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Effects of Inhalation Exposure to a High-Boiling (288 to 454°C) Coal Liquid[1]

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Effects of Inhalation Exposure to a High-Boiling (288 to 454°C) Coal Liquid. SPRINGER, D. L., MILLER, R. A., WEIMER, W. C., RAGAN, H. A., BUSCHBOM, R. L., AND MAHLUM, D. D. (1986). *Toxicol. Appl. Pharmacol.* **82,** 112–131. Coal liquids have been evaluated in a variety of short-term toxicological assays; however, few studies have been conducted to determine the systemic effects after inhalation exposure to these materials. To extend the data base on potential health effects from coal liquefaction materials, we performed a study with solvent refined coal (SRC)-II heavy distillate (HD). Fischer-344 rats were exposed for 6 hr/day, 5 days/week for 5 or 13 weeks to an aerosol of HD (boiling range, 288 to 454°C) at concentrations of 0.69, 0.14, 0.03, or 0.0 mg/liter of air for the high, middle, low, and control groups, respectively. Survival through 13 weeks of exposure was greater than 90% for all groups; body weights for exposed animals were decreased in a dose-dependent manner. Significant increases in liver weights and decreases in thymus and ovary weights were observed for treated animals compared with controls. There were also significant treatment-related decreases in erythrocytes, hemoglobin, volume of packed red blood cells, lymphocytes, eosinophils, and total white blood cells. After 5 weeks of exposure serum cholesterol concentrations increased in a dose-dependent manner for both sexes and serum triglyceride amounts decreased for males but not for females. After 13 weeks of exposure, high-dose animals had significant increases in cholesterol (males only), triglycerides, blood urea nitrogen, and serum glutamic pyruvic transaminase (SGPT; males) and significant decreases in albumin, SGPT (females), and lactate dehydrogenase (LDH). Examination of bone-marrow preparations from exposed animals demonstrated consistent decreases in the degree of cellularity, suggesting that this organ is a target for HD. Microscopic evaluation of organ sections indicated exposure-related changes for nasal mucosa, pulmonary macrophages, thymus, liver, kidney, bone marrow, ovaries, and cecum. Results from this study indicated dose-dependent increases in the severity of the lesions observed, with few effects in the low-exposure group that were attributable to the exposure.

Coal is being considered as an alternative energy source since it is an abundant natural resource in North America, but its use raises environmental and health concerns. Several processes, including solvent refined coal (SRC)-I and -II (Glasstone, 1982a), EDS (Vick and Epperly, 1982), and H-Coal (Glasstone, 1982b), have been developed to convert coal to liquid products. These processes remove a large portion of the ash and sulfur from the coal, thereby reducing the emissions released to the environment. Although these changes have enhanced the acceptability of coal as an energy source, potential health effects associated with the new technologies need investigation. For this reason, we are conducting toxicological studies in animals, using samples obtained from the SRC processes.

Studies by Pelroy and co-workers (Pelroy and Petersen, 1981; Wilson *et al.*, 1981) have shown that process solvent (PS) and heavy

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distillate (HD), the high-boiling liquids from the SRC-I and -II processes, are mutagenic to *Salmonella typhimurium*. Moreover, these materials are also active in mammalian-cell mutation and transformation assays (Frazier and Andrews, 1983; Frazier and Mahlum, 1984). In addition to these *in vitro* assays, which are often used to screen materials for potential carcinogenicity, mouse skin-painting assays demonstrated that both PS and HD were carcinogenic (Mahlum, 1983; Renne *et al.*, 1981). In other studies with low- and middle-boiling-range materials, neither mutagenic (Pelroy and Petersen, 1981) nor carcinogenic activity (Renne *et al.*, 1981) was observed.

Occupational exposure to coal liquids will most likely occur either by inhalation or through skin contact. Limited information is available on the systemic effects of coal liquids following inhalation exposure although we have shown that inhalation exposure of pregnant rats to HD (0.66 mg/liter) on Days 12 through 16 of gestation resulted in reduced body weight gain, decreased thymus weight, and increased lung, and spleen weights for the dams; in addition, cleft palates, small lungs, and growth retardation were observed in fetuses taken from these dams at 21 days of gestation (Springer *et al.*, 1982b). To expand the toxicological data base for coal liquids, we therefore conducted a subchronic (13-week) exposure to SRC-II HD. Results from this study are reported for survival, growth, organ weights, hematology, clinical chemistry, and histopathology.

METHODS

Material. The coal liquid used in this study was HD (boiling range, 288–454°C), obtained from the Ft. Lewis, Washington plant, which was operated by the Pittsburg & Midway Coal Mining Company (P&M Co.). The sample was collected on April 3 and 4, 1980 during operation in the SRC-II mode using Powhatan No. 6 coal and was stored at 4°C under nitrogen in Teflon-lined drums. Samples of the stored material were recently evaluated for changes in chemical composition and mutagenicity to determine if changes had occurred since this study was conducted, between April and July 1982. These data indicate that the composition and biological activity for HD have remained constant for up to 4 years after collection (Wright and Weimer, 1984).

Chemical Analysis. Concentrations of the major HD components are listed in Table 1; more detailed chemical analysis for an SRC-II HD sample has recently been reported by Wilson *et al.* (1984) and Wright *et al.* (1984). High-resolution gas chromatography (GC) was used to quantify the concentrations of these constituents: a Hewlett–Packard (HP) 5880A equipped with a 25 m × 0.25-mm (i.d.) fused silica capillary column coated with SE-52 (J. & W. Scientific) and flame ionization detection. The oven was temperature-programmed from 50 to 300°C at 3°C min^{-1}, and helium carrier gas was used, with splitless injection. Quantitative results were determined from the response factor of an internal standard (*n*-nonadecane). Identification of the components was achieved by GC/mass spectrometry (MS) procedures; an HP 5992 benchtop GC/MS system equipped with a 30 m SE-52 fused silica column was used for these identifications.

The HD sample was also fractionated into chemical classes, according to the method of Later *et al.* (1981). This separation yields aliphatic hydrocarbon (AH), neutral polycyclic aromatic hydrocarbon (PAH), nitrogen-containing polycylic aromatic compound (NPAC), and hydroxylated polycyclic aromatic hydrocarbon (HPAH) fractions. For the SRC-II HD, the fractional composition is as follows: AH = 1%; PAH = 46%; NPAC = 36%; and HPAH = 25% (overall recovery ~108%).

Exposure system. The inhalation exposure system used in this study has been described previously (Moss *et al.*, 1982) but has been modified for exposure of animals to coal liquids (Springer *et al.*, 1982a). Stainless-steel multitiered chambers (Hazelton Systems, Inc., Aberdeen, Md.) with a volume of 2.3 m^3 were used. The HD aerosol was generated with two Solo-sphere nebulizers (McGaw Respiratory Therapy, Irvine, Calif.) housed in a 65°C water bath and was introduced into an orifice-controlled manifold delivery system constructed from 7.61 cm (3 in.)-diameter stainless-steel pipe and fittings. An appropriate volume of the aerosol was diluted with filtered room air to give the desired concentration within each of the three exposure chambers. A fourth chamber, supplied with filtered air, was used for sham-exposure of control animals.

Chambers were operated at a flow rate of approximately 283 liter/min, with air movement from top to bottom. After each daily exposure, the flow rate for each chamber was increased to approximately 340 liter/min to reduce the time required to clear aerosol from the chamber. To reduce ammonia to less than 5 ppm, Deotized animal cage board (Shepherd Specialty Papers, Kalamazoo, Mich.) was placed on catch pans under each cage unit after the exposure and removed the next morning prior to startup. Temperatures in the chambers were continuously monitored; relative humidity (RH) was recorded three times daily. Means ($\bar{x} \pm$ SD) for temperature and RH during the 13 weeks of exposure were 23 ± 1°C and 68 ± 7%, respectively.

