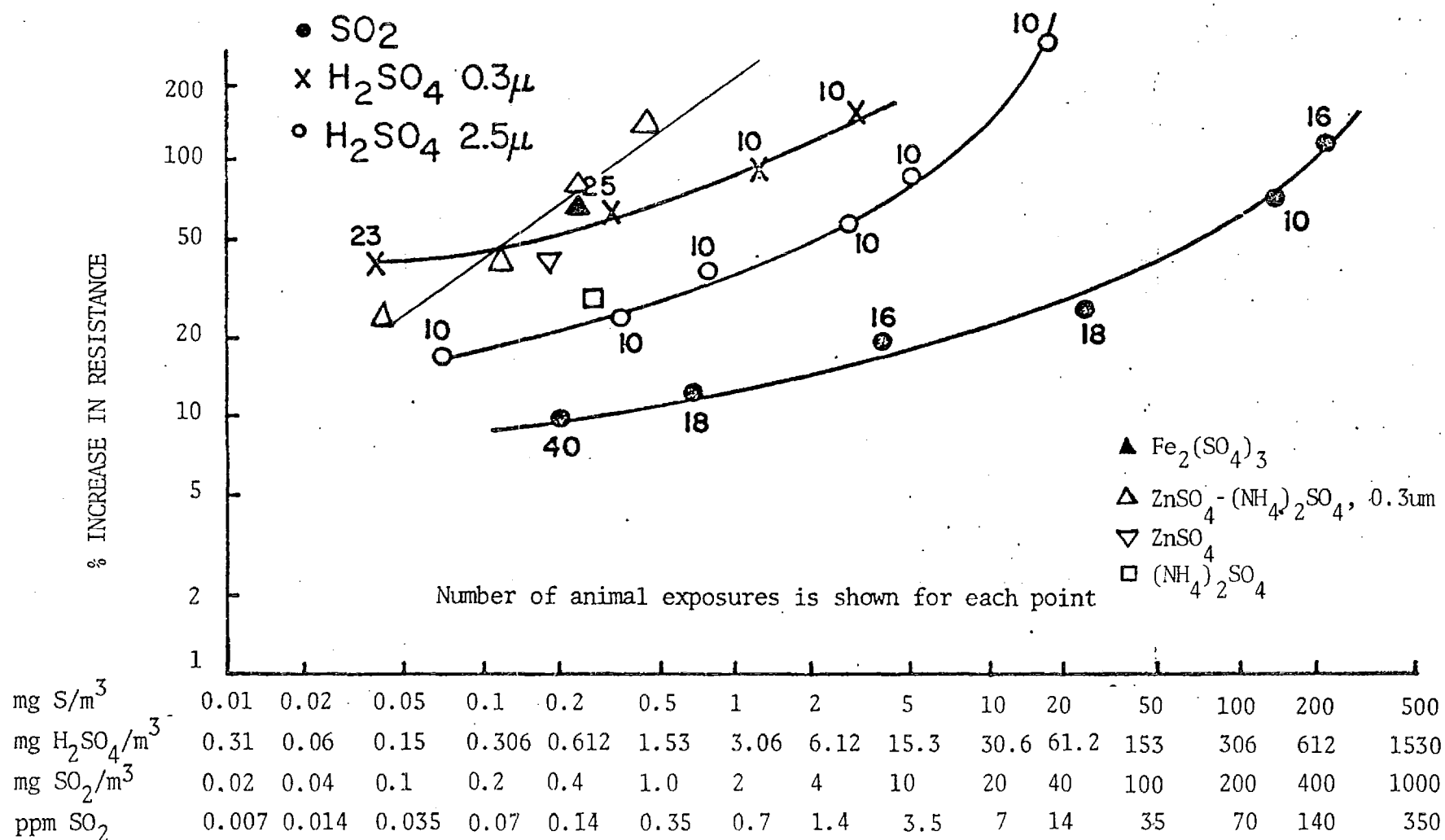
FIGURE 5

*from Amdur, 6.

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PHYSIOLOGICAL EFFECTS OF AIR POLLUTANTS IN

HUMANS SUBJECTED TO SECONDARY STRESS

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Presented at the Conference on Health Effects of

Atmospheric Salts and Gases of Sulfur and Nitrogen

in Association with Photochemical Oxidant

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CHAPTER IV.

Table of Contents

Abstract

Adult male volunteers were exposed to purified air or to ozone, alone or in combination with nitrogen dioxide and carbon monoxide, in an investigation of physiological effects of photochemical air pollution. Exposure conditions simulated those of a smoggy Southern California summer, including the secondary stresses of heat, exercise and repeated exposure. Pulmonary function, blood biochemistry, psychomotor performance capability, and symptoms experienced by the subjects were evaluated. Ozone exposures similar to those expected during pollution episodes produced significant decrement in pulmonary function, symptoms sufficient to restrict normal activity, and oxidative changes in erythrocytes. Psychomotor tracking ability and measures of attention were adversely affected by heat, but not by ozone exposure. Subjects with a history of cough, chest discomfort, or wheezing associated with allergy or exposure to air pollution, were more reactive than subjects without such a history. Addition of nitrogen dioxide and carbon monoxide to ozone in exposures did not produce additional detectable effects except for slight increases in carboxyhemoglobin levels and small variable decrements in psychomotor performance with carbon monoxide exposure. It is concluded that in sensitive subjects, exposures to photochemical oxidants at concentrations sometimes achieved in California urban areas may produce physiological dysfunction and inability to carry on normal activities.

Conclusions

The results show that exposures to ozone at 0.37 to 0.50 parts per million (ppm) for two hours or more with intermittent light exercise can have significant deleterious effects on health. Some subjects thus exposed not only developed measurable physiological and biochemical changes, but felt physically ill and were unable to perform their normal jobs during exposure and for several hours afterward. The most sensitive subjects tested experienced respiratory symptoms after a single two-hour exposure to 0.37 ppm ozone and developed measurable physiological changes after a second similar exposure the following day. The

least sensitive subjects tested developed no respiratory symptoms or physiological changes even after five-hour exposures to 0.5 ppm ozone on two successive days; however, biochemical changes were observed even in these subjects. The more reactive subjects were generally those with history of asthma, allergy, or previous subjective adverse reactions to smog exposure. No additional effects of exposure were detected when 0.30 ppm nitrogen dioxide was added to ozone. Addition of 30 ppm carbon monoxide to the ozone-nitrogen dioxide mixtures produced no additional effects other than slight increases in blood carboxyhemoglobin levels and small decrements in psychomotor performance, which were not consistent in different subject groups.

Tentative inferences concerning threshold levels for ozone exposure may be drawn from the finding that the most sensitive of these subjects did not show significant changes when exposed to 0.25 ppm ozone for two hours. First approximation mean dose-response curves, generated by analysis of observed changes in stable physiological parameters plotted as a function of ozone concentration, suggest a "zero-effect" level of 0.25 to 0.3 ppm. It must be emphasized, however, that these findings relate to relatively healthy, young to middle-aged adult men performing light exercise. Other groups such as children, older adults, pulmonary disease patients, or workers performing heavy exercise may be at risk at even lower ozone levels. In addition, the findings relate to ozone exposure against a background of highly purified air. Actual ambient exposures involve additional photochemical oxidants and other gaseous and particulate pollutants which may have additive or synergistic effects.

I. Experimental Studies on Human Health Effects of Air Pollutants: Design Considerations (1)

Because of the possible threat to public health posed by photochemical air pollution, a need exists for experimental studies of short-term respiratory effects of air pollutant exposure in humans. Such studies require rigorous control of the experimental air environment and exposure conditions to ensure that results are both reliable and relevant to public-health questions. In addition to biochemical and behavioral measures, a comprehensive battery of pulmonary tests is required to assure that effects at different levels of the respiratory tract are detected. We have developed a core protocol based on the foregoing principles. Findings from a series of studies using this protocol indicate that a wide range of sensitivity to photochemical pollutants exists and that more sensitive individuals develop significant symptoms, biochemical changes and respiratory function decrement under exposure conditions similar to those experienced during ambient pollution episodes.

Facilities: Environmental Chamber

Studies are performed in the Rancho Los Amigos Clinical Environmental Stress Testing Laboratory. This facility consists of a stainless steel-sheathed controlled environment chamber, approximately 28 square meters in area, accessible through a five square meter double-door lock compartment which contains lavatory facilities and through which air is exhausted. The main chamber contains physiological test equipment and can hold four or five subjects at the same time. Data recording and monitoring equipment are located outside the chamber in an adjacent laboratory area. Air flows in an approximately laminar manner through the main chamber from ceiling to floor at a rate that provides a complete change of air every five minutes, and is then exhausted without recirculation. The air is highly purified and can be adjusted to simulate a wide range of meteorological conditions (Table 1). The air purification unit (Mine Safety Appliances, Inc., Evans City, Pa.) contains high-efficiency particulate filters, a catalytic oxidation unit for conversion of carbon monoxide and hydrocarbons to carbon dioxide, and chemical filters containing activated charcoal (Barnebey-Cheney, Inc., Columbus, Ohio) and aluminum oxide pellets impregnated with potassium permanganate (Purafil, Inc., Chamblee, Ga.). The air conditioning unit consists of refrigerant coils for cooling and dehumidification, followed by intermittently operating heaters

TABLE 1

CHAMBER ENVIRONMENTAL CONTROL FACTORS

PARAMETER	DESIGN SPECIFICATION AMBIENT[f]	DESIGN SPECIFICATION CHAMBER	ACTUAL PERFORMANCE AMBIENT	ACTUAL PERFORMANCE CHAMBER[h]
Temperature, $^\circ$F	25-110	Within ± 1, range 14-110	25-110	Within ± 1, range 15-130
Relative Humidity	10%-100%	Within ± 4%, range 10%-100%	10%-100%	Within ± 5%, range 20%-100%
Total Number[a] Particles per ft.^{3}	$\geq 10^6$	$< 10^5$	$\geq 10^6$	200-400[b] $2 \times 10^3 - 10^4$ [c] $2 \times 10^4 - 10^5$ [d]
CO, parts per million (ppm)	30	2	20[g]	1
NO, ppm	2	.01	1[g]	0.02
NO_2, ppm	1	.01	0.7[g]	< 0.01
O_3, ppm	0.7	.01	0.4[g]	< 0.01
SO_2, ppm	1	.05	1[e]	0.01[e]
Hydrocarbons, ppm	30	5	100[e]	5[e]

(a) Particles with diameter $\geq$ 0.5 μm
(b) No one in chamber
(c) One to four subjects at rest
(d) One to four subjects exercising
(e) When deliberately challenged at or above the ambient level
(f) Extreme ambient conditions experienced in Los Angeles area
(g) Maximum concentration typically found outside of the environmental control facility
(h) Over at least a six-hour period

and steam injectors controlled automatically to maintain desired levels of temperature and humidity.

Pollutant Generation

Each pollutant gas is introduced through its own stainless steel inlet line into the purified air in the chamber inlet duct. Complete mixing occurs before the air reaches the main chamber producing uniform concentrations throughout the chamber (within five percent of the mean value). Carbon monoxide, nitric oxide, nitrogen dioxide and ozone have been studied. CO may be introduced directly from a cylinder of pure gas through a flowmeter system with a solenoid-actuated shutoff valve, which operates automatically in case of power failure. NO is similarly introduced from a cylinder containing 10 percent of the gas in nitrogen. The diluted mixture minimizes air oxidation of NO entering the chamber duct. NO_2 is introduced by bubbling nitrogen gas through a cylinder of liquid N_2O_4 and metering the resulting N_2-NO_2 mixture through a specially designed flow control apparatus. Ozone is generated using an ozonator (Welsbach T-408) which ionizes oxygen in purified air flowing between two charged plates. No contaminating nitrogen oxides are produced using this technique. All pollutants can be generated in concentration ranges realistically simulating ambient conditions and concentrations can be controlled to within 10 percent of the expected value.

Environmental Monitoring

The chamber air environment is monitored continuously utilizing instruments and techniques equivalent to those used in ambient air monitoring networks. Instruments are calibrated as recommended by the California Department of Public Health and California Air Resources Board (2), and cross comparisons are made with analytical laboratories of the latter agency. Two monitoring instruments, each operating on a different principle, are used for each gaseous pollutant under study. Ozone and nitrogen oxides are monitored using chemiluminescent analyzers (Models 612 and 642, REM Scientific, Santa Monica, Ca.), which provide fast response and freedom from interference by other pollutants. Total oxidants (i.e., ozone) and nitrogen oxides are also monitored by the neutral potassium iodide solution and Saltzman reagent methods, respectively, using continuous-flow colorimetric analyzers (Model K-76, Beckman Instruments, Fullerton, Ca.). Carbon monoxide is monitored by a nondispersive infrared analyzer (Mine Safety Appliances) and by an oxidative electrochemical analyzer (Model 2100, Energetics Science, Inc., Elmsford, N.Y.). A light-scattering, single particle counter (Royco Instruments, Model 225, Menlo Park, Ca.) monitors particulates in five subranges between 0.5

and 10 microns in diameter.

Methods: Physiological Testing

An initial target of any air pollutant challenge is the respiratory tract, which is thus the center of attention in tests of effects of exposure. Other areas of interest include hematology, blood enzyme biochemistry and psychomotor performance. Insult by pollutants can be manifested at various sites in the respiratory system. Bronchoconstriction in the large airways, maldistribution of ventilation due to hypersecretion in small airways, constriction of alveolar units, and diffusion impairment due to edema are possible effects. A variety of pulmonary tests is required to examine the various possibilities. The tests employed in this study are described below.

Flow-volume curves are recorded using a low-resistance spirometer (Electro-Med 780). Partial and maximum forced expiratory maneuvers are performed. Partial forced expirations are initiated at 65 percent of vital capacity. These tests may be affected more by mild broncho-constriction than are full-vital capacity forced expirations (3). The parameters measured are forced vital capacity (FVC), one-second forced expiratory volume (FEV_1), peak expiratory flow rate (Vmax), and flow rates at 50 percent and 25 percent FVC ($\dot{V}_{50}$, $\dot{V}_{25}$) for partial and maximum flow-volume curves. These measurements give an easily obtained, relatively reproducible evaluation of overall pulmonary mechanical performance, but provide little information on the mechanisms responsible for any observed changes.

Airway resistance (R_{aw}) and thoracic gas volume (TGV) are determined in a whole-body plethysmograph using the method of DuBois et al (4,5). The measurement of R_{aw} is more sensitive to bronchoconstriction than maximum-flow measurements, but it is also more difficult to perform and less stable. These problems similarly affect the measurement of TGV which, however, may be useful for detecting gas trapped as a consequence of airways dysfunction (in combination with a gas-dilution lung-volume determination).

Total respiratory resistance (R_t) is determined by the forced oscillation technique (6). The method of Goldman (7) is used to eliminate the need to achieve or simulate resonance. To eliminate the phase shift introduced by the Fleisch pneumotachograph at higher frequencies, a new phase-compensation technique (8) is used to ensure correct relationships of the flow and pressure signals. Resistance is measured at pressure perturbation frequencies of 3, 6 9 and 12 hertz. This measurement is affected by changes in upper airway

configuration, which may complicate detecting changes in pulmonary airways per se. The method is believed to be capable of detecting asynchronous mechanical behavior (unequal regional ventilatory time constants) as predicted by Otis (9) which otherwise can be documented only by the considerably more difficult measurement of dynamic lung compliance.

Closing volume (CV) is determined by the single-breath nitrogen washout method (10) using a linear nitrogen analyzer (Med-Science 505). This test is believed to be sensitive to changes in small airways in dependent lung regions. It determines the lung volume at which closure of a significant number of small airways presumably occurs and also provides an estimate of residual volume (RV) and total lung capacity (TLC) through the expired nitrogen concentration (11) and an estimate of the uniformity of ventilation distribution through the slope of the alveolar plateau.(12)

Static and Dynamic Lung Compliance (C_{st}, C_{dyn}) are measured from recordings of transpulmonary pressure and respiratory flow and volume. Transpulmonary pressure is measured by the esophageal balloon method of Milic-Emili, et al (13). Flow at the mouth is measured by a pneumotachograph (Fleisch) and volume by a spirometer (Electro-Med 780). Adequate dynamic response of the system has been verified at frequencies up to 100 breaths/minute. Dynamic compliance in the tidal range is measured in a series of at least 10 breaths each at normal frequency and at 20, 40, 60, 80 and 100 breaths/minute with tidal volume monitored and kept constant at 0.75 liter. Static compliance is measured by closing a mouth shutter intermittently during an inspiration from functional residual capacity (FRC) to TLC, followed by an expiration to RV. Static compliance determinations are made in triplicate and each is preceded by an inspiration to TLC to give a consistent volume history.

Compliance measurements are indispensable for documentation of changes in the mechanical characteristics of the lung, particularly the development of unequal time constants. Unfortunately, the measurements are somewhat unstable and require considerable effort on the part of subjects and investigators. In this study these tests are performed only on a subgroup of subjects selected for motivation and performance.

Pulmonary Diffusing Capacity ($D_{L_{Co}}$) is determined by the single-breath carbon monoxide method. (14) A test gas containing 0.15 percent CO and 10 percent helium in air is used. In the calculation of $D_{L_{Co}}$ correction was made for back

pressure of CO due to significant levels of blood carbon monoxide hemoglobin. Helium is analyzed using a thermal conductivity meter (W.E. Collins, Inc., Braintree, Massachusetts) and CO using an electrochemical analyzer (Energetics Science, Inc., Model 2700). Reproducibility of this test is poor under conditions of this study (heat and intermittent exercise), but the test offers the potential to detect changes in the blood-air interface (such as alveolar edema) which might otherwise go undetected.

Oxygen consumption is measured at rest and during exercise on a constant-load bicycle ergometer (Model 844, Quinton Instruments, Seattle, Wash.) at a level yielding 65 percent of predicted maximum oxygen consumption (15). Expired air is collected in meteorological balloons and emptied into a spirometer (Collins 120-liter) to determine total expired volume. Gas samples are analyzed for oxygen using a paramagnetic analyzer (Beckman E-2) and for carbon dioxide using a gas chromatograph (Beckman GC-M). Oxygen consumptions are calculated after the method of Consolazio, et al. (16) A telemetry system (Spacelabs, Inc., Chatsworth, Ca.) records an exercise electrocardiogram during this test.

Carboxyhemoglobin concentration (COHb) is estimated using the method of Gaensler and co-workers. (17) The subject holds a breath for 20 seconds to allow equilibration of CO between alveolar air and blood, then expires a sample of alveolar air into a container. CO is measured with an electrochemical analyzer (Model 2100, Energetics Science). The air CO concentration may be directly related to carboxyhemoglobin concentration. The test is performed prior to exposure in the chamber to verify that the subject has not received an inordinate ambient pollutant exposure and performed again at the conclusion of the chamber exposure period.

Experimental Protocol

The exposure protocol has been designed to simulate as realistically as possible the ambient exposure of a person working outdoors on a smoggy summer day. A two-hour exposure period is realistic in that high ambient pollutant concentrations usually persist about that length of time. Intermittent light exercise (sufficient to approximately double minute volume) during exposure gives a realistic level of ventilation (to which pollutant dose is proportional) during work. Elevated temperature is an additional stress factor frequently present during air pollution episodes, and consequently introduced into the experimental situation. The design provides for successive days' exposures, as deleterious effects of

exposure may be cumulative. These requirements are incorporated into the proto-
col in a cost-effective manner that tests several subjects on a given day and
requires staggered exposure and testing periods, precluding blind studies or
control measurements on the same day. Thus, two or three days of sham control
runs (exposures to purified air) precede the pollutant exposure so reliable
baseline values of the measured parameters can be obtained.

A test series may be reasonably designed to support or reject the null
hypothesis that no effects of pollutant exposure at realistic levels will be
detected in volunteer subjects. Results supporting the null hypothesis are use-
ful to regulatory agencies in setting air pollution standards. This approach
provides a simple method to test for combined effects of two or more pollutants:
a single pollutant is tested initially, a second pollutant is added to the first
in the next test cycle, etc. Exposure times may also be increased to simulate
two days of pollutant exposure in one day's testing. If no effects are found,
even under the "worst" exposure conditions, valuable information relevant to
standard-setting may be obtained. If effects are found at some point, additional
studies will be required to determine if they are attributable to a single
pollutant, to a combined effect, or to cumulative effects of repeated exposures.
If minimal effects are found in a group of "normal" subjects, the experimental
plan provides for testing a group of well-specified "hyper-reactive" subjects
as the next step. These subjects are characterized by a pre-study history of
cough, chest discomfort or wheezing, associated with allergy or exposure to
air pollution.

Table 2 describes the detailed experimental protocol incorporating the
features described. A shorter protocol, eliminating certain tests (marked with
an asterisk) is also used. This protocol retains tests considered relatively
simple to perform and likely to detect effects of exposure. The shorter test
series requires a briefer training period for subjects and thus results in
subject groups more representative of larger populations. Some tests from the
comprehensive protocol may be added to the short protocol when subjects have
sufficient performance ability.

Statistical analysis

Experimental data are subjected to repeated one-way variance analyses. Post
hoc comparisons using the Newman-Kuels test (18) are made when significant F
values are found. For each parameter comparisons are made among controls,
first day of exposure, and second day of exposure; for each subject as well as

TABLE 2

EXPERIMENTAL PROTOCOL

OVERALL EXPOSURE SCHEDULE

Week 1 - O_3;   Week 2 - O_3 + NO_2;   Week 3 - O_3 + NO_2 + CO

WEEKLY SCHEDULE

| Monday, Tuesday (Wednesday)* | Control (clean air) - 4 subjects |
| Thursday, Friday | Pollutant exposure - 4 subjects |

DAILY SCHEDULE

Subject No.	Begin Exposure	Begin Test Cycle
1	0700 Hr.	0845 Hr.
2	0800	0945
3	0900	1045
4	1000	1145

INDIVIDUAL SUBJECT SCHEDULE

TIME (Hr., min.)	Procedure
-0:01	COHb
0:00	Enter chamber, exercise first 15 min. of each half hour
1:45	Last rest period; psychomotor performance test*
2:00	Respiratory resistance (forced oscillation)
2:05	Flow volume maneuvers
2:10	Closing volume
2:15	Body plethysmography*
2:22	Lung compliance*
2:40	Exercise testing*
2:58	COHb
3:00	DL_{co}*
3:15	Exit chamber, venous blood sample
3:20	Physician interview and examination

* Deleted in abbreviated test protocol

each subject group. A few significant differences due to random variation may be found because of the number of statistical comparisons being made; therefore, all observed statistically significant changes must be examined critically for physiological significance.

Results

Several general observations which are documented in Part II are of interest and will be described here:

1. We have found that at least some lightly exercising individuals develop discomfort and measurable effects when exposed to realistic concentrations of ozone.

2. It is apparent from studies made to date that some individuals are not noticeably affected by ozone doses two or three times greater than those at which other individuals experience symptoms and measurable respiratory dysfunction. Thus, if only group comparisons are made, risks to more sensitive individuals may go undetected--a matter of concern in experimental and in epidemiologic studies.

3. One striking result in exposure studies conducted to date is that, generally, pulmonary tests that are simplest to perform are most reliable in demonstrating changes. "Reliability" in this sense means that changes observed after exposure are significant compared to the normal test-to-test variability under control conditions. These tests include FVC, FEV_1, $\dot{V}_{50}$, total respiratory resistance by the oscillatory method and the slope of the alveolar plateau of the closing-volume tracing. This finding implies that more complex tests are not essential to studies concerned primarily with documenting exposure effects. Although these tests are simple to perform, unfortunately interpretation of underlying physiological mechanisms is complicated; therefore, when the goal of testing is analysis of such mechanisms, more complex test procedures such as C_{st} and C_{dyn} are required.

