

**Consequences of Prolonged Inhalation of  
Ozone on F344/N Rats: Collaborative Studies**

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**Part XII. Atrophy of Bone in Nasal Turbinates**

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**Includes the Commentary of the Institute's  
Health Review Committee**

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# HEI HEALTH EFFECTS INSTITUTE

The Health Effects Institute, established in 1980, is an independent and unbiased source of information on the health effects of motor vehicle emissions. HEI studies all major pollutants, including regulated pollutants (such as carbon monoxide, ozone, nitrogen dioxide, and particulate matter), and unregulated pollutants (such as diesel engine exhaust, methanol, and aldehydes). To date, HEI has supported more than 150 projects at institutions in North America and Europe.

Typically, HEI receives half its funds from the U.S. Environmental Protection Agency and half from 28 manufacturers and marketers of motor vehicles and engines in the United States. Occasionally, funds from other public or private organizations either support special projects or provide resources for a portion of an HEI study. For this study, the Institute acknowledges the cooperation and support of the National Toxicology Program (NTP), which consists of four charter agencies of the U.S. Department of Health and Human Services. The NTP sponsored the inhalation component of this project as part of its studies on the toxicologic and carcinogenic effects of ozone. However, in all cases HEI exercises complete autonomy in setting its research priorities and in reaching its conclusions. An independent Board of Directors governs HEI. The Institute's Research and Review Committees serve complementary scientific purposes and draw distinguished scientists as members. The results of HEI-funded studies are made available as Research Reports, which contain both the Investigators' Report and the Review Committee's evaluation of the work's scientific quality and regulatory relevance.

# HEI Statement

Synopsis of Research Report Number 65 Part XII

## Prolonged Ozone Exposure Leads to Structural Changes in the Rat Nose

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### BACKGROUND

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Ozone, a common outdoor air pollutant, is a highly reactive gas and a major component of smog. The U.S. Environmental Protection Agency (EPA) has set a one-hour National Ambient Air Quality Standard for ozone of 0.12 parts per million (ppm) that should not be exceeded more than once per year. This standard, which is based largely on data documenting the adverse effects of short-term exposure on lung function in humans, is currently being reevaluated by the EPA.

Because ozone can damage cells, prolonged or repeated exposures could increase the risk of cancer. For this reason, the National Toxicology Program (NTP) recently evaluated ozone's carcinogenicity in rodents. Another public health concern is that prolonged exposure to ozone might damage the structural components of the airways and contribute to developing noncancerous respiratory diseases. To examine this issue, the Health Effects Institute collaborated with the NTP to provide HEI-funded investigators access to animals that underwent the same rigorously controlled ozone inhalation protocol and quality assurance processes along with the NTP animals. In this NTP/HEI Collaborative Ozone Project, rats were exposed to 0, 0.12, 0.50, or 1.0 ppm ozone for six hours per day, five days per week, for 20 or 24 months.

In one of the original NTP/HEI collaborative studies, Dr. Jack Harkema and his colleagues found a number of structural and functional changes in the nasal region of the rats exposed to 0.5 or 1.0 ppm (but not 0.12 ppm) ozone. (In the rat nose, turbinates are structures that project from the walls of nasal passages and filter potentially harmful substances from the inhaled air.) One of the more provocative findings after ozone exposure was that bony areas of the nasal turbinates had atrophied. This was an intriguing finding because investigators in Mexico have reported alterations in the cells lining the nasal passages of people living in areas with high levels of mixed air pollutants, including ozone.

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### APPROACH

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HEI funded this follow-on study to allow Dr. Harkema and his colleagues the opportunity to employ sophisticated microscopic and quantitative techniques to examine the effects of ozone exposure on the nasal passages of F344/N rats. The tissues used in this study came from the NTP/HEI animals that had been exposed to ozone for 20 months and from the NTP animals that had continued the exposure protocol for 24 months.

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### RESULTS AND IMPLICATIONS

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The investigators confirmed and extended their original results. They found no effects in the nasal turbinates of male or female rats exposed to 0.12 ppm ozone, but the loss of turbinate bone in rats exposed to the two higher ozone concentrations (0.5 and 1.0 ppm) was a major finding. Dr. Harkema and colleagues also found that ozone exposure caused inflammation in an area adjacent to turbinate bone. The investigators proposed that ozone-induced inflammation contributed to the loss of turbinate bone.

The damage to nasal turbinate bone caused by ozone inhalation may have implications for nasal defense mechanisms. Reducing the surface area of the nasal turbinates could decrease their ability to filter potentially harmful dusts, irritant gases, bacteria, or viruses from inhaled air, a mechanism that protects the respiratory tract against infection and injury. These findings are provocative; however, they are difficult to extrapolate to humans because of marked differences between the nasal structures of rodents and humans and because rodents breathe only through the nose, whereas humans use both the nose and mouth. Nevertheless, they do point to the need for additional research on the effects of ozone on the structure and function of the human nasal passages.

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This Statement, prepared by the Health Effects Institute and approved by its Board of Directors, is a summary of a research project sponsored by HEI from 1995 to 1996. This study was conducted by Dr. Jack R. Harkema and colleagues of Michigan State University, East Lansing, MI. The following Research Report contains both the detailed Investigators' Report and a Commentary on the study prepared by the Institute's Health Review Committee.

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Jack R. Harkema, Paul J. Catalano, and Jon A. Hotchkiss

**I. STATEMENT Health Effects Institute . . . . . i**  
This Statement, prepared by the HEI and approved by the Board of Directors, is a nontechnical summary of the Investigators' Report and the Health Review Committee's Commentary.

**II. INVESTIGATORS' REPORT . . . . . 1**  
When an HEI-funded study is completed, the investigators submit a final report. The Investigators' Report is first examined by three outside technical reviewers and a biostatistician. The Report and the reviewers' comments are then evaluated by members of the HEI Health Review Committee, who had no role in selecting or managing the project. During the review process, the investigators have an opportunity to exchange comments with the Review Committee and, if necessary, revise the report.

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# INVESTIGATORS' REPORT

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## Consequences of Prolonged Inhalation of Ozone on F344/N Rats: Collaborative Studies

### Part XII: Atrophy of Bone in Nasal Turbinates

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#### ABSTRACT

As part of the National Toxicology Program/Health Effects Institute collaborative study of the health effects of prolonged ozone exposure, it was observed that rats chronically exposed to ozone had marked histopathologic changes in the upper respiratory tract, including atrophy of the nasal turbinates. The principal objective of the present study was to morphometrically assess the severity of the ozone-induced changes in the bony tissue of the maxilloturbinates in these chronically exposed rats. Male and female F344/N rats were exposed to 0, 0.12, 0.5, or 1.0 part per million (ppm)\* ozone, 6 hours/day, 5 days/week for 20 or 24 months. Rats were killed one week after the end of the exposure, and nasal tissues were processed for light and electron microscopy. Using image analysis and standard morphometric techniques, the amounts of bone, surface epithelium, and lamina propria comprising the maxilloturbinates were estimated by measuring the cross-sectional area of each tissue compartment at a defined location in the proximal nasal passage. Both male and female rats had significant morphologic and morphometric changes in the maxilloturbinates after prolonged exposures to 0.5 or 1.0 ppm ozone, but not to 0.12 ppm ozone. Ozone-exposed rats

had significant reductions in the cross-sectional area of turbinate bone, reflecting the loss of bone in the maxilloturbinate after prolonged exposure. This ozone-induced bony atrophy was more severe in male than in female rats. Using electron microscopy, numerous bone-resorption sites were identified on the outer, periosteal, surface of the turbinate bone in ozone-exposed animals. Rats with bony atrophy also had a conspicuous influx of mixed inflammatory cells into the lamina propria surrounding the turbinate bone. In addition, ozone exposures caused reductions in the area of lamina propria, due to blood vessel constriction, and increases in the area of the surface epithelium, due to hyperplasia and metaplasia. The results of the present study demonstrated that prolonged exposure of rats to ozone can cause marked loss of turbinate bone. The severity of this ozone-induced bony atrophy in rats is dependent on both concentration and gender.

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#### INTRODUCTION

Ozone is an irritating gas and the major oxidizing component in photochemical smog that is present in the tropospheric atmosphere of many urban areas during the summer months. This air pollutant is a common summertime problem not only in southern California, but also in many central, northeastern, and southeastern cities in the United States. Of the major air pollutants for which National Ambient Air Quality Standards (NAAQS) have been designated under the Clean Air Act Amendments of 1990, ozone presents the most pervasive problem (Steinfeld 1991). It has been estimated that during 1989, 67 million people lived in areas that were in violation of the NAAQS for ozone (Friedman 1989). The episodic high concentrations of ozone in the troposphere of large metropolitan areas in the United States and other large urban centers in the world, like Mexico City, Mexico, may pose significant threats to the health of their inhabitants.

Epithelial cells lining the respiratory airways are the primary cellular targets for ozone-induced injury (Dung-

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\* A list of abbreviations appears at the end of the Investigators' Report.

This Investigators' Report is Part XII of Health Effects Institute Research Report Number 65, which also includes a Commentary by the Health Review Committee, and an HEI Statement about the research project. Correspondence concerning the Investigators' Report may be addressed to Dr. Jack R. Harkema, Michigan State University, B336 Life Sciences Building, East Lansing, MI 48824-1317.

This study was supported by HEI funds from the U.S. Environmental Protection Agency and the motor vehicle industry. The inhalation component of this project was sponsored by the National Toxicology Program as part of its studies on the toxicologic and carcinogenic effects of ozone.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Award R824835 to the Health Effects Institute, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency, and no official endorsement should be inferred. The contents of this document also have not been reviewed by private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views or policies of these parties, and no endorsement by them should be inferred.

worth 1989). The response of airway epithelium to ozone exposure has been studied most extensively at the bronchiolar and alveolar level of the respiratory tract in laboratory animals (Evans et al. 1976; Schwartz et al. 1976; Plopper et al. 1979, 1994; Boorman et al. 1980, 1994; Castleman et al. 1980; Barry et al. 1985; Fujinaka et al. 1985; Barr et al. 1988; Harkema et al. 1993). Even though it has been recognized for several years that considerable amounts (40% to 70%) of inhaled ozone can be absorbed by nasal tissues (Yokoyama and Frank 1972; Miller et al. 1979), the possible deleterious effects of ozone on the tissues lining the nasal passages have only recently been investigated in inhalation studies of laboratory animals (Harkema et al. 1987a,b, 1989, 1994; Hotchkiss et al. 1989; Johnson et al. 1990; Henderson et al. 1993; Boorman et al. 1994). In addition, nasal lesions thought to be related to exposure to air pollution have been recently described in people living in ozone-polluted atmospheres in Mexico City (Calderon-Garcidueñas et al. 1992).

As part of the National Toxicology Program/Health Effects Institute (NTP/HEI) collaborative study of the effects of prolonged ozone inhalation in laboratory rats, we demonstrated that rats chronically exposed to 0.5 or 1.0 ppm ozone for 20 months, 6 hours/day, 5 days/week had marked alterations in the airway epithelium lining the nasal passages with concomitant functional alterations in the nasal mucociliary apparatus (Harkema et al. 1994). In that same study, we also noted that rats exposed to ozone had lesions in the subepithelial tissues of the nasal turbinates (e.g., conspicuous bony atrophy in the maxilloturbinates). That was the first study to suggest that exposure to an oxidant air pollutant could lead to loss of turbinate bone in the nasal cavity. Although a histologic description of the ozone-induced bony atrophy was provided in that initial report, a morphometric analysis of the severity of the lesions was not conducted. Loss of turbinate bone could lead to marked and permanent reduction in the surface area of turbinates and subsequently alter the nasal mechanisms (e.g., filtration, humidification, and warming) that are important in conditioning the inhaled air before it reaches the lung and in defending the lung from excessive burdens of harmful agents (e.g., xenobiotics and bacteria).

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#### SPECIFIC AIMS

The present study investigated the loss of turbinate bone in the noses of F344/N rats that had been exposed to 0, 0.12, 0.5, or 1.0 ppm ozone, 6 hours/day, 5 days/week for 20 or 24 months. The principal objectives were (1) to determine if the severity of the ozone-induced bony atrophy of the maxilloturbinates was dependent on the exposure concen-

tration and if it was different between the genders, and (2) to determine the severity of concomitant ozone-induced alterations in other tissue compartments of the turbinate (i.e., lamina propria and surface epithelium).

This study was designed to quantify the ozone-induced alterations in the amount of turbinate bone in the proximal nasal airways of chronically exposed rats. In addition, we wanted to examine the effects of ozone exposure on the other principal tissue components of the maxilloturbinates (i.e., surface epithelium and lamina propria) using image analysis and standard morphometric techniques. Alterations in the ultrastructure of subepithelial tissues in the exposed nasal turbinates (e.g., blood vessels or bone) along with the cellular components of the concomitant inflammatory response (i.e., rhinitis) would be further examined using transmission electron microscopy (TEM). Our purpose for conducting this research was to provide structural data that would illustrate the severity of the nasal injury resulting from prolonged ozone exposure in both male and female rats. To our knowledge, this would be the first study designed to morphometrically determine the structural effects of prolonged ozone exposure on the bony tissues of the nose.

