RESEARCH REPORT

Respiratory Tract Toxicity in Rats Exposed to Mexico City Air

Owen R Moss, Elizabeth A Gross, R Arden James, Derek B Janszen, Paul W Ross, Kay C Roberts, Andrew M Howard, Jack R Harkema, Lilian Calderón-Garcidueñas, and Kevin T Morgan

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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 200 projects at institutions in North America and Europe and has published over 100 research reports.

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HEI STATEMENT

Synopsis of Research Report 100

Effects of Mexico City Air on Rat Nose

BACKGROUND

Residents of southwestern Mexico City are exposed to a mixture of air pollutants (ozone, formaldehyde, acetaldehyde, particulate matter, polycyclic aromatic hydrocarbons, and other hydrocarbons) not found in other areas of the city. Pathologists have found evidence of cell damage and inflammation in nasal tissue from some residents of this highly polluted area that was not present in people living in areas of the country with cleaner air. This finding prompted HEI to support a collaborative study to determine whether the effects of Mexico City air on humans could be replicated in rats. If so, rats could serve as sentinels to detect the effects of air pollutants on human nasal tissue. The collaboration involved researchers from the Chemical Industry Institute of Toxicology (CIIT; original Principal Investigator Dr Kevin Morgan, later Dr Owen Moss), Michigan State University (Dr Jack Harkema), and the Instituto Nacional de Pediatria (INP) in Mexico City (Dr Lilian Calderón-Garcidueñas, who had conducted the human studies).

APPROACH

Most studies of the health effects of air pollutants on laboratory animals are performed by controlled laboratory exposures to a single pollutant. In this study, the laboratory was Mexico City and its ambient air was the exposure atmosphere. Moss and coworkers at CIIT constructed mobile exposure chambers designed to expose rats to either ambient southwestern Mexico City air or to air from which pollutants would be removed by a filtration system. The investigators exposed rats to unfiltered or filtered Mexico City air for 23 hours/day for 21, 35, or 49 consecutive days. They examined the animals' respiratory tract for evidence of tissue damage using histopathology and state-of-the-art morphometry.

RESULTS AND INTERPRETATIONS

Moss and colleagues demonstrated the feasibility of using specially designed exposure chambers to study the effects of ambient air on laboratory animals. They found no significant differences in the nasal tissue of rats exposed to unfiltered or filtered air for up to seven weeks. Several possible interpretations could explain the lack of effect of Mexico City air on rats compared with humans. Pollutant levels may have been too low (or the exposure times too short) to affect the rats. In this study, the mean levels of ozone and formaldehyde during the daytime period of highest pollution were lower than those inducing nasal lesions in controlled exposures of laboratory animals. Alternatively, because the anatomy and physiology of rodent and human nasal passages differ significantly, human nasal tissue may be more susceptible to the pollutant mixture than rat tissue. Further research should explore the validity of each interpretation to determine whether bioassays using rat tissue are appropriate for assessing the effects of air pollutants on humans.

This Statement, prepared by the Health Effects Institute, summarizes a research project sponsored by HEI from October 1995 to November 1998 and conducted by Dr Owen R Moss of the Chemical Industry Institute of Toxicology, Research Triangle Park NC. The following Research Report contains both the detailed Investigators' Report and a Critique on the study prepared by the Institute's Health Review Committee.

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HEI STATEMENT

This Statement is a nontechnical summary of the Investigators' Report and the Health Review Committee's Commentary.

INVESTIGATORS' REPORT

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.

CRITIQUE Health Review Committee

The Critique about the Investigators' Report is prepared by the HEI Health Review Committee and Staff. Its purpose is to place the study into a broader scientific context, to point out its strengths and limitations, and to discuss remaining uncertainties and implications of the findings for public health.

RELATED HEI PUBLICATIONS

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INVESTIGATORS' REPORT

Respiratory Tract Toxicity in Rats Exposed to Mexico City Air

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ABSTRACT

The rat has been used extensively as a health sentinel, indicator, or monitor of environmental health hazards, but this model has not been directly validated against human exposures. Humans in Mexico City show upper respiratory tract lesions and evidence of pulmonary damage related to their environmental inhalation exposure. In this study, male and female F344 rats were exposed (23 hr/day) in Mexico City to local Mexico City air (MCA)* for up to seven weeks.

Controls were maintained at the same location under filtered air. Prior to these exposures, several steps were taken. First, the nasal passages of normal male rats shipped from the United States and housed in Mexico City were examined for mycoplasma infection; no evidence of infection was found. In addition, a mobile exposure and monitoring system was assembled and, with an ozone (O_3) exposure atmosphere, was tested along with supporting histopathology techniques and analysis of rat nasal and lung tissues. Last, the entire exposure model (equipment and animals) was transported to Mexico City and validated for a three-week period.

During the seven-week study there were 18 one-hour intervals during which the average O_3 concentration of MCA in the exposure chamber exceeded the US National Ambient Air Quality Standard (NAAQS) of 0.120 ppm O_3 (hourly average, not to be exceeded more than once per year). This prolonged exposure of healthy F344 rats to MCA containing episodically low to moderate concentrations of O_3 (as well as other urban air pollutants) did not induce inflammatory or epithelial lesions in the nasal airways or lung as measured by qualitative histologic techniques or quantitative morphometric techniques. These findings agree with those of previous controlled O_3 inhalation studies, but they are in contrast to reports indicating that O_3-polluted MCA causes significant nasal mucosal injury in adults and children living in southwestern Mexico City. Taken together, these findings may suggest that human airways are markedly more susceptible to the toxic effects of MCA than are the airways of the F344 rat.

INTRODUCTION

Rats are used extensively in the United States to assess potential risks to human health after exposure to air pollutants, but little information is available comparing changes in rats with changes seen in humans under similar inhalation exposure. Seldom are concentrations of pollutants high enough or environmental exposures of human populations long enough to result in clearly measurable effects. One exception appears to be the incidence of nasal tissue damage in humans exposed to MCA, a correlation for which there is a growing database. The research documented in this report addresses the following hypothesis: "Rats exposed to MCA exhibit respiratory tract lesions similar to those observed in humans, with differences attributable to species-specific, airflow-driven dosimetry patterns of inhaled air pollutants, regional metabolism, or other definable factors."

An extensive database documents nasal toxicity in rats after inhalation exposure to toxic chemicals (Reznik and Stinson 1983; Barrow 1986; Feron and Bosland 1989; Uraih et al 1990), and much of this information was generated for the purpose of human risk assessment. Because

*A list of abbreviations and other terms appears at the end of the Investigators' Report.

This Investigators' Report is one part of Health Effects Institute's Research Report 100, which also includes a Critique by the Health Review Committee and an HEI Statement about the research project. Correspondence concerning the Investigators' Report may be addressed to Dr Owen R Moss, Chemical Industry Institute of Toxicology, 6 Davis Drive, PO Box 12137, Research Triangle Park NC 27709.

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; therefore, it may not necessarily reflect the views of the Agency, and no official endorsement by it 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.

of anatomic, physiologic, behavioral, and biochemical differences between rats and humans, however, the relevance of rodent data for human risk assessment is problematic (DeSesso 1993). Recent studies of human subjects exposed to the urban environment of Mexico City revealed considerable injury of the nasal mucosa, including epithelial hyperplasia and dysplasia, in both adults and children (Calderón-Garcidueñas et al 1992, 1995, 1996, 1997, 1999; Calderón-Garcidueñas and Roy-Ocotla 1993). The lesions were attributed, at least in part, to changes in MCA quality due to the increased use of motor vehicles and associated increased air pollution during the prior decade; the extensive use of liquid propane gas in the city was also considered a possible factor. The relative contribution of each of these pollutant sources to nasal mucosa injury is still a matter for debate (Guzman et al 1996), although Calderón-Garcidueñas and colleagues (1992) expressed concern that the lesions they saw may relate to increases in airborne O_3 and formaldehyde (CH_2O). Both are gases for which there is a great deal of information on nasal histopathology in rats following inhalation exposure (Morgan et al 1986; Harkema et al 1987a,b, 1989, 1997a,b; Harkema 1990; Heck et al 1990; Monticello 1990; Morgan and Monticello 1990b).

Nasal lesions induced by inhaled chemicals occur in specific regions of the nose in both rats (Morgan et al 1986; Johnson et al 1990; Monticello et al 1991) and nonhuman primates (Harkema et al 1987a,b; Monticello et al 1989). This site specificity is attributable to regional gas dosimetry, local tissue susceptibility, or a combination of these factors (Morgan and Monticello 1990a). In the case of CH_2O, airflow-driven regional dosimetry is considered responsible for the location of nasal lesions both in rats (Morgan et al 1991; Kimbell et al 1993a) and rhesus monkeys (Morgan et al 1991). In regions of the lower respiratory tract exposed to inhaled CH_2O, a relatively soluble reactive gas, local metabolism also plays a major role in the toxic outcome (Heck et al 1990). In contrast, nasal airflow does not appear to play as important a role in the distribution of nasal lesions induced by O_3, a relatively insoluble reactive gas (Kimbell et al 1993b). Mucociliary function, however, may have a major impact on toxic responses to this important environmental air pollutant (Pryor 1992; Harkema et al 1994). Chemicals such as O_3 and CH_2O are ideal candidates for attempts to incorporate classic mechanistic data into the human risk assessment process (Conolly and Andersen 1991).

The work presented here is intended not only to address the validity of rat nasal passages as a model for human risk assessment (DeSesso 1993) but also to provide information on the use of rats as monitors for environmental health hazards in many situations. The issue of animals as health sentinels, indicators, or monitors has been extensively reviewed by O'Brien and colleagues (1993). The general aim of the work reported here was to assess the value of the rat, specifically the rat nose, as an indicator of human disease risk associated with inhalation exposure to air pollutants. The known toxicity of MCA on the human nose (Calderón-Garcidueñas et al 1992, 1994, 1996, 1997; Calderón-Garcidueñas and Roy-Ocotla 1993) was used to compare responses in rats and humans.

Our approach involved multiple steps. We examined the nasal passages of normal male rats housed in Mexico City for evidence of active mycoplasma infection or other conditions that might adversely affect nasal passages. We assessed the biological and physical efficacy of an exposure system that could protect animals from or expose animals to O_3, and then the equipment and monitors supporting two 1-m^3 exposure chambers were shipped to Mexico City. After the efficacy of the exposure system was tested, we evaluated the basic response of rats to filtered and unfiltered MCA. Finally, we exposed rats for up to seven weeks to filtered or unfiltered MCA.

Based on our starting hypothesis, we expected rats exposed to MCA to show a range of nasal lesions, as well as possible additional respiratory tract responses, whereas rats exposed to filtered MCA (the controls) would be unaffected. The lesions would resemble changes previously reported for one or more nasal toxicants; the locations of the lesions would be an indication of which air pollutants induced toxicity. On the other hand, the absence of lesions in both the control and exposed groups of rats would imply that the rat may not be a good model for estimating human risk to such inhalation exposures.

MATERIALS AND METHODS

All experimental work involving animals was conducted with approval from the Institute Animal Care and Use Committee (IACUC) of the Chemical Industry Institute of Toxicology (CIIT). The work reviewed and approved by the Committee included all experimental work related to this project, including work conducted at CIIT and work conducted by CIIT staff at other sites.

EXPERIMENTAL DESIGN

Protocol: Prepilot Study

The objective of the Prepilot Study was to examine the nasal passages of normal male rats housed in Mexico City for evidence of active mycoplasma infection or other

infectious condition. Any pathogenic agents identified might be endemic to the Instituto Nacional de Pediatria (INP) building and might adversely affect identification of lesions expected to occur in the nasal passages following exposure to MCA.

From a group of 20 eight-week-old male F344 rats, 10 rats were shipped directly from the supplier to Mexico City, Mexico, and 10 rats were sent to CIIT, Research Triangle Park, North Carolina. Upon arrival at their respective locations, 5 rats from each group were immediately examined and killed, blood was collected for serology, and the entire respiratory tract was examined with histopathologic techniques. The remaining 5 rats at each location were held under standard animal husbandry methods for four weeks and then killed. Samples were collected from all 10 remaining animals for serology and for histopathologic examination of the nasal passages.

Protocol: Ozone Study

The objective of the Ozone Study was to use a positive nasal-tissue response to O_3 to evaluate the design, assembly, and operation of the mobile exposure and monitoring system. The system was designed to monitor rat exposure to MCA while protecting control rats from exposure to MCA. A critical feature of this study was to demonstrate that control animals could be housed in the same (namely, urban, rural, or workplace) environment and simultaneously be protected from exposure to the pollutants of interest. Because O_3 was to be the primary indicator of exposure or nonexposure to MCA, this experimental model (including histopathologic preparation and analysis of rat nasal and lung tissue) was evaluated through the use of an O_3 exposure protocol.

We used three groups of animals for the Ozone Study: a room control group, and, sequentially in the same chamber, an O_3 atmosphere group followed by a filtered O_3 atmosphere group (Table 1). The rats were exposed to a target concentration of 1.0 ppm O_3, 23 hr/day, for six consecutive days. Weekly and terminal body weights were collected. At the end of the last 23-hour exposure period, the respiratory tracts from all of the animals were prepared for histopathologic examination for O_3-induced lesions.

