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Effects of Inhalation Exposure to SRC-II Heavy and Middle Distillates

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November 1984

Prepared for the U.S. Department of Energy
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Pacific Northwest Laboratory
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LIST OF ABBREVIATIONS

AH	= Aliphatic hydrocarbon(s)
BAP	= Benzo[a]pyrene
F-344	= Fischer-344 rat
GC	= Gas chromatography
GSD	= Geometric standard deviations
HD	= Heavy distillate
Hgb	= Hemoglobin
H&E	= Hematoxylin and eosin
HP	= Hewlett-Packard
HPAH	= Hydroxylated polycyclic aromatic hydrocarbon(s)
LDH	= Lactate dehydrogenase
MD	= Middle distillate
MMAD	= Mass median aerodynamic diameter
MS	= Mass spectrometry
NBF	= Neutral buffered formalin
NPAC	= Nitrogen containing polycylic aromatic compound(s)
PAH	= Polycyclic aromatic hydrocarbon(s)
P&M Co.	= Pittsburg & Midway Coal Mining Company
PNL	= Pacific Northwest Laboratory
PS	= Process solvent
RAM	= Real-time aerosol monitor
RBC	= Red blood cells
RH	= Relative humidity
SD	= Standard deviation
SGPT	= Serum glutamic pyruvic transaminase
SRC	= Solvent refined coal
TLV	= Threshold limit value
UV	= Ultraviolet
VPRC	= Volume of packed red cells
WBC	= White blood cells

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EXECUTIVE SUMMARY

Coal liquids have been evaluated in a variety of short-term toxicological assays; however, few studies have been conducted to determine the effects after inhalation exposure to these materials. To expand the data base on potential health effects of coal liquefaction materials, we have performed studies with both solvent refined coal (SRC)-II heavy distillate (HD) and middle distillate (MD). For the first study, Fischer-344 rats were exposed for 6 hours/day, 5 days/week for 5 or 13 weeks to an aerosol of HD (boiling range, 288-454°C) at concentrations of 0.69, 0.14, 0.03 or 0.0 mg/L for the high, middle, low and control groups, respectively. Aerosol particle sizes in the three chambers ranged from 1.7 to 1.8 μm mass median aerodynamic diameter (MMAD), with geometric standard deviations (GSD) of approximately 2.0.

Weight gain for exposed animals was less than that of controls and was dose-related, ranging from no significant difference for animals in the low-exposure group to failure to gain in the high-dose animals. Ninety-four percent of the males and 91% of the females survived 13 weeks of exposure. Liver weights increased significantly over controls, and thymus weights decreased for animals sacrificed at 5 and 13 weeks. After both exposure periods, there were significant treatment-related decreases in erythrocyte parameters and in certain types of white blood cells (WBC). By 26 weeks after exposure, effects on red blood cells (RBC) were less pronounced than immediately following exposure, but the magnitude of effects on WBC were similar at both times. Serum cholesterol levels in rats exposed for 5 weeks were higher than in controls. Significant changes in clinical chemistry parameters for the 13-week high-dose group included increased serum cholesterol concentrations for males and middle-dose females. Bone marrow cellularity and numbers of megakaryocytes consistently decreased, suggesting that bone marrow is a target tissue for high-boiling coal liquids. In addition, microscopic evaluation of tissue indicated exposure-related changes, including inflammation and squamous metaplasia of the nasal mucosa, diffuse pulmonary alveolar macrophage infiltrates, thymic atrophy, hepatopathy, decreases in bone marrow and spleen megakaryocytes, renal pelvic epithelial hyperplasia, a decrease in ovarian luteal tissue; and cecal ulceration and epithelial hyperplasia. Most lesions were dose-dependent, with few effects in the low exposure group.