We hypothesized that male and female rats exposed to 0.5 or 1.0 ppm ozone for 20 or 24 months would have marked bony atrophy of the maxilloturbinates resulting in significantly less turbinate bone compared with that in rats exposed to 0 or 0.12 ppm ozone. Because no significant nasal lesions had been identified in rats exposed to 0.12 ppm ozone in the previous NTP 24-month study or the NTP/HEI 20-month collaborative study, we anticipated that this concentration of ozone would not induce losses in the turbinate bones in these animals.

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#### METHODS

##### ANIMALS, MAINTENANCE, AND EXPOSURES

Nasal tissues analyzed in the present study were from animals that had been part of the NTP/HEI collaborative study of the health effects of prolonged (20-month) ozone inhalation (Harkema et al. 1994) or an NTP 24-month inhalation study of ozone (Boorman et al. 1994). The overall experimental designs for both the NTP/HEI and NTP studies have been previously described in detail (Kaden et al. 1996; Boorman et al. 1994, respectively). Only tissues from animals that survived these scheduled exposure periods were used in the present study. We obtained 37 F344/N rats (4 or 5 males and 4 or 5 females from each of four exposure groups) from the NTP/HEI collaborative study, and 127 F344/N rats (4 to 8 males and 23 to 28 females from each of

four exposure groups) from the NTP inhalation study. The disparity in the numbers of male and female rats available for analysis in the present study resulted from a high incidence of early unscheduled deaths in male rats from mononuclear cell leukemia. This is one of the most commonly occurring neoplastic diseases in old, untreated F344/N rats, and the reported incidence is greater in male rats (34%) than in female rats (Haseman et al. 1990). The incidence of unscheduled deaths due to mononuclear cell leukemia was not affected by ozone exposure in either study. A detailed description of the incidence of animal survival for the entire 24-month NTP ozone exposure has been previously reported (Boorman et al. 1994).

The methods of animal housing and exposure conditions were the same in both studies and have been previously reported in detail (Boorman et al. 1994; Harkema et al. 1994). In brief, these male and female F344/N rats (Simonson Laboratories, Gilroy, CA) were randomly assigned at four to five weeks of age to control or ozone exposure groups after a 10- to 14-day quarantine period. The rats were housed during the exposures in individual wire cages within 2.0-m<sup>3</sup> inhalation exposure chambers (H2000, Hazelton Systems, Aberdeen, MD). The chambers were maintained at approximately 24°C, 59% relative humidity, and an air flow rate providing 10 air changes per hour. The exposure rooms were lighted on a 12-hour cycle (from 0600 to 1800). Untreated paper cage board beneath the cages was changed twice daily, and chambers were washed weekly. The rats were provided with a pelleted ration (NIH-07, Zeigler Bros., Gardner, PA) *ad libitum* outside exposure hours and with water *ad libitum* at all times.

Ozone was generated from 100% oxygen by corona discharge (OREC Model 03V5-0, Ozone Research and Equipment Corp., Phoenix, AZ). Ozone concentrations were measured with multiplexed ultraviolet spectrophotometric analyzers (Model 1003-AH, Dasibi Environmental Corp., Glendale, CA) calibrated by a chemical method using neutral-buffered potassium iodide. Ozone in the control atmosphere was below the limit of detection (0.002 ppm).

All the rats from the NTP/HEI collaborative study were exposed to 0, 0.12, 0.5, or 1.0 ppm ozone for 6 hours/day, 5 days/week for 20 months. The animals in the NTP inhalation study were exposed to the same regimen except these rats were exposed for 24 months. Animals in both studies were killed one week after the end of the exposure. After death, the entire nasal cavity of each animal in the NTP/HEI study was fixed in 2% glutaraldehyde and decalcified with 10% ethylenediaminetetraacetate in 0.1 M cacodylate buffer in preparation for light microscopy and TEM. Nasal tissues from rats in the NTP study were fixed in 10% neutral-buffered formalin, decalcified, embedded in paraffin, sectioned to a thickness of 5 to 6 µm, and stained with

hematoxylin and eosin for light microscopic examination. In the present study, all of the nasal tissues used for morphometric examination by light microscopy were selected from a transverse tissue section immediately posterior to the upper incisor teeth (Figure 1).

Only nasal tissues from rats in the NTP/HEI 20-month study were ultrastructurally examined using TEM. A transverse tissue section of the maxilloturbinate from one nasal passage at the level of the posterior aspect of the incisor tooth (immediately anterior to the tissue section taken for light microscopy) was selected for further processing by TEM. Sampled tissues were postfixed in 1% osmium tetroxide, dehydrated in a graded series of alcohols, infiltrated with propylene oxide solutions, embedded in epon araldite, and thin-sectioned with a diamond knife on an Ultracut E microtome (Leica, Inc., Deerfield, IL). These sections were mounted on Formvar-coated, slotted grids, stained with uranyl acetate and lead citrate, and examined with an Hitachi H7000 scanning TEM (Hitachi Instruments, Inc., Mountain View, CA).

#### MORPHOMETRIC ANALYSIS OF MAXILLOTURBINATES

We morphometrically analyzed the nasal tissues of 19 male and 18 female F344/N rats from the NTP/HEI 20-month study, and 25 male and 107 female F344/N rats from the NTP 24-month study. A semiautomatic image analysis system was used to morphometrically determine the total cross-sectional area of the maxilloturbinate in the proximal nasal airway. The individual areas of its three major tissue compartments (i.e., surface epithelium, lamina propria, and bone; Figure 1) also were determined using this imaging system and standard morphometric techniques. The image analyzer system consisted of a light microscope (Olympus BX-60; Olympus Corp., Lake Success, NY) connected to a high-resolution couple-charged device camera (VE-1000CCD; Dage-MTI, Inc., Michigan City, IN), a Scion LG-3 digital image processing board (Scion Corp., Frederick, MD), a color monitor, and a Power Macintosh 7100/66 computer running NIH Image, the public-domain image analysis program (Rasband 1996).

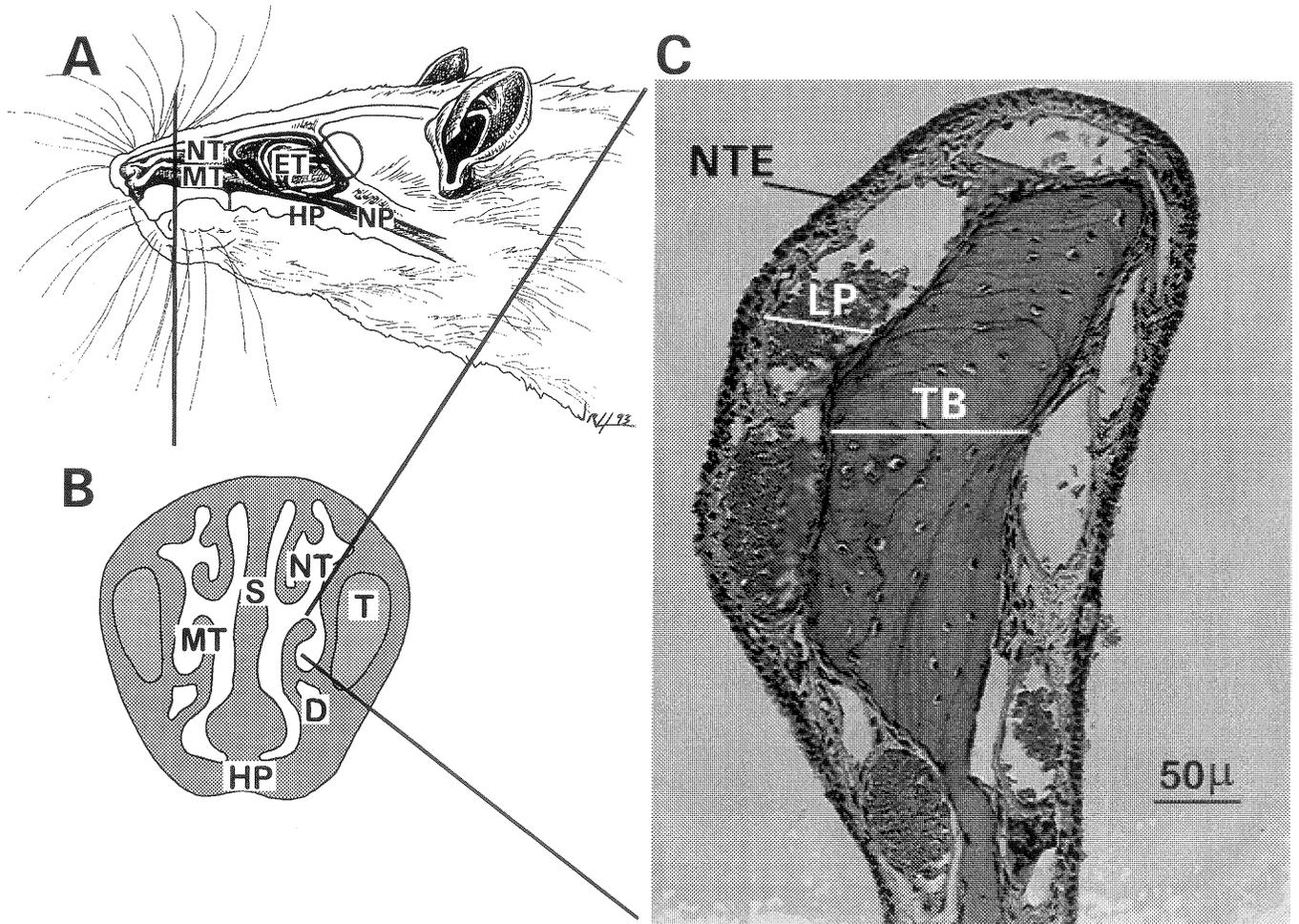
The digitized image of the maxilloturbinate was displayed on the video monitor at a final magnification of × 450. The luminal surface of the epithelium covering the maxilloturbinate was traced using a pointing device and the medial and lateral surfaces were connected with a straight line at the point where the turbinate protrudes from the lateral wall. The total area (mm<sup>2</sup>) of the turbinate was calculated by the NIH Image program from the circumference of the enclosed area. The perimeter of the turbinate

bone within the previously outlined maxilloturbinate was traced and the area of bone was calculated (Figure 2). The area of the lamina propria also was calculated from its perimeter measurement. The area of the surface epithelium was derived by subtracting the area of the turbinate bone and the lamina propria from the total maxilloturbinate area.

The arithmetic means of the cross-sectional areas of the two maxilloturbinates and the individual tissue compartments (e.g., bone) were determined for each animal and expressed as the area/turbinate. Approximately 25% of the nasal tissue sections had only one maxilloturbinate that could be measured due to tissue folding or sectioning artifacts; the area measurements from these animals were derived from the single usable maxilloturbinate.

#### MORPHOMETRIC ANALYSIS OF NASAL AIRWAY LUMEN

The image analysis system also was used to determine the total luminal area and perimeter of the nasal passages in the transverse nasal section from a subgroup of male and female rats exposed for 24 months ( $n = 4$  to 6 rats of each gender from each exposure group). Rats were randomly chosen for this morphometric analysis. Only nasal tissue sections that contained intact cross sections of both nasal passages with no observable tissue artifacts (e.g., partial loss of a turbinate due to processing) were used in this analysis. The digitized image of the transverse nasal section from each animal was displayed on the monitor at a magnification of  $\times 450$ , and the contours of the entire luminal surfaces



**Figure 1.** (A) Diagram of the exposed lateral wall of the nasal airway of the F344/N rat. The vertical line represents the location of the transverse tissue block in the proximal nasal airway that was selected for morphologic and morphometric analysis. NT = nasal turbinate; MT = maxilloturbinate; ET = ethmoid turbinate; HP = hard palate; NP = nasopharynx. (B) Diagram of the anterior face of the transverse nasal tissue selected for analysis. S = septum; T = root of the incisor tooth in the lateral wall; D = nasolacrimal duct. (C) Computer-digitized image of the transverse histologic section of the maxilloturbinate. NTE = nasal transitional epithelium lining the luminal surface of the maxilloturbinate; LP = lamina propria containing large venous blood vessels; TB = turbinate bone.

from both the right and left nasal passages were outlined (Figure 3). The luminal area for each nasal airway was automatically calculated from its perimeter measurement. For every animal, the cross-sectional area of the nasal lumina (mm<sup>2</sup>) and the airway perimeters (mm) were determined by summing the individual values from each nasal passage.

### STATISTICAL ANALYSIS

The effects of gender, chamber ozone concentration, and the interaction of these factors on the measured parameters were tested using a two-way analysis of variance (ANOVA). Dunnett's criterion for comparing several exposure groups to a control group was used to account for multiple comparisons. An overall value of  $p < 0.05$  was used to determine statistical significance in all tests.

