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SUBCHRONIC INHALATION STUDY IN RATS USING AGED AND DILUTED SIDESTREAM SMOKE FROM A REFERENCE CIGARETTE

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Male Sprague–Dawley rats were exposed 6 hr/day, 5 days/week for up to 13 weeks to aged and diluted sidestream smoke (ADSS), used as a surrogate for environmental tobacco smoke (ETS), at concentrations of 0.1 ("typical"), 1 ("extreme"), or 10 ("exaggerated") mg of particulates/m^3. Subgroups of animals were killed after 1 and 4 weeks of exposure. Animals were exposed nose-only, inside whole-body chambers, to ADSS from the 1R4F reference cigarette. End points included histopathology, CO oximetry, plasma nicotine and cotinine, clinical pathology, and organ and body weights. The target particulate concentrations were achieved; at the exaggerated exposure they resulted in CO concentrations in excess of 50 ppm. Particle size distributions showed that the aerosols were completely respirable: the mass median diameter values were less than 1 μm. The only pathological response observed was slight to mild epithelial hyperplasia in the rostral nasal cavity, in the exaggerated exposure group only. No effects were noted at low (typical of measured real-world ETS concentrations) or extreme exposures. The changes were similar in animals killed after 4, 28, or 90 days, and were also similar to those noted in an earlier experiment with only 14 days duration, indicating that the change does not progress with increased exposure duration from 4 to 90 days. The nasal change was absent in a subgroup of animals kept without further smoke exposure for an additional 90 days, indicating complete reversibility. Overall, the end points used in the study demonstrated that (1) there was no detectable biological activity of ADSS at typical or even 10-fold ETS concentrations, and (2) the activity was only minimal at exaggerated concentrations in one region of one organ only. Based on the nasal histopathology, the NOEL for the 90-day study is >1 mg/m^3.

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INTRODUCTION

Numerous statements have been made in the scientific literature on the biological activity of environmental tobacco smoke (ETS) (Department of Health and Human Services, 1986; National Research Council, 1986). Recent work with animals exposed 6 hr/day for 14 consecutive days to aged and diluted sidestream smoke (ADSS) reported minimal histopathological changes in such studies, even when smoke concentrations many times higher than those reported in the field were used (Coggins et al., 1992). ADSS has been shown to be a suitable surrogate for ETS (Baker and Proctor, 1990; Gori and Mantel, 1991; Guerin et al., 1992).

The purpose of the present study was to determine for histopathology (1) if the changes seen in the above study of 14 days duration were modified in subchronic (90-day) exposures, (2) whether any new changes were observed in subchronic exposures, (3) whether the dose–response relationship was different after the subchronic exposure (using the same target ADSS concentrations as used previously), and (4) whether the 90-day changes (if any were noted) were reversible in a further 90-day period without any exposure.

Target ADSS concentrations were 0.1, 1, and 10 mg/m^3, the same as those used in the 14-day study. Respectively, these correspond to "typical," "extreme," and "exaggerated" field measurements (Guerin et al., 1992).

The primary end point of this study was the histopathology of the respiratory tract and related organs, as assessed by pathologists with experience in rodent inhalation studies with cigarette smoke. Secondary end points included those suggested by regulatory authorities (Organisation for Economic Co-Operation, 1981); other end points (CO-oximetry, plasma nicotine and cotinine) were added to the basic protocol to verify that smoke inhalation did occur (dosimetry).

MATERIALS AND METHODS

Experimental Design

The experimental design was based on published guidelines (Organisation for Economic Co-Operation, 1981). Three groups of animals were exposed to ADSS; there was a sham-exposed group exposed only to filtered air. Animals were exposed to smoke inside whole-body chambers (Chen et al., 1989) using nose-only restraint tubes. A further group of animals was kept as sentinels for the detection of disease. There were 106 male animals in each of the four groups. Animals were exposed 6 hr/day, with animals killed after 4, 28, or 90 days of exposure. The 4-day exposures were performed with only 10 animals per chamber; the chambers were refilled with the remaining 96 animals per group for the 28-

and 90-day exposures. Animals in satellite groups were kept for a further 90 days without treatment to assess reversibility.

Experimental Animals

A total of 506 male animals, weighing 125–150 g, was purchased from Charles River Laboratories (Raleigh, NC). Animals were housed individually in transparent polycarbonate cages and acclimated to laboratory conditions for 14 days prior to the first exposure.

The Sprague–Dawley rat (Crl:CD/BR, VAF/Plus) was chosen as the experimental animal because it has frequently been used in inhalation studies and there is a large amount of background inhalation information available in the scientific literature. Males only were used, to allow sufficiently large group sizes for the many different end points studied. Earlier work (Coggins et al., 1992) showed no difference between the responses shown by males and females.

Within 5 days of delivery, five animals were randomly chosen and killed for collection of sera, which were tested for the following antibodies to disease: reovirus type 3, cilia associated respiratory bacillus, Kilham's rat virus, Toolan's H-1 virus, pneumonia virus of mice, Sendai, rat coronavirus/sialodacryoadenitis virus, lymphocytic choriomeningitis virus, and *Mycoplasma pulmonis*. Antibody testing was made on sera obtained from five animals at the beginning, midpoint, and end of the inhalation part of the experiment, and at reversibility (total of 25 animals). The lungs from the sentinel animals were taken and examined histopathologically to ascertain health status.

Within a week of delivery, the animals were allocated into four groups of 106 animals each, such that the body weights in the groups were as homogeneous as possible. The mean ($\pm$SD, n = 106) weights in the sham, low, mid, and high groups at randomization were 157.7 $\pm$ 6.6, 157.5 $\pm$ 6.7, 157.6 $\pm$ 6.3, and 157.7 $\pm$ 6.6 g, respectively.

During the week after allocation into groups, animals were tail-tattooed (Animal Identification and Marking Systems, Piscataway, NJ) with their permanent identification number.

The animals were housed and cared for in accordance with the Animal Welfare Act of 1970 and amendments (Public Law 91-579), as set forth in CFR Title 9, Part 3 Sub-part E, *Specifications for the humane handling, care, treatment, and transportation of warm-blooded animals other than dogs, cats, rabbits, hamsters, guinea pigs and non-human primates.* Reference was also made to the DHHS document *Guide for the Care and Use of Laboratory Animals* (NIH publication 86-23). Animals were housed in a vivarium that had controlled lighting (12 hr of darkness, from 18:00 hr), temperature (20–24°C), and humidity (40–60% RH). Seven-day continuous recordings were kept of RH and temperature. Empty animal rooms

were certified (Certek, Raleigh, NC) as being class 100 (less than 100 particles/m^3.