II. Experimental Studies on Human Health Effects of Air Pollutants: Four-Hour
     Exposure to Ozone Alone and in Combination with Other Pollutant Gases (19)

Eight adult male volunteers were exposed to ozone singly and in combination
with nitrogen dioxide and carbon monoxide under conditions simulating ambient
air pollution exposures. Four "normal" men showed few or no effects in repeated
exposures. Four male volunteers with a history of "hyper-reactive" airways,
but with normal baseline pulmonary function spirometric studies, developed
definite symptoms and pulmonary function decrement after ozone exposure.

Results

Few significant pulmonary function changes or respiratory symptoms were
detected in Group 1. The physiological significance of the changes in pulmonary
function data is in doubt, since the changes occurred in the less stable parame-
ters, (R_t, C_{dyn}, $D_{L_{Co}}$), were small in magnitude and did not occur consistently
throughout all exposures. Statistical analyses for this group are given in
Table 3 . In Group 2, numerous changes were observed. Significant changes in
group data are displayed in Figures 1-4 and in Table 4 . Because of the marked
differences between "normal" and "reactive" subjects, individual responses are
discussed below. Height, weight, age, and other characteristics are given in
Table 5 .

Although individual responses of the asthmatic subjects to the exposures
were variable, several consistent patterns emerged; these are discussed below.

Forced vital capacity, forced expired volume at one-second, and maximum
expiratory flow rates. These parameters were consistently reduced in sensitive
subjects. Contributing factors appeared to be increased airway resistance,
reduced inspiratory capacity due to substernal pain, and tendency to cough
during forced expirations. As these measurements were highly reproducible under
control conditions, observed changes with exposure attained higher levels of
statistical significance than with most other tests.

Lung volumes. Total lung capacity was consistently reduced in sensitive
subjects. Residual volume, as determined by three-breath nitrogen dilution (20),
single-breath nitrogen dilution (21), or plethysmography (22), increased signifi-
cantly in one subject and was unchanged in the others. These changes appear to
be due to pain produced by attempting to reach extremes of lung volume.

TABLE 3

RESULTS OF ANALYSIS OF VARIANCE FOR PHYSIOLOGICAL PARAMETERS, GROUP 1
Exposures: O=0.5 ppm O_3; N=0.3 ppm NO_2; C=30 ppm CO

F=statistic for analysis of variance
dF=degrees of freedom for analysis of variance
P=probability of control-exposure difference being due to chance

PARAMETER	EXPOSURE	F	dF	P	PARAMETER	EXPOSURE	F	dF	P
FVC	O	2.04	2,6	NS	CV	O	<1.0	2,22	NS
	ON	4.17	2,6	NS		ON	3.12	2,22	NS
	ONC	<1.0	2,6	NS		ONC	<1.0	2,22	NS
$\dot{V}_{50}$	O	1.25	2,6	NS	Cdyn	O	<1.0	2,78	NS
	ON	<1.0	2,6	NS	(norm. freq)	ON	<1.0	2,72	NS
	ONC	<1.0	2,6	NS		ONC	1.79	2,72	NS
$\dot{V}_{25}$	O	<1.0	2,6	NS	Cdyn	O	5.62	2,78	<.01(
	ON	<1.0	2,6	NS	(60/min)	ON	<1.0	2,72	NS
	ONC	n/a	n/a	n/a		ONC	2.42	2,72	NS
RV	O	1.18	2,6	NS	Cdyn	O	1.45	2,78	NS
(single br)	ON	<1.0	2,6	NS	(100/min)	ON	<1.0	2,78	NS
	ONC	7.73	2,6	<.05(a)		ONC	<1.0	2,78	NS
TLC	O	6.79	2,6	<.05(b)	Cstat	O	<1.0	2,22	NS
(single br)	ON	<1.0	2,6	NS		ON	1.06	2,22	NS
	ONC	<1.0	2,6	NS		ONC	<1.0	2,22	NS
DL_{co}	O	11.1	2,22	<.01(c)	TGV	O	5.67	2,16	<.05(
	ON	<1.0	2,22	NS	(RV)	ON	4.07	2,16	<.05(
	ONC	15.9	2,22	<.01(b)		ONC	<1.0	2,16	NS
Rt	O	<1.0	2,6	NS	TGV	O	5.47	2,16	<.05(
(3 Hz)	ON	<1.0	2,6	NS	(TLC)	ON	5.11	2,16	<.05(
	ONC	7.95	2,6	<.05(b,d)		ONC	2.06	2,16	NS

(a) Decreased first day of exposure   (b) Increased second day of exposure

(c) Decreased second day of exposure   (d) Increased first day of exposure

TABLE 4　　　　　IV-14

MEAN VALUES AND RESULTS OF VARIANCE ANALYSIS FOR PHYSIOLOGICAL PARAMETERS

GROUP 2

F=statistic for analysis of variance
dF=degrees of freedom for analysis of variance
P=probability of control-exposure difference being due to chance

PARAMETER	O_3 Exposure	Control	Exposure 1	Exposure 2	F	dF	P
FVC	.50	5.03	4.55*	4.69*	5.17	2,22	<.05
	.25	5.08	4.94	5.13	<1.0	2,22	NS
	.37	5.07	5.04	none	<1.0	1,11	NS
FEV_1	.50	3.96	3.54**	3.68	(a)		
	.25	4.07	4.03	4.11	<1.0	2,22	NS
	.37	4.07	3.95	none	2.15	1,10	NS
$\dot{V}_{50}$	.50	4.32	3.42*	3.27*	4.06	2,14	<.05
	.25	4.09	4.24	4.33	2.01	2,14	NS
	.37	4.15	4.10	none	<1.0	1,8	NS
$\dot{V}_{25}$	.50	1.99	1.50	1.20*	6.06	2,6	<.05
	.25	1.72	1.87	2.42	2.26	2,10	NS
	.37	1.80	1.78	none	<1.0	1,7	NS
$\dot{V}_{50}$ (partial)	.50	4.55	3.14*	3.05*	5.34	2,16	<.05
	.25	4.66	4.56	4.60	<1.0	2,18	NS
	.37	4.42	3.52**	none	8.49	1,9	<.01
$\dot{V}_{25}$ (partial)	.50	1.83	1.15**	1.19**	7.67	2,22	<.01
	.25	2.00	1.71	1.72	1.39	2,20	NS
	.37	1.62	1.39**	none	2.22	1,11	<.01
CC	.50	2.35	2.27	2.47	3.08	2,20	NS
	.25	2.33	2.36	2.20	1.05	2,22	NS
	.37	2.21	2.28	none	2.07	1,11	NS

(a) Insufficient data, some subjects unable to complete specified number of tests
satisfactorily
*=significant change from control, p <.05　　**=significant change from control, p <.01

TABLE 4

(continued)

PARAMETER	O_3 Exposure	Control	Exposure 1	Exposure 2	F	dF	P
ΔN_2	.50	0.73	1.60*	1.03	4.21	2,20	<.05
	.25	0.67	0.71	0.63	2.07	2,22	NS
	.37	0.69	0.84	none	2.13	1,11	NS
TLC	.50	6.84	6.42	6.18*	3.96	2,20	<.05
(single br)	.25	6.91	6.85	6.77	<1.0	2,22	NS
	.37	6.85	6.83	none	<1.0	1,11	NS
RV	.50	1.69	1.88	1.76	1.23	2,20	NS
(single br)	.25	1.66	1.64	1.58	1.51	2,22	NS
	.37	1.60	1.74*	none	8.17	1,11	<.05
R_{aw}	.50	1.26	1.70	1.65	2.50	2,22	NS
(FRC)	.25	1.09	1.24	1.11	<1.0	2,22	NS
	.37	1.35	1.49	none	2.75	1,11	NS
Cdyn	.50	.196	.187	none	<1.0	1,32	NS
(norm. freq)	.25	.241	.227	.266	<1.0	2,68	NS
	.37	.224	.222	none	<1.0	1,36	NS
Cdyn	.50	.179	.153	none	1.68	1,30	NS
(100/min)	.25	.184	.189	.172	<1.0	2,60	NS
	.37	.173	.127	none	3.07	1,39	NS
DL_{co}	.50	41.8	42.4	38.8	2.18	2,20	NS
	.25	41.4	40.4	49.8**	57.8	2,16	<.01
	.37	40.1	45.4**	none	19.4	1,11	<.01

TABLE 5

SUBJECT CHARACTERISTICS

	I.D. NO.	AGE, YR.	HT., IN.	WT., LB.	SMOKING HISTORY PACK-YEARS[1]	PRE-STUDY HISTORY OF SMOG SENSITIVITY[2]	HISTORY OF ASTHMA[3]	HISTORY OF ALLERGY[4] PERSONAL	FAMILY
GROUP 1	01	49	70	185	17	0	0	0	0
	03	36	70	185	34	0	0	0	0
	04	44	74	195	4 †	0	0	+	0
	06	42	69.5	185	50*‡	0	0	+	0
	MEAN	43	71	188	‡				
	(S.D.)	(5.4)	(2.1)	(5)					
GROUP 2	07	36	68.5	155	0	+	0	+	+
	08	29	68	157	30	+	+	+	+
	09	41	74	177	20*	+	+	+	+'
	10	30	68	172	0	+	0	0	0
	MEAN	34	69.5	165					
	(S.D.)	(5.6)	(2.9)	(11)					

* - Still smoking:
all others have been non-smokers
for at least four years prior to study.

†=now smokes pipe
‡=also smokes and
inhales cigars

1 - 1 pack year = 1 pack of cigarettes per day for 1 year.
2 - Defined by symptoms of cough, wheezing, or chest discomfort when outside on high pollution days.(L.A. area)
3 - Defined by spontaneous attacks of bronchospasm requiring bronchoidilator therapy separated by asymptomatic intervals.
4 - Defined by symptoms of wheezing, gastroenteritis, or dermatitis when in contact with specific antigens or a history of chronic rhinitis, post nasal drip, or hayfever.
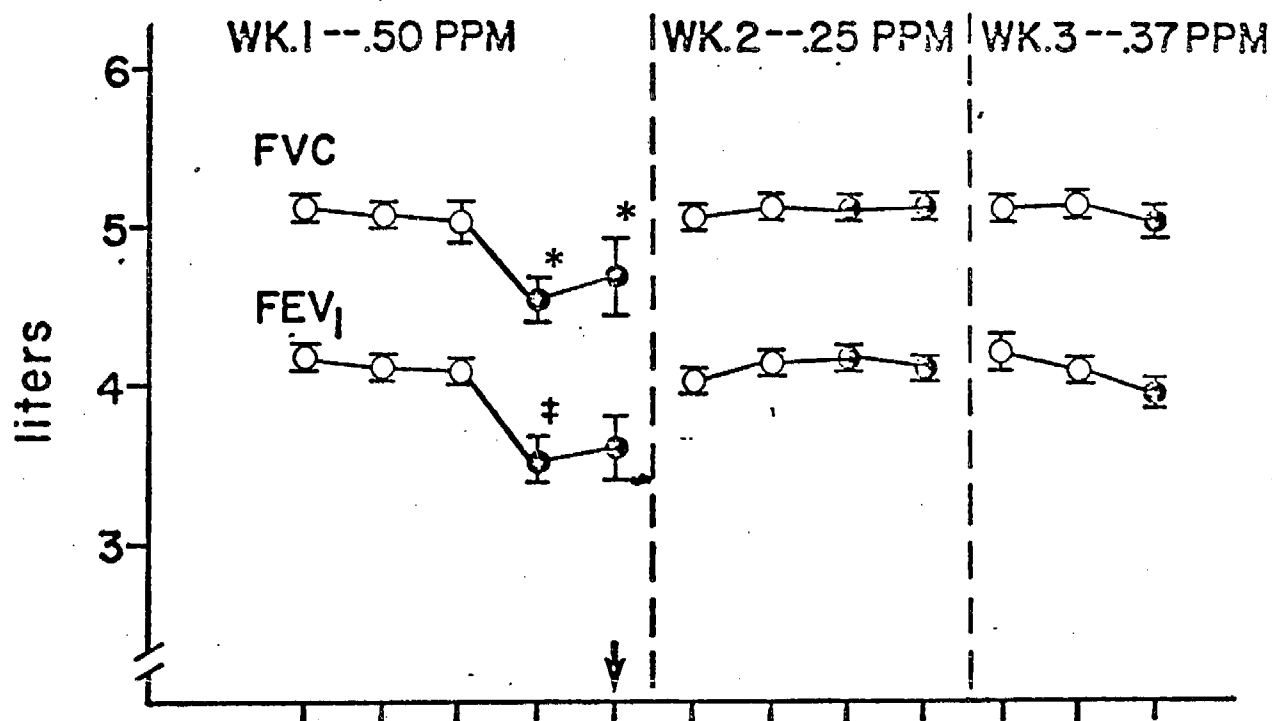
FIGURE 1

Daily group means (± one standard error) for FVC and FEV_1, Group 2. Open circle - control; black circle = ozone exposure. * = significant change from control, p <.05; ‡ = significant change from control, p <.01.

↓ Exposure time decreased from 4 to 2 hours in 3 of the 4 subjects.
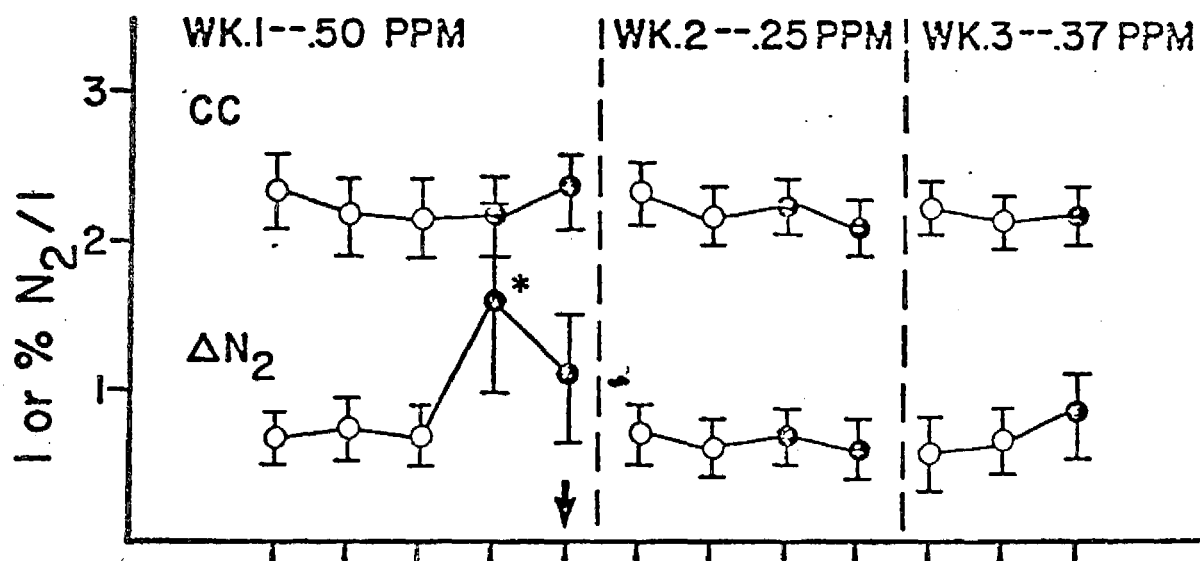
FIGURE 2

Daily group means (± one standard error) for closing capacity and delta nitrogen, Group 2. Open circle = control; black circle = ozone exposure. * = significant change from control, p <.05.

+ Exposure time decreased from 4 to 2 hours in 3 of the 4 subjects.

FIGURE 3

Daily group means (± one standard error) for lung volumes calculated from single-breath nitrogen tracings. Open circle = control; black circle = ozone exposure. * = significant change from control, p < .05.
✚ Exposure time decreased from 4 to 2 hours in 3 of the 4 subjects.

FIGURE 4

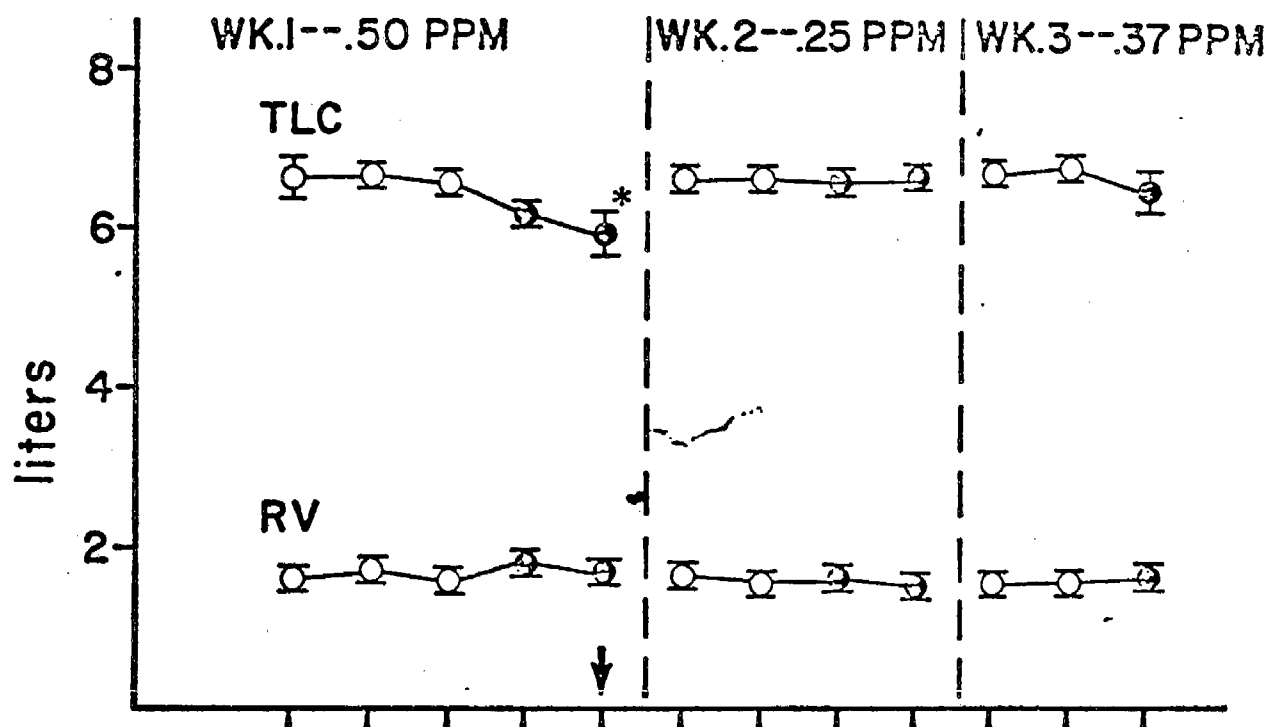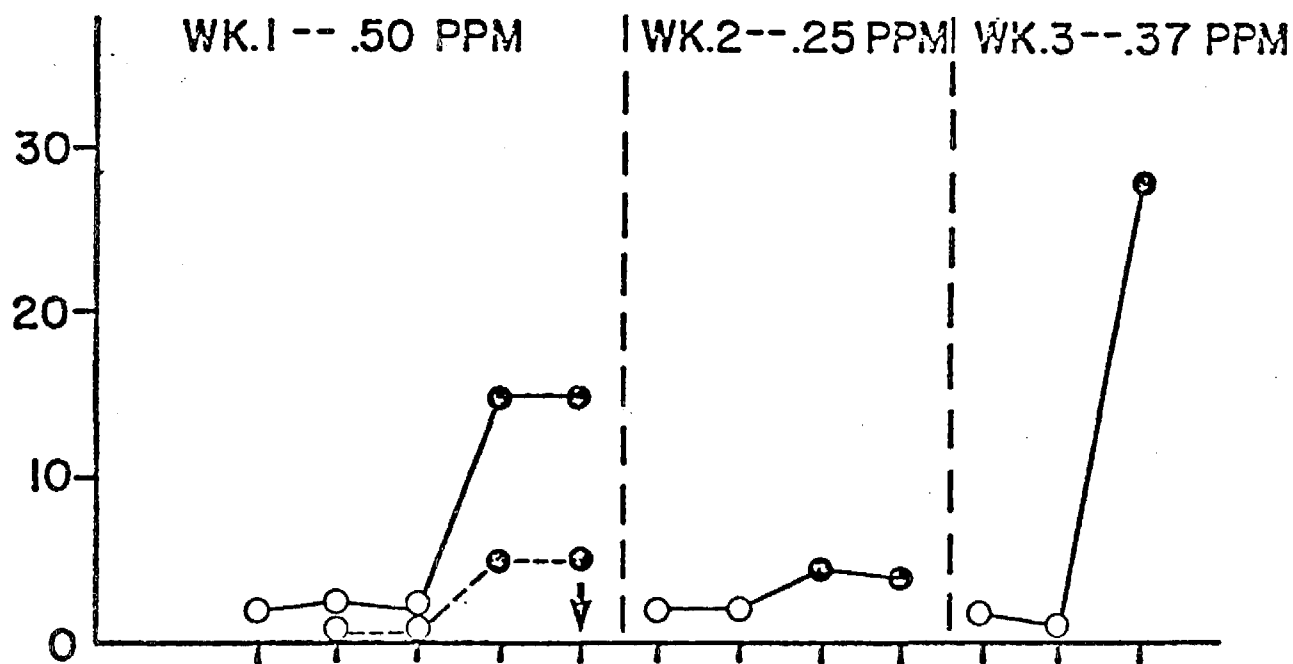Daily mean symptom scores related to ozone exposure
for Group 1 (dotted line, week 1 only) and Group 2
(solid lines). Open circle = control; black circle =
ozone exposure.
+ Exposure time decreased from 4 to 2 hours in 3 of
  4 subjects of Group 2.

Single-breath nitrogen test. This test determines phase-4 volume (closing volume), presumably affected by changes in dependent peripheral airways; and phase-3 or alveolar plateau slope (delta nitrogen), presumably affected by significant changes in ventilation distribution anywhere in the lung. No significant changes in closing volume or closing capacity (CV plus RV) were found in any subject in this study, whereas delta nitrogen values increased with exposure in all sensitive subjects - slightly in some and markedly in others.

Total pulmonary resistance. Further investigation is required before results of this test can be evaluated in depth, since normal limits and test-to-test variability have not been fully worked out. Significant changes definitely occurred with ozone exposure, however. Resistance determined by forced oscillation increased at all oscillation frequencies in three of four sensitive subjects and in one "normal" (Group 1) subject in whom no other significant physiological changes were found. The increases in oscillatory resistance in the sensitive subjects generally correlated with increases in airway resistance measured plethysmographically. In the fourth sensitive subject, total resistance, which was already elevated in comparison to other subjects, did not increase overall but became markedly frequency-dependent, as did dynamic lung compliance, strongly suggesting significant small-airways dysfunction resulting in nonuniform regional ventilatory time constants under exposure conditions.

Lung compliance. One sensitive subject showed a reduction in static compliance with exposure, and a corresponding drop in dynamic compliance at all frequencies without significant frequency-dependence. Another subject developed frequency dependence as previously indicated. No other statistically significant changes in compliance were found.

Exercise testing. No consistent changes in resting or exercise oxygen consumption were found with exposure. One "normal" subject developed possible angina and minimal EKG changes during exercise when exposed to CO plus oxidants, as previously described.

Pulmonary diffusing capacity. Reproducibility of this test was poor in this study as compared to normal conditions (resting subjects, normal room temperature). In both Groups 1 and 2, slight decreases in diffusing capacity occurred on the second day of exposure as compared to the first day of exposure, when results were essentially unchanged from control values.