Summary Table 1 (1) provides a list of specific nasal tissues and airways we examined morphometrically, (2) identifies the total number of animals from each of the two studies, and (3) allocates the animals according to tissue obtained, gender, and exposure group.

## RESULTS

### MORPHOLOGIC CHANGES IN NASAL TISSUES AFTER OZONE EXPOSURE

Similar ozone-induced histopathologic changes were evident in the nasal tissues of rats exposed for 20 or 24 months. No conspicuous time-related differences were apparent in the nature or the severity of the exposure-related lesions. Both male and female rats exposed to 1.0 ppm

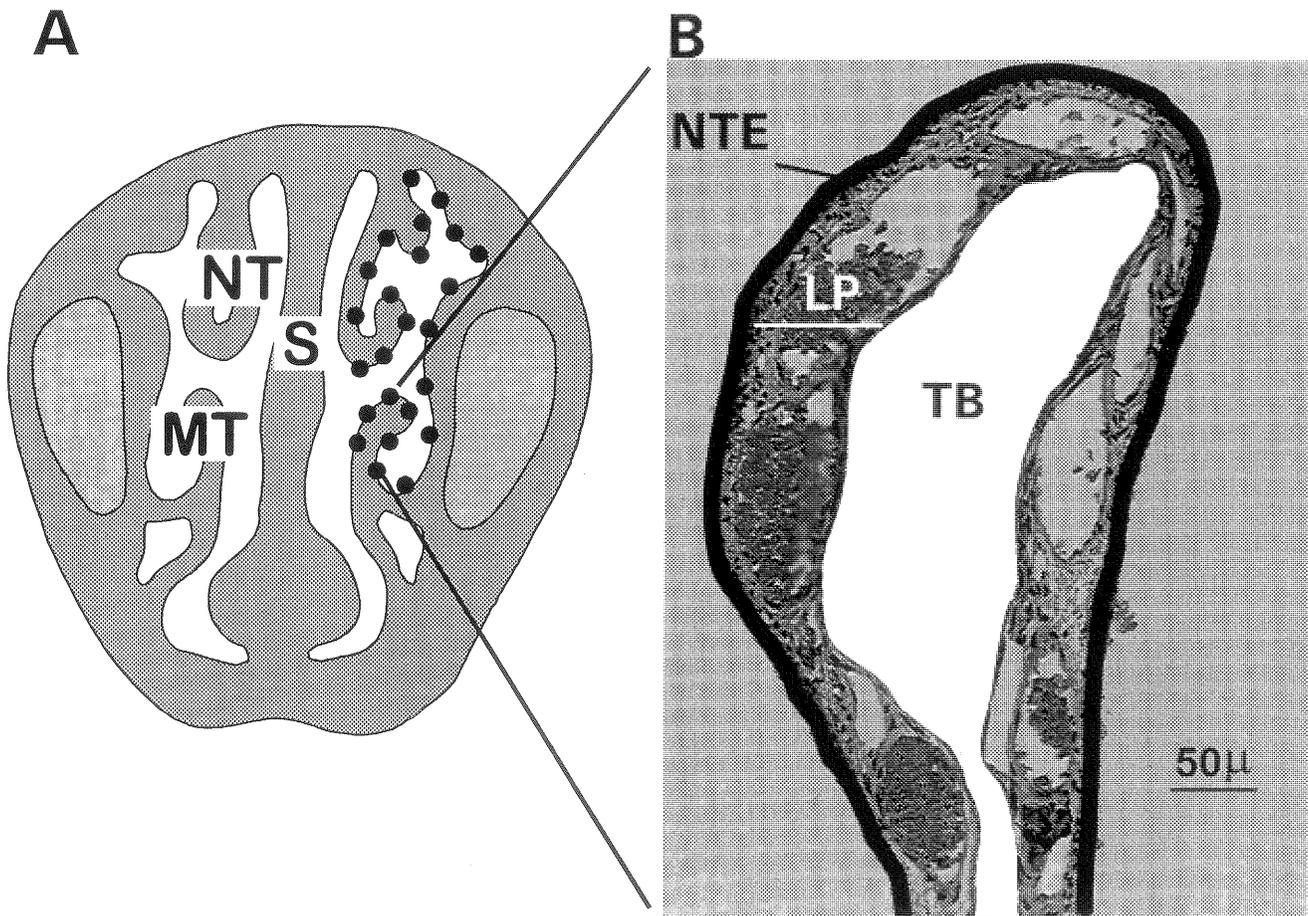


Figure 2. (A) Diagram of the distribution of ozone-induced nasal injury in the examined nasal tissue section. The black dots mark the region of ozone-induced abnormalities that were restricted to nasal tissue surrounding the lateral meatus. NT = nasoturbinates; S = septum; MT = maxilloturbinates. (B) Computer-digitized image of the transverse histologic section of the maxilloturbinate. The areas of the nasal transitional epithelium (NTE) and turbinates (TB) that were morphometrically analyzed are highlighted in black (NTE) and white (TB). LP = lamina propria.

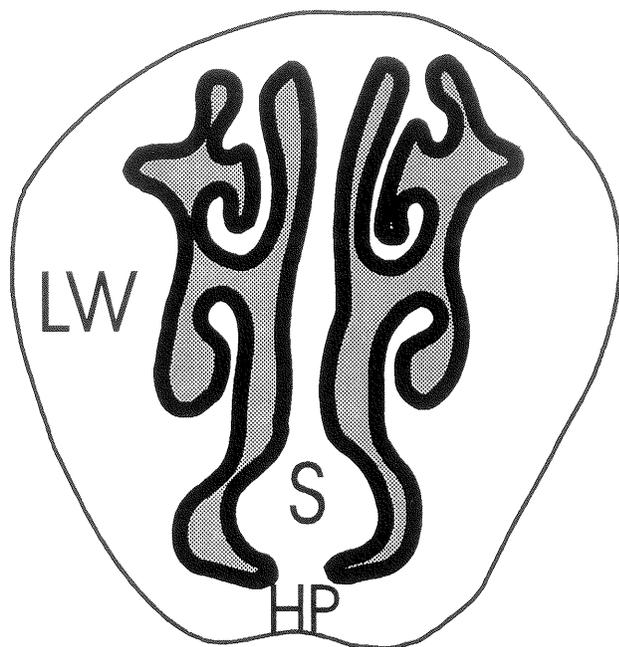


Figure 3. Diagram of the transverse tissue section and the nasal airway lumina (right and left nasal passages) that were morphometrically analyzed. The luminal areas (gray) and perimeters (black) of the right and left nasal passages are shown. LW = lateral wall; S = septum; HP = hard palate.

ozone had significant morphologic alterations that were detected by light microscopy. Similar but less severe alterations were evident in rats exposed to 0.5 ppm ozone. No exposure-related lesions were evident in the nasal airways of rats exposed to 0.12 ppm ozone. No microscopically detectable differences were noted in the nasal mucosa of rats exposed to 0 ppm ozone (control animals) and those exposed to 0.12 ppm ozone.

The most severe alterations induced by 0.5 or 1.0 ppm ozone occurred in the nasal mucosa of the lateral wall, the nasoturbinate, and the maxilloturbinate lining the lateral meatus in the examined section from the proximal nasal passages (Figure 2). No significant histologic alterations were identified in the nasal respiratory epithelium lining the nasal septum in the proximal nasal airways of rats exposed to 0.5 or 1.0 ppm ozone.

The principal ozone-induced lesions in this proximal region of the nasal airways included marked thickening of the luminal surface epithelium, bony atrophy of the nasal turbinates (i.e., maxilloturbinates and lateral ridge of nasoturbinates), and a conspicuous thinning of the lamina propria (the tissue between the outer surface epithelium and the inner bone of the turbinates). Atrophy of the osseous tissue of the maxilloturbinates resulted in a conspicuous dorsoventral shortening of the maxilloturbinate in each nasal passage (Figure 4). The epithelial thickening was due to

Table 1. Summary of the Nasal Tissues and Airways That Were Morphometrically Examined from Rats in the 20-Month NTP/HEI and the 24-Month NTP Studies

Nasal Tissue or Airway <sup>a</sup>	Morphometric Parameter	Study (months)	Ozone Concentration (ppm)							
			Male Rats				Female Rats			
			0	0.12	0.5	1.0	0	0.12	0.5	1.0
Maxilloturbinate	Area	NTP/HEI (20)	5	4	5	5	4	5	4	5
		NTP (24)	8	4	7	6	28	23	27	24
Bone	Area	NTP/HEI (20)	5	4	5	5	4	5	4	5
		NTP (24)	8	4	7	6	28	23	27	24
Lamina propria	Area	NTP/HEI (20)	5	4	5	5	4	5	4	5
		NTP (24)	8	4	7	6	28	23	27	24
Surface epithelium	Area	NTP/HEI (20)	5	4	5	5	4	5	4	5
		NTP (24)	8	4	7	6	28	23	27	24
Nasal airway lumen	Area	NTP (24)	6	4	5	5	6	6	6	6
	Perimeter <sup>b</sup>									

<sup>a</sup> All tissues of airways were taken from cross sections of the rat nose at the level of the incisor tooth (see text for details). The bone, lamina propria, and surface epithelium are tissue components that compose the maxilloturbinate.

<sup>b</sup> The same animals were used to examine area and perimeter. They were randomly chosen from the group of 127 animals.

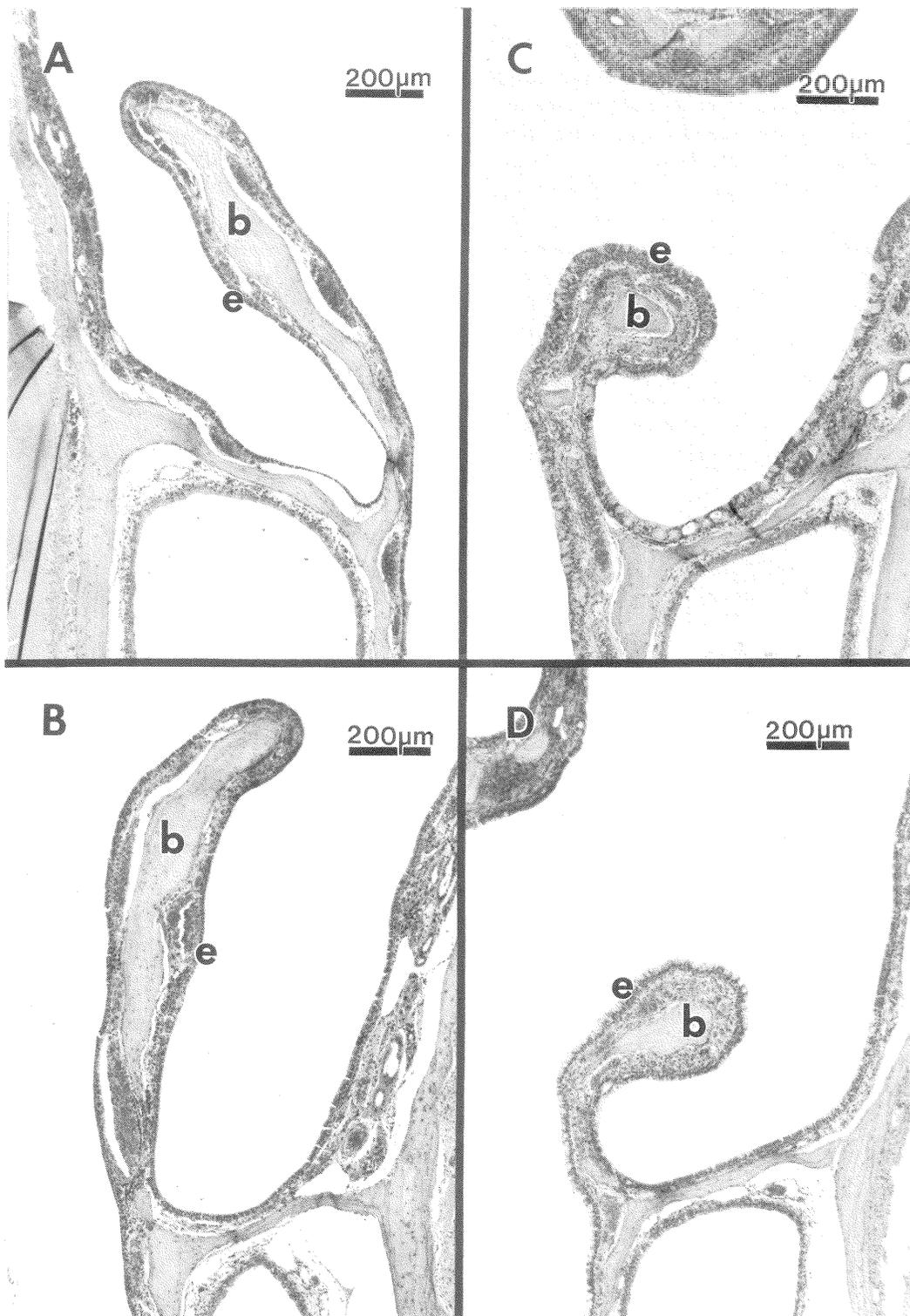


Figure 4. Light photomicrographs of maxilloturbinates from male F344/N rats exposed for 20 months to (A) 0 ppm ozone, (B) 0.12 ppm ozone, (C) 0.5 ppm ozone, or (D) 1.0 ppm ozone. Because of severe atrophy of the bone matrix, the dorsoventral lengths of the turbinates in panels C and D are markedly shorter than those in panels A and B. e = luminal surface epithelium; b = turbinate bone.

mucous cell metaplasia and epithelial hyperplasia in the nasal transitional epithelium lining the entire surface of the lateral meatus (Figure 5). In rats exposed only to filtered air (0 ppm ozone), the nasal transitional epithelium was only one to two cells thick and was composed predominantly of nonciliated cuboidal or columnar cells with only an occasional ciliated or mucous (goblet) cell. In contrast, the nasal transitional epithelium in rats exposed to 0.5 or 1.0 ppm ozone was four to six cells thick and contained numerous mucous cells. In addition, small focal areas of nonkeratinizing squamous cell metaplasia were occasionally found on the dorsal aspect of the maxilloturbinates, on the lateral scroll and lateral ridge of the nasoturbinates, and on the midaspect of the lateral wall.

