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Fraunhofer Institute for Toxicology and Aerosol Research, Hannover, Federal Republic of Germany

Includes the Report of the Institute's Health Review Committee

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INVESTIGATORS' REPORT


Uwe Heinrich, Ulrich Mohr, Rainer Fuhst, and Carsten Brockmeyer

ABSTRACT

Syrian golden hamsters (480 males and 480 females) allocated into 24 groups were exposed 19 hours per day and 5 days per week for 6, 10.5, 15, or 18 months to total diesel exhaust, diesel exhaust without particles, a mixture of nitrogen dioxide (5 parts per million [ppm]) and sulfur dioxide (10 ppm), or clean air. Two exposure groups from each test atmosphere were also treated by a single subcutaneous injection of either 3 mg or 6 mg of diethylnitrosamine/kg of body weight to evaluate an enhancing effect of diethylnitrosamine on exposure-related changes. Morphological evaluation was done by histopathology. Minor changes of the larynx and trachea were investigated by scanning electron microscopy, which showed a loss of ciliated cells in all exhaust-exposed groups. After exposure to diesel exhaust with or without particles, focal metaplasia and dysplasia of the respiratory epithelium were seen in the oldest animals by scanning electron microscopy. In the same specimens, attached mucous droplets indicated changes in mucous cells and mucous viscosity.

Only the exposure to total diesel exhaust significantly increased the tumor rate in the upper respiratory tract of male hamsters treated with 6 mg of diethylnitrosamine per kg of body weight. At the lower diethylnitrosamine dose, no exposure-related effects on the tumor rates could be observed.

The results from this study and from our other inhalation experiments appear to be insufficiently conclusive to demonstrate that diesel-engine exhaust should be classified as a cocarcinogen or enhancer for the test system used.

INTRODUCTION

Diesel-engine-exhaust emissions contain a variety of trace components. Gaseous compounds like carbon monoxide (CO), oxides of nitrogen [nitric oxide (NO) and nitrogen dioxide (NO2)], and volatile hydrocarbons, or vaporized products of incomplete combustion like aldehydes, organic oxidants, and polycyclic aromatic hydrocarbons (PAHs), are actually common to all kinds of combustion-engine exhaust. In addition to these traces, however, diesel engines characteristically produce and discharge carbonaceous particles and, to a small extent depending on the quality of the fuel, sulfur dioxide (SO2). Some of the less volatile PAHs are known carcinogens and adsorb easily, along with other vaporized organics of low volatility, on the surface of the diesel soot particles. There are suggestions from trends in epidemiologic investigations (Heinrich et al. 1983) and indications from laboratory experiments (Heinrich et al. 1982, 1986; Brightwell et al. 1986; Ishinishi et al. 1986; Iwai et al. 1986; Mauderly et al. 1986) that exposure to diesel soot might increase the risk of lung cancer in humans. Recently, long-term inhalation studies on rats have shown that diesel-engine exhaust, if inhaled in sufficiently high concentrations, induces lung tumors (bronchioloalveolar adenomas, adenocarcinomas, and squamous cell tumors) in rats, but not in hamsters (Brightwell et al. 1986; Heinrich et al. 1986).

These hamster results confirmed findings of an earlier long-term inhalation study (Heinrich et al. 1982) in which we did not observe any carcinogenic effects of diesel exhaust in Syrian golden hamsters; only female Syrian golden hamsters were used in that study. However, in satellite groups of hamsters that received subcutaneous injections of diethylnitrosamine (DEN) during the early phase of that long-term study of diesel-exhaust exposure, there was a cotumorigenic effect, that is, an enhanced tumor induction. A 45 percent tumor rate in the upper respiratory tract of hamsters treated with 4.5 mg DEN/kg body weight (bw) was significantly increased when the animals were also exposed to either total diesel exhaust or filtered diesel exhaust containing no soot particles. However, using a DEN dose of 1.5 mg/kg bw, all test groups showed a tumor rate of 13 percent and no diesel-exhaust-related tumor-induction-enhancing effect could be observed.

These results apparently indicate that total diesel exhaust, as well as its gaseous components, facilitates an increase of the DEN-induced tumor rate. Data reported in the literature (Laskin et al. 1970; Dalbey 1982; Pauluhn et al. 1983) indicate that tumor induction in the respiratory tract of hamsters, caused by either DEN (upper airway papillomas) or benzo[a]pyrene (BaP) (upper airway carcinomas and papillomas), will be enhanced by the inhalation of irritant gases like NO2, SO2, ozone, and formaldehyde. Thus,

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2 A list of abbreviations appears at the end of this report for your reference.
Potential Cotumorigenic Effect of NO₂, SO₂, and Diesel-Engine Exhaust

after tumor initiation and induction by DEN, the promotion and the enhancement of the tumor incidence could have been affected either by the traces of vaporized organics of incomplete combustion or by the inorganic irritants.

AIMS

The specific aims of the study were (1) to determine if inhalation of total diesel-exhaust emissions enhances the carcinogenic effects of subcutaneously injected DEN in Syrian golden hamsters; (2) to determine if such enhancing effects are produced by inhalation of diesel-exhaust emissions from which the particulate fraction is removed; (3) to determine if such enhancing effects are produced by inhalation of a mixture of the irritant gases NO₂ (5 ppm) and SO₂ (10 ppm); and (4) to study any pathological effects on the respiratory tract of the exposed animals, whether or not they are related to neoplasia, that may result from inhalation of the above materials.

MATERIALS AND METHODS

ANIMALS AND HOUSING CONDITIONS

A total of 480 male and 480 female 10-week-old Syrian golden hamsters (Hoe: SYHK [SPF Ars]) were purchased from the breeder (Hoechst Pharmaceutical Company, Hattersheim, FRG) and used in this study. The animals were kept in four 6-m³ chambers and were exposed to four different test atmospheres: slightly diluted genuine diesel exhaust; the same diesel exhaust devoid of particulate matter; an NO₂/SO₂ trace gas mixture; and clean air.

Each chamber was occupied by three differently treated groups of 80 animals (Table 1). The number of hamsters used in each exposure group was based on the results of a previous study (Heinrich et al. 1982). Each of these groups included 32 animals that were assigned for scheduled sacrifices in order to facilitate an intermediate light and electron microscopic inspection of the respiratory tract, predominantly for preneoplastic lesions. According to the original research proposal, four sacrifices of eight animals per group and date were scheduled after six, nine, 12, and 15 months of exposure (the animals were assigned before treatment was initiated). However, a mean animal lifetime shorter than expected, primarily in the females, forced a reduction to only three sacrifices after 6, 10.5, and 15 months.

During the exposure, the animals were kept in stainless-steel wire-mesh cages with excreta trays supplied with wood-chip bedding. The bottoms of the cages were partly covered with a solid polyvinylchloride plate, in order to improve the housing conditions of the animals. Each cage (37.5 cm long × 21 cm wide) was subdivided into four compartments by wire-mesh partitions. Each animal was kept in a separate compartment.

Beginning at noon, the animals were exposed for 19 hours per day, 5 days per week, for up to 18 months. They were weighed every four weeks. After 18 months, the female hamsters had reached a mortality rate of nearly 90 percent, and the exposure was terminated for males and females.

At the end of the 18-month exposure period, the surviving hamsters were transferred individually to polycarbonate (Macrolon®) cages containing wood-chip bedding. The animals were then kept in clean air for up to six months prior to being killed.

The exposure chambers and animal rooms were maintained at 22° to 24°C and 40 to 60 percent relative humidity, under a 12-hour light cycle starting at 7 a.m. Food (RMHGS, Hope Farms, Woerden, The Netherlands) and water were available ad libitum throughout the exposures.

Animals from all groups were killed if they were found in a moribund condition.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NO₂/SO₂ Mixture (5 ppm/10 ppm)</th>
<th>Total Diesel Exhaust</th>
<th>Diesel Exhaust Without Particles</th>
<th>Clean Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mg DEN²/kg bw</td>
<td>48 + 32b</td>
<td>48 + 32</td>
<td>48 + 32</td>
<td>48 + 32</td>
</tr>
<tr>
<td>3 mg DEN²/kg bw</td>
<td>48 + 32</td>
<td>48 + 32</td>
<td>48 + 32</td>
<td>48 + 32</td>
</tr>
<tr>
<td>Without DEN treatment</td>
<td>48 + 32</td>
<td>48 + 32</td>
<td>48 + 32</td>
<td>48 + 32</td>
</tr>
</tbody>
</table>

² DEN was administered subcutaneously.

b From each group, 32 animals were assigned to serial sacrifice.
DIESEL-EXHAUST EXPOSURE

The diesel-exhaust emission used for this study was generated by a 2.4-liter Daimler-Benz engine mounted on a test bench and connected directly to an eddy-current-type brake. The engine was operated at a constant load of about 15 kW and a uniform speed of about 2,000 rpm. The fuel used in the engine was a European reference fuel with a sulfur content of about 0.36 percent by weight. A guide channel blower drew the exhaust from the tail pipe into the mixing boxes. The stainless-steel pipe delivering the exhaust to the mixing boxes was heated and insulated to prevent the condensation of exhaust components in the conduit. Down the duct, the delivery pipe was divided into two lines. One line connected directly to a mixing box above the inhalation chamber, and the other line fed the exhaust first through a filter device (FP-65 HT 610, Luwa, Frankfurt/M, FRG) heated to 80°C to remove the soot particles before entry into the mixing box. In the mixing boxes, both total and filtered exhaust were diluted with filtered (FP-85, Luwa; NS-22, Luwa), dry, cooled air at an exhaust:clean-air ratio of about 1:13. The exhaust devoid of soot particles passed through a backup filter system (FP-85, Luwa; NS-30, Luwa; and CH63302 Dräger, Lübeck, FRG) before it entered the inhalation chamber.

The diluted diesel exhaust moved (Figure 1) from the upper-left entry port through a compartment connected to the inhalation chamber by a perforated wall. After penetrating this diaphragm, the flow continued horizontally across the chamber and exited through another perforated wall on the opposite side, into a spent-air pipe ('exhaust,' Figure 1). The perforated diaphragms provided a horizontal air flow and facilitated an even distribution of the soot particles in the chamber and, thus, a uniform soot burden for the animals.

Several exhaust components (CO, carbon dioxide [CO₂], SO₂, NO, NO₂, methane, and total hydrocarbons) were measured simultaneously and continuously by means of a computer-operated compact measuring cabinet (UPK, Bad Nauheim, FRG). The average diesel soot concentration was determined by gravimetric measurements of samples using Sartorius (Göttingen, FRG) glass-fiber filters (SM 13400, 47-mm diameter). Some particle-associated PAHs were measured in batches. The method of extracting PAHs from the particles, and the measuring procedure by gas chromatography, were published elsewhere (König et al. 1981).

The average concentration of some gaseous exhaust components, and the mass concentrations of the particulate matter measured in the inhalation chambers during the whole exposure time, are shown in Table 2. By comparison, the concentrations for CO₂, CO, and NO in the chamber with diesel exhaust devoid of soot particles are somewhat lower than for the chamber with unfiltered diesel exhaust. The chamber with diluted total diesel exhaust contained approximately 4 mg soot/m³.

The mass median aerodynamic diameter of the diesel soot particles was measured by means of a 10-stage low-pressure impactor (model LPI 30/006, Hauke KG, Gmunden, Austria). A value of 0.16 ± 0.06 (mean ± SD) μm was obtained in the exposure chamber.

The concentrations of some PAHs adsorbed on diesel-exhaust particles are listed in Table 3. The concentrations of BaP and benz[a]pyrene (BeP) measured in the exposure atmosphere with diluted total diesel exhaust were approximately 37.5 and 61.9 ng/m³, respectively.