Symptomatology. Symptoms were absent in Group 1 during control runs, but some were reported during exposures. A low frequency of occurrence of symptoms in Group 2 during control runs increased dramatically with ozone exposure at 0.5 and 0.37 ppm. Symptom scores for each subject on each day were assigned by the project physician based on an interview using a standard questionnaire. Symptoms of cough, wheezing, sputum production, substernal pain, dyspnea, fatigue, headache, laryngitis, and nasal discharge were scored. Each was given from 0 to 4 points, based on severity, during each of the three time periods--during exposure, remainder of the exposure day, and the following morning. Less specific symptoms such as malaise and muscular aches were not scored. In Group 2, symptom scores were strongly correlated with observed physiological changes except in the 0.37 ppm O_3 exposure, in which symptoms were more severe than would be expected, and out of proportion on the basis of physiological findings. The 0.37 ppm exposure was during the third week and the severity of these symptoms was greater than for the 0.5 ppm exposure of the first week.

III. Experimental Studies on Human Health Effects of Air Pollutants: Two-Hour Exposure to Ozone Alone and in Combination with Other Pollutant Gases (23)

Adult male volunteers were exposed to ozone (O_3) at 0.25, 0.37 or 0.50 parts per million (ppm), and to ozone in combination with nitrogen dioxide (NO_2) and carbon monoxide (CO), with secondary stresses of heat, intermittent light exercise and repeated exposure. Few significant physiological changes, and only mild symptoms, were found with 0.25 ppm O_3, with 0.25 ppm O_3 plus 0.30 ppm NO_2 or when 30 ppm CO was added to the latter mixture. With 0.37 ppm O_3, more symptoms were present and some subjects developed significant pulmonary function decrement. With 0.50 ppm O_3, most subjects had symptoms and about half showed significant pulmonary function decrement. In reactive subjects exposed on two successive days, changes were usually significantly greater the second day, indicating that effects of successive exposures were cumulative.

Discussion

The foregoing results together with those reported previously from this laboratory (24) allow formulation of dose-response curves for effects of ozone exposure (Figures 5-7). Responses are expressed in terms of changes in the
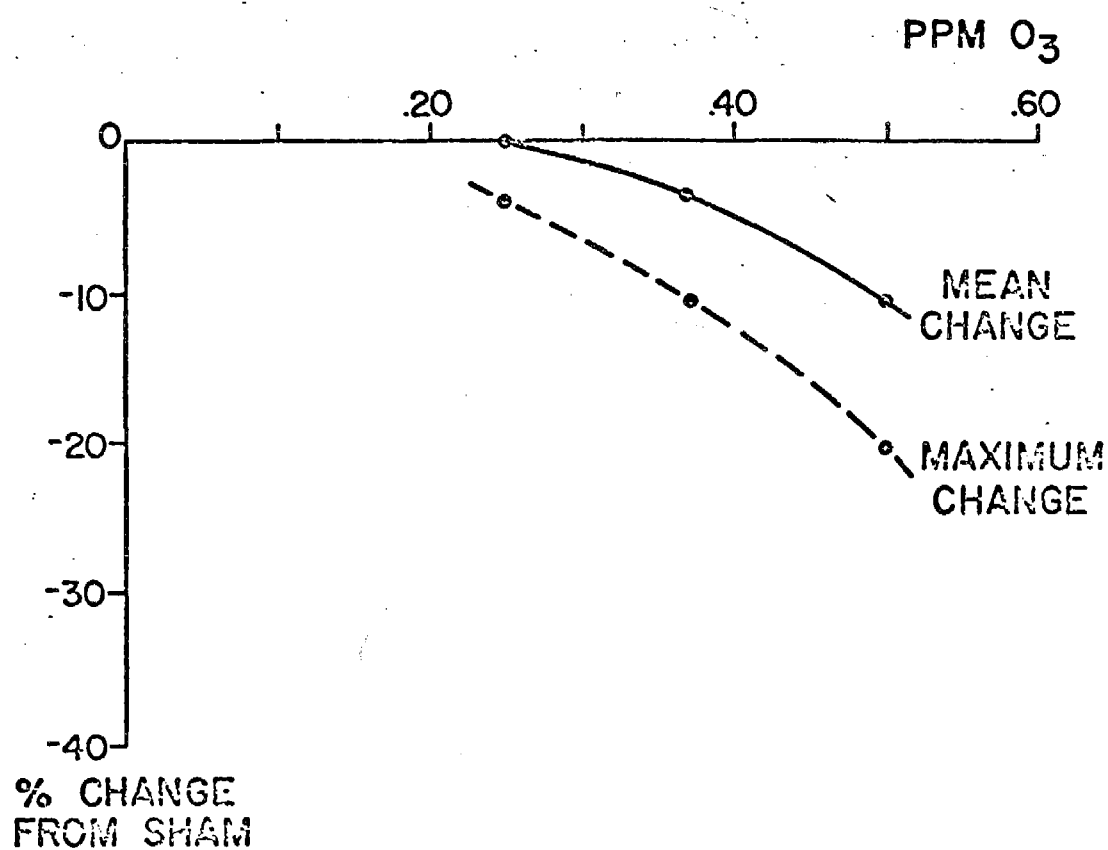
FIGURE 5

Dose-response behavior of FEV_1 in subjects exposed to O_3. Mean and maximum changes from control values observed in all subjects tested at given concentrations.

FIGURE 6

Dose-response behavior of delta nitrogen in subjects exposed to O_3. Mean and maximum changes from control values observed in all subjects tested at given concentrations.

FIGURE 7

Dose-response behavior of selected erythrocyte parameters in subjects exposed to O_3. Mean and maximum changes from control val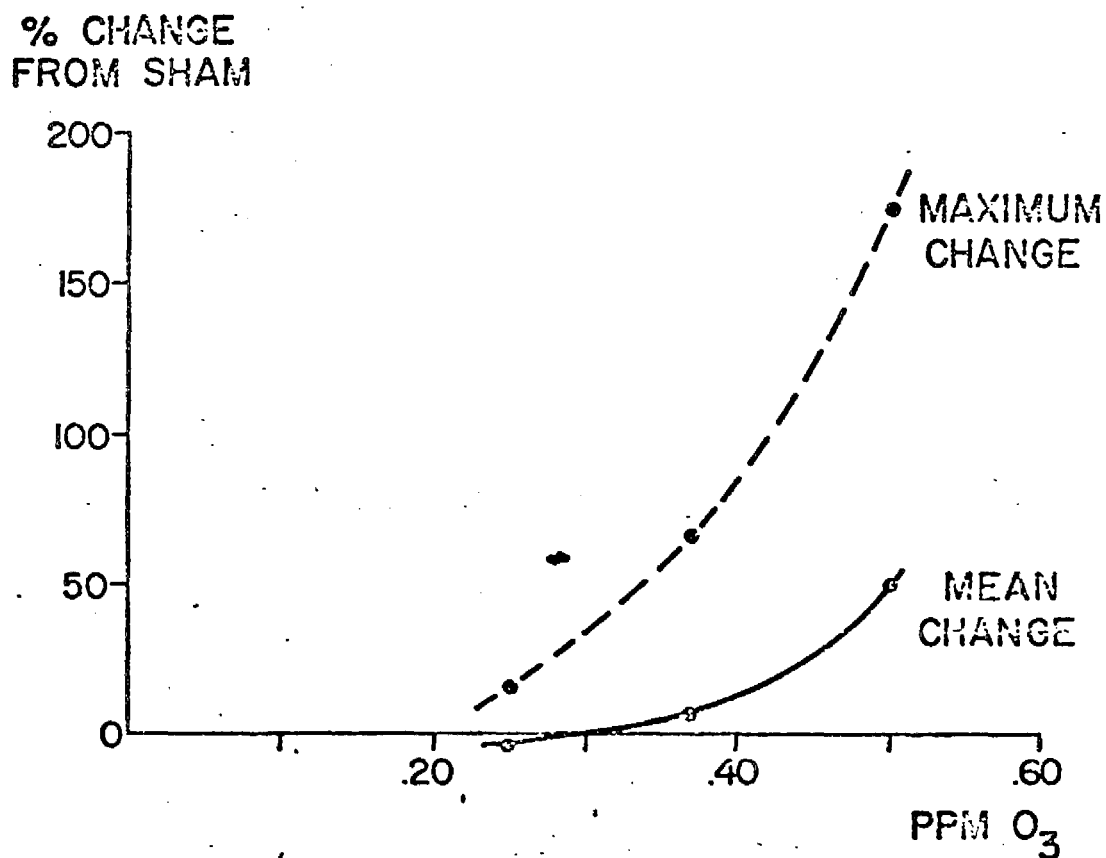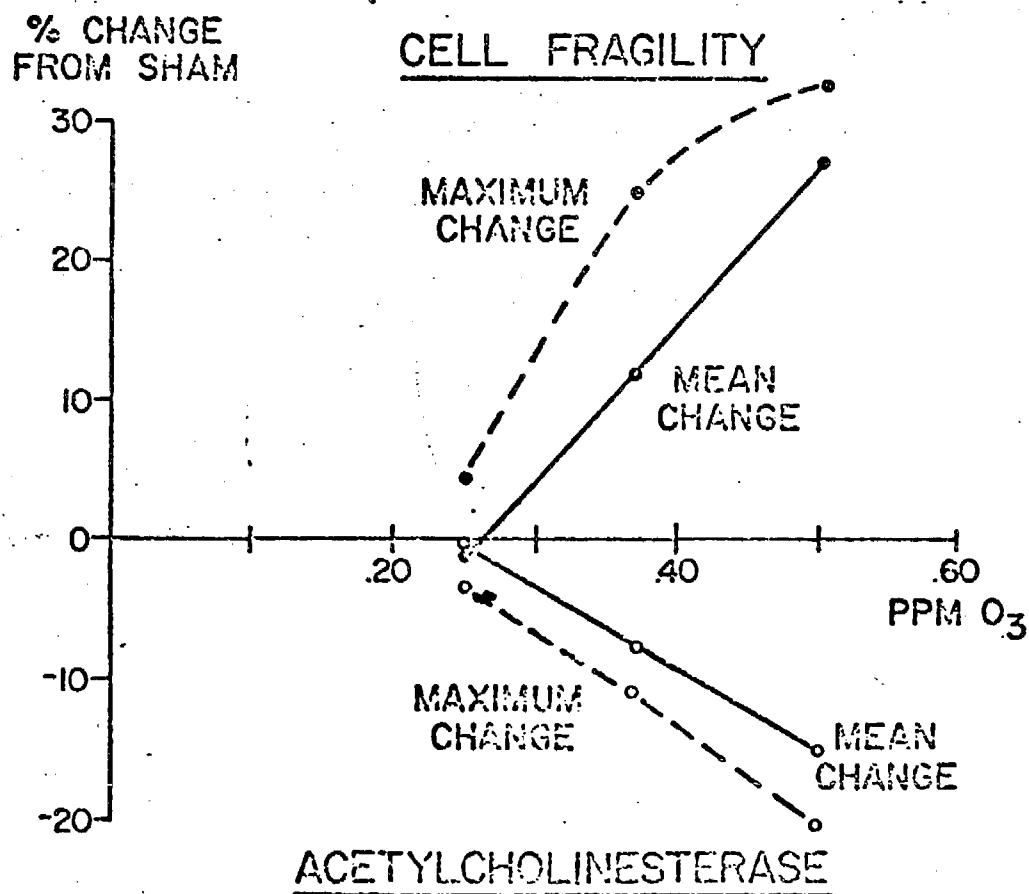ues observed in all subjects tested at given concentrations. Black circles = osmotic fragility of cells; open circles = cell membrane acetylcholinesterase level.

more stable physiological and biochemical parameters. Equating these observed responses with "significant deleterious effects on health" is not completely straightforward, since the underlying mechanisms are not fully understood. However, from a practical standpoint the observed physiological changes may reasonably be considered to represent significant health effects, since (a) the changes are qualitatively similar to those observed in certain pulmonary disease states, and (b) in studies to date, significant physiological changes have always been accompanied by significant clinical illness (respiratory symptoms severe enough to inhibit normal activity). While biochemical changes have been observed in asymptomatic as well as symptomatic subjects, the dose-response curves for those biochemical parameters expected to show an immediate response to oxidant exposure are remarkably similar to the curves for the physiological parameters.

The given dose-response curves are to be considered first approximations only since they are based on small samples, neglect differences in exposure time, and do not distinguish between initial and cumulative exposures. Two methods have been used for deriving curves from individual dose-response data. In the first, a best-fit straight line is derived from all individual data points using the method of least squares. In the second, the mean observed response is plotted for each concentration studied and a smooth curve drawn through the resulting three points (Figures 5-7   ). A "maximum response" curve is obtained similarly by plotting for each concentration the response of the most reactive individual studied at that concentration. For the physiological parameters, the maximum individual response is taken as the difference between the mean of all measurements made during the exposure in which the subject showed the most severe reaction, and the mean of all control measurements for the same week. For the biochemical parameters, only one measurement can be made per exposure, so the maximum response is taken as the largest individual percentage difference between an exposure value and the immediately preceeding control value. The mean dose-response curves generated by either of the above methods suggest a "zero-effect threshold" concentration of 0.25 to 0.30 ppm. This level is exceeded for one hour or more at least 20 days per year in parts of the Los Angeles area (25) and is not uncommon in other metropolitan areas, such as Toronto (26). Since the exposure conditions which were studied simulate light, outdoor, physical work, a significant and widespread public-health risk related to ozone or other photochemical oxidant pollution is implied. Furthermore, many individuals are considerably

more sensitive than the average, and thus may be at risk at significantly lower levels. The degree of risk to populations not studied, such as children, the elderly, or chronic pulmonary disease patients remains to be determined.

IV. Experimental Studies on Human Health Effects of Air Pollutants: Biochemical Observations (27)

Statistically significant changes ($p \leq 0.05$) were observed in erythrocytes (RBC) and sera of young adult human males following a single acute exposure to 0.50 ppm ozone (O_3) for 2 3/4 hours. RBC membrane fragility, glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) enzymes activities were increased, while RBC acetylcholinesterase (AcChase) activity and reduced gluta- thione (GSH) levels were decreased. RBC glutathione reductase (GSSRase) activities were not significantly altered. Serum GSSRase activity, however, was significantly decreased while serum vitamin E, and lipid peroxidation levels were significantly increased. These alterations tend to disappear gradually but were still detecta- ble two weeks following exposure.

Results

Data in Figures 8-11 are means and standard errors of the means, of paired- group analyses of seven experimental subjects.

The evidence indicating O_3-induced changes in the RBC membrane is shown in Figure 8. The single O_3 exposure resulted in a significant increase ($p < 0.001$) in RBC fragility to H_2O_2, while the activity of the membrane-bound enzyme AcChase was decreased ($p < 0.001$). Figure 9 shows that the activity of G6PDH was significantly increased ($p < 0.001$) while RBC levels of GSH were decreased ($p < 0.01$). Ozone inhalation also stimulated an increase in LDH activity ($p < 0.001$) although red cell GSSRase activities were not altered (Figure 10). Serum GSSRase activity levels are shown on Figure 11 to be significantly decreased ($p < 0.05$). Vitamin E levels were also increased ($p < 0.025$) at the same time that increased oxidation of unsaturated fatty acids ($p < 0.025$) were observed.

V. Experimental Studies on Human Health Effects of Air Pollutants: Psycho- physiological and Psychomotor Assessment (28)

Human subjects have been tested in an environmental control chamber under conditions simulating a smoggy summer in the Los Angeles Coastal Basin. The

FIGURE 8

HUMAN RED CELL RESPONSE
TO INHALED OZONE
(0.5 ppm x 2 hr 45 min.)
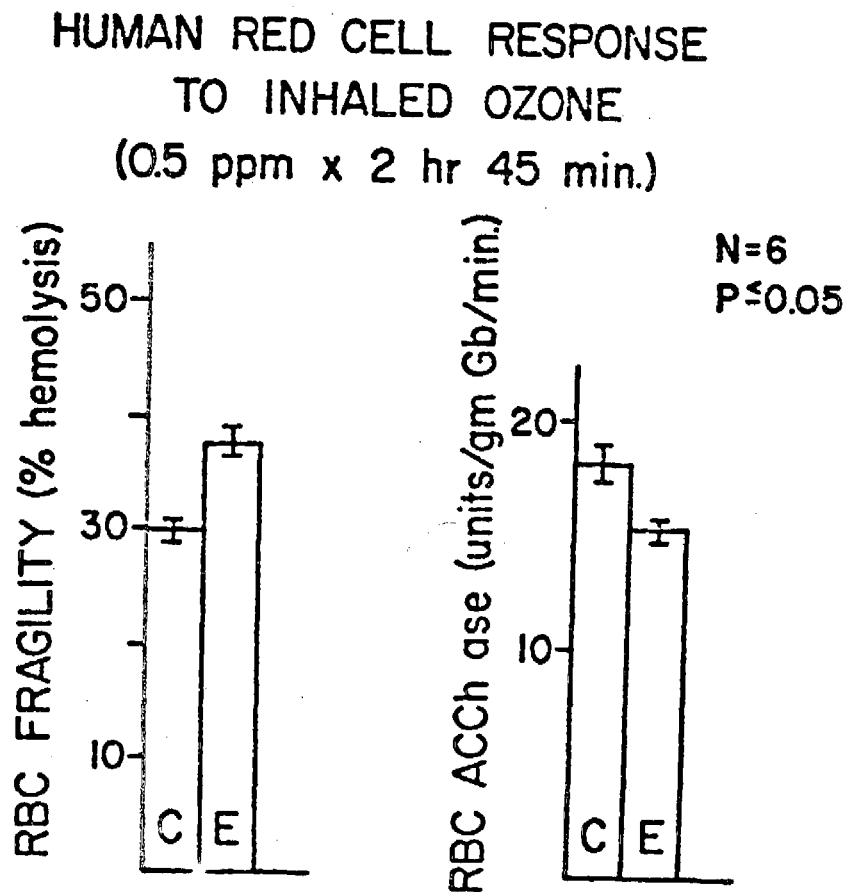
Fragility was measured as % hemolysis in 2% hydrogen peroxide and incubated for 1 hour at pH 7.4 in Krebs Ringer bicarbonate buffer.

Acetylcholinesterase was measured at pH 8.0 in 0.1 M. phosphate buffer employing acetylthiocholine as substrate. Activity is expressed as mM/ml blood/min.

FIGURE 9

HUMAN RED BLOOD CELL
RESPONSE TO OZONE
(0.5 ppm x 2 hr 45 min)
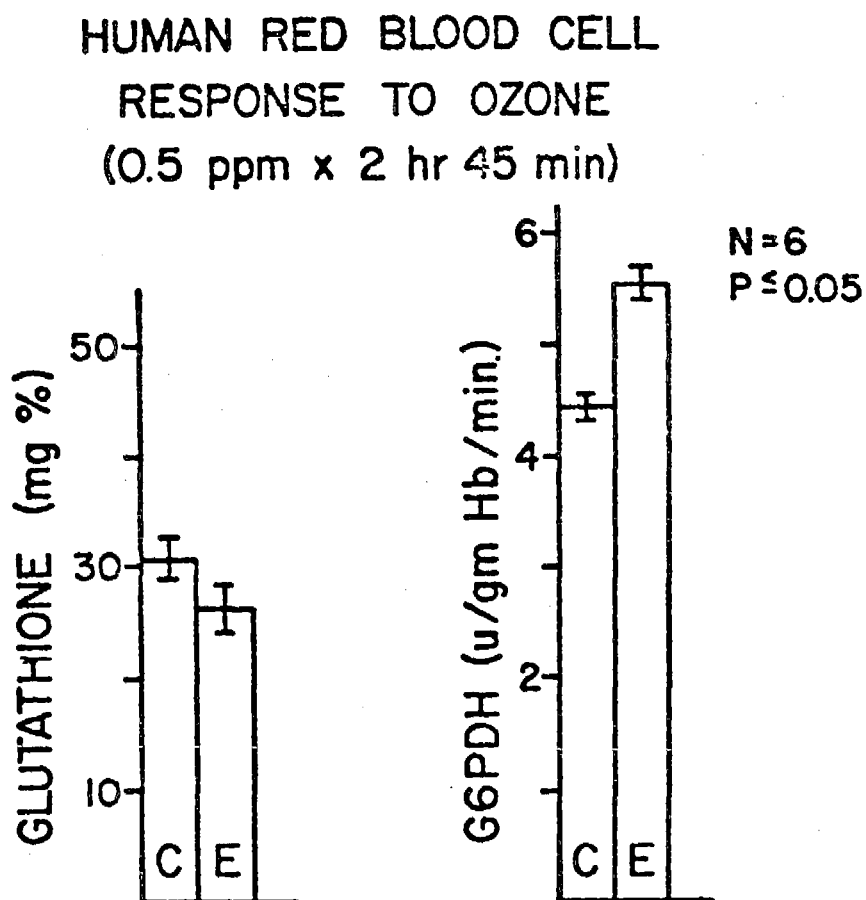
Glutathione assay detects soluble GSH employing 5, 5' dithiobis
(2-nitrobenzoic acid) as coupling reagent.

FIGURE 10

HUMAN RED BLOOD CELL
RESPONSE TO OZONE
(0.5 ppm x 2 hr 45 min)

N=6
P≤0.05
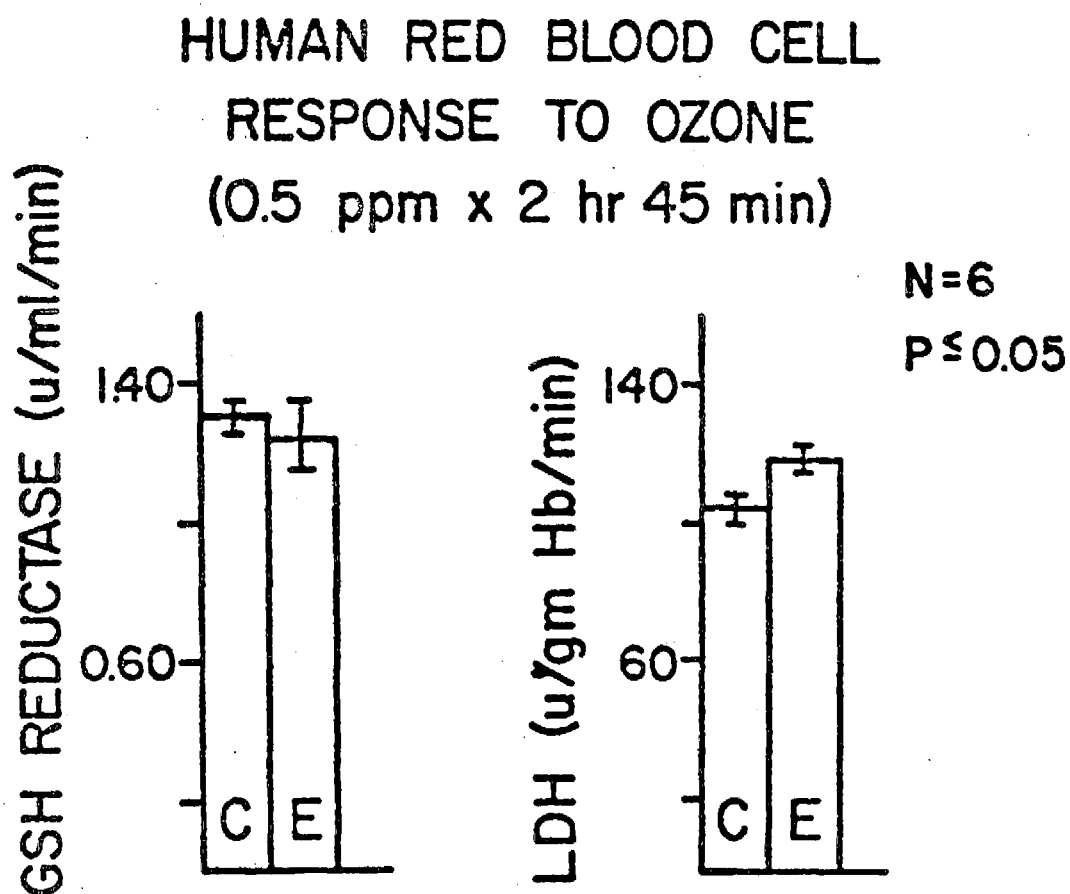
Glutathione reductase activity was expressed as international units/ml of blood/min. Oxidized glutathione was used as substrate.

