UC Irvine

UC Irvine Previously Published Works

Title

Effects of exercise exposure on toxic interactions between inhaled oxidant and aldehyde air pollutants.

Permalink

<https://escholarship.org/uc/item/8nf5z9xq>

Journal

Journal of toxicology and environmental health, 25(2)

ISSN

0098-4108

Authors

Mautz, WJ
Kleinman, MT
Phalen, RF
et al.

Publication Date

1988

DOI

10.1080/15287398809531198

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at

<https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

EFFECTS OF EXERCISE EXPOSURE ON TOXIC INTERACTIONS BETWEEN INHALED OXIDANT AND ALDEHYDE AIR POLLUTANTS

William J. Mautz, Michael T. Kleinman, Robert F. Phalen,
T. Timothy Crocker

Department of Community and Environmental Medicine,
University of California, Irvine

Respiratory tract injury resulting from inhalation of mixtures of ozone (O_3) and nitrogen dioxide (NO_2) and of O_3 and formaldehyde (HCHO) was studied in Sprague-Dawley rats under exposure conditions of rest and exercise. Focal inflammatory injury induced in lung parenchyma by O_3 exposure was measured morphometrically and HCHO injury to the nasal respiratory epithelium was measured by cell turnover using tritium-labeled thymidine. Mixtures of O_3 (0.35 or 0.6 ppm) with NO_2 (respectively 0.6 or 2.5 ppm) doubled the level of lung injury produced by O_3 alone in resting exposures to the higher concentrations and in exercising exposures to the lower concentrations. Formaldehyde (10 ppm) mixed with O_3 (0.6 ppm) resulted in reduced lung injury compared to O_3 alone in resting exposures, but exercise exposure to the mixture did not show an antagonistic interaction. Nasal epithelial injury from HCHO exposure was enhanced when O_3 was present in a mixture. Mixtures of O_3 and NO_2 at high and low concentrations formed respectively 0.73 and 0.02 ppm nitric acid (HNO_3) vapor. Chemical interactions among the oxidants, HNO_3 , and other reaction products (N_2O_5 and nitrate radical) and lung tissue may be the basis for the O_3 - NO_2 synergism. Increased dose and dose rate associated with exercise exposure may explain the presence of synergistic interaction at lower concentrations than observed in resting exposure. No oxidation products were detected in O_3 -HCHO mixtures, and the antagonistic interaction observed in lung tissue during resting exposure may result from irritant breathing pattern interactions.

INTRODUCTION

Exercise is an important exposure variable acting to enhance the toxic effects of inhaled pollutants. Exercise increases minute ventilation and inhaled dose rate and is likely to extensively modify the internal dose distribution of inhaled toxicants. It is a common observation in exercise studies that toxic effects of inhaled compounds can be observed at considerably lower concentrations in exercising as opposed

This study was supported by the California Air Resources Board (A0-129-32, A2-129-33), the U.S. Environmental Protection Agency (R811494-02-0), and the Electric Power Research Institute (RP 1962-1).

We thank M. Azizian, S. Bucher, C. Bufalino, D. Daniels, R. Lejnieks, R. Mannix, T. McClure, T. Nguyen, and R. Zamora for technical assistance, and J. Cantonwine for typing the manuscript.

Requests for reprints should be sent to Dr. William J. Mautz, Department of Community and Environmental Medicine, University of California, Irvine, California 92717.

to resting exposures (DeLucia and Adams, 1977; Folinsbee et al., 1978; McDonnell et al., 1983; Adams and Schlegele, 1983; Mautz et al., 1985a).

Respiratory tract injury by air pollutants can also be modified by interactions when other compounds are inhaled in a mixture. Pulmonary biochemical and morphological changes induced in rodents by ozone (O_3) or nitrogen dioxide (NO_2) inhalation have been reported to be enhanced by the presence of acidic aerosols as copollutants (Last et al., 1983, 1984, 1986). Other studies have demonstrated synergistic interactions between inhaled O_3 and H_2SO_4 using allergic sensitization and resistance to bacterial infection as end points (Gardner et al., 1974; Osebold et al., 1980). The combination of O_3 and NO_2 also shows evidence of synergism in producing biochemical alterations in lung tissue and depressing resistance to bacterial infection (Goldstein, 1976; Mustafa et al., 1984; Graham et al., 1987). While the mechanisms underlying these interactions are as yet poorly known, it is clear that interactions between air pollutant compounds in mixtures are potentially important in the evaluation of toxicity of these compounds.

Possible toxic interactions between O_3 and formaldehyde (HCHO) have not been examined. While the acute toxic effects of the oxidants, O_3 and NO_2 , occur in lung tissues, HCHO primarily affects epithelial tissues of the upper respiratory tract (Swenberg et al., 1983; Buckley et al., 1984; Morgan et al., 1986a,b; Maronpot et al., 1986). This is likely the consequence of relatively high aqueous solubility and preferential deposition of HCHO in the nasal cavity (Egle, 1972; Heck et al., 1983). The presence of oxidants could, however, modify HCHO effects in the nasal cavity through either chemical or physiological mechanisms.