The normal turbinate bone from control rats (0 ppm ozone) and from those exposed to 0.12 ppm ozone consisted of lamellar bone with smooth external, periosteal surfaces

(Figure 5). These outer surfaces were predominantly lined by flat bone-lining cells. The underlying bone matrix contained widely scattered lacunae filled with osteoclasts. In contrast, the atrophic turbinate bones in the maxilloturbinates of rats exposed to 0.5 or 1.0 ppm ozone had marked loss of bone matrix, and irregular periosteal surfaces due to conspicuous areas of bone resorption (i.e., bone resorption pits) and remodeling. Bone resorption was most prominent in the dorsal ends of the turbinates. The outer surfaces of the atrophic turbinate bones were lined by plump polyhedral cells (active osteoblasts), or mononuclear phagocytic cells, or both (Figure 5). Multinucleated osteoclasts were only occasionally evident in these areas. A layer of osteoid sometimes was present between the surface osteoblasts and the underlying mineralized bone matrix. osteoclasts within the remaining bone matrix appeared histologically normal in these ozone-exposed rats. Though both male and female

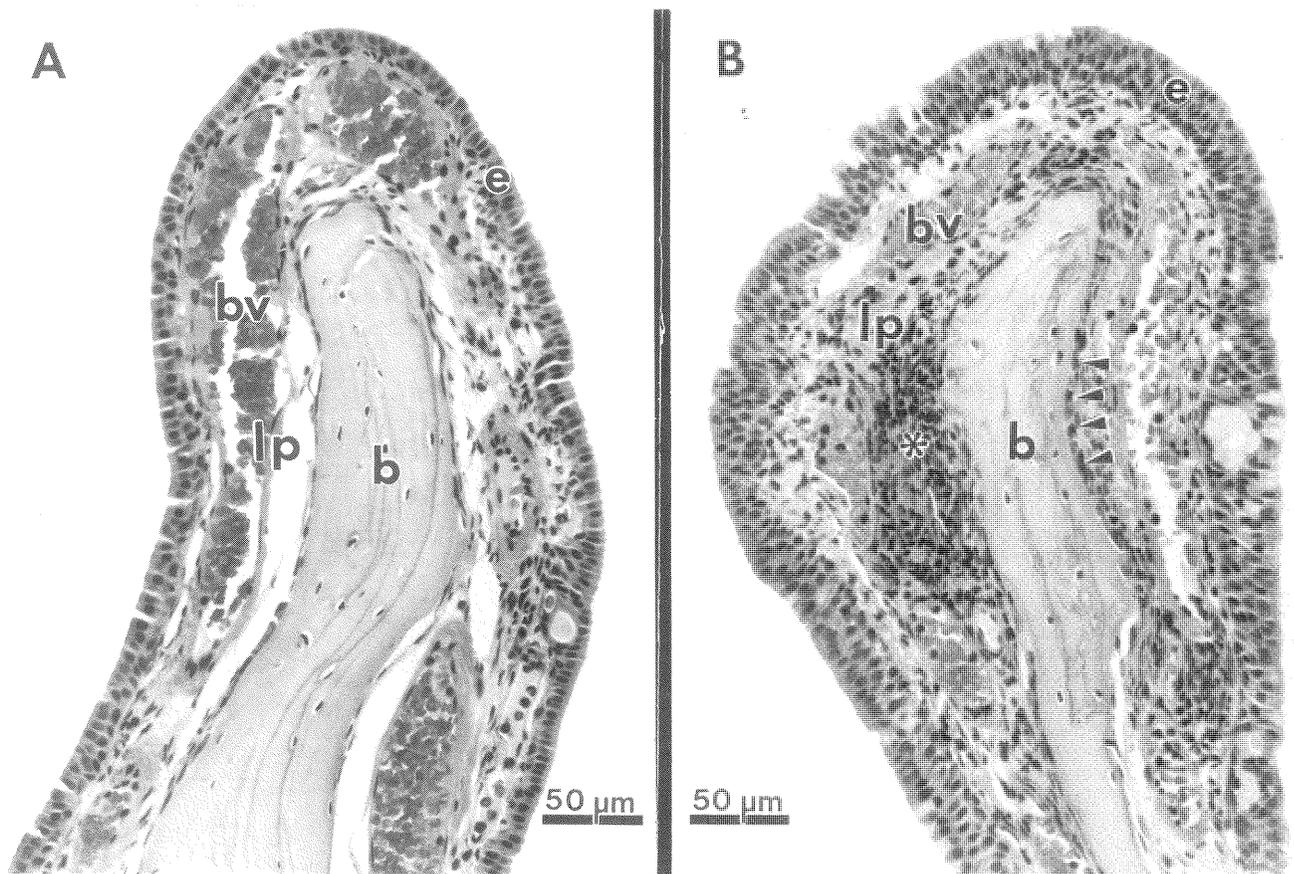


Figure 5. Light photomicrographs of maxilloturbinates from the proximal nasal passages of rats exposed for 20 months to (A) 0 ppm ozone or (B) 1 ppm ozone. In panel B, the nasal transitional epithelium (e) is thick due to mucous cell metaplasia and epithelial hyperplasia. The underlying lamina propria (lp) contains a mild inflammatory cell influx and constricted blood vessels (bv). The turbinate bone matrix (b) in panel B is conspicuously smaller than that in panel A. Arrowheads = osteoblasts lining outer bone surface; \* = inflammatory cell influx.

rats exposed to 0.5 or 1.0 ppm ozone had bony atrophy, this alteration was more conspicuous in the turbinates of male rats.

A mild to moderate influx of mixed inflammatory mononuclear cells (e.g., lymphocytes, monocytes) and neutrophils was present in the nasal mucosa of the lateral walls, maxilloturbinates, and nasoturbinates of rats exposed to 1.0 ppm ozone (Figure 5). A similar, but less severe, inflammatory response was evident in rats exposed to 0.5 ppm ozone. The ozone-induced chronic rhinitis also was more prominent in male than female animals exposed to either 0.5 or 1.0 ppm ozone. In addition, the normally large, dilated, capacitance blood vessels (i.e., "swell bodies") in the lamina propria of these same affected regions were markedly constricted with small luminal profiles and thick walls after ozone exposure (Figure 5).

Ultrastructurally, the smooth external surfaces of turbinate bone in rats exposed to 0 or 0.12 ppm ozone were lined by bone-lining cells that were very flat and elongated with a spindle-shaped nucleus and few cytoplasmic organelles (Figure 6). In contrast, the enlarged, polyhedral-shaped osteoblasts lining the outer surface of the turbinate bone in the rats exposed to 0.5 or 1.0 ppm ozone were characterized by an eccentrically placed nucleus, abundant rough endoplasmic reticulum, free ribosomes, a well-developed Golgi zone, and numerous mitochondria. Mononuclear phagocytic cells in these areas of bone resorption and remodeling had numerous cytoplasmic extensions and lysosomes (Figure 6). In addition, the periosteum surrounding the osseous tissue in these ozone-exposed turbinates was often thicker than that of control animals due to an apparent increase in the numbers of fibroblast-like or osteoprogenitor cells.

#### MORPHOMETRY OF MAXILLOTURBINATES AFTER A 20-MONTH EXPOSURE TO OZONE

Statistically significant alterations in the total cross-sectional area of the maxilloturbinates, and the areas of the individual tissue compartments comprising the maxilloturbinates (i.e., turbinate bone, lamina propria, and surface epithelium) were found only in animals that were exposed to 0.5 or 1.0 ppm ozone. Morphometric data from the maxilloturbinates of rats exposed to 0 ppm ozone (control group) or 0.12 ppm ozone were not statistically different. Table 2 describes the significant changes (increase, decrease, none) in the amount (cross-sectional area) of maxilloturbinate tissues after a 20-month exposure to ozone.

#### Morphometry of the Turbinate Bone

Female and male rats exposed to 1.0 ppm ozone for 20 months had significantly less cross-sectional area of bone in the maxilloturbinates than the control rats (Figure 7). Male rats exposed to 1 ppm ozone had approximately 64% less bony tissue compared with male control rats (0 ppm ozone), whereas female rats exposed to 1 ppm ozone had 50% less turbinate bone compared with female control animals. Male and female rats exposed to 0.5 ppm ozone also had significant losses in turbinate bone after the end of the 20-month exposure to ozone (i.e., 52% less bone area than control rats for both genders).

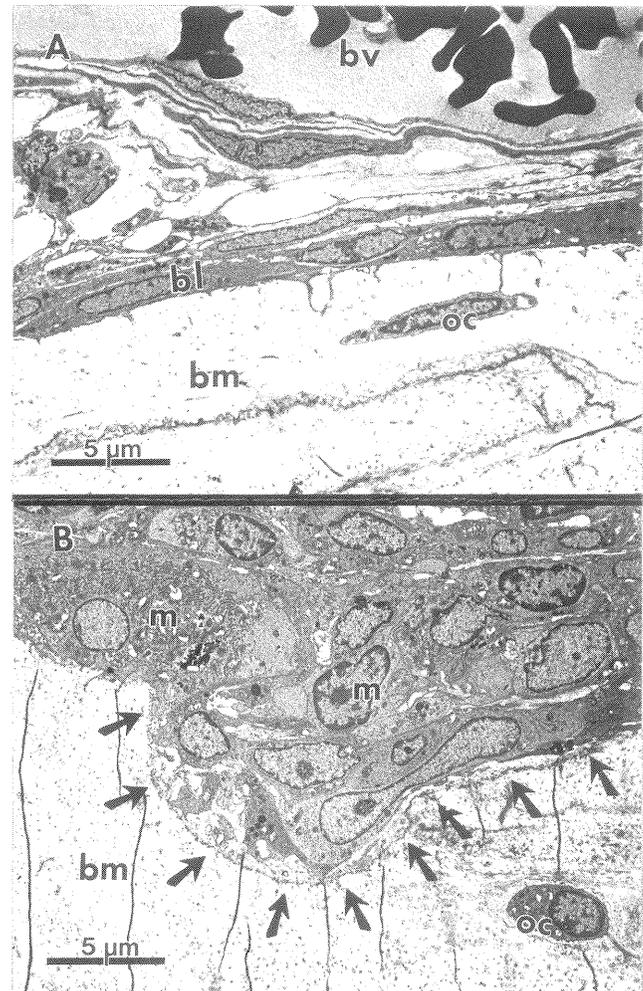


Figure 6. Transmission electron photomicrographs of turbinate bone from male rats exposed for 20 months to (A) 0 ppm ozone or (B) 1.0 ppm ozone. bv = blood vessel in lamina propria; bl = bone-lining cells; bm = bone matrix; oc = osteocyte; m = mononuclear phagocytic cells; arrows = bone resorption pit.

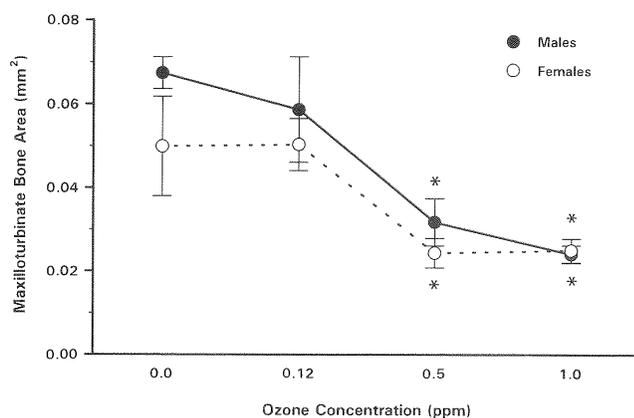
### Morphometry of the Lamina Propria

Exposure-related morphometric changes in the area of the lamina propria (i.e., mucosal tissue between the inner bone and outer surface epithelium) were evident only in male rats exposed to 0.5 or 1.0 ppm ozone (Figure 8). No significant differences were noted in the lamina propria areas between air-exposed controls (0 ppm ozone) and female rats exposed to ozone at all concentrations. Compared with the lamina propria area in male control rats, 45% or 40% less lamina propria area was evident in the maxilloturbinates of male rats exposed to 0.5 or 1.0 ppm ozone, respectively.