In contrast to the reported mutagenic and carcinogenic effects observed for the high-boiling coal liquids, middle-boiling-range materials lacked activity in these assays. However, this material contains a high percentage of polyaromatic hydrocarbons (PAH), and some of these compounds are known to cause adverse effects on bone-marrow cells and other tissues. To extend our inhalation studies, we exposed animals for 4 weeks to MD (176-288°C) at doses of 0.73, 0.20, or 0.04 or 0.0 mg/L; particle sizes for the study ranged from 1.4 to 2.0 μm MMAD and increased as concentration increased. All rats and 55% of the mice survived the exposure. Rats exposed to the high dose failed to gain weight during exposure but returned to control levels by 4 weeks after exposure. Immediately after exposure, weights of liver, kidney and adrenals increased relative to controls; thymus and ovary weights decreased. By 4 weeks after exposure the weights of these tissues had returned to control levels. Blood samples collected at the end of exposure had decreased levels of RBC and hemoglobin and increased numbers of reticulocytes and segmented neutrophils.

These data demonstrate a great deal of similarity in the kinds of effects observed following exposure to middle- and high-boiling-range coal liquids. However, the significance of changes in organ weights and peripheral blood parameters are not always readily apparent following a subchronic study. Because of this, we exposed animals to HD in a manner similar to that for the subchronic experiment and have followed these animals throughout their lives for the development of adverse effects such as reduced longevity and the appearance of tumors. Results from this study will be available for mice in Fiscal Year 1985 and for rats in Fiscal Year 1986.

I. INTRODUCTION

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 coworkers (Pelroy and Petersen, 1981; Wilson et al., 1981) have shown that process solvent (PS) and heavy distillate (HD), the high-boiling liquids from the SRC-I and -II processes, are highly mutagenic to *Salmonella typhimurium*. Moreover, these materials are also active in mammalian-cell mutation and transformation assays (Frazier and Andrews, 1983; Frazier and Mahlum, in press). 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. The studies reported here were designed to determine whether systemic effects occur following inhalation exposure.

In a previous study (Springer et al., 1982b) we found that inhalation exposure of pregnant rats to HD (0.66 mg/L) on days 12 through 16 of gestation resulted in reduced body weight gain, decreased thymus weight, and increased adrenal, lung, and spleen weights for the dam. In addition, malformations were observed in the offspring (Hackett et al., 1984). To expand the toxicological data base for coal liquids, we therefore conducted a subchronic (13-

week) exposure to SRC-II HD and a 4-week exposure to SRC-II middle distillate (MD). Results from these studies are reported for survival and growth, organ weights, hematology and clinical chemistry. In addition, histopathology data are presented for animals exposed to HD; preserved organs from MD-exposed animals await microscopic examination.

II. 13-WEEK INHALATION EXPOSURE TO HD

METHODS

Material

The coal liquid used in this study was HD, (boiling range, 288-454°C), obtained from the Ft. Lewis, WA 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 #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. (in press). High-resolution gas chromatography (GC) was used to quantitate the levels of these constituents: a Hewlett-Packard (HP) 5880A equipped with a 25 m x 0.25-mm (ID) 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 that were quantified was achieved through retention-time measurements using GC/mass spectrometry (MS) procedures; an HP 5992 bench-top 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

TABLE 1. Concentration of Major Components of SRC-II HD

Components*	Concentration (parts per Thousand)**
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

*Identified by retention time and GC/MS

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

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 (Springer et al., 1982a) but has been modified for exposure of animals to coal liquids. Stainless-steel multitiered chambers (Hazelton Systems, Inc., Aberdeen, MD) with a volume of 2.3 m^3 (Moss et al., 1982) were used. The HD aerosol was generated with two Solo-sphere nebulizers (McGaw Respiratory Therapy, Irvine, CA; housed in a 65°C water bath) and 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 L/min, with air movement from top to bottom. After each daily exposure, the flow rate for each chamber was increased to approximately 340 L/min to maintain the time required to clear aerosol from the chamber. To reduce ammonia levels to less than 5 ppm, Deotized animal cage board (Shepherd Specialty Papers, Kalamazoo, MI) 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 for temperature and RH during the 13 weeks of exposure were 76 ± 2°F and 68 ± 7%, respectively.