Table 1. Number of Rats per Ozone Study Exposure Group

Exposure Group	Females	Males
Room control	5	5
Filtered O_3 atmosphere	8	8
O_3 atmosphere	8	8
Total number of rats	21	21

Protocol: Pilot Study

The objective of the Pilot Study was to transport the exposure and monitoring system to Mexico City and to validate efficacy of the experimental model at INP during exposure to filtered or unfiltered MCA atmospheres. This objective included operating the system with a full load of rats (Table 2), evaluating lungs and nasal tissue for O_3-induced lesions, and responding to recommendations from an on-site study review.

The efficacy of the experimental model depended on demonstrating that control animals (namely, rats exposed to filtered MCA) could be successfully isolated from the local environment, thus addressing issues of potential confounding variables such as effects of local altitude, water supply, and noise pollution.

We used four groups of animals in the Pilot Study: a health screen group, a room control group, a filtered MCA group, and an unfiltered MCA group. Additional reserve animals were included in case casualties occurred during transit. The room controls were used as an indicator of whether any viral or bacterial infections in animals housed elsewhere in INP had broken through the procedural and physical barriers established for the conduct of this study. The rats in the latter two groups were exposed to the target exposure atmosphere for approximately 23 hr/day for

Table 2. Number of Rats per Pilot Study Exposure Group

Exposure Group		Females	Males
Health screen (sent directly to CIIT)		5	5
Room control (at INP)	(7 days)	9	9
	(21 days)	9	9
Filtered MCA (at INP)	(7 days)	9	9
	(21 days)	9	9
	(Reserve)[a]	6	6
Unfiltered MCA (at INP)	(7 days)	9	9
	(21 days)	9	9
	(Reserve)[a]	6	6
Total number of rats		71	71

[a] Reserve animals were treated the same as exposed animals in each chamber.

Table 3. Number of Rats per Main Study Exposure Group

Group	Completion Time Point	Histopathology/ Morphometry		Molecular Pathology		Reserve		Total Animal
		Males	Females	Males	Females	Males	Females	
Health screen[a]	Week 0	5	5	—	—-	—	—	10
Room control[b]	Week 3	9	9	—	—	—	—	18
	Week 7	9	9	—	—	2	2	22
Filtered MCA	Week 3	9	9	—	—	—	—	18
	Week 5	9	9	4	4	—	—	26
	Week 7	9	9	4	4	4	4	34
Unfiltered MCA	Week 3	9	9	—	—	—	—	18
	Week 5	9	9	4	4	—	—	26
	Week 7	9	9	4	4	2	2	30
2-Week recovery[c]	Week 7	9	9	—	—	2	2	22
Total animals per completion time point		86	86	16	16	10	10	224
Total animals in study		172		32		20		

[a] Conducted at INP.

[b] Animals exposed to 5 weeks of MCA followed by 2 weeks of filtered MCA.

[c] Conducted at CIIT.

either 7 or 21 consecutive days. At the end of 7 or 21 days, a group of animals was killed and their respiratory tracts prepared for histopathologic examination for evidence of air pollutant–induced lesions.

Protocol: Main Study

The objective of the Main Study was to expose animals to unfiltered and filtered MCA for at least 7 weeks and, if possible, for up to 13 weeks. This would test the hypothesis that rats would develop nasal lesions similar to those reported in humans exposed to MCA.

We used five groups of animals in the Main Study: a health screen group, a room control group, a filtered MCA group, an unfiltered MCA group, and a 2-week recovery group (Table 3). Reserve animals were included in all groups sent to Mexico City in case of casualties during transit. The rats were exposed to the target exposure atmosphere for approximately 23 hr/day for either 21, 35, or 49 consecutive days. At the end of the last exposure day for each time period, a group of animals (Tables 3 and 4) was killed and their respiratory tracts prepared for histopathologic examination for evidence of air pollutant–induced lesions. Four additional male and female rats per 5- and 7-week completion time points were included in the filtered and unfiltered MCA groups; these animals enabled us to establish archival storage of nasal and lung tissue for possible future evaluation with molecular pathology techniques. The final decision concerning allocation of the reserve animals in the Pilot and Main Studies was made by

Table 4. Number of Rats Removed from Exposure Chamber at Each Time Point of Main Study

Exposure Group	Weeks of Exposure			
	0	3	5	7
Room control		18		22
Filtered MCA		18	26	34
Unfiltered MCA		18	26	30
2-Week recovery				22
Health screen	10			
Total number of animals coming off study at each time point	10	54	52	108
Total number of animals in study				224

the principal investigator during the necropsy procedure conducted on-site in Mexico City.

ANIMALS AND ANIMAL CARE

All F344 rats (CDF[F-344]/CrIBR) used in this study were purchased from Charles River Breeding Laboratories, Raleigh NC. Eight-week-old male and female rats were purchased for the Prepilot Study. Seven-week-old male and female rats were purchased for the Ozone Study. Nine-week-old male and female rats were purchased for the Pilot and Main Studies.

For the Prepilot Study, rats at INP and CIIT were group housed in shoebox cages with cellulose bedding and fed ad libitum an NIH-07 diet (Zeigler Bros, Gardener PA) with filtered water (at CIIT) or distilled water (in Mexico). For the Ozone Study, rats during acclimation were housed singly in polycarbonate shoebox cages with cellulose bedding (ALPHA-dri, Sheperd Specialty Papers, Kalamazoo MI). During exposure periods, rats were individually housed in stainless-steel suspended cages contained in glass and stainless-steel inhalation chambers. The rats were fed ad libitum an NIH-07 diet with deionized, filtered water. Lighting was on a 12-hour light, 12-hour dark cycle with the dark cycle at night.

In the Pilot Study, all animals assigned to exposure groups were initially housed together for several days in the filtered MCA chamber for the conduct of a prestudy heat-loading test. The animal load anticipated during the next phase of the project (the Main Study) was duplicated. The prestudy heat-loading test was conducted to assure that the environmental parameters in the exposure room would allow for the delivery of adequately cooled and dehumidified air to the filtered MCA chamber. During the heat-loading test, room and chamber parameters were monitored and adjusted as needed to balance airflow, temperature, and relative humidity.

For the Pilot and Main Studies, the male and female rats were shipped directly to Mexico City and housed in the INP laboratory of Dr Lilian Calderón-Garcidueñas, under local accreditation and laboratory animal care guidelines. For the Main Study, five additional male and female rats were shipped directly to CIIT for an immediate health screen. In both studies, rats were blindly eartagged by the supplier, and they were assigned to treatment groups as they were taken out of the shipping crates in Mexico City. Throughout these studies, the room controls were housed five per cage on direct bedding in polycarbonate shoebox cages. The cages were in the same room as the exposure chambers. The animals to be exposed to filtered or unfiltered MCA were individually housed in stainless-steel suspended cages contained in glass and stainless-steel inhalation chambers. All rats were provided ad libitum with Purina Rodent Laboratory Chow-(5001, Purina Mills, St Louis, MO) and distilled water. Rats in the exposure chambers were provided water via an automatic watering system. Lighting was on a 12-hour light, 12-hour dark cycle, with the dark cycle from 9:00 AM to 9:00 PM, in contrast to the dark only cycles used in the ozone study.

Weekly and terminal body weights and daily cage-side observations were made. Fresh diet was provided daily. At scheduled time points, rats were killed by pentobarbital overdose followed by exsanguination.

In-Life Parasitology

The presence of *Syphacia obvelata,* or pinworm, was estimated by scanning tape lifted from the perianal area of the rodent for the presence of eggs or migrating adult worms. Adult *S. obvelata* or *Aspicularis tetraptera* worm presence was estimated from the contents of a cecal wash. Fecal flotation was used to check for ova of *Syphacia*. In order to check for the ectoparasites *Myocoptes*, *Myobia,* and *Polyplax*, the cooling pelage of the rat was observed following necropsy for mite migration. Due to site constraints, parasitology data were not collected in Mexico City.

Serology

Blood samples for serology were collected by heart puncture, allowed to coagulate, and centrifuged for three to five minutes. Serum was decanted, frozen, and shipped to CIIT. Serum samples from the CIIT rats, as well as the single sample from Mexico City, were heat inactivated and sent to Microbiological Associates (Rockville MD) for analysis. The Mexico City sample was a pooled sample from all of the rats in the health screen group.

INHALATION

Chamber System

Animals exposed to control air (filtered MCA) or unfiltered MCA were permanently housed in two Hazelton 1-m^3 rodent inhalation exposure chambers (H-1000 chambers, Lab Products, Maywood NJ), which had glass, silicon, and stainless-steel components. A pump (Fluid

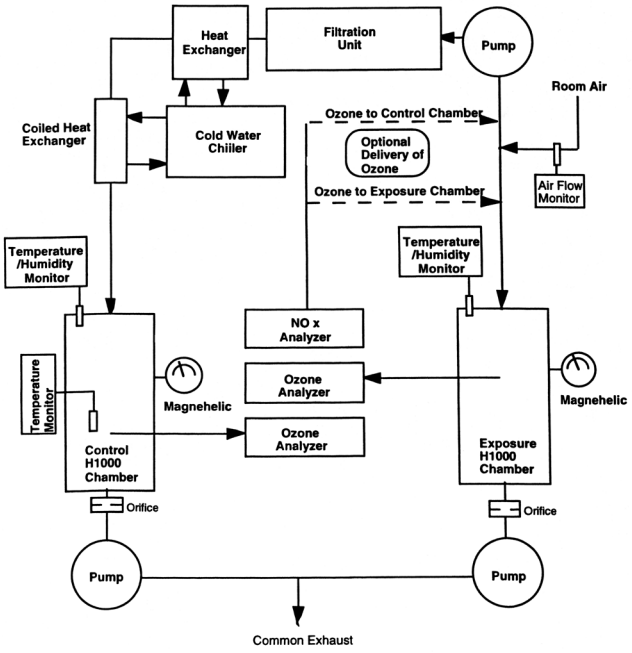

Figure 1. Exposure system for ozone study.

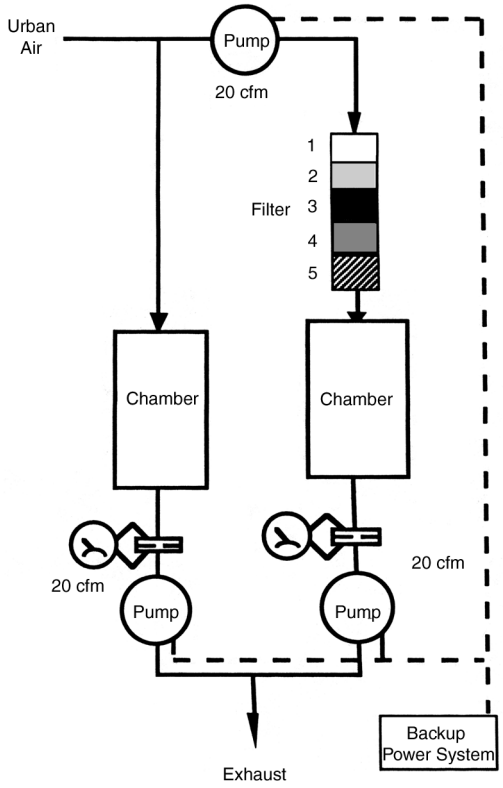

Figure 2. Schematic of basic exposure system. The filter system consists of Stage 1 (a glass-filter prefilter), Stage 2 (an aluminum oxide and potassium permanganate bed), Stage 3 (a Carulite 200 bed for O_3), Stage 4 (a Carulite 300 bed for CO), and Stage 5 (a HEPA filter). Abbreviation: cfm = cubic feet per minute.

Energy, Charlotte NC, for the Ozone Study; model VB-001, Spencer Turbine Co, Windsor CT, for the Pilot and Main Studies) pulled air at a rate up to 500 L/min (approximately 30 air changes/hr) through each exposure chamber. Flow through each chamber was restricted by the 2.71 cm (1.068 inch) orifice for the Ozone Study (Figure 1) and by the chamber exhaust fitting (4.32 cm [1.70 inch] inside diameter) for the Pilot and Main Studies (Figure 2).

The major concern for the O_3 generation system and the MCA generation system was the reactivity of O_3. The first precautionary measure was to construct the system from materials that would not tend to act as a chemical sink for O_3 and other chemically reactive air pollutants. All system components were constructed from stainless steel, silicon, Teflon, and chlorinated polyvinyl chloride (CPVC). The airflow was increased up to 500 L/min, or approximately 30 air changes/hr, in order to reduce the impact of chemicals reacting with animal fur or chamber components.