EXPOSURE TO A NITROGEN DIOXIDE/ SULFUR DIOXIDE MIXTURE

The NO₂ and SO₂ were obtained from gas cylinders containing 99.5 percent dinitrogen tetroxide (N₂O₄) or 99.98
Potential Cotumorigenic Effect of NO₂, SO₂, and Diesel-Engine Exhaust

Table 2. Some Gaseous Compounds and Total Particulate Matter of the Various Exposure Atmospheres

<table>
<thead>
<tr>
<th>Compound</th>
<th>Unit of Measure</th>
<th>Clean Air</th>
<th>NO₂/SO₂</th>
<th>Diesel Exhaust Without Particles</th>
<th>Total Diesel Exhaust</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>Vol %</td>
<td>0.08 ± 0.02³</td>
<td>0.07 ± 0.02</td>
<td>0.27 ± 0.07</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>CO</td>
<td>ppm</td>
<td>—</td>
<td>—</td>
<td>9.4 ± 2.3</td>
<td>15.7 ± 3.7</td>
</tr>
<tr>
<td>NO</td>
<td>ppm</td>
<td>—</td>
<td>—</td>
<td>9.1 ± 2.5</td>
<td>16.0 ± 3.3</td>
</tr>
<tr>
<td>NO₂</td>
<td>ppm</td>
<td>—</td>
<td>4.5 ± 0.7</td>
<td>1.1 ± 0.2</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>SO₂</td>
<td>ppm</td>
<td>—</td>
<td>9.6 ± 1.2</td>
<td>1.3 ± 0.9</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>C₇H₸²⁶</td>
<td>ppm</td>
<td>4.5 ± 0.5</td>
<td>4.2 ± 0.7</td>
<td>7.2 ± 1.2</td>
<td>7.5 ± 1.4</td>
</tr>
<tr>
<td>CH₄⁶</td>
<td>ppm</td>
<td>3.3 ± 0.4</td>
<td>3.1 ± 0.4</td>
<td>4.2 ± 0.5</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>Particles</td>
<td>μg/m³</td>
<td>88 ± 52</td>
<td>119 ± 111</td>
<td>97 ± 47</td>
<td>3,750 ± 650</td>
</tr>
</tbody>
</table>

³ Values are given with SD.

Table 3. Concentration of Some Polycyclic Aromatic Hydrocarbons Adsorbed onto Diesel Exhaust Particles

<table>
<thead>
<tr>
<th>PAH</th>
<th>μg PAH/g Particle³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibenz[a]anthracene</td>
<td>28.3 ± 14.3</td>
</tr>
<tr>
<td>Chrysene</td>
<td>53.0 ± 19.0</td>
</tr>
<tr>
<td>Benzo[a]fluoranthene</td>
<td>42.8 ± 11.2</td>
</tr>
<tr>
<td>Benzo[k]pyrene</td>
<td>16.5 ± 4.1</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>10.0 ± 5.5</td>
</tr>
<tr>
<td>Indeno[123-cd]pyrene</td>
<td>12.0 ± 3.1</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>15.1 ± 4.8</td>
</tr>
<tr>
<td>Coronene</td>
<td>6.0 ± 1.7</td>
</tr>
</tbody>
</table>

³ Values are given with SD.

At the outset of the study, a high ammonia concentration occurred in the chambers, leading to the formation of ammonium sulfate and ammonium nitrate, predominantly in the NO₂/SO₂ exposure chambers. This effect was eliminated by (1) increasing the air changes in all chambers from about 12 to approximately 22 times per hour; (2) changing the urine-collection trays three times a week and the cages and racks once a week; and (3) cleaning the exposure chamber every other day.

With these measures, the ammonia concentrations could be kept below an average of 1 ppm. The highest ammonia concentration was then 0.8 ± 0.59 ppm, measured in the clean-air chamber, and the lowest concentration was 0.05 ± 0.02 ppm in the NO₂/SO₂ chamber. In the latter case, the low concentration of gaseous ammonia was caused by...
the reaction of NO₂ and SO₂ with ammonia to form particles of ammonium sulfate and ammonium nitrate.

The average concentrations of NO₂ and SO₂ in the irritant gas atmosphere were 4.5 ± 0.7 ppm and 9.6 ± 1.2 ppm, respectively.

**DIETHYL-NITROSAMINE TREATMENT**

After approximately two weeks of exposure, two groups of 80 animals in each exposure chamber were treated once by subcutaneous injection of 3 and 6 mg DEN/kg bw, respectively (Table 1). The decision to employ two dose levels of DEN was based on the fact that, in the previous study (Heinrich et al. 1982), the tumor increase by long-term diesel exposure, in comparison to the basic DEN-induced tumor rate, was statistically significant only after raising the DEN dose from 1.5 to 4.5 mg/kg bw.

Diethylnitrosamine (purity greater than 99 percent by gas chromatography, Fluka GmbH, Neu-Ulm, FRG) was diluted with a 0.9 percent saline solution and injected subcutaneously into the side of the hamsters when they were lightly anesthetized with halothane. The injection cannula was pushed as far as possible into the scapula before a volume of about 0.05 ml/10 g bw was injected. To prevent reflux of the injected solution, slight pressure was exerted with the fingertips on the syringe needle and the skin of the injection site, particularly when withdrawing the syringe.

**HISTOPATHOLOGY**

The larynx and two-thirds of the attached proximal trachea of each sacrificed animal were removed. The tracheas were fixed by immersion in Karnovsky's fixative diluted to 440 mOsm (pH 7.2 to 7.4; 22°C) after the lumen had been flushed by syringe with fixative. After midsagittal section, the mucosal surfaces of both halves were examined using a dissecting microscope. One half was then processed for scanning electron microscopy, and the other for light microscopy.

The sample used for scanning electron microscopy investigation was rinsed for one hour in cacodylate buffer (pH 7.2; 0.15 M; without other additives). After passing the increasing alcohol and freon row, the specimens were dried in freon by the "critical point method" and fixed on studs with Leitcarbone (Gökke, Münster, FRG). They were then sputtered with gold (SCD 030, Balzers, Liechtenstein), and the total sample was examined with a scanning electron microscope using 40 kV (PSEM 500, Philips, Eindhoven, The Netherlands).

The findings of the scanning electron microscopy investigations were noted on an extra protocol sheet for each speci- men, and the distribution patterns of the ciliated cells in the trachea were documented on a low-magnification micrograph or on a sketch.

Excised lungs were fixed by infusion with diluted Karnovsky's fixative at 25-cm fluid pressure (20°C). The trachea was ligated distally to the end of the cannula after fixation under pressure for three hours, and the lungs were stored in fixative until processed. Blocks of lung for morphological examination were sampled as follows:

1. **Left lung.** Vertical transverse sectioning at the center of the craniocaudal axis provided one block for light microscopy, and the complementary face provided a block for scanning electron microscopy.
2. **Right lung.** Sectioning of the right caudal lobe in a vertical plane along the axis of the main bronchus provided a slice that was divided vertically into two blocks for light microscopy. The complementary slice provided two blocks for scanning electron microscopy; a 1-mm-wide slice of tissue for transmission electron microscopy was taken where the slice for scanning electron microscopy had been divided into two before being processed.

Pertinent pulmonary changes were graded to facilitate the establishment of correlations between lesions, pathological changes, and dose groups. The terms "mild" to "marked" apply to this study only and should not be used with other studies. Pneumoconiosis, in the context of this study, refers to the incorporation of carbonaceous soot particles into the lungs. The amount of this particulate matter in the lungs was estimated as not present (0), mild (1), moderate (2), or marked (3). "Mild" was defined as less than 33 percent of alveoli containing soot particles or macrophages laden with particles. "Moderate," similarly, was defined as 34 to 65 percent of alveoli, and "marked" as 66 percent or more of the alveoli. For the categories mild to marked, alveoli containing less than three particles or particle-laden macrophages, each less than 10 μm in greatest dimension, were excluded from the estimation.

The term "pneumonitis" was used in this study to indicate a thickening of the pulmonary alveolar walls greater than 15 μm. The extent of pneumonitis was graded as none (0), mild (1), moderate (2), or marked (3). "Mild" pneumonitis was defined as affecting 3 to 19 percent of alveoli. "Moderate" indicated that 20 to 40 percent of alveolar walls were involved, and "marked" pneumonitis affected over 40 percent of alveolar walls. Finally, the proliferation of bronchiolar-type epithelium in alveoli adjacent to bronchioles was graded as not present (0), mild (1), moderate (2), or marked (3). The degree of change was estimated from the number of cells lining alveoli around the average bronchiole, and was graded as mild, 5 to 19 cells; moderate, 20 to 40 cells; or marked, more than 40 cells.
The organs taken from dead or moribund sacrificed animals were nasal cavity, sinuses, larynx, trachea, esophagus, thyroid, heart, spleen, lungs, stomach, liver, kidney, adrenal gland, and urinary bladder. Other than the lungs, which were fixed by infusion, all organs were fixed by immersion in 10 percent buffered formalin. Histological sections stained with hematoxylin-eosin were prepared mainly from the respiratory tract (nasal cavity, larynx, trachea, lung).

Tissue samples from each lung lobe were carefully trimmed and then sectioned at two different levels. A total of two to three sections were prepared for each lung lobe. The tracheas were divided in half longitudinally, and before further histological preparation was started, these trachea halves were examined under the dissecting microscope for tumors. A histological section was prepared from each of the two halves. Four histological sections were prepared from the nasal cavity and three from the larynx.

STATISTICAL ANALYSIS

Only the tumor rates in the upper respiratory tract of DEN-treated control and exposed animals were analyzed statistically. This was done by comparing the contingency table and the $\chi^2$ statistics. Also, life-table analysis of respiratory tract tumor incidence and estimates of survival distributions by the product-limit method (Kaplan and Meier 1958) was used; the generalized Wilcoxon (Breslow) statistic was used for testing the equality of survival curves (Dixon 1981). Differences between groups were considered statistically significant at $p<0.05$.

RESULTS

HEALTH STATUS OF THE ANIMALS

The body weight development in all groups was typical for Syrian golden hamsters kept in wire-mesh cages (Heinrich et al. 1982, 1986). The males showed a 15 to 20 percent increase in weight within six to nine months, but this weight increase disappeared in the subsequent nine to 12 months. In females, a 10 to 15 percent increase in body weight was observed after one to one-and-a-half months, but from then on, their weight decreased continuously and showed values clearly below their body weight at the beginning of the experiment. No exposure-related differences could be found by comparing the various exposure groups with the clean-air-exposed control group.

SURVIVAL TIME

The 50 and 75 percent survival time was lower in females than in males in all treatment and exposure groups. In clean-air-exposed control animals not treated with DEN, this gender-specific difference in survival time amounted to about 10 weeks: in males, mortality levels of 50, 75, and 100 percent were reached after 70, 83, and 102 weeks, respectively; in females these levels were reached after 62, 72, and 92 weeks, respectively (Figures 3 and 4, Appendix A). The shorter lifetime of the female hamster compared to the male is well known, and is probably caused by the kidney amyloidosis that is more pronounced and occurs at an earlier age in females than in males.

Slightly shortened survival times were seen in total-diesel-exhaust- and NO$_2$/SO$_2$-exposed animals (Figures 3 and 4), but this effect was meaningful only for NO$_2$/SO$_2$-exposed females. However, no statistically significant differences at the 5 percent level were found when we compared the survival curves of females exposed to the various chamber atmospheres and treated with the same dose of DEN. This result also held true for the males. But, as already mentioned, the survival curves of males and females were significantly different.

HISTOPATHOLOGICAL AND SCANNING ELECTRON MICROSCOPY FINDINGS IN THE RESPIRATORY TRACT AFTER VARIOUS EXPOSURES TO NO$_2$/SO$_2$ GAS OR DIESEL EXHAUST

Table 4 shows the disposition of all animals by group; Table 5 summarizes the exposure atmospheres and DEN treatments.

For the six-month serial sacrifice, four males and four females from each experimental group were investigated. After 10.5 months, four males and three females were killed in all but one group. In the NO$_2$/SO$_2$ group not treated with DEN, only two out of four males designated for this scheduled sacrifice date were still alive and could be used for this investigation. After 15 months of exposure, at least one, usually two, and in some groups even three or four animals

Figure 3. Mortality rate of male hamsters [without additional treatment].
were still available for the scheduled sacrifice. There were no animals still alive for the 15-month sacrifice in two groups of females (NO₂/SO₂ and NO₂/SO₂ + 3 mg DEN/kg bw).

**Larynx and Trachea**

**Histopathology.** Only one clearly exposure-related lesion was found histopathologically. A papilloma was observed in the trachea of one hamster exposed to a particle-free phase of diesel exhaust and also treated with DEN. Other lesions of the trachea and larynx were limited to local distension of submucosal glands with inspissated secretion and focal infiltrates of lymphocytes. These findings were observed to about the same extent in control animals as in test animals, and are therefore thought to occur spontaneously.