In view of the broadly potentiating effects of exercise on responses to single inhaled pollutants, we investigated the effects of exercise exposure on interactions between O_3 and NO_2 and between O_3 and HCHO. Rats were exposed at rest and during exercise to these compounds alone and in combination, and the effects on respiratory tract tissues were quantified for analysis of interactions between exposure variables.

MATERIALS AND METHODS

Animals and Exposure Systems

Experimental subjects were male barrier-reared Sprague-Dawley rats (Hilltop Laboratory Animals, Inc., Scottsdale, Pa.). Rats were shipped in filter barrier containers, and housed at the laboratory in a laminar-flow air barrier caging system using high-efficiency particle filters and $KMnO_4$ on alumina (Purafil, H. E. Burroughs, Inc.) filters. Rats were received at age 6 wk and held for 1 wk before exposure. Ten percent of the rats in each shipment were autopsied immediately to

verify that the animals were free from lung disease. In experiments involving exercise exposure, rats were trained to achieve an exposure exercise capability of running at 15 m/min at 20% grade for 3 h. Training on d 1 was continuous running for 3 h at 8 m/min, 20% grade; d 2 was 30 min running at 15 m/min 20% grade alternating with 5 min rest for 2.9 h; and d 3 was continuous running for 3 h at 15 m/min, 20% grade. Training was designed to acquaint the rats with the treadmill and to ensure that they could complete the exposure protocol. Trained animals were randomly assigned to experimental groups.

Exposures of rats at rest were conducted in 1-m³ stainless steel chambers (Walters et al., 1982). Rats were held in nose-only exposure tubes. Exercising exposures were performed in a Quinton 42-15 rodent treadmill modified to contain the exposure atmosphere delivered from the 1-m³ chamber (Mautz et al., 1985a,b). Rats exposed during exercise ran at 15 m/min, 20% grade for 3 h, and this exercise level raised metabolic gas exchange by a factor of about two over resting metabolism (Mautz et al., 1985a,b).

Pollutant Exposures

Exposure experiments with a single batch shipment of rats aged 7 wk were limited to 4–5 treatment groups, and it was not possible to include all combinations of single and mixed pollutant exposures at both rest and exercise in a single experiment. Therefore, five exposure experiments were performed to test a restricted set of treatment effects.

1. Resting exposure for 4 h to (a) clean air, (b) 0.6 ppm O₃, (c) 0.6 ppm O₃ + 2.5 ppm NO₂.
2. Exercise exposure for 3 h to (a) clean air, (b) 0.35 ppm O₃, (c) 0.6 ppm NO₂, (d) 0.35 ppm O₃ + 0.6 ppm NO₂.
3. Exposure for 3 h to (a) clean air at rest, (b) 0.6 ppm O₃ during exercise, (c) 0.6 ppm O₃ + 2.5 ppm NO₂ at rest, and (d) 0.6 ppm O₃ + 2.5 ppm NO₂ during exercise.
4. Resting exposure for 4 h to (a) clean air, and (b) 10 ppm HCHO.
5. Exposure for 3 h to (a) clean air at rest, (b) 0.6 ppm O₃ at rest, (c) 0.6 ppm O₃ + 10 ppm HCHO at rest, (d) 10 ppm HCHO during exercise, and (e) 0.6 ppm O₃ + 10 ppm HCHO during exercise.

Exposure atmospheres were generated by mixing pollutants with purified air. Outdoor air was compressed to 100 psig with a liquid water ring compressor (Nash Engineering Co., Norwalk, Conn.), then filtered through a bed of KMnO₄ on alumina, a Del-Monox gas scrubbing filter (Deltech, Newcastle, Del.), and, after decompression, through a HEPA (high-efficiency particle absolute) air filter. Air was then humidified by injection of water vapor to 40 ± 2% or 85 ± 2% relative humidity. Test

gases were injected into the air stream, which then passed through a conical diffuser-mixer (Walters et al., 1982) and to the 1-m³ exposure chamber.

Ozone was generated from medical-grade oxygen passed through an electrostatic discharge O₃ generator (Sander Ozonizer, Type III, Osterburg, West Germany), and NO₂ was metered into the air stream from a compressed gas cylinder (1% NO₂ in nitrogen). Formaldehyde was produced by passing dry N₂ through a bed of paraformaldehyde maintained at 60°C and then metering the vapor into the air stream. Exposure concentrations were monitored from fluoro carbon or stainless steel sample lines placed in the rats' breathing zone either in the exposure chamber or in the treadmill (Mautz et al., 1985a). Because pollutant mixtures could involve chemical reactions of constituents, generation of test compounds was controlled to support target concentrations at the breathing zone of the exposed animals. Ozone was measured by ultraviolet spectrophotometry (Dasibi model 1003-AH, Dasibi Environmental Corp., Glendale, Calif.), and NO₂ was measured with a chemiluminescent detector (Monitor Labs 8840, San Diego, Calif.). Formaldehyde was collected into solution using midjet impingers and analyzed using a chromotropic acid colorimetric method (Altshuller et al., 1961; Katz, 1977).