Animals had unrestricted access to certified feed (Purina Rodent Chow no. 5002, presented as pellets) and distilled water. No feed was available during inhalation exposures. Feed was withheld overnight prior to necropsy. Chemical analyses of feed, water, or bedding were not performed because it was deemed unlikely that contaminants would adversely affect the experiment.

Cigarettes

The 1R4F reference cigarettes were purchased from the Tobacco and Health Research Institute (Lexington, KY). A full description of the mainstream, sidestream, and ETS chemistry of the 1R4F cigarette has been published elsewhere (R. J. Reynolds, 1988), as has a description of the physical and chemical characteristics of mainstream and environmental tobacco smoke (Gori and Mantel, 1991). A description of the origins of ETS has also been published (Baker and Proctor, 1990).

Aerosol Generation Apparatus

Methods have been described in detail elsewhere (Coggins et al., 1992); the following constitutes a basic description only. A 30-port smoke generator (CH Technologies, Westwood, NJ), similar to that described by Baumgartner and Coggins (1980) and by Ayres et al. (1990), was fitted with an aluminum cone for collection of sidestream smoke (SS). Mainstream smoke was generated under Federal Trade Commission conditions (except butt length: 7 puffs were taken instead of burning to a fixed butt length) and was discarded. Sidestream smoke was drawn from the cone into a common plenum used for aging and diluting the smoke, using 3-in. (76.2-mm)-diameter polyvinylchloride (PVC) tubing throughout. Different amounts of SS were drawn from the plenum for each chamber and mixed with further amounts of dilution air drawn from the room through HEPA filters. Room air was HEPA-filtered "upstream." The sham and sentinel animals were kept in chambers that were not attached to the plenum.

Animal Exposure Apparatus

The whole-body inhalation chamber (Moss et al., 1982) is available commercially (Lab Products, Maywood, NJ). Each chamber was operated at a flow rate of 16 ft^3/min (in excess of 15 air changes/hr).

The conical nose-only restraint tubes (Baumgartner and Coggins, 1980) are also commercially available (CH Technologies, Westwood, NJ); they were used to minimize contamination of the pelt with deposited ADSS which could then be absorbed dermally or ingested during preen-

ing (Langård and Nordhagen, 1980). Tubes were 73 mm in diameter and 263 mm long; the inlet was 22 mm in diameter. The ventilation slots on the restraint tubes were covered with duct tape.

On exposure days, individual animals were taken from their cage in the chamber, placed inside a nose-only restraint tube, and replaced in the tube into the same cage. The positions of racks within the chambers were changed daily in order to minimize any effects of cage position within the chamber.

Daily Characterization of Inhalation Exposures

During animal exposures, probes were used to monitor the aerosol presented. This monitoring was by collection of aerosol on 25-mm Teflon pads (TF-450, Gelman, Ann Arbor, MI), followed by gravimetric determination of collected particulates using Cahn C-31 microbalances (Cahn, Cerritos, CA). The RAM-1 instrument (MIE Corp., Bedford, MA) was used to give an on-line estimate of particulate mass concentrations.

The main analytical instrument used for estimating CO and CO_2 concentrations was the Horiba PIR-2000 (Horiba Instruments, Irvine, CA), calibrated daily with certified gas mixtures (AIRCO Welding Supply, Greensboro, NC). Oxygen concentrations (%) were monitored by a Horiba PMA-200 instrument, also calibrated with a certified gas mixture. Very low concentrations of CO were measured with the Miran 80 gas analyzer (Foxboro Instruments, South Norwalk, CT). Data from the on-line instruments were logged manually every 60 min.

Measurement of nicotine and its pyrolysis product 3-ethenylpyridine (Guerin et al., 1992) was by gas chromatography with thermionic-specific detection. Chamber atmospheres were sampled with XAD-4 sorbent tubes (SKC Inc., Eighty-Four, PA) that were extracted for analysis with ethyl acetate containing 0.01% triethylamine (Ogden, 1989).

Solanesol measurements were made by liquid chromatography with UV detection at 205 nm. After gravimetric determination of particulate mass, the pads were extracted with 3 ml methanol for solanesol analyses (Ogden and Maiolo, 1992).

Measurements of particle size distribution were made with Mercer-style cascade impactors (Mercer et al., 1970; In-Tox Products, Albuquerque, NM). The impactor had cutoff diameters in the range of 0.4–2.5 μm under the conditions of use (1.2 l/min); calculation of mass median diameter and associated standard geometric deviation was by probit analysis. The cover slips (uncoated) used to collect the aerosol for impactor analysis were weighed using Cahn C-31 microbalances.

Temperature and % RH of the exposure atmosphere were measured with a condensation dew point hygrometer (model 1100 DP, General Eastern Instrument Co., Watertown, MA).

Clinical Observations

Animals were inspected visually for signs of overt toxicity as they were being transferred from their cages to the restraint tubes and when being transferred back to their cages. More detailed clinical observations were made on each animal once every 4 days, before the exposure and within 2 hr of the end of the exposure.

Body Weights

Individual body weights were determined within 48 hr of receipt, at randomization, and every week thereafter, using Mettler PM 2000 balances.

Dosimetry

Blood samples were obtained after 6 hr of smoke exposure, on different exposure days throughout the experiment. Blood was drawn from the retro-orbital sinus, using anesthesia with 70% CO_2 in air and heparinized micropipettes, and held on ice in plastic cuvettes containing disodium edetate (Na_2-EDTA) during the time between sampling and analysis.

Blood carboxyhemoglobin (COHb) concentrations were determined on 0.5 ml of the total sample, using a model 482 CO-oximeter (Instrumentation Laboratories, Hartford, CT). Subsamples of the blood collected for COHb were taken for the determination of plasma nicotine and cotinine. The latter analyses were performed by an enzyme-linked immunosorbent assay (ELISA) method (Chang et al., 1992).

Necropsy

The following numbers of animals were allocated to necropsy and subsequent histopathology in each of the four exposure groups: 10 for the 4-day necropsy, 10 for the 28-day necropsy, 25 for the 90-day necropsy, and 15 for the reversibility necropsy. Remaining animals were used for end points other than histopathology (no data presented here).