Filtration System

A commercially available filtration unit (Riley Equipment Company, Houston TX) was used to clean the air entering one of the two exposure chambers (that of the filtered MCA groups of the Pilot and Main Studies). The system consisted of five filters sealed in a stainless-steel housing (Figure 2) as follows:

- pleated microglass panel (8 × 8 × 2 inches) with 98% efficiency for removal of particles 5 µm or larger in aerodynamic diameter;
- media cell (8 × 8 × 3 inches) filled with aluminum oxide and potassium permanganate to filter hydrogen sulfide, sulfur dioxide, sulfur compounds, mercaptans, CH_2O, ethylene, and other similar corrosive gases;
- media cell (8 × 8 × 3 inches) filled with Carulite 200 catalyst for 99+% removal of O_3;
- media cell (8 × 8 × 3 inches) filled with Carulite 300 catalyst and heated to 450°F for 99% efficiency removal of carbon monoxide(CO); and
- high-efficiency particulate air (HEPA) microglass fiber filter (8 × 8 × 3 inches) for 99.97% efficiency removal of total suspended particulates (TSP).

The filtration system was designed to run at 0.57 m^3/min (20 cubic feet per minute [cfm]) and to be effective for greater than six months of continuous operation in filtering air bearing concentrations twice those of the then-current air quality standards. (Standard concentrations at the time were 0.12 ppm O_3, 0.13 ppm sulfur dioxide (SO_2), 3.0 mg/m^3 CH_2O, 13 ppm CO, 1.0 mg/m^3 sulfuric acid, and 0.275 mg/m^3 TSP mass.)

The filter assembly heated the air to approximately 232°C (450°F). Two heat exchangers were placed in line to bring air temperature within the limits of the 1985 National Institutes of Health (NIH) guidelines for animal housing, 26°C (78.8°F). Chilled water was supplied to the two heat exchangers through a water recirculator (model 12000, SPI Steadi Cool, Structure Probe Supplies, West Chester PA). The first heat exchanger was a 30 cm × 30 cm square radiator that was placed in line within one meter of the outlet of the filter assembly. A second heat exchange coil was placed inside the air delivery line approximately three meters downstream of the first heat exchanger. The second heat exchange coil consisted of a 0.64-cm diameter line coiled inside approximately one meter of the 5-cm diameter air delivery line. This combination of heat exchangers reduced the temperature in the filtered-MCA exposure chamber to the same temperature found in the MCA exposure chamber. After the heat from the filter system was dissipated, the temperature in the chambers was maintained in normal fashion by controlling room temperature.

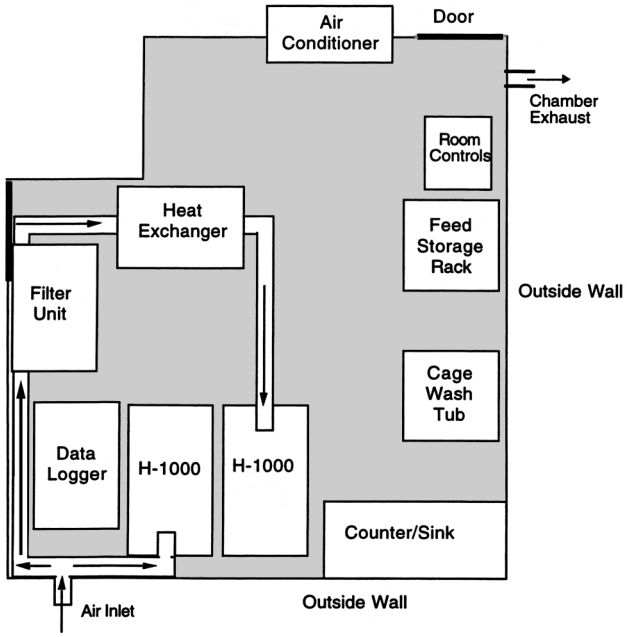

Figure 3. Layout of the exposure system at Instituto Nacional de Pediatria, Mexico City, Mexico.

Ozone Generation

For the Ozone Study, O_3 was generated using the exhaust of a chemiluminescence analyzer (model 42C, TEI, Franklin MA) for oxides of nitrogen (NO_x). The NO_x analyzer's sample port was plugged in order to generate concentrations of O_3 sufficiently high to elicit nasal pathology in rats (approximately 1.0 ppm in 500 L/min of total airflow). An O_3 flow of approximately 0.1 L/min from the generator was diluted to target concentration by counter-current injection into the air inlet line at a point approximately one meter upstream from the 1-m^3 exposure chamber inlet. The NO_x/ozone generator lasted six days before the calcium sulfate used in the generation of O_3 expired. Because of this limitation in the O_3 generator, the two six-day exposures were scheduled in series.

Some of the pollutants expected to be present in MCA were O_3, NO_x, TSP, CH_2O, SO_2, hydrogen sulfide (H_2S), and CO. The urban air was pulled from the outdoor environment through a 2-inch CPVC pipe located at the third-floor level of INP at a point approximately 40 cm perpendicular to the outside wall (Figures 2 and 3). This sampling location was approximately 1.7 miles (2.7 km) from the Pedregal air monitoring station. This station is operated by the Mexican government and is located in the southwest region of Mexico City, which is downwind from major diurnal emissions in metropolitan Mexico City.

The extreme outermost end of the pipe at INP was turned down (facing the ground) to prevent entry of unwanted foreign substances such as rainwater or bird droppings. A 1 mm × 1 mm stainless-steel screen was sealed into the end of the pipe to prevent entry of unwanted foreign substances such as insects or flying plant or animal debris.

The air was pulled into the pipe to a common "T" point (Figures 2 and 3). Half of the airflow was diverted directly into the unfiltered MCA exposure chamber. The other half passed through the filter assembly and heat exchangers prior to entering the filtered MCA exposure chamber. Air from both exposure chambers was exhausted outdoors through a vent located near the corner of the room diagonally opposite from the inlet.

The filtration system in the Main Study was modified from its previous configuration in the Ozone and Pilot Studies (Figure 2) by the addition of a charcoal filter prior to the five-stage filter.

Exposures

For the Prepilot Study, animals were maintained in either CIIT or INP laboratories for four weeks. No effort was made to expose the animals to any specific atmosphere. For the Ozone Study, a Study Day was defined as a 24-hour period from 9:00 AM until 9:00 AM the following day; animal care procedures occurred around 8:30 AM each day. The Study Days were numbered consecutively, with O_3 atmosphere exposures occurring on Study Days 1 to 6 and filtered O_3 atmosphere exposures occurring on Study Days 8 to 13.

The Pilot Study exposures were originally scheduled to begin in November 1996. The direct approach of shipping equipment, supplies, and animals to Mexico City became bogged down because of misconstrued arrangements related to importing such equipment for temporary use in Mexico. The help of Dr German de la Garza Estrada (Bioterio Mexico SA de CV, Mexico DF, Mexico) was key in meeting the import requirements. The Pilot Study was conducted from February 20, 1997, to March 15, 1997. Study Days were consecutively numbered as Exposure Days 1 to 22 with February 22, 1997, as Day 1.

Exposures for the Main Study were originally scheduled to begin in April 1997. Even with the previous experience of conducting the Pilot Study, there were significant difficulties in shipping animals and needed chemicals to Mexico. In addition, our temporary permit for use of the exposure system within Mexico had inadvertently been scheduled to end before the completion date of the Main Study exposures. Again, it was the assistance of Dr German de la Garza Estrada that enabled us to conduct even a truncated version of the Main Study. The Main Study was conducted from May 3, 1997, to June 21, 1997, and the equipment was allowed to remain in Mexico without penalty one month longer than originally approved. The Study Days were consecutively numbered as Exposure Days 1 to 50 with Exposure Day 1 on May 3, 1997.

A Study Day for exposures in the Pilot and Main Studies was defined as a 23-hour exposure, generally from midnight until midnight the following day; animal care procedures required approximately 1 hour between 6 and 9 AM each day.

Monitoring System

Environmental Parameter Sampling Locations For the Prepilot Study, no specific environmental samples were collected. For the Ozone Study, the sampling point for O_3 was the center of the chamber. For the Pilot Study, the sampling point for all components of the exposure atmosphere was the chamber inlet. For the Main Study, the sampling point for O_3, nitric oxide (NO), nitrogen dioxide (NO_2), and NO_x was the center of the chamber. The sampling points for CH_2O, SO_2, TSP mass, particulate matter (PM) smaller than 10 µm (PM_{10}), and PM smaller than 2.5 µm ($PM_{2.5}$) were located in the exposure chamber inlet line (Figure 4). All sample lines were made from ¼-inch Teflon or stainless steel.

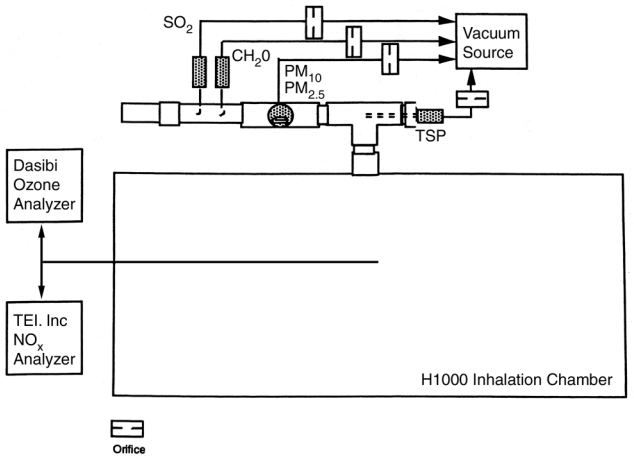

Figure 4. Sampling points in the exposure system. Sampling points are shown for ozone (O_3), total oxides of nitrogen (NO_x), formaldehyde (CH_2O), sulfur dioxide (SO_2), total suspended particulates (TSP), particulate matter (PM) smaller than 10 µm (PM_{10}) and smaller than 2.5 µm ($PM_{2.5}$).

Temperature and relative humidity in both control and exposure chambers were monitored continuously with a combination temperature-relative humidity probe positioned through a port located in the back door of each chamber in order to be near the animals. (The probes used were model HX11, OMEGA Engineering, Stamford CT, for the Ozone Study and model HT205, Rotronics Instrument Corp, Huntington NY, for the Pilot and Main Studies.)

Total airflow through each exposure chamber was monitored in the 2-inch inlet pipe of the chamber by a hot-wire anemometer (Kurz Instruments, Monterey CA, for the Ozone Study; Sierra Instrument, Monterey CA, for the Pilot and Main Studies).

Data Logger In the Ozone Study, the temperature and relative humidity probes were connected to an exposure control system (Infinity building automation control system, Andover Controls, Andover MA), and an average was calculated once per hour for each 24-hour environmental monitoring period.

In the Pilot and Main Studies, a data logger (model 8800, Environmental Systems Corporation, Knoxville TN) was used to record voltages or currents from the monitoring

equipment. The data logger was verified and used as a microprocessor-based data accumulation system capable of acquiring, processing, storing, and outputting electronic information. The two O_3 analyzers, NO_X analyzer, and temperature and humidity probes sent electronic information to the data logger for the duration of the studies. The information stored in the data logger was downloaded on each business workday from Mexico City via a modem to CIIT.

Ozone The O_3 exposure atmospheres were measured with two calibrated O_3 analyzers (model 1008-AH, Dasibi Instruments, Carlsbad CA). These analyzers were calibrated against each other and also against an audited O_3 slide calibrator at the EPA Health Effects Research Laboratory (Research Triangle Park NC). The two O_3 analyzers agreed to within 1.4% of each other and within 3.5% of the calibration unit.

Each O_3 analyzer acquired samples at a flow rate of approximately 2.0 L/min. Voltage from the O_3 analyzers that corresponded to the exposure concentration was continuously transmitted to a data logger. An average O_3 concentration was calculated and recorded once per hour for a 23-hour period (Ozone and Pilot Studies) or 21-hour period (Main Study). Each O_3 analyzer was zeroed daily on charcoal-filtered room air.

The reactivity of O_3 with the stainless-steel and glass exposure system and its components was examined by comparing the concentration of O_3 in the exposure chamber with the concentration of O_3 in the exhaust air that had passed through the chamber.

Total Oxides of Nitrogen The NO_X concentration was monitored by an NO_x analyzer (model 42C, TIE, Franklin MA). The analyzer acquired samples at a flow rate of approximately 0.5 L/min. Voltage from the NO_x analyzer that corresponded to the exposure concentration was continuously transmitted to a data logger. An average NO_x concentration was calculated and recorded once per hour for a 23-hour period (Pilot Study) or for a 21-hour period (Main Study). The Teflon solenoid valve was used to switch sampling every 60 minutes from one exposure chamber to the other. The analyzer was zeroed on zero air and calibrated with NO prior to study start.

Formaldehyde A portion of the exposure atmosphere was pulled through a glass tube containing a dinitrophenylhydrazine-impregnated (DNPH-impregnated) substrate that trapped aldehydes. Samples were collected at a flow rate of approximately 0.20 L/min for a 23-hour period for the Pilot Study, whereas samples were collected at a flow rate of 0.25 L/min for a 23-hour period for the Main Study. The glass sample tubes were sealed with Teflon tape and refrigerated immediately after the end of each collection period. The samples were sent to Daniel Grosjean Associates (Ventura CA), where the aldehydes were extracted and analyzed by DNPH chemical assay to determine the concentrations of C^1 to C^{14} carbonyls present over 1 day or 3 days (Grosjean et al 1996).