Mixtures of O₃ with NO₂ and with HCHO were sampled for the possible formation of oxidation products HNO₃ and formic acid (HCOOH) during the 2.5-min passage time between gas mixing and exposure of rats. Vapor-phase HNO₃ was analyzed in the rat breathing zone by collection onto a nylon filter (Membrane Nylasorb) preceded by a 1-μm pore size fluorocarbon particle filter (Gelman Zefluor). Filters were extracted in distilled water and analyzed for NO₃⁻ and HCOO⁻ with an ion-exchange chromatograph (Dionex Model 10, Sunnyvale, Calif.).

Histopathology

Acute exposure to oxidants results in lung tissue injury and an inflammation repair process that is revealed as focal lesions in terminal bronchioles and alveolar regions (Evans, 1984; Bils and Christie, 1980). At 18 or 48 h after a single 2- to 4-h O₃ exposure, scattered centriacinar units in the alveolar (parenchymal) zone of the lung develop are infiltrated by macrophages in response to death of alveolar epithelial cells. One or more adjacent involved centriacinar units appear as a discrete inflammatory focus surrounded by otherwise normal parenchymal structures. These focal inflammations are referred to as lesions because they represent sites of lung injury with cell killing and repair. The lesions differ in numbers and types of infiltrating cells, and the extent of lung injury can be quantified morphometrically as described by Mautz et al. (1985b). Rats were anesthetized 48 h post exposure with

sodium pentobarbital (250 mg/kg) and killed by exsanguination. The trachea and lungs were removed and fixed by airway perfusion with 10% neutral buffered formalin at 30 cm fluid pressure for 72 h (McClure et al., 1982). The volume of the fixed right middle lobe was measured by displacement and the lobe was cut along the center line of the lobar bronchus. A block consisting of half of the lobe was embedded in paraffin, sectioned at 6 μm , and stained with hematoxylin and eosin. The use of one half of the right middle lobe as the tissue sample from each rat is based on preliminary quantitative evaluation of the volume density of inflammatory lesions in all lobes of lungs from rats exposed to a range of O_3 concentrations (Mautz et al., 1985b). Variation among sections within a lobe and among lobes was small compared to variation among animals and among pollutant treatment groups. The volume density of the lung parenchymal region (excluding major vessels and bronchi) in a section of the lobe was measured using a dissecting microscope and a 100-square ocular grid at a magnification of 12.5 in a systematic scan of the entire section. Each grid division was regarded as a "point" in the morphometric approach described by Cruz-Drive and Weibel (1981). On the low-power scan, the section was evaluated only for the number of points represented by parenchyma or nonparenchyma (major vessels and bronchi). The section was then systematically examined at a magnification of $\times 100$ using an ocular grid; at this magnification, each grid square covers $0.1 \times 0.1 \mu\text{m}$. In the 100-square ocular grid, one grid square of $100 \times 100 \mu\text{m}$ overlies all or parts of about 2.5 alveoli with a mean diameter of about 70 μm . Each "point" contains a population of cells and/or an anatomic structure large enough to be assigned a descriptive category that defines the histologic state of the tissue within that point. Histologic states were designated as:

- a. Normal: typically delicate walls of alveolar septae, alveolar ducts and terminal bronchioles without free cells in the alveolar lumen nor excess nuclei in the walls of these structures.
- b. Type 1: as above but with one or more free cells, usually macrophages or shed epithelial cells, in the lumen. This state occurs at low incidence in lungs of control rats; however, the number of sampling "points" that exhibit the type 1 state increases markedly at 24 and 48 h after exposure to O_3 , so the appearance of free cells in the air spaces of the alveolar zone of the lung is useful as an indicator of the effect of exposure.
- c. Type 2: represented by increased numbers of nuclei in the walls of alveolar ducts and septae with or without shed epithelial cells or free macrophages in air spaces.
- d. Type 3: like type 2 but, in addition, the walls of bronchioles, ducts, and alveoli have increased proteinaceous eosinophilic desposits

consistent with interstitial accumulation of plasma proteins. Discrete areas of the lung parenchyma contain a confluent area made up of one or more adjacent centriacinar units that exhibit the type 1, 2, or 3 histologic state. This focus, or area, is surrounded by normal parenchymal structures, so that the acute inflammatory response to O_3 appears as a focal lesion.

The acute inflammatory response of rat lung parenchyma to O_3 alone or to O_3 combined with other air pollutants was quantified by dividing the number of "points" recorded as $100 \times 100 \mu\text{m}$ grid divisions that fell over histologic states 1, 2, or 3 in the parenchymal zone of the section by the total number of parenchymal "points" recorded in the first scan at $\times 12.5$, adjusted to the same units of area as were represented in the second scan at $\times 100$. The quotient represents the percent of the parenchyma of the lung lobe that was occupied by structures exhibiting the histologic states described above. In an earlier study (Mautz et al., 1985b) we found this analysis effective in distinguishing acute ozone treatment effects for the variables exercise, O_3 concentration, and exposure duration.