Animals were killed on the day following their last exposure, and the time interval recorded. Feed was not available to the animals during this interval.

At necropsy, animals were weighed and then killed first by anesthetization with 70% CO_2 in air and then exsanguination via the vena cava prior to cessation of heartbeat. Blood samples for the various assays to be performed were collected from the vena cava.

Animals were subjected to a complete gross examination in the presence of a board-certified veterinary pathologist, with special attention paid to the respiratory tract.

Clinical Pathology

The following assays were performed on whole blood obtained at each necropsy: red blood cell count, hemoglobin, hematocrit, mean red cell volume, mean red cell hemoglobin, mean red cell hemoglobin concentration, white cell count, differential white cell count, reticulocyte count, and platelet count. The anticoagulant Na_2-EDTA was used; standard hematological methods were used.

The following assays were performed on serum obtained from animals at each necropsy: calcium, phosphorus, chloride, sodium, potassium, glucose, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, urea nitrogen, albumin, creatinine, total bilirubin, total cholesterol, triglycerides, and total protein. The time of blood sampling was recorded. Sure-Sep II serum separators (Organon-Teknika, Durham, NC) were used to minimize hemolysis; the time between blood collection and serum collection was kept as short as possible. Standard analytical methods were used.

Organ Weights

The lungs (complete with trachea but excluding the larynx), brain, liver, testes (pair), kidneys (pair), and heart (excluding major vessels) were weighed at each necropsy using Mettler PM 460 balances. Organ weights and the (fasted) body weight recorded immediately before death were used to calculate organ/body weight ratios. The time from removal of the organ until weighing was minimized and tissues were kept in saline until they were weighed.

Tissue Collection

Tissues were removed from each animal and fixed in 10% neutral buffered formalin (NBF), at a volume dilution of 1 part tissue to at least 15 parts formalin (Feldman and Seely, 1988). The fixative contained 20 ml of 1% eosin per 20 l of 37% formalin as a precaution to identify the fluid as fixative. Lungs were infused with NBF at a volume that ensured proper distention and fixation. The trachea was ligated after distention.

The following tissues were collected: adrenals, aorta, bone (sternum, femur), brain, cecum, colon, cranium, duodenum, epididymides, esophagus, eyes/optic nerve, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (various), nasopharynx, nose/turbinates, pancreas, parathyroid, pituitary, prostate, rectum, salivary gland, seminal vesicle, skeletal muscle (thigh), skin (abdominal), spinal cord (lumbar), spleen, stomach, tail, testes, thymus, thyroid, tongue, trachea, urinary bladder, and Zymbal's gland.

Histopathology

Respiratory tract tissues (nasal passages, larynx, trachea, conducting airways, deep lung), heart and related lymph nodes (thymic and peribronchial), and gross changes were examined in each of the animals allocated to histopathology.

The nasal tissues were cut at three different locations to obtain representative sections of the different epithelia, as described previously (Young, 1981). The lungs were sectioned so as to provide a section along the main stem bronchus of each lung lobe. A precise anatomic site for cutting the larynges is required: serial step sections were taken to reach this site (Burger et al., 1989; Sagartz et al., 1992).

Tissues were stained with hematoxylin and eosin (H&E); duplicate slides of a representative section of the anterior nasal tissues, larynx, lung, and trachea were stained with periodic acid-Schiff/Alcian blue (PAS-AB) to facilitate evaluations of mucus-secreting cells. Tissues were read by an ACVP board-certified veterinary pathologist with knowledge of the exposure groups.

Statistical Analyses

Statistical evaluations were made using Bartlett's test of homogeneity of variance, followed by analysis-of-variance (ANOVA) techniques. The statistical evaluation of incidence and severity data for histopathology was made by the Kolmogorov–Smirnov test (Siegel, 1956). Statistical tests were carried out to 5%, two-sided criteria.

RESULTS

Inhalation Exposures

The mean concentrations (±SD, $n = 69$) of wet total particulate matter (WTPM) for the low-, medium-, and high-exposure groups were 0.105 ± 0.013, 1.01 ± 0.071, and 10.3 ± 0.691 mg/m^3, respectively. Figure 1 shows the within- and between-day variation in WTPM concentrations. These WTPM exposures resulted in CO concentrations (ppm) of 2.9 ± 0.59, 9.3 ± 1.87, and 55.1 ± 5.0.

Nicotine concentrations (±SD, $n = 14$) at the medium and high exposures were 272 ± 65 and 2377 ± 393 μg/m^3. Although nicotine could be detected in the low-exposure chambers, the values were unexpectedly low: the mean was 0.39 ± 0.19 μg/m^3. The values for 3-ethenylpyridine at the low, medium, and high exposures were 0.26 ± 0.39, 39.0 ± 8.7, and 320 ± 40 μg/m^3. Solanesol concentrations (±SD, $n = 14$) in the low, medium, and high exposures were 1.9 ± 0.36, 22.9 ± 4.4, and 192 ± 18 μg/m^3, respectively.