Sulfur Dioxide Filter samples of SO_2 were collected on 47-mm cellulose filters impregnated with potassium carbonate. Samples were collected at a flow rate of approximately 0.5 L/min for either a 23-hour or a 47-hour period. The filters were removed from the filter holders immediately following the end of sampling and kept in a refrigerator until returned to the Desert Research Institute (DRI) (Reno NV) for analysis with use of ion chromatography (EPA 1994).

Total Suspended Particulates For the Pilot and Main Studies, filter samples for TSP mass were collected over 23-hour periods onto 47-mm Teflon filters at a flow rate of approximately 0.5 L/min. Samples were collected approximately every other day in each chamber. The filters were removed from the filter holders immediately following the end of sampling and kept in a refrigerator until returned to DRI for gravimetric analysis.

PM_{10} and $PM_{2.5}$ Samples from the inlet of each chamber were collected through a single-stage cascade impactor. PM_{10} or $PM_{2.5}$ were collected on 37-mm Teflon filters at a flow rate of approximately 2.0 L/min for a 47-hour period. The sample device (model 200 Personal Environmental Monitor, MSP Corp, Minneapolis MN) had impaction stages that served as size cutoffs for particles entering the sampling device. The filters were removed from the filter holders immediately following the end of sampling, sealed, and kept in a refrigerator until returned to DRI for gravimetric analysis.

Other Equipment A power surge protector and an uninterrupted power supply (Fortress, Best Power Technology, Necedah WI) provided transient voltage surge suppression circuitry to prevent data loss due to power surges, brownouts, or outages.

PATHOLOGY

Pathology: Histopathology

The entire respiratory tract and any gross lesions that were noted were included in necropsy. Except as indicated for the Main Study, the nasal airways were flushed and the trachea and lungs instilled using a syringe filled with fixative (10% neutral buffered formalin [NBF]). Tissue samples for histopathology were fixed in 10% NBF for at least one week. The noses were then decalcified in 5% to 10% formic acid, and all tissues were gross trimmed and processed to paraffin. A single section of left lung was prepared; six levels of the nasal passages, selected to include the maxilloturbinate (Méry et al 1994), were prepared as a standard set. All sections were cut at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) (Luna 1968). All tissue sections were examined by bright-field light microscopy.

Tissues from the Prepilot Study were examined by the study pathologist. The slides were not blinded for examination. For the Ozone Study, the severity of nasal lesions in the maxilloturbinates was assessed subjectively in female rats using a simple scoring system (zero = no lesions; one = minimal lesion; two = mild lesion; three = severe lesion; four = very severe lesion). Nasal tissues from males, being similar in this respect to those of females, were observed but the lesions were not scored. In the Pilot Study, H&E-stained tissues from the upper and lower respiratory tract included nose, larynx, trachea, and lung of male and female rats in each exposure group.

During the Main Study, a complete (full screen) necropsy was performed by the study pathologist; a few additional nonrespiratory tract tissues (that is, liver, spleen, testes, kidney, and any tissues with observed gross lesions) were removed from the carcass and immersed and stored in NBF. Immediately after exsanguination the trachea was cannulated, the thorax opened, and the block of trachea-heart-lung was removed from the carcass. The lung lobes were fixed via tracheal infusion of 10% NBF at 25-cm fixative pressure for at least 2 hours. After inflation fixation, the trachea was ligated, and the tissue block was stored in a large volume of the same fixative until further tissue processing. Sagittal tissue blocks from the distal trachea and carina, right cranial and caudal lung lobes, and transverse tissue blocks (cross sections) from the right middle and accessory lung lobes and larynx were prepared. These tissues were microscopically examined and subjectively graded as minimal, mild, moderate or severe, based on the severity and distribution of the specific anatomic alteration (for example, minimal nasal inflammation).

Morphometry

Tissue Preparation The lung lobes of rats designated for morphometric analysis were intratracheally fixed with a buffered 2% glutaraldehyde fixative. The maxilloturbinates from the nasal airways fixed in 2% glutaraldehyde were microdissected from the head, decalcified in 10% ethylene diaminetetracetate (EDTA), embedded in eponaraldite, cut to 1- to 2-µm thickness, and stained with toluidine blue. All of the nasal tissues for morphometric evaluation were selected from a transverse section through the nose immediately posterior to the incisor teeth as previously described (Harkema et al 1997a,b).

Numeric Cell Density The nasal transitional epithelium (NTE) overlying the maxilloturbinates in the proximal nasal airway of exposed rats was morphometrically examined using light microscopy and image analysis. The numbers of total epithelial cells, mucous cells, and inflammatory cells (that is, neutrophils and mononuclear leukocytes) in the NTE were determined by methods previously described in detail (Harkema et al 1997a,b).

The numeric cell densities of total nasal epithelial cells, mucous cells stained with Alcian Blue (pH 2.5)/Periodic Acid Schiff (AB/PAS), and intraepithelial inflammatory cells were determined by counting the number of nuclear profiles of these individual cell types in the surface epithelium covering the maxilloturbinates and then dividing by the length of basal lamina underlying the surface epithelium. The length of the basal lamina was calculated from the contour length of the digitized image of the basal lamina on a Power Macintosh 7100/66 computer using a public domain NIH IMAGE image analysis program. The mean number of cells/mm basal lamina ± the standard error of the mean (SEM) was reported. Cells in the NTE were identified by distinct morphologic criteria for each cell type.

Mucosubstance Volume Density Sections of eponaraldite–embedded tissue from the maxilloturbinates were also stained with AB/PAS for the detection of acidic and neutral mucosubstances stored in the NTE cells.

In order to estimate the amount of intraepithelial mucosubstances in the surface epithelium lining the maxilloturbinate, the volume density of AB/PAS-stained mucosubstances within the airway epithelium was quantified using the computerized image analysis system and standard morphometric techniques that have been previously described (Harkema et al 1997a,b). The areas of the AB/PAS-stained, intraepithelial mucosubstances were calculated by the image analysis software program, with use of either the manually or automatically circumscribed perimeter of the

stained material. The method of Harkema and colleagues (1987a) was used to estimate the volume of stored mucosubstance per unit surface area of epithelial basal lamina. Each datum was expressed as the mean volume density (Vs; nL/mm^2 basal lamina) of AB/PAS-positive mucosubstances within the epithelium ± SEM.

Statistical Procedures

The values measured for the continuously and semicontinuously monitored parameters (namely, O_3 concentration, NO concentration, NO_2 concentration, NO_x concentration, chamber airflow, chamber temperature, and chamber relative humidity) were averaged and recorded on an hourly basis. In the Pilot and Main Studies, a daily geometric mean and geometric standard deviation (GSD) was calculated for each 21-hour to 23-hour monitoring period.

Differences in effects between experimental groups were evaluated using a two-sample *t* test statistical analysis; analyses were performed using a commercial statistical analysis package (JMP, SAS Institute, Cary NC, Version 3.2.2, or SigmaStat, Jandel Scientific Software, San Rafael CA). All tests were two-tailed and had a significance level of 0.05.

Morphometric data obtained from the NTE of rats killed immediately following the last day of exposure were evaluated for potential effects of gender, exposure, and exposure atmosphere on stored intraepithelial mucosubstances, epithelial cell density, mucous secretory cell density, and inflammatory cell density. A separate three-way analysis of variance test was used for each of the experimental end points.

RESULTS

IN-LIFE

Prepilot Study

For the Prepilot Study, clinical samples collected at CIIT indicated no evidence of endoparasites or ectoparasites either immediately upon arrival or after the animals were held for four weeks. The serology testing was negative. Rats delivered to CIIT or Mexico City and killed immediately exhibited no clinical evidence of any disease process. In addition, rats exposed to the Mexico City laboratory environment or the CIIT animal facility for four weeks exhibited no clinical evidence of disease.

Table 5. Rat Weights During O_3 Exposures

		Body Weight (g)[a]		
Exposure Group	*N*	Day 3	Day 7	Day 14
Female				
Room control	5	152 ± 15	155 ± 14	162 ± 15[b]
Filtered O_3	8	NA	159 ± 6	162 ± 5[b]
O_3	8	149 ± 5	154 ± 3[b]	NA
Male				
Room control	5	232 ± 14	242 ± 15	257 ± 18[b]
Filtered O_3	8	NA	246 ± 15	259 ± 15[b]
O_3	8	226 ± 12	235 ± 12[b]	NA

[a] Presented as mean ± SD.

[b] Terminal body weight data.

Ozone Study

There were no significant body weight changes among the three groups of animals in the Ozone Study (Table 5).

Pilot Study

In the Pilot Study, animals were shipped to CIIT or INP on February 17, 1997. The animals arriving at CIIT on February 17, 1997, were in apparent good health. Animal health necropsies were conducted on these animals. The serology and parasitic exams were negative. Tissues were examined microscopically and no abnormalities were observed. The animals shipped to Mexico City cleared customs and arrived at INP in the evening of February 20, 1997. The animals were dehydrated, active, and very aggressive. One crate was broken, and two animals were dead.

During the 24 days of the Pilot Study, there was no significant differences in body weight among the groups of animals at INP (Table 6). Daily cage-side observations were unremarkable.

Main Study

In the Main Study, animals were shipped to CIIT or INP on April 28, 1997. Animals arrived at CIIT on April 28, 1997. These animals appeared to be in good health. Animal health necropsies were conducted; the serology and parasitic exams were negative. Tissues were examined microscopically and no abnormalities were observed. Animals shipped to Mexico cleared customs and arrived at INP on May 2, 1997. The rats were dehydrated, active, and very aggressive. During the 49 days of the Main Study, body weights did not differ significantly among the groups

Table 6. Rat Weights During Pilot Study

Exposure Group	N[b]	Body Weight (g)[a]			
		Day 1	Day 6	Day 12	Day 22
Female					
Room control	18, 18, 8, 9	124 ± 5	132 ± 10	150 ± 7	143 ± 7
Filtered MCA	24, 24, 13, 15	111 ± 12	124 ± 9	136 ± 10	150 ± 9
Unfiltered MCA	20, 21, 11, 18	123 ± 8	132 ± 9	147 ± 10	148 ± 7
Male					
Room control	18, 18, 9, 9	153 ± 7	164 ± 8	171 ± 7	223 ± 8
Filtered MCA	24, 24, 15, 13	148 ± 11	163 ± 12	173 ± 8	227 ± 11
Unfiltered MCA	23, 23, 18, 11	158 ± 4	173 ± 8	181 ± 4	233 ± 14

[a] Presented as mean ± SD.

[b] N is the number of animals weighed at each time point (study days 1, 6, 12, and 22, respectively).

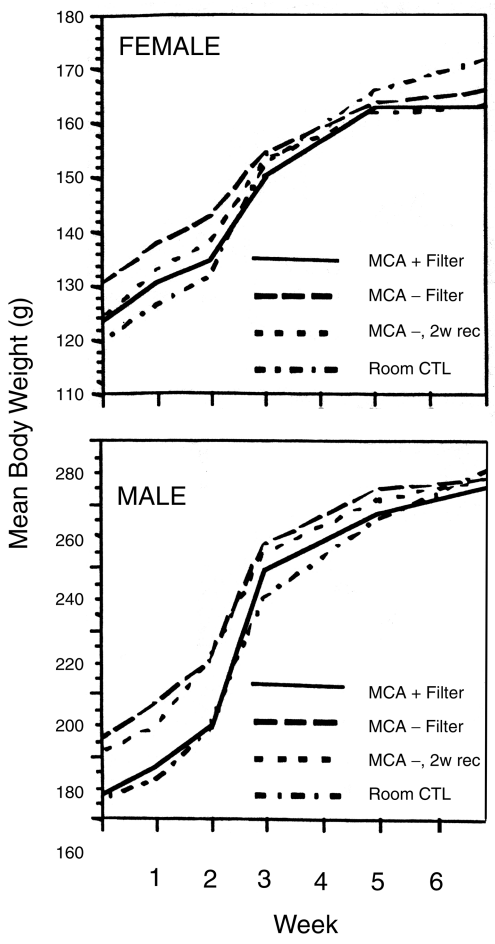

Figure 5. Rat body weights during the Main Study. Weights were collected during the 7 weeks of the study for the room control, filtered MCA, unfiltered MCA, and 2-week recovery groups of rats.

of animals at INP (Figure 5). Daily cage-side observations were unremarkable.

INHALATION

Prepilot Study

No experimental characterization of the INP atmosphere was conducted in conjunction with the Prepilot Study.

Ozone Study

For the Ozone Study, the overall mean concentration of O_3 (± standard deviation [SD]) in the O_3 atmosphere was 0.74 ± 0.05 ppm. The overall mean concentration of O_3 in the filtered O_3 atmosphere was 0.11 ± 0.01 ppm. These levels were maintained 23 hours per day for 6 consecutive days.

Approximately 41% of the O_3 reaching each chamber was absorbed onto, or inhaled by, the rats.