Nasal epithelial injury was assessed following HCHO exposure experiments by measurement of cell turnover rates using incorporation of tritium-labeled thymidine into DNA of dividing cells. Labeled thymidine was injected intraperitoneally ($2 \mu\text{Ci/g}$ body mass) 24 h post exposure. Rats were killed at 48 h post exposure, and that portion of the head containing the nasal cavity was fixed in formalin and decalcified for three weeks in Tris-buffered EDTA. Sections of nasal cavity were prepared by freehand cutting a $2\text{--}3 \mu\text{m}$ slice through the hard palate at the level of the incisive papillae perpendicular to the plane of the hard palate and nasal septum. Slices were embedded in glycol methacrylate, sectioned at $2 \mu\text{m}$, dipped in Kodak NTB 2 emulsion, air dried, and stored refrigerated in light-tight boxes for 30 d. Multiple sequential, but not serial, sections were prepared by discarding $50 \mu\text{m}$ between sections; the $50\text{-}\mu\text{m}$ separation between sections eliminated the possibility of counting the same cell population. After photographic development, slides were stained lightly with toluidine blue. Formaldehyde injury is evident in respiratory epithelium of the anterior nasal passages, specifically at the tips of the nasal and maxillary turbinates and lateral wall of level II nasal cavity sections (Chang et al., 1983; Buckley et al., 1984; Morgan et al., 1986a,b). We observed the most prominent injury in a subdivision of respiratory epithelium characterized by non-ciliated or sparsely ciliated cuboidal epithelium—the same locale observed as a focus for development of polypoid adenomas in chronic HCHO exposures of rats (Morgan et al., 1986c). The percentage of labeled cells was determined by cell counts of sections of this epithelium. No consistent changes occurred in other epithelial zones (gob-

TABLE 1. Effects of Exercise on Lung Injury Induced by Exposures to O₃ and NO₂ Alone and in Combination^a

Experiment	Exposure group	Exposure conditions	n	Focal lung lesions (% ± SE of parenchyma)
1	Clean air control	Rest 4 h	6	1.63 ± 0.037 ^b
	0.6 ppm O ₃	Rest 4 h	6	6.77 ± 0.69 ^c
	0.6 ppm O ₃ + 2.5 ppm NO ₂	Rest 4 h	6	14.33 ± 1.83 ^d
2	Clean air control	Exercise 3 h	10	1.69 ± 0.20 ^b
	0.6 ppm NO ₂	Exercise 3 h	9	1.87 ± 0.17 ^b
	0.35 ppm O ₃	Exercise 3 h	8	6.11 ± 1.03 ^c
	0.35 ppm O ₃ + 0.6 ppm NO ₂	Exercise 3 h	9	10.76 ± 1.63 ^d
3	Clean air control	Rest 3 h	6	2.24 ± 0.38 ^b
	0.6 ppm O ₃	Exercise 3 h	9	20.77 ± 2.85 ^c
	0.6 ppm O ₃ + 2.5 ppm NO ₂	Rest 3 h	8	10.22 ± 1.00 ^d
	0.6 ppm O ₃ + 2.5 ppm NO ₂	Exercise 3 h	9	29.95 ± 2.98 ^e

^aRelative humidity was 85% (experiments 1 and 3) or 45% (experiment 2). Exercise level was 15 m/min at 20% grade.

^{b-e}Treatment groups with different superscripts within an exposure experiment were significantly different from one another (ANOVA and Tukey multiple comparisons, *p* < 0.05).

let-cell-bearing respiratory epithelia or squamous or olfactory epithelia).

RESULTS

Exposure Atmospheres

O₃ and NO₂ concentrations were controlled at target values with coefficients of variation less than 5%. The HCHO vapor concentrations during exposures were controlled with coefficients of variation less than 12%. Mixtures of O₃ with NO₂ produced HNO₃ vapor; 0.35 ppm O₃ + 0.6 ppm NO₂ at 45% relative humidity formed 49 ± 9 SD μg/m³ HNO₃ (0.02 ppm), and mixtures of 0.6 ppm O₃ + 2.5 ppm NO₂ at 85% relative humidity formed 1900 ± 620 SD μg/m³ HNO₃ (0.73 ppm). Mixtures of 0.6 ppm O₃ and 10 ppm HCHO did not produce detectable HCOO⁻.

Toxicology

There were strong interactions between the oxidant and HCHO gases and exercise versus resting exposure influencing lung parenchymal and nasal epithelial damage. Oxidant exposures inducing significant lung inflammatory responses involved all three histological response states in focal lesions with no clear patterns of differential responses among the histological categories. Consequently a single combined category measure of percent of parenchyma occupied by focal lesions is given. Lung parenchymal lesions induced by exposures to O₃ and NO₂ alone and in combination are shown Table 1. Single

resting exposure to 0.6 ppm O₃ induced a significant inflammatory lesion response, and the combination 0.6 ppm O₃ + 2.5 ppm NO₂ resulted in lesion percentages significantly elevated over those from 0.6 ppm O₃ alone. Exercising exposures to lower concentrations of both O₃ and NO₂ demonstrated the synergistic interactive effect between the oxidants. Exposure to 0.6 ppm NO₂ alone did not induce significant inflammatory response. Exposure to 0.35 ppm O₃ induced a response, and exposure to the combination of O₃ and NO₂ resulted in an approximate doubling of inflammatory lesions compared to O₃ exposure alone. The interaction was highly significant (O₃, NO₂ interaction $p < 0.0005$, ANOVA). A comparison of rest versus exercise exposure to 0.6 ppm O₃ in combination with 2.5 ppm NO₂ showed a significant increase in parenchymal lesions for the exercise group. Resting exposure to the higher concentrations of experiment 3 gave a lesion response similar to exercise exposure to the lower concentrations (experiment 2), and exercising exposure to the higher concentrations elevated lesions by a factor of 3 over resting exposure.