None of the above analytes could be detected at significant concen-

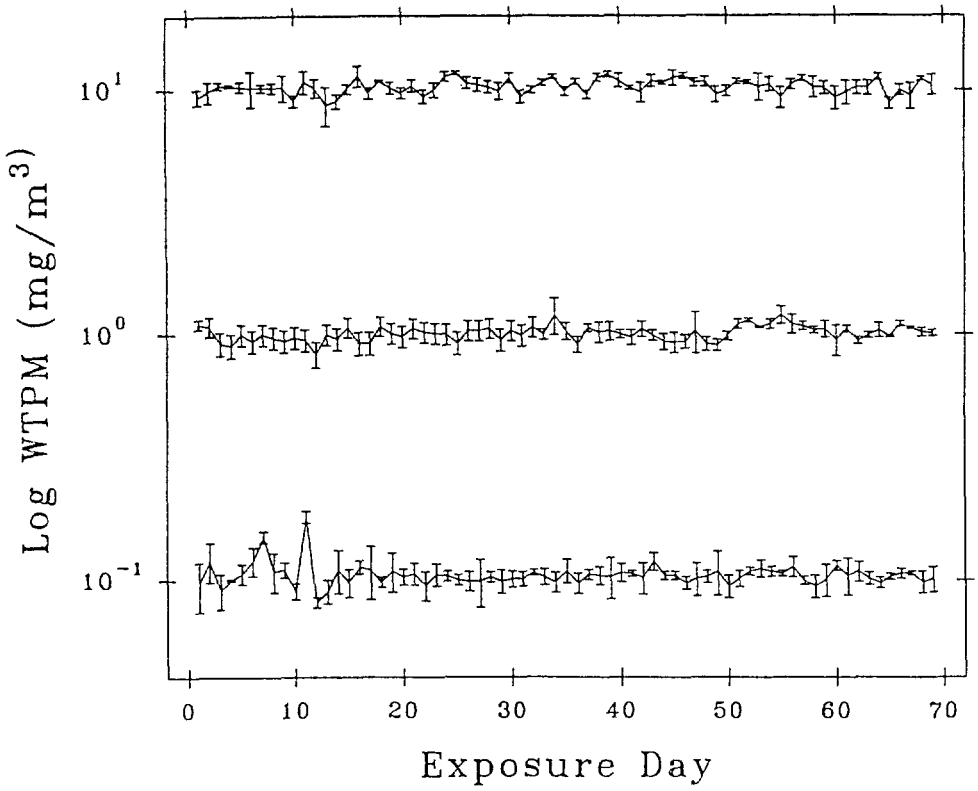

FIGURE 1. Variation in daily concentrations of wet total particulate matter in aged and diluted sidestream smoke presented to experimental animals. Mean ± standard deviations. Targets were 0.1, 1, and 10 mg/m^3.

trations in the sham chamber or in the exposure room. In the exposure room, the mean CO_2 concentration (±SD, n = 15) was 401 ± 50 ppm; in the sham chamber the mean (±SD, n = 14) concentration was 1569 ± 286 ppm. The mean concentrations (±SD, n = 14) in the low, medium, and high chambers were 1430 ± 140, 1702 ± 382, and 1745 ± 257 ppm, respectively.

Particle size distributions at the low, medium, and high exposures were similar, with the average values (±SD, n = 14) being 0.32 ± 0.08, 0.43 ± 0.06, and 0.53 ± 0.03 μm, respectively. The standard geometric deviations were 1.94 ± 0.55, 1.59 ± 0.17, and 1.51 ± 0.11.

In-Life Observations

There were no exposure-related clinical signs or mortalities and the serology results were negative. Animals in the smoke-exposed groups

showed body weights that were not different from those in the sham group (Fig. 2); at no point did animals lose weight (Fig. 3).

Blood COHb concentrations at the end of the exposures were negligible in the sham and low-exposure groups. The means for the medium and high groups (±SD, n = 30) were 0.4 ± 0.4 and 4.9 ± 0.9%, respectively.

The sham and low-exposure groups had plasma nicotine and cotinine concentrations at or below the limit of detection. The mean plasma nicotine for the mid- and high-exposure groups (±SD, n = 30) were 20.5 ± 5.1 and 62.8 ± 9.8 ng/ml, respectively. The mean cotinine concentrations (±SD, n = 29) were 26.6 ± 2.3 and 165 ± 41 ng/ml, respectively.

Necropsy Data

The mean terminal body weight (±SD, n = 10) for sham, low, medium, and high exposures at the 4-day necropsy were 243 ± 10.9, 254.9 ± 10.1, 243.5 ± 9.3, and 247.6 ± 10.8 g, respectively. The mean

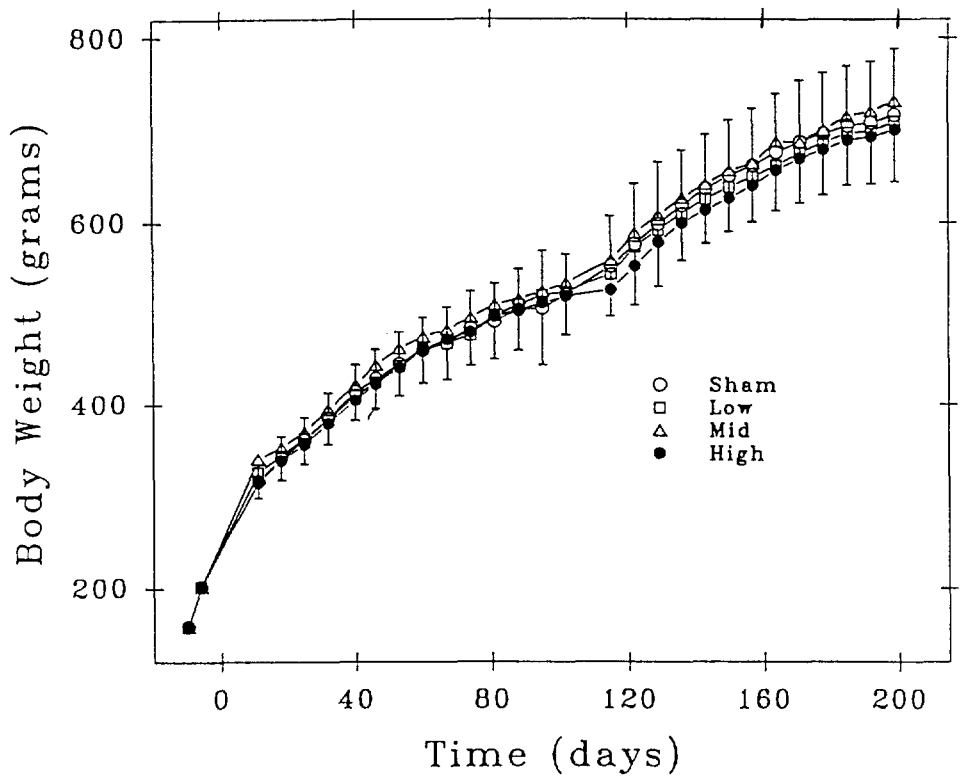

FIGURE 2. Change in body weight of animals exposed to aged and diluted sidestream smoke. Means ± standard deviations.