Target concentrations, as indicated by previous work (Springer et al., 1982b; Loscutoff et al., 1983), were 0.70 mg/L 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, MI; 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 was determined by collecting triplicate samples at three equal intervals throughout the 6-hour exposure period. Mean aerosol concentrations ($\bar{x}$ ± SD) during the 13-week exposure were 0.69 ± 0.030, 0.14 ± 0.012, 0.03 ± 0.003, and 0.0 mg HD/L 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, MA), 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, NY) 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 animals was composed of 128 male and 128 female Fischer-344 (F-344) rats, all distributed equally over the three treatment and the control groups. They were exposed for 6 hour/day,

5 days/week for either 5 or 13 weeks. Ten male and 10 female rats from each treatment and the control group were sacrificed during the fifth week of exposure; other animals were sacrificed 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, IL; 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 terminal sacrifice 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 sacrificed animals.

A complete necropsy (35 tissues) was performed on the rats after 5 or 13 weeks exposure, and the weights of heart, liver, kidney, 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, microtomed at 4-6 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 using 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 using Dunnett's test (Dunnett, 1955). Lesion data for each treatment group were compared with control lesion data using Fisher's Exact Test (Siegel, 1956).

RESULTS

Weekly body-weight data obtained during the 13-week exposure are shown in Figures 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, 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.