Formaldehyde inhalation had no discernible effect on lung parenchyma, but there was a strong interaction between HCHO and O₃ in a combination exposure (Table 2). Parenchymal inflammation following exercising or resting exposures to HCHO alone did not differ from clean air control. While a resting exposure to 0.6 ppm O₃ gave a strong lesion response, 0.6 ppm O₃ in combination with 10 ppm HCHO did not differ from the clean air control. When the exposure to the combination was performed during exercise, however, a severe lung lesion response was induced.

The direct effects of HCHO exposure were apparent in cell turnover indices in nasal respiratory epithelium (Table 3). The response following exposure to 0.6 ppm O₃ alone at rest did not differ from that of the clean air control, but exposure to HCHO alone or in combination

TABLE 2. Effects of Exercise on Lung Injury Induced by Exposures to O₃ and HCHO Alone and in Combination*

Experiment	Exposure group	Exposure conditions	n	Focal lung lesions (% $\pm$ SE of parenchyma)
4	Clean air control	Rest 4 h	4	2.25 $\pm$ 0.44 ^b
	10 ppm HCHO	Rest 4 h	5	2.28 $\pm$ 0.37 ^b
5	Clean air control	Rest 3 h	10	2.58 $\pm$ 0.25 ^b
	0.6 ppm O ₃	Rest 3 h	10	8.93 $\pm$ 0.89 ^c
	10 ppm HCHO	Exercise 3 h	10	3.16 $\pm$ 0.28 ^b
	0.6 ppm O ₃ + 10 ppm HCHO	Rest 3 h	10	3.45 $\pm$ 0.40 ^b
	0.6 ppm O ₃ + 10 ppm HCHO	Exercise 3 h	9	35.18 $\pm$ 2.89 ^d

*Relative humidity was 85% Exercise level was 15 m/min at 20% grade.

^{b-d}Treatment groups with different superscripts were significantly different (ANOVA and Tukey multiple comparisons, $p < 0.05$).

TABLE 3. Effects of Exercise on Nasal Epithelial Injury Induced by Exposure to O₃ and HCHO Alone and in Combination^a

Exposure group	Exposure conditions	<i>n</i>	Percent labeled cells in low columnar respiratory epithelium (mean ± SE)
Clean air control	Rest 3 h	7	1.03 ± 0.26 ^b
0.6 ppm O ₃	Rest 3 h	10	0.47 ± 0.15 ^b
10 ppm HCHO	Exercise 3 h	7	6.67 ± 2.35 ^c
0.6 ppm O ₃ + 10 ppm HCHO	Rest 3 h	9	9.88 ± 1.86 ^c
0.6 ppm O ₃ + 10 ppm HCHO	Exercise 3 h	6	15.27 ± 1.71 ^d

^aRelative humidity was 85%. Exercise level was 15 m/min at 20% grade.

^{b-d}Treatment groups with different superscripts were significantly different from one another (ANOVA and Tukey multiple comparisons, *p* < 0.05).

with O₃ induced a significant cell turnover response. Exercise exposure to the combination produced a response that was significantly elevated above that seen for resting exposure, and the presence of 0.6 ppm O₃ in combination with HCHO significantly elevated cell turnover as compared to HCHO alone in exercise-exposed rats.

DISCUSSION

Exercise poses an important challenge to respiratory defenses against the toxic effects of air pollutants. This study has demonstrated that exercise exposure can greatly modify the interactive toxic effects of combined air pollutants on respiratory tract tissues. While human pulmonary function studies have not found evidence for strong interactions between the oxidants O₃ and NO₂ (Hackney et al., 1975; Folinsbee et al., 1981; Kagawa, 1983), synergistic and additive interactions have been demonstrated at the biochemical level and in resistance to bacterial infection in animal studies (Ehrlich et al., 1977; Watanabe et al., 1980; Mustafa et al., 1984; Graham et al., 1987). In the present study, synergism was demonstrated at the tissue level as lung parenchymal inflammatory lesions. The effect of exercise exposure was to reveal the toxic interaction at lower exposure concentrations. Early studies of O₃ interactions with NO₂ or with respirable aerosols used O₃ concentrations greater than 0.45 ppm and NO₂ concentrations greater than 4 ppm and employed multiple daily exposures (Watanabe et al., 1980; Last et al., 1983; Mustafa et al., 1984). A recent study using the bacterial infectivity model (Graham et al., 1987) found significant interactions between O₃ and NO₂ using exposure protocols with superimposed concentration peaks to twice baseline concentrations of 0.5 ppm NO₂ and 0.05 ppm O₃. No interaction was observed in concentration regimes where exposure to each gas alone failed to have significant

effects. Strong interactions were observed in the present study with single exercising exposures to 0.35 ppm O_3 and 0.6 ppm NO_2 (Table 1). This synergism was observed at an NO_2 concentration that had no detectable effect when tested alone. These levels are in the range of concentrations occurring during urban air pollution episodes (Pitts, 1983), and under exposure conditions of mild activity for realistic durations.