**Scanning Electron Microscopy Investigations After Six Months of Exposure.**

**Exposure to Clean Air.** The larynx and trachea of the clean-air-exposed control animals were lined with epithelial cells with cilia or microvilli. The trachea, in particular, showed

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**Table 4. Disposition of All Animals by Group**

<table>
<thead>
<tr>
<th>Exposure and DEN Treatment</th>
<th>Number of Animals in Main Group</th>
<th>Number of Animals in Serial Sacrifice Group</th>
<th>Died Spontaneously 6 Months</th>
<th>10.5 Months</th>
<th>15 Months</th>
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<tbody>
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<td>Clean air</td>
<td>Gender</td>
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<td>Investigated Histologically</td>
<td>16</td>
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<td>F</td>
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<tr>
<td>Particle-free diesel exhaust + 3 mg/kg bw</td>
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*Includes animals from the serial sacrifice groups that died spontaneously before the scheduled sacrifice date.*
Table 5. Summary of Tumor Data for the Animals Available for Histological Investigation

<table>
<thead>
<tr>
<th>Exposure and DEN Treatment</th>
<th>Gender</th>
<th>Total</th>
<th>No. of Animals Died/Killed</th>
<th>Tumor-Bearing Animals Died/Killed</th>
<th>Tumor Type^a/Location^b</th>
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<td>16/14</td>
<td>2/4</td>
<td>P/3 XL, 3 XT</td>
</tr>
</tbody>
</table>

^a P = papilloma; C = carcinoma; A = adenoma.
^b N = nasal cavity; L = larynx; T = trachea.

A typical distribution pattern of noncornified ciliated or microvilli-covered cells (Figure 5). In addition, cornified cells with some desquamation were present in the epiglotal area. Hamsters that were subcutaneously injected with 3 mg DEN/kg bw had a slight increase in cilia-free areas of the trachea, in comparison with the control animals. The number of mucous cells was increased.

An injection of 6 mg DEN/kg bw caused a further increase of cilia-free areas in the trachea. In one of four females, two large protrusions of the tracheal mucosa were present (Figure 7). The injection of 6 mg DEN/kg bw produced no further changes.

**Exposure to Diesel Exhaust Without Particles.** Compared with the control animals, the inhalation of filtered diesel exhaust led to a decrease of ciliated cells and low mucous cell hyperplasia in the tracheas of all hamsters. Mucous drops (Figure 8) covered the entire trachea. In contrast to the animals of the NO₂/SO₂ group, the cilia were not stuck together. Increased mucous cell hyperplasia occurred in hamsters given DEN at both doses.

**Exposure to Total Diesel Exhaust.** Focal loss of cilia and mucous cell hyperplasia occurred in the tracheas of hamsters exposed to total diesel exhaust. Mucous cell hyperpla-
Sia was more pronounced than with the NO\textsubscript{2}/SO\textsubscript{2} mixture. In the region of the cilia-free respiratory epithelium, small particles, possibly soot (Figure 9), became visible. Mucous drops were deposited in the entire trachea. In the trachea of one of four females injected with 3 mg DEN/kg bw, a “giant cell” (Figure 10) was observed. One of four females treated with 6 mg DEN/kg bw had slight elevations in the vestibular epithelium (Figure 11).

**Scanning Electron Microscopy Investigations After Ten-and-a-Half Months of Exposure.**

**Exposure to Clean Air.** In comparison with the findings for the control animals at six months, the number of ciliated cells in the trachea was reduced. Marked increase of mucous cells occurred in the cranial part of the trachea. Large epithelial protrusions were observed in the trachea of one of three females. Another animal had a slight elevation in the vocal cord area (Figure 12).

Because of the injection of 3 mg DEN/kg bw, the decrease in ciliated cells was more pronounced, and the surface of the mucous cells protruded into the lumen of the trachea. An exophytic neoplasia, surrounded by an area of mucous cell hyperplasia, was detected in the trachea of one of three females (Figure 13). Five out of seven specimens from animals injected with 6 mg DEN/kg bw could not be evaluated, in part due to incomplete fixation. One animal had a pleomorphic, possibly dysplastic, area in the vocal cord region (Figure 14).
Figure 9. High magnification of the tracheal epithelium of a hamster exposed to diesel exhaust for six months. Small particles (arrows) are numerous in cilia-free regions. Magnification = ×5,000.

Figure 10. High magnification of the tracheal epithelium of a hamster exposed to diesel exhaust for six months, with an intermediate injection of 3 mg DEN/kg bw. Note a 'giant' cell, which was probably produced by the fusion of several neighboring cells. Magnification = ×2,500.

Figure 11. Vestibular epithelium of a hamster exposed to diesel exhaust for six months, with an intermediate injection of 6 mg DEN/kg bw. The arrow points to a slightly elevated portion of the mucosa. Magnification = ×160.

Figure 12. Cornified epithelium of vocal cord area of a hamster exposed to clean air for 10.5 months, showing cells with irregular microvilli in a slightly elevated position. They are possibly leukocytes. Magnification = ×2,500.

Figure 13. Exophytic neoplasia in the trachea of a hamster exposed to clean air for 10.5 months, with an intermediate injection of 3 mg DEN/kg bw. The surrounding epithelium is covered with mucus. Magnification = ×160.

Figure 14. Vocal cord epithelium of a hamster exposed to clean air for 10.5 months, with an intermediate injection of 6 mg DEN/kg bw. The epithelium shows enlarged cells with smooth surface slightly elevated above the epithelial surface level. Magnification = ×320.
Exposure to Nitrogen Dioxide/Sulfur Dioxide. Extensive mucous deposits were present, indicating a mucous cell hyperplasia that was more obvious than in the six-month exposure animals. The cilia were stuck together. Three out of seven hamsters given 3 mg DEN/kg bw had slight elevations in the larynx similar to those shown in Figure 12; two were in the vocal cord area and one in the supraglottis.

Two out of three females injected with 6 mg DEN/kg bw had laryngeal metaplasia and dysplasia in the vocal cord area, and one of these females also showed an exophytic neoplasia in the supraglottic area. For the first time in the NO$_2$/SO$_2$ groups, pleomorphic surface areas (Figure 15) were observed in the tracheas of two of seven hamsters.

Exposure to Diesel Exhaust Without Particles. The mucosa of the vocal cord was more cornified than in the animals of the corresponding group at six months of exposure. Loss of cilia and increase in mucous cells were present, but the extent of these changes cannot be differentiated from those in the 10.5-month NO$_2$/SO$_2$-exposed group. There was a general deposit of mucous drops, as was seen in the group exposed to diesel exhaust without particles for six months. In one of four males, epithelial protrusions were present similar to those shown in Figure 7.

In hamsters given 3 mg DEN/kg bw, a further increase in mucous cells and loss of ciliated cells were observed. One of three female hamsters showed a dysplastic lesion of the trachea, and a slight elevation in the vocal cord area was observed in the trachea of another female.

In animals injected with 6 mg DEN/kg bw, dysplasia was generally not found in the larynx, in contrast to the 10.5-month NO$_2$/SO$_2$-exposed group.

A mass of epithelial cells that was, in our opinion, an exophytic neoplasia was seen in the subglottis of one female (Figure 16).

Exposure to Total Diesel Exhaust. In comparison with the control animals and the six-month diesel-exhaust-exposed group, the vocal cords appeared to be more severely cornified. Loss of ciliated cells and increase in mucous cells were observed to the same extent as in the corresponding groups exposed to NO$_2$/SO$_2$ and particle-free diesel exhaust. Deposits of mucous drops and more particles similar to those shown in Figures 8 and 9 occurred.

Five out of seven animals injected with 3 mg DEN/kg bw showed, in addition to an increase in mucous cells and loss of ciliated cells in the trachea, focal pleomorphic or dysplastic changes in the glottis and subglottis. Two animals could not be evaluated because of incomplete fixation.

After an additional injection of 6 mg DEN/kg bw, no further increase in the effects was determined. The number of observed pleomorphic surface changes and dysplastic lesions was also reduced, in comparison with the 3-mg-DEN group of the same age.

Scanning Electron Microscopy Investigations After Fifteen Months of Exposure.

Exposure to Clean Air. In the trachea, the number of mucous cells had further increased. They clearly protruded farther into the lumen than at 10.5 months.

One of three males injected with 3 mg DEN/kg bw had exophytic neoplasia of the trachea. Two of five animals injected with 6 mg DEN/kg bw had cellular masses that were
diagnosed as exophytic neoplasia, one in the subglottis and one in the trachea. The mucosa in the area surrounding these lesions always showed pleomorphic surface areas.

**Exposure to Nitrogen Dioxide/Sulfur Dioxide.** The number of ciliated cells was further reduced, in comparison with the corresponding 10.5-month NO₂/SO₂-exposed group. There was mucous cell hyperplasia, and the number of plant particles present in the trachea was increased. Apart from these changes, one of three male animals injected with 3 mg DEN/kg bw had metaplasia in the glottis. Exophytic neoplasia occurred in the subglottis of one of two males, and epithelial protrusions were seen in the trachea of one female given 6 mg DEN/kg bw.

**Exposure to Diesel Exhaust Without Particles.** Focal or extensive mucous cell hyperplasia and focal metaplasia were present in the tracheas of all animals. A slight elevation similar to that shown in Figure 7 was found in the vocal cord area of one of two females.

In addition, cellular masses (Figure 17), which we assumed reflected exophytic neoplasia, were detected in the trachea of one of two males following injection of 3 mg DEN/kg bw. The surrounding epithelium partly showed cells with pleomorphic surface areas.

Metaplasia and dysplasia had spread through the tracheas of hamsters injected with 6 mg DEN/kg bw. In two of six animals, cellular masses, which we assumed reflected exophytic neoplasia, were observed in the subglottis of the larynx. One of four females had exophytic neoplasia of the trachea. All these exophytic epithelial masses were surrounded by cells with pleomorphic surface areas.

**Exposure to Total Diesel Exhaust.** Focal metaplasia, and focal or extensive mucous cell hyperplasia, were present in the tracheas of all animals. Deposits of mucous drops and small particles were found. Metaplasia was present in the glottis of one of five hamsters (Figure 18). Dysplasia occurred in the trachea of the one female injected with 3 mg DEN/kg bw. Treatment with 6 mg DEN/kg bw led to no further recognizable increase in effects.

**Summary.** In summary, in addition to the already macroscopically diagnosed papillomas, we observed minor changes in the tracheal mucosa. In general, pleomorphic surface areas, like metaplasia and dysplasia, surrounded the observed exophytic neoplasias. Slight elevations of the mucosa occurred in both genders in almost all groups. They were covered with flat microvilli-bearing cells and may be signs of degenerative alterations.

The inhalation of NO₂/SO₂ irritant gas, in comparison with clean air, caused a greater decrease in the number of ciliated cells than was the case after a DEN injection. The cilia of the ciliated epithelium stuck together, and the number of mucous cells increased. The intermediate administration of 6 mg DEN/kg bw induced pleomorphic surface areas in the trachea of one of seven six-month-old hamsters. The inhalation of particle-free or total diesel exhaust caused more pronounced alterations in the tracheal mucosa. Although the loss of cilia was similar to that in the corresponding NO₂/SO₂-exposed groups, there were more mucous drops covering the tracheal epithelium in all hamsters. Metaplasia and focal dysplasia occurred in complete and filtered diesel exhaust atmospheres after a 15-month inhalation period, even in the lower DEN-dose groups. After the inhalation of total diesel exhaust, small particles, possibly soot particles, reformed as a deposit on the mucosa.
Lungs

**Histopathology After Six Months of Exposure.** The lungs from clean-air-exposed control animals treated with DEN were indistinguishable from those from clean-air-exposed control animals without DEN treatment. After six months, the only clear effect was observed in lungs exposed to the total exhaust. They contained large numbers of carbonaceous particles that usually occurred in loose or dense aggregates. Most of the aggregates could not be recognized as being within macrophages, although there were often sharp boundaries around the aggregates, and sometimes associated cell debris indicated that phagocytosis had often occurred. Where the particles were fewer, they could be seen within the cytoplasm of foamy alveolar macrophages.

The distribution of particles was not uniform. Most were in alveolar lumens, and the heaviest accumulations tended to be in alveoli adjacent to alveolar ducts or pulmonary veins. They were especially common between bifurcations of alveolar ducts and the centrally placed blood vessels. Clusters of particles were also present in the interstitium adjacent to bronchioles and vessels, but this was far less extensive than the intraalveolar distribution.