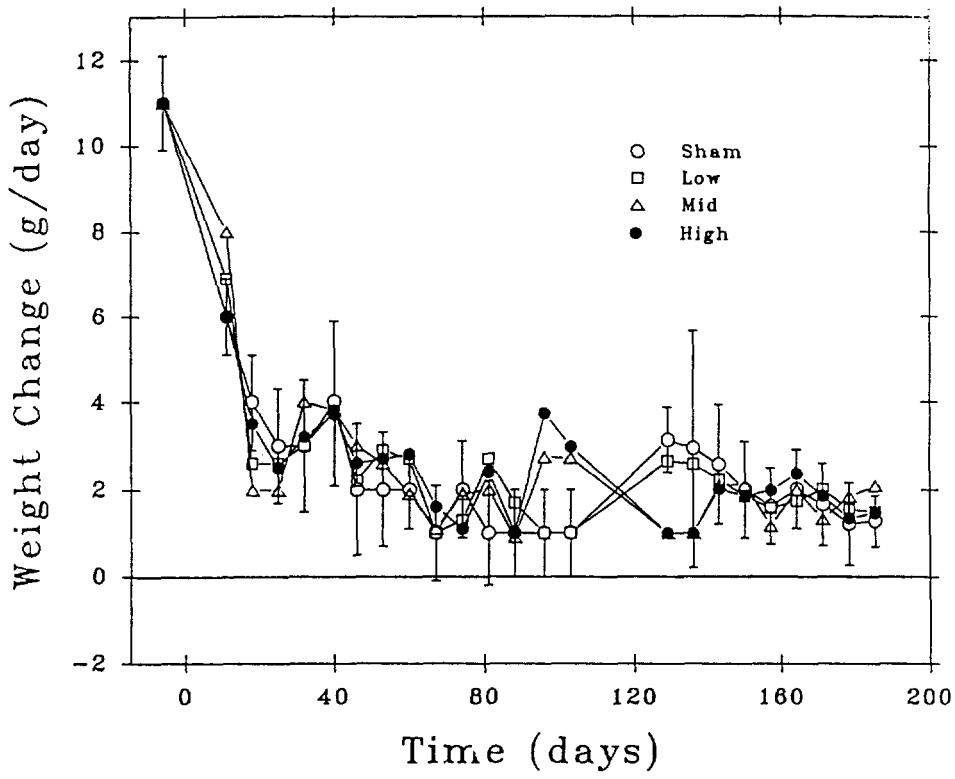

FIGURE 3. Rate of changes in body weight in animals exposed to aged and diluted sidestream smoke. Means ± standard deviations.

terminal body weight (±SD, n = 15–20) for sham, low, medium, and high exposures at the 28-day necropsy were 433 ± 31.8, 428.6 ± 33.5, 447 ± 43.7, and 441.4 ± 35.9 g, respectively.

The mean terminal body weight (±SD, n = 25) for sham, low, medium, and high exposures at the 90-day necropsy were 493.2 ± 38.1, 519.3 ± 41.3, 545.9 ± 58.5, and 508.4 ± 42.8 g, respectively. The mean value for the medium exposure group was 10% greater than that for the sham ($p < .01$), and 5% ($p < .05$) and 7% ($p < .01$) higher than the means for the low and high groups, respectively. At the reversibility necropsy, the means (±SD, n = 12–21) were 738.7 ± 81.9, 712.6 ± 68.9, 734.2 ± 87.9, and 704.7 ± 106.7 g for the sham, low, mid, and high groups, respectively. There were no significant group differences at the reversibility necropsy.

There were no significant differences between the groups for any clinical pathology parameters at any of the necropsies.

There were no exposure-related gross observations at any of the necropsies, nor were there any differences in organ weights.

Histopathology

In the nasal I section (transverse, immediately caudal to the incisor teeth, Fig. 4), there was minimal to mild epithelial hyperplasia, in the high-exposure group only. In this change, there was hypercellularity and thickening of the respiratory epithelium of the dorsal nasal conchae (nasoturbinates: Figs. 5 and 6) and the adjacent wall of the middle meatus. Figures 7–9 show the distributions of the different severities of this change at the 4-, 28-, and 90-day necropsies, respectively. At each of these necropsies the distribution of the change in the high-exposure group was significantly different from that in the other groups; no such effect was noted at the low or medium exposures.

There were no other histopathological changes noted in animals killed at any of the necropsies, nor were any changes noted in the animals killed at the reversibility necropsy (i.e., the minimal changes noted at the end of the exposure were totally reversible).

The above results were made by a pathologist (JWS) with knowledge of the exposure group. The slides and scores from these readings were reviewed by a second pathologist (Dr. D. L. Dungworth) without knowledge of the exposure.

Because of the very similar incidences and severities seen in the high-exposure groups at the 4-, 28-, and 90-day necropsies, and because these latter results were also similar to those noted in the earlier 14-day study (Coggins et al., 1992), "blind" rereads on a combined set of slides for the high-exposure group only were performed. In the blind rereads, the pathologists (operating independently) thus had knowledge of the exposure group, but not of the duration of the exposure. Only the male animals were used from the 14-day study, mixed with the 4-, 28-, and 90-day slides of the present study. The unmixed data from one of the pathologists (JWS) are presented as Fig. 10: there were similar responses at each of the time points, indicating no progression of the hyperplasia with continued exposure.

DISCUSSION

The inhalation data presented here show that the target concentrations were met and that the aerosols presented to the animals were respirable by them. As in the earlier work (Coggins et al., 1992), we have no explanation for the low concentrations of both nicotine and 3-ethenylpyridine in the low chamber, although concentrations were above those noted in the sham chamber. Inhalation of presented aerosols was confirmed in the medium and high groups through blood COHb and plasma nicotine and cotinine measurements. Consequently, comparisons can validly be made of the histopathological changes in the different groups. The three biological markers of dosimetry could not

FIGURE 4. Low-power view of a transverse section of the rat nasal passages, immediately caudal to the incisor teeth. The arrow marks the position of the change shown in Figs. 5 and 6. H&E, original magnification ×45.