The basis for the toxic interactions between O_3 and NO_2 may lie in chemical reactions between the oxidants, and in biochemical interactions between the reactants and products at the tissue level. The mixture of O_3 and NO_2 formed significant quantities of HNO_3 vapor, adding another potentially toxic compound to the mixture. Observations that the toxic effects of O_3 are enhanced by the presence of particle acids (Last et al., 1986) suggests that the O_3 - NO_2 synergism could stem from the formation of the acid, HNO_3 . However, reactions between O_3 and NO_2 are expected to form other products in addition to HNO_3 , including N_2O_5 and nitrate radical (Diggle and Gage, 1955; Mustafa et al., 1984; Pitts, 1983), and these products could also modify toxicity of the mixture of pollutants. The effects of exercise in enhancing the toxic effects of the mixture may be a consequence of the increased inhalation dose rate associated with increased respiratory ventilation and decreased effectiveness of upper airway scrubbing (Yokoyama and Frank, 1972).

The inhibitory effect of HCHO on O_3 -induced lung injury in resting exposures to the combination (Table 2) was unexpected. We are not aware of chemical properties of HCHO that could act to inhibit the free radical peroxidation effects of O_3 on epithelial tissue, and the high aqueous solubility of HCHO makes it unlikely that substantial quantities could penetrate to the lung and participate in biochemical interactions (Egle, 1972; Heck et al., 1983). An alternative mechanism that could explain the antagonistic interaction between these pollutants is based on their irritant effects on respiration. Exposure to the pulmonary irritant O_3 induces a reflex shift in breathing pattern to rapid and shallow respiration (Lee et al., 1979; McDonnell et al., 1983). Formaldehyde, however, is an upper-airway irritant (Alarie, 1973; Kane and Alarie, 1977) and induces reflex depression of breathing frequency and minute ventilation by up to 30–50% of control in resting exposures of rats and mice to 7–15 ppm HCHO (Chang et al., 1981; Jaeger and Gearhart, 1982). Such depression of ventilation will reduce the inhaled dose of O_3 in an exposure to the mixture, and a shift in breathing pattern and possible increase in respiratory resistance (Amdur, 1960), could further affect the distribution of inhaled O_3 in the lungs. It is interesting in this regard that exercise exposure to the combination of O_3 and HCHO appeared to remove the antagonistic effects of HCHO on O_3 and induced massive lung tissue damage. A rat group exposed during

exercise to 0.6 ppm O_3 alone was not available for direct comparison in experiment 5; however, this exposure was performed in experiment 3 (Table 1) and did not result in greater lung injury than exercise exposure to O_3 + HCHO in experiment 5. The exercise demand for gas exchange may override the irritant inhibitory effects of HCHO on breathing pattern and ventilation. The interactive effects between O_3 and HCHO in the lung may be primarily mediated by physiological responses to the irritant HCHO, rather than to direct chemical interactions in lung tissue.

Acidic compounds, such as HNO_3 vapor, are also upper-airway irritants (Alarie, 1973), and reflex effects on breathing pattern could play a role in the interaction between O_3 , NO_2 , and associated reaction products. However, enhancement of oxidant effects occurred for the mixtures in both exercise and rest exposures, and this is consistent with a chemical interaction mediating the synergism and exercise increasing the dose rate to the deep lung.

Turnover of cells in nasal respiratory epithelium reflected injury due primarily to HCHO; 0.6 ppm O_3 alone at rest had no significant effect on nasal epithelium (Table 3). HCHO induced nasal epithelial injury, and there was evidence that the presence of O_3 as a copollutant enhanced the effect of HCHO. Exercise exposure to the combination of HCHO and O_3 resulted in a greater labeling index than exercise exposure to HCHO alone. Whether this interaction is related to chemical interactions between the pollutant gases in the epithelium or to different changes in breathing pattern and dose distribution invoked by irritancy of the pollutants is not clear. Variations in ventilatory responses to HCHO in rats and mice have been implicated as affecting the magnitude of epithelial injury (Chang et al., 1983). Breathing pattern responses to O_3 exposure are present both at rest and during exercise in dogs and humans (DeLucia and Adams, 1977; Lee et al., 1980). Establishment of the basis for O_3 and HCHO interactions will require concurrent measurement of breathing pattern to control for possible changes in dose distribution.

This study has shown that the toxic responses of pollutant compounds inhaled in mixtures can be complex. Interactions may be mediated by chemical reactions forming new toxic products, by biochemical interactions of compounds at the tissue level, or by physiological interactions influencing breathing pattern and ventilation. Exposure of people to airborne toxic agents usually involves exposure to multiple compounds in mixtures and frequently occurs under conditions of exercise respiratory ventilation. The toxicity of pollutants is not fully appreciated by tests of single compounds, and identification of the relative importance of modifying factors remains an important topic for investigation.