**Table 2.** Summary of the Ozone-Related Changes in the Cross-Sectional Area of Maxilloturbinate Tissues After a 20-Month Exposure to Ozone (NTP/HEI Study)<sup>a</sup>

Nasal Tissue	Ozone Concentration (ppm)					
	Male Rats			Female Rats		
	0.12	0.5	1.0	0.12	0.5	1.0
Bone	↔	↓	↓	↔	↓	↓
Lamina propria	↔	↓	↓	↔	↔	↔
Surface epithelium	↔	↔	↔	↔	↑	↑
Total turbinate	↔	↓	↓	↔	↔	↔

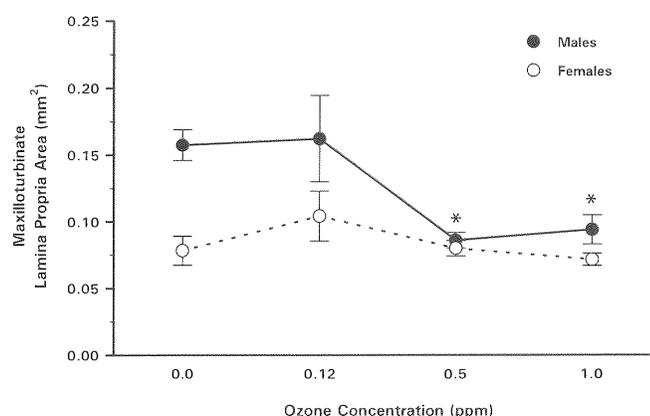
<sup>a</sup> ↔ = Not significantly different from, ↑ = significantly greater than, and ↓ = significantly less than control group (0 ppm).



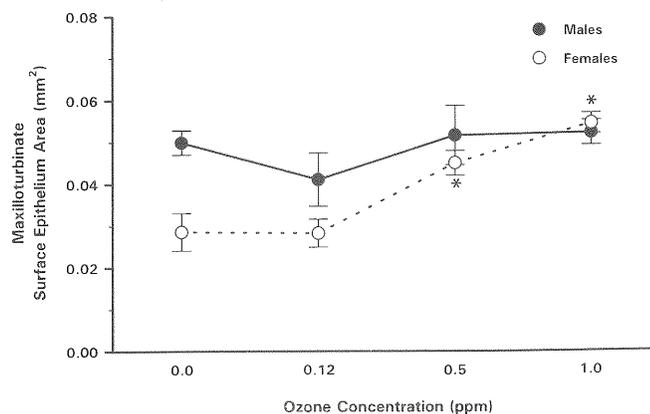
**Figure 7.** Changes in the cross-sectional area of the maxilloturbinate bone in male and female rats exposed for 20 months to one of four concentrations of ozone. Data points are means ± SEMs. \* = Significantly different ( $p < 0.05$ ) from control group.

### Morphometry of the Surface Epithelium

Ozone-related changes in the area of the surface epithelium lining the maxilloturbinates occurred only in female rats exposed to 0.5 or 1.0 ppm ozone (Figure 9), where the epithelial area measured was 55% or 90% (respectively) more than in control animals after the 20-month exposure. Like the females, male rats exposed to the same concentrations and exposure regimen exhibited a markedly thickened surface epithelium due to hyperplastic and metaplastic changes; and yet, in contrast to females, the measurements in males of total cross-sectional area of the surface epithelium lining the maxilloturbinates were similar to those in control animals. This was due to a concomitant atrophy of the total maxilloturbinate. Therefore, although these ozone-exposed males and females both had hyperplastic and metaplastic



**Figure 8.** Changes in the cross-sectional area of the lamina propria in the maxilloturbinates of male and female rats exposed for 20 months to one of four concentrations of ozone. Data points are means ± SEMs. \*Significantly different ( $p < 0.05$ ) from control group.



**Figure 9.** Changes in the cross-sectional area of surface epithelium in the maxilloturbinates of male and female rats exposed for 20 months to one of four concentrations of ozone. Data points are means ± SEMs. \*Significantly different ( $p < 0.05$ ) from control group.

changes in the surface epithelium, it appeared that the surface epithelium in females had a greater response to ozone because it was not counteracted by the atrophy of the total turbinate seen in male rats.

**Morphometry of the Total Turbinate**

Ozone-related loss in the total turbinate area (i.e., turbinate atrophy) was morphometrically evident in male rats exposed to 0.5 or 1.0 ppm ozone (Figure 10). The reduction in the total cross-sectional area of the maxilloturbinates was similar in these two groups of ozone-exposed male rats (i.e., 38% less area, compared to that of controls, in both groups). No morphometric evidence of turbinate atrophy was apparent in female rats exposed to ozone.

**MORPHOMETRY OF MAXILLOTURBINATES AFTER A 24-MONTH EXPOSURE TO OZONE**

As in the rats exposed for 20 months, exposure-associated changes in turbinate morphometry were found only in rats exposed to 0.5 or 1.0 ppm ozone. The degrees of these ozone-induced changes were sometimes different between the genders. A summary of the types of ozone-related changes in the amount of maxilloturbinate tissues after a 24-month exposure to ozone (i.e., significant differences from the 0-ppm control group) is presented in Table 3.

**Morphometry of the Turbinate Bone**

Male rats exposed to 0.5 or 1.0 ppm ozone, but not those exposed to 0.12 ppm ozone, for 24 months had significantly less area of bone in the maxilloturbinates compared with control animals (0 ppm ozone) (Figure 11). Male rats ex-

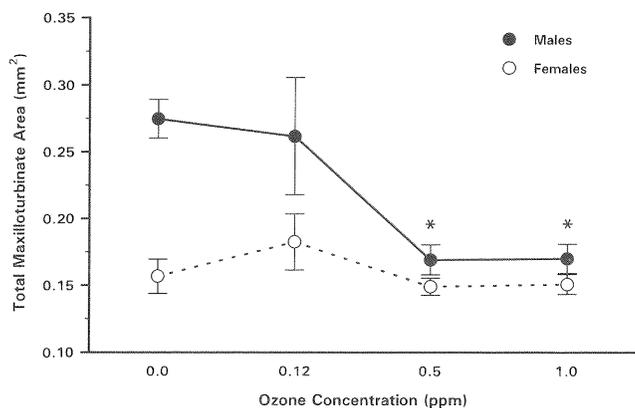
posed to 0.5 or 1.0 ppm ozone had 49% or 52% less bony tissue, respectively, compared with control rats. Approximately 24% of the tissue comprising the maxilloturbinates was bony tissue in air-exposed male rats (controls). Bony tissue comprised only 15% or 18% of the maxilloturbinates in male rats exposed to 0.5 or 1.0 ppm ozone, respectively.

No significant difference was noted in the area of the turbinate bone among female rats exposed to 0, 0.12, or 0.50 ppm ozone. Only female rats exposed to 1.0 ppm ozone had significant ozone-induced losses of the bone matrix (38% less area of bone compared with control animals). Approximately 27% of the total area of the maxilloturbinates was

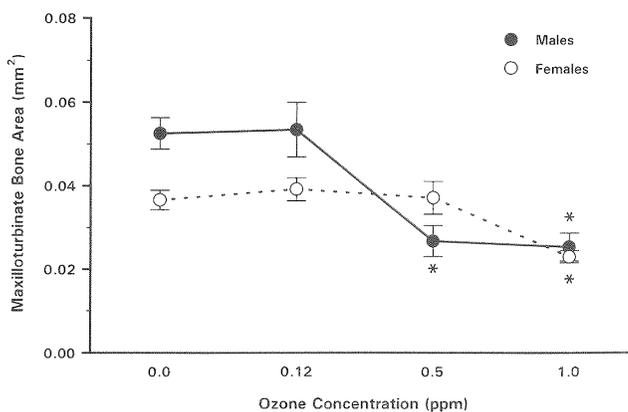
**Table 3.** Summary of the Ozone-Related Changes in the Cross-Sectional Area of Maxilloturbinate Tissues After a 24-Month Exposure to Ozone (NTP Study)<sup>a</sup>

	Ozone Concentration (ppm)					
	Male Rats			Female Rats		
Nasal Tissue	0.12	0.5	1.0	0.12	0.5	1.0
Bone	↔	↓	↓	↔	↔	↓
Lamina propria	↔	↓	↓	↔	↓	↓
Surface epithelium	↔	↑	↑	↔	↑	↑
Total turbinate	↔	↓	↓	↔	↔	↔

<sup>a</sup> ↔ = Not significantly different from, ↑ = significantly greater than, and ↓ = significantly less than control group (0 ppm).



**Figure 10.** Changes in the total cross-sectional area of the maxilloturbinates of male and female rats exposed for 20 months to one of four concentrations of ozone. Data points are means ± SEMs. \*Significantly different ( $p < 0.05$ ) from control group.



**Figure 11.** Changes in the cross-sectional area of the maxilloturbinate bone of male and female rats exposed for 24 months to one of four concentrations of ozone. Data points are means ± SEMs. \*Significantly different ( $p < 0.05$ ) from control group.

bony tissue in air-exposed (0 ppm ozone) female rats; in contrast, only 18% of the total area of the maxilloturbinates was comprised of bone in females exposed to 1.0 ppm ozone.

### Morphometry of the Lamina Propria

Ozone-induced alterations in the amount of lamina propria in the maxilloturbinates were evident only in rats exposed to 0.5 or 1.0 ppm ozone. Like the responses observed in turbinate bone, areas of lamina propria were significantly reduced in these ozone-exposed rats compared with air-exposed control animals (Figure 12). These changes were predominantly reflections of marked reductions in the profile areas of blood vessel lumina (i.e., constriction of blood vessel lumina). Female rats exposed to 0.5 or 1.0 ppm ozone had 16% or 22% less area of lamina propria, respectively, compared with controls. Female rats in the control group (0 ppm ozone) had a lamina propria area of  $0.074 \pm 0.003 \text{ mm}^2$  (mean  $\pm$  SEM) compared with only  $0.058 \pm 0.002 \text{ mm}^2$  in female rats exposed to 1.0 ppm ozone. Only 46% or 48% of the total area of maxilloturbinates was lamina propria in female rats exposed to 0.5 or 1.0 ppm ozone, respectively.

Compared with female rats, male rats had even more severe reductions in the amounts of lamina propria after exposure to ozone. Male rats exposed to 0.5 or 1.0 ppm ozone had approximately 28% or 43% less area of lamina propria, respectively, than that of air-exposed control animals (Figure 12). The lamina propria in male control rats comprised approximately 58% of the total area of the maxilloturbinates. The lamina propria in male rats exposed to 0.5 or 1.0 ppm ozone comprised only 53% or 48% of the total area of the maxilloturbinates, respectively.

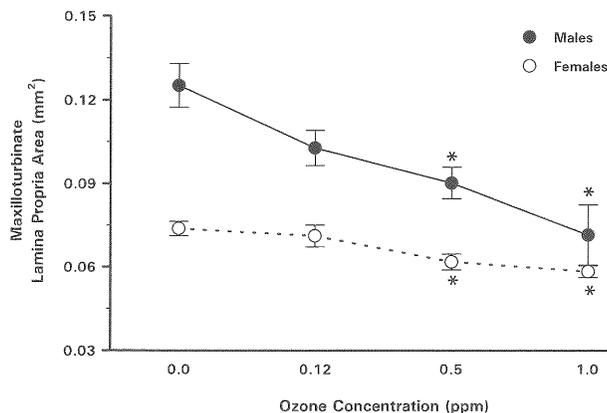


Figure 12. Changes in the cross-sectional area of the lamina propria in the maxilloturbinates of male and female rats exposed for 24 months to one of four concentrations of ozone. Data points are means  $\pm$  SEMs. \*Significantly different ( $p < 0.05$ ) from control group.

### Morphometry of the Surface Epithelium

The surface epithelial area of the maxilloturbinates increased after exposure to 0.5 or 1.0 ppm ozone for 24 months in both male and female rats (Figure 13). Female animals exposed to either 0.5 or 1.0 ppm ozone for 24 months had approximately 60% more epithelial area compared with control animals. Male rats exposed to these same concentrations of ozone had only a 25% to 40% increase in epithelial area. Increases in the area of the epithelial compartment in both genders were reflections of the marked, ozone-induced, hyperplastic and metaplastic changes in these tissues. The smaller increases in the amount of surface epithelium in male rats reflected a marked atrophy of the total turbinate in males but not in females (see Morphometry of the Total Turbinate, below).