TABLE 1

CONCENTRATION OF MAJOR COMPONENTS OF SOLVENT REFINED COAL-II HEAVY DISTILLATE

Components[a]	Concentration (parts per thousand)[b]
Benzene	0.16
Fluorene	6.78
C_1-Acenaphthene, C_2-biphenyl	7.88
C_4-Naphthalene	7.06
Xanthene	5.17
C_1-Fluorene	5.03
C_1-Fluorene	6.04
C_1-Fluorene	12.2
C_1-Fluorene	4.14
Dibenzothiophene	7.97
Tetrahydrophenanthrene	4.52
Phenanthrene	42.5
C_2-Fluorene	3.33
C_2-Fluorene	2.53
C_2-Fluorene	2.68
Carbazole	9.73
3-Methylphenanthrene	8.73
2-Methylphenanthrene	15.7
1-Methylphenanthrene	2.57
C_2-Xanthene	3.67
Dihydrofluoranthene	4.36
C_2-Phenanthrene	2.92
Fluoranthene	3.82
Pyrene	18.9
Dihydropyrene	2.27
2- or 4-Methylpyrene, benzo[b]fluorene	11.2
C_2-Pyrene, C_1-benzo[b]fluorene	2.13
C_1-Chrysene	3.07
Benzo[a]pyrene	0.55
Benzo[e]pyrene	0.92

[a] Identified by retention time and GC/MS.

[b] Based on 1 mg/ml *n*-nonadecane internal standard, 1:50 dilution, integration of peak areas.

Target concentrations, as indicated by previous work (Springer *et al.*, 1982b; Loscutoff *et al.*, 1983), were 0.70 mg/liter for the high-dose treatment group, with a factor of five between each consecutive treatment group. The concentration in each chamber was determined by collecting aerosol on a Metricel filter (Gelman Science, Ann Arbor, Mich.; 0.45 μm), eluting the HD into chloroform, and determining the ultraviolet (uv) absorbance at 254 nm (Springer *et al.*, 1981). Mean daily concentrations of the aerosol from each chamber were determined by collecting triplicate samples at three equal intervals throughout the 6-hr exposure period. Mean aerosol concentrations ($\bar{x} \pm$ SD) during the 13-week exposure were 0.69 ± 0.030, 0.14 ± 0.012, 0.03 ± 0.003, and 0.0 mg HD/liter of air for the high-, middle-, low-exposure, and control groups, respectively.

In addition to the filter samples, the aerosol concentrations in each chamber were continuously monitored and recorded by a real-time aerosol monitor (RAM; GCA Corporation, Bedford, Mass.), which responds to changes in aerosol concentration by measuring the amount of light scattered by the aerosol particles. If readings from this on-line monitor indicated that concentrations were outside the range specified (±10%), adjustments were made by increasing the air pressure to the generator, adjusting the amount of dilution air, or increasing the temperature of the water bath containing the nebulizer. Only minor adjustments were required.

During the 13-week study, at least two Mercer cascade impactor samples of aerosol were collected per chamber per week for particle-size analysis. The amount of aerosol present on each stage of the impactor was determined by uv absorption at 254 nm. Since flow rates were uniform, the amount of aerosol on the same stages of the impactors was summed for all runs to give a composite weight for each stage. Using the computer program NEWCAS (Pacific Northwest Laboratory [PNL], 1977), the calculated mass median aerodynamic diameters (MMAD) were 1.7, 1.7, and 1.8 μm, respectively, for the low-, middle-, and high-exposure chambers; geometric standard deviations (GSD) ranged between 2.0 and 2.3.

Animals. Animals were obtained from Charles River (Charles River, Kingston, N.Y.) at 9 weeks of age and were quarantined at our laboratory for 3 weeks prior to exposure. Animals were individually identified by ear tags and randomly assigned by weight to an exposure group. The group of 128 male and 128 female Fischer-344 (F-344) rats were distributed equally over the three treatment and the control groups. They were continuously housed in the chambers and exposed for 6 hr/day, 5 days/week for either 5 or 13 weeks. Ten male and 10 female rats from each treatment and the control group were killed during the fifth week of exposure; other animals were killed during Week 13. Another group of animals, exposed for 13 weeks, were maintained for 26 weeks after exposure for hematology studies. Animals were observed twice daily for signs of toxicity and weighed weekly. Feed (Wayne Lab Blox, Chicago, Ill.; 24.5% protein) was available *ad libitum* during nonexposure hours; animals had free access to water at all times.

Serologic tests for common viral agents and *Mycoplasma pulmonis* were performed on blood samples collected at the end of the study from 15 rats randomly selected from each exposure chamber. Serologic assays were performed by Microbiological Associates (Bethesda, Md.).

Blood samples for hematology and clinical chemistry evaluations were obtained from rats after 5 or 13 weeks of exposure. In addition, blood samples were collected from male rats 26 weeks after completion of 13 weeks of exposure. Samples for hematology were collected from free-flowing tail vein blood of unanesthetized rats into tubes containing EDTA; the samples were assayed using a Coulter Counter Model S. Blood samples for serum chemistry measurements were obtained without anticoagulants, by heart puncture of anesthetized rats. These samples were evaluated using an Abbott VP bichromatic chemistry analyzer. Bone marrow smears were prepared from femurs taken from animals that were killed.

A necropsy (35 tissues) was performed on the rats after 5 or 13 weeks exposure, and the weights of heart, liver, kidneys, spleen, thymus, liver, gonads, and adrenals were recorded. All tissues were fixed in 10% neutral buffered Formalin (NBF). Tissues were obtained from 10 randomly selected rats of each sex from each treatment and the control group after 5 or 13 weeks of exposure and examined histologically. Tissues examined include: brain, pituitary gland, skin, mammary gland, trachea, esophagus, thyroid gland, parathyroid gland, larynx, thymus, tracheobronchial lymph node, liver, lung, spleen, pancreas, heart, aorta, kidney, adrenal glands, stomach, duodenum, colon, testes, salivary gland, mandibular lymph node, urinary bladder, prostate, ovaries, uterus, nasal structures, bone marrow, cecum, mesenteric lymph node, and grossly abnormal tissues. The nasal sections were taken at three levels: (1) immediately posterior to the incisor teeth, (2) midway between the incisor teeth and first molars, and (3) through the second molar. Tissues were embedded in paraffin, cut at 4 to 6 μm, and stained with hematoxylin and eosin (H&E). Bone marrow smears were stained with Wright–Giemsa stain. Selected special stains were used when necessary, and transmission electron microscopy was conducted on selected liver and cecal samples from randomly selected rats. All tissues from high-dose and control groups were examined by light microscopy; target organs identified in the high-dose group were trimmed and examined for the next lower dose groups until either no effect was observed or the lowest dose group specimens were examined.

Statistics. Body weight growth curves were analyzed by a randomization test (Lindgren, 1963); this test is based on the absolute area between two curves defined by connecting successive mean weights (Steel and Torrie, 1960). Analysis of variance was used to analyze tissue weight, hematology, and clinical pathology data. Treatment means were compared with control means by Dunnett's test (Dunnett, 1955). Lesion data for each treatment group were compared with control lesion data by Fisher's exact test (Siegel, 1956).

RESULTS

Weekly body weight data obtained during the 13-week exposure are shown in Figs. 1A and B. While control males grew at a steady rate during the exposure period, gaining a total of approximately 120 g, animals exposed to the highest aerosol concentration lost weight during the first 2 weeks of exposure, and then failed to gain weight throughout the remainder of the period. Male rats in the middle-dose group consistently gained significantly less weight than controls ($p < 0.01$); weight gain for males in the low-dose group was not statistically different from that of the control group. Similar effects on weight gain were observed for female rats, although the absolute differences in body weight between exposed and control groups were less dramatic than for males. The effects of exposure on both males and females were clearly dose related.

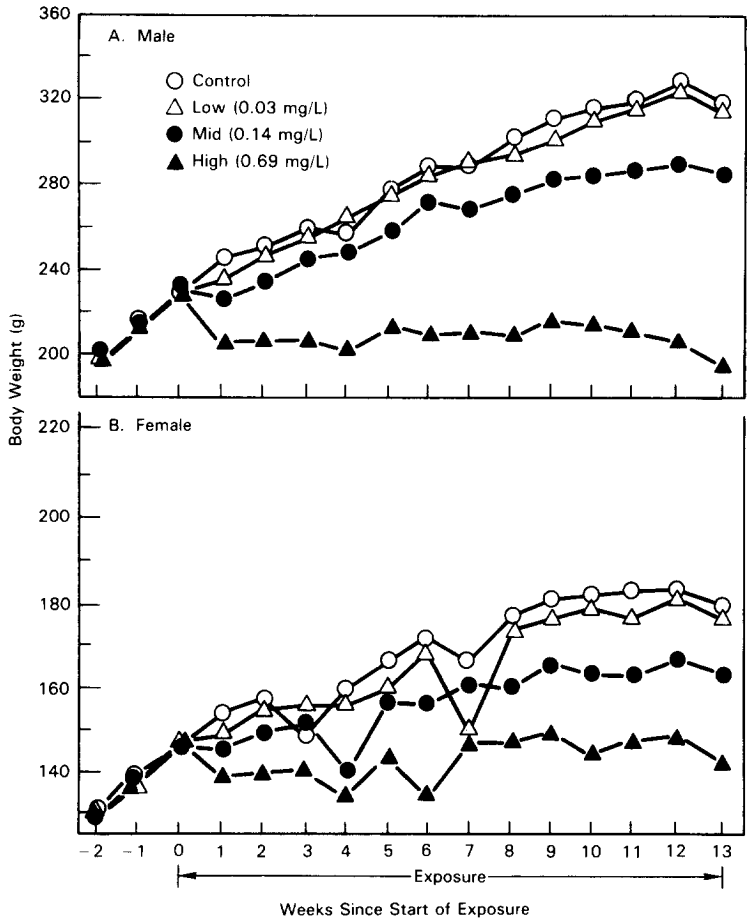

FIG. 1. Body weights for male and female rats during 13-week inhalation exposure to SRC-II HD: (A) male rats; (B) female rats.