Lactate dehydrogenase activity was measured by following the disappearance of NADH at 340 nm and was expressed as international units/gl hemoglobin/min.

FIGURE 11

HUMAN INHALATION OF OZONE-SERUM
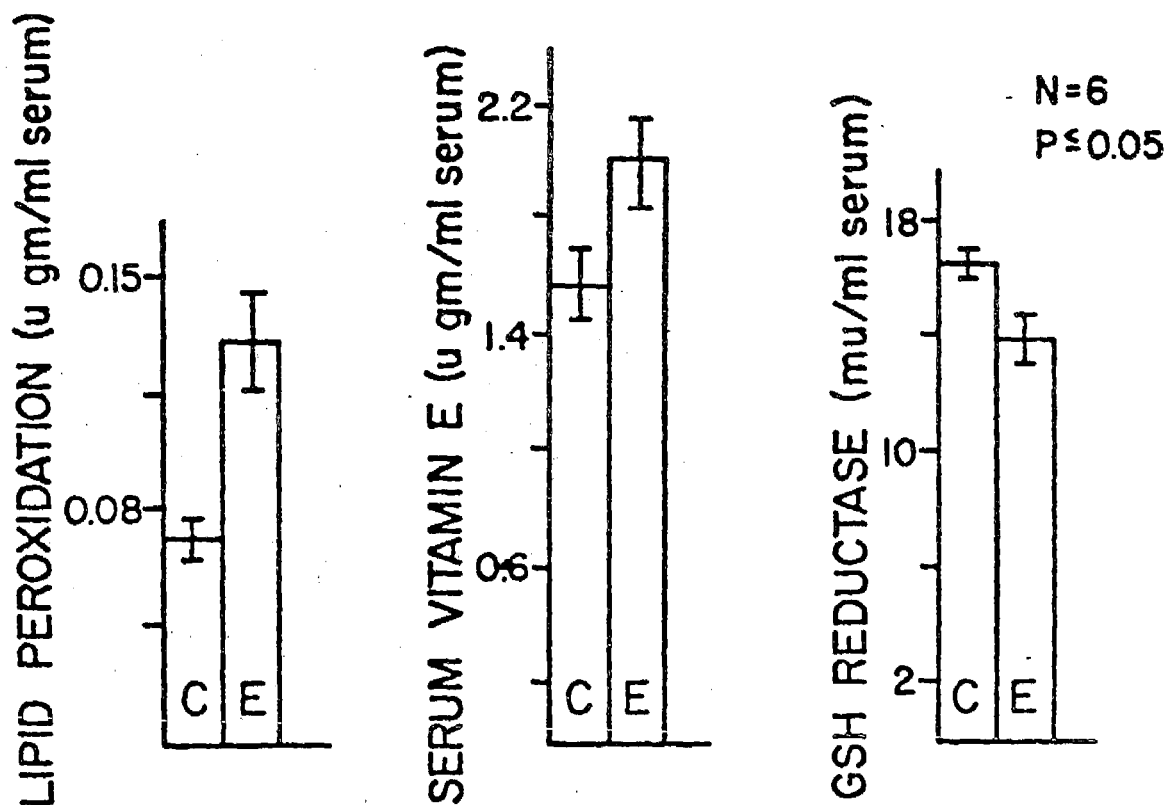
, Lipid peroxidation is expressed as micrograms malonaldehyde per ml serum.

Vitamin E is expressed as microgram alpha tocopheral per ml serum.

Glutathione (GSH) reductase activity was expressed as m units/ml serum/min.

pollutants studied were ozone (O_3), nitrogen dioxide (NO_2) and carbon monoxide (CO) together with elevated temperature. A divided attention task was given at the end of the exposure period. The subjects' heart rate variability, a potential psychophysiological measure of attention, was also evaluated. Subjects were run in three different groups. (Groups 1, 2, and 4)

Subjects displayed a significant decrement in peripheral attention associated with elevated ambient temperature. Effects attributable to pollutant gases were variable. Subjects in Group 1 (4-5 hr exposure to 0.5 ppm O_3 + 0.5 ppm NO_2 + 30 ppm CO) showed some decreased attention when exposed to the mixed pollutants. This occurred in the ability to detect stimuli in the periphery. Subjects in the fourth group (2 hr exposure to 0.25 ppm O_3, 0.30 ppm NO_2, and 30 ppm CO) displayed a decreased ability to perform the central attention task when exposed to the mixed pollutants. However, a decrement in peripheral attention was not shown. Subjects in the second group (4-5 hr exposure to 0.5 ppm O_3) showed only marginal effects. These subjects, however, were not exposed to the mixed pollutants with CO.

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CHAPTER V.

HEALTH CONSEQUENCES OF SULFUR OXIDES

Carl M. Shy, M.D. [*]

CHAPTER V.

Table of Contents

INTRODUCTION

In this final paper of the CHESS monograph, we will summarize results

from the different CHESS areas, make comparisons of data obtained for each

health indicator, and attempt to draw conclusions concerning the level of

pollutant exposure associated with undesirable health effects. Epidemiologic

studies grouped into the CHESS program provided dose-response information

relating short-term and long-term sulfur oxide exposures to adverse health

effects.[1] The individual research reports presented in the monograph often

suggested pollutant-disease associations but left a number of problems

unanswered. These problems include (1) the relative contribution of various

air pollutants, especially sulfur dioxide, total suspended particulates and

suspended sulfates to observed disease frequencies; (2) the importance of

intervening influences, or covariates, such as occupational exposures, socio-

economic status, residential mobility, cigarette smoking; (3) the association

between chronic disease prevalence and current vs. past pollutant exposures;

(4) the precise pollutant threshold for excess disease in exposed communities.

Obviously, epidemiologic studies alone cannot resolve all, or any one,

of the above problems. The findings of this monograph must be substantiated

by replicated observations in different years and under different circumstances.

Well controlled human and animal studies are required to isolate several of

the important intervening variables which are inherent to studies of free

living populations, and to elucidate the precise nature of the pollutant-

disease relationship. Hence, the conclusions put forth in this overview

cannot be definitive, but are offered in the sense of developing more refined

quantitative and scientific hypotheses concerning pollutant-health effect associations in a real-life environment. In the CHESS program itself, we are repeating our observations, using essentially the same health indicators in the same (and more) communities.[1] These results will provide one form of data verification required for scientifically defensible air quality standards.

In relating observed health effects to possible pollutant thresholds, wherever practical and possible, three threshold estimates were provided: a "worst case estimate," which attributes an observed adverse health effect to the lowest pollution exposure suggested by the epidemiologic studies after considering only the strongest and most established covariates; a "least case estimate," which attributes an observed adverse health effect to the highest pollution exposure level suggested by the epidemiologic studies after considering effects of all covariates and a "best judgment estimate" based upon a synthesis of several studies. The best judgment estimate duly considers interactions between pollutants and is at times based upon special analyses that were necessary when individual studies raised questions regarding interactions involving pollutants or intervening variables.

A. Responses to Long-Term Pollutant Exposures

1. Summary of Chronic Respiratory Disease Studies

Chronic bronchitis prevalence rates observed in four CHESS areas are given in Table 1.[2-5] In each of the four studies, a very consistent pattern of excess chronic bronchitis (as defined in this monograph) was found among residents of more polluted communities. In each case, these differences were statistically significant. Mean respiratory symptom scores, which

take into account less severe as well as more classical chronic respiratory symptoms, are tabulated for each CHESS area in Table 2. Symptom scores substantiate the consistent pattern of excess respiratory symptoms among participants from more polluted neighborhoods.

In the New York,[5] Utah,[2] and Idaho-Montana[3] surveys where parents of school children were studied, several consistent findings were observed. For both smokers and nonsmokers there was a male excess of chronic respiratory disease, whether defined in terms of bronchitis prevalence or of symptom scores. Chronic bronchitis rates and symptom scores were higher among male and female smokers. Finally, male and female nonsmokers, exsmokers and current smokers had higher chronic bronchitis rates and symptom scores in the high as opposed to the low exposure areas.

Despite considerable variation in the population characteristics and pollutant exposures of the above three CHESS areas, the relative contribution of cigarette smoking alone was greater than the effect of the air pollution gradient (Tables 3 and 4), with the exception of males in New York. Among males in the Salt Lake and Rocky Mountain CHESS areas, and among all females, air pollution alone was associated with an excess bronchitis rate (when compared with nonsmokers of low exposure neighborhoods) ranging from 1.5 to 3.8 percent. Among New York City males, this excess was 11.3 percent - an unusually high figure requiring verification in subsequent study years. Cigarette smoking alone accounted for an excess bronchitis rate of 9.3 to 16.6 percent in the three CHESS areas. Thus, the relative

contribution of air pollution alone ranged from one-third to one-seventh

as strong as that of cigarette smoking as a determinant of chronic

bronchitis prevalence in communities (with the exception of males in

New York, where air pollution appeared to make a slightly larger contribution

than smoking - a finding difficult to accept in the light of other evidence).

The range of observed differences in the relative contributions of smoking

and pollution is not surprising, in view of the quantitative and qualitative

differences in pollution profiles of the communities studied, as well as the

community differences in smoking patterns. The sum of the evidence suggests

that, while personal cigarette smoking is the largest determinant of

bronchitis prevalence among parents of school children, air pollution

itself is a significant and consistent contributing factor, leading to

increased bronchitis rates in nonsmokers as well as smokers from polluted

communities.

Among young white military recruits studied in the Chicago area,[4]

air pollution was associated with a considerably smaller excess in bronchitis

rates (Table 3) than was found in the other CHESS areas, and the contribution

of air pollution was relatively much less than that of cigarette smoking.

However, there was evidence that even among these young (18-24 year old)

inductees, respiratory symptoms were more prevalent among persons from

more polluted communities. Black and white inductees showed similar

effects of pollution on bronchitis rates (Tables 1 and 3). In the case

of blacks, these effects were superimposed on higher base rates among

persons residing in relatively clean outstate areas. Whether these high

rates are attributable to sources of indoor pollution or other environmental

factors, whether these baseline rates are indeed verifiable remains to be studied. Strangely, cigarette smoking alone was associated with no excess bronchitis in blacks, while cigarette smoking and air pollution combined accounted for more bronchitis than the additive effect of both pollution and smoking. Among whites, air pollution and cigarette smoking were generally additive in their effect on bronchitis rates among smokers within polluted communities of each of the four CHESS areas.

Attempts were made to assess the length of residence in polluted areas required for development of excess bronchitis rates.[2-4] These findings should be accepted in a very preliminary vein because relatively small sample sizes were available for analysis after populations were finely subdivided into smoking and residence-duration specific groups. The overall evidence suggests that immigrants into polluted areas reported excess chronic bronchitis after two to seven years of exposure. Further evidence from the New York study[5] indicated that movement from polluted to clean communities could effect a substantial decline in bronchitis rates, while migration into a polluted community seems to result in high bronchitis rates like those of the long-time residents of high exposure neighborhoods. These conclusions should be taken as hypotheses for further testing, but they justify some optimism about current efforts to improve air quality. An important feature of the CHESS program is the plan to re-survey residents of the high exposure neighborhoods during and after achievement of desirable air quality.[1] These studies can provide considerably more firmness to the conclusions stated in this monograph.

Other covariates such as age, race, sex, socioeconomic status and occupational exposure were controlled, insofar as possible, by the selection of study areas and by appropriate adjustments in statistical analyses. Covariates other than occupational exposure played a relatively minor role as determinants of bronchitis prevalence. Participants with known occupational exposure were analyzed separately, and in none of the above quantitative assessments concerning air pollution and cigarette smoking was the occupationally exposed group included. Occupational exposure to irritating dusts, fumes and aerosols added to the effects of ambient air pollution and cigarette smoking in producing a higher prevalence of chronic bronchitis among exposed workers.[2,3] In general, occupational exposures made a quantitative contribution somewhat larger than that of air pollution and one-half as large as cigarette smoking.

Table 5 lists current (i.e. during the year of the survey) and past exposures[6-9] (within 10 years) estimated for those communities in which excess bronchitis was observed in the four CHESS studies. The precise exposure or dose which should be associated with excess respiratory symptoms could not be determined because accurate measures of past exposures were not made and the duration of exposure required to produce excess respiratory disease is not known. Current exposures may be taken as a worst case estimate for the chronic bronchitis effect, and past exposures as a least case estimate. In the best judgment of the investigators, excess chronic bronchitis in the Salt Lake Basin could be reasonably attributed to sulfur dioxide levels of 92 to 95 $\mu g/m^3$ and/or suspended sulfate levels of 15 $\mu g/m^3$. This was the

only CHESS area in which low concentrations of total suspended particulates occurred in the face of elevated sulfur oxide pollution. Pollutant concentrations measured in 1971 were unlikely determinants of the excess bronchitis rates in the high exposure Salt Lake Basin community. In the other CHESS areas, combinations of particulate matter and sulfur oxide exposures occurred, and the investigators judged that the lowest pollutant concentrations which could reasonably be associated with excess chronic bronchitis were past exposures to 100-177 $\mu g/m^3$ sulfur dioxide, 80-118 $\mu g/m^3$ total suspended particulates and 9-14 $\mu g/m^3$ suspended sulfates. The individual contribution of each pollutant could not be identified.

From these data, it appeared that excess bronchitis may be reasonably associated with community exposures to sulfur oxides alone, in the form of annual levels of 92 to 95 $\mu g/m^3$ SO_2 and 15 $\mu g/m^3$ suspended sulfates. When higher levels of particulate matter are present, annual exposures to 100 $\mu g/m^3$ SO_2, 120 $\mu g/m^3$ total suspended particulate and 14 $\mu g/m^3$ suspended sulfate are reasonably associated with excess bronchitis. None of the CHESS areas experienced elevated exposures to total suspended particulates without concomitant increases in sulfur oxide levels. Overall, these data support the existing primary national standards of 80 $\mu g/m^3$ annual mean (arithmetic) for SO_2 and 75 $\mu g/m^3$ annual mean (geometric) for total suspended particulates. A national standard for suspended sulfates has not been established.

2. Summary of Lower Respiratory Disease Studies of Children

In the lower respiratory disease (LRD) studies conducted in the Salt Lake Basin and Rocky Mountain CHESS areas,[10,11] three findings were consistently observed. First, for all combinations of disease and numbers of illness episodes, no significant association between total LRD and pollution was found for children whose parents had been residents of their communities for less than three years. Second, for single and repeated episodes of croup and repeated episodes of any LRD, families of children who had lived three or more years in the high exposure communities reported more illness across all ages of children from 0 to 12 years than did their counterparts in the less polluted communities. Third, for single and repeated illness episodes and for residence duration, there was no association of pollution exposure with pneumonia or number of hospitalizations for total LRD. The only inconsistencies noted were that for children who had lived three or more years in their community both single and repeated episodes of bronchitis and single episodes of any LRD were significantly associated with pollution exposure in the Utah study, whereas these associations were not found in the Rocky Mountain study.

The effects of the age and socioeconomic covariates were very consistent in the two LRD studies. In almost every instance, significantly higher illness rates occurred at younger ages, while there were very few significant associations with socioeconomic levels of the household. In Utah, no significant differences in illness rates of males and females were

observed. In the Rocky Mountain study, male and female children who had lived in their communities for less than three years had similar illness rates with the exception of single episodes of croup. But, in every instance, male children who had been residents of their community for three or more years had higher illness rates than females. The reason for this inconsistent sex effect is unknown.

The increase in the rates of single or repeated episodes of LRD, croup, and bronchitis attributable to high air pollution exposure can be determined from the data in Table 6. This table gives the illness rates over a three year reporting period for children who had been residents of their communities for three or more years. During the three year periods covered by the two studies, the mean annual SO_2 concentrations in the high exposure communities were 92 $\mu g/m^3$ in the Salt Lake Basin study and as high as 177 $\mu g/m^3$ in the Rocky Mountain study. Hence, a "worst case" estimate of the annual SO_2 concentration associated with increased LRD is 92 $\mu g/m^3$ while the "least case" estimate is 177 $\mu g/m^3$. (The national primary standard for SO_2 is 80 $\mu g/m^3$ annual arithmetic mean). During the same periods, mean annual suspended sulfate concentrations in the high exposure communities were 15 $\mu mg/m^3$ in the Salt Lake Basin study and as low as 7.2 $\mu g/m^3$ in the Rocky Mountain study. For suspended sulfates, a "worst case" estimate of the annual concentration associated with increased LRD is 7.2 $\mu g/m^3$ while the "least case" estimate is 15 $\mu g/m^3$. (A national standard for this pollutant does not exist.) Total suspended particulate levels in the Rocky Mountain communities ranged from 65 to 102 $\mu g/m^3$, representing the worst case and least case estimates respectivel for this pollutant. (The national primary standard for total suspended particu- lates is 75 $\mu g/m^3$ annual geometric mean.)

It is interesting to note that larger increases in total LRD and two of its components were observed in the high pollution community of the Salt Lake Basin study than in the corresponding communities in the Rocky Mountain study. Also, the mean annual suspended sulfate concentration was higher in the high pollution community in the Salt Lake Basin study than in the Rocky Mountain study. The opposite was true for SO_2. This suggests that increases in LRD frequency are probably associated with suspended sulfates rather than SO_2.

Several cautions should be remembered when interpreting the results of the LRD studies. First, the data were collected by asking the childrens' parents about illness frequency over a three year period. Hence, the recall ability of the parents could affect the validity of the data as could the degree of cooperation of the parents. However, it does not seem likely that this source of error affected the communities differently and thereby affected the community comparisons. Second, there could be differences in diagnostic criteria among the communities in a study. In both LRD studies, a sample of physicians were asked to diagnose six respiratory syndromes so that a determination of differences in diagnostic criteria could be made. No differences were found in either study. Third, the communities observed in the studies were mainly white and middle class. Therefore, the results of these studies may not apply to other ethnic or socioeconomic groups. Fourth, a majority of the pollution exposure data in both studies was estimated from emissions data. The degree to which these give reasonable estimates of individual exposures may be questionable.

On the basis of the two LRD studies in the monograph, and in the best judgment of the investigators, it seems reasonable to conclude that there is a positive association between lower respiratory disease frequency in children and pollution exposure, and that excess respiratory disease may reasonably be associated with community exposures to approximately 95 $\mu g/m^3$ SO_2 and 15 $\mu g/m^3$ suspended sulfates. From these studies, there is no evidence that elevated levels of total particulate matter are required to produce the adverse effect.

3. Summary of Acute Respiratory Disease Studies of Families

Table 7 summarizes findings for total acute respiratory disease (combined upper and lower tract disease) among family members in the Chicago[12] and New York studies.[13] With the exception of fathers, who often have greater occupational exposures and daily changes of exposure due to place of work, a consistent excess acute respiratory disease rate was reported among family members living in more polluted neighborhoods. The relative excess in acute respiratory illness rates within more polluted neighborhoods varied from 3 to 40 percent. A range estimate of 5 to 20 percent relative excess includes all but the most extreme values. Unfortunately, the low exposure community for the Chicago study also had elevated pollutant concentrations of sulfur oxides and particulates; this community does not therefore afford a satisfactory baseline illness rate. In New York, the "High I" (or "Intermediate I") community consistently reported considerably higher illness rates in all

family segments than in the "High II" community, even though measured pollutant concentrations were somewhat lower in the "High I" neighborhood. Other environmental factors, including the proximity of a large international airport, may have influenced the illness reporting of residents in the "High I" New York community. For these reasons it was difficult to determine the magnitude of excess illness associated with specific pollutant levels in these two studies. A conservative estimate (i.e. closer to the least case than the worst case estimate) would be that exposures (see Table 8) to 210 $\mu g/m^3$ SO_2, with 104 $\mu g/m^3$ total suspended particulates and approximately 16 $\mu g/m^3$ suspended sulfates was associated with a 5 to 20 percent excess of acute respiratory illness in various family members. This estimate largely discounts the high illness experience of the "High I" New York community and the relatively low current SO_2 levels in New York and Chicago. Further observations of acute respiratory illness in these and other CHESS areas are being made and should considerably refine the quantitative estimates given above.

The Chicago study[12] also provided evidence of increased susceptibility to epidemic A_2/Hong Kong influenza among otherwise healthy families exposed during the previous three years to atmospheric levels of 106 to 119 $\mu g/m^3$ SO_2, 151 to 159 $\mu g/m^3$ total suspended particulates, and 14 $\mu g/m^3$ suspended sulfates.

The effects of other factors on the incidence of ARD were most interesting (Table 9). In both Chicago and in the New York City areas, socioeconomic status was a significant factor in the incidence of upper

respiratory disease; more illness was reported by respondents of the upper-middle socioeconomic level. No effect of socioeconomic level on reporting of lower respiratory disease was observed.

Personal cigarette smoking apparently had little effect on the initial contracting of respiratory infections but was a significant determinant in the development of lower tract illness as a result of the initial infection.

Parental smoking also was a significant factor in the development of lower tract illness among nonsmoking young members of their households. This latter is a most significant association and provides additional evidence that smoking is more than a means of self pollution and affects other individuals in the immediate environment as well.

Data collected during studies of ARD are difficult to interpret because in addition to the effects of all other environmental factors, the incidence of illness depends first of all on exposure to infectious agents. The fact that many of these agents are more virulent than others in itself may affect the ease with which infections are recognized and reported. Also a mild attenuated agent may protect against infection with a more virulent one, thus the recognizable illness rate may depend upon the sequence in which successive exposures to infectious agents occur. These difficulties must be recognized, but controlling for them is impossible without a prohibitively large and expensive laboratory activity.

Consequently, the credibility of the results depends not only on the careful

collection and analysis of data, but also on the reproducibility of associations

between increased illness and higher pollution exposure. This latter factor

provides the greatest strength to the data reported in this monograph. The

consistency with which increased illness rates were observed to be associated

with higher pollution exposure levels in different parts of the country and

in the various segments of the population add greatly to the credibility of

the results.

Differences observed between metropolitan areas, e.g. between New York and

Chicago, were anticipated to be greater than those that would be found between

neighborhoods within a single area. These differences can be accounted for

by the fact that data were collected in each metropolitan area by different

survey groups; thus techniques were consistent within the same area but may

have varied somewhat from area to area as a result of differences in execution

of the same study protocol. Furthermore, a more conservative definition of

lower respiratory symptoms was employed in the Chicago than in the New York

surveys. As a result lower respiratory diseases were reported at lower

frequencies in Chicago than in New York. These definitions have since

been standardized for all CHESS areas.

4. Summary of Pulmonary Function Studies

Ventilatory function of elementary school children, measured by the

three-quarter second forced expiratory volume ($FEV_{0.75}$), was diminished in

areas of elevated exposure to sulfur oxides. In all cases, observed

decrements were subtle. In the New York study,[14] only the older children (age nine to 13 years) who had been exposed to substantially elevated pollutant concentrations for the first five to ten years of life suffered reduced ventilatory function. The best available estimates of these remote annual average exposures were as follows: sulfur dioxide, 131-435 $\mu g/m^3$; total suspended particulates, 75-200 $\mu g/m^3$; suspended sulfates, 18-28 $\mu g/m^3$.

From the New York study, the authors could not determine the relative importance of specific pollutants in reducing ventilatory function. From the Cincinnati study,[15] however, suspended sulfates emerged as a pollutant of particular concern. In all Cincinnati neighborhoods, sulfur dioxide concentrations were at or below the moderate level of 57 $\mu g/m^3$ during the time of testing, permitting suspended sulfates to be assessed in the relative absence of sulfur dioxide. Ventilatory function in white children exposed to suspended sulfate concentrations of about 9.5 $\mu g/m^3$ was lower than that of white children exposed to concentrations of about 8.3 $\mu g/m^3$. Black children in Cincinnati were all exposed to suspended sulfate concentrations of about 8.9 $\mu g/m^3$, and these children demonstrated no differences in ventilatory function.