REFERENCES

- Adams, W. C., and Schelegle, E. S. 1983. Ozone and high ventilation effects on pulmonary function and endurance performance. *J. Appl. Physiol.* 55:805–812.
- Alarie, Y. 1973. Sensory irritation by airborne chemicals. *CRC Crit. Rev. Toxicol.* 2:299–363.
- Amdur, M. O. 1960. The response of guinea pigs to inhalation of formaldehyde and formic acid alone and with a sodium chloride aerosol. *Int. J. Air Pollut.* 3:201–220.
- Altshuller, A. P., Miller, D., and Sleva, S. 1961. Determination of formaldehyde in gas mixtures by the chromotropic acid method. *Anal. Chem.* 33:621–625.
- Bils, R. F., and Christie, B. R. 1980. The experimental pathology of oxidant and air pollutant inhalation. *Int. Rev. Exp. Pathol.* 21:195–293.
- Buckley, L. A., Jiang, X. Z., James, R. A., Morgan, K. T., and Barrow, C. S. 1984. Respiratory tract lesions induced by sensory irritants at the RD50 concentration. *Toxicol. Appl. Pharmacol.* 74:417–429.
- Chang, J. C. F., Steinhagen, W. H., and Barrow, C. S. 1981. Effect of single or repeated formaldehyde exposure on minute volume of B6C3F1 mice and F-344 rats. *Toxicol. Appl. Pharmacol.* 61:451–459.
- Chang, J. C. F., Gross, E. A., Swenberg, J. A., and Barrow, C. S. 1983. Nasal cavity deposition, histopathology, and cell proliferation after single or repeated formaldehyde exposures in B6C3F1 mice and F-344 rats. *Toxicol. Appl. Pharmacol.* 68:161–176.
- Cruz-Drive, L. M., and Weibel, E. R. 1981. Sampling designs for stereology. *J. Microsc.* 122:235–257.
- DeLucia, A. J., and Adams, W. C. 1977. Effects of O₃ inhalation during exercise on pulmonary function and blood biochemistry. *J. Appl. Physiol.* 43:75–81.
- Diggle, W. M., and Gage, J. C. 1955. The toxicity of ozone in the presence of oxides of nitrogen. *Br. J. Ind. Med.* 12:60–64.
- Egle, J. L. 1972. Retention of inhaled formaldehyde, propionaldehyde and acrolein in the dog. *Arch. Environ. Health* 25:119–124.
- Ehrlich, R., Findlay, J. C., Fenters, J. D., and Gardner, D. E. 1977. Health effect of short-term inhalation on nitrogen dioxide and ozone mixtures. *Environ. Res.* 14:223–231.
- Evans, M. J. 1984. Oxidant gases. *Environ. Health Perspect.* 55:85–95.
- Folinsbee, L. J., Drinkwater, B. L., Bedi, J. F., and Horvath, S. M. 1978. The influence of exercise on the pulmonary function changes due to exposure to low concentrations of ozone. In *Environmental Stress*, eds. L. J. Folinsbee, J. A. Wagner, J. F. Borgia, B. L. Drinkwater, J. A. Gliner, and J. F. Bedi, pp. 125–145. New York, Academic Press.
- Folinsbee, L. J., Bedi, J. F., and Horvath, S. M. 1981. Combined effects of ozone and nitrogen dioxide on respiratory function in man. *Am. Ind. Hyg. Assoc. J.* 42:534–541.
- Gardner, D. E., Illing, J. W., Miller, F. U. and Coffin, D. L. 1974. Enhancement of effect of exposure of O₃ and NO₂ by exercise. *Toxicol. Appl. Pharmacol.* 29:129–130 (abstr.).
- Goldstein, B. O. 1976. Combined exposure to ozone and nitrogen dioxide. *Environ. Health Perspect.* 13:107–110.
- Graham, J. A., Gardner, D. E., Blommer, E. J., House, D. E., Menache, M. G., and Miller, F. J. 1987. Influence of exposure patterns of nitrogen dioxide and modifications by ozone on susceptibility to bacterial infectious disease in mice. *J. Toxicol. Environ. Health* 21:113–125.
- Hackney, J. D., Linn, W. S., Mohler, J. G., Pedersen, E. E., Breisacher, P., and Russo, A. 1975. Experimental studies on human health effects of air pollutants. *Arch. Environ. Health* 30:379–384.
- Heck, H. d'A., Chin, T. Y., and Schmitz, M. C. 1983. Distribution of (¹⁴C) formaldehyde in rats after inhalation exposure. In *Formaldehyde Toxicity*, ed. J. E. Gibson, pp. 26–37. Washington, D.C.: Hemisphere.
- Jaeger, R. J., and Gearhart, J. M. 1982. Respiratory and metabolic response of rats and mice to formalin vapor. *Toxicology* 25:299–309.
- Kane, L. E., and Alarie, Y. 1977. Sensory irritation to formaldehyde and acrolein during single and repeated exposures in mice. *Am. Ind. Hyg. J.* 38:509–522.
- Kagawa, J. 1983. Respiratory effects of two-hour exposure with intermittent exercise to ozone,