Even though body weight gain was severely depressed for rats in the high-dose group, 91% of the females and 94% of the males from the exposure groups survived 13 weeks of exposure; all but one of the deaths occurred during the 11th and 12th week of exposure. Two females from the high- and two from the middle-exposure groups died near the end of the exposure period from undetermined causes. In addition, one female rat from the low-dose group died, as did one from the control group; the cause of these deaths was not clearly identified, although both animals may have been injured during handling. Prior to the 13-week termination, high-dose-group animals appeared listless; in addition, the eyes of many lacked their normal pinkish color, suggesting that they were anemic.

Organ weights, expressed as organ to body weight ratio (%) for 5- and 13-week-exposed animals are shown in Table 2. After 5-weeks exposure significant increases were observed in relative weights of liver for high-dose males and for females from all three treatment groups. Relative thymus weights were significantly less than those of controls for females from the high exposure group. Absolute kidney weights for high-dose male rats were significantly lower than those for controls; conversely, kidney weights for female rats from the same exposure group were significantly higher than for controls. When the weights for

TABLE 2

SUMMARY OF BODY WEIGHT AND ORGAN TO BODY WEIGHT RATIO (% $\bar{x} \pm$ SEM) FOR RATS EXPOSED BY INHALATION FOR 5 OR 13 WEEKS TO SOLVENT REFINED COAL-II HEAVY DISTILLATE

Sex	Measurement	Exposure group			
		Control	Low	Middle	High
		After 5-week exposure			
Male	*N*	10	10	10	10
	Body	269 ± 3.93	259 ± 5.78	249 ± 4.55[a]	196 ± 3.42[a]
	Liver	3.610 ± 0.088	3.804 ± 0.102	3.864 ± 0.121	5.640 ± 0.169[a]
	Kidney	0.714 ± 0.009	0.753 ± 0.009[a]	0.771 ± 0.011[a]	0.911 ± 0.013[a]
	Thymus	0.091 ± 0.009	0.113 ± 0.017	0.085 ± 0.010	0.075 ± 0.011
	Brain	0.646 ± 0.016	0.671 ± 0.030	0.677 ± 0.026	0.872 ± 0.022[a]
Female	*N*	10	10	10	10
	Body	154 ± 1.88	155 ± 2.59	145 ± 2.36[a]	132 ± 2.97[a]
	Liver	3.213 ± 0.054	3.525 ± 0.047[a]	3.837 ± 0.069[a]	5.490 ± 0.099[a]
	Kidney	0.761 ± 0.007	0.786 ± 0.010	0.836 ± 0.004[a]	0.966 ± 0.014[a]
	Thymus	0.152 ± 0.014	0.132 ± 0.007	0.131 ± 0.007	0.054 ± 0.010[a]
	Brain	1.029 ± 0.029	1.020 ± 0.045	1.096 ± 0.043	1.161 ± 0.038
		After 13-week exposure			
Male	*N*	22	22	22	20
	Body	319 ± 3.03	314 ± 3.97	286 ± 3.88[a]	194 ± 5.37[a]
	Liver	3.354 ± 0.049	3.735 ± 0.076[a]	4.279 ± 0.097[a]	6.425 ± 0.153[a]
	Kidney	0.670 ± 0.009	0.688 ± 0.010	0.732 ± 0.011[a]	1.047 ± 0.028[a]
	Thymus	0.089 ± 0.005	0.082 ± 0.006	0.065 ± 0.003[a]	0.039 ± 0.004[a]
	Testis	0.930 ± 0.010	0.952 ± 0.010	0.999 ± 0.012[a]	1.237 ± 0.036[a]
	Brain	0.591 ± 0.006	0.603 ± 0.011	0.645 ± 0.012[a]	0.912 ± 0.024[a]
Female	*N*	21	21	20	19
	Body	180 ± 1.61	176 ± 1.99	163 ± 2.93[a]	142 ± 2.33[a]
	Liver	3.545 ± 0.086	3.548 ± 0.077	4.185 ± 0.122[a]	6.368 ± 0.112[a]
	Kidney	0.744 ± 0.012	0.767 ± 0.024	0.807 ± 0.012[a]	0.968 ± 0.019[a]
	Thymus	0.127 ± 0.009	0.110 ± 0.005	0.090 ± 0.005[a]	0.049 ± 0.006[a]
	Ovaries	0.038 ± 0.002	0.036 ± 0.001	0.037 ± 0.001	0.026 ± 0.002[a]
	Brain	0.988 ± 0.019	1.001 ± 0.009	1.075 ± 0.017[a]	1.148 ± 0.028[a]

[a] Significantly different from control mean ($p < 0.05$).

kidneys were evaluated on a percentage-of-body-weight basis, they were significantly greater than those of controls for both males and females from the middle- and high-dose groups. Absolute brain weights were relatively constant across all treatment groups. The weights of heart, spleen, and adrenals were lower than those of controls on an absolute basis; however, on a relative-weight basis, they were higher than those of controls.

After 13 weeks of exposure, changes in organ weights were similar to those described for the 5-week-exposed animals. Liver weights for male rats were signficantly elevated, relative to controls, for animals in the three treatment groups; this increase was dose dependent. For female rats, there was a significant increase in liver weights for animals in the high-exposure group and a significant trend for increased weight with increasing dose. Absolute kidney weights for male rats from the high-dose group were significantly lower than

those for controls; however, weights of this organ for high-dose female rats were not altered relative to those of controls. When kidney weights were evaluated on percentage-of-body-weight basis, they were elevated relative to those of controls for both sexes from the middle- and high-exposure groups. Thymus weights decreased in a dose-dependent manner, with significant decreases for animals in the middle- and high-dose groups. The higher liver weights and lower thymus and ovary weights were also statistically significant when analyzed as percentage of body weight. Heart, spleen, testis, and adrenal weights were significantly lower on an absolute-organ-weight basis and were significantly elevated on relative-weight bases when compared to those for controls.

After 5-weeks exposure to HD, animals from the middle- and high-exposure groups had significantly decreased volumes of packed red cells (VPRC), hemoglobin (Hgb) concentrations, and number of red blood cells (RBC), relative to controls. The number of reticulocytes was significantly increased compared to controls for these groups (Table 3). After 13 weeks of exposure, decreases in erythrocyte parameters were significant for high-exposure group females and for males from all three treatment groups; quantitatively, these effects were more severe than after 5 weeks of exposure. In addition, the magnitude of increase in the number of reticulocytes was smaller after 13 weeks of exposure than was observed after 5 weeks of treatment. By 26 weeks after exposure, there were small but significant differences in the numbers of RBC in samples taken from male rats in the middle- and high-exposure groups; however, these differences were much smaller than that observed immediately after 13 weeks of exposure (Table 3). By 2 to 4 weeks after exposure, the animals' eyes were sufficiently pink that high-dose animals were no longer distinguishable from controls on this basis.

The total numbers of white blood cells (WBC) were similar for control and treated female rats after 5 weeks of exposure (Table 4). Although WBC appeared to increase for male rats in the 5-week high-dose group, this difference was attributed to one animal with an extraordinarily large neutrophil count. When data for this animal were eliminated, the mean for this group decreased so that it was no longer different from the control group. After 5-weeks exposure, numbers of eosinophils from treated animals decreased significantly relative to controls. After 13-weeks exposure, total WBC for high-exposure-group animals was significantly less than that for controls, primarily as a result of decreased numbers of lymphocytes. Numbers of eosinophils and monocytes from exposed animals were also significantly lower, relative to controls. These decreases were usually dose related with the most pronounced effects in the middle- and high-dose groups. By 26 weeks after exposure, the total number of WBC, lymphocytes, and eosinophils from the high-exposure group were still significantly below those of controls. The magnitudes of these decreases were similar to those observed for animals sampled immediately after 13-weeks exposure.