There was relatively little reaction to the particles, and what reaction there was occurred inconsistently. The alveolar response was minimal: a slight increase in numbers of type II cells in a few regions and an occasional sprinkling of polymorphonuclear and mononuclear cells. Often alveoli containing dense clumps of particles appeared normal. There was rare good evidence of interstitial fibrosis. Occasionally, a minimal amount was present in the pleura, or associated with perivascular-peribronchiolar accumulations of particles. Bronchiolar epithelial responses were difficult to detect. The epithelium of the bronchioles themselves appeared slightly thickened and Clara cells were more prominent; only occasionally was there a lining of a few alveoli adjacent to terminal bronchioles with bronchiolar epithelium.

No difference could be detected between the total exhaust alone and the exhaust with DEN.

The lungs of animals exposed to particle-free exhaust, with or without DEN treatment, showed no changes in comparison with control animals.

The lungs of animals exposed to NO2 and SO2, with or without DEN treatment, had a minimal lesion consisting of one to several very small foci in each section. The lesion displayed a few alveoli adjacent to terminal bronchioi, which were lined with bronchiolar epithelium, and usually several pigment-containing macrophages were visible in the affected lumens.

**Histopathology After Ten-and-a-Half Months of Exposure.** The lungs from clean-air-exposed control animals showed no significant lesions, whether or not they were treated with DEN.

The lungs of hamsters exposed to total diesel exhaust for 10.5 months showed clusters of carbonaceous particles that were more numerous and larger than those found after six months of exposure. There were also more particles accumulated in the pulmonary interstitium and in macrophages. In heavily affected areas, there was an associated thickening of alveolar septa. Type II cells showed a slight increase in number, with thickening of the interstitium. No differences were seen between lungs exposed to total diesel exhaust and those also treated with DEN.

The lungs of hamsters exposed to particle-free diesel exhaust, with or without additional treatment with DEN, showed no changes in comparison with control animals.

The lungs of hamsters exposed to NO2/SO2 gas showed minimal changes in a few alveoli adjacent to terminal bronchioles. These alveoli were lined by bronchiolar epithelium and usually contained several pigment-loaded macrophages. This centriacinar change was more prominent than in the six-month exposure group.

**Histopathology After Fifteen Months of Exposure.** The soot particles in the lungs of hamsters exposed to total diesel exhaust for 15 months were found in aggregates of various sizes within alveoli, and especially in alveoli near bronchi. The particles were also more numerous surrounding blood vessels and in foci just under the pleura. The vast majority of particles were neither surrounded nor engulfed by macrophages. Some accumulations of macrophages containing fine black pigment were observed, especially near bronchioles. Occasionally, small groups of macrophages appeared to form spherical cell masses within alveoli. The particulate matter, while varying somewhat in amount from animal to animal, was generally more plentiful in the 15-month-exposed animals than in those exposed to total exhaust for 10.5 months.

Inflammatory reaction to the total exhaust was not prominent. There were few inflammatory cells present other than macrophages. There was, however, an interstitial pneumonitis of a focal nature, which varied in degree from mild to marked. The reaction was characterized by a thickening of alveolar septa, which were lined by enlarged alveolar cells, and a diminution of alveolar lumina containing soot and macrophages. In some of the foci, early interstitial fibrosis was suspected. The degree of pneumonitis appeared to be directly related to the number of particles and was more advanced in the animals exposed for 15 months than in those exposed for 10.5 months. There was no obvious difference
between animals exposed to total exhaust with doses of DEN and without DEN.

The lungs of hamsters exposed to particle-free diesel exhaust for 15 months, with or without additional treatment with DEN, were not distinguishable from those of the clean-air-exposed control animals.

The lungs of hamsters exposed to atmospheres of NO$_2$/SO$_2$ gas for 15 months, with or without additional treatment with DEN, did not differ from those of the control animals, except in one respect: the presence or degree of centriacinar proliferative lesions. All animals exposed to NO$_2$/SO$_2$ had alveoli adjacent to terminal bronchioles lined with bronchiolar-type epithelium. Some of these affected areas also contained a few pigmented macrophages. The degree of centriacinar change was marked in seven animals, moderate in one, and mild in another. The lesions appeared to be progressive, as most animals in this group were more severely affected than those exposed for 10.5 months.

The centrilobular proliferative change described in NO$_2$/SO$_2$-exposed hamsters was also observed in animals in other groups, including the control group, after an experimental time of 15 months. In clean-air-exposed control animals, there was one moderately affected hamster and 11 mild cases. Four animals had no lesions. Hamsters exposed to particle-free exhaust showed a similar degree of change to that seen in the control hamsters: 12 animals had mild changes and four had no lesions. All hamsters exposed to total diesel exhaust had similar lesions; they were of only a mild nature in 11 animals, but were marked in two animals. There was no obvious correlation between the degree of pneumoconiosis or pneumonitis and centrilobular proliferative change in the latter group, but in the NO$_2$/SO$_2$-exposed group, the lesions were a dominant, and apparently progressive, feature.

HISTOPATHOLOGICAL FINDINGS IN THE RESPIRATORY TRACT OF ANIMALS NOT ASSIGNED TO SERIAL SACRIFICE

At the start of the experiment, each treatment and exposure group, apart from the 32 animals assigned to serial sacrifices, comprised 48 animals that were exposed to the different chamber atmospheres for a maximum of 18 months, followed by a clean-air period of up to six months. These animals were used to assess exposure-related morphological changes in the upper respiratory tract.

Nasal Cavity

Lesions within the nasal cavity and paranasal sinuses were limited to purulent inflammation, focal subepithelial calcification, and one neoplasm. Purulent rhinitis and sinusitis were generally associated with foreign matter (plant material, hair, displaced teeth) or were secondary to periodontitis. Purulent periodontitis was associated with necrosis of teeth and with foreign bodies (plant material or hair) wedged into the pulp cavity or periodontal tissues. Periodontal inflammation spread through the maxilla into the nasal cavity and sinuses, probably often via infected roots of teeth.

Calcification occurred alongside the epichondrol membranes, sometimes focally in the subepithelial connective tissue and, less commonly, in the nasal epithelium. Calcification of the subepithelial connective tissue was associated with calcification at the same location in the trachea, and often in the surrounding bronchioles in the lungs.

Lesions of the facial tissues outside the nasal cavity were nearly always inflammatory, except for one fibrosarcoma of the subcutis. Abscesses in the subcutaneous tissue and deep tissues around the nose or orbits were associated with foreign bodies (hair, teeth, plant material) or fine basophilic material interpreted as bacterial colonies, or both. There were no nasal lesions associated with a particular exposure atmosphere or DEN treatment.

Larynx, Trachea, and Major Bronchi

Papillomas were observed in the larynx, trachea, and bronchi. The incidences of these tumors will be reported and discussed later.

The papillomas were associated with DEN treatment in a dose-dependent way, and were not seen in hamsters not given DEN. The benign lesions arose from the respiratory epithelium and had a typical frond-like pattern, with no signs of invasion. Large eosinophilic inclusion bodies were commonly seen in the squamous epithelium. Most lesions were small, although some nearly occluded the larynx, and often appeared in only one of three or more step sections.

Focal collections of lymphocytes, with a few plasma cells and macrophages, were observed just under the laryngeal epithelium in nearly all hamsters. These collections were considered to be normal lymphoid tissues and were, therefore, not recorded histologically. Their state seemed to bear no relation to the exhaust-dose group. Occasionally, a purulent laryngitis was associated with foreign particles (plant material or hair) or with the presence of bacterial colonies.

Squamous metaplasia was observed infrequently and in small patches. Occasionally, it was observed over dilated submucosal glands or calcific lesions that bulged into the lumen, but generally, the ciliated epithelium was merely attenuated over these elevations. The small areas of squamous metaplasia often contained cellular eosinophilic inclusion...
bodies similar to papillomas, and may have been precursors to papillomatous lesions.

Lungs

Chronic passive congestion was characterized primarily by a diffuse infiltration of macrophages, some of which contained hemosiderin. In addition, congested blood vessels with focal hemorrhages, perivascular edema, infiltration of mononuclear cells, and perivascular accumulation of basophilic material resembling ground substance were present. This lesion was not only present whenever thrombi were observed in the cardia atria or in the heart valves, but also was found, in some cases, when no lesion was seen microscopically in the heart. Because the pulmonary lesions are also characteristic of left-sided heart failure or obstruction, it is believed that obstructive lesions were present in these cases, even though they could not be demonstrated in histological sections.

Atrial or valvular thrombosis is a common age-related affliction of hamsters that can result in death. In this study, a number of hamsters died with this lesion and the associated chronic passive congestion of the lungs. In many cases, bronchopneumonia was superimposed on the edematous lungs. Pulmonary changes secondary to the heart lesions made study of the pulmonary changes resulting from exhaust inhalation difficult or impossible. In some cases, exhaust-related lesions were entirely obscured; in others, pulmonary lesions were observed, but not well enough to be graded. Other incidental lesions of the lungs were metastatic tumors. Calcification of bronchial, and especially bronchial epithelium occurred in all dose groups. This change was characterized by focal (especially in bronchi) deposition of dark blue staining material on the epithelial cells in the submucosa. The change was often more diffuse in bronchioles, and extended into alveolar ducts and sometimes alveoli, especially those adjacent to the affected bronchioles. The calcific change was more prominent in the submucosa, especially in the capillary walls just under the epithelium, and often occurred adjacent to apparently healthy cells. Calcification of the tissues distal to the bronchi and bronchioles appeared to precede mineralization of the epithelial cells. The process showed no infiltration of inflammatory cells, and since no necrosis of cells was seen (except for those already calcified), it appears that the process is one of metastatic, rather than dystrophic, calcification.

Proliferation of Alveolar Cells. The alveoli adjacent to the bronchioloalveolar junctions often became lined with bronchiolar-type epithelial cells. This proliferation of epithelium in peribronchiolar acini is here referred to as a proliferation of alveolar cells. A mild degree of this centriacinar reaction is apparently normal, or at least common, in aging hamsters. The change was usually accompanied by a thickening of the bronchiolar epithelium. The acinar proliferation, especially when moderate or marked, was frequently accompanied by a mild increase in nearby macrophages, some of them containing a fine yellow pigment. This lesion, when severe, contained some squamous epithelial cells, referred to in rare cases as squamous metaplasia. Not commonly, a focus of alveoli, with no apparent connection to a bronchiole, was found to be lined with cuboidal cells. A few of these foci, referred to as adenomas, measured up to 1 mm in their greatest dimension.

Pneumoconiosis. These lesions were limited to hamsters that received total diesel exhaust. The soot particles were fine black specks largely phagocytosed by macrophages, and larger irregular aggregates that were generally found to be most numerous around bronchioles, blood vessels, and just under the pleura. Accumulations of soot particles were associated with an infiltration of macrophages, but no other inflammatory cells. A gradual accumulation of soot particles could be observed from six months of exposure to sacrifice. There was a direct association between location and degree of pneumoconiosis, and interstitial pneumonitis.

Pneumonitis. Interstitial pneumonitis was characterized by thickening of alveolar walls. Alveolar walls were increased in thickness due to enlarged type II pneumocytes, an infiltration of mononuclear inflammatory cells, and sometimes an apparent intramural deposition of eosinophilic material. The lesions were generally focal and were usually associated with the deposition of soot particles and infiltration with macrophages. Thus, interstitial pneumonitis was most severe where most of the particulate matter appeared. In areas of pneumonitis, the alveoli were smaller and apparently unable to dilate because of the postmortem infiltration of formalin. There was no case of advanced interstitial fibrosis, although deposits of various collagen fibers between alveoli were occasionally observed.

A mild, more diffuse thickening of alveolar septa appeared to be associated with inhalation of NO₂/SO₂ exhaust. The thickening was believed to be largely due to thickened type II pneumocytes.

TUMORS IN THE RESPIRATORY TRACT

All animals that were not scheduled for sacrifice and that could be examined histologically were included to evaluate the treatment- and exposure-related tumor rates. The number of animals investigated, the number of animals that died spontaneously or were killed, the number of tumor-bearing
Potential Cotumorigenic Effect of $NO_2$, $SO_2$, and Diesel-Engine Exhaust

Table 6. Tumors in the Upper Respiratory Tract of Male Hamsters Treated with 3 mg Diethylnitrosamine/kg Body Weight

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Tumor-Bearing Animals (%)</th>
<th>Mean Lifetime&lt;sup&gt;a&lt;/sup&gt; ± SD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean air</td>
<td>29</td>
<td>13.8</td>
</tr>
<tr>
<td>$NO_2/\text{SO}_2$</td>
<td>28</td>
<td>14.3</td>
</tr>
<tr>
<td>Diesel exhaust without particles</td>
<td>30</td>
<td>30.0</td>
</tr>
<tr>
<td>Total diesel exhaust</td>
<td>29</td>
<td>17.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Maximum lifetime was 99 to 105 weeks.