### Morphometry of the Total Turbinate

Marked reductions in the total area of the maxilloturbinates (i.e., turbinate atrophy) were observed in male rats exposed to 0.5 or 1.0 ppm ozone (Figure 14). The total area of the maxilloturbinates was  $0.22 \pm 0.01 \text{ mm}^2$  (mean  $\pm$  SEM) in male control animals, and only  $0.17 \pm 0.01 \text{ mm}^2$  and  $0.14 \pm 0.01 \text{ mm}^2$  in male rats exposed to 0.5 or 1.0 ppm ozone, respectively. Female rats exposed to the same concentrations of ozone did not have a significant loss of total turbinate area.

### Morphometry of the Nasal Airway Lumen and Perimeter

Rats exposed to 1.0, 0.5 or even 0.12 ppm ozone for 24 months had significantly greater nasal luminal areas compared with control rats (0 ppm) (Figure 15). Male and female rats in all of the ozone-exposed groups had approxi-

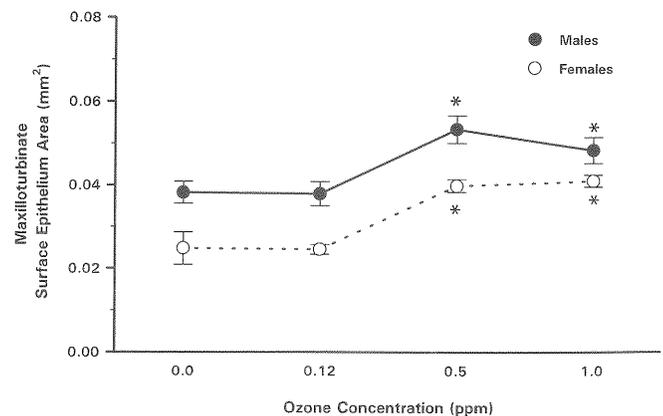


Figure 13. Changes in the cross-sectional area of the surface epithelium in the maxilloturbinates of male and female rats exposed for 24 months to one of four concentrations of ozone. Data points are means  $\pm$  SEMs. \*Significantly different ( $p < 0.05$ ) from control group.

mately 9% to 11% more cross-sectional area of nasal airway lumen compared with the air-exposed control group. However, only male and female rats chronically exposed to 0.5 ppm ozone had statistically smaller nasal luminal perimeters compared with control animals (Figure 16). Females in this exposure group had only 2.5% less perimeter compared with female control rats, whereas males had 11% less luminal perimeter compared with male control rats.

## DISCUSSION AND CONCLUSIONS

The results of this study indicate that F344/N rats exposed for 20 or 24 months to 0.5 or 1.0 ppm ozone, but not those exposed to 0.12 ppm ozone, had significant quantitative changes in both the epithelial and subepithelial tissue compartments comprising the maxilloturbinates. As we hypothesized, marked ozone-related losses of turbinate bone were found in male and female rats. This is the first study to morphometrically demonstrate that ozone can alter bone in the nasal turbinates of the upper airway. Loss of turbinate bone could lead to marked and permanent changes in the normal structure of the tissues lining the nasal passages and subsequently alter nasal airway flow patterns, air filtration, and warming and humidification of the inhaled air.

Although this is the first study to demonstrate that rhinitis with marked bony atrophy of the nasal turbinates can be experimentally induced in laboratory rodents by prolonged exposure to a common air pollutant, atrophic rhinitis is a naturally occurring pathologic condition in humans and in laboratory and domestic animals. Primary atrophic rhinitis, or ozena, is a rarely reported chronic human disease characterized by progressive atrophy of the nasal mucosa and the underlying bone of the turbinates (Goodman and

De Souza 1986; Zohar et al. 1990). The etiology of this nasal disease has not yet been determined. Human atrophic rhinitis can also occur as a secondary condition to chronic granulomatous nasal infections, chronic sinusitis, radical nasal surgery, trauma, and irradiation.

Naturally acquired rhinitis with concomitant turbinate atrophy also has been reported in laboratory rabbits infected with *Pasteurella multocida* (DiGiacomo et al. 1989, 1991). In addition, severe atrophy of turbinate bone associated with chronic rhinitis is a common and widespread disease in pigs that have intranasal infections of toxigenic strains of *Pasteurella multocida*, *Bordetella bronchiseptica*, or both (Switzer 1981; Rutter 1985; Ackermann et al. 1991). The loss of turbinate bone in this swine disease is due to a

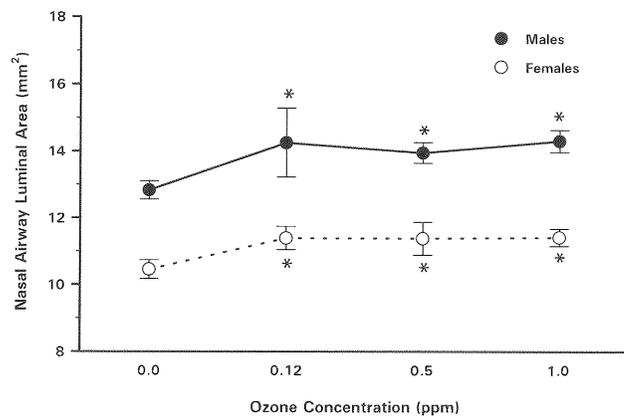


Figure 15. Changes in the cross-sectional area of the nasal airway lumina of male and female rats exposed for 24 months to one of four concentrations of ozone. Data points are means  $\pm$  SEMs. \*Significantly different ( $p < 0.05$ ) from control group.

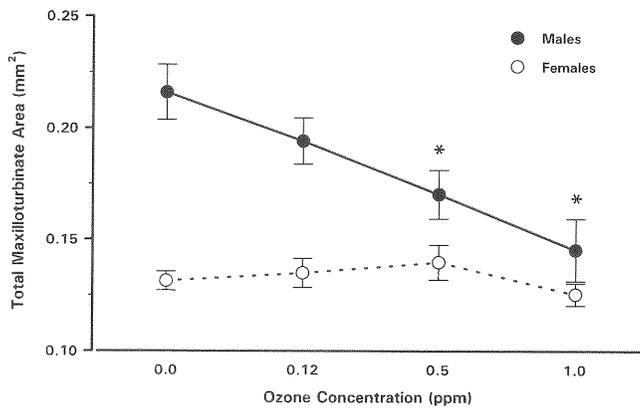


Figure 14. Changes in the total cross-sectional area of the maxilloturbinates in male and female rats exposed for 24 months to one of four concentrations of ozone. Data points are means  $\pm$  SEMs. \*Significantly different ( $p < 0.05$ ) from control group.

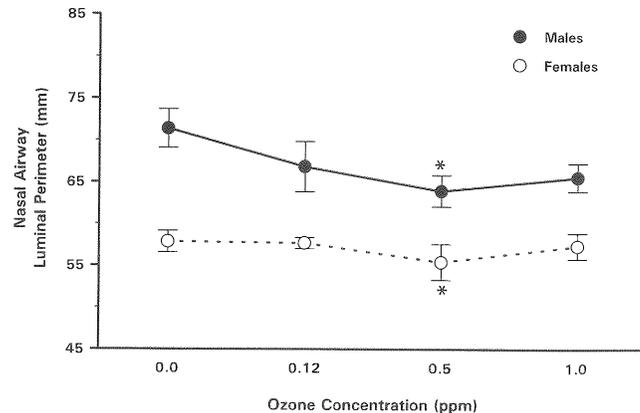


Figure 16. Changes in the cross-sectional perimeter of the nasal airway lumina of male and female rats exposed for 24 months to one of four concentrations of ozone. Data points are means  $\pm$  SEMs. \*Significantly different ( $p < 0.05$ ) from control group.

bacterial toxin-induced increase in osteoclastic resorption of the turbinate bone followed by an apparent disruption of osteoid synthesis by osteoblasts (Pedersen and Elling 1984).

The mechanism or mechanisms responsible for the ozone-induced bony atrophy in our study are not known. Ozone is very reactive, and therefore so short-lived that it is thought to be destroyed as it passes through the airway lining fluids or the surface epithelium it meets in the respiratory airways (Pryor and Church 1991; Pryor 1992). Consequently, it is highly unlikely that the subepithelial tissues of the nasal turbinate (i.e., lamina propria and bone) are exposed to ozone itself, but they may come in contact with longer-lived, secondary byproducts of ozone (e.g., aldehydes, hydroxyhydroperoxides) that can diffuse further into the tissue. Ozone exposure also is known to initiate the production and release of cytokines from airway epithelium that can initiate inflammatory reactions in the subepithelial airway tissues (Leikauf et al. 1995). Therefore, we speculate that the loss of turbinate bone caused by ozone in the present study may be closely related to the chronic inflammation observed in the adjacent nasal mucosa. The close proximity of the inflammatory cell influx in the lamina propria surrounding the atrophic turbinate bone in ozone-exposed animals, along with the known relationship between chronic inflammation and bone resorption in other chronic diseases make this the most plausible hypothesis.

Persistent inflammatory processes are central to bone destruction in other human diseases such as rheumatoid arthritis, periodontitis, and osteomyelitis (Harvey 1988). In addition, inflammatory cell mediators (e.g., prostaglandins, interleukin 1) have been implicated in the pathogenesis of bone resorption in these chronic diseases (Duncan 1983; Harvey 1988; Robinson 1989; Birkedal-Hansen 1993; Raisz et al. 1993). It has been suggested that generating large amounts of prostaglandins (mainly prostaglandin E<sub>2</sub>) in the inflamed rheumatoid synovium adjacent to bone may stimulate osteoclastic activity and lead to bone resorption (Harvey 1988). It has also been suggested that activating multinucleated osteoclasts or mononuclear phagocytes may be mediated by osteoblasts that respond directly to bone-resorbing agents by exposing the bone mineral to osteoclasts and preosteoclasts, or by releasing a soluble factor or factors that activate these cells, or both (Vaes 1988).

In the present study, multinucleated osteoclasts were rarely observed on the osseous surfaces of ozone-induced atrophic turbinates. The principal cells lining these areas of bone remodeling were mononuclear phagocytes, osteoblasts, and osteoprogenitor cells. Previous morphologic studies of resorption sites tend to support the hypothesis that normal bone resorption is a two-phase process initiated by osteoclasts dissolving bone mineral, which is followed by

mononuclear cells digesting the organic matrix (Jee 1988). This is then followed closely by osteoblastic production of new bone. This close linkage of bone resorption and bone formation along the bone surface is referred to as trabecular bone remodeling (Jee 1988). In the present study, male and female rats chronically exposed to 0.5 or 1.0 ppm ozone and killed after 20 or 24 months of exposure had morphologic evidence of marked trabecular remodeling of the bone in the dorsal aspects of the maxilloturbinates. Most of the bone-resorption sites were found in a cellular phase between mononuclear phagocytic cell resorption and early osteoblastic bone formation. Our morphometric data suggest that in rats chronically exposed to 0.5 or 1.0 ppm ozone, turbinate bone formation was less than resorption resulting in marked loss of the bone matrix. Because the normal maintenance of bone is dependent on both production of new bone and resorption of old bone, ozone exposure also may have caused a reduction in normal bone production. Our study was not designed to answer these mechanistic questions; therefore future studies must be specifically designed to further elucidate the possible mechanisms involved in the pathogenesis of ozone-induced bony atrophy.

Interestingly, the severity of the ozone-induced atrophy of turbinate bone was gender-dependent. Male rats exposed to either 0.5 or 1.0 ppm ozone had greater loss of turbinate bone than did female rats exposed to the same concentrations of ozone. The reason for this gender-specific difference in severity is unknown. It may have been related to the degree of the inflammatory response in the surrounding nasal mucosa, which appeared to be slightly greater (i.e., more inflammatory cells in the nasal mucosa) in male rats than in female rats after exposure to the same concentration of ozone.

In a parallel study, Pinkerton and colleagues morphometrically examined ozone-induced lung lesions in rats from the same exposure groups in the 20-month NTP/HEI collaborative study (Pinkerton et al. 1995). Interestingly, they found gender-related differences in the severity of the airway epithelial lesions in the pulmonary centriacinar regions similar to those we found in the nasal airways of ozone-exposed animals. As in our study, male rats had more severe alterations in the centriacinar airways compared with those in female rats. These results, along with our findings, demonstrate that the male rats were more susceptible than the female rats to ozone-induced alterations in both the nasal and pulmonary airways. The reasons for these gender-specific differences in response to ozone exposure are unknown, but may be related to differences in the amount of ozone delivered to airway tissues in male and female rats. Because male rats were considerably larger in body size, with larger lung volumes (and presumably larger

nasal volumes), than the female rats in the NTP/HEI study (Harkema and Mauderly 1994), it is conceivable that these targeted upper and lower airways in male rats received a greater cumulative dose of ozone compared with those airway tissues in female rats exposed to the same ozone chamber concentration. Our study, however, was not designed to examine or predict the doses of ozone at specific nasal airway tissue sites. In addition, there is not yet enough information in the literature to adequately estimate the amount of ozone to which the maxilloturbinates were exposed in our study. Therefore it may be premature at this time to speculate on how the size of the nasal cavity and the respiratory functions of the animal may affect the dosimetry of ozone in the rat nasal airway. We also cannot rule out the possibility that the gender-associated differences in response were due to inherent differences in the sensitivity of airway tissues to ozone between male and female rats. Future innovative studies must be specifically designed to determine if the gender-related differences in ozone-induced nasal turbinate atrophy (and other nasal tissue responses) are due to the dose of ozone to the targeted tissue, the tissue's inherent susceptibility to injury from exposure to this oxidant pollutant, or a combination of these factors.