After 5-weeks exposure, cholesterol concentrations for males from the high-dose group and females from the middle- and high-dose group were elevated relative to controls (Table 5). Triglyceride concentrations were significantly below controls for males from the middle- and high-exposure groups. Concentrations of albumin, globulin, blood urea nitrogen, glucose, and bilirubin, and activities of SGPT and LDH were similar for control and treated animals after 5-weeks exposure. After 13-weeks exposure (Table 5), triglycerides and blood urea nitrogen had significantly increased, and total protein, albumin, globulin, and lactate dehydrogenase (LDH) had decreased; these effects were present primarily in animals from the high-exposure group. Cholesterol concentration significantly increased for high-dose males, with a statistically significant dose-related trend. In addition, an increase in cholesterol concentration was apparent for females from the middle-exposure group but not the high-exposure group. Serum

TABLE 3

ERYTHROCYTE MEASUREMENTS ($\bar{x} \pm$ SEM) FOR RATS EXPOSED BY INHALATION TO SOLVENT REFINED COAL-II HEAVY DISTILLATE

Measurement	Male				Female			
	Control	Low	Middle	High	Control	Low	Middle	High
				5-week exposure				
VPRC (ml/dl)	42.3 ± 0.56	40.6 ± 0.51	38.6 ± 0.92[a]	33.4 ± 1.29[a]	40.7 ± 0.63	39.6 ± 0.21	37.6 ± 0.35[a]	33.9 ± 0.37[a]
Hgb (g/dl)	16.7 ± 0.24	16.2 ± 0.16	15.1 ± 0.36[a]	12.9 ± 0.54[a]	16.1 ± 0.24	15.6 ± 0.14	14.6 ± 0.13[a]	13.2 ± 0.16[a]
RBC ($10^6/\mu l$)	8.90 ± 0.11	8.62 ± 0.09	8.17 ± 0.20[a]	7.06 ± 0.32[a]	8.08 ± 0.13	7.83 ± 0.06	7.47 ± 0.10[a]	7.16 ± 0.09[a]
Reticulocytes ($10^3/\mu l$)	185 ± 22	178 ± 15	236 ± 16	684 ± 108[a]	167 ± 8	176 ± 15	261 ± 20[a]	423 ± 23[a]
				13-week exposure				
VPRC (ml/dl)	44.0 ± 0.16	40.4 ± 1.69[a]	39.2 ± 0.23[a]	18.4 ± 1.65[a]	44.2 ± 0.26	42.6 ± 0.40	40.7 ± 0.60	27.0 ± 3.05[a]
Hgb (g/dl)	17.1 ± 0.06	16.3 ± 0.13	15.2 ± 0.08[a]	7.06 ± 0.60[a]	17.2 ± 0.09	16.6 ± 0.17	15.7 ± 0.18[a]	10.3 ± 1.18[a]
RBC ($10^6/\mu l$)	8.93 ± 0.04	8.86 ± 0.08	8.29 ± 0.06[a]	3.28 ± 0.36[a]	8.34 ± 0.05	8.04 ± 0.09	7.84 ± 0.11	5.24 ± 0.71[a]
Reticulocytes ($10^3/\mu l$)	112 ± 25	87 ± 23	242 ± 126	168 ± 40	90 ± 26	117 ± 23	86 ± 28	294 ± 70[a]
				26 weeks after exposure				
VPRC (ml/dl)	44.0 ± 0.38	44.0 ± 0.29	43.6 ± 0.39	43.7 ± 0.16				
Hgb (g/dl)	17.5 ± 0.11	17.5 ± 0.12	17.3 ± 0.12	17.2 ± 0.07				
RBC ($10^6/\mu l$)	9.2 ± 0.06	9.1 ± 0.05	8.9 ± 0.09[a]	8.5 ± 0.05[a]				
Reticulocytes ($10^3/\mu l$)	157 ± 11	173 ± 11	157 ± 13	133 ± 10				

[a] Significantly different from control mean ($p < 0.05$).

TABLE 4

WHITE BLOOD CELL COUNTS ($\bar{x} \pm$ SEM) FOR RATS EXPOSED BY INHALATION TO SOLVENT REFINED COAL-II HEAVY DISTILLATE

	Male				Female			
Measurement[a]	Control	Low	Middle	High	Control	Low	MIddle	High
				5-week exposure				
Total WBC	7.06 ± 0.40	7.05 ± 0.38	6.97 ± 0.33	9.78 ± 1.65	6.50 ± 0.27	5.80 ± 0.29	5.99 ± 0.46	6.03 ± 0.36
Lymphocytes	5.28 ± 0.34	5.33 ± 0.29	5.05 ± 0.32	5.45 ± 0.69	4.84 ± 0.28	4.65 ± 0.26	4.46 ± 0.27	4.26 ± 0.19
Neutrophils	1.68 ± 0.13	1.64 ± 0.12	1.87 ± 0.27	4.22 ± 0.97[b]	1.58 ± 0.15	1.09 ± 0.10	1.48 ± 0.31	1.75 ± 0.29
Eosinophils	85 ± 17	35 ± 14[b]	30 ± 14[b]	10 ± 6[b]	65 ± 21	26 ± 9	27 ± 12	8 ± 4[b]
Monocytes	13 ± 7	33 ± 11	14 ± 7	35 ± 18	17 ± 9	25 ± 10	19 ± 8	5 ± 3
				13-week exposure				
Total WBC	9.30 ± 0.52	9.60 ± 0.43	8.38 ± 0.64	6.35 ± 0.33[b]	7.72 ± 0.43	7.77 ± 0.37	7.09 ± 0.28	5.81 ± 0.48[b]
Lymphocytes	6.55 ± 0.47	6.64 ± 0.45	6.06 ± 0.46	4.35 ± 0.20[b]	6.00 ± 0.33	6.12 ± 0.28	5.80 ± 0.19	4.22 ± 0.34[b]
Neutrophils	2.52 ± 0.14	2.69 ± 0.24	2.19 ± 0.44	1.97 ± 0.21	1.52 ± 0.14	1.55 ± 0.18	1.19 ± 0.19	1.56 ± 0.20
Eosinophils	104 ± 14	98 ± 16	41 ± 18[b]	5 ± 3[b]	90 ± 12	44 ± 11[b]	31 ± 9[b]	2 ± 2[b]
Monocytes	120 ± 25	175 ± 49	80 ± 25	14 ± 6[b]	115 ± 20	59 ± 14[b]	66 ± 12[b]	30 ± 8[b]
				26 weeks after exposure				
Total WBC	9.68 ± 0.51	9.06 ± 0.32	10.3 ± 1.14	7.17 ± 0.41[b]				
Lymphocytes	5.66 ± 0.46	6.34 ± 0.30	6.45 ± 0.52	4.34 ± 0.25[b]				
Neutrophils	3.77 ± 0.65	2.41 ± 0.23	3.60 ± 0.70	2.71 ± 0.41				
Eosinophils	116 ± 33	134 ± 29	71 ± 44	14 ± 9[b]				
Monocytes	108 ± 37	136 ± 45	122 ± 38	71 ± 30				

[a] Total WBC, lymphocytes, and neutrophil data times 10^3 gives the number of cells/μl; eosinophils and monocytes are shown as cells/μl.

[b] Significantly different from control mean ($p < 0.05$).