- sulfur dioxide and nitrogen dioxide alone and in combination in normal subjects. *Am. Ind. Hyg. Assoc. J.* 44:14–20.
- Katz, M. 1977. *Methods of Air Sampling Analysis*, pp. 300–307. Washington, D.C.: American Public Health Association.
- Last, J. A., Gerriets, J. E., and Hyde, D. M. 1983. Synergistic effects on rat lungs of mixtures of oxidant air pollutants (ozone and nitrogen dioxide) and respirable aerosols. *Am. Rev. Respir. Dis.* 128:539–544.
- Last, J. A., Hyde, D. M., and Chang, D. P. Y. 1984. A mechanism of synergistic lung damage by ozone and a respirable aerosol. *Exp. Lung Res.* 7:223–235.
- Last, J. A., Hyde, D. M., Guth, D. J., and Warren, D. L. 1986. Synergistic interaction of ozone and respirable aerosols on rat lungs. 1. Importance of aerosol acidity. *Toxicology* 39:247–257.
- Lee, L. Y., Dumont, C., Djokic, T. D., Menzel, T. E., and Nadel, J. A. 1979. Mechanism of rapid, shallow breathing after ozone exposure in conscious dogs. *J. Appl. Physiol.* 46:1108–1114.
- Lee, L. Y., Djokic, T. D., Dumont, D., Graf, T. D., and Nadel, J. A. 1980. Mechanism of ozone-induced tachypneic response to hypoxia and hypercapnia in conscious dogs. *J. Appl. Physiol.* 48:163–168.
- Maronpot, R. R., Miller, R. A., Clarke, W. J., Westerburg, R. B., Decker, J. R., and Moss, O. R. 1986. Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. *Toxicology* 41:253–266.
- Mautz, W. J., McClure, T. R., Reischl, P., Phalen, R. F., and Crocker, T. T. 1985a. Enhancement of ozone-induced lung injury by exercise. *J. Toxicol. Environ. Health* 16:841–854.
- Mautz, W. J., Phalen, R. F., McClure, T. R., and Bufalino, C. 1985b. A rodent treadmill for inhalation toxicological studies and respirometry. *J. Appl. Physiol.* 58:673–679.
- McClure, T. R., Diller, P. J., and Phalen, R. F. 1982. An airway perfusion apparatus for whole lung fixation. *Lab. Anim. Sci.* 32:195–196.
- McDonnell, W. F., Horstman, D. H., Hazucha, M. J., Seal, E., Haak, E. D., Salaam, S. A., and House, D. E. 1983. Pulmonary effects of ozone exposure during exercise: Dose response characteristics. *J. Appl. Physiol.* 54:1345–1352.
- Morgan, K. T., Patterson, D. L., and Gross, E. A. 1986a. Response of the nasal mucociliary apparatus of F-344 rats to formaldehyde gas. *Toxicol. Appl. Pharmacol.* 82:1–13.
- Morgan, K. T., Gross, E. A., and Patterson, D. L. 1986b. Distribution progression, and recovery of acute formaldehyde-induced inhibition of nasal mucociliary function in F-344 rats. *Toxicol. Appl. Pharmacol.* 86:448–456.
- Morgan, K. T., Jiang, X., Starr, T. B., and Kearns, W. D. 1986c. More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. *Toxicol. Appl. Pharm.* 82:264–271.
- Mustafa, M. G., Elsayed, N. M., von Dohlen, F. M., Hassett, C. M., Postlethwait, E. M., Quinn, C. L., Graham, J. A., and Gardner, D. E. 1984. A comparison of biochemical effects of nitrogen dioxide, ozone and their combination in mouse lung. 1. Intermittant exposures. *Toxicol. Appl. Pharmacol.* 72:82–90.
- Osebold, J. W., Gershwin, L. J., and Zee, Y. C. 1980. Studies on the enhancement of allergic lung sensitization by inhalation of ozone and sulfuric acid aerosol. *J. Environ. Pathol. Toxicol.* 3:221–234.
- Pitts, J. N. 1983. Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmosphere. *Environ. Health Perspect.* 47:115–140.
- Swenberg, J. A., Gross, E. A., Martin, J., and Popp, J. A. 1983. Mechanisms of formaldehyde toxicity. In *Formaldehyde Toxicity*, ed. J. E. Gibson, pp. 132–147. Washington, D.C.: Hemisphere.
- Walters, R. B., Phalen, R. F., Mannix, R. C., and Smart, G. L. 1982. An aerosol and gas aging line suitable for use in inhalation toxicological research. *Am. Ind. Hyg. Assoc. J.* 43:218–222.
- Watanabe, H., Fukase, O., and Isomura, K. 1980. Combined effects of nitrogen oxides and ozone on mice. In *Nitrogen Oxides and Their Effects on Health*, ed. S. D. Lee, pp. 181–189. Ann Arbor, Mich.: Ann Arbor Science.
- Yokoyama, E., and Frank, R. 1972. Respiratory uptake of ozone in dogs. *Arch. Environ. Health* 25:132–138.

Received June 8, 1987

Accepted April 5, 1988