The environmental parameters of temperature and relative humidity in the exposure chambers were maintained at or near the target set points (between 18°C [64°F] and 26°C [79°F] and between 30% and 70% relative humidity) throughout the entire study. Average temperatures for the two chambers (for the filtered O_3 and O_3 groups) were identical: 25 ± 0.6°C (77 ± 1°F). Average relative humidity values for the filtered O_3 group and the O_3 group were 46% ± 1% and 51% ± 1%, respectively.

Pilot Study

Prior to the start of the Pilot Study, we observed 1-hour average O_3 concentrations as high as 0.617 ppm in the

Table 7. Concentrations of Air Pollutants Measured in Filtered MCA and Unfiltered MCA Exposure Chambers During Pilot Study

		Filtered MCA Chamber				Unfiltered MCA Chamber			
Pollutant[a]	Sample Period (hr)	*N*	Geometric Mean Concentration	GSD	Maximum Daily Mean Concentration	*N*	Geometric Mean Concentration	GSD	Maximum Daily Mean Concentration
O_3	8	20	0.021 ppm	1.37	0.035 ppm	20	0.073 ppm	1.90	0.130 ppm
O_3	23	20	0.010 ppm	1.45	0.015 ppm	20	0.021 ppm	1.38	0.032 ppm
NO	21	20	0.000 ppm	3.96	0.009 ppm	0	—	—	—
NO_2	21	20	0.004 ppm	1.31	0.010 ppm	0	—	—	—
NO_x	21	20	0.000 ppm	5.11	0.014 ppm	0	—	—	—
CH_2O	23	2	3.3 ppb	1.43[b]	3.9 ppb	2	3.3 ppb	1.95[b]	4.6 ppb
SO_2	23	2	0.007 mg/m^3	1.50[b]	0.009 mg/m^3	2	0.032 mg/m^3	1.56[b]	0.039 mg/m^3
TSP	23	9	0.002 mg/m^3	15.12	0.046 mg/m^3	9	0.068 mg/m^3	1.33	0.106 mg/m^3
PM_{10}	23	1	0.004 mg/m^3	N/A	0.004 mg/m^3	1	0.035 mg/m^3	N/A	0.035 mg/m^3

[a] The parameters sampled in the exposure atmospheres were ozone (O_3), total oxides of nitrogen (NO_x), formaldehyde (CH_2O), sulfur dioxide (SO_2), particulate matter smaller than 10 microns (PM_{10}) and TSP. Abbreviations: GSD = geometric standard deviaton; — = no data available; N/A = not applicable.

empty exposure chambers. (The concentration of 0.617 ppm was observed from 2 PM to 3 PM on February 4, 1997.) Over the same 9-day preexposure monitoring period, the O_3 concentration in the outdoor environment around the INP site appeared to be representative, but not tightly coupled, with the outdoor environment around the Pedregal monitoring station. For example on February 3, 1997, from 10 AM to 6 PM, the average O_3 concentration of the eight hourly measurements was 0.179 ppm in the exposure chamber and 0.077 ppm at the Pedregal monitoring station. The respective hourly average readings were 0.122, 0.163, 0.243, 0.166, 0.185, 0.225, 0.202, and 0.124 ppm in the exposure chamber and 0.022, 0.053, 0.072, 0.098, 0.089, 0.078, 0.100, and 0.103 ppm at the Pedregal monitoring station. The O_3 concentrations for the same eight-hour periods in the exposure chamber remained higher than the O_3 concentrations measured at the Pedregal site for the next five days before they reversed in relation to each other. On February 9, 1997, from 10 AM to 6 PM the average O_3 concentration of the eight hourly measurements was 0.019 ppm in the exposure chamber and 0.097 ppm at the Pedregal monitoring station. The respective hourly average readings were 0.014, 0.017, 0.021, 0.023, 0.021, 0.021, 0.020, and 0.017 ppm in the exposure chamber and 0.027, 0.052, 0.101, 0.110, 0.114, 0.116, 0.133, and 0.119 ppm at the Pedregal monitoring station.

On the last day of exposures (Exposure Day 21), the concentration of O_3 in the inlet of the MCA exposure chamber was compared with the concentration of O_3 in the exhaust line after all animals were removed from the chamber. During that day, the average concentration of O_3 in the inlet of the unfiltered MCA chamber was 0.031 ppm O_3 compared with 0.015 ppm O_3 in the exhaust, a 52% loss of O_3. These losses were approximately 10% higher than those determined during similar comparisons made during the Ozone Study.

The geometric mean concentration (and GSD) of all daily 8-hour periods of highest O_3 level in the filtered MCA and unfiltered MCA groups were 0.021 (1.37) and 0.073 (1.90) ppm O_3, respectively (Table 7). The grand mean (and GSD) for the daily 23-hour O_3 monitoring periods for the filtered MCA and unfiltered MCA groups were 0.010 (1.45) and 0.021 (1.38) ppm O_3, respectively. There were 46 one-hour intervals during which the average O_3 concentration in the unfiltered MCA chamber exceeded the NAAQS standard of 0.120 ppm O_3.

Because of failure in the switching valve after the start of exposures, only the filtered MCA exposure chamber was monitored for NO_x. On only three days, March 12, 14, and 15, 1997, during the course of these three exposures, did the daily average concentration of NO_x in the filtered MCA chamber exceed 0.001 ppm. The maximum observed daily mean concentration was 0.013 ppm NO_x.

The geometric mean (and GSD) of the nine filter TSP samples taken from the filtered MCA or the unfiltered MCA exposure chamber was 0.002 (15.1) mg/m^3 and 0.068 (1.33) mg/m^3, respectively. In the filtered MCA chamber, the maximum concentration measured over a

Table 8. Concentrations of Air Pollutants Measured in Filtered and Unfiltered MCA Exposure Chambers During Main Study

		Filtered Mexico City Air				Unfiltered Mexico City Air			
Pollutant	Sample Period (hr)	*N*	Geometric Mean Concentration	GSD	Maximum Daily Mean Concentration	*N*	Geometric Mean Concentration	GSD	Maximum Daily Mean Concentration
O_3	8	49	0.002 ppm	1.92	0.009 ppm	50	0.018 ppm	2.77	0.150 ppm
O_3	21	49	0.001 ppm	2.77	0.009 ppm	49	0.004 ppm	4.95	0.038 ppm
NO	21	23	0.001 ppm	11.5	0.051 ppm	43	0.005 ppm	3.47	0.047 ppm
NO_2	21	23	0.004 ppm	1.82	0.015 ppm	43	0.001 ppm	15.8	0.015 ppm
NO_x	21	23	0.009 ppm	2.84	0.054 ppm	43	0.010 ppm	2.95	0.045 ppm
CH_2O	69	16	8.0 ppb	1.31	12.1 ppb	16	3.3 ppb	1.50	5.3 ppb
SO_2	47	24	0.002 mg/m^3	1.31	0.003 mg/m^3	24	0.020 mg/m^3	1.39	0.042 mg/m^3
TSP	47	25	0.008 mg/m^3	2.64	0.032 mg/m^3	25	0.068 mg/m^3	1.48	0.164 mg/m^3
PM_{10}	47	12	0.006 mg/m^3	1.76	0.019 mg/m^3	12	0.032 mg/m^3	3.39	0.058 mg/m^3
$PM_{2.5}$	47	12	0.002 mg/m^3	8.51	0.015 mg/m^3	12	0.016 mg/m^3	5.92	0.047 mg/m^3

23-hour period was 0.046 mg/m^3. This represented one of only three of nine samples that exceeded the mean concentration. For the unfiltered MCA exposure chamber, the maximum concentration measured was 0.106 mg/m^3. The PM_{10} concentrations, based on one sample from each chamber, were 0.004 and 0.035 mg/m^3, respectively. The geometric means of two SO_2 filter samples taken over a 23-hour period from the filtered or unfiltered MCA exposure chamber were 0.007 mg/m^3 and 0.032 mg/m^3, respectively. The geometric means of the two CH_2O samples collected over a 23-hour period from the filtered or unfiltered MCA exposure chambers were the same: 3.3 ppb.

Chamber airflows (± SD) were maintained at 518 ± 19 and 501 ± 11 L/min for the filtered MCA and unfiltered MCA exposure chambers, respectively. Over the course of the exposures, temperature was 24.4 ± 1.1°C (76 ± 2°F) and 24.4 ± 1.1°C (76 ± 2°F) and relative humidity was 33% ± 5% and 32% ± 7% for the filtered MCA and unfiltered MCA exposure groups, respectively. When the filtered MCA chamber, which was downstream from the heated filter, was fully loaded with animals (the expectation for the Main Study), the environmental parameters (23.9 ± 1.1°C [75 ± 2°F], 27% ± 3% relative humidity) were within acceptable levels (NIH 1985).

Main Study

The major concern for the generation system was the reactivity of O_3. All system components were constructed from stainless steel, glass, silicon, Teflon, or CPVC. In addition, we increased the total airflow through both exposure chambers to approximately 500 L/min/chamber, or 30 air changes/hr, to reduce the likelihood of chemicals reacting with either the animals or the chamber. The reactivity of O_3 with animal fur and with the glass and stainless-steel exposure system and components was examined by comparing the concentrations of O_3 in different locations and under different exposure scenarios. There was a 23% to 31% loss of O_3 going from the outdoor environment to the center of the unfiltered MCA exposure chamber (loaded with rats and caging); there was a 5% loss of O_3 going from the outdoor environment to the center of the exposure chamber (with no rats or caging). This appeared to indicate that the animals and caging were an O_3 sink for approximately 25% of the incoming O_3.

In spite of the improvement over the performance of the exposure system during the Pilot Study, the concentrations of air pollutants in the exposure chamber were lower than anticipated. The filter for the filtered MCA chamber appeared to be effective in removing the air pollutants that were monitored. The addition of the charcoal filter prior to the main filter reduced the concentrations of the pollutants in the filtered MCA chamber compared with concentrations in the Pilot Study. However, in the Main Study there were fewer one-hour periods during which the mean concentration of O_3 exceeded 0.120 ppm than in the Pilot Study: 18 times versus 46 times.

The geometric mean concentration (with GSD) of all of the mean concentrations for the daily period of highest O_3 levels (10 AM to 6 PM) in the filtered MCA and unfiltered MCA groups were 0.002 ppm (1.92) and 0.018 ppm (2.77), respectively (Table 8). There were 18 one-hour intervals

during which the average O_3 concentration of MCA in the unfiltered MCA chamber exceeded 0.120 ppm O_3. The geometric mean concentration (with GSD) of all of the mean concentrations for the daily 21 one-hour periods of highest NO_x levels in the filtered MCA and unfiltered MCA groups were 0.009 ppm (2.84) and 0.010 ppm (2.95), respectively.

In the control and exposure air, 21 C^1 to C^{14} carbonyls were detected: formaldehyde, acetaldehyde, propanal, butanal, acetone, 2-butanone, benzaldehyde, glyoxal, methylglyoxal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, tridecanal, tetradecanal, acrolein, biacetyl, o-tolualdehyde, and (m+p)-tolualdehyde. The concentration of formaldehyde was consistently higher than the others and showed the greatest difference between exposure chambers. When CH_2O samples were pooled into three-day blocks, each representing a total of 69 hours of sampling, the filtered MCA chamber and unfiltered MCA chamber concentrations were 8.0 ppb (1.31) and 3.3 ppb (1.50), respectively. By comparison, in the Pilot Study there was no difference between the CH_2O concentrations in the two chambers (3.3 ppb versus 3.28 ppb). The only difference made in the filtered chamber side of the exposure system between the time of the Pilot and Main Studies was the instillation of a charcoal filter assembly. The increase in the trace amounts of CH_2O in the filtered MCA chamber was probably due to glue used in this assembly.

The geometric mean concentrations of SO_2 for the 48 sampling periods (each 47 hours long) in the filtered MCA and unfiltered MCA chambers were 0.002 mg/m^3 (1.31) and 0.020 mg/m^3 (1.39), respectively. The geometric mean concentration of TSP for the 25 sampling periods (each 46 hours long) was 0.008 mg/m^3 (2.64) for the filtered MCA chamber and 0.068 mg/m^3 (1.48) for the unfiltered MCA chamber. Likewise, the respective geometric mean concentrations of PM_{10} from 12 such sampling periods were 0.006 mg/m^3 (1.76) and 0.32 mg/m^3 (3.39). For 12 similarly obtained samples of $PM_{2.5}$ the respective concentrations were 0.002 mg/m^3 (8.51) and 0.016 mg/m^3 (5.92).

The environmental parameters—temperature, relative humidity, and airflow—for the Main Study were maintained at or near the target set points throughout the entire study. The exposures were performed May 3, 1997, through June 21, 1997. Chamber airflows (average ± SD) were maintained at 457 ± 6 and 457 ± 33 L/min for the filtered MCA and unfiltered MCA groups, respectively. Over the course of the exposures, temperature was held at 24.4 ± 1.1°C (76 ± 2°F) to 26.7 ± 1.1°C (80 ± 2°F); relative humidity was maintained at 46% ± 4% and 48% ± 5% for the filtered MCA and unfiltered MCA groups, respectively. During this same time period, the temperature and relative humidity in the laboratory containing the exposure system and the room controls was 22 ± 2°C (71.6 ± 3.6°F) and 39% ± 4% relative humidity.