TABLE 5

VALUES ($\bar{x} \pm$ SEM) FOR CLINICAL CHEMISTRY DATA FOR RATS EXPOSED BY INHALATION TO SOLVENT REFINED COAL-II HEAVY DISTILLATE FOR 5 OR 13 WEEKS

	Male				Female			
Measurement	Control	Low	Middle	High	Control	Low	Middle	High
After 5-week exposure								
Cholesterol (mg/dl)	64.6 ± 4.52	54.7 ± 4.06	51.0 ± 3.10	95.0 ± 6.30[a]	71.4 ± 4.42	79.4 ± 3.43	92.4 ± 2.25[a]	117 ± 6.75[a]
Triglycerides (mg/dl)	227 ± 11.1	200 ± 12.7	180 ± 13.6[a]	183 ± 11.0[a]	137 ± 9.99	163 ± 12.0	149 ± 9.26	152 ± 8.08
After 13-week exposure								
Albumin (g/dl)	3.80 ± 0.08	3.88 ± 0.08	4.10 ± 0.09	3.32 ± 0.10[a]	3.84 ± 0.04	3.89 ± 0.07	3.91 ± 0.06	3.35 ± 0.09[a]
Globulin (g/dl)	1.86 ± 0.08	1.91 ± 0.04	1.80 ± 0.06	1.69 ± 0.05	1.80 ± 0.05	1.78 ± 0.07	1.68 ± 0.05	1.46 ± 0.07[a]
Cholesterol (mg/dl)	55.5 ± 2.59	57.1 ± 1.82	68.9 ± 4.18	92.2 ± 7.53[a]	80.5 ± 3.21	81.6 ± 2.41	99.3 ± 4.40[a]	83.1 ± 4.12
Triglycerides (mg/dl)	134 ± 13.4	151 ± 15.1	144 ± 10.6	185 ± 13.9[a]	114 ± 7.57	126 ± 7.22	107 ± 4.26	147 ± 6.25[a]
Blood urea nitrogen (mg/dl)	20.5 ± 0.79	20.3 ± 0.97	20.1 ± 0.62	36.2 ± 4.43[a]	25.1 ± 1.41	24.7 ± 0.98	25.1 ± 1.02	31.9 ± 3.32[a]
Glucose (mg/dl)	203 ± 12.4	204 ± 8.20	200 ± 5.17	180 ± 13.6	172 ± 5.47	171 ± 4.12	173 ± 5.76	182 ± 11.56
Bilirubin (mg/dl)	0.12 ± 0.03	0.08 ± 0.01	0.06 ± 0.01	0.12 ± 0.03	0.07 ± 0.02	0.08 ± 0.02	0.06 ± 0.01	0.04 ± 0.01
SGPT (IU/liter)	49.9 ± 3.41	50.3 ± 4.24	37.4 ± 1.63	126 ± 33.79[a]	90.7 ± 13.0	64.2 ± 8.09	55.2 ± 5.58[a]	49.1 ± 8.83[a]
LDH (IU/liter)	1128 ± 90	1233 ± 99	1070 ± 116	422 ± 70[a]	789 ± 84	743 ± 20	909 ± 108	326 ± 55[a]

[a] Significantly different from control mean ($p < 0.05$).

glutamic pyruvic transaminase (SGPT) activity increased for males from the high-dose group and decreased for females from the middle- and high-treatment groups. Glucose and bilirubin concentrations for exposed animals were not significantly different from those for controls.

Microscopic examination of liver sections revealed lesions in high-dose males and females after 5 and 13 weeks of exposure (Tables 6 and 7); there was also evidence of similar changes in the middle-dose group, although this lesion was less prominent than those for the high-dose group. Hepatic lesions were

TABLE 6

LESIONS (NUMBER OF LESIONS/NUMBER EXAMINED) FOR RATS KILLED AFTER 5-WEEK INHALATION EXPOSURE TO SOLVENT REFINED COAL-II HEAVY DISTILLATE

	Control		High		Middle		Low	
Measurement	Male	Female	Male	Female	Male	Female	Male	Female
Liver								
Hepatopathy	0/10	0/10	9/10[a]	10/10[a]	2/10	0/10	0/10	NE[b]
Necrosis	0/10	0/10	2/10	0/10	0/10	0/10	2/10	NE
Kidney								
Pigmentation of cortical tubules	0/10	0/10	0/10	0/10	0/10	0/10	NE	NE
Pelvic epithelial hyperplasia	0/10	0/10	4/10[a]	0/10	0/10	0/10	NE	NE
Thymus								
Atrophy	0/10	0/7	8/8[a]	2/3	0/8	0/10	NE	NE
Ovary								
Decreased luteal tissue		0/10		5/10[a]		0/10		NE
Bone marrow								
General hypocellularity	0/9	0/10	0/10	0/10	NE	NE	NE	NE
Decrease in megakaryocytes	0/9	1/10	0/10	0/10	NE	NE	NE	NE
Nasal sections (most anterior section)								
Squamous metaplasia	0/10	0/10	3/10	0/10	0/10	NE	1/10	NE
Suppurative inflammation	0/10	0/10	0/10	1/10	0/10	NE	0/10	NE
Lung								
Histiocytosis	0/10	0/10	10/10[a]	10/10[a]	10/10[a]	9/10[a]	10/10[a]	10/10[a]
Spleen								
Decrease in megakaryocytes	0/10	0/10	6/10[a]	7/10[a]	0/10	0/10	NE	NE
Cecum								
Epithelial hyperplasia	0/9	1/10	4/10[a]	0/9	0/10	0/10	NE	NE
Ulcer	0/9	0/10	3/10	0/9	0/10	0/10	NE	NE
Chronic active inflammation	0/9	0/10	5/10[a]	3/9	0/10	0/10	NE	NE

[a] Significantly different from control ($p < 0.05$).
[b] Not examined.

TABLE 7

LESIONS (NUMBER OF LESIONS/NUMBER EXAMINED) FOR RATS KILLED AFTER 13-WEEK INHALATION EXPOSURE TO SOLVENT REFINED COAL-II HEAVY DISTILLATE

	Control		High		Middle		Low	
Measurement	Male	Female	Male	Female	Male	Female	Male	Female
Liver								
Hepatopathy	0/10	0/10	10/10[a]	9/10[a]	3/10	1/10	0/10	0/10
Necrosis	0/10	0/10	1/10	2/9	0/10	0/10	1/10	1/10
Kidney								
Pigmentation of cortical tubules	0/10	0/10	9/10[a]	10/10[a]	0/10	0/10	0/10	NE[b]
Pelvic epithelial hyperplasia	0/8	0/10	6/10[a]	8/10[a]	6/9[a]	0/10	0/10	NE
Thymus								
Atrophy	0/10	0/10	6/6[a]	8/8[a]	0/8	0/8	NE	NE
Ovary								
Decreased luteal tissue		0/10		5/10[a]		0/10		NE
Bone marrow								
General hypocellularity	0/10	0/10	6/10[a]	4/10[a]	0/8	0/9	NE	NE
Decrease in megakaryocytes	0/10	0/10	8/10[a]	5/10[a]	0/8	0/9	NE	NE
Nasal sections (most anterior section)								
Squamous metaplasia	0/10	0/10	2/10	7/10[a]	1/10	2/10	0/10	0/10
Suppurative inflammation	0/10	0/10	6/10[a]	4/10[a]	0/10	1/10	0/10	0/10
Lung								
Histiocytosis	0/10	0/10	10/10[a]	10/10[a]	8/10[a]	10/10[a]	7/10[a]	7/10[a]
Spleen								
Decrease in megakaryocytes	2/10	0/10	10/10[a]	10/10[a]	0/10	2/10	NE	NE
Cecum								
Epithelial hyperplasia	0/10	0/10	8/10[a]	6/10[a]	0/10	0/10	NE	NE
Ulcer	0/10	0/10	7/10[a]	3/10	0/10	0/10	NE	NE
Chronic active inflammation	0/10	0/10	8/10[a]	7/10[a]	0/10	0/10	NE	NE

[a] Significantly different from control ($p < 0.05$).
[b] Not examined.

characterized by a slight increase in cytoplasmic basophilia, increased variability in hepatocellular size, hepatomegalocytes, increased numbers of cells with double nuclei, increased variability in nuclear size, and loss of cording and lobular patterns of the liver with apparent sinusoidal compression (Fig. 2). Focal hepatic necrosis and single-cell necrosis were observed in a few treated animals; however, the incidence was low and the lesions were of minimal severity. Transmission electron microscopy of liver samples from high-dose animals demonstrated an increase in smooth endoplasmic reticulum, a decrease in