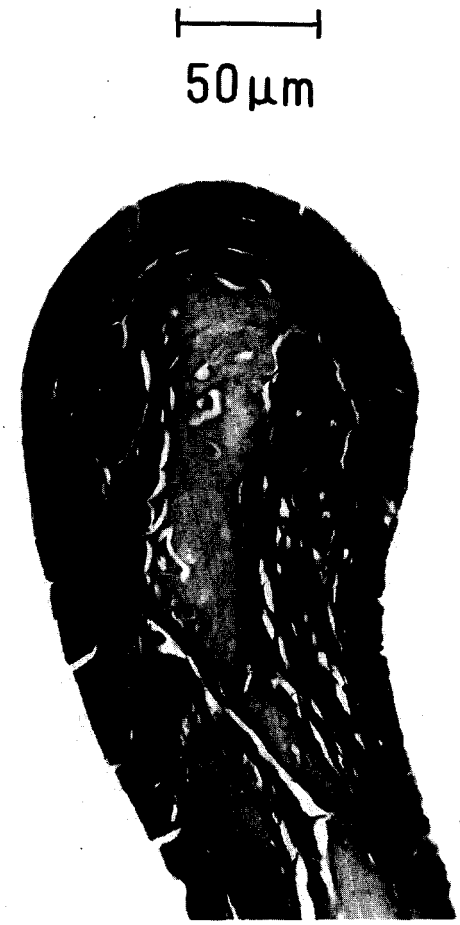

FIGURE 5. High-power view of the tip of a nasal concha (nasoturbinate) from a typical sham-exposed rat. The epithelium is 2–3 cell layers thick. H&E, original magnification ×500.

confirm exposure in the low group, where ADSS concentrations were considered to be at or near "real-world" (Guerin et al., 1992). It is thus very unlikely that these markers could be considered as being acceptable for characterizing human exposures to ETS.

The increased terminal body weights in the medium exposure group at the 90-day necropsy are considered to be a statistical anomaly and are not considered to be biologically significant.

The histopathology results obtained here at 90 days are in agreement with earlier work (von Meyerinck et al., 1989), where only a single concentration of ADSS was used (4 mg/m^3) for 13 weeks at 10 hr/day. In the earlier work, the main histopathological change noted was also in the

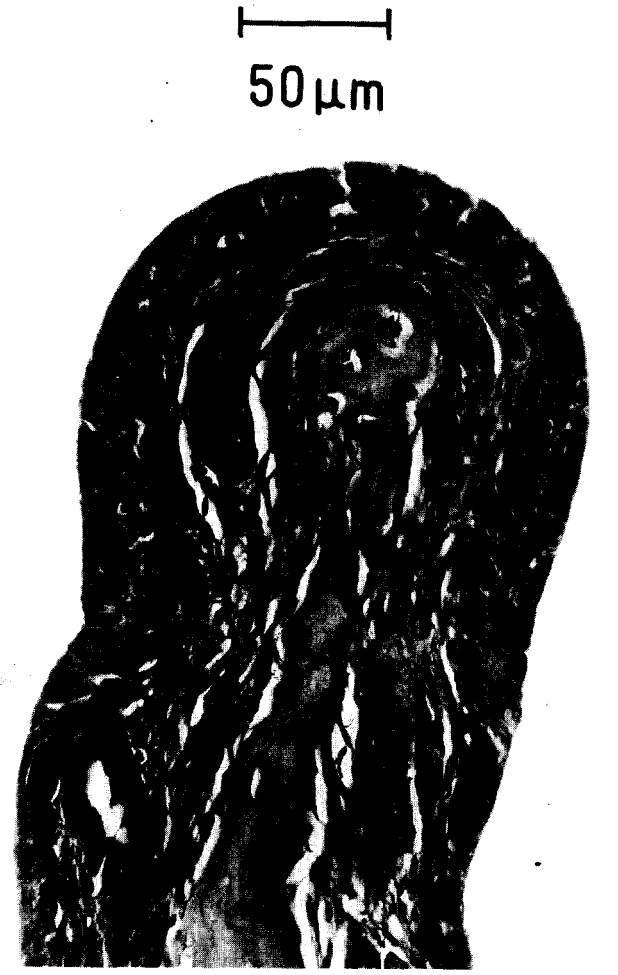

FIGURE 6. High-power view of the tip of a nasal concha (nasoturbinate) from a typical high-exposure rat. The epithelium is 5–6 cell layers thick (epithelial hyperplasia). H&E, original magnification × 500.

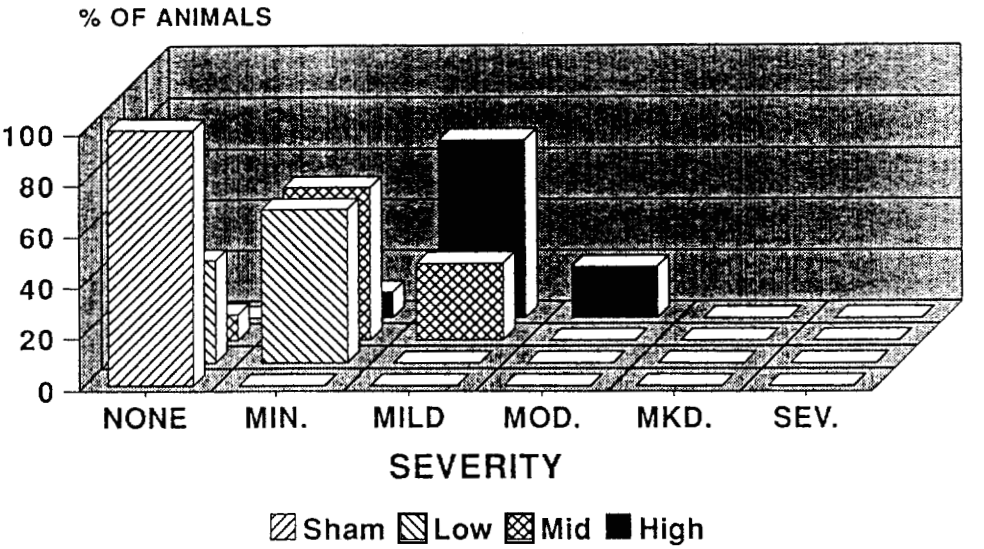

FIGURE 7. Distribution of epithelial hyperplasia in the rostral nasoturbinates at the 4-day necropsy of animals exposed to aged and diluted sidestream smoke. MIN, minimal change; MILD, mild change; MOD, moderate change; MKD, marked change; SEV, severe change.