PATHOLOGY

Prepilot Study

Rats maintained for four weeks in the INP laboratory of Dr Calderón-Garcidueñas in Mexico City exhibited characteristic nasal lesions, including edema of the lamina propria in the anterior nose with associated hemorrhage and local inflammatory response (Figure 6). These lesions are not consistent with any infectious disease process or expected environmental exposure with which the investigators are familiar, nor do they appear to resemble O_3-induced lesions seen by the investigators in experimental material. However, these lesions may have been induced by a volatile organic chemical generated by workers in the adjacent laboratory. This is based upon the airflow-driven pattern of lesion location and the similarity of these lesions to those induced by low concentrations (2 to 10 ppm) of chloroform (Larson et al 1994). By the time of the Main Study, the work under suspicion had moved from the adjacent laboratory. Rats delivered to CIIT remained in good health with no evidence of nasal disease or any infectious disease process.

Ozone Study

Both male and female rats in the O_3 atmosphere group showed mucous metaplasia of the transitional epithelium of the anterior lateral meatus and medial maxilloturbinate. The severity of the nasal lesions in the female rats in the O_3 atmosphere chamber was subjectively assessed to be minimal to mild (Table 9). No such lesions were detected in the filtered O_3 atmosphere group or in the room control group. Only the 16 O_3-exposed animals (8 males, 8 females) were affected, indicating that the filter assembly was working to filter out O_3.

Table 9. Incidence and Mean Severity Scores for Nasal Lesions Among Exposure Groups in Ozone Study

	Females	
Exposure Group	Mean Severity Score[a]	Incidence/ (Affected Total)
Room control	0.0 ± 0.0	0/5
Filtered O_3 atmosphere	0.0 ± 0.0	0/8
O_3 atmosphere	1.9 ± 0.6	8/8

[a] Mean ± SD.

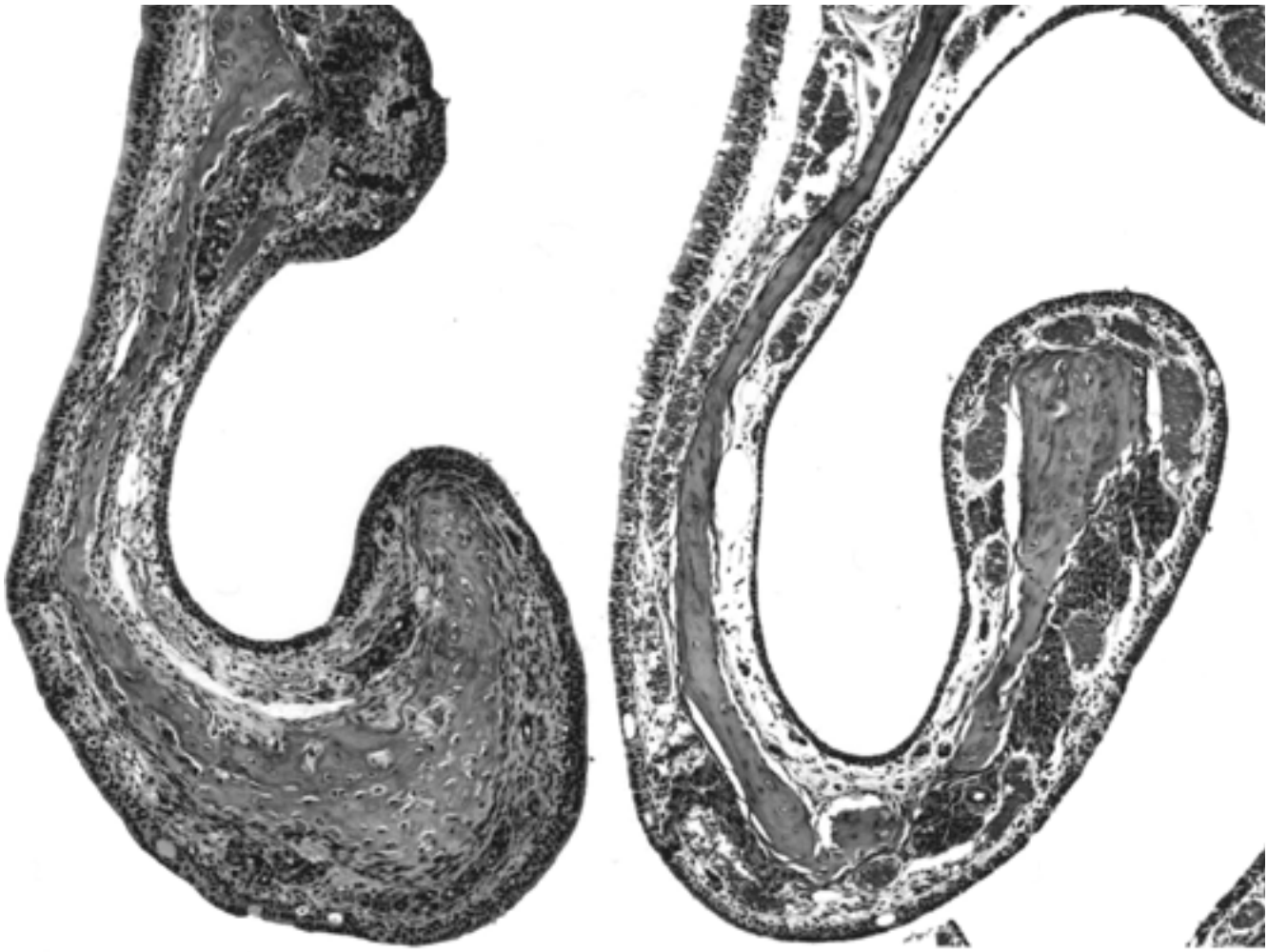

Figure 6. Photomicrographs of nasal turbinates taken from rats in the prepilot study. The control micrograph on the left is from the anterior nasoturbinate of a rat held for four weeks in the CIIT animal care facility. Note the compact nature of the lamina propria. The micrograph on the right is from the anterior nasoturbinate of a rat held for 4 weeks in the INP facility. Note the edematous swelling of the lamina propria.

Pilot Study

There were no histologic findings to suggest that the health-screen animals at CIIT or the animals held at INP had any infectious or noninfectious respiratory diseases related to their housing or maintenance conditions during the course of the Pilot Study. There were no underlying nonexposure-related lesions (or "background" changes) in the nasal, tracheal, or pulmonary airways that interfered with the microscopic interpretation of the response of these animals to the various exposure regimes. Using H&E-based light microscopy analysis, there were no exposure-related histologic lesions in the nose, larynx, trachea, or lung that were induced in the rat by exposure to unfiltered MCA.

Nasal sections showed unremarkable findings in all three study groups (room control, filtered MCA, and unfiltered MCA) across different study time periods and in both sexes. Nonolfactory nasal epithelium composed of goblet cells, ciliated cells, nonciliated columnar cells, cuboidal cells, brush cells, and basal cells revealed no abnormalities. Both squamous and mucous metaplasia were absent. There was no evidence of inflammatory infiltrates, ciliary abnormalities, or alterations in the olfactory epithelium.

Tracheal sections in animals from all three study groups showed patchy areas of basal cell hyperplasia with occasional polymorphonuclear leukocytes (PMNs) infiltrating the epithelial layer. In some areas there was focal absence of cilia and occasional globular eosinophilic neutrophils. A blind reading of the nasal slides for basal cell hyperplasia, deciliated areas, PMNs, and globular eosinophilic neutrophils revealed no statistical differences among different groups or time points.

Lung sections were characterized by the presence of scattered macrophages in alveolar spaces. An occasional

binucleated alveolar macrophage (AM) was seen. No changes were present in terminal bronchioles or acini. A blind reading of the lung slides revealed no differences in the numbers of AMs for the two exposure groups (filtered MCA and unfiltered MCA), nor was there a difference between groups in the amount of collagenous material around the small bronchioles and alveolar septae. Esophageal sections, salivary glands, lymph nodes, and fatty tissue showed no abnormalities.

Main Study

The random histologic alterations observed in only a few rats in each group (including the room control group) were similar in morphologic character and minimal in severity. Consequently, these minor changes were not interpreted as adverse effects and were not found to be dependent on exposure.

No significant adverse gross or microscopic lesions were found in the respiratory tract of male or female rats. There were no differences among the three groups of rats (room control, filtered MCA, and unfiltered MCA) or among time points (exposures for three, five, or seven weeks, or five weeks of exposure to unfiltered MCA plus 2-week recovery with filtered MCA). By routine light microscopy analysis, there were no exposure-related lesions in the respiratory tract.

Morphometry: Main Study

Neither exposure duration nor exposure atmosphere affected observed cell numeric density in the NTE. The numeric density values of all male rats and all female rats were grouped separately because gender was the only significant factor identified as contributing to group mean variances of cell density in the maxilloturbinates.

In the unfiltered MCA, filtered MCA, and 2-week recovery groups, the NTE consisted predominantly of luminal nonsecretory, cuboidal-columnar cells and nonluminal basal cells. Mucous cells and ciliated cells were less frequently observed in this nasal epithelium. Mucous cells had morphologic characteristics including a high cuboidal to columnar profile, AB/PAS-stained secretory granules, and a basal nucleus that distinguished these cells from other epithelial cell types. Inflammatory cells within the NTE were rare and consisted predominantly of neutrophils. Mononuclear leukocytes (for example, monocytes or lymphocytes) were even less frequently observed in the NTE. Neutrophils were small ovoid-to-round cells with a highly segmented nucleus and clear cytoplasm containing dust-like granules. Though similar in relative size and shape, mononuclear leukocytes in the NTE had round-to-ovoid hyperchromatic nuclei with moderate-to-scant amounts of cytoplasm.

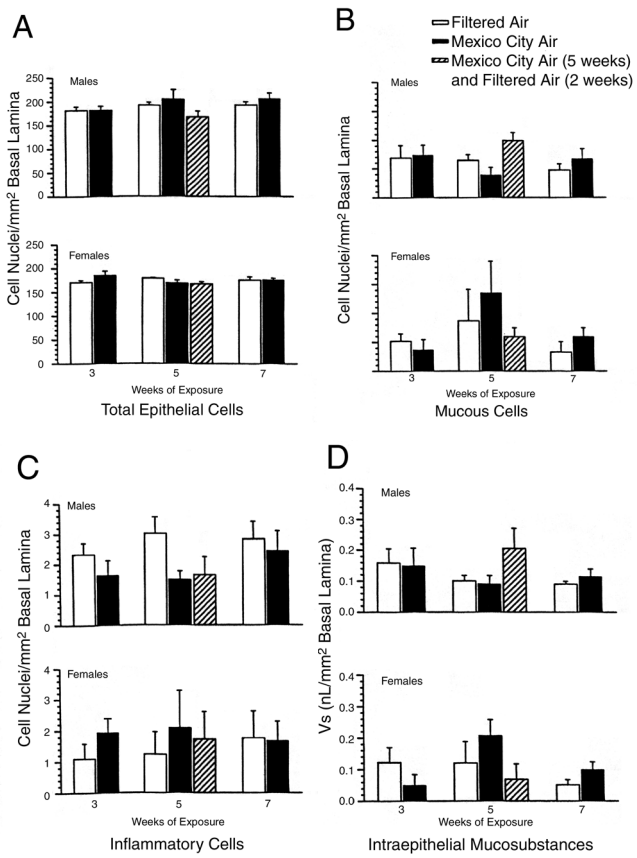

Figure 7. Numeric cell density and volume density of intraepithelial mucosubstances in NTE of rats in the Main Study. The graphs in A–D represent morphometry data regarding total epithelial cells, mucous cells, inflammatory cells, and intraepithelial mucosubstances in the male and female rats of the three exposed groups (filtered MCA, unfiltered MCA, 2-week recovery). Sample sizes were 4 males and 4 females per filtered MCA and unfiltered MCA group.

There were no statistical differences in the numeric cell densities of total epithelial cells, mucous cells, or inflammatory cells in the NTE of male or female rats among the exposure groups (filtered MCA, unfiltered MCA, 2-week recovery) (Figure 7). Similarly, there were no gender-related differences in the number of mucous cells or intraepithelial inflammatory cells in the NTE (Table 10). In general, rats contained approximately four mucous cells and two inflammatory cells per millimeter of basal lamina regardless of their exposure or their gender. There was, however, a significant gender-related difference in the numeric cell densities of total epithelial cells in the NTE. Male rats had a greater density of total epithelial cells compared with the density in female rats. There were approximately 8.5% more cells/mm

Table 10. Abundance of Cells and Mucosubstances in Nasal Transitional Epithelium of F344 Rats in the Main Study[a]

Epithelial Component	Male Rats[a]	Female Rats[a]
Total Epithelial Cells (nuclei/mm^2 basal lamina)	192.7 ± 4.5	175.7 ± 2.8[b]
Mucous Cells (nuclei/mm^2 basal lamina)	3.6 ± 0.4	4.0 ± 0.9
Inflammatory Cells (nuclei/mm^2 basal lamina)	2.3 ± 0.2	1.6 ± 0.3
Intraepithelial Mucosubstances (nL/mm^2 basal lamina)	0.12 ± 0.01	0.11 ± 0.02

[a] Data are expressed as mean ± SEM.