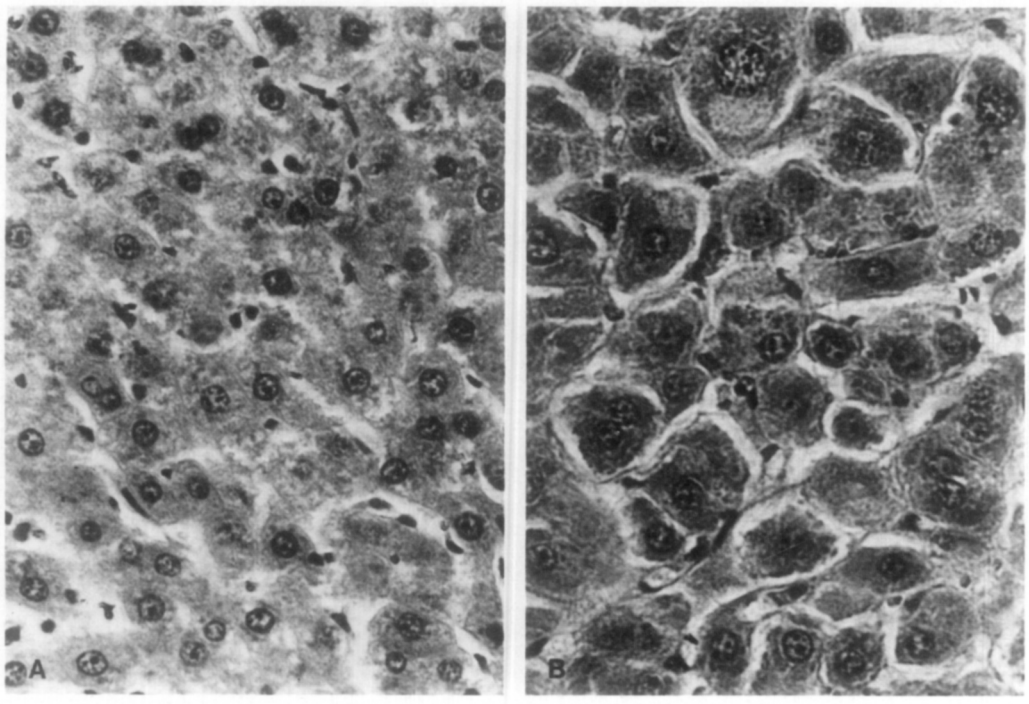

FIG. 2. Photomicrographs of liver sections from a F-344 rat, demonstrating an increase in variabilty of hepatocyte and nucleus size, hepatomegalocytes, and loss of cording pattern of the liver. (A) Control; (B) section from a rat exposed by inhalation to 0.69 mg/liter of HD for 13 weeks. (H&E stain; original magnification, ×250).

glycogen, and an apparent increase in secondary lysosomes.

Renal pelvic epithelial hyperplasia, observed in exposed animals, was characterized by proliferation of cells usually along most of the pelvic epithelial surface; this effect was more prominent at the junction of the pelvic and papillary epithelium. The papillary epithelial hyperplasia did not extend to the tip of the papillae. The thickened epithelium contained vacuoles, a few degenerative cells, and some mitotic figures (Fig. 3). These hyperplastic lesions were present in both sexes from the high-dose group after 13 weeks of exposure, in high-dose male rats after 5 weeks of exposure, and in male rats from the middle-dose group after 13 weeks of exposure. Minimal to mild unidentified brown pigmentation occurred in the cortical renal tubular epithelium of male and female rats from the high-dose group after 13 weeks of exposure. When kidney sections were stained for hemosiderin and lipofuscin, the brown pigment failed to take up stain.

Atrophy of the thymus was characterized by prominent lymphoid depletion and loss of normal cortical-medullary architecture. This effect was apparent only in the high-dose male and female animals after 5 or 13 weeks of exposure.

An apparent decrease in the number of corpora lutea relative to controls was observed in high-dose animals; other ovarian structures appeared normal.

Examination of bone marrow smears from controls and high-dose rats showed that high-dose animals had hypocellular marrows with a marked decrease in the number of megakaryocytes. These changes were characterized by a reduction of both myeloid and erythroid cells, with a marked shift in the myeloid/ery-

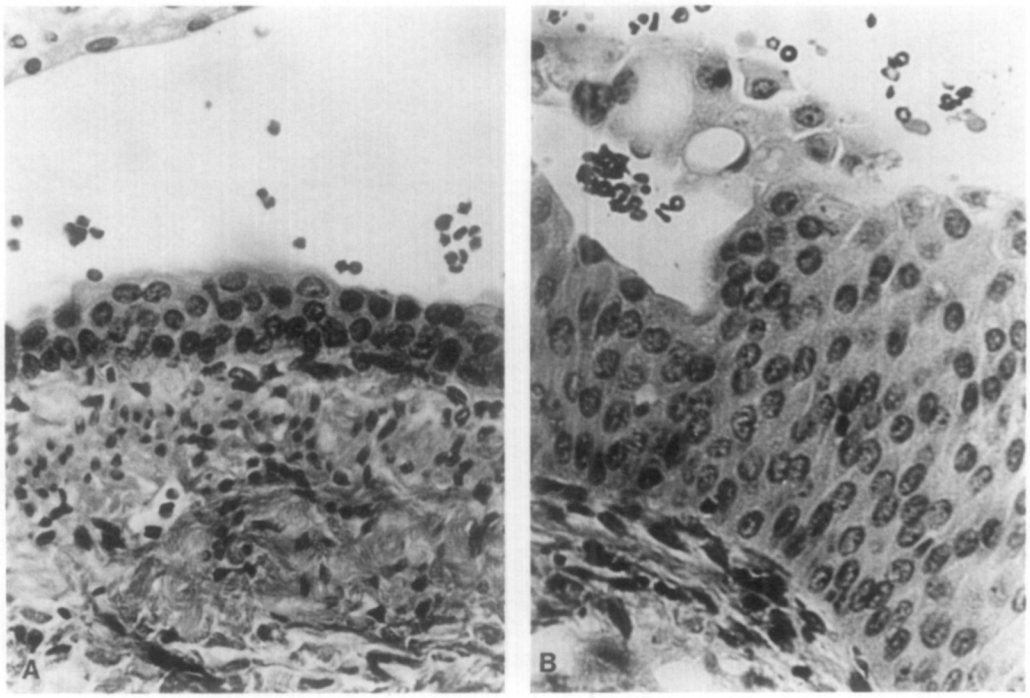

FIG. 3. Photomicrographs of the renal pelvic epithelium of a F-344 rat, demonstrating an increase in the number of layers of cells and vacuolar degenerative changes. Both sections are from the same plane of sectioning and same location of pelvic epithelium. (A) Control; (B) section from a rat exposed by inhalation to 0.69 mg/liter of HD for 13 weeks. (H&E stain; original magnification, ×250).

throid ratio from 1:1 for controls to 1:8 for high-dose animals after 13-weeks exposure. The sternal bone marrow sections of several high-dose male and female rats from the 13-week-exposure group showed an overall decrease in most cell types (Fig. 4). Some bone marrow sections with otherwise adequate cellularity had decreased numbers of megakaryocytes. This change was not seen in 5-week exposed animals. The number of megakaryocytes was decreased, relative to controls, in spleens of high-dose male and female rats after 5 or 13 weeks of exposure. Control rats averaged five to six megakaryocytes per cross-section of spleen; similar preparations from high-dose animals contained either none or one megakaryocyte.

Examination of nasal tissue sections revealed exposure-related lesions, primarily restricted to the most anterior section, which were characterized by mild squamous metaplasia and mild suppurative inflammation of the respiratory epithelial cells (Fig. 5). Squamous metaplasia was present at the dorsolateral aspects of the most anterior nasal cavity between the maxillary and nasoturbinates and at the dorsal tip of the maxillary turbinates. Squamous metaplasia was apparent in the high-dose, 13-week exposure, male and female rats; less obvious in the middle-dose, 13-week exposure, male and female rats; and possibly an effect in the high-dose, 5-week exposure male rats. Suppurative inflammation of the most anterior nasal cavity was prominent only in the 13-week, high-dose, male and female rats. Only minimal brown pigment was apparent in the mucosa and submucosa of exposed animals; staining for iron and lipofuscin was negative.