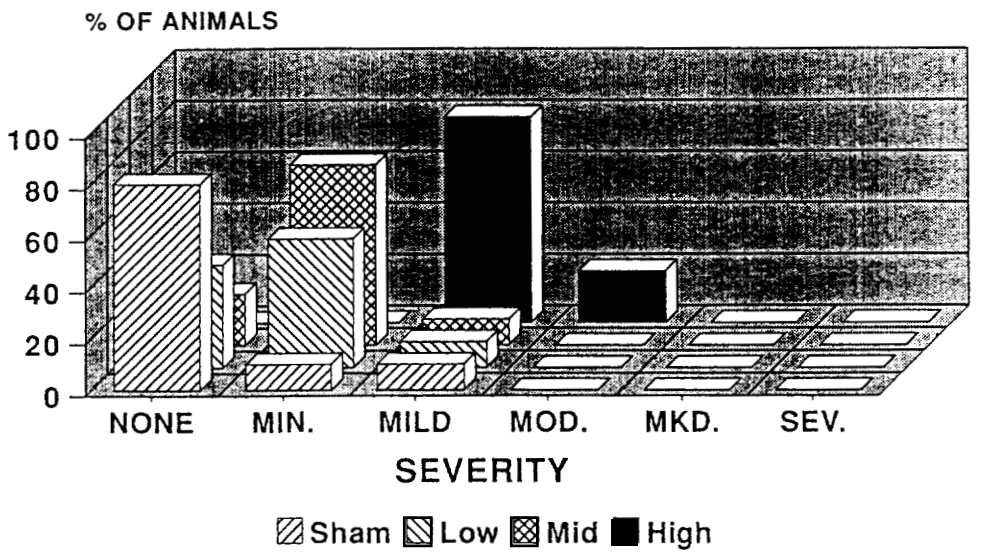

FIGURE 8. Distribution of epithelial hyperplasia in the rostral nasoturbinates at the 28-day necropsy of animals exposed to aged and diluted sidestream smoke. Abbreviations as in Fig. 7.

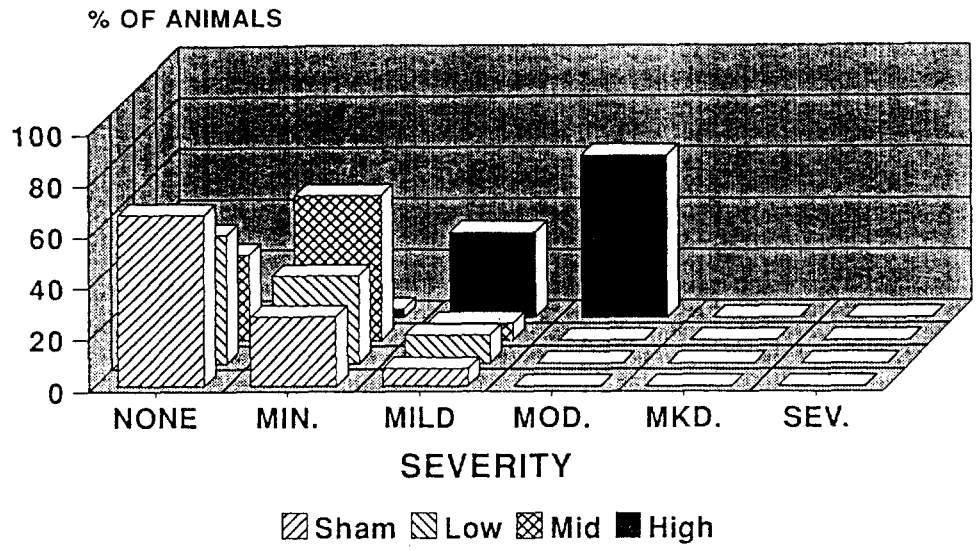

FIGURE 9. Distribution of epithelial hyperplasia in the rostral nasoturbinates at the 90-day necropsy of animals exposed to aged and diluted sidestream smoke. Abbreviations as in Fig. 7.

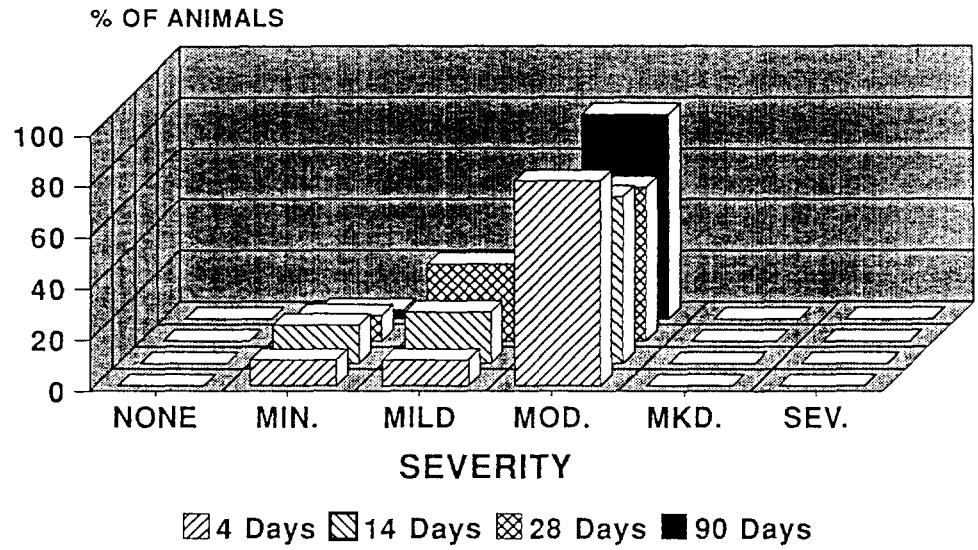

FIGURE 10. Distribution of epithelial hyperplasia in the rostral nasoturbinates in the high-exposure groups at the 4-, 28-, 14-, and 90-day necropsies of animals exposed to aged and diluted sidestream smoke. Abbreviations as in Fig. 7.

rostral nasal cavity of both rats and hamsters—a change that appears morphologically to be very similar to that noted in the high-exposure group in the present study. The results presented here at 4, 28, and 90 days of exposure are similar and are also in agreement with the data presented in a 14-day study using the same concentrations and daily durations (Coggins et al., 1992). This finding suggests a complete lack of progression of the histopathology change with continued exposure. In this and the Coggins et al. (1992) study the histopathology changes noted were completely reversible once the exposures had been stopped. For these reasons, the epithelial hyperplasia in the nasoturbinates is considered to be an adaptive and reactive response to repeated irritation.