From the study results, the authors developed "worst case," "least case," and best judgment estimates of pollution exposures required to reduce ventilatory function (Table 10). From the Cincinnati study, it was conceivable that one year's exposure to 9 $\mu g/m^3$ of suspended sulfates, in the presence of moderate levels of sulfur dioxide and total suspended

particulates (57 μg/m^3 and 96 μg/m^3, respectively) might alone account for reduced ventilatory function. The New York study strongly indicated a more moderate interpretation, however. It was the authors' best judgment that eight to nine years of exposure to about 10 to 13 μg/m^3 of suspended sulfates might reduce ventilatory function. If these suspended sulfate exposures were accompanied by exposures to about 200-250 μg/m^3 of sulfur dioxide and about 100-150 μg/m^3 of total suspended particulates, further reductions in FEV$_{0.75}$ might be expected.

Clearly, these best judgments are based on suggestive, not conclusive, evidence. In Cincinnati, for example, the socioeconomic-racial patterns of exposure to suspended nitrates were very similar to suspended sulfate exposure patterns. Though absolute levels of suspended nitrates were much lower than suspended sulfates, possible effects of suspended nitrates could not be ruled out. Also, the ventilatory performance of black children in Cincinnati remains somewhat confusing. At present, it is impossible to disentangle the effects of objective environmental factors from these childrens' possible subjective responses to the all-white testing teams.

The contribution of other covariates to pulmonary function results in school children is summarized in Table 11. Height, age, sex and race are well recognized in the literature as significant determinants of pulmonary function in children, and these variables were taken into account in analyzing the CHESS data. Table 12 summarizes the CHESS pulmonary function findings to date. These studies have been repeated in New York and other CHESS areas for two successive years and will be reported in subsequent papers.

B. Responses to Short-Term Pollutant Exposures

    1. Summary of Studies on Panels of Asthmatics and Cardiopulmonary
        Subjects

    In contrast to the health indicators previously summarized in this paper, studies on panels of subjects gave the investigators the opportunity to relate daily changes in symptom status to daily changes in pollutant levels. One pattern immediately emerged from the asthma studies conducted in the Salt Lake Basin[16] and New York.[17] As shown in Table 13, daily asthma attack rates in the Salt Lake Basin were more consistently correlated with colder outdoor temperature than with any measured pollutant. Therefore, an analysis of asthma attack rates against daily pollutant concentrations was carried out within two temperature ranges: 30 to 50° F and greater than 50° F. These dara are summarized, for SO_2, total suspended particulates (TSP) and suspended sulfates (SS) in Figures 1 and 2 for Salt Lake and in Figures 3 and 4 for New York. Inspection of these figures reveals one quite consistent finding: asthma attack rates were most closely related to stepwise increases in the levels of suspended sulfates. Virtually no relationship between SO_2 and attack rates appeared. Total suspended particulates (with the exception of Figure 4) and suspended sulfates (with the exception of Figure 1) were positively and stepwise correlated with daily asthma attack rates. In the Salt Lake Basin, where the effects of total particulates and suspended sulfates were partitioned, a higher frequency of asthma attacks was observed at the same daily TSP concentration when a high sulfate fraction was present in the atmosphere (Figure 5). Thus, it appeared that sulfate levels were a stronger determinant than TSP of asthma attack rates in the Salt Lake Basin. However,

in the cold dry climate of the Basin, the effect of cold temperatures was considerably stronger than that of sulfates (Figure 6), and the pollutant threshold for the asthma response was much higher in colder than in more moderate temperatures.

In New York, asthma attack rates were more consistently associated with daily suspended sulfate levels than with either SO_2 or TSP (Figures 3 and 4). As in Utah, the pollutant threshold for the asthma response was higher in colder than in more moderate temperatures (Figure 7), but unlike Utah, attack rates were generally higher on days with more moderate than with colder temperatures.

Thus, the effect of temperature was somewhat inconsistent between the two study areas; colder temperatures were associated with higher attack rates in the Salt Lake Basin but not in New York. It is difficult to compare the temperature effect in the two studies because they extended over different seasons of the year. In each case, the sulfate threshold was higher on colder days; and, in each case, elevated daily sulfate levels were quite consistently associated with increased asthma attack rates.

The pattern of daily aggravation of symptoms in cardiopulmonary subjects in New York[16] was very similar to that of asthma with respect to temperature and pollutants. In each of the three New York neighborhoods, cold temperatures were directly related to increased symptom rates in subjects with combined heart and lung disease (Table 14). Elevated suspended sulfates were the only pollutant consistently associated with symptom

aggravation, as shown in Table 14 and Figures 8 and 9. Daily SO_2 and TSP could not be associated with symptom aggravation in the heart and lung panel, which was the most sensitive to variations in daily pollutant concentrations.

The pollutant thresholds for SO_2, TSP and suspended sulfates among the several cardiopulmonary and asthmatic panels at different temperature ranges are summarized in Table 15. Although this table presents pollutant thresholds for SO_2 and TSP, the above discussion should make it clear that suspended sulfate levels demonstrated the only consistent relationship with daily aggravation of symptoms in these diseased panelists. Thus, while adverse effects were occurring at daily concentrations below the short-term (24-hour) primary standard for SO_2 and TSP, the investigators would attribute these effects to suspended sulfate concentrations on those days rather than to SO_2 or TSP. It was the best judgment of the investigators that significant aggravation of cardiopulmonary symptoms could be attributed to 24-hour suspended sulfate levels as low as 8-10 $\mu g/m^3$ on cooler days (20-40°F) or warmer days (41+°F). The investigators intuitively felt that the chemical composition and particle size involved in sulfate exposures were critical determinants of the threshold for the adverse response. Since these sulfate-symptom relationships were manifested even in the low exposure communities of the Salt Lake and New York CHESS areas, there was evidence that suspended sulfates emanating from point or urban sources penetrated well beyond the suburban ring and adversely affected persons living in more distant communities. Such penetration might involve smaller respirable particles,

acid mist or other atmospheric transformation products of sulfur oxide emissions. The magnitude of such remote exposures and their overall health importance cannot yet be quantified.

C. Conclusions

In this CHESS monograph, we have brought together an original series of studies describing a variety of pollutant relationships with several health indicators. The pollutants of main concern were sulfur dioxide, total suspended particulates and suspended sulfates. We have examined the impact of community differences in exposure to these pollutants on chronic respiratory disease in adults, acute lower respiratory disease in children, acute respiratory illness in families, aggravation of symptoms in subjects with pre-existing asthma and cardiopulmonary disease, and lung function of school children. These health indicators were selected because past studies by many investigators indicated that the frequency of these responses in a community was affected by sulfur oxides and particulates. Our studies more than substantiate these findings.

Our results can be divided into two groups: (1) health indicators responsive to cumulative, long-term pollutant exposures and (2) health indicators sensitive to daily or shorter-term variations in pollutant exposure. Least case, worst case, and best judgment estimates concerning the pollutant thresholds for long-term exposures are given in Table 16. Best judgment estimates are recapitulated for long-term exposures in Table 17. Our findings support the existing national primary standard for long-

term, or annual average, exposures, insofar as we have measured the desirability of these standards in terms of chronic respiratory disease in adults, acute lower respiratory disease in children, acute respiratory disease in families, and lung function of children. With regard to short-term exposures, least case, worst case, and best judgment estimates were given in Table 15, and best judgment estimates are recapitulated in Table 18. Our data indicate that adverse effects on elderly subjects with heart and lung disease, and on panels of asthmatics, are being experienced even on days below the national primary standard for 24-hour levels of SO_2 and total suspended particulates. However, as is evident from the presentation given above, these adverse health effects should be attributed to suspended sulfate levels rather than to the observed concentrations of SO_2 and TSP. The consistency of the relationship between symptom aggravation and sulfate levels, and the lack of consistency for this relationship with other pollutants, leads us to this conclusion.

Having identified atmospheric suspended sulfates as an environmental pollutant of present concern to health, we by no means have acquired sufficient intelligence to establish a national standard for this pollutant. We know little about the environmental determinants of atmospheric suspended sulfates, and less about the means to control sulfate levels or to bring about significant reductions in sulfate concentrations in urban, suburban and rural areas (particularly of the northeastern U.S.). In identifying the need for control of sulfates, we have raised a series of unstated questions and issues. Are all sulfates equally biologically reactive?

Are sulfates reactive because of the chemical properties associated with specific chemical compounds, or because of physical properties such as particle size or pH? Are sulfates equally reactive in humid and dry air, at warm and cold temperatures? These biological issues must be addressed and satisfactorily resolved, because our strategies to control sulfate levels may be critically dependent on the nature of the sulfate-biologic response relationship. If acid mist is the problem, we may be able to neutralize the sulfur oxides emitted at the source, without more stringent reductions in sulfur oxide emissions than are presently required to achieve primary national standards. On the other hand, if atmospheric transformation products of SO_2 are implicated, we may be forced to restrict even more severely the sulfur content of fossil fuels. Until more definition of these issues is achieved, however, our findings strongly argue against any measures that would allow more sulfur loading of the atmosphere.

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TABLE 1

SMOKING AND SEX SPECIFIC CHRONIC BRONCHITIS PREVALENCE RATES (PERCENT) BY
COMMUNITY EXPOSURE IN FOUR CHESS AREAS

CHESS Area Pollutant Exposure *	Percent Chronic Bronchitis					
	Nonsmoker		Exsmoker		Smoker	
	Male	Female	Male	Female	Male	Female
Salt Lake Basin						
Low	3.0	2.3	2.6	5.3	19.9	17.8
Intermediate I	3.6	2.0	3.4	4.0	18.6	14.7
Intermediate II	2.3	4.7	5.4	7.0	20.1	15.3
High	6.8	5.2	6.0	7.1	26.8	22.2
Rocky Mountain						
Pooled Low	1.25	1.08	1.45	3.12	17.05	11.78
Pooled High	3.47	2.54	4.82	2.80	18.63	12.88
Chicago						
Black						
Low	8.8				8.8	
Intermediate	7.8				12.7	
High	9.3				13.0	
White						
Low	4.2				17.6	
Intermediate	5.4				18.8	
High	5.4				17.8	
New York						
Low	4.6	2.0	13.9	3.8	13.9	13.9
High I	18.0	7.5	18.0	9.0	21.3	19.8
High II	14.2	4.9	18.7	4.5	22.1	16.6

*Refer to previous reports in this monograph (References 2-5) for numerical
data on current and past exposures. The pollutant gradients presented in
this column do not represent equal class intervals.

TABLE 2

MEAN RESPIRATORY SYMPTOM SCORES FOR ALL STAGES OF CHRONIC RESPIRATORY SYMPTOMS IN FOUR CHESS AREAS

CHESS Area Pollutant Exposure*	Mean Symptom Score					
	Nonsmoker		Exsmoker		Smoker	
	Male	Female	Male	Female	Male	Female
Salt Lake Basin						
Low	1.54	1.36	1.48	1.65	2.73	2.57
Intermediate I	1.56	1.37	1.54	1.79	2.88	2.53
Intermediate II	1.48	1.55	1.82	1.83	2.99	2.44
High	1.94	1.73	1.87	2.02	3.32	2.98
Rocky Mountain						
Pooled Low	1.33	1.23	1.41	1.30	2.65	2.20
Pooled High	1.38	1.29	1.58	1.45	2.72	2.34
Chicago						
Black						
Low	2.10				2.47	
Intermediate	2.24				2.61	
High	2.34				2.71	
White						
Low	1.76				2.90	
Intermediate	1.82				2.93	
High	1.84				2.91	
New York						
Low	1.81	1.29	2.05	1.37	2.48	2.30
High I	2.41	1.76	2.56	2.00	2.76	2.68
High II	2.35	1.61	2.51	1.72	2.76	2.53

*Refer to previous reports in this monograph (References 2-5) for numerical data on current and past exposures. The pollutant gradients presented in this column do not represent equal class intervals.

TABLE 3

MALES: EXCESS CHRONIC BRONCHITIS ATTRIBUTABLE TO AIR POLLUTION AND SMOKING, AND RELATIVE CONTRIBUTION OF EACH FACTOR

Factor	Excess Chronic Bronchitis Prevalence (Percent)*				
	New York	Salt Lake	Rocky Mountain	Chicago	
				Whites	Blacks
Air Pollution Alone	11.3	3.8	2.2	1.2	0.7
Smoking Alone	9.3	16.6	15.8	13.4	0.0
Air Pollution + Smoking	17.1	23.8	17.4	13.6	4.2
$\dfrac{\text{Air Pollution Alone}}{\text{Smoking Alone}}$	$\dfrac{1}{0.82}$	$\dfrac{1}{4.4}$	$\dfrac{1}{7.2}$	$\dfrac{1}{11.2}$	NA

*Excess prevalence:absolute excess above rate experienced by nonsmokers in the low exposure community of the same CHESS area.

NA: not ascertainable

TABLE 4

FEMALES: EXCESS CHRONIC BRONCHITIS ATTRIBUTABLE TO AIR POLLUTION AND
SMOKING, AND RELATIVE CONTRIBUTION OF EACH FACTOR

Factor	Excess Chronic Bronchitis Prevalence (Percent)*		
	New York	Salt Lake	Rocky Mountain
Air Pollution Alone	4.0	2.2	1.5
Smoking Alone	11.9	12.8	10.7
Air Pollution + Smoking	16.1	19.2	11.8
$\dfrac{\text{Air Pollution Alone}}{\text{Smoking Alone}}$	$\dfrac{1}{3.0}$	$\dfrac{1}{5.8}$	$\dfrac{1}{7.1}$

* Excess prevalence absolute excess above rate experienced by nonsmokers in the low
exposure community of the same CHESS area.

TABLE 5

RANGE OF POLLUTANT EXPOSURES ASSOCIATED WITH
EXCESS CHRONIC BRONCHITIS

CHESS Area	Current Exposures (annual average)			Exposures Within Past 10 Years* (annual average)		
	SO_2 $\mu g/m^3$	TSP $\mu g/m^3$	SO_4 $\mu g/m^3$	SO_2 $\mu g/m^3$	TSP $\mu g/m^3$	SO_4 $\mu g/m^3$
Salt Lake	62	66	12.4	92-95	53-70	15.0
Rocky Mountain	177-374	65-102	7.2-11.3	177-374	62-179	6.9-19.9
Chicago	96-217	103-155	14.5	100-282	118-177	14.1-17.3
New York	50-144	63-104	13.2-16	144-404	80-203	9-26
National Primary Air Quality Standard	80	75	-	80	75	-

*Estimated from emissions data and pollutant trends.

TABLE 6

AGE-SEX-SOCIOECONOMIC STATUS ADJUSTED THREE YEAR LRD ATTACK RATES
PER 100 CHILDREN BY POLLUTION EXPOSURE, NUMBER OF EPISODES,
AND STUDY AREA

Disease Category	Pollution Exposure *	Number of Episodes	Study Area	
			Rocky Mountain	Salt Lake Basin**
LRD	Low High	≥ 1	21.3 24.2 (NS)***	27.7 38.2 (p <.001)
	Low High	≥ 2	9.2 12.1 (p <.001)	15.7 23.4 (p <.001)
CROUP	Low High	≥ 1	6.2 11.2 (p <.001)	15.9 26.4 (p <.001)
	Low High	≥ 2	3.1 4.8 (p <.05)	7.2 13.8 (p <.001)
BRONCHITIS	Low High	≥ 1	14.9 15.8 (NS)	16.8 23.6 (p <.001)
	Low High	≥ 2	5.1 6.7 (NS)	7.0 10.8 (p <.001)

*Consult references 10 and 11 for numerical data on air pollution exposures.

**Rates given for low pollution exposure are weighted averages of the age-sex-socioeconomic status adjusted rates for the Low, Intermediate I, and Intermediate II communities.

***Probability that the difference between the two rates is as large or larger than that observed under the hypothesis of no difference. NS denotes p > .05.

TABLE 7

RELATIVE RISK OF TOTAL ACUTE RESPIRATORY ILLNESS IN FAMILIES LIVING
IN NEW YORK AND CHICAGO CHESS AREAS

Family Segment	Community Air Pollution Exposure*	Relative Risk of Total Acute Respiratory Illness	
		Chicago	New York
Preschool Children	Low High I** High II***	1.00 (9.37) 1.06 1.09	1.00 (7.88) 1.40 1.03
School Children	Low High I High II	1.00 (4.56) 1.39 1.06	1.00 (6.22) 1.20 1.03
Mothers	Low High I High II	1.00 (5.00) 1.19 1.19	1.00 (4.45) 1.14 1.05
Fathers	Low High I High II	1.00 (3.09) 0.95 1.19	1.00 (3.44) 1.17 0.88

*Consult references 12 and 13 for numerical data on air pollution exposures.

**High I: In Chicago, equivalent to "High" neighborhood
In New York, equivalent to "Intermediate I" neighborhood

***High II: In Chicago, equivalent to "Highest" neighborhood
In New York, equivalent to "Intermediate II" neighborhood

TABLE 8

RANGE OF POLLUTANT EXPOSURES IN NEW YORK AND CHICAGO NEIGHBORHOODS
HAVING EXCESS ACUTE RESPIRATORY DISEASE RATES IN FAMILIES

CHESS Areas	Current Exposures (annual average)			Previous 2 Years (annual average)		
	SO_2 $\mu g/m^3$	TSP $\mu g/m^3$	SO_4 $\mu g/m^3$	SO_2 $\mu g/m^3$	TSP $\mu g/m^3$	SO_4 $\mu g/m^3$
Chicago						
High I	51	126	(14.5)*	83	135	(14.1)*
High II	106	151	(14.5)	119	159	(14.1)
New York						
High I	50-63	63-84	13.2	144	80	13.4
High II	50-58	87-104	14.3	210	104	16.2
National Primary Air Quality Standard	80	75	-	80	75	-

*Estimate from Chicago Stations of the National Air Surveillance Network.

TABLE 9
EFFECTS OF SELECTED FACTORS
ON ACUTE RESPIRATORY DISEASE

FACTOR	STATISTICAL SIGNIFICANCE OF FACTOR			
	UPPER TRACT DISEASE		LOWER TRACT DISEASE	
	CHICAGO	N.Y.	CHICAGO	N.Y.
AIR POLLUTION	< 0.05	NS	< 0.05	< 0.005
SOCIOECONOMIC STATUS	< 0.001	< 0.005	NS	NS
PERSONAL CIGARETTE SMOKING	NS	NS	< 0.10	< 0.005
PARENTAL SMOKING EFFECT ON CHILDREN	< 0.10	NS	< 0.001	< 0.001

TABLE 10

LEAST CASE, WORST CASE, AND BEST JUDGMENT ESTIMATES OF AIR POLLUTION EXPOSURE
SUFFICIENT TO PROMOTE IMPAIRMENT OF CHILDHOOD VENTILATORY FUNCTION

Type of Estimate	Duration of Exposure (years)	Pollutant (Annual Average in $\mu g/m^3$)		
		Sulfur Dioxide	Total Suspended Particulates	Suspended Sulfates
Worst Case	1	57	96	9
Least Case	9	435	200	28
Best Judgment	8-9	200-250	100-150	10-13
National Primary Air Quality Standard		80	75	-

TABLE 11

Summary of Effects of Covariates Observed in
CHESS Pulmonary Function Studies

Covariate	Effect
1. Height	• In all studies, height was the most significant determinant of $FEV_{0.75}$, being somewhat less important in children aged 5 through 8 years (0.045 liters per inch) than in children aged 9 through 13 years (0.063 liters per inch).
2. Age	• In New York, children aged 9 through 13 years demonstrated area differences in $FEV_{0.75}$ while children aged 5 through 8 years did not. • In all children tested age was a significant determinant of $FEV_{0.75}$, being somewhat more important in children aged 5 through 8 years (0.045 liters per year) than in children aged 9 through 13 years (0.019 liters per year).
3. Sex	• In New York, area differences in $FEV_{0.75}$ were statistically significant for boys aged 9 through 13 years, but not for girls of the same age. • The $FEV_{0.75}$ of boys was consistently higher than that of girls of the same age and height.
4. Race	• In Cincinnati the $FEV_{0.75}$ of black children was consistently lower than that of white children. • In Cincinnati, white children demonstrated area differences in $FEV_{0.75}$, while black children did not.

TABLE 12

Summary of Findings in CHESS Pulmonary Function Studies

Location	Time	Age Group Tested	Findings
Cincinnati:	1967-68	Second Grade	●White children exposed to average suspended sulfate levels of 9.5 $\mu g/m^3$ had lower $FEV_{0.75}$ than white children exposed to average suspended sulfate levels of 8.3 $\mu g/m^3$ ●The $FEV_{0.75}$ of black children did not vary with air pollution exposure ●The $FEV_{0.75}$ of black and white children was lowest in winter. ●The $FEV_{0.75}$ of black children was consistently lower than that of whites.
New York	1970-71	All Elementary Grades	●The $FEV_{0.75}$ of white children aged 9 through 13 years, who had been exposed to high levels of sulfur oxides and particulates during the first decade of life, was lower than that of children who had not been so exposed. ●This finding was statistically significant in males, but not in females. ●The $FEV_{0.75}$ of children aged 5 through 8 years did not vary consistently with pollution exposure. ●The $FEV_{0.75}$ of children in all grades was lowest in winter.