Table 7. Tumors in the Upper Respiratory Tract of Female Hamsters Treated with 3 mg Diethylnitrosamine/kg Body Weight

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Tumor-Bearing Animals (%)</th>
<th>Mean Lifetime&lt;sup&gt;a&lt;/sup&gt; ± SD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean air</td>
<td>30</td>
<td>6.7</td>
</tr>
<tr>
<td>$NO_2/\text{SO}_2$</td>
<td>33</td>
<td>12.1</td>
</tr>
<tr>
<td>Diesel exhaust without particles</td>
<td>30</td>
<td>10.0</td>
</tr>
<tr>
<td>Total diesel exhaust</td>
<td>32</td>
<td>15.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Maximum lifetime was 83 to 92 weeks.

animals, and the types of tumor and tumor locations are listed in Table 5.

Because there were no lung tumors, either in exhaust-exposed or in DEN-treated hamsters, the tumor rates given are related to the upper respiratory tract alone (nasal cavities, larynx, trachea). Only two animals, both treated with the higher DEN dose, showed a tumor (carcinoma) of the nasal cavity. Apart from two animals (treated with DEN and exposed to total diesel exhaust), one of which had a tracheal carcinoma and the other of which had a laryngeal carcinoma, all other tumor-bearing animals showed benign neoplastic lesions in the larynx or trachea, or both. All but one of these lesions were diagnosed as papillomas. One tumor of the larynx was an adenoma.

No tumors were detected in the upper respiratory tracts of clean-air control animals, or in $NO_2/\text{SO}_2$ gas-exposed or particle-free diesel-exhaust-exposed animals not treated with DEN. Of 57 animals exposed to total diesel exhaust without an additional DEN injection, only one developed a tracheal tumor. All the other animals with tumors of the larynx or trachea had been injected with DEN.

In Tables 6 through 13, three different tumor rates for each DEN treatment are given. The calculation of the first tumor rate (Tables 6, 7, 10, 11) was based on the total number of animals per group investigated for tumors, and therefore includes animals that died during the first month of the experiment as well as animals that lived a relatively long time. In the second approach (Tables 8, 9, 12, 13), the total number of animals per group was divided into two subgroups, characterized by their different mean and maximum lifetimes. The first subgroup includes all animals that did not live beyond the time when a 50 percent mortality rate was reached. Thus, all animals in the second subgroup had longer lifetimes than the animals in the first subgroup. The tumor rates and number of animals investigated histologically, as well as the mean lifetimes of the first and second subgroups, are always listed together in one table.

The tumor rate in clean-air-exposed control animals induced by a 3-mg dose of DEN was below 15 percent in both males and females (Tables 6 and 7). No significant changes of these tumor rates occurred after exposure to diesel exhaust or irritant gases. Although the mean lifetime of the animals that died before the 50 percent survival date was 20 to 30 weeks shorter than the mean lifetime of the corresponding animals that died after the 50 percent survival date, the tumor rates in these two groups were not different (Tables 8 and 9).

The administration of 6 mg of DEN induced a higher tu-

Table 8. Tumors in the Upper Respiratory Tract of Male Hamsters, Treated with 3 mg Diethylnitrosamine/kg Body Weight, That Died Before or After the 50 Percent Survival Date (72nd to 74th Week)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>n Before</th>
<th>After</th>
<th>Tumor-Bearing Animals (%) Before</th>
<th>After</th>
<th>Mean Lifetime ± SD (weeks) Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean air</td>
<td>15</td>
<td>14</td>
<td>13.3</td>
<td>14.3</td>
<td>56 ± 14</td>
<td>88 ± 9</td>
</tr>
<tr>
<td>$NO_2/\text{SO}_2$</td>
<td>14</td>
<td>14</td>
<td>14.3</td>
<td>14.3</td>
<td>52 ± 17</td>
<td>91 ± 8</td>
</tr>
<tr>
<td>Diesel exhaust without particles</td>
<td>15</td>
<td>15</td>
<td>26.7</td>
<td>33.3</td>
<td>58 ± 13</td>
<td>87 ± 9</td>
</tr>
<tr>
<td>Total diesel exhaust</td>
<td>16</td>
<td>13</td>
<td>12.5</td>
<td>23.1</td>
<td>57 ± 13</td>
<td>88 ± 8</td>
</tr>
</tbody>
</table>
mor rate in clean-air-exposed control females than in males (Tables 10 and 11). The two different DEN dosages resulted in no dose-dependent increase of tumor rates, even in clean-air-exposed control males. After exposure to total diesel exhaust, a clear, but not statistically significant, increase of the tumor rate was observed in males (Table 10).

Aside from the animals exposed to total diesel exhaust, nearly all tumors in those males treated with 6 mg of DEN were found after a minimum lifetime of 68 to 73 weeks and a mean lifetime of 85 to 88 weeks (Table 12).

Contrary to these findings, after exposure to total diesel exhaust, male hamsters that died before or after the 50 percent survival date showed clearly increased tumor rates (Table 12). This was the only group of the whole experiment that showed a significant exposure-related alteration of the tumor rate using the life-table analysis.

In females treated with 6 mg of DEN, higher tumor rates were also found in the animals with longer mean lifetimes. However, exposure to the NO₂/SO₂ gas appeared to have an inhibiting effect on the DEN-induced tumors using the χ² statistics (Table 13). However, the life-table analysis of respiratory tract tumor incidence data did not reveal a significant difference between clean-air- and NO₂/SO₂-exposed females at the 5 percent level.

### DISCUSSION

During the course of this study, it was shown elsewhere that long-term inhalation of diesel-engine exhaust with high concentrations of soot particles induces lung tumors in rats but not in hamsters (Heinrich et al. 1982, 1986; Brightwell et al. 1986; Ishinishi et al. 1986; Iwai et al. 1986; Mauderly et al. 1986). When the soot particles were removed from the exhaust, the lung tumors did not develop (Brightwell et al. 1986; Heinrich et al. 1986). However, the unconfirmed results of a study of mice with a relatively high spontaneous tumor rate showed a significant increase in lung tumor incidence after inhalation of diesel exhaust, even when devoid of soot particles (Heinrich et al. 1986).

Studies showing no carcinogenic effects of inhaled diesel exhaust in hamsters were confirmed repeatedly. However, in an earlier study with Syrian golden hamsters, a single injection of 4.5 mg DEN/kg bw given subcutaneously about

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**Table 9. Tumors in the Upper Respiratory Tract of Female Hamsters, Treated with 3 mg Diethylnitrosamine/kg Body Weight, That Died Before or After the 50 Percent Survival Date (57th to 62nd Week)**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>n Before</th>
<th>After</th>
<th>Tumor-Bearing Animals (%) Before</th>
<th>After</th>
<th>Mean Lifetime ± SD (weeks) Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean air</td>
<td>15</td>
<td>15</td>
<td>6.7</td>
<td>6.7</td>
<td>48 ± 11</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>NO₂/SO₂</td>
<td>17</td>
<td>16</td>
<td>0</td>
<td>25.0</td>
<td>40 ± 13</td>
<td>70 ± 7</td>
</tr>
<tr>
<td>Diesel exhaust without particles</td>
<td>15</td>
<td>15</td>
<td>13.3</td>
<td>6.7</td>
<td>53 ± 7</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>Total diesel exhaust</td>
<td>16</td>
<td>16</td>
<td>12.5</td>
<td>18.8</td>
<td>40 ± 13</td>
<td>70 ± 7</td>
</tr>
</tbody>
</table>

*Maximum lifetime was 86 to 99 weeks.

**Table 10. Tumors in the Upper Respiratory Tract of Male Hamsters Treated with 6 mg Diethylnitrosamine/kg Body Weight**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>n</th>
<th>Tumor-Bearing Animals (%)</th>
<th>Mean Lifetime ± SD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean air</td>
<td>28</td>
<td>14.3</td>
<td>68 ± 24</td>
</tr>
<tr>
<td>NO₂/SO₂</td>
<td>28</td>
<td>25.0</td>
<td>71 ± 18</td>
</tr>
<tr>
<td>Diesel exhaust without particles</td>
<td>28</td>
<td>17.9</td>
<td>74 ± 19</td>
</tr>
<tr>
<td>Total diesel exhaust</td>
<td>30</td>
<td>46.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67 ± 17</td>
</tr>
</tbody>
</table>

<sup>a</sup> Maximum lifetime was 86 to 99 weeks.

<sup>b</sup> p < 0.05.

**Table 11. Tumors in the Upper Respiratory Tract of Female Hamsters Treated with 6 mg Diethylnitrosamine/kg Body Weight**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>n</th>
<th>Tumor-Bearing Animals (%)</th>
<th>Mean Lifetime ± SD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean air</td>
<td>32</td>
<td>37.5</td>
<td>61 ± 18</td>
</tr>
<tr>
<td>NO₂/SO₂</td>
<td>31</td>
<td>12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54 ± 19</td>
</tr>
<tr>
<td>Diesel exhaust without particles</td>
<td>31</td>
<td>41.9</td>
<td>58 ± 16</td>
</tr>
<tr>
<td>Total diesel exhaust</td>
<td>30</td>
<td>20.0</td>
<td>59 ± 14</td>
</tr>
</tbody>
</table>

<sup>a</sup> Maximum lifetime was 82 to 84 weeks.

<sup>b</sup> p < 0.05.
Potential Cotumorigenic Effect of NO₂, SO₂, and Diesel-Engine Exhaust

Table 12. Tumors in the Upper Respiratory Tract of Male Hamsters, Treated with 6 mg Diethylnitrosamine/kg Body Weight, That Died Before or After the 50 Percent Survival Date (68th to 73rd Week)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>n</th>
<th>Tumor-Bearing Animals (%)</th>
<th>Mean Lifetime ± SD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Clean air</td>
<td>14</td>
<td>14</td>
<td>7.0</td>
</tr>
<tr>
<td>NO₂/SO₂</td>
<td>14</td>
<td>14</td>
<td>7.0</td>
</tr>
<tr>
<td>Diesel exhaust without particles</td>
<td>14</td>
<td>14</td>
<td>7.0</td>
</tr>
<tr>
<td>Total diesel exhaust</td>
<td>15</td>
<td>15</td>
<td>40.0a</td>
</tr>
</tbody>
</table>

*p < 0.05.

Table 13. Tumors in the Upper Respiratory Tract of Female Hamsters, Treated with 6 mg Diethylnitrosamine/kg Body Weight, That Died Before or After the 50 Percent Survival Date (49th to 63rd Week)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>n</th>
<th>Tumor-Bearing Animals (%)</th>
<th>Mean Lifetime ± SD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Clean air</td>
<td>15</td>
<td>17</td>
<td>20.0</td>
</tr>
<tr>
<td>NO₂/SO₂</td>
<td>16</td>
<td>15</td>
<td>12.5</td>
</tr>
<tr>
<td>Diesel exhaust without particles</td>
<td>16</td>
<td>15</td>
<td>25.0</td>
</tr>
<tr>
<td>Total diesel exhaust</td>
<td>15</td>
<td>15</td>
<td>13.3</td>
</tr>
</tbody>
</table>

*p < 0.05.

two weeks after starting the exhaust exposures induced a basic tumor rate in the upper respiratory tract of almost 45 percent (Table 14) in the control animals, and long-term exposure to both filtered and unfiltered diesel exhaust caused increases in tumor incidence up to 66 and 70 percent, respectively. Apparently, diesel exhaust acted as a cofactor in the tumor induction with DEN and, thus, could be considered as causing cotumorigenic effects.

In the HEI study presented here, an attempt was made to find out whether the cotumorigenic effect of both types of diesel exhaust could be related to the organic trace constituents of diesel exhaust or to the influence of NO₂ and SO₂ as traces of irritant gases present in diesel exhaust. Cotumorigenic effects of irritants are described in the literature (Laskin et al. 1970; Dalbey 1982; Pauluhn et al. 1985). Thus, long-term inhalation experiments were conducted with hamsters exposed to an NO₂/SO₂ mixture at a concentration comparable to the occurrence of the two irritants in diesel exhaust. The results, however, indicated substantial variations in the tumor-inducing capability of the applied single DEN doses.