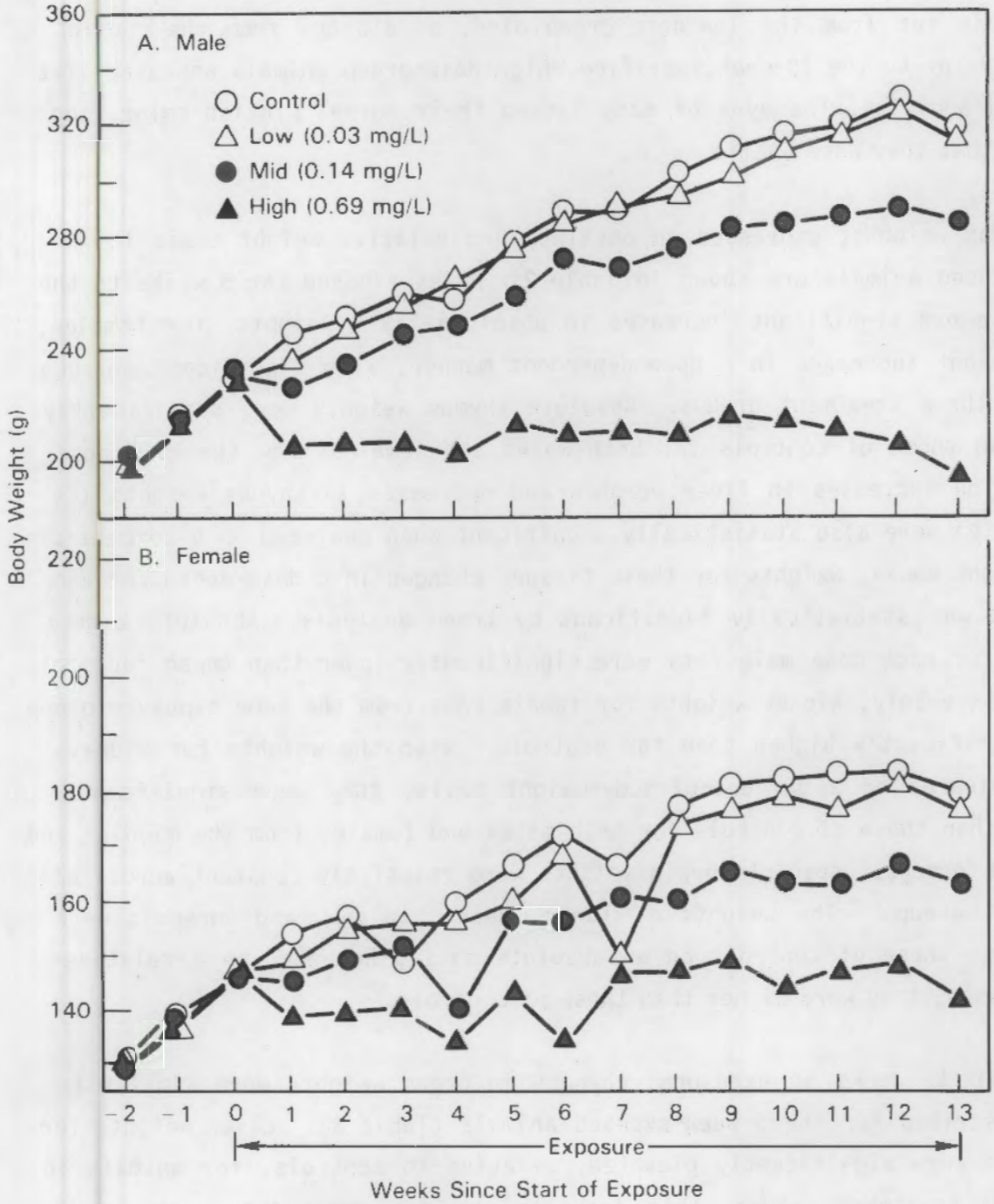

FIGURE 1. Body Weights for Male and Female Rats During 13-Week Inhalation Exposure to SRC-II HD: A) Male Rats; B) Female Rats

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. In addition, one female rat from the low-dose group died, as did one from the control group. Prior to the 13-week sacrifice, 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 on absolute and relative weight basis for 5-week-exposed animals are shown in Table 2. Males exposed for 5 weeks to the high dose had significant increases in absolute liver weights; for females, liver weight increased in a dose-dependent manner, with significant changes for all three treatment groups. Absolute thymus weights were significantly less than those of controls for both males and females from the high-dose group. The increases in liver weights and decreases in thymus weights (females only) were also statistically significant when analyzed on a percent-of-body-weight basis; weights for these tissues changed in a dose-dependent manner that was statistically significant by trend analysis. 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 kidneys were evaluated on a percent-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 lungs, 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 (Table 3). Liver weights for male rats were significantly 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 increas-

TABLE 2. Summary of Weight Data ($\bar{x}$ ± SEM) for Rats Exposed by Inhalation for 5 Weeks to SRC-II HD

Sex	N	Dose (mg/L): Measurement	Exposure Group 0.0 Control	0.03 Low	0.14 Middle	0.69 High
			Weight (g)			
Male	10	Body	269 ± 3.93	259 ± 5.78	249 ± 4.55*	196 ± 3.42*
		Liver	9.72 ± 0.25	9.87 ± 0.41	9.62 ± 0.35	11.04 ± 0.38*
		Kidney	1.92 ± 0.03	1.95 ± 0.06	1.92 ± 0.04	1.78 ± 0.04*
		Thymus	0.25 ± 0.03	0.29 ± 0.04	0.21 ± 0.03	0.15 ± 0.02*
		Brain	1.74 ± 0.05	1.72 ± 0.05	1.68 ± 0.05	1.71 ± 0.05
Female	10	Body	154 ± 1.88	155 ± 2.59	145 ± 2.36*	132 ± 2.97*
		Liver	4.94 ± 0.09	5.48 ± 0.12*	5.55 ± 0.17*	7.23 ± 0.20*
		Kidney	1.17 ± 0.01	1.22 ± 0.02	1.21 ± 0.02	1.27 ± 0.02*
		Thymus	0.23 ± 0.02	0.20 ± 0.01	0.19 ± 0.01*	0.07 ± 0.01*
		Brain	1.58 ± 0.04	1.58 ± 0.05	1.58 ± 0.05	1.52 ± 0.04
			Organ/Body Weight Ratio (%)			
Male	10	Liver	3.610 ± 0.088	3.804 ± 0.102	3.864 ± 0.121	5.640 ± 0.169*
		Kidney	0.714 ± 0.009	0.771 ± 0.011*	0.753 ± 0.009*	0.911 ± 0.013*
		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*
Female	10	Liver	3.213 ± 0.054	3.525 ± 0.047*	3.837 ± 0.069*	5.490 ± 0.099*
		Kidney	0.761 ± 0.007	0.786 ± 0.010	0.836 ± 0.004*	0.966 ± 0.014*
		Thymus	0.152 ± 0.014	0.132 ± 0.007	0.131 ± 0.007	0.054 ± 0.010*
		Brain	1.029 ± 0.029	1.020 ± 0.045	1.096 ± 0.043	1.161 ± 0.038*

*Significantly different from control mean ($P < 0.05$)