[b] Significantly different than value for male rats, $P \leq 0.05$.

basal lamina in the NTE of male rats compared with the number of cells in the NTE in female rats (see Table 10).

No exposure-related differences in the amount of AB/PAS-stained mucosubstances in the NTE were found in this study (Figure 7). In addition, there were no gender-related differences in stored mucosubstances. Both male and female rats had approximately 0.1 nL/mm^2 basal lamina of stored mucosubstances in the NTE (Table 10).

DISCUSSION AND CONCLUSIONS

The long-term objective of this work was to clarify the relevance of rodent data from nasal and lung lesions for use in human risk assessment. Our focus was on improving the interpretation of responses in the nasal passages of rats exposed to inhaled chemicals, including components of vehicle emissions present as air pollutants. Our intent was to select an approach that would allow for the following:

- development of a mobile system for exposure of rodents to outdoor environments;
- application of this mobile exposure system to the study of the effect of the Mexico City urban environment on rats; and
- direct comparison of responses in humans and rats similarly exposed to the Mexico City urban environment and determination of the air pollutants most likely to be responsible for upper respiratory tract diseases seen in humans exposed to Mexico City air.

The husbandry procedures used in this study worked well to keep the rats healthy in the INP laboratory environment. The work in the Ozone Study demonstrated the efficacy and limits of the exposure system. The inlet air filtration unit reduced O_3 concentration in the filtered O_3 atmosphere chamber to 15% of the concentration in the O_3 atmosphere chamber. This level of efficiency was due to a portion of the air being able to bypass the central heated region as well as the activated pellet regions of the filter assembly. This condition was not readily modified in the commercial unit. However, the resulting difference in exposure levels was sufficient to allow a clear distinction to be made between the slight nasal lesions in the O_3 atmosphere group and the absence of nasal lesions in the filtered O_3 and room control groups. The observed lesion of mucous metaplasia of the transition epithelium of the anterior lateral meatus and medial maxilloturbinate is characteristic of O_3 exposure (Hotchkiss et al 1989). On the basis of these observations it was decided to ship the exposure system to Mexico.

The objective of the Pilot Study was to validate the efficacy of the experimental model on-site in Mexico City. This latter objective included operating the exposure system with a full load of rats for three weeks and evaluating lung and nasal tissue for lesions. The two-chamber exposure system was successfully transported, set up, and operated in Mexico City. This work demonstrated that control animals (that is, rats exposed to filtered MCA) could successfully be isolated from the local environment at the INP during the conduct of exposures to filtered and unfiltered MCA. However, by H&E-based light microscopy analysis, there were no significant differences in nasal or lung tissue between the two exposure groups. Based on a review of these findings, the exposure system, and the expected environmental conditions, we decided to proceed with the Main Study. There appeared to be a reasonable possibility of seeing significant differences between groups following longer exposure times even though the concentrations in the exposure chamber were lower than anticipated during this Pilot Study.

The atmosphere around INP appeared to be a unique outdoor environment; it did not track identically on an hourly basis with the environment at the Pedregal monitoring station operated by the Mexican government. This difference was probably due to source locations as well as air and traffic movements around the buildings at INP compared with those at the Pedregal monitoring station. However, because the two sites were within 2.7 km of each other it could be expected that on a monthly or longer basis both sets of measurements would be representative

of regional O_3 concentrations. At both sites, the O_3 concentrations were high during the day and low at night.

During the seven-week period of the Main Study, the addition of a charcoal filter assembly prior to the main filter reduced pollutant concentrations in the filtered chamber compared with concentrations in the Pilot Study data with the exception of CH_2O. The increase in the trace amounts of CH_2O in the filtered chamber during the Main Study was probably due to off-gassing from components of this additional assembly.

Routine histologic examination of nasal and lung tissues from the Main Study indicated two important points. First, there were no histologic findings that suggested that the laboratory rats had any infectious or noninfectious respiratory diseases related to their housing or maintenance. Therefore, there were no underlying nonexposure-related lesions (or "background" changes) in the nasal, tracheal, or pulmonary airways that interfered with microscopic interpretation of animal response to the various exposure regimes.

Secondly, no exposure-related histologic lesions in the nose, larynx, trachea, or lungs were induced by filtered or unfiltered MCA after three, five, or seven weeks of exposure.

The results of the quantitative analyses of NTE substantiated the initial subjective findings. There were no exposure-related differences in any of the examined parameters of the NTE. Repeated exposures to MCA did not cause changes in the number of intraepithelial inflammatory cells, numbers of epithelial cells, or increases in mucous cells in the NTE even after seven weeks of daily exposure.

These additional, more sensitive and quantitative (that is, morphometric) analyses of the NTE were conducted to confirm and extend the findings from the initial qualitative histopathologic examination. These state-of-the-art morphometric techniques have been routinely used to assess quantitatively the severity and distribution of epithelial alterations in the nasal airways of O_3-exposed F344 rats (Hotchkiss et al 1991; Harkema et al 1997a,b). Importantly, each previous study has demonstrated that these morphometric methods are able to detect concentration-dependent differences in O_3-induced nasal lesions in F344 rats.

There are several plausible explanations for the observed lack of response of NTE in rats from this study. A principal oxidant pollutant in the photochemical smog of Mexico City is O_3. The daily O_3 concentrations in the unfiltered MCA exposures in this study were considerably lower than the O_3 concentrations that caused NTE lesions in previous controlled inhalation studies using the single oxidant pollutant, O_3. During the seven weeks of the Main Study there were only 18 one-hour periods during which the O_3 concentration exceeded 0.120 ppm; this is compared with 46 such one-hour periods for the three-week-long Pilot Study.

The surface epithelium lining the proximal nasal airways of the rat has been shown in previous inhalation studies of air pollutants (for example, O_3 and aldehydes) to be particularly sensitive to toxicant-induced injury (Dungworth 1989). The principal nasal lesions in laboratory rats exposed to O_3 are located in the NTE lining the proximal nasal airway (Harkema et al 1997a). These O_3-induced alterations to the NTE include the following: epithelial hyperplasia (that is, increase in the number of airway-lining cells), mucous cell metaplasia (that is, change in nasal epithelium from epithelium devoid of mucus-secreting cells to an epithelium with numerous mucous cells containing copious amounts of mucosubstances), and rhinitis (that is, influx of inflammatory cells into the exposed nasal mucosa). Harkema and colleagues (1997b) have demonstrated that exposure of F344 rats to 0.12 ppm O_3 for as long as 20 months does not cause NTE alterations. Rats in the same study that were exposed to 0.5 or 1.0 ppm O_3 for 20 months did have marked exposure-related cellular changes in NTE.

Nonhuman primates (that is, macaque monkeys) exposed to O_3 concentrations as low as 0.15 ppm O_3 (8 hr/day for 6 or 90 days) did have marked alterations in the NTE and respiratory epithelium lining the proximal nasal passages (Harkema et al 1987a,b). Hyde and colleagues (1994) have suggested, based on morphometric data from these and other reported studies of monkeys and rats, that nonhuman primates are approximately 10 times more sensitive to O_3-induced injury than are rodents. The reasons for these species differences in response to O_3 exposure are unknown. Because the nasal and pulmonary airways of monkeys are more similar in structure to the airways of humans than are those of rats (Harkema 1991; DeSesso 1993), it is possible that humans, like monkeys, are more susceptible to O_3 toxicity than are laboratory rats.

Interestingly, nasal epithelial lesions thought to be related to exposure to air pollution have been described in people living in the O_3-polluted atmosphere of southwestern Mexico City (Calderón-Garcidueñas et al 1992, 1994, 1995, 1996, 1997, 1999; Calderón-Garcidueñas and Ocotla 1993). Therefore, even though the F344 rat did not have nasal epithelial injury after unfiltered MCA exposure, this does not exclude the possibility that exposure to unfiltered MCA would have induced nasal alterations in nonhuman primates or another more sensitive mammalian species. In fact, Calderón-Garcidueñas and her colleagues have reported in several recent studies that both adults

and children living in the southwestern communities of Mexico City have conspicuous nasal mucosal lesions (that is, inflammatory, epithelial, or vascular). These lesions appear to be associated with the ambient air pollution in MCA (Calderón-Garcidueñas et al 1992, 1994, 1995, 1996, 1997, 1999; Calderón-Garcidueñas and Ocotla 1993). These findings, along with those in the present study, suggest that human nasal airways may be considerably more susceptible to the toxic effects of unfiltered MCA than are the nasal airways of F344 rats. However, this suggestion is still speculative because of obvious differences in experimental design, exposure regimes, living environments, nutrition, and other factors that make it difficult to directly compare the responses of these two species. More specifically designed studies are needed to determine similarities and differences in the toxic effects of urban air pollutants among mammalian species and to understand the underlying mechanisms of toxicity in these species. Such research is important to use data from animal studies more accurately to estimate the human risk from inhaled urban air pollutants.

The air in Mexico City is a chemical mixture composed of several potentially toxic chemical agents including O_3. The absence of nasal lesions in the exposed rats in this study also suggests that chemical components in this urban air other than O_3 (for example, aldehydes or PM) were not toxic to the respiratory tract of the exposed F344 rats at the measured ambient concentrations. Like the concentrations of O_3 measured in this study, the ambient concentrations of aldehydes, PM, and other potentially toxic agents in unfiltered MCA were considerably lower than the concentrations shown to induce respiratory tract injury in laboratory rodents after controlled inhalation exposures. Although it is often hypothesized that chemical mixtures will induce greater toxic responses than any single component of the mixture, it is also plausible that the mixture could be less toxic, due to chemical interactions, than its individual constituents. Therefore, it would be inappropriate to conclude that any chemical constituent of MCA alone would not have had an adverse effect on rodent airways.

IMPLICATIONS OF FINDINGS

The long-term objective of this work was to clarify the relevance of rodent nasal and lung lesion data for use in human risk assessment. Our focus was on improving the interpretation of responses in the nasal passages of rats exposed to inhaled chemicals, including components of vehicle emissions, that are part of air pollution.

We have demonstrated that repeated exposure of healthy F344 rats to unfiltered MCA containing episodically low to moderate concentrations of O_3 (and other urban air pollutants) did not induce inflammatory or epithelial lesions in the nasal airways or lungs. These findings are in agreement with previous O_3 inhalation studies demonstrating dose-dependent induction of rhinitis and nasal epithelial alterations in F344 rats. However, the lack of responses of F344 rats to unfiltered MCA is in contrast to previous reports that indicate that O_3-polluted MCA does cause significant nasal mucosal injury in adults and children living in southwestern Mexico City. Taken together, these findings may suggest that human airways are markedly more susceptible to the toxic effects of MCA than are the airways of the F344 rat. Other studies, however, are needed to test this hypothesis.

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ABOUT THE AUTHORS

Owen R Moss is head of the CIIT Inhalation Facility and holds a PhD from the University of Rochester in toxicology with special focus on aerosol physics. He has many years of experience in developing and troubleshooting rodent exposure systems, and he has published extensively in the field of inhalation toxicology. His research interests focus on the true relations among inhalation exposure, inhalation dosimetry, and biologic response.

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Paul W Ross is a laboratory animal facility manager with a BS from the University of Florida and certification as a laboratory animal technologist from the American Association for Laboratory Animal Science. He currently manages the animal care facility at CIIT. His research interests include rodent breeding and production, xenobiotics, and animal response to exposure conditions independent of the chemical challenge.

Kay C Roberts is a lead inhalation technical specialist with an AS from Sandhills Community College and training in automation controls, on-chemical detection, and whole-body inhalation exposure technology. Her research contributions include the design, creation, operation, and reporting of inhalation toxicology exposure protocols specific to whole-body and nose-only exposures.

Andrew M Howard is a senior maintenance/electrical controls technician with training in electrical and electronic controls and telecommunications. His research contributions include the creation and validation of the electrical-mechanical components of complex exposure systems.

Jack R Harkema is a Professor of Pathology at the College of Veterinary Medicine at Michigan State University. He received his PhD in comparative pathology from the University of California, Davis, and is a diplomate of the American College of Veterinary Pathologists. He is internationally known for his work on the adverse effects of ozone and other inhaled toxicants on the airway epithelium of laboratory animals. Dr Harkema's primary research interests are in the understanding of the cellular and molecular mechanisms responsible for the pathogenesis of airway epithelial injury, adaptation, and repair after acute or chronic toxicant exposure. Currently, his laboratory is investigating the roles of inflammatory cells and their mediators in the pathogenesis of airway epithelial alterations (epithelial cell death, epithelial hyperplasia, and mucous cell metaplasia) in the upper and lower respiratory tract after exposure to various inhaled xenobiotic agents (airborne PM, ozone, and bacterial endotoxin).