TABLE 13

SIMPLE CORRELATION OF ASTHMA ATTACKS
WITH ENVIRONMENTAL FACTORS

ENVIRONMENTAL FACTORS	CORRELATION IN CHESS AREAS					
	SALT LAKE BASIN			NEW YORK		
	LOW	MID	HIGH	LOW	MID. I	MID. II
MIN. TEMP.	−SS	−SS	−SS	N	N	+S
TSP	N	N	+SS	N	N	N
SO_2	N	N	N	N	N	−SS
SUSP. SO_4	−S	N	+SS	+S	N	N
NO_2	N	−S	N	+SS	N	N

S = p < 0.05   SS = p < 0.01   N = NOT SIGNIFICANT

− = INVERSE CORRELATION   + = POSITIVE CORRELATION

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TABLE 14

NEW YORK HEART AND LUNG PANELS:
CORRELATION OF DAILY EXPOSURES WITH SYMPTOM AGGRAVATION

COMMUNITY EXPOSURE	EXPOSURE TO				
	SO_2	TSP	SS	NO_2	MAX. TEMP.
LOW	0.23*	0.16*	0.28**	0.15*	−0.33**
INTERMEDIATE-I	−0.09	−0.05	0.26**	−0.04	−0.15*
INTERMEDIATE-II	0.08	0.09	0.18*	−0.06	−0.19**

* $p < 0.05$ ** $p < 0.01$

TABLE 15

Summary of CHESS Studies Relating Pollutant Thresholds for Adverse Health Effects to Short-Term Air Quality Standards
for Sulfur Dioxide, Total Suspended Particulates and Suspended Sulfates

Adverse Effect on Human Health	Type of Estimate	Minimum 24-Hour Temperature °F	24-Hour Pollutant Threshold Levels for Adverse Health Effects ($\mu g/m^3$)			
			National Standard	Sulfur Dioxide	Total Suspended Particulates	Suspended Sulfates
			Primary Air Quality Standard	365	260	No Standard
			Significant Harm Level	2620[a]	1000[a]	No Standard
Aggravation of Chronic Heart and Lung Disease Symptoms in the "Well"[11]	Worst Case Least Case Best Judgment	20-40°	Between 81 and 365 / No Effect Below Primary Standard / No Proven Effect Below Primary Standard		No Effect Below Primary Standard / No Effect Below Primary Standard / No Proven Effect Below Primary Standard	0 / No Effect / 8-10
	Worst Case Least Case Best Judgment	>40°	Between 81 and 365 / No Effect Below Primary Standard / No Proven Effect Below Primary Standard		68 / No Effect Below Primary Standard / 80-100	2 / 10 / 8-10
Aggravation of Cardio-Respiratory Symptoms in Elderly Patients With Heart Disease[11]	Worst Case Least Case Best Judgment	>40°	No Effect Below Primary Standard / No Effect Below Primary Standard / No Proven Effect Below Primary Standard		Between 76 and 260 / No Effect Below Primary Standard / No Proven Effect Below Primary Standard	10 / 10-20 / 10
Aggravation of Chronic Lung Disease Symptoms in Elderly Patients with Chronic Lung Disease[11]	Worst Case Least Case Best Judgment	20-40°	No Effect Below Primary Standard / No Effect Below Primary Standard / No Proven Effect Below Primary Standard		Between 76 and 260 / No Effect Below Primary Standard / No Proven Effect Below Primary Standard	6 / No Effect / 10
	Worst Case Least Case Best Judgment	>40°	No Effect Below Primary Standard / No Effect Below Primary Standard / No Proven Effect Below Primary Standard		Between 76 and 260 / No Effect Below Primary Standard / No Proven Effect Below Primary Standard	11 / 12 / 12
Aggravation of Cardio-Respiratory Symptoms in Elderly Patients With Combined Heart and Lung Disease[11]	Worst Case Least Case Best Judgment	20-40°	181 / No Effect Below Primary Standard / No Proven Effect Below Primary Standard		47 / No Effect Below Primary Standard / 80-100	9 / 10 / 10
	Worst Case Least Case Best Judgment	>40°	No Effect Below Primary Standard / No Effect Below Primary Standard / No Proven Effect Below Primary Standard		76 / No Effect Below Primary Standard / No Proven Effect Below Primary Standard	6 / 17 / 10
Aggravation of Asthma Manifest by Higher Attack Rates[9,10]	Worst Case Least Case Best Judgment	30-50°	No Effect Below Primary Standard / No Effect Below Primary Standard / No Proven Effect Below Primary Standard		Between 61 and 75 / No Effect Below Primary Standard / 107	8 / No Effect / 9-10
	Worst Case Least Case Best Judgment	>50°	23 / No Effect Below Primary Standard / 180-250[b]		Between 61 and 75 / No Effect Below Primary Standard / 71	1 / 10 / 8

a/ Significant Harm Levels also consider the probable interaction between sulfur dioxide and suspended particulates by setting as a 24-hour significant harm level the following value: Concentration of Sulfur Dioxide ($\mu g/m^3$) x Concentration of Total Suspended Particulates ($\mu g/m^3$) x A Constant (490×10^3).

b/ This judgment estimate based on the presently reported studies and on the CHESS study of asthma in New Cumberland, W. Virginia which was previously reported. [17]

TABLE 16

SUMMARY OF CHESS STUDIES RELATING LONG-TERM POLLUTANT EXPOSURES INVOLVING SULFUR DIOXIDE, TOTAL
SUSPENDED PARTICULATES AND SUSPENDED SULFATES TO ADVERSE EFFECTS ON HUMAN HEALTH

Adverse Effect on Human Health	Type of Estimate	Duration of Exposure (Years)	Annual Average Levels Linked to Adverse Health Effects ($\mu g/m^3$)		
			Sulfur Dioxide (80)*	Total Suspended Particulates (75)*	Suspended Sulfates (No Standard)
Increase in Prevalence of Chronic Bronchitis in Adults	Worst Case	3	62	65	12
	Least Case	10	374	179	20
	Best Judgment	6	95	100	15
Increases in Acute Lower Respiratory Tract Infections in Children	Worst Case	3	92	65	7.2
	Least Case	3	177	102	15
	Best Judgment	3	95	102	15
Increase in Frequency or Severity of Acute Respiratory Illness in Otherwise Healthy Families	Worst Case	1	50	104	14
	Least Case	3	210	159	16
	Best Judgment	3	106	151	15
Subtle Decreases in Childhood Ventilatory Function	Worst Case	1	57	96	9
	Least Case	9	435	200	28
	Best Judgment	8-9	200	100	13

*National primary ambient air quality standard in parentheses. The particulate standard is a geometric mean and the equivalent arithmetic mean would be about 85 $\mu g/m^3$.

TABLE 17

LONG-TERM EXPOSURE: POLLUTANT THRESHOLDS FOR ADVERSE EFFECTS
(BEST JUDGMENT)

Effect	Threshold, Annual $\mu g/m^3$		
	SO_2	TSP	SS
Increased Prevalence of Chronic Bronchitis	95	100	15
Increased Acute Lower Respiratory Disease in Children	95	102	15
Increased Frequency of Acute Respiratory Disease in Families	106	151	15
Decreased Lung Function of Children	200	100	13
Present Standard	80	75 (Geometric)	–

TABLE 18

SHORT-TERM EXPOSURES:
POLLUTANT THRESHOLDS FOR ADVERSE EFFECTS
(BEST JUDGMENT)

EFFECT	THRESHOLD, 24-hour $\mu g/m^3$		
	SO_2	TSP	SS
AGGRAVATION OF SYMPTOMS IN ELDERLY	>365	80-100	8-10
AGGRAVATION OF ASTHMA	180-250	70	8-10
PRESENT STANDARD	365	260	—

Figure 1

SALT LAKE BASIN: ASTHMA
(MINIMUM TEMPERATURE 30 TO 50° F)

RELATIVE
RISK
OF
ASTHMA
ATTACK
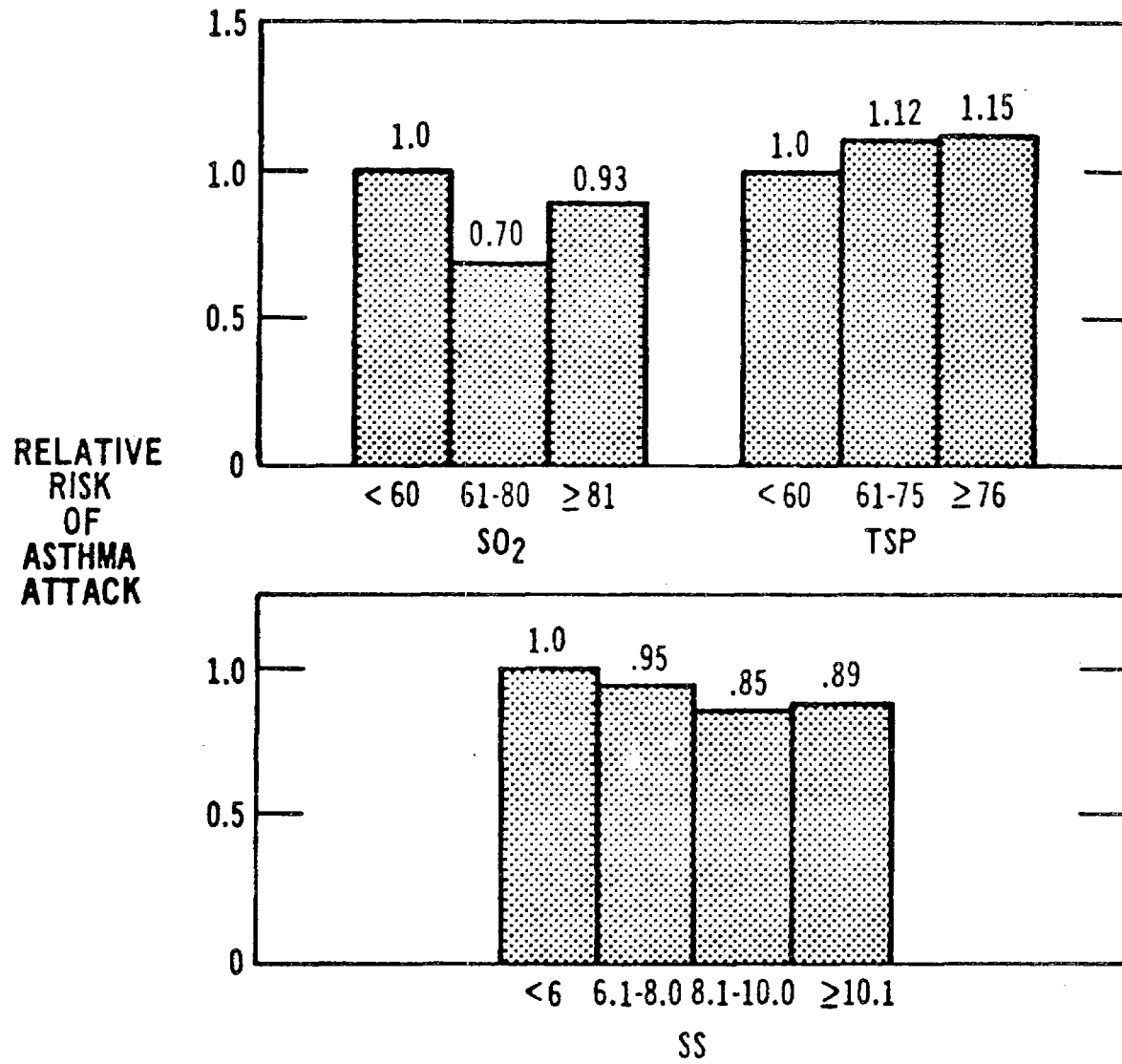
Figure 2

SALT LAKE BASIN: ASTHMA
(MINIMUM TEMPERATURE $\geq 51°F$)
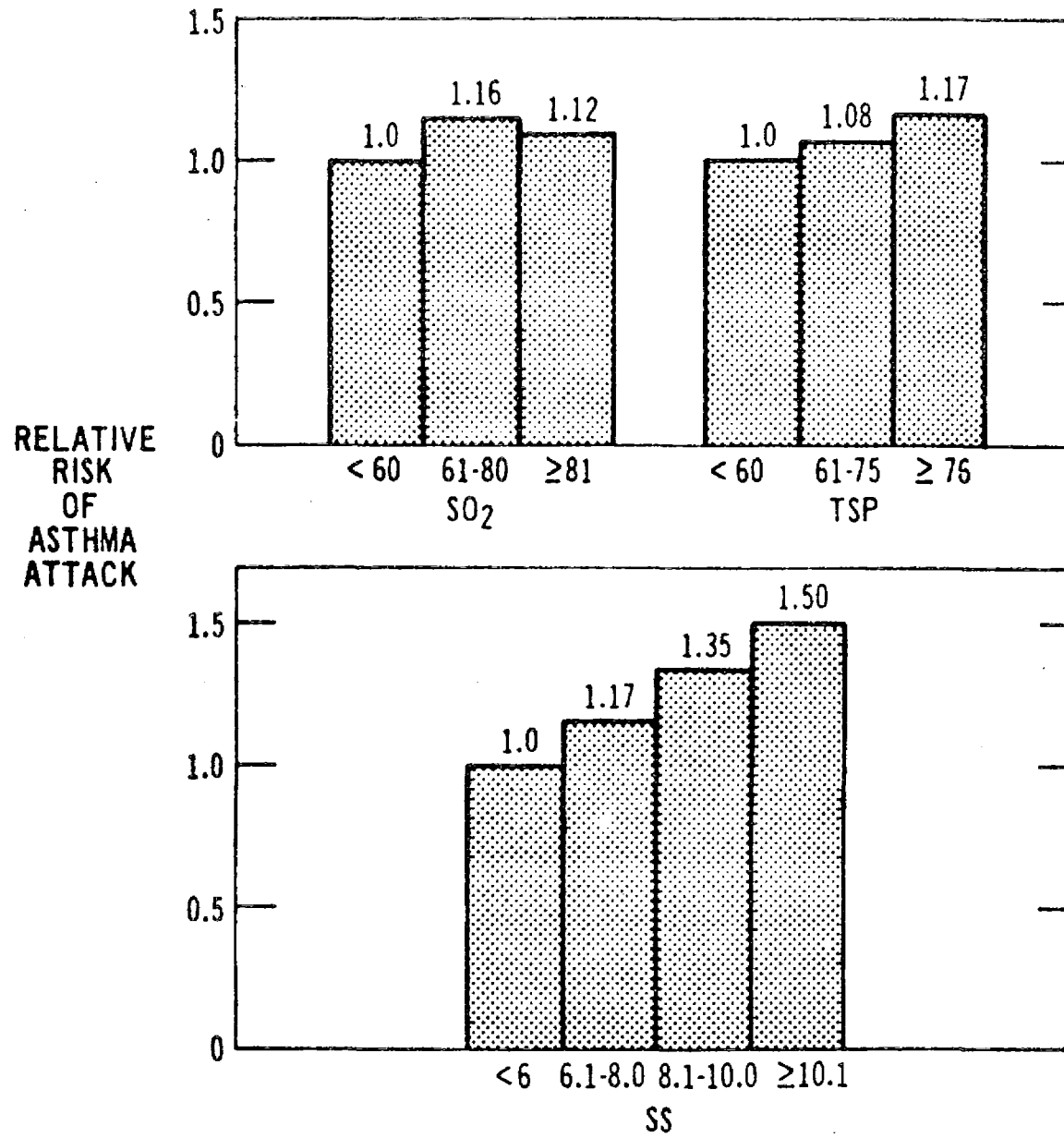
Figure 3
NEW YORK: ASTHMA
(MINIMUM TEMPERATURE 30 TO 50°F)
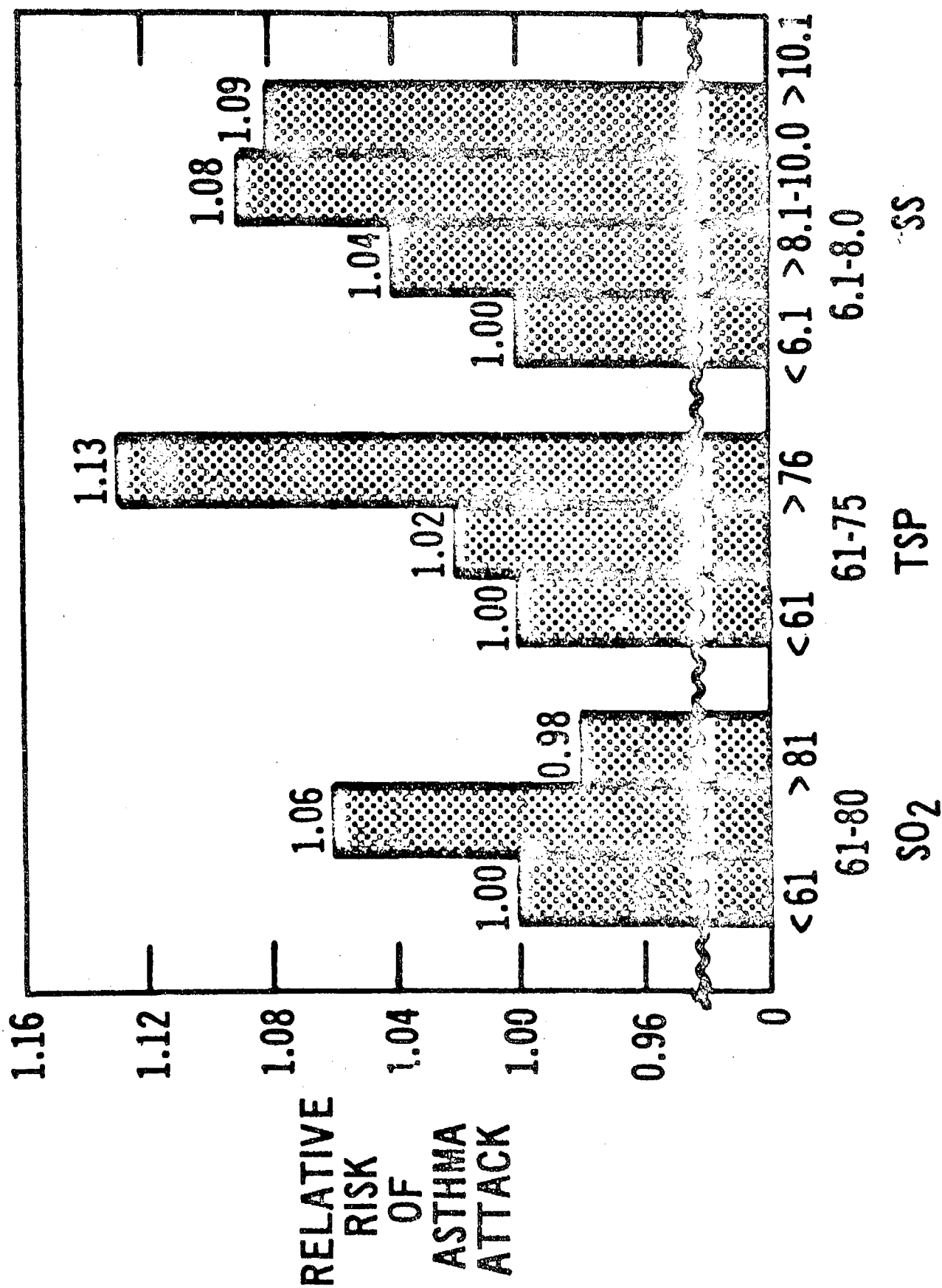
Figure 4

NEW YORK: ASTHMA
(MINIMUM TEMPERATURE ≥51°F)
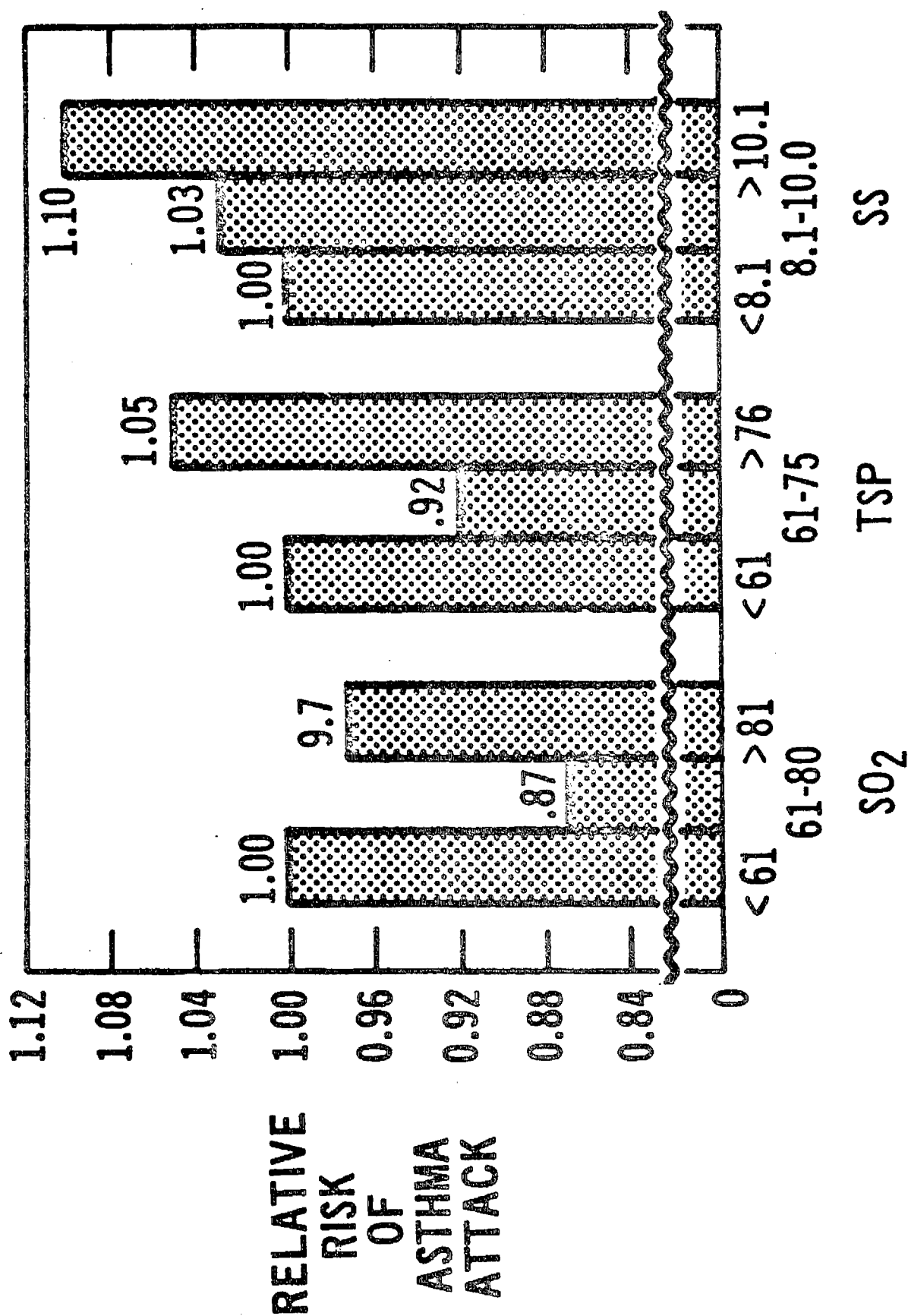
Figure 5

SALT LAKE BASIN: ASTHMA ATTACKS RATES
(MINIMUM TEMPERATURE ≥ 51°F)

ASTHMA ATTACK RATE PER 100

WITH HIGH SULFATES

WITHOUT HIGH SULFATES

(26)

(71)