The results of this study also indicated that ozone exposures at 0.5 or 1.0 ppm caused significant reductions in the area of the lamina propria in the maxilloturbinates of male and female rats exposed for 24 months and of male rats exposed for only 20 months. This appeared to be due to a marked reduction in the profiles of blood vessels in these tissues. These normally large, dilated, thin-walled vessels were markedly constricted with thick walls in both male and female rats after exposure to 0.5 or 1.0 ppm ozone. Like the ozone-induced bony atrophy, the ozone-induced reduction in the area of the lamina propria was significantly greater in male rats than in female rats. In addition, female rats exposed to 0.5 or 1.0 ppm ozone for only 20 months had no measurable reduction in the cross-sectional area of the lamina propria. The reason for little or no response in female rats compared with male rats exposed to ozone also is unknown.

These normally cavernous venous plexuses in the lamina propria of the nasal mucosa are referred to as "swell bodies" (Negus 1958; Harkema and Morgan 1996). Congestion of these capacitance vessels can cause thickening of the mucosa, which subsequently may restrict the air flow within regions of the nasal passages. Therefore, the marked reduction in the area of these mucosal blood vessels in the ozone-exposed rats of our study may reflect a physiologic, adaptive response of the animal to further open the nasal

airways. Rats, like other laboratory rodents, are obligate nose breathers and are dependent on a patent upper airway to breathe (Harkema and Morgan 1996).

In contrast to the ozone-induced losses in the area of subepithelial tissues (i.e., bone and lamina propria), significant increases in the cross-sectional area of the outer surface epithelium were noted in male and female rats exposed to 0.5 or 1.0 ppm ozone for 24 months and in female rats exposed to the same concentrations of ozone for only 20 months. The conspicuous increase in epithelial areas in these animals was the result of marked epithelial hyperplasia and mucous cell metaplasia. Although male rats exposed for 20 months to 0.5 or 1.0 ppm ozone did not exhibit statistically significant increases in the cross-sectional area of the surface epithelium, these animals did have conspicuously thickened, hyperplastic and metaplastic epithelium similar to the animals in the other groups exposed to 0.5 or 1.0 ppm ozone. As mentioned previously, these male rats also had an overall reduction in total turbinate area due to marked loss of subepithelial tissues (e.g., bone). This turbinate atrophy resulted in a reduction in the luminal surface area of the epithelium covering the atrophic turbinate, negating any ozone-induced increase in cross-sectional area due to epithelial thickening.

The nasal transitional epithelium that lines the maxilloturbinates, lateral wall, and medial aspects of the nasoturbinates in the proximal nasal airways of rats is usually a thin, nonciliated, cuboidal epithelium that is normally devoid of secretory cells (Harkema and Morgan 1996). We have previously reported in detail on the cellular changes that occurred in this epithelium after prolonged exposure to ozone (Harkema et al. 1994). Briefly the transitional epithelium in rats exposed to 0.5 or 1.0 ppm ozone for 20 months had marked mucous cell metaplasia characterized by numerous mucous cells and intraepithelial mucosubstances. It has been reported that mucus is an effective antioxidant (Cross et al. 1984, 1994). Therefore, more mucous cells contributing increased amounts of luminal mucus could significantly reduce the direct or indirect toxic effects of ozone on airway tissues.

The results of the morphometric analyses conducted in the present study provided some new information concerning the nasal transitional epithelium in rats and its response to prolonged ozone exposure. We found that the normal cross-sectional area of this epithelium covering the proximal maxilloturbinates is gender-dependent. Male rats have significantly more area of surface epithelium compared with females of similar age. This was due to the overall larger size of the maxilloturbinates in male rats compared with female rats, and did not appear to be due to a gender-specific difference in the relative thickness of the epithelium.

lium. In addition, female rats exposed to 0.5 or 1.0 ppm ozone usually had a greater epithelial response (i.e., increase in cross-sectional area of surface epithelium) compared with similarly exposed male rats. This was in contrast to the responses of the other tissue compartments of the maxilloturbinate, which were usually greater in males than in females (i.e., ozone-induced reductions in the area of bone and lamina propria).

In addition, the degree of the ozone-induced atrophy of the subepithelial tissues in male rats exposed to 1.0 ppm ozone was significantly greater than the ozone-induced increase in their epithelial tissues. This resulted in an overall atrophy of the entire maxilloturbinate (i.e., turbinate atrophy) in these ozone-exposed male rats. In contrast, the loss of subepithelial tissue in female rats exposed to 1.0 ppm was compensated by an increase in surface epithelial tissue. Therefore, there was no overall loss of cross-sectional area of the entire maxilloturbinate in these female rats. These differences in the degree of the responses of the different tissue compartments also suggest that the severity of ozone-induced atrophy of subepithelial tissues (bone and lamina propria) may be partially dependent on the degree of the metaplastic or hyperplastic changes in the surface epithelium lining the turbinate.

Gender-specific differences in the cross-sectional areas and perimeters of the nasal lumina reflected the differences in the sizes of the heads between adult male and female F344/N rats. Male F344/N rats have a much larger body size compared with that of female F344/N rats of similar age (Boorman and Morgan 1990). We were surprised to find that both male and female rats had measurable increases in their nasal luminal areas after ozone exposure, and that the magnitude of the response was not concentration-dependent. We do not know the reason or reasons for this generalized dilatation of the nasal passages after these prolonged exposures to both low and high concentrations of ozone, but we hypothesize that these observed changes reflect a generalized physiologic, rather than a pathologic, response to the pollutant.

In conclusion, 20- and 24-month exposures to ozone induced significant concentration-dependent alterations in the principal tissue compartments (surface epithelium, lamina propria, and bone) of the maxilloturbinates in F344/N rats. The severity of ozone-induced subepithelial tissue injury in the maxilloturbinates was gender-dependent. Future studies must be specially designed to determine the mechanisms responsible for the bone and lamina propria changes observed after prolonged exposure to ozone. In addition, it will be important to determine how long these ozone-induced nasal alterations persist after the end of exposure. Finally, the nature and severity of these lesions

suggest that repeated exposures to ozone may adversely affect the normal defense mechanisms of the nose (e.g., filtration and humidification of the inhaled air) leaving the lower respiratory tract vulnerable to excessive burdens of potentially harmful, inhaled agents (e.g., xenobiotics, bacteria).

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#### ABBREVIATIONS

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CASAC	Clean Air Scientific Advisory Committee
ppm	parts per million
NAAQS	National Ambient Air Quality Standard
NTP	National Toxicology Program
TEM	transmission electron microscopy



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## INTRODUCTION

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Clinical and epidemiologic studies provide evidence that children and young adults exposed, while exercising, to low levels of ozone for short periods experience transient decrements in lung function and an influx of inflammatory cells in the nose and lungs (reviewed by Lippmann 1992; U.S. Environmental Protection Agency 1996a). The current National Ambient Air Quality Standard for ozone promulgated by the U.S. Environmental Protection Agency (0.12 parts per million [ppm]\*; a level not to be exceeded for more than one hour, once per year) relies heavily on data obtained from these short-term clinical and epidemiologic studies. However, these studies do not address the potential for repeated or prolonged inhalation of ozone that may produce long-term decrements in respiratory function, or may contribute to or aggravate existing chronic respiratory diseases in humans.

During its last review of the ozone criteria and standards, the Clean Air Scientific Advisory Committee (CASAC) concluded that the existing standards provided little, if any, margin of safety. The ozone standards are being reevaluated and CASAC has completed its review of a new Ozone Criteria Document and an Ozone Staff Paper (U.S. Environmental Protection Agency 1996b; Wolff 1996). In a notice of proposed rulemaking, the EPA has proposed changing the level and the form of the standard to 0.08 ppm based on an eight-hour, rather than a one-hour, average. The Administrator, acknowledging the range of views regarding the appropriate level for the standard, sought public comment on two alternatives for an eight-hour primary standard, 0.07 and 0.09 ppm ozone. The EPA is also soliciting comment on retaining the current standard. The final regulation will be issued in June 1997.

Because exposure to ozone is widespread and little information is available regarding the health risks of long-term exposure to this pollutant, the National Toxicology Program (NTP) and the Health Effects Institute established a collaboration to evaluate how laboratory rats were affected by prolonged inhalation of ozone. The results of this collaboration, including an Integrative Summary of the results of nine individual studies and a description of the Project Design, were published as Parts I through XI of HEI Research Report Number 65.

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\* A list of abbreviations appears at the end of the Investigators' Report for your reference.

This document has not been reviewed by public or private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views of these parties, and no endorsements by them should be inferred.

One part of the respiratory tract targeted for study in the NTP/HEI Collaborative Ozone Project was the nasal region (Harkema et al. 1994a). The nasal passages are the first line of defense against inhaled pathogens, dusts, or irritant gases, and the first site where injury might occur. Therefore, changes in its normal defense capabilities could lead to increased nasal infections and increased susceptibility to lower respiratory tract diseases.

As part of the original NTP/HEI Collaborative Ozone Project, Dr. Harkema and colleagues conducted detailed morphologic and functional examinations of ozone's effect on the nasal mucosa of F344/N rats. They observed alterations in the epithelial layers lining the nasal passages in rats exposed to 0.5 or 1.0 ppm (but not to 0.12 ppm) ozone for 20 months, and concomitant functional changes in the nasal mucociliary clearance system. One unexpected finding was changes, including bony atrophy, in the subepithelial tissues of the nasal turbinates (Harkema et al. 1994a). After the study was completed, Dr. Harkema requested additional funds to confirm and extend these observations. The HEI Health Research Committee viewed the proposed research as an opportunity to derive additional information from tissue samples that remained from the earlier study. Dr. Harkema's study began in February 1995, and total expenditures were \$58,187. The Investigators' Report was received at HEI in April 1996 and was accepted for publication by the Health Review Committee in July 1996. The following Commentary is intended to aid the sponsors of HEI and the public by highlighting both the strengths and limitations of the study and by placing the Investigators' Report into scientific and regulatory perspective.

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## SCIENTIFIC BACKGROUND

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Ozone is a highly reactive oxidant gas with the potential to damage the epithelial cells that line the respiratory tract. A great deal of attention has been focussed on ozone's effects on the lower respiratory tract (Plopper et al. 1991; Lippmann 1992, 1993; Chang et al. 1995; Pinkerton et al. 1995). Only recently has much attention been paid to the nose, which might be expected to be a site of ozone-induced injury. Part VII of Research Report Number 65 provides a detailed discussion of the structural and functional effects of ozone on the nasal mucosa. This section focusses on some key points that are related to the interpretation of the present study.

The nasal passages of humans exposed to polluted ambient air show signs of structural alterations and inflammation. Calderon-Garcidueñas and associates (1992) reported

marked structural changes in the nasal epithelia of Mexico City residents living in areas where ozone levels range from 0.1 to 0.4 ppm for several hours each day at certain times of the year, compared with residents of another Mexican city with low ozone levels. The changes included mild to moderate epithelial dysplasia (abnormal tissue development), a marked loss of normal respiratory epithelium, basal cell hyperplasia, and squamous metaplasia. Although ozone is the major oxidant gas in Mexico City's smog, high levels of sulfur dioxide and elevated levels of suspended particles containing nitrates and sulfates are also present. Thus, these results are generally interpreted as implicating a mix of air pollutants in damage to nasal epithelia.

Inflammatory changes are found in the nasal passages of normal and asthmatic subjects after acute or short-term exposure to ozone. Humans exposed to 0.4 or 0.5 ppm ozone for two or four hours (Graham et al. 1988; Graham and Koren 1990) and subjects with asthma exposed to 0.24 ppm ozone for 90 minutes (McBride et al. 1994) showed an inflammatory cell influx in nasal lavage fluid immediately after exposure ceased that lasted for 18 to 24 hours. Frischer and colleagues (1993) found that children exposed for five months to ambient ozone levels ranging from 0.04 to 0.09 ppm also showed signs of nasal inflammation. These investigators reported an increased content of inflammatory mediators in nasal lavage as ozone levels increased from spring to summer, and a decrease as summer progressed into fall. After testing for sensitivity to aeroallergens, the investigators considered it unlikely that pollen exposure accounted for their findings because markers of inflammation increased in both allergic and nonallergic children on days following high ozone exposure.