TABLE 3. Summary of Weight Data ($\bar{x}$ ± SEM) for Rats Exposed by Inhalation for 13 Weeks to SRC-II HD

Sex	N	Dose (mg/L): Measurement	Exposure Group 0.0 Control	0.03 Low	0.14 Middle	0.69 High
			Weight (g)			
Male	22	Body	319 ± 3.03	314 ± 3.97	286 ± 3.88*	194 ± 5.37*
		Liver	10.71 ± 0.22	11.74 ± 0.32*	12.25 ± 0.32*	12.42 ± 0.37*
		Kidney	2.14 ± 0.03	2.16 ± 0.04	2.10 ± 0.04	2.01 ± 0.03*
		Thymus	0.28 ± 0.02	0.26 ± 0.02	0.18 ± 0.01*	0.08 ± 0.01*
		Testis	2.96 ± 0.02	2.98 ± 0.02	2.85 ± 0.03	2.41 ± 0.10*
		Brain	1.88 ± 0.02	1.89 ± 0.02	1.84 ± 0.02	1.75 ± 0.02*
Female	21	Body	180 ± 1.61	176 ± 1.99	163 ± 2.93*	142 ± 2.33*
		Liver	6.40 ± 0.17	6.26 ± 0.17	6.80 ± 0.21	9.08 ± 0.23*
		Kidney	1.34 ± 0.02	1.35 ± 0.04	1.31 ± 0.02	1.37 ± 0.02
		Thymus	0.23 ± 0.02	0.19 ± 0.02	0.15 ± 0.02*	0.07 ± 0.01*
		Ovaries	0.09 ± 0.003	0.063 ± 0.002	0.060 ± 0.002*	0.037 ± 0.003*
		Brain	1.78 ± 0.03	1.76 ± 0.01	1.74 ± 0.01	1.63 ± 0.03*
			Organ/Body Weight Ratio (%)			
Male	22	Liver	3.354 ± 0.049	3.735 ± 0.076*	4.279 ± 0.097*	6.425 ± 0.153*
		Kidney	0.670 ± 0.009	0.688 ± 0.010	0.732 ± 0.011*	1.047 ± 0.028*
		Thymus	0.089 ± 0.005	0.082 ± 0.006	0.065 ± 0.003*	0.039 ± 0.004*
		Testis	0.930 ± 0.010	0.952 ± 0.010	0.999 ± 0.012	1.237 ± 0.036*
		Brain	0.591 ± 0.006	0.603 ± 0.011	0.645 ± 0.012	0.912 ± 0.024*
Female	22	Liver	3.545 ± 0.086	3.548 ± 0.077	4.185 ± 0.122*	6.368 ± 0.112*
		Kidney	0.744 ± 0.012	0.767 ± 0.024	0.807 ± 0.012*	0.968 ± 0.019*
		Thymus	0.127 ± 0.009	0.110 ± 0.005	0.090 ± 0.005*	0.049 ± 0.006*
		Ovaries	0.038 ± 0.002	0.036 ± 0.001	0.037 ± 0.001	0.026 ± 0.002*
		Brain	0.988 ± 0.019	1.001 ± 0.009	1.075 ± 0.017*	1.148 ± 0.028*

*Significantly different from control mean ($P < 0.05$)

ing 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 percent-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 percent of body weight. Changes in heart, spleen, testes, and adrenals 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 volume of packed red cells (VPRC), hemoglobin (Hgb) concentration, and number of red blood cells (RBC), relative to controls. The number of reticulocytes was significantly increased compared to controls for these groups (Table 4). The RBC parameters for animals in the low-exposure group were not statistically different from those of the control group. After 13 weeks of exposure, decreases in erythrocyte parameters were significant for animals in the middle- and high-exposure groups relative to controls; 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. Effects on both RBC parameters and numbers of reticulocytes were independent of sex.

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. 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, suggesting that they were recovering from the anemia.