TSP, μg/m³
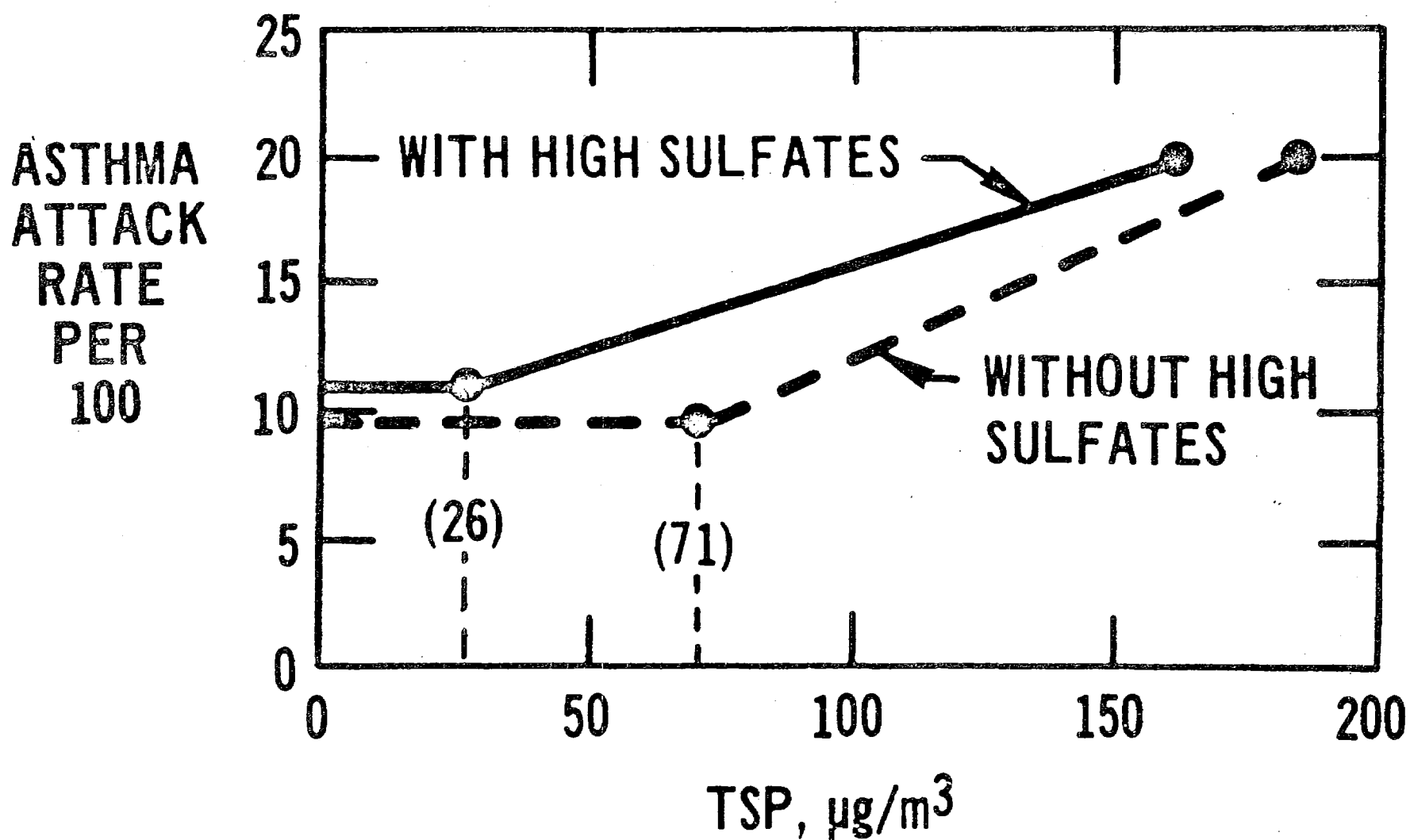
Figure 6

SALT LAKE BASIN: ASTHMA ATTACK RATES
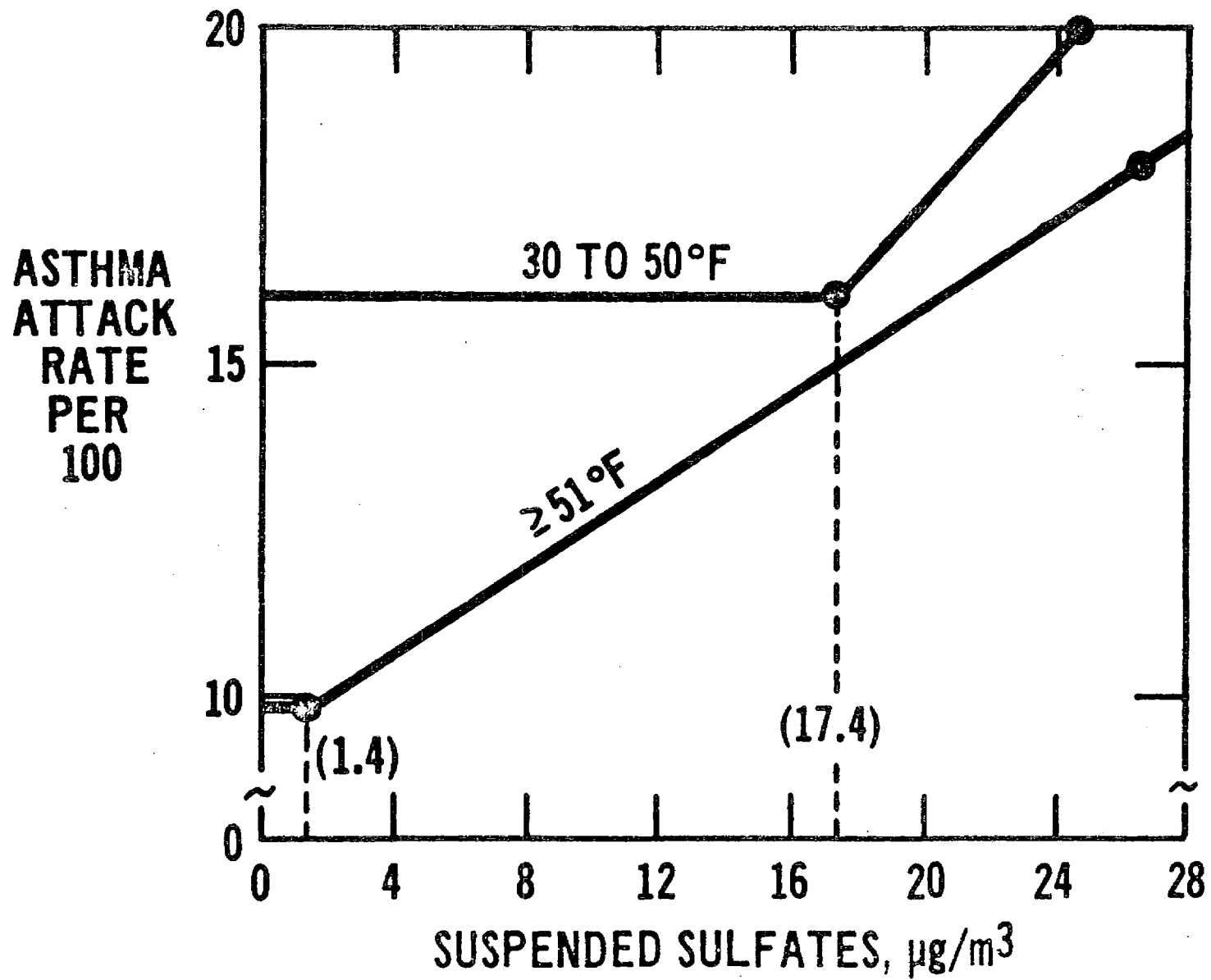
FIGURE 7

NEW YORK: ASTHMA ATTACK RATES
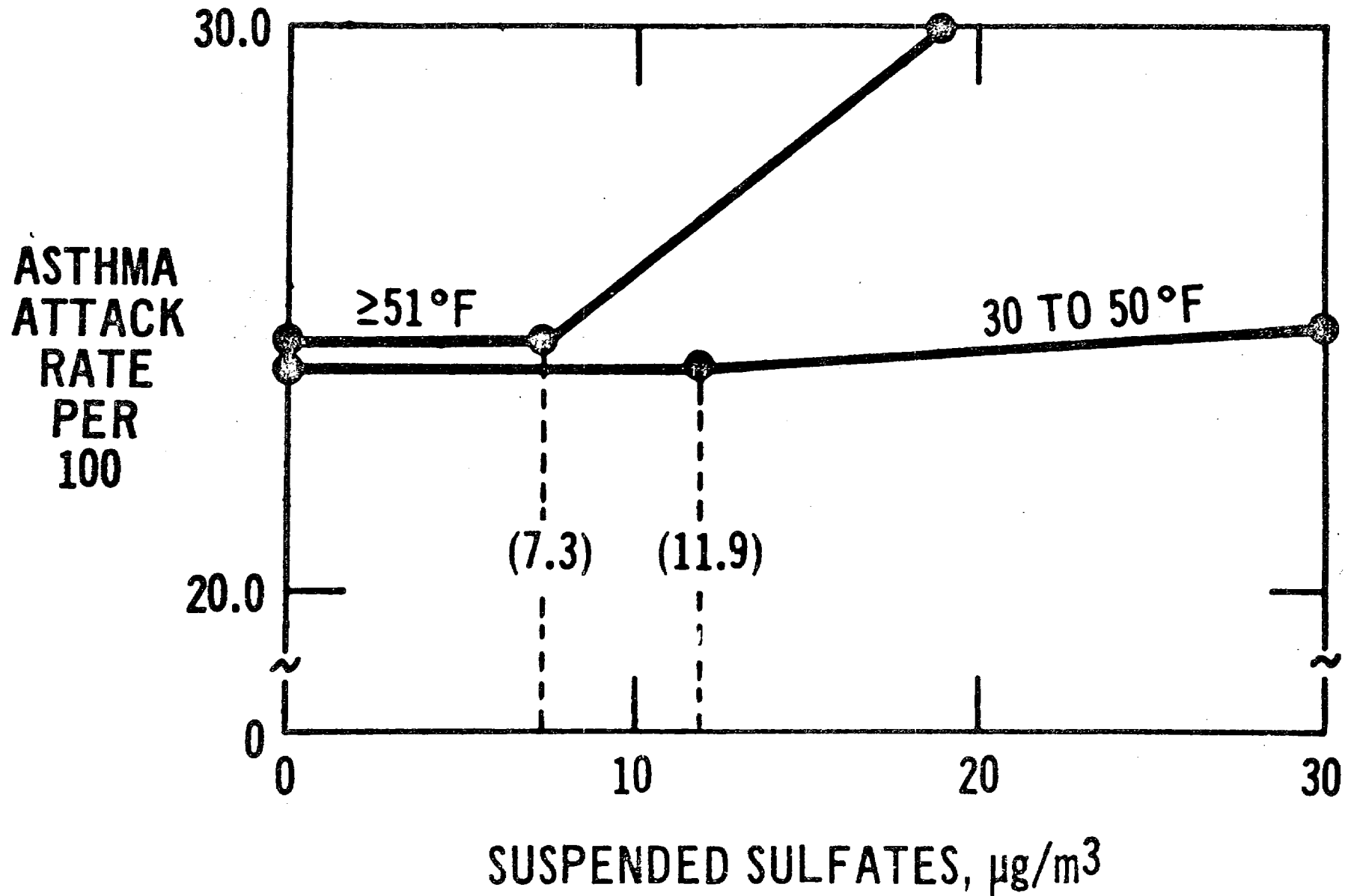
Figure 8

NEW YORK HEART AND LUNG PANELS:
RELATIVE SYMPTOM RISK ON HIGH AND LOW EXPOSURE DAYS
(MINIMUM TEMPERATURE 20 TO 40 °F)
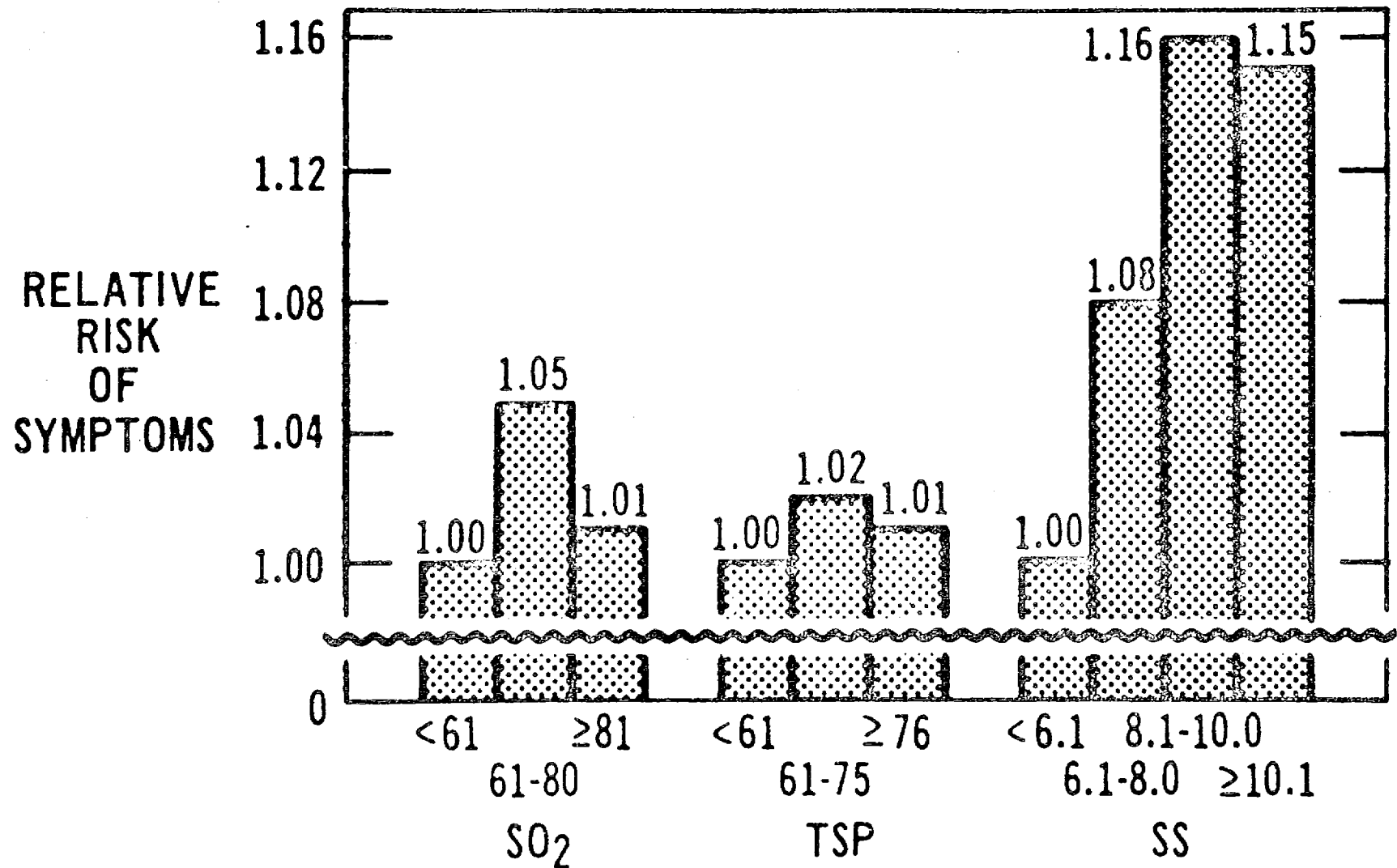
Figure 9

NEW YORK HEART AND LUNG PANELS:
RELATIVE SYMPTOM RISK ON HIGH AND LOW EXPOSURE DAYS
(MINIMUM TEMPERATURE $\geq 41°F$)
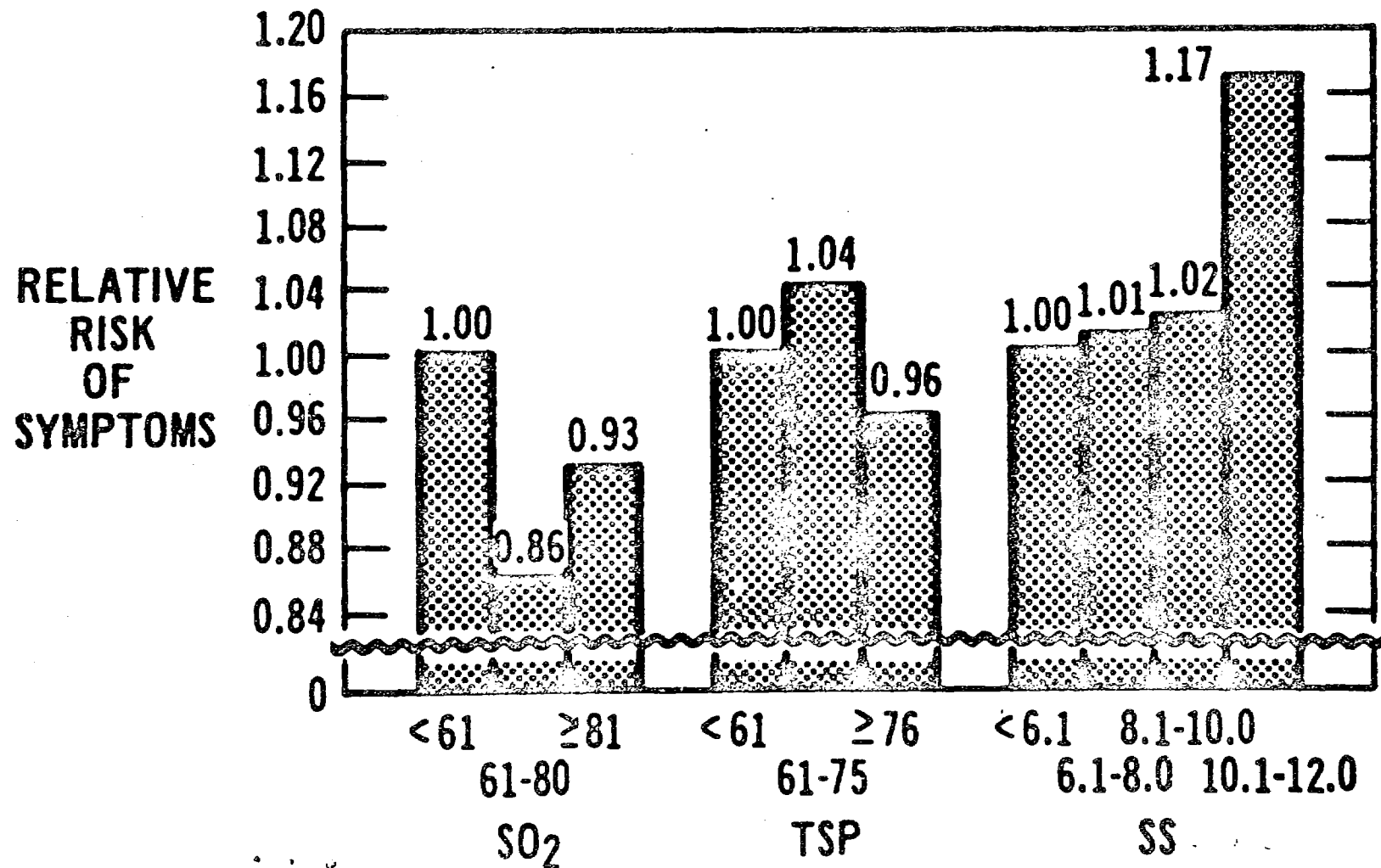
CHAPTER V-A

HEALTH CONSEQUENCES OF NITRATES

A STUDY OF AGGRAVATION OF ASTHMA
IN TWO CHESS AREAS.   1971 - 1972

Jean G. French, Dr. P.H.*

Introduced by Carl Shy, M.D.

Presented at the Conference on Health Effects of

Atmospheric Salts and Gases of Sulfur and Nitrogen

in Association with Photochemical Oxidant

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CHAPTER V-A

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INTRODUCTION

It has been recognized for some time that complex urban air pollution mixtures aggravate asthma. Earlier studies have show that asthmatic attacks were increased during periods of elevated pollution exposuree, but these studies failed to identify the harmful agent of agents or find those levels of pollution at which health effects first became marked.[1,2,3,4]

Studies conducted by the Environmental Protection Agency have attempted to quantify the relationship between short term relatively high dose pollution exposure and frequency of asthma episodes. Preliminary findings from some of these studies suggest that suspended sulfates and possibly suspended nitrates associated with aggravation of asthma attacks than other pollutants, but the findings are not entirely con sistent. A study of asthma and air pollution from a coal fueled power plant showed that temperature had the strongest effect on asthma attack rates but after the temperature effect was removed, suspended sulfates showed the strongest effect.[5] A study of asthmatics in four western communities linked excess asthma attacks with elevated levels of suspended sulfates and suspended nitrates and decreased ambient temperature.[6] Another study of asthmatics conducted in three northeastern communities in 1971 and 1972 showd an association between excess asthma attacks and elevated suspended sulfates, but the ambient temperature effect was less clear.[7]

Laboratory studies of relative toxicity carried out in guinea pigs have shown that particulate sulfates are more irritating than

sulfur dioxide gas at similar concentrations. However, these studies
have also demonstrated that the chemical compositon of the sulfate
compound is an important factor in its toxicity as well as relative
humidity and particle size. Ferric sulfate was found to be more
toxic than ferrous sulfate, and within the range of 0.3 to 2.5 MMD,
the smaller the particle size, the greater the irritant potency.[8]

On the basis of this laboratory evidence, it is possible that the
irritating effects of sulfates may vary among communities depending
upon the physical and chemical properties of the sulfate particles pre-
sent, as well as differences in meteorologic conditions in the various
communities.

There is a need to conduct additional community studies to determine
if previous findings can be replicated and to determine the circum-
stances under which sulfates exert a significant toxic effect.

It is now possible to measure the level of fine particles in
ambient air and although this information does not provide a direct
measure of the size of specific compounds. It does permit an assessment
of associations between the asthma attack rate and particle size.

Two EPA studies are reported here which replicated the methods used
in previous studies, but also take particle size and relative humidity
into condsideration. The study in the southeastern communities permitted
as assessment of exposures to high levels of total suspended particulates
and suspended sulfates accompanied by low levels of SO_2, a combination
which has not been considered in previous CHESS studies.

STUDY A - <u>Aggravation of Asthma</u>
<u>by Air Pollutants: 1972-73</u>
New York and New Jersey Studies

In a previous study conducted by the CHESS program of EPA in the
New York metropolitan area, increased asthma attack rates were linked
to suspended sulfate particulate air pollution.
Pollution exerted an effect only on days in which the temperature was
above freezing (T. min. 30°-50°F). [1] It is important to continue these
studies to determine if previous findings can be replicated. The fol-
lowing is a report based on the second year of study of asthmatics in
three New York communities as well as the results from the first year
of study of asthmatics in four New Jersey communities. Measurements of
respirable particules as well as relative humidity are included in the
present study but were not in the previous study.

Methods

The methhods used in the present study are essentially the same
as in the previous CHESS study.

Setting and Study Population

The New York City - New Jersey metropolitan areas were initially
chosen because they were thought to represent an air pollution gradient
for exposures involving sulfur dioxide, suspended sulfates and total
suspended particles. Other pollutants including nitrates, nitrogen
dioxide, organus, metals, carbon monoxide and gaseous hydrocarbons were
also present. The three New York communities are Queens, Bronx, and

Riverhead. The four New Jersey communities are Elizabeth, Matawan, Ridgewood and Fairfield.

Rosters of possible asthmatic panelists were compiled from hospital clinic records and records of practicing physicians. Each prospective subject was interviewed by trained interviewers to obtain information regarding the nature, frequency and severity of asthma. Information was obtained on smoking habits, occupational exposure to respiratory irritants and socioeconomic status. Subjects were selected for admission to the study who had been diagnosed as an asthmatic by a physician, had symptoms of wheezing accompanied by dyspnea and had experienced at least three such episodes during the previous year. In addition, panelists had to live within a 1 1/2 mile radius of an air monitoring station.

Diary Coverage

Each panelist received a diary each week by mail, recorded attacks as they occurred each day and returned the completed diary by mail at the end of the week. Non response and diaries requiring clarification were investigated by telephone. Diaries received more than 12 days after the last day covered by the diary were not accepted for data processing. The information collected in the first two weeks of the study was not used in the analysis because previous studies have shown that panelists tended to over report during the first two weeks of study. Throughout the study, appropriate contacts were made with the panelists to improve performance and repeated non respondents were deleted from the study.

Monitoring Air Pollution Exposures

Air monitoring stations were located in each New York community on
the roof of a two story building approximately 25 feet above the ground.
In the four New Jersey communities air monitoring stations were installed
approximately six feet above ground level. The New York stations had been
installed a year before the New Jersey stations, and when the New Jersey
stations were installed it was considered more desirable to locate measure-
ment stations at ground level. The New York stations were not changed
because differences in collection methods would make it difficult
to compare results with previous New York CHESS studies. Measurement
devices at each station included high volume samplers for measuring total
suspended particulates, cyclone separators developed by the Atomic Energy
Commision for measuring the respirable fraction of suspended particulates,
dustfall buckets and sulfur dioxide bubblers. Continuous 24 hour monitor-
ing for SO_2 and TSP was maintained throughout the study period. Sample
filters and bubblers were replaced daily and sent for laboratory analysis.
Dustfall buckets were replaced monthly. Minimum and maximum temperatures
and relative humidity were recorded at the nearby airport serving the
respective communities. In the laboratory SO_2 gas bubblers were analyzed
by the EPA standard method modified after the West Gaeke Method. The NO_2 was
analyzed by the Jacob Hockheiser Method. This method has since been found
unreliable for measuring NO_2 levels particularly at lower levels. There-
fore NO_2 data is included in this study for purposes of general information
only. Its contribution to any observed effects will not be considered.
High volume sampler filters were measured gravimetrically and sulfate and
nitrate fractions were determined by an automated analysis reduction-diazo
coupling method. For the sulfate fraction, a turbidimetric method was

used and turbidity measured by spetro-photometer. More detailed descrip-
tions of exposure monitoring are presented elsewhere.[2]

Statistical Analysis

The primary hypothesis to be tested was that in addition to
meteorological factors, air pollutants exert a significant influence on
the aggravation of asthma attacks. The second hypothesis was that a
pollution level exists below which there is little or no effect and above
which a significant effect can be found.

Data for a single day of the study consisted of a daily number of
asthma attacks, a 24 hour average level for each pollutant measure, a mini-
mum and a maximum temperature and a 24 hour relative humidity.