In spite of the fact that this study utilized a slightly increased DEN dose of 6 mg/kg bw instead of the 4.5 mg/kg bw dose of the previous investigation, the tumor rate in the clean-air-exposed control females given 6 mg/kg bw was even slightly lower than before (Tables 11 and 14), although the difference between 44.7 and 37.5 percent was not statistically significant. But in a comparison of the tumor rates of animals that had died before reaching the 50 percent survival time, it was surprising that the animals in the previous study (administered 4.5 mg DEN/kg bw) showed a very high tumor rate, exceeding that of the animals living beyond the 50 percent survival time (Table 15). The corresponding animals in the 6-mg-DEN group had substantially lower tumor rates (Table 13). One reason for the higher tumor rate in the 4.5-mg-DEN study could be the longer mean and

Table 14. Tumors in the Upper Respiratory Tract of Female Hamsters Treated with 4.5 mg Diethylnitrosamine/kg Body Weighta

<table>
<thead>
<tr>
<th>Exposure</th>
<th>n</th>
<th>Tumor-Bearing Animals (%)</th>
<th>Mean Lifetime ± SD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean air</td>
<td>47</td>
<td>44.7</td>
<td>69 ± 21</td>
</tr>
<tr>
<td>Diesel exhaust</td>
<td>47</td>
<td>66.0c</td>
<td>70 ± 18</td>
</tr>
<tr>
<td>without particles</td>
<td>57</td>
<td>70.2c</td>
<td>68 ± 17</td>
</tr>
</tbody>
</table>

a Based on data reported by Heinrich and associates (1982).
b Maximum lifetime was 112 to 119 weeks.
c p < 0.05.
maximum lifetimes of the animals. The tumor rates of the 4.5-mg-DEN female hamsters that had died before the 65th or 71st experimental week were relatively high in all exposure groups, and not significantly different. The diesel-exhaust-related increase of the tumor rates occurred only in those animals that lived for at least 65 to 71 weeks and to a maximum of 112 to 119 weeks. The inhibiting effect of NO_2/SO_2 on tumor induction in females treated with 6 mg DEN/kg bw may to some extent be explained by the higher mortality rate in this group (Table 13).

The only significant influence of the exhaust exposure on the DEN-induced tumor rate, which, in principle, is comparable to the result of the earlier study using 4.5 mg DEN/kg bw, is the increased tumor rate in males treated with 6 mg DEN/kg bw and exposed to total diesel exhaust (Table 12).

However, none of the other exposure groups showed a similar effect. Apart from the group of males exposed to particle-free exhaust, the tumor rate of all other groups treated with 3 mg DEN/kg bw was not higher than the tumor rate induced in the earlier study with 1.5 mg DEN. In both the earlier and the present study, no exposure-related effects with the lower DEN-induced tumor rate could be observed.

Low doses of DEN administered subcutaneously induced tumors only in the upper respiratory tract of hamsters. Therefore, until now, the lungs were not included in the investigation of a potential cotumorogenic effect of an NO_2/ SO_2 gas mixture or diesel-engine exhaust.

The scanning electron microscopy investigation of the larynx and trachea clearly showed exhaust-dependent epithelial changes, at least in the 15-month groups. Loss of ciliated cells occurred in all exhaust-exposed groups.

After inhalation of particle-free, as well as total, diesel exhaust, focal metaplasia and dysplasia occurred in the oldest animals. The larynxes were probably more strongly affected, but exact quantitation was impossible. Attached mucous droplets may indicate changes in mucous cells that lead to an altered mucous viscosity, which could affect ciliary activity adversely. This effect was also described by Barnes and associates (1983), who cultivated hamster tracheal explants under elevated oxygen pressure.

The observed calcification of tracheal or alveolar connective tissue occurred in all groups. It resembles the generalized calcinosis disease, first described by Pour and Birt (1979), which may occur spontaneously in hamster colonies.

Other substances have proved to be better tumor inducers in the hamster respiratory tract than DEN. For example, vinylenitrile induced lung tumors as well as upper respiratory tract tumors after subcutaneous injection in Syrian golden hamsters (Althoff et al. 1977; Green and Althoff 1982). Dipentylamine was used in rats to induce tumors of the lung and the upper respiratory tract. After exposure to total diesel exhaust, the occurrence of squamous cell carcinoma in the rat lungs increased significantly. On the other hand, the tumor incidence in the upper respiratory tract, mainly in the nasal cavities, was significantly reduced, not only after inhalation of total diesel engine exhaust but also after exposure to particle-free exhaust (Heinrich et al. 1986).

When the histopathological findings in the lungs of hamsters and rats exposed to total diesel exhaust containing approximately 4 mg soot/m^3 were compared, serious differences became obvious. Figures 19 and 20 are light photomicrographs showing hamster and rat lungs, both exposed to diesel exhaust for 18 months. Although the loads of soot particles in the hamster and rat lungs appeared to be very similar, the hamster lungs, unlike the rat lungs, showed only very slight reactions to the particle deposits. Therefore, the terms mild, moderate, and marked used to describe these slight changes in hamster lungs can only be usefully applied to the hamster study. In Table 16, the pulmonary changes found in rats and hamsters are compared on the ba-
sis of a grading system that also applies to the findings in rat and hamster lungs.

CONCLUSIONS

Including this HEI study, five different inhalation experiments were conducted in our institute to investigate the influence of inhaled pollutants on the tumor-inducing effect of subcutaneously injected DEN in Syrian golden hamsters. These five studies comprised, besides 11 clean-air-exposed control groups, 24 groups exposed to total and particle-free diesel exhaust, total gasoline-engine exhaust, PAH-enriched coal-oven flue gas, and an NO\textsubscript{2}/SO\textsubscript{2} gas mixture. Five doses of DEN and two different strains of hamsters were used (Table 17). The following conclusions can be drawn from these experiments:

1. The spontaneous tumor rate in the upper respiratory tract of the Syrian golden hamster (as well as in the lungs) is very low. Averaging the results from all our control animals, we found a tumor rate of 0.2 percent.

2. Diethylnitrosamine dosages as low as approximately 1.5 mg/kg bw, administered only once at the beginning of the experiment, induced a significantly increased tumor rate in the upper respiratory tract.

3. In females, comparable DEN doses appeared to induce higher tumor rates than in males.

4. A hamster-strain-specific effect cannot be excluded, but, on the basis of these data, appears to be unlikely.

5. In the low-dose range, including 3 to 4.5 mg DEN/kg bw in females and 6 mg DEN/kg bw in males, the tumor rate in most groups appeared to be no higher than 15 percent, except the one group (from the study sponsored by the Umweltbundesamt) that reached 44.7 percent with 4.5 mg DEN/kg bw (Table 17).
Figure 20. Rat lung after exposure to total diesel exhaust, showing bronchioloalveolar hyperplasia, slight squamous metaplasia, septal thickening, and mild fibrosis. Magnification = ×160.

6. Although formaldehyde (Dalbey 1982), SO₂ (Pott and Heinrich 1982), and cigarette smoke (Hoffmann 1983) increased the DEN-induced tumor rate in the upper respiratory tract of Syrian golden hamsters, the exhausts tested in our institute showed no consistent effects. However, the differences in the mean and maximum lifetimes of the animals in the various studies may be one reason for these, at times, inconsistent results.

7. Only one out of eight groups exposed to total diesel exhaust, and two out of eight groups exposed to particle-free diesel exhaust, showed a statistically significant increase in the DEN-induced tumor rate. The results available so far from our inhalation experiments appear to be not sufficiently conclusive to demonstrate that diesel-engine exhaust should be classified as a carcinogen or enhancer for the test system used.

### Table 16. Pulmonary Changes After Exposure to Total Diesel Exhaust

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Hamster</th>
</tr>
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<tbody>
<tr>
<td>Particle deposits,</td>
<td>+++</td>
<td>+ (+)</td>
</tr>
<tr>
<td>accumulation of macrophages</td>
<td>+ +</td>
<td>+ (+)</td>
</tr>
<tr>
<td>Inflammatory reactions</td>
<td>+ +</td>
<td>(+)</td>
</tr>
<tr>
<td>Bronchioloalveolar hyperplasia</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Squamous metaplasia</td>
<td>++</td>
<td>No</td>
</tr>
<tr>
<td>Tumor</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*(+)= very slight; + = slight; ++ = moderate; + (+) = moderate to severe; +++ = severe.*

**ACKNOWLEDGMENTS**

Our thanks are due to Prof. D.L. Dungworth, Department of Veterinary Pathology, University of California, Davis, USA for his valuable help, numerous stimulating discus-
Table 17. Hamsters with Upper Respiratory Tract Tumors from Various Studies Using Diethylnitrosamine Treatment and Exposure to Diesel- or Gasoline-Engine Exhaust, Nitrogen Dioxide/Sulfur Dioxide Gas Mixture, or Polycyclic-Aromatic-Hydrocarbon-Enriched Coal-Oven Flue Gas

<table>
<thead>
<tr>
<th>Study</th>
<th>DEN [mg/kg bw]</th>
<th>Gender and Strain</th>
<th>Clean Diesel Exhaust Without Particles</th>
<th>Total Diesel Exhaust</th>
<th>Other Exposure</th>
<th>50 Percent Survival (weeks)</th>
<th>Maximal Survival (weeks)</th>
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<tr>
<td>UBA</td>
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<td>F/H</td>
<td>13.4</td>
<td>13.0</td>
<td>15.1</td>
<td>79</td>
<td>120</td>
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<tr>
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<td>F/T</td>
<td>12.8</td>
<td>13.6</td>
<td></td>
<td>67</td>
<td>94</td>
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<tr>
<td>FAT</td>
<td>3.0</td>
<td>F/T</td>
<td>6.4</td>
<td>6.4</td>
<td></td>
<td>66</td>
<td>86</td>
</tr>
<tr>
<td>HEI</td>
<td>3.0</td>
<td>F/H</td>
<td>6.7</td>
<td>10.0</td>
<td>15.6</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>VW</td>
<td>4.5</td>
<td>F/T</td>
<td>14.9</td>
<td>10.0</td>
<td>13.0</td>
<td>62</td>
<td>92</td>
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<td>UBA</td>
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<td>70.2</td>
<td>71</td>
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<td>30.0</td>
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<td>105</td>
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<tr>
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<td>M/T</td>
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<tr>
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<td>M/H</td>
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<td>17.9</td>
<td>46.7</td>
<td>25.0</td>
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</tr>
<tr>
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<td>M/T</td>
<td>41.7</td>
<td>46.7</td>
<td></td>
<td>110</td>
<td>126</td>
</tr>
</tbody>
</table>

All studies referenced were conducted at the Fraunhofer-Institut für Toxikologie und Aerosolforschung.

UBA = sponsored by Umweltbundesamt, Berlin, FRG; FAT = sponsored by Forschungsvereinigung Automobiltechnik, Frankfurt/M, FRG; HEI = sponsored by Health Effects Institute; VW = sponsored by Volkswagen AG, Wolfsburg, FRG.

DEN was administered subcutaneously.

Strain H = Hoe SYHK (SPF Ars); strain T = Cpb-SHGAs1.

Diesel-engine exhaust.

Gasoline-engine exhaust.

NO₂/SO₂ gas mixture.

p < 0.05.

PAH-enriched coal-oven flue gas.
sions, and interest in the problem. We also wish to express our thanks to Prof. B.C. Zook, Department of Pathology, George Washington University Medical Center, Washington, DC, USA for his never failing interest and constructive criticism. Finally, we would like to thank the many individuals on the staff of the Department of Experimental Pathology and Aerosol Research of our Institute who contributed to this study.

REFERENCES


APPENDIX A. Cumulative Proportion of Surviving Animals and Animals Dying Without a Respiratory Tract Tumor

Figure A.1. Male hamsters exposed to total diesel exhaust with 6 mg DEN/kg bw.

Figure A.2. Female hamsters exposed to total diesel exhaust with 6 mg DEN/kg bw.

Figure A.3. Male hamsters exposed to NO$_2$/SO$_2$ with 6 mg DEN/kg bw.

Figure A.4. Female hamsters exposed to NO$_2$/SO$_2$ with 6 mg DEN/kg bw.

Figure A.5. Male hamsters exposed to NO$_2$/SO$_2$ with 3 mg DEN/kg bw.