TABLE 4. Erythrocyte Measurements ($\bar{x}$ ± SEM) for Rats Exposed by Inhalation to SRC-II HD

Measurement	Male				Female			
	Control	Low	Middle	High	Control	Low	Middle	High
	5-Week Exposure				5-Week Exposure			
VPRC (ml/dl)	42.3 ± 0.56	40.6 ± 0.51	38.6 ± 0.92*	33.4 ± 1.29*	40.7 ± 0.63	39.6 ± 0.21	37.6 ± 0.35*	33.9 ± 0.37*
Hgb (g/dl)	16.7 ± 0.24	16.2 ± 0.16	15.1 ± 0.36*	12.9 ± 0.54*	16.1 ± 0.24	15.6 ± 0.14	14.6 ± 0.13*	13.2 ± 0.16*
RBC (10^6/μl)	8.90 ± 0.11	8.62 ± 0.09	8.17 ± 0.20*	7.06 ± 0.32*	8.08 ± 0.13	7.83 ± 0.06	7.47 ± 0.10*	7.16 ± 0.09*
Reticulocytes (10^3/μl)	185 ± 22	178 ± 15	236 ± 16	684 ± 108*	167 ± 8	176 ± 15	261 ± 20*	423 ± 23*
	13-Week Exposure				13-Week Exposure			
VPRC (ml/dl)	44.0 ± 0.16	42.5 ± 0.54	39.2 ± 0.23*	18.4 ± 1.65*	44.2 ± 0.26	42.6 ± 0.40	40.7 ± 0.60	27.0 ± 3.05*
Hgb (g/dl)	17.1 ± 0.06	16.3 ± 0.13	15.2 ± 0.08*	7.06 ± 0.60*	17.2 ± 0.09	16.6 ± 0.17	15.7 ± 0.18*	10.3 ± 1.18*
RBC (10^6/μl)	8.93 ± 0.04	8.86 ± 0.08	8.29 ± 0.06*	3.28 ± 0.36*	8.34 ± 0.05	8.04 ± 0.09	7.84 ± 0.11	5.24 ± 0.71*
Reticulocytes (10^3/μl)	112 ± 25	87 ± 23	242 ± 126	168 ± 40	90 ± 26	117 ± 23	86 ± 28	294 ± 70*
	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/μl)	9.2 ± 0.06	9.1 ± 0.05	8.9 ± 0.09*	8.5 ± 0.05*				
Reticulocytes (10^3/μl)	157 ± 11	173 ± 11	157 ± 13	133 ± 10				

*Significantly different from control mean ($P < 0.05$)

The total numbers of white blood cells (WBC) were similar for control and treated female rats after 5 weeks of exposure (Table 5). 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 magnitude of these decreases were similar to those observed for animals sampled immediately after 13 weeks exposure.

After 5 weeks exposure, cholesterol levels for males from the high-dose group and females from the middle- and high-dose group were elevated relative to controls (Table 6). Triglyceride levels were significantly below controls for males from the middle- and high-exposure groups. After 13 weeks exposure (Table 7), 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 glutamic pyruvic transaminase (SGPT) activity increased for males from the high-dose group and decreased for females from the same group. Generally, the kinds and magnitude of effects on clinical chemistry parameters were similar between males and females.

Microscopic examination of liver sections revealed lesions in high-dose males and females after 5 and 13 weeks of exposure (Tables 8 and 9); there was

TABLE 5. White Blood Cell Counts ($\bar{x}$ ± SEM) for Rats Exposed by Inhalation to SRC-II HD

Measurement*	Male				Female			
	Control	Low	Middle	High	Control	Low	Middle	High
	5-Week Exposure				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 ± 0.28	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**	1.58 ± 0.15	1.09 ± 0.10	1.48 ± 0.31	1.75 ± 0.29
Eosinophils	85 ± 17	35 ± 14	30 ± 14	10 ± 6**	65 ± 21	26 ± 9**	27 ± 12**	8 ± 4**
Monocytes	13 ± 7	33 ± 11	14 ± 7	35 ± 18	17 ± 9	25 ± 10	19 ± 8	5 ± 3
	13-Week Exposure				13-Week Exposure			
Total WBC	9.30 ± 0.52	9.60 ± 0.43	8.38 ± 0.64	6.35 ± 0.33**	7.72 ± 0.43	7.77 ± 0.37	7.09 ± 0.28	5.81 ± 0.48**
Lymphocytes	6.55 ± 0.47	6.64 ± 0.45	6.06 ± 0.46	4.35 ± 0.20**	6.00 ± 0.33	6.12 ± 0.28	5.80 ± 0.19	4.22 ± 0.34**
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**	5 ± 3**	90 ± 12	44 ± 11**	31 ± 9**	2 ± 2**
Monocytes	120 ± 25	175 ± 49	80 ± 25	14 ± 6**	115 ± 20	59 ± 14**	66 ± 12**	30 ± 8**
	26 Weeks After Exposure							
Total WBC	9.68 ± 0.51	9.06 ± 0.32	10.3 ± 1.14	7.17 ± 0.41**				
Lymphocytes	5.66 ± 0.46	6.34 ± 0.30	6.45 ± 0.52	4.34 ± 0.25**				
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**				
Monocytes	108 ± 37	136 ± 45	122 ± 38	71 ± 30				