The exposure levels used in this study were carefully chosen with respect to typical concentrations in real-life environments. The low exposure used is typical of exposure concentrations of particulate matter in public places where smoking is allowed without restriction, although the contribution of tobacco smoke to such exposures has been shown to be less than 50% (Guerin et al., 1992). In the present work, the contribution of tobacco smoke to the aerosol presented to the animals was clearly 100%. The exaggerations of field values should then in reality be 2-, 20-, and 200-fold rather than 1-, 10-, and 100-fold. Results of a recent survey (Turner et al., 1992) showed an overall mean value of 46 $\mu g/m^3$ for respirable suspended particulates in areas where smoking was allowed, suggesting that the figure of 200 for the exaggeration of field exposures may be an underestimate.

The results again show only a minimal effect of exposure to very high concentrations of ADSS, the only effect being the completely reversible and nonprogressive changes seen in the rostral nasal cavity at the high exposure only. Even with the extended durations (5 times those of the 14-day study) no changes were reported in any organ other than the rostral nasal cavity, and even here the change was described as being very mild. No histopathological findings of any kind were noted at the medium or low exposures. Since the concentrations of smoke used were gross exaggerations (at least 100-fold) of any reasonable field situation for ETS (Guerin et al., 1992), we conclude that ETS is unlikely to have any significant toxicological activity in humans. The NOEL for the 90-day study, based again on nasal histopathology, is at least 1 mg/m^3.

REFERENCES

Ayres, P. H., Mosberg, A. T., and Coggins, C. R. E. 1990. Modernization of nose-only smoking machines for use in animal inhalation studies. *J. Am. Coll. Toxicol.* 9:441–446.

Baker, R. R., and Proctor, C. J. 1990. The origins and properties of environmental tobacco smoke. *Environ. Int.* 16:231–245.

Baumgartner, H., and Coggins, C. R. E. 1980. Description of a continuous smoking inhalation machine for exposing small animals to tobacco smoke. *Beit. Tabakforsch. Int.* 10:169–174.

Burger, G. T., Renne, R. A., Sagartz, J. W., Ayres, P. H., Coggins, C. R. E., Mosberg, A. T., and Hayes,

A. W. 1989. Histologic changes in the respiratory tract induced by inhalation of xenobiotics: physiologic adaptation or toxicity? *Toxicol. Appl. Pharmacol.* 101:521–542.

Chang, K.-M., Gentry, G., Davis, R., Stiles, M., and Coggins, C. R. E. 1992. Determination of plasma nicotine and cotinine in rats exposed to aged and diluted sidestream smoke, using an enzyme-linked immunosorbent assay (ELISA). *Toxicologist* 12:1003.

Chen, B. T., Bechtold, W. E., Barr, E. B., Cheng, Y.-S., Mauderly, J. L., and Cuddihy, R. G. 1989. Comparison of cigarette smoke exposure atmospheres in different exposure and puffing modes. *Inhal. Toxicol.* 1:331–347.

Coggins, C. R. E., Ayres, P. H., Mosberg, A. T., Ogden, M. W., Hayes, A. W., and Sagartz, J. W. 1992. Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. I. Inhalation toxicology; histopathology. *Fund. Appl. Toxicol.* 19:133–140.

Department of Health and Human Services. 1986. *The Health Consequences of Involuntary Smoke: A Report of the Surgeon General.* DHHS Publ. No. (PHS) 87-8398. Rockville, Maryland.

Feldman, D. B., and Seely, J. C. 1988. *Necropsy Guide: Rodents and the Rabbit,* pp. 1–50. Boca Raton: CRC Press.

Gori, G. B., and Mantel, N. 1991. Mainstream and environmental tobacco smoke. *Regul. Toxicol. Pharmacol.* 14:88–105.

Guerin, M. R., Jenkins, R. A., and Tomkins, B. A. 1992. *The Chemistry of Environmental Tobacco Smoke: Composition and Measurement.* Chelsea, Michigan: Lewis.

Langård, S., and Nordhagen, A.-L. 1980. Small animal inhalation chambers and the significance of dust ingestion from the contaminated coat when exposing rats to zinc chromate. *Acta Pharmacol. Toxicol.* 46:43–46.

Mercer, T. T., Tillery, M. I., and Newton, G. J. 1970. A multi-stage low flow rate cascade impactor. *Aerosol Sci.* 1:9–15.

Moss, O. R., Decker, J. R., and Cannon, W. C. 1982. Aerosol mixing in an animal exposure chamber having three levels of caging with excreta pans. *Am. Ind. Hyg. Assoc. J.* 43:244–249.

National Research Council. 1986. *Environmental Tobacco Smoke. Measuring Exposures and Assessing Health Effects.* Washington, DC: National Academy Press.

Organisation for Economic Co-Operation and Development. 1981. *Guidelines for Testing of Chemicals. No. 413, Repeated-dose inhalation toxicity: 90-day study.* Paris: OECD.

Ogden, M. W. 1989. Gas chromatographic determination of nicotine in environmental tobacco smoke: Collaborative study. *J. Assoc. Off. Anal. Chem.* 72:1002–1006.

Ogden, M. W., and Maiolo, K. C. 1992. Comparison of GC and LC for determining solanesol in environmental tobacco smoke. *LC/GC* 10:459–462.

R. J. Reynolds Tobacco Co. 1988. *Chemical and Biological Studies on New Cigarette Prototypes that Heat Instead of Burn Tobacco.* Winston-Salem, NC: R. J. Reynolds Tobacco Co.

Sagartz, J. W., Madarasz, A. J., Forsell, M. A., Burger, G. T., Ayres, P. H., and Coggins, C. R. E. 1992. Histological sectioning of the rodent larynx for inhalation toxicity testing. *Toxicol. Pathol.* 20:118–121.

Siegel, S. 1956. *Non-parametric Statistics for the Behavioral Sciences,* pp. 47–52. New York: McGraw-Hill.

Turner, S., Cyr, L., and Gross, A. R. 1992. The measurement of environmental tobacco smoke in 585 office environments. *Environ. Int.* 18:19–28.

von Meyerinck, L., Scherer, G., Adlkofer, F., Wenzel-Hartung, R., Brune, H., and Thomas, C. 1989. Exposure of rats and hamsters to sidestream smoke from cigarettes in a subchronic inhalation study. *Exp. Pathol.* 37:186–189.

Young, J. T. 1981. Histopathologic examination of the rat nasal cavity. *Fund. Appl. Toxicol.* 1:309–312.