Figure A.6. Female hamsters exposed to NO$_2$/SO$_2$ with 3 mg DEN/kg bw.
Figure A.7. Male hamsters exposed to diesel exhaust without particles and with 3 mg DEN/kg bw.

Figure A.10. Female hamsters exposed to diesel exhaust without particles and with 6 mg DEN/kg bw.

Figure A.8. Female hamsters exposed to diesel exhaust without particles and with 3 mg DEN/kg bw.

Figure A.11. Male hamsters exposed to total diesel exhaust with 3 mg DEN/kg bw.

Figure A.9. Male hamsters exposed to diesel exhaust without particles and with 6 mg DEN/kg bw.

Figure A.12. Female hamsters exposed to total diesel exhaust with 3 mg DEN/kg bw.
Potential Cotumorigenic Effect of NO₂, SO₂, and Diesel-Engine Exhaust

Figure A.13. Male and female hamsters exposed to NO₂/SO₂ with 3 mg DEN/kg bw.

Figure A.14. Male and female hamsters exposed to NO₂/SO₂ with 6 mg DEN/kg bw.

Figure A.15. Male and female hamsters exposed to diesel exhaust without particles and with 3 mg DEN/kg bw.

Figure A.16. Male and female hamsters exposed to diesel exhaust without particles and with 6 mg DEN/kg bw.

Figure A.17. Male and female hamsters exposed to total diesel exhaust with 3 mg DEN/kg bw.

Figure A.18. Male and female hamsters exposed to total diesel exhaust with 6 mg DEN/kg bw.
ABOUT THE AUTHORS

Dr. rer. nat. U. Heinrich studied biology at the University of Tübingen, and graduated in 1977. From 1976 to 1977 he worked as a scientific staff member in the Institute for Toxicology and Pharmacology, Bayer AG, Wuppertal, and from 1977 to 1978 in the Medizinisches Institut für Umwelthygiene, Düsseldorf. Since 1978 he has been Senior Scientist and the Head of the Department of Environmental Hygiene at the Fraunhofer Institute for Toxicology and Aerosol Research, Hannover, with special interest in inhalation studies and other projects on toxicological, carcinogenic, and cocarcinogenic effects.

Prof. Dr. U. Mohr studied at the Ludwig-Maximilians University Medical School in Munich, and in 1960 he was awarded his Board and Licenses as an M.D. In 1967 his venia legendi in experimental pathology was published through the Medical Faculty at Rupprecht-Karl-University in Heidelberg. Since 1968 he has been Professor and Director of the Section of Experimental Pathology at the Hannover Medical School. In 1982 he became a senior member of the staff of the Department of Pathology and Inhalation, Fraunhofer Institute for Toxicology and Aerosol Research, Hannover, and since 1988 he has been the Director of this institute.

Dr. med. vet. R. Fuhst studied at the Justus-Liebig-University Veterinary School in Giessen and at the Hannover Veterinary School. He graduated in 1978. In 1979 he worked as Scientific Research Assistant in the animal laboratory of the Medical School of the University of Frankfurt, and from 1980 to 1981 at the Institute of Experimental Pathology, Hannover Medical School. Since 1982, he has been a member of the scientific staff of Fraunhofer Institute for Toxicology and Aerosol Research, Hannover, with special interest in inhalation studies and other investigations on toxicological, carcinogenic, and cocarcinogenic effects with laboratory animals.

Dr. rer. biol. hum. C. Brockmeyer studied at Georg-August-University in Göttingen, where he received his Dipl.-Biol. degree in 1986. From 1986 to 1989 he was associated with the Department of Pathology and Inhalation, Fraunhofer Institute for Toxicology and Aerosol Research, Hannover, conducting studies in ultrastructural pathology. He graduated in 1988 at the Hannover Medical School. In 1989 he was approved as a Visiting Fellow at the Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, Maryland, USA.

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Name</th>
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<tbody>
<tr>
<td>BaP</td>
<td>benzo[a]pyrene</td>
</tr>
<tr>
<td>BeP</td>
<td>benzo[e]pyrene</td>
</tr>
<tr>
<td>bw</td>
<td>body weight</td>
</tr>
<tr>
<td>CO</td>
<td>carbon monoxide</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>DEN</td>
<td>diethylnitrosamine</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NO₂</td>
<td>nitrogen dioxide</td>
</tr>
<tr>
<td>N₂O₄</td>
<td>dinitrogen tetroxide</td>
</tr>
<tr>
<td>PAHs</td>
<td>polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>SO₂</td>
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INTRODUCTION

A Request for Applications (RFA 82-4), which solicited proposals for "Inhalation Carcinogenesis Studies in Rodents: Multifactor Interactions," was issued by the Health Effects Institute (HEI) in 1982. In response to the RFA, Drs. U. Mohr and U. Heinrich from the Fraunhofer Institute for Toxicology and Aerosol Research in Hannover, Federal Republic of Germany, submitted a proposal entitled, "Investigation of a Potential Cocarcinogenic Effect of Nitrogen Dioxide and Sulfur Dioxide on the Respiratory Tract of Syrian Golden Hamsters." The HEI approved the two-and-a-half-year project, which began in 1984, and authorized a total expenditure of $363,330. The Investigators' Report was received at the HEI in June 1987 and accepted by the Health Review Committee in January 1988. The Health Review Committee's Report is intended to place the Investigators' Report in perspective as an aid to the sponsors of the HEI and to the public.

THE CLEAN AIR ACT

The Environmental Protection Agency (EPA) sets standards for diesel (and other) emissions under Section 202 of the Clean Air Act, as amended in 1977. Section 202(a)(1) directs the Administrator to "prescribe (and from time to time revise) ... standards applicable to the emissions of any air pollutant from any class or classes of motor vehicles or motor vehicle engines, which in his judgment cause, or contribute to, air pollution which may reasonably be anticipated to endanger public health or welfare." Section 202(a)(3)(A)(i) specifically directs the Administrator to "prescribe regulations ... applicable to emissions of carbon monoxide, hydrocarbons, and oxides of nitrogen from classes... of heavy-duty vehicles or engines." Section 202(a)(3)(A)(iii) similarly requires regulations applicable to emissions of particulate matter from classes or categories of vehicles.

Under these provisions, the EPA has taken regulatory actions with respect to diesel engines. In 1980, the EPA set light-duty diesel particulate matter standards, and, in 1984, granted a two-year delay in their effective date. The EPA established emissions-averaging in 1983, and it set nitrogen oxides standards in 1985. For heavy-duty diesel engines, the EPA set hydrocarbon and carbon monoxide standards in 1983, and nitrogen oxides and particulate matter standards in 1985. In addition, under Section 109 of the Act, the EPA has established National Ambient Air Quality Standards for particulate matter. Those standards were most recently revised in July 1987.

Research on the tumorigenic effect of components of diesel-engine exhaust can contribute to an increased understanding of the risks to humans from exposure to diesel-engine-exhaust emissions, and can thereby contribute to informed decision-making with respect to standards under the Clean Air Act.

BACKGROUND

An important environmental issue of the past decade has been the potential health effects from emissions resulting from the use of diesel as a fuel in vehicles (for review see McClellan 1986a). Such vehicles emit relatively large amounts of particulate matter, 30 to 100 times as much as gasoline-fueled vehicles. Diesel-emission particles are submicronic in size and contain a carbon core. The particles have a large surface-to-volume ratio, which allows for ample adsorption of chemical products derived from incomplete combustion to the particle surface. Diesel-engine exhaust thus consists of respirable particles with adsorbed organic materials that are potentially bioavailable after the particles have been deposited in the lungs. Furthermore, diesel-engine exhaust contains gaseous components that may influence any effects of the particulate material.

Early findings, which showed that the polar organic fraction of diesel-engine particle extracts was tumorigenic in the mouse skin initiation assays (Kotin et al. 1955; Nesnow et al. 1984) and was genotoxic in several bacterial, as well as a few mammalian, cell short-term assays (reviewed in Lewtas 1983), aroused concern about the potential carcinogenic risk from exposure to diesel-engine exhaust. To address this concern, several chronic studies using different laboratory species (rats, mice, and hamsters) were undertaken to investigate the tumorigenicity of inhaled diesel-engine exhaust. The more recent studies demonstrated that if rats are exposed to high levels of diesel-engine exhaust (2 to 7 mg/m$^3$) over their lifetimes, lung tumors (adenomas, adenocarcinomas, adenosquamous carcinomas, and squamous cell carcinomas) are induced (Brightwell et al. 1986; Heinrich et al. 1986; Ishinishi et al. 1986; Iwai et al. 1986; Mauderly et al. 1986).

Although inhalation of diesel-engine exhaust results in carcinomas in laboratory animals, difficulties arise when attempting to extrapolate these findings to carcinogenic risk for human exposure. One major consideration, is the use of the appropriate animal model. During the 1970s, the Syrian
golden hamster became one of the most widely used species for studies of respiratory carcinogenesis (reviewed in Nettesheim 1972). In this species, the histology of the respiratory tract is similar to that in humans; pulmonary infections are infrequent; spontaneous lung tumors are rare; and carcinomas similar to the type found in humans (bronchogenic squamous cell) can be induced. Using the hamster, a successful model of respiratory carcinogenesis was developed (Saffiotti et al. 1968), whereby carcinomas in the trachea and bronchi could be induced by the intratracheal instillation of polycyclic aromatic hydrocarbons adsorbed onto iron oxide particles.

In contrast to the results with rats, exposure of hamsters to diesel-engine exhaust by inhalation has failed to produce malignant tumors (Wehner et al. 1979; Heinrich et al. 1982; Brightwell et al. 1986; Heinrich et al. 1986). With the exception of the induction of carcinomas in the hamster larynx with cigarette smoke (Dontenwill et al. 1973; Bernfield et al. 1974), inhalation studies with other potential carcinogenic agents have met with limited success (Laskin et al. 1970; Wehner et al. 1979; Thyssen et al. 1981). A closer examination of the hamster as a model for respiratory carcinogenesis reveals peculiarities that may influence its usefulness. First, there are specific strain differences in the susceptibility of the hamster to experimentally induced carcinogenesis. Outbred strains appear unsuitable for some carcinogenic agents (Wehner et al. 1979), and in some inbred strains certain carcinogens exhibit strain-specific organotropism (Homburger et al. 1979). Second, it appears that the induction of pulmonary and bronchial tumors has occurred only by intratracheal instillation of carcinogens or by systemic administration of nitrosamines (Homburger 1972). There is some recent evidence that intratracheal instillation results in tracheal lesions, which affect the proliferative and carcinogenic response of the airways: carcinogen administration by intralaryngeal instillation does not produce respiratory tract tumors (Keenan et al. 1987).

Related to the question of using the most appropriate animal model is the issue of the type of tumor induced. Specific segments of the respiratory tract of different species appear to be selectively sensitive to different carcinogens (Jones et al. 1985). The neoplastic response may vary considerably, ranging from benign papillomas or adenomas to malignant squamous cell carcinomas or adenocarcinomas. Whether or not benign pulmonary neoplasms constitute preneoplastic lesions has not been established. In addition, the reporting in the literature of tumor type and location has not always been precise; hence, the actual carcinogenic potential of a compound to the lung may be difficult to ascertain.

Most animal studies attempt to associate excess tumors with different exposures; this approach, however, does not provide insight into the mechanisms of carcinogenesis (Kaufman 1988). Because diesel-engine exhaust is a complex mixture, it may contain, in addition to potential carcinogens, initiators, promoters, cocarcinogens, and inhibitors of carcinogenesis. Collectively, these constituents determine the ultimate carcinogenic response, and do so through different mechanisms of action. Some components, such as polycyclic aromatic hydrocarbons, may alter genetic material, whereas others may stimulate a proliferative response of the pulmonary epithelium, thus increasing the susceptibility of the tissue to cancer induction. The relative importance of these mechanisms depends, in part, on the level of exposure or dose (McClellan 1986a).