Inflammation has been implicated in the bone resorption process observed in several diseases, including rheumatoid arthritis, periodontitis, and osteomyelitis (Harvey 1988). Moreover, bone loss occurs in atrophic rhinitis, a condition characterized by progressive atrophy of the nasal mucosa and underlying turbinate bone, enlargement of the nasal cavities, and the formation of thick, dry, nasal crusts. Atrophic rhinitis can occur as either a primary or secondary condition, both of which have similar characteristics. The etiology of primary atrophic rhinitis is unknown and it is comparatively rare in developed countries (Zohar et al. 1990). However, secondary atrophic rhinitis has become more common, possibly as a result of chronic granulomatous nasal infections, chronic sinusitis, trauma, irradiation, or as a consequence of intranasal surgical procedures (Bertrand et al. 1996).

Several of the ozone-induced changes in rat nasal mucosa reported by Harkema and colleagues (1994a) have the potential to contribute to the development of atrophic rhin-

itis. In that study, they found that rats exposed to 0.5 or 1.0 ppm ozone for 20 months showed decreased ciliary beating, a reduced rate of mucous flow, and an influx of inflammatory cells into the nasal turbinates. Lesions that reduce ciliary beating and mucous flow can allow microorganisms to remain in the airway and proliferate on stagnant mucus, exacerbating crust formation and inflammation-driven bone resorption (Harvey 1988; Ferguson et al. 1990; Birkedal-Hansen 1993).

In their earlier study, Dr. Harkema and colleagues (1994a) identified the presence of atrophied turbinate bone by qualitative histopathology. The present study extends these observations by quantifying changes in rat nasal turbinates after prolonged exposure to ozone and relating these changes to inflammation in nasal mucosal tissue.

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## OBJECTIVES AND STUDY DESIGN

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The major objective of this study was to quantify ozone-induced alterations in the maxilloturbinate bone of F344/N rats and to determine if there was a dose-response relationship. Additional objectives were to measure ozone's effects on the other principal components of the maxilloturbinates (the surface epithelium and the lamina propria [mucosal tissue that lies between the outer surface epithelium and the inner turbinate bone]), characterize the ozone-induced ultrastructural changes in the maxilloturbinates, and examine ozone's effect on the surface area of the nasal lumen and the perimeter of the nasal passages.

To achieve these objectives, Dr. Harkema and colleagues studied tissue sections from 37 rats (4 or 5 males and 4 or 5 females per exposure group) from their earlier NTP/HEI study (20-month exposure of male and female rats to 0, 0.12, 0.50, or 1.0 ppm ozone for six hours per day, five days per week) and a second group of tissue sections from 127 rats of the same strain (25 males and 102 females) exposed to the same ozone regimen at the same facility for 24 months. Animals were killed approximately one week after exposure ceased to allow time for transient ozone-induced changes to reverse. The preponderance of female rats available for analysis was due to the high incidence of early deaths in male rats from mononuclear cell leukemia.

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## TECHNICAL EVALUATION

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### ATTAINMENT OF STUDY OBJECTIVES

The investigators successfully attained their objectives. They obtained morphometric measurements of turbinate bone, lamina propria, and surface epithelium in rats ex-

posed to ozone or clean air. Dr. Harkema and colleagues also determined changes in nasal passage lumina and perimeters.

#### ASSESSMENT OF METHODS AND STUDY DESIGN

The investigators used state-of-the-art imaging techniques and standard morphometric analysis to estimate the amounts of turbinate bone, lamina propria, and surface epithelium by measuring the cross-sectional area of each tissue compartment at a defined location in the proximal nasal passage. The area of the nasal airway lumen and the perimeter of the nasal passages were also measured by image analysis. Dr. Harkema employed light microscopy to identify inflammatory cell types and alterations in blood vessels, and transmission electron microscopy to identify sites of bone resorption.

#### STATISTICAL METHODS

The investigators used two-way analysis of variance to test the effects of gender, ozone concentration, and their potential interaction on the development of lesions in the maxilloturbinates. Dunnett's criterion for comparing several exposure groups to a control group was used to account for multiple comparisons. A  $p < 0.05$  was considered to be statistically significant.

#### RESULTS AND INTERPRETATION

Compared with control rats that breathed clean air, F344/N rats exposed to 0.5 or 1.0 ppm ozone for 20 or 24 months developed dose-dependent, and gender-dependent quantitative changes in the turbinate bone, lamina propria, and surface epithelium that compose the maxilloturbinate. The maxilloturbinates of rats exposed to 0.12 ppm ozone did not differ from control animals. Thus, the threshold of ozone's effect appears to be between 0.12 and 0.5 ppm. The morphometric data and increased number of tissue samples provided convincing evidence that the earlier histologic evidence of bony atrophy was correct. The main findings are summarized below.

##### Turbinate Bone

The loss of turbinate bone was greater in male rats than in female rats at the two higher ozone concentrations and at each exposure time. Male rats exposed to 0.5 or 1.0 ppm ozone for 20 or 24 months lost between 49% and 64% of their turbinate bone. In contrast, the response in female rats ranged from no change to a 52% loss of turbinate bone. Atrophic turbinate bone in rats exposed to 0.5 or 1.0 ppm ozone showed conspicuous areas of bone resorption. Thus,

the original finding of bony atrophy (Harkema et al. 1994a) was supported by two independent analytical methods, quantitative image analysis, and transmission electron microscopy.

##### Lamina Propria

The investigators observed a change in the lamina propria adjacent to the turbinate bone that may be related to bone loss. Rhinitis (an influx of inflammatory cells consisting of neutrophils, lymphocytes, and monocytes) in the lamina propria of rats exposed to 0.5 or 1.0 ppm ozone was greater in males than in females. The authors interpreted this finding as suggesting that bone resorption was due to inflammation that may have been initiated by the interaction of ozone with the epithelial layer of the maxilloturbinate. Because male rats lost more turbinate bone than females, the greater inflammatory response in males strengthens this hypothesis.

A second change in the lamina propria of rats exposed to ozone was the replacement of the normally large, dilated, thin-walled blood vessels by thick-walled vessels with constricted lumina. Blood vessel constriction caused a greater reduction in cross-sectional area in male rats than in females. Male rats exposed to 0.5 or 1.0 ppm ozone for 20 or 24 months lost 28% to 45% of their lamina propria area; responses in female rats ranged from no change to a 22% loss. The authors suggest that blood vessel congestion can cause the lamina propria to thicken, which may reduce air flow. Blood vessel constriction may be an adaptive response of the organism to open the nasal airway by decreasing the volume taken by blood vessels. Because rats are obligate nose breathers, they require an unobstructed upper airway to breathe optimally.

##### Surface Epithelium

In contrast to the decreased cross-sectional areas of turbinate bone and lamina propria, exposure to 0.5 or 1.0 ppm ozone increased the cross-sectional area of the outer surface epithelium, and these changes were greater in female rats than in males. For example, the surface epithelium of female rats exposed to 0.5 or 1.0 ppm ozone for 20 or 24 months increased by 55% to 90%. The responses in male rats ranged from no change to a 40% increase. The thickened surface epithelium was caused by increases in epithelial cells, mucous cells, and mucosubstances in rats exposed to 0.5 or 1.0 ppm ozone (Harkema et al. 1994a). Because mucus has been reported to have antioxidant properties (Cross et al. 1994), the authors suggest that more mucous cells producing increased amounts of mucus might have partially protected female rats against the toxic effects of ozone. Thus, differences in the severity of ozone-induced

injury to bone and lamina propria may have been dependent on the different degrees of epithelial cell increase in males and females. The reasons for these disparate changes in epithelial thickening are unclear. Other factors that might be responsible for the more severe damage in males is their greater head size, and possibly greater activity level, both of which might cause a greater flux of ozone to the nose, and thus a larger target dose.

#### **Total Maxilloturbinate Area**

The degree of epithelial thickening affected the total cross-sectional area of the maxilloturbinates. The ozone-induced losses of turbinate bone and lamina propria in male rats were greater than the increase in epithelial tissue. Thus, the total cross-sectional area of the maxilloturbinates was reduced by 23% to 38% after exposure to 0.5 or 1.0 ppm ozone for 20 or 24 months. In contrast, because the increased amount of epithelial tissue in female rats compensated for the ozone-induced reduction in turbinate bone and lamina propria, the cross-sectional area of their maxilloturbinates did not differ from controls.

#### **Nasal Airway Lumina**

Rats exposed to ozone had measurable changes in the luminal area and perimeter of their nasal passages. Exposure to each concentration of ozone produced statistically significant increases in luminal area; however, the increases were not dependent on dose. The increased luminal area in rats exposed to 0.12 ppm ozone is surprising because their total turbinate area was unchanged. It would be expected that additional luminal area would be formed at the expense of the turbinates. Rats exposed to 0.5 ppm ozone showed a statistically significant decrease in the perimeters of their nasal passages, whereas rats exposed to 0.12 or 1.0 ppm ozone did not differ from controls. The causes of these effects are not understood.

#### **OVERALL INTERPRETATION**

The atrophy of nasal turbinate bone and the lamina propria caused by ozone inhalation may have implications for nasal defense mechanisms. First, reducing the surface area of the turbinates (which compose the primary air filtration network in the nasal cavity) could decrease the filtration of potentially harmful pathogens, dusts, or irritant gases from inhaled air, a mechanism that protects the respiratory tract against infection and injury. Second, atrophic rhinitis can result from the combination of reduced mucociliary clearance, caused by mucosal atrophy, and bacterial infection. Although the response of the nasal passages of F344/N rats to prolonged exposure to ozone levels of 0.5

ppm or greater raises concerns that similar effects may occur in people, it is difficult to extrapolate the results of this study to humans for several reasons. First, rats and primates have different nasal architecture, and possibly different susceptibility of their epithelial cell populations. Second, because rodents are obligate nose breathers and primates are oronasal breathers, one would expect that exposing both species to similar levels of ozone might result in a greater dose (and thus greater injury) to the rodent nose. However, the results of Plopper and colleagues (1991) suggest that nonhuman primates may be more sensitive than rodents to ozone's toxic effects on nasal epithelia. Thus, it is possible that the results with rats may underestimate the possible effects on humans. Finally, in addition to interspecies differences, rat strains and substrains also differ in ozone sensitivity. For example, male Sprague-Dawley rats develop a greater inflammatory response in the lungs after ozone exposure than the Wistar strain (Pino et al. 1991). Also, ozone produces a higher degree of secretory metaplasia in the nasal mucosa of F344/NTac and Wistar rats than in F344/N or Sprague-Dawley rats (Harkema et al. 1994b).

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#### **IMPLICATIONS FOR FUTURE RESEARCH**

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Further research is needed to identify the mechanisms responsible for turbinate bone resorption after prolonged exposure to ozone. An important avenue of investigation is the investigators' hypothesis that ozone-induced inflammation in the lamina propria contributes to this process. It is also important to determine whether ozone-induced nasal alterations persist for more than the one-week period after exposure that was examined in this study. However, if future research with rodents is planned to extend this study's findings, the study design must carefully take into account strain and substrain variability in ozone sensitivity.

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#### **CONCLUSIONS**

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In an earlier study, which was part of the NTP/HEI Collaborative Ozone Project, Dr. Harkema and colleagues (1994a) used qualitative histochemical techniques to identify bony atrophy in nasal turbinates of F344/N rats exposed to 0.5 or 1.0 ppm ozone for 20 months. The present study confirms and extends this unexpected finding by providing morphometric measurements and ultrastructural observations on the nasal turbinates of rats exposed to 0, 0.12, 0.5, or 1.0 ppm ozone for 20 or 24 months.

Prolonged inhalation of 0.12 ppm ozone did not affect the structure of the maxilloturbinates in F344/N rats. How-

ever, changes in the turbinate bone, lamina propria, and surface epithelium that compose the maxilloturbinates were dose-dependent, and quantifiable in rats exposed to 0.5 or 1.0 ppm ozone, compared with control rats breathing filtered air.

The major finding in this study was the loss of turbinate bone in male and female rats exposed to 0.5 or 1.0 ppm ozone. Rats exposed to these ozone concentrations developed a mixed inflammatory cell influx (rhinitis) in the lamina propria adjacent to the turbinate bone, and the area of the lamina propria decreased due to marked blood vessel constriction. Bone loss, the inflammatory response, and the loss of lamina propria were greater in male rats than in females, possibly because the greater proliferative response of the surface epithelium in female rats provides a greater degree of protection from ozone.

The results of this study provide the first quantitative evidence that prolonged exposure to ozone at concentrations equal to or greater than 0.5 ppm causes bone loss in the nasal turbinates of F344/N rats. Although these findings are provocative, they are difficult to extrapolate to humans because of species differences in breathing characteristics (nasal versus oronasal), nasal architecture, and possible differences in susceptible epithelial cell populations. However, the production of lesions in a component of the rodent nose that filters inspired air suggests the possibility that long-term exposure to ozone may compromise the ability of the nose to protect against harmful inhaled microorganisms and other unhealthy agents.

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