Lilian Calderón-Garcidueñas is an MD and medical pathologist with extensive experience in human neurology, including pediatric neuropathology. Dr Calderón-Garcidueñas has obtained research experience on mechanisms of respiratory tract carcinogenesis at University of Toronto and Harvard University and the Toxicology Program at the University of North Carolina. Her recent studies of human nasal pathology have provided important new information on the effects of pollutants.

Kevin T Morgan is an experimental pathologist who received his PhD in veterinary pathology from the University of Edinburgh, Scotland. He is a diplomate of the American College of Veterinary Pathologists and a member of the Royal College of Pathologists. Dr Morgan has published more than 100 peer-reviewed articles, book chapters, and reviews on the nose of the rat and rhesus monkey, including numerous articles on responses to inhaled and parenterally administered xenobiotics. This work has included a detailed description of normal nasal mucociliary function in the nose of the rat and responses of this mucociliary system to inhaled gases such as ozone. Dr Morgan and collaborators also generated the first report of nasal airflow patterns in the nose of the rat and rhesus monkey. This work led to the development of numerical procedures for the prediction of mass flux patterns for inhaled gases. Other publications include multiple articles on the application of nasal histochemistry and biochemistry to toxicology and on the development of mapping strategies for nasal toxicology.

ABBREVIATIONS AND OTHER TERMS

AB/PAS	Alcian Blue/Periodic Acid Schiff staining
AM	alveolar macrophage
cfm	cubic feet per minute
CH_2O	formaldehyde
CIIT	Chemical Industry Institute of Toxicology
CO	carbon monoxide
CPVC	chlorinated polyvinyl chloride
DNPH	dinitrophenylhydrazine
EDTA	ethylene diaminetetracetate
GSD	geometric standard deviation
H&E	hematoxylin and eosin
HEPA	high-efficiency particulate air (filter)
INP	Instituto Nacional de Pediatria
MCA	Mexico City air
NAAQS	US National Ambient Air Quality Standard
NBF	neutral buffered formalin
NIH	National Institutes of Health
NO	nitric oxide
NO_2	nitrogen dioxide
NO_x	total oxides of nitrogen
NTE	nasal transitional epithelium
O_3	ozone
PM	particulate matter
PM_{10}	PM smaller than 10 μm in aerodynamic diameter
$PM_{2.5}$	PM smaller than 2.5 μm in aerodynamic diameter
PMN	polymorphonuclear leukocyte
SO_2	sulfur dioxide
TSP	total suspended PM
EPA	US Environmental Protection Agency

CRITIQUE
Health Review Committee

INTRODUCTION

Mexico City is located in a valley, more than 2,000 meters above sea level, and is surrounded by mountains that form a closed basin. The combination of year-round sunshine, 4 million cars, urban leakage of liquified petroleum gas, and industrial activity are ideal for formation of oxidant chemicals and other toxic air pollutants. Wind currents from the industrial northern and central areas of the city transport pollutants to its southwestern part. Thus, residents of this section of the city are exposed to a complex mixture of air pollutants, including ozone, formaldehyde, acetaldehyde, particulate matter, polynuclear aromatic hydrocarbons, and other hydrocarbons such as propane, isobutane, and *n*-butane (described by Calderón-Garcidueñas et al 1998).

The study described in this report originated from a series of reports from Calderón-Garcidueñas and coworkers demonstrating that the air in southwestern Mexico City is toxic to human nasal tissue. They reported that nasal tissue from area residents showed signs of inflammation (Calderón-Garcidueñas et al 1995), pathologic mucosal cellular changes (Calderón-Garcidueñas et al 1992, 1994, 1998), and changes in DNA (Calderón-Garcidueñas et al 1996, 1997). These findings suggested that Mexico City air provided a natural inhalation atmosphere for testing animal models that might serve as sentinels for early detection of harmful effects of air pollutants in humans.

In 1994, Dr Kevin Morgan, then of the Chemical Industry Institute of Toxicology (CIIT)*, planned a collaboration with Dr Calderón-Garcidueñas of the Instituto Nacional de Pediatria (INP) in Mexico City. Through its Requests for Preliminary Applications process, HEI supports studies in areas that are compatible with its overall research program but lie outside the areas defined by its focused Requests for Applications. After evaluating Morgan's preliminary application, entitled "Studies of Respiratory Tract Toxicity in Rats Exposed to Mexico City Urban Air," the HEI Research Committee requested and approved a full application. The Committee thought that Morgan described an innovative approach that would help assess the usefulness of the rat as a model for human respiratory disease and that the collaboration with Calderón-Garcidueñas could yield important results. The study, which was designed for a two-year period, began in October 1995.† In March 1997, Morgan left CIIT and Dr Owen Moss (a researcher at CIIT with expertise in exposure design and monitoring) became the Principal Investigator. Dr Jack Harkema of Michigan State University replaced Morgan as the study pathologist. Harkema proposed to extend the analyses of nasal tissue to include morphometric measurements. Unforseen delays in performing the exposures in Mexico City and the additional analyses extended the study period to two and a half years.

OBJECTIVE

The major objective was to determine whether rats exposed to air in the southwestern part of Mexico City developed nasal lesions similar to those identified in residents of this area (Calderón-Garcidueñas et al 1992, 1994, 1995, 1996, 1997, 1998). Thus, the study would help assess the value of the rat as a model for human nasal disease.

STUDY DESIGN AND RESULTS

A strength of this study was the careful, step-by-step approach the investigators used to validate their final results.

First, the investigators determined whether rats housed in the animal quarters at the INP developed nasal infections that might interfere with the subsequent identification of lesions produced by exposure to outdoor Mexico City air. Male F344 rats obtained in the United States were housed at the INP or at CIIT in Research Triangle Park, North Carolina. Comparison of serum and nasal tissue from both groups of rats indicated no evidence of infectious disease or pollutant-induced nasal lesions in animals at either facility. Thus, the investigators concluded that the animals could be maintained in good health at the INP.

Second, Moss and coworkers at CIIT constructed mobile exposure chambers designed to expose rats to either ambient southwestern Mexico City air or to air from which

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

† Dr Owen R Moss's 3-year study, *Studies of Respiratory Tract Toxicity in Rats Exposed to Mexico City Urban Air,* began in October 1995 with total expenditures of $434,382. The Investigators' Report was received for review in November 1998. A revised report, received in July 1999, was accepted for publication in August 1999. During the review process, the HEI Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the Review Committee's Critique.

pollutants had been removed. They designed a five-stage filtration unit to remove ozone, formaldehyde, sulfur dioxide, carbon monoxide, and particulate matter. Exposures to ozone (Johnson et al 1990; Hotchkiss et al 1991; Plopper et al 1991; Harkema et al 1994, 1997a,b) or formaldehyde (Monticello et al 1989, 1991) are known to induce pathologic changes in the nasal cavity of rats and monkeys. The investigators tested the chambers at CIIT by exposing rats in the filtered and unfiltered chambers to 0.74 ppm ozone for 23 hours/day for six consecutive days. A histopathologist graded the severity of nasal lesions from zero (no lesions) to 4 (very severe lesions). Female rats exposed to ozone in the unfiltered chamber showed minimal to mild nasal lesions that were not present in females exposed in the chamber with filtered air. Results with male rats (not reported) were similar. The filtration system removed 85% of the incoming ozone; therefore, the investigators concluded that the filtration system protected control rats from the external atmosphere.

Moss and colleagues shipped the exposure chambers to Mexico City and conducted the third step in their study, a pilot study that tested the efficiency of their exposure chambers on site. They exposed male and female rats (shipped from the United States) to unfiltered or filtered Mexico City air for 23 hours/day for 7 or 21 days. Another control group of rats breathed room air to ensure that viral or bacterial infections endemic to locally obtained rats housed elsewhere in the INP did not add a confounding factor to the study rats. Because rats are nocturnal animals, the room housing the exposure chambers (and the rats that breathed room air) was darkened between 9 am and 9 pm. This feature of the study design caused the rats to be active when ozone levels are highest (late morning to early evening) and matched the period when humans are most active.

The investigators measured levels of ozone, oxides of nitrogen, aldehydes, sulfur dioxide, total suspended particulates, and particulate matter with an aerodynamic diameter smaller than 10 μm (PM_{10}) in both exposure chambers. Rats in the unfiltered chamber were exposed to ambient southwestern Mexico City air for 46 one-hour intervals when the average ozone concentration exceeded 0.120 ppm, but the mean pollutant concentrations in the unfiltered chamber were generally lower than expected. For example, in the pilot study, the geometric mean concentration of all daily 8-hour periods of highest ozone levels was 0.073 ppm. With the exception of formaldehyde, however, aldehyde levels in both unfiltered-air chambers were below the limit of detection. Formaldehyde concentrations were similar in both chambers (3.3 ppb), but the filtration system lowered the concentrations of ozone, total suspended particulates, PM_{10}, and sulfur dioxide. (Equipment failure prevented measurement of nitrogen oxide levels in the unfiltered chamber.)

Histopathology examination revealed no nasal lesions in exposed or control rats; tracheal and lung tissue from both study groups showed similar levels of minor abnormalities. This short pilot study was an excellent precautionary step to look for problems that might compromise the investigators' main study of extended exposure to Mexico City air. Because the pilot study demonstrated that the filtration system successfully reduced levels of most pollutants in the control chamber, the investigators anticipated that longer exposure times might reveal differences between exposed and control animals.

In their fourth step (called the Main Study in the Investigators' Report), Moss and colleagues exposed male and female rats to unfiltered or filtered Mexico City air for 23 hours/day for 21, 35, or 49 consecutive days. (The investigators were unable to extend the exposure period to 13 weeks as originally planned.) Two groups of rats were exposed to unfiltered air for 35 days; one group was examined at the end of the exposure period and one group was allowed to recover for two weeks in filtered air. A room air exposure group was also included. As in the pilot study, pollutant concentrations in the exposure chamber were lower than the investigators anticipated. There were also fewer occasions when ambient ozone levels exceeded 0.12 ppm (18 versus 46) compared with the pilot study. In addition to histopathology examination of the airways, the investigators examined sections of nasal tissue by quantitative morphometry to evaluate more subtle signs of injury such as differences in the numbers of epithelial, mucous, and inflammatory cells or mucosubstances. (An increase in mucosubstances is a characteristic nasal protective response to inhaled toxicants.)

Moss and colleagues detected random histologic alterations in the respiratory tract in a few control and exposed rats. These changes, which were minimal and similar in morphologic character, were not considered to be exposure-related. Neither exposure duration or exposure atmosphere (unfiltered air, filtered air, unfiltered air followed by clean air for two weeks) caused changes in nasal tissue morphometry. The investigators concluded that: (1) constructing mobile chambers for exposing rats to the outdoor environment and protecting control rats from that environment were feasible, and (2) exposure of rats to ambient air in southwestern Mexico City air for seven weeks did not produce lesions in the nose or lower respiratory tract.

DISCUSSION AND CONCLUSIONS

Rats and mice are the most frequently used animals in inhalation bioassays of chemical toxicity or carcinogenicity (DeSesso 1993). The tissues of the lower respiratory tract of most mammals (including humans) are composed of similar cell types that perform similar functions; consequently, the results of lung bioassays can be extrapolated to humans. However, the anatomy and physiology of rodent nasal passages differ significantly from humans in several ways: the overall geometry of the nasal passages, relative nasal surface areas, proportions of nasal surfaces lined by various epithelia, mucociliary clearance patterns, and inspiratory airflow routes (DeSesso 1993). In contrast, the nasal passages of nonhuman primates, such as monkeys, exhibit many similarities to humans. Studies of rats and monkeys indicate that the respiratory system of monkeys (including the nasal cavity) is more susceptible to ozone-induced injury than that of rats (reviewed by Plopper et al 1991). These differences call into question the use of rats as sentinels for nasal injury in humans and suggest that nonhuman primates may be better models for this purpose.

Moss and colleagues found no effects of exposure to ambient Mexico City air on rat respiratory tract histopathology or nasal tissue morphometry. These findings allow several possible interpretations. Pollutant levels may have been too low and/or the exposure periods too short to affect the rats. For example, the mean ozone concentration in the unfiltered chamber during the main study (0.018 ppm) was lower than those producing nasal lesions in controlled laboratory exposures of rats (0.5 to 1.0 ppm) (Johnson et al 1990; Hotchkiss et al 1991; Harkema et al 1994, 1997a,b). Similarly, the concentration of formaldehyde that produced nasal lesions in controlled laboratory exposures of rats and monkeys (6 ppm) (Monticello et al 1989, 1991) was much higher than the mean formaldehyde concentration in the unfiltered chamber during the main study (3.3 ppb). Alternatively, anatomic differences in rat nasal passages may make them less susceptible than human tissues to the pollutant mixture. Further research should explore the validity of each interpretation to determine whether bioassays using rat tissue are appropriate for assessing the effects of air pollutants on humans.

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