For example, long-term exposure of rats to diesel-engine exhaust is associated with the progressive accumulation of particles in the lungs; the relation of high particulate burden to tumor incidence is not fully understood. At high particulate concentrations, long-term particle clearance rates are decreased (Heinrich et al. 1982; Wolff et al. 1986), thus increasing the residence time of carcinogenic material in the lung. Particle accumulation has also been associated with increased influx of inflammatory cells in the lungs, enlarged lung-associated lymph nodes, and bronchiolar metaplasia (McClellan 1986a). These effects, in a secondary manner, may facilitate the development of cancerous lesions. It has been suggested that the differences between rats and hamsters in susceptibility to diesel-engine exhaust may be due, in part, to species differences in the reaction of the lung to excess particulate material (Heinrich et al. 1986). The role of particulates has become one of the major uncertainties in the extrapolation of the results from animal studies to the assessment of human risk. Because the general population is exposed to relatively low levels of diesel-engine-exhaust particulate, in contrast to the high levels used in animal studies, it is important to determine what influence high particle concentrations may have on biological responses (Vostal 1986).

One methodology that is employed to assess the cocarcinogenic, promotional, or inhibitory potential of a compound is to test the compound in conjunction with a known carcinogen. The premise is that the known carcinogen will initiate a specific tumor incidence rate, which will be altered in an additive, synergistic, or inhibitory manner, depending on the nature of the test compound. For respiratory carcinogenesis studies, various protocols have been used, the most popular being the instillation of a polycyclic aromatic hydrocarbon or the injection of a nitrosamine. The type of tumor initiated, as well as its anatomical location, exhibits species-specific differences (reviewed by Montesano et al. 1970). For example, in hamsters, diethylnitros-
amine induces primarily papillomas of the larynx and tra-
chea; carcinomas have been observed most often in the
nasal cavities. Therefore, it is important to use the appro-
priate initiating protocol for the end-point of interest.

In addition to particulate material, diesel-engine exhaust
consists of a gaseous phase that contains volatile organic
compounds, irritant gases, and carbon monoxide. Exposure
of rats or hamsters to particle-free diesel-engine exhaust has
failed to produce an increase in respiratory tract tumors
(Brightwell et al. 1986; Heinrich et al. 1986; Ishinishi et al.
1986; Iwai et al. 1986). In contrast, exposure of mice to
filtered exhaust resulted in an increase in adenocarcinomas
(Heinrich et al. 1986). When, however, hamsters were ex-
posed to filtered exhaust and treated concurrently with a
known carcinogen, increases in papillary tumor incidence
rates were reported (Heinrich et al. 1982). (Later experi-
ments [Heinrich et al. 1986], as well as studies reported in
the Investigators' Report, were unable to reproduce these
findings of an effect of filtered exhaust on hamsters.)

The role of irritant gases has also been evaluated in
cotumorigenic systems. The results have been variable. Rats
exposed to sulfur dioxide (3.5 ppm) in conjunction with
benzo[a]pyrene (10 mg/m³) aerosol showed an increase in
squamous cell carcinomas; results in hamsters were nega-
tive (Laskin et al. 1970). In contrast, Pauluhn and coworkers
(1985) reported increases in carcinomas and papillary
tumors in hamsters exposed to high levels of sulfur dioxide
(127 ppm) and particles coated with benzo[a]pyrene (2 or 10
mg/m³). How various components of the gaseous phase of
engine exhaust influence a carcinogenic response remains
unknown.

JUSTIFICATION FOR THE STUDY

The HEI was interested in supporting studies designed to
investigate exposures to combinations of diesel-engine emis-
sions, or specific components, and known initiators,
promoters, or complete carcinogens. Additive, synergistic,
or inhibitory effects of these combinations could then be
evaluated. To approximate human exposure conditions, in-
halation studies were of primary interest to the Institute.

Drs. Mohr and Heinrich proposed to investigate whether
or not exposure to different components of diesel-engine
exhaust enhances the tumorigenicity of systemic nitro-
amines. The investigators had conducted an earlier study
(Heinrich et al. 1982) that suggested a cotumorigenic effect
of whole diesel exhaust with diethylnitrosamine; however,
the components of the exhaust that were responsible for
this observation were unknown. The investigators had the
appropriate facilities to do long-term inhalation studies and
had considerable experience conducting such studies.

In the original proposal, the investigators planned to use
two different nitrosamines on separate groups of animals:
diethylnitrosamine and its α,β-unsaturated derivative, vinyl-
ethylnitrosamine. Because of the large scope of the project,
the HEI Research Committee recommended that the investi-
gators limit their study to a protocol similar to their previ-
ous work, which meant using diethylnitrosamine as their
inducing agent.

It is important to note that at the time the HEI approved
Drs. Mohr and Heinrich's proposal, the positive findings
from carcinogenicity studies in rats, and the negative
results from studies in hamsters, had not yet been pub-
lished.

OBJECTIVES OF THE STUDY

This study was undertaken to determine if the cotumori-
genic effect of inhaled diesel-engine exhaust, observed in
an earlier study (Heinrich et al. 1982), was due to organic
components or irritant gases present in the exhaust. The
specific aims of the study were:

1. To confirm that inhalation of whole diesel-engine ex-
haust enhances the carcinogenic effects of subcutane-
ously injected diethylnitrosamine;
2. To determine if such enhancing effects are produced by
inhalation of diesel-engine exhaust from which the par-
ticulate fraction is removed;
3. To investigate whether or not such enhancing effects are
produced by inhalation of a mixture of nitrogen dioxide
and sulfur dioxide;
4. To study any pathological effects on the respiratory tract
of the exposed animals, whether or not related to neo-
plasia, that may result from inhalation of the above
materials.

STUDY DESIGN

Syrian golden hamsters (480 males, 480 females) were
chronically exposed to one of four different test atmo-
spheres: diluted diesel-engine exhaust (3.75 ± 0.65 mg/m³);
diluted diesel-engine exhaust after removal of particulates;
nitrogen dioxide (4.5 ± 0.7 ppm) and sulfur dioxide (9.6 ± 1.2
ppm) mixture; or clean air. Each exposure group was
divided into three treatment groups: one group received a
single subcutaneous injection of 3 mg diethylnitrosa-
mine/kg body weight; another group received 6 mg dieth-
ylnitrosamine/kg body weight; and a third group received
no injection. Animals were exposed 19 hours per day, 5 days
per week, for up to 18 months. Animals were killed for
histopathological assay either according to a predeter-
mined serial-sacrifice schedule (at 6, 10.5, and 15 months),
at the termination of the study, or at any time during the
study if found in a moribund condition. Hamsters that died
during the course of the study were also examined.

TECHNICAL EVALUATION

ATTAINMENT OF STUDY OBJECTIVES

All of the specific aims were addressed by the investi-
gators.

METHODS AND STUDY DESIGN

The design of the protocol was reasonable with respect
to the numbers and types of exposure groups to be included
for comparison. The number of animals in each exposure
group was based on the results of previous experiments by
these investigators (Heinrich et al. 1982), but no statistical
rationale for the size of the groups was provided.

The Syrian golden hamster was selected as the experi-
mental animal for this study based upon previous experi-
ments by the investigators that suggested cotumorigenic ef-
fects of diesel-engine exhaust in this species. However, the
failure of this study to confirm such effects, along with ob-
servations by other investigators of carcinogenic effects of
diesel-engine exhaust in rats but not in hamsters (see Back-
ground section), raises questions about the suitability of the
hamster for future studies of this type.

Diethylnitrosamine was used as the initiator of tumor-
genesis, with the aim of assessing the synergistic or addi-
tive effects of the test atmospheres. It should be pointed out
that in hamsters, diethylnitrosamine produces primarily
benign papillomas of the upper airways; the relevance of
this to the assessment of the carcinogenic potential of
diesel-engine exhaust is not clear. Furthermore, extensive
papilloma formation may compromise the longevity of lab-
orary animals by occluding their airways (Montesano et
al. 1970). The use of an initiating agent, such as vinylethyl-
nitrosamine, might have been more appropriate. Under
conditions of chronic administration in hamsters, vinyl-
ethylnitrosamine induces tumors in all segments of the re-
spiratory tract. Furthermore, in addition to papillomas, the
agent produces carcinomas (Althoff et al. 1977; Green and
Althoff 1982).

RESULTS AND INTERPRETATION

A single subcutaneous injection of 3 or 6 mg diethyl-
nitrosamine/kg body weight caused an increase in the cu-
mulative overall incidence of benign tumors of the trachea
and upper respiratory tract in air-exposed male and female
hamsters. In those animals surviving more than 60 weeks
after injection of the 6-mg/kg dose, tumors were observed
in 21 percent of the males and 53 percent of the females,
whereas no tumors were observed in the noninjected con-
trols. Tumors of the lung, as opposed to tumors of the upper
respiratory tract, were not observed in any of the animals.

Some inconsistencies in the tumor-response rate pro-
duced by diethylnitrosamine should be noted; hence, these
experiments should be interpreted with caution. For exam-
ple, in the investigators’ previous study, the tumor inci-
cidences in females were 13.5 percent and 44.7 percent for the
1.5-mg/kg and 4.5-mg/kg dosage groups, respectively. The
Corresponding values for the current study were only 13.8
percent for the 3-mg/kg group and 37.5 percent for the 6-mg/
kg group. These differences in tumor-response rates could
be attributable to experiment-to-experiment variation; they
may also be due to the shorter survival time of the female
hamsters in the current study. In addition, when the car-
cinogen dose was doubled from 3 to 6 mg/kg, the incidence
of papillomas in males was unchanged. Gender differences
were also inconsistent. At the 3-mg/kg dose, male hamsters
had a higher tumor incidence than female animals, where-
as at the 6-mg/kg dose, females had a higher incidence than
males.

The overall incidence of respiratory tract tumors was not
consistently affected by exposure to whole diesel-engine ex-
haust, particle-free diesel-engine exhaust, or a mixture of
nitrogen dioxide and sulfur dioxide. Hence, intergroup
differences that were occasionally noted (for example, an
increased incidence of tumors in males exposed to 6 mg
diethylnitrosamine/kg body weight and whole diesel-
engine exhaust) may have been fortuitous.

The shorter survival time of the hamsters in the current
study (50 percent survival time of air-exposed, untreated
animals: males, 70 weeks; females, 62 weeks), compared to
that in the investigators’ previous study (females, 72 weeks),
may have contributed to the failure of this study to confirm
the previously observed cotumorigenic effects of diesel-
engine exhaust. Although this possibility is mentioned in
the Investigators’ Report, the reasons for the differences in
survival time were unfortunately not discussed.

Degenerative, inflammatory, and dysplastic lesions,
which were observed in the respiratory epithelium of aging
hamsters, were generally more pronounced in the hamsters
exposed to whole diesel-engine exhaust or to the irritant gas
mixture than in the animals exposed to clean air; however,
the data are not presented in a form that facilitates either
quantitative comparisons among the groups or inferences
about the pathogenic mechanisms of the lesions. Because
the investigators obtained considerable, detailed data on
nonneoplastic pathological changes, this information could have contributed to their discussion of species differences in the development of pulmonary neoplasms.

REMAINING UNCERTAINTIES AND IMPLICATIONS FOR FUTURE RESEARCH

Because diesel-engine exhaust particles adsorb genotoxic compounds, and under certain experimental conditions whole exhaust is carcinogenic in rats, additional research should be undertaken to help understand the potential for adverse health effects in humans.

Although the rat may be a more suitable animal model for future inhalation experiments on the carcinogenicity of diesel-engine exhaust, the resistance of the hamster could possibly be exploited to explore mechanisms of the carcinogenic response.

The complex nature of diesel-engine exhaust, which is a mixture of many substances, complicates the analysis of its carcinogenic potential. Of immediate concern is the determination of the role of inhaled particulate matter in carcinogenesis. Because the induction of respiratory tract cancer in rats has been reported under conditions of high diesel-particulate dose, this observation should receive additional study. Also, the contribution of various components of the exhaust to carcinogenesis would be clarified by obtaining a better general understanding of the way in which factors such as cellular injury, proliferative responses, impaired clearance mechanisms, and metabolic capacity modulate cancer induction.

CONCLUSIONS

No consistent cotumorigenic effects resulted from the chronic inhalation of diesel-engine exhaust, particle-free diesel-engine exhaust, or a mixture of nitrogen dioxide and sulfur dioxide. However, inconsistencies in the observed dose-responses cast doubt on the validity of the diethylnitrosamine-induced tracheal papilloma model as a reproducible model for studies of tumor initiation. Furthermore, since the initiation of this study, it has become apparent through other investigations that the hamster may not be an appropriate test animal for studies of the carcinogenicity of inhaled diesel-engine exhaust. These problems may explain why it was not possible to test adequately the proposed hypothesis that organics or irritant gases in diesel engine exhaust have promotional or cocarcinogenic activity in the respiratory tract.

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