Nasal respiratory epithelial cell hyperplasia was more pronounced in the high- and middle-dose groups of the 13-week exposure male and

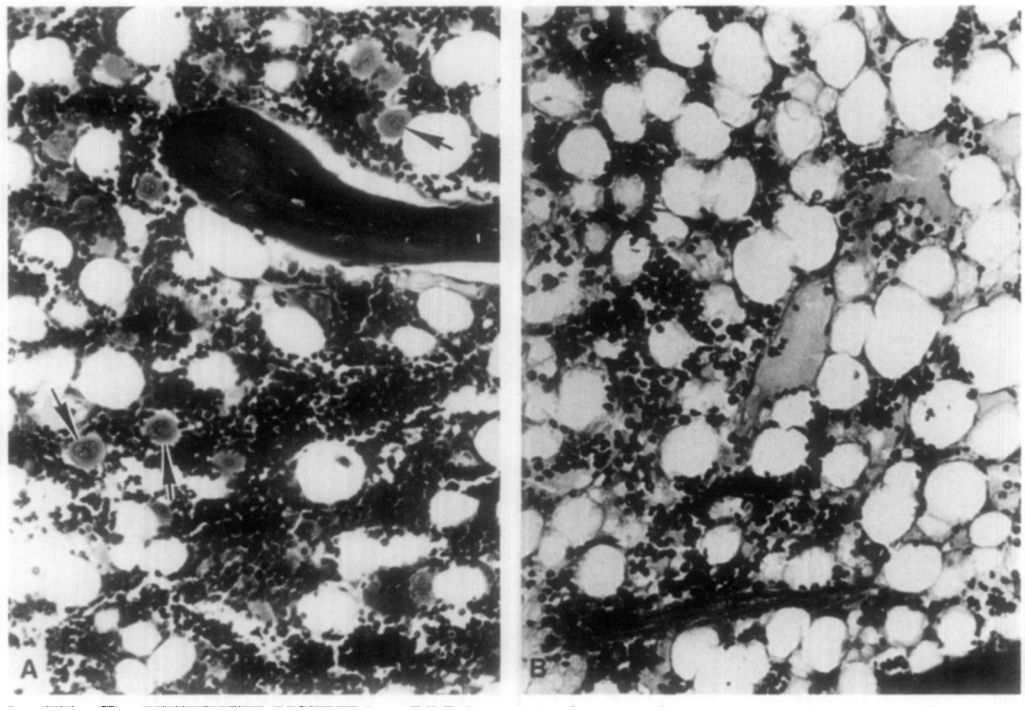

FIG. 4. Photomicrographs of the sternal bone marrow from an F-344 rat, demonstrating an overall decrease in cellularity and lack of megakaryocytes. (A) Control animal with abundant megakaryocytes (arrows); (B) section from an F-344 rat exposed by inhalation to 0.69 mg/liter of HD for 13 weeks. (H&E stain; original magnification, ×125).

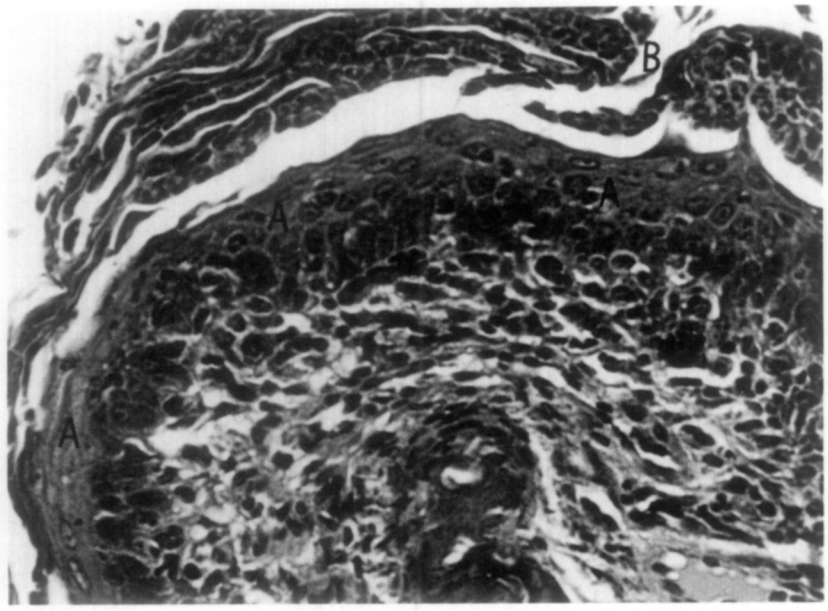

FIG. 5. Photomicrograph of a nasal section from the dorsal tip of the maxilloturbinate of an F-344 rat exposed by inhalation to 0.69 mg/liter of HD for 13 weeks. Demonstrates prominent squamous metaplasia of respiratory epithelium (A) and exudate containing neutrophils (B) on the surface of the metaplastic epithelium. (H&E stain; original magnification, ×250).

female rats than in the controls or low-dose groups; however, the presence of minimal to mild epithelial hyperplasia in control and low-dose group animals after 5 or 13 weeks of exposure complicated interpretation of this lesion. Nonspecific mononuclear inflammation was present and scattered throughout all treatment groups.

Lesions were not detected in the larynges or tracheas of rats of any dose group. Lungs of high-dose male and female rats had minimal to mild diffuse increases in alveolar macrophages (histiocytosis) in both the 13- and 5-week exposure high-dose groups; this response was much less pronounced in the middle- and low-dose groups after 5- or 13-weeks exposure. There was no evidence of pigment in the lungs of exposed animals.

A prominent, treatment-related lesion present near the ileocecal orifice consisted of deep, focal, mucosal ulcerations and focal epithelial proliferation (Fig. 6). Frequently, the proliferating epithelium was seen beneath the muscularis mucosa. In addition, the lesion was covered with a zone of necrotic tissue, contained a mixed inflammatory cell reaction that extended into the submucosa and muscularis, and had a peripheral zone of granulation tissue. Occasionally, epithelial hyperplasia and inflammation were present without ulceration. Warthin–Starry silver stains and electron microscopic examination failed to demonstrate the presence of microorganisms that could be implicated in the etiology of the lesion. These lesions were most pronounced in both sexes after 13-week exposure and, to a lesser degree, in males from the high-dose group after 5-week exposure. Females from the 5-week exposure, high-dose group had evidence of only the chronic inflammatory infiltrate.

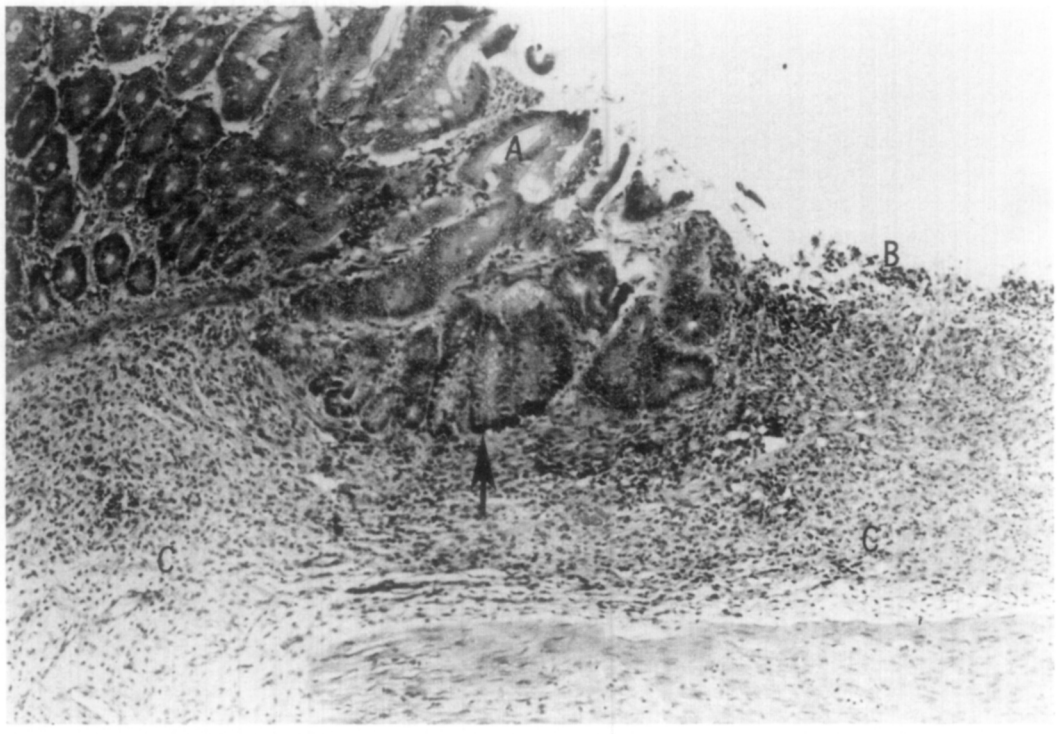

FIG. 6. Photomicrograph of a section of cecum from an F-344 rat exposed by inhalation to 0.69 mg/liter of HD for 13 weeks, demonstrating hyperplastic epithelium (A) next to an ulcer (B). Hyperplastic epithelium is also seen below the muscularis mucosa (arrow), and prominent chronic inflammation of the submucosa (C) is evident. (H&E stain; original magnification, ×50).