A series of steps were involved in the analysis:

First, studying the demographic and reporting patterns of the
panelists within each community. Panelists who were in the study for less
than 10 weeks, as well as those reporting no attacks or daily attacks
during their participation in the study, were dropped from the analysis.
The varying length of time of participation for those remaining in the
analysis was adjusted by computing an expected value for each individual
based on the total number of days he was in the study. The daily expected
attack rate for a given panel was then derived by computing the total
expected value for all members reporting an attack on that day. This value
served as the denominator and the total number of observations
on that day (based on no more than one attack per person) served as the
numerator. The ratio of the observed over the expected values was then

used as the asthma attack rate throughout the study.

Second, plotting weekly averages of asthma attack rates, pollutant levels and minimal temperature to look for seasonal patterns.

Third, calculating a simple correlation matrix for daily asthma attack rates, pollutant levels temperature and humidity.

Fourth, performing multiple regression analyses to look for pollutant effects after removal of temperature effects. To further identify the contribution of each pollutant the "F to remove" stepdown multiple regression is performed. In this procedure the contribution of each pollutant is tested after allowing for the contribution of temperature aaand other pollutants.

Fifth, construct temperature specific relative risk and excess risk for various pollutant levels.

RESULTS

Environmental Exposure

Among the three New York communities, Bronx had consistently higher pollution levels than the other communities. The levels of total suspennded particles (TSP) and Sulfur Dioxide (SO_2) were below the mean annual primary standard as well as the short term standard in each community during each quarter of the study period. The levels of respirable particulates (RSP), total suspended particulate, suspended nitrates (NO_x) and sulfur dioxide were highest during the fall quarter and lowest during the spring quarter. Suspended sulfates (SO_x) followed a similar pattern except that the highest maximum values for sulfates were found during the spring quarter. These results are shown in Table 1.

In general the pollution levels in New Jersey were lower than those in New York as shown in Table 2. The levels of SO_2 and TSP were well below the annual mean primary standard and the short term standard in each community. The pollution levels among the four New Jersey communities were similar and the highest levels for each pollutant generally occurred in the fall.

Meteorological Conditions

During the study period the weekly average minimum temperature reached as low as 13°F but the weekly average maximum temperature seldom exceeded 75°F. The temperature fluctuation within a 24 hour period averaged about 20°F. There was very little temperature difference among the seven communities. The relative humidity never dropped below 50% and averaged about 65% throughout the study (Table 3).

Temporal Pattern

The asthma attack rate when plotted for each week during the study period is shown in Figures 1 and 2. In general the highest attack rates tend to occur in the fall and spring. The fall was also the period of highest pollution levels but the peak of attacks in this time frame might also reflect "a starting up effort" in the study.

The pattern of high attack rates in the early fall does not correspond with the pollen season in the New York - New Jersey season. Ragweed pollens are at high levels in August and September while the elevated attack rates in this study occurred in early November. However the increased attack rate at the end of the late spring does correspond with the grass pollen season in that area.

Charateristics of Study Population

The characteristics of the study population are shown in Table 4. There was a total of 373 panelists included in the analysis all of whom represented white middle class individuals. The panels varied in size and Fairlawn, New Jersey was the largest with 66 panelists and Riverhead , Long Island the smallest with 44 panelists. There was a higher percentage of children than adults in each panel but the age distribution among the seven panels was comparable. The number of smokers in each panel was relatively small ranging from 17.4% to 25%. The sex composition and history of severity of illness varied among the panels. The differences in the distribution of characteristics for each panel was obviated by conducting most of the analysis within each panel rather than across panels.

Sample Correlation Matrix

A simple correlation matrix was constructed to determine the degree of association of asthma attack rates with pollutants and meteorological variables (Table 5). The correlation of the asthma attack rate in children with that in adults ranged from $r=0.040$ in Bronx and $r=0.349$ in Riverhead, New York.

Elevated levels of suspended nitrates were significantly correlated with a high asthma attack rate in each of the seven panels.

A higher attack rate in the adult panel members in Fairlawn was significantly correlated with all pollutants and the attack rate in adults in Riverhead was significantly correlated with elevated levels of RSP, TSP, and NO_x.

Elevated minimum and maximum temperatures were significantly associated with the higher asthma attack rates in each community and high relative humidity was significantly correlated with increased asthma attacks in Bronx, Riverhead, Fairlawn and Ridgewood.

All the pollutants were significantly correlated with each other (Table 6) and the highest correlation was total suspended particulates with respirable particulates. The correlation between SO_x and SO_2 was less than that between NO_x and SO_x in each community.

Minimum and maximum temperatures were more closely correlated with TSP and RSP than with nitrates and sulfates but relative humidity was more closely correlated with nitrates and sulfates than with other pollutants.

Multiple Regression Analysis

A multiple regression analysis was done to separate temperature effect from pollutant effect. The results of this analysis are shown Elevated temperature levels were significantly associated with an increase in the asthma attack rate in each of the seven panels.

After temperature effects were removed, elevated levels of suspended nitrates were significantly associated with the asthma attack rate in six of the seven communities. The exception was the Bronx in New York. None of the other pollutants showed the same consistent significant association with increased aggravation of asthma as suspended nitrates. Elevated levels of respirable particulates were significantly associated with the asthma attack rate in the total non smoking asthma panel in Mattawan and Riverhead and in the adult panel in Elizabeth and Fairlawn. The adult panelists in Fairlawn showed a significant correlation with all of the pollutants.

In the previous New York asthma ,study it was difficult to separate
the effect of suspended sulfates from suspended nitrates. In an attempt
to disentangle these pollutants the multiple regression analysis was re-
peated with suspended nitrates placed in the regression analysis alone
and then in conjunction with minimum temperature and suspended sulfates
(Table 8). This analysis showed that suspended sulfates did not contri-
bute significantly to the asthma attack rate but suspended nitrates alone
and in conjunction with temperature continued to demonstrate a significant
association with the asthma attack rate. In another analysis the combina-
tion of suspended nitrates and suspended sulfates were entered into the
multiple regression analysis with minimum temperature but no significant
association was seen with the asthma attack rate.

Temperature Specific Relative Risk Models
Relating to Asthma Attack Rates and Pollution Levels

To determine if certain temperature ranges were more critical than
others, specific relative risks were computed within specific temperature
ranges combined with different levels of pollutants. The strongest
and most consistent finding oin the analysis was that of excess risks of
asthma attacks with elevated elvels of suspended nitrates when accompanied
by temperatures of 50°F or above. Both children and adult panelists in
each of the seven communities demonstarated this effect ranging from 14%
in Bronx to 110% in Elizabeth.

Excess risk was also observed with elevated levels of suspended sulfates at temperatures of 50°F or above in six of the seven communities but the risk was not as high as that seen with suspended nitrates. The combination of NO_x and SO_x produced relative risks comparable to that of nitrates alone.

In general the excess risk observed with total suspended particulates and respirable particulates followed the same pattern as that observed with suspended nitrates and suspended sulfates. There was little excess risk observed with elevated levels of sulfur dioxide except in Riverhead at temepratures of 30 - 50°F and in Fairlawn at temperatures of 50°F or above.

Threshold and Linear Dose Relationships

The Hockey Stick method of Hasselblad et al.[3] was used to look for threshold levels at which pollutants such as nitrates aggravate asthma attacks.

Temperatures above 40°F were used in the Hockey Stick analysis since the other analysis in this study demonstrated effects predominately at higher temperatures.

The comparability of pollution levels in the New Jersey communities permitted the pooling of these communities for this analysis. However there were sufficient differences among the three New York communities to warrant treating them separately.

The threshold estimates for suspended nitrates in the four New Jersey communities was 3.77 μg/m^3 when temperatures were below 40°F and 2.16 μg/m^3 when accompanied by temperatures of 40°F or above. In Queens the estimated threshold for suspended nitrates was 7.63 μg/m^3 at temperatures above 40°F and 14.93 μg/m^3 when temperatures were below 40°F. The estimated threshold in the Bronx was substantially higher at 14.98 μg/m^3 with temperatures below 40°F and 20.4 μg/m^3 with temperatures of 40°F or above. In Riverhead no estimated threshold was fund for colder temperatures but when temperatures were 40°F or above the estimated threshold for suspended nitrates was 6.45 μg/m^3.

In the case of suspended sulfates the estimated threshold levels on colder days ranged from 5.79 μg/m^3 in Riverhead to 14.98 μg/m^3 in Queens. When temperatures were above 40°F the estimated effect threshold was 14.03 μg/m^3 in New Jersey, 20.4 μg/m^3 in Queens and 28.5 μg/m^3 in Bronx. No effect was found in Riverhead.

DISCUSSION

There is consistent evidence in this study that elevated levels of
suspended nitrates on warmer days significantly contributed to the
aggravation of asthma.

In the previous New York asthma study, suspended sulfates appeared
to have a more significant effect on asthma than suspended nitrates but
the contribution of nitrates could not be completely disentangled from the
effect of sulfates. Nitrates may have been exerting more of an influence
on the asthma attack rate in the previous study than could be identified or it m
or it may be that the nitrates in the present study were more irritating
than those in the previous study. Compounds of sulfates and nitrates
may vary in a community over time and some compounds are more irritating
than others. The work of Amdur[4] had demonstrated that the irritating
effects of sulfates may vary based on the metallic cation which appears in
the compound. Unfortunately we are presently unable to determine the
chemical composition of the nitrates and sulfates which are collected on
the filters.

In this present study suspended sulfates continued to exert some
influence on the asthma attack rate as evidenced by excess risk of asthma
attacks with elevated levels of suspended sulfates at higher temperatures
in six of the seven communities.

The observations that suspended nitrates and/or suspended sulfates
may contribute to the aggravation of asthma raises the possibility
that precursors of these compounds such as nitric acid and sulfuric acid
mists may be the true irritants. Present technology does not yet permit
the measurement of acid aerosols in ambient air on a community wide basis.

The compounds of nitrates and sulfates which are currently measured on the filter may reflect transformation products of nitric and sulfuric acid aerosols in ambient air. The high correlation of nitrates and sulfates with the relative humidity in this study suggests that the moisture in the air might react with some precursor to form a transformation product which we currently measure as nitrates and sulfates. The observation that nitrates consistently exerted an effect at higher temperatures might reflect the fact that the transformation of pollutants is more apt to occur at higher temperatures.

The failure of respirable particles alone to contribute significantly to the aggravation of asthma shows the limitation of considering particle size alone rather than as an integral part of the physical and chemical properties of each pollutant.

The relatively low levels at which suspended nitrates were shown to exert an effect on the asthma attack rate in this study would pose problems in planning a control strategy for nitrates alone. Before such measures are considered it is essential to determine what suspended nitrates fully represent in terms of primary pollutants and their transformation products.

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TABLE 1

Daily Air Pollutant Exposure Patterns in New York Distributed by Season

Pollutant $\mu g/m^3$	Comm.	Mean			Median			90th Percentile			Maximum		
		Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring
RSP	Riverhead	35.5	21.2	24.9	31.3	21.1	22.8	72.6	35.0	42.0	93.1	54.1	71.3
	Queens	37.2	28.5	37.1	36.5	27.8	38.0	73.5	41.3	52.4	96.3	92.5	58.1
	Bronx	48.4	38.7	47.3	40.4	35.6	47.7	82.8	70.2	69.9	195.8	87.9	99.0
TSP	Riverhead	42.6	28.6	31.0	38.5	28.2	28.6	86.5	46.2	48.3	116.1	62.7	92.7
	Queens	52.7	41.4	54.5	42.4	35.8	54.0	94.6	65.1	78.3	126.6	116.0	103.7
	Bronx	71.5	62.1	79.2	64.3	51.0	69.6	134.4	113.9	133.5	235.3	160.3	182.2
SO_x	Riverhead	11.3	11.5	10.6	10.1	10.1	8.4	19.9	19.9	19.5	30.3	30.9	54.9
	Queens	13.5	14.1	12.1	12.2	12.4	10.5	22.4	21.8	21.8	33.8	38.2	29.5
	Bronx	34.1	14.0	13.0	12.8	13.1	11.0	25.0	20.3	24.5	34.1	32.7	39.7
NO_x	Riverhead	3.1	2.6	1.4	2.8	2.1	1.1	5.8	5.0	2.4	8.7	9.3	7.0
	Queens	5.2	4.0	3.0	4.1	3.2	2.6	10.6	8.4	6.4	23.5	12.2	9.0
	Bronx	5.1	3.7	3.3	4.0	3.0	2.7	10.4	6.9	5.6	18.3	15.7	14.4
NO_2	Riverhead	29.0	26.8	17.0	25.5	22.2	14.8	56.2	51.7	32.1	86.7	106.4	56.0
	Queens	64.7	59.6	50.7	60.4	60.8	49.3	97.7	84.3	79.5	142.5	118.7	116.9
	Bronx	73.9	73.6	81.4	68.8	65.5	82.8	115.3	117.3	130.0	190.2	681.9	158.1
SO_2	Riverhead	30.1	40.2	12.5	23.8	32.8	10.0	61.2	88.8	28.8	144.9	146.5	61.1
	Queens	59.9	62.6	20.0	54.5	57.4	16.7	100.4	127.3	42.1	246.9	172.8	62.1
	Bronx	70.1	88.0	29.1	60.7	75.7	21.4	133.4	178.6	68.2	204.4	272.9	103.7

TABLE 2

Daily Air Pollutant Exposure Patterns in New Jersey by Season

Pollutant ug/m³	Community	Mean			Median			90th Percentile			Maximum		
		Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring	Fall	Winter	Spring
RSP	Ridgewood		34.8	34.4		28.9	30.8		56.1	50.3		185.0	72.9
	Fairlawn	41.9	38.2	37.8	33.6	34.2	35.1	92.1	65.5	58.5	138.7	101.8	114.4
	Matawan	47.0	31.8	39.7	46.1	29.3	37.9	66.4	53.6	60.6	120.1	66.2	83.7
	Elizabeth	16.2	6.5	23.6	11.5	5.3	16.0	33.7	12.6	31.8	35.3	28.7	136.7
TSP	Ridgewood	55.3	53.3	42.5	44.5	46.8	38.8	105.6	99.7	63.3	127.3	149.1	77.7
	Fairlawn	55.3	55.0	47.8	45.3	49.0	43.1	105.8	93.7	78.8	177.3	178.3	96.2
	Matawan	48.2	44.1	59.0	43.8	40.9	53.5	72.3	66.7	91.7	158.1	82.4	176.9
	Elizabeth	63.8	59.9	46.7	61.2	55.7	48.4	102.9	91.4	66.4	131.4	168.4	84.6
SO_x	Ridgewood	12.8	12.5	11.0	11.4	10.9	9.5	21.9	19.9	20.0	43.3	32.5	54.7
	Fairlawn	14.2	12.2	13.1	12.6	10.9	10.6	22.2	18.6	21.0	48.8	33.0	56.4
	Matawan	13.0	12.0	12.6	11.5	11.2	10.2	21.3	17.8	21.3	32.2	38.7	53.8
	Elizabeth	11.7	12.9	12.5	10.7	12.3	9.8	17.5	19.1	24.4	20.8	27.7	40.7
NO_x	Ridgewood	4.0	2.9	1.5	3.2	2.5	1.0	7.0	6.9	3.1	21.5	10.9	6.2
	Fairlawn	4.7	3.1	2.1	3.4	2.6	1.5	8.3	6.9	4.3	17.0	11.7	7.6
	Matawan	3.7	2.6	1.5	3.2	2.1	1.2	6.9	5.3	2.7	12.4	8.9	5.7
	Elizabeth	3.9	3.0	2.3	3.0	2.3	1.5	6.8	5.3	3.3	10.7	10.8	7.1
NO_2	Ridgewood	54.8	43.2	53.6	53.8	48.6	52.4	84.6	91.3	109.6	129.9	147.2	168.7
	Fairlawn	53.6	48.3	46.8	50.7	44.5	52.7	91.5	82.1	74.1	147.1	127.8	116.8
	Matawan	41.6	42.8	43.0	36.3	41.2	39.2	74.0	71.0	72.3	117.0	87.2	102.8
	Elizabeth	67.2	58.2	60.1	66.4	54.6	55.9	98.1	88.6	87.9	154.5	110.8	155.3
SO_2	Ridgewood	31.8	42.6	17.7	27.5	36.0	16.0	65.6	82.7	33.1	102.5	114.8	72.7
	Fairlawn	43.6	49.5	24.2	39.2	43.5	21.5	77.7	88.3	42.0	139.9	149.5	67.2
	Matawan	36.6	41.5	20.3	31.2	36.4	17.9	67.2	38.3	33.5	199.9	80.0	58.8
	Elizabeth	62.7	55.4	24.4	52.2	45.9	20.8	105.1	55.4	24.4	142.3	45.9	20.8

TABLE 4

Distribution of Characteristics of Asthma Panelists in the Seven Communities

Characteristic	Bronx #	Bronx %	Elizabeth #	Elizabeth %	Fairlawn #	Fairlawn %	Matawan #	Matawan %	Queens #	Queens %	Ridgewood #	Ridgewood %	Riverhead #	Riverhead %
Age <15	29	63.0	32.	59.3	41	62.1	40	64.5	27	57.4	34	63.0	27	61.4
>15	17	37.0	22	40.7	25	37.9	22	35.5	20	42.6	20	37.0	17	38.6
Total	46		54		66		62		47		54		44	
Female	17	37.0	29	53.7	25	37.9	28	45.2	21	44.7	21	38.9	21	47.7
Male	29	63.0	25	46.3	41	62.1	34	54.8	26	55.3	33	61.1	23	52.3
White	43	93.5	50	92.6	66	100.0	62	100.0	47	100.0	54	100.0	44	100.0
Black	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Age of Onset >45	1	2.2	0	0	0	0	0	0	0	0	0	0	2	4.5
Smokers	8	17.4	14	25.9	13	19.7	17	27.4	8	17.0	12	22.2	10	22.7
Exsmokers	3	6.5	8	14.8	8	12.1	6	9.7	3	6.4	7	13.0	4	9.1
Hx of Allergy	23	50.0	20	37.0	35	53.0	19	30.6	20	42.6	18	33.3	23	52.3
Hx of <10 Attacks per Study	19	41.3	10	18.5	30	45.5	21	33.9	26	55.3	27	50.0	28	63.6

TABLE 5

Simple Correlation Matrix of Asthma Attacks With Pollutants and Meteorological Variables by Age and Panel for Each City

Community	Age	RSP	TSP	NO_x	SO_x	SO_2	Max. Temp.	Min. Temp.	Humidity
Bronx	<15	.051	.112	.031	-.064	-.063	.135*	.138*	.074
	>15	.045	?082	.137*	-.023	.043	-.0003	.089	.232
	Total	.046	.114	.084	-.098	-.031	.080	.147*	.185**
Elizabeth	<15	.310**	-.184**	.138*	.0191	-.166*	-.012	.033	.005
	>15	-.213**	.103*	.029	.108	-.090	.201**	.166**	.046
	Total	.145	0.027	.153*	.097	-.145	.132*	.138*	.066
Fairlawn	<15	-.013	.091	.058	.095	-.140*	.239**	.247*	-.026
	>15	.180**	.178**	.205**	.175**	.136*	.046	.144*	.172**
	Total	.103**	.165**	.189**	.178**	.022	.140*	.211**	.107*
Matawan	<15	.220**	.158**	.049	.003	-.162**	.369**	.361**	.093
	>15	.050	-.121	.195**	.020	.199**	-.077	-.005	.110
	Total	.212**	.129*	.151*	.004	-.059	.286	.292**	.092
Queens	<15	.079	.083	.121*	.042	.075	-.057	.008	.009
	>15	-.076	.063	.081	.051	-.054	.203**	.273**	.076
	Total	.028	.131*	.161**	.081	.040	.102*	.190**	.065
Ridgewood	<15	.072	.107	.066	.042	.134*	.183**	.211**	.075
	>15	-.014	.069	.098	.099	-.027	.047	.134*	.142*
	Total	.066	.145*	.135*	.144*	-.030	.164**	.257*	.173**
Riverhead	<15	.235**	.249**	.131*	.088	-.042	.269	.267**	.078
	>15	.119*	.257**	.171**	.111	.040	.161**	.148*	.139*
	Total	.187**	.304	.155**	.085	.000	.250	.233*	.133*

* p = $\leq$ 0.05
** p = $\leq$ 0.01

Table 6. Simple Correlation Matrix of Pollutants with Each Other and Meteorological Factors in Each Community

| | RSP | | | | | | | TSP | | | | | | |
	B	E	F	M	Q	RW	RH	B	E	F	M	Q	RW	RH
RSP								.63	.09	.61	.48	.72 ·	.51	.37
TSP														
NO_x														
SO_x														
SO_2														
Max. Temp.														
Min. Temp.														
Humidity														

* - P = ≤ 0.05

** - P = ≤ 0.01

B - Bronx   Q - Queens
E - Elizabeth   RW - Ridgewood
F - Fairlawn   RH - Riverhead
M - Matawan

Table 6.   Simple Correlation Matrix of Pollutants with Each Other and Meteorological Factors in Each Community

	NO$_x$							SO$_x$						
	B	E	F	M	Q	RW	RH	B	E	F	M	Q	RW	RH
RSP	.52	.37	.45	.27	.43	.40	.25	.45	.24**	.43	.31	.42	.31	.23**
TSP	.52	.51	.61	.26	.47	.70	.42	.38	.41	.43	.34	.43	.52	.59
NO$_x$								.59	.30	.38	.19**	.40	.41	.46
SO$_x$														
SO$_2$														
Max. Temp.														
Min. Temp.														
Humidity														

* - P = ≤ 0.05
** - P = ≤ 0.01

B - Bronx
E - Elizabeth
F - Fairlawn
M - Matawan

Q - Queens
RW - Ridgewood
RH - Riverhead

Table 6. Simple Correlation Matrix of Pollutants with Each Other and Meteorological Factors in Each Community

	SO$_2$							Max. Temp.						
	B	E	F	M	Q	RW	RH	B	E	F	M	Q	RW	RH
RSP	.17*	-.14	.41	.11	.39	.43	.25	.29	.31	.12	.22**	.27**	.10	.09
TSP	.07	.41	.58	.15*	.43	.41	.29	.43	.23**	.28	.38	.07	.18**	.22
NO$_x$	.23	.43	.49	.50	.38	.44	.32	.11	-.02	.07	-.13*	.09	.01	-.01
SO$_x$	.21**	.23**	.37	.18**	.32	.23**	.24	.05	.18**	.10	.10	-.01	.12	-.07
SO$_2$								-.49	-.25	-.21**	-.36	-.42	-.33	.41
Max. Temp.														
Min. Temp.														
Humidity														

* - P = ≤ 0.05
** - P = < 0.01

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Table 6. Simple Correlation Matrix of Pollutants with Each Other and Meteorological Factors in Each Community

	Min. Temp.							Humidity						
	B	E	F	M	Q	RW	RH	B	E	F	M	Q	RW	RH
RSP	.28	.33	.12	.22**	.28**	.05	.08	.21**	.16	.27	.08	.14	.06	.10
TSP	.40	.16*	.22	.28	.04	.15*	.17**	.12	.13	.21	-.08	.11	.24	.12
NO$_x$	.18**	-.02	.10	-.16**	.12	.02	-.03	.27	.21**	.26	.16**	.19**	.26	.16**
SO$_x$	.11	.25	.12	.14*	.07	.16**	-.01	.22	.17**	.22	.04	.19**	.18**	.07
SO$_2$	-.48	-.25	-.22**	-.35	-.39	-.35	-.44	-.16**	.27	.24	.05	.07	.20**	-.05
Max. Temp.	.90	.88	.88	.87	.89	.88	.89	.20**	-.01	.02	.05	.18**	.02	.18**
Min. Temp.								.34	.16	.18**	.20**	.19**	.18**	.15**
Humidity														

* - P = ≤0.05
** - P = ≤0.01

B - Bronx   Q - Queens
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MONITORING FOR OXIDES OF SULFUR AND

NITROGEN AND RELATED SALTS

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in Association with Photochemical Oxidant

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