Epithelial hyperplasia of the squamous portion of the stomach was observed in two male and two female high-dose rats after 13-weeks exposure and in two male rats from the high-dose group after 5-weeks exposure. Urinary bladder epithelial hyperplasia was present in two female rats from the high-dose group after 13-weeks exposure and in one female rat from the high-dose group after 5 weeks of exposure; although these lesions were few in number and minimal in severity, they may represent changes attributable to the exposure.

Serologic tests on blood samples collected at the 13-week termination were negative for Kilham rat virus, H-1, rat coronavirus/sialodacryoadenitis virus, and *M. pulmonis;* however, titers for Sendai virus were positive. Since mice, which were housed with the rats during the exposure, had negative titers for Sendai virus, it was concluded that this infection occurred prior to beginning of the exposure.

DISCUSSION

Since HD is an extremely complex mixture, it is impossible to ascertain the determinant compounds with regard to toxicity, due to possible antagonisms and/or synergisms. HD is a high-boiling coal liquid (288 to 454°C) with essentially all components of the aerosol present in the liquid state in the oil droplets (Schirmer *et al.,* 1985). Comparison of the material used in this study with those of other studies provides a better perspective to our data. Carpenter *et al.* (1977) conducted a series of subchronic inhalation exposures to determine the effects of petroleum hydrocarbons. For those studies, aerosol concentrations ranged between 0.1 and 10 mg/liter. The materials boiled from 66° to 260°C, which was considerably lower than the boiling range for HD. Those petroleum-derived hydrocarbons were of lower molecular weight than our material and the aromatic portion was composed of a high percentage of alkylated benzene compounds. Significant biological effects attributable to the test material were rare and usually not dose related. For the 16 materials that they evaluated, there is little evidence for effects on survival, growth, tissue weights, hematology, or clinical pathology, even though the concentrations for most of the exposures were considerably higher than those in our study.

Comparison of the effects seen with HD to those known to occur following exposure to individual compounds present in HD revealed both similarities and differences in the observed toxic effects. For example, exposure of animals to benzo[a]pyrene (BaP) at a dose of approximately 100 mg/kg resulted in decreased numbers of primary oocytes in the ovaries (Mattison and Thorgeirsson, 1979) and hypocellularity of the bone marrow, followed by aplastic anemia (Nebert and Jensen, 1979). For comparison, we estimated the amount of HD deposited in the lung of the rat during inhalation exposure and calculated the weight of benzene, BaP, and PAH received by each rat per day of the exposure. Assuming a minute volume of 150 ml (Guyton, 1947), and that one-half the inhaled material was deposited in the lung or gastrointestinal tract, a 6-hr exposure to the high concentration (0.69 mg/liter) would result in a dose of about 60 mg/kg body wt/day. Since benzene and BaP are present in HD at 0.016 and 0.055%, the animal would receive about 0.010 and 0.034 mg/kg/day, respectively, a daily dose much lower than that observed to produce effects on bone marrow and oocytes. However, PAH other than BaP could produce similar kinds of effects. The PAH fraction of HD is composed primarily of parent and alkylated three- to six-fused-ring compounds, i.e., phenanthrene, pyrene, chrysene, and benzopyrene. Major nitrogen-containing PAH present in HD are parent and alkylated three- to five-fused-ring systems based on the carbazole and quinoline structures. Since the neutral PAH represents about 46% of the HD (Wilson *et al.,* 1984), the estimated dose of PAH was 28 mg/kg/day, indicating that it is near the range of a single dose of BaP known to result in effects on the ovary and bone marrow. The amount of PAH taken in by the animal through the skin or ingested because of

grooming was not measured and therefore not considered in the calculation; it is possible that this noninhaled dose may make a significant contribution to the total exposure.

Changes in organ weights were accompanied by cellular changes that were frequently apparent upon microscopic examination. Increased liver weights correlated well with observed light microscopic features of hepatocytomegaly and sinusoidal compression. Electron microscopy revealed increased amounts of smooth endoplastic reticulum, which is compatible with cytomegaly. In addition, decreased serum protein and albumin quantities suggest hepatocellular dysfunction whereas increased SGPT activity for males may indicate hepatocellular necrosis.

Although the changes in kidney weights and the appearance of lesions in this organ indicated that exposure to HD resulted in adverse effects, clinical chemistry parameters did not demonstrate clear changes in the function of this organ. Comparison of these effects with those observed following exposure to other lighter hydrocarbon mixtures indicated that the type of lesions produced by HD were dissimilar. For example, Bruner (1983) reported that inhalation exposure to jet fuel (JP-5) produced dilated and cystic renal tubules filled with eosinophilic granular debris near the corticomedullary junction. This lesion was observed only in male rats. The renal tubular epithelium had increased eosinophilic cytoplasmic droplets and was compressed in areas where there was abundant tubular debris. Tubular regeneration was also observed, and renal papillary epithelial hyperplasia was present in animals held 20 months after exposure. In another study, Phillips (1983) reported that Stoddard solvent produced (in male rats only) signs similar to those reported for JP-5. The types of kidney lesions observed by those and other workers (Carpenter *et al.*, 1977; Gaworski *et al.*, 1983; Halder *et al.*, 1984; Phillips and Egan, 1984; Phillips and Cockrell, 1984) were produced by hydrocarbon mixtures boiling between 60 and 220°C; the effect appears to be due to branched chain aliphatics and cycloparaffinic compounds.

Nasal lesions (suppurative inflammation and respiratory epithelial squamous metaplasia) observed for the rats were similar to those observed following exposure to other compounds affecting nasal tissue (Young, 1981). Lung changes in the form of increased numbers of alveolar macrophages, were apparent in all treated groups. Although pigmented material of HD origin was not observed in lung sections, the pulmonary alveolar macrophage response observed was compatible with the effects reported for another inhalation study using a coal tar aerosol (McConnell and Specht, 1973).

The cecal lesions observed in this study are not unlike those of hamster ileitis (Frisk and Wagner, 1977) and other animal intestinal diseases associated with the intracellular bacterium *Campylobacter* species (Fox *et al.*, 1982). Examination of cecal tissue, using transmission electron microscopy or light microscopy of silver-stained sections, did not identify similar organisms. This lesion appears to be related to exposure and future long-term studies may provide information as to its significance.

The fact that weights for heart, brain, testes, and adrenals decreased on an absolute-weight basis and increased on a relative basis indicates that they probably do not reflect biologically significant changes. This interpretation was substantiated by microscopic evaluation of these organs. Decreases in thymus weight may be due to stress associated with exposure (Riley, 1981) or the result of a direct toxic effect of HD.

Treatment-related changes in erythrocyte parameters following 5-weeks exposure progressed so that by 13 weeks of exposure, the number of RBC and the VPRC were about one-half (40% reduction for females and 60% for males) of that for the control animals. A contributing cause of anemia might be blood loss from cecal ulceration. Bone marrow preparations collected from animals immediately after 13-weeks exposure demonstrated substantial reductions, relative to controls, in the degree of cellularity, suggesting that components of HD produced adverse effects on

marrow stem cells. The lack of megakaryocytes in spleen and in bone marrow sections as well as bone marrow smears was further evidence of toxicity to stem cells. After a 6-month recovery period, values for erythrocyte parameters had nearly returned to control values.

In addition to the effects on red cells, leukocyte counts decreased with duration of exposure. However, unlike erythrocytes, the number of leukocytes from treated animals had not returned to control values 6 months after exposure ended, suggesting that effects on these stem cells were less reversible. Decreased number of leukocytes after 13 weeks of exposure might be the result of reduced lymphopoiesis; however, a contributing cause may be reductions mediated by increased amounts of adrenal corticosteroids due to chronic stress. This latter speculation is supported by the fact that the numbers of eosinophils, another cell type susceptible to corticosteroids, were also significantly reduced.

Although the aerosol concentration used for the high-exposure group of this study is high relative to anticipated human exposures, the fact that certain effects were observed in the low-exposure group and clear toxic effects were observed at 0.14 and 0.69 mg/liter indicate that these doses must be considered in setting exposure limits for humans. For comparison, 0.03 mg/liter is six times the threshold limit value (TLV) for exposure to nuisance dust and a factor of 150 times greater than the TLV for coal tar pitch volatiles (American Conference of Governmental Industrial Hygienists, 1971). Even though inhalation exposure to HD clearly results in systemic effects in rats, conformance to limits similar to those for coal tar pitch volatiles should result in human doses below those which we have found to cause effects in animals.

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