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

**Significantly different from control mean ($P < 0.05$)

TABLE 6. Values ($\bar{x}$ ± SEM) for Clinical Chemistry Data for Rats Exposed by Inhalation to SRC-II HO for 5 Weeks

Measurement	Male				Female			
	Control	Low	Middle	High	Control	Low	Middle	High
Protein	6.10 ± 0.28	6.16 ± 0.16	5.88 ± 0.17	5.65 ± 0.23	5.22 ± 0.17	5.37 ± 0.17	5.34 ± 0.10	5.61 ± 0.12
Albumin	4.17 ± 0.16	4.26 ± 0.11	4.17 ± 0.13	3.89 ± 0.13	3.74 ± 0.13	3.97 ± 0.11	3.88 ± 0.09	4.10 ± 0.10
Globulin	1.93 ± 0.14	1.90 ± 0.06	1.71 ± 0.06	1.76 ± 0.17	1.48 ± 0.05	1.40 ± 0.07	1.46 ± 0.04	1.51 ± 0.04
Cholesterol	64.6 ± 4.52	54.7 ± 4.06	51.0 ± 3.10	95.0 ± 6.30*	71.4 ± 4.42	79.4 ± 3.43	92.4 ± 2.25*	117 ± 6.75*
Triglycerides	227 ± 11.1	200 ± 12.7	180 ± 13.6*	183 ± 11.0*	137 ± 9.99	163 ± 12.0	149 ± 9.26	152 ± 8.08
Blood urea nitrogen	25.4 ± 1.11	24.6 ± 1.30	22.9 ± 1.25	22.8 ± 1.08	24.4 ± 2.64	23.7 ± 2.27	22.4 ± 1.51	25.3 ± 1.76
Glucose	179 ± 20.1	156 ± 4.05	163 ± 19.8	158 ± 10.6	160 ± 6.46	147 ± 5.03	159 ± 5.20	156 ± 8.45
Bilirubin	0.31 ± 0.04	0.29 ± 0.05	0.20 ± 0.05	0.47 ± 0.20	0.09 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	0.19 ± 0.03
SGPT	48.7 ± 5.20	55.9 ± 5.95	158 ± 108	33.4 ± 2.56	38.8 ± 2.87	45.8 ± 4.27	39.4 ± 4.45	41.0 ± 3.13
LDH	583 ± 140	491 ± 98	1093 ± 539	598 ± 141	320 ± 39	396 ± 58	511 ± 52	424 ± 81

*Significantly different from control mean ($P < 0.05$)

TABLE 7. Values ($\bar{x}$ ± SEM) for Clinical Chemistry Data for Rats Exposed by Inhalation to SRC-II HD for 13 Weeks

Measurement	Male				Female			
	Control	Low	Middle	High	Control	Low	Middle	High
Protein	5.66 ± 0.12	5.79 ± 0.10	5.90 ± 0.13	5.02 ± 0.14*	5.64 ± 0.05	5.67 ± 0.12	5.59 ± 0.09	4.81 ± 0.13*
Albumin	3.80 ± 0.08	3.88 ± 0.08	4.10 ± 0.09	3.32 ± 0.10*	3.84 ± 0.04	3.89 ± 0.07	3.91 ± 0.06	3.35 ± 0.09*
Globulin	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*
Cholesterol	55.5 ± 2.59	57.1 ± 1.82	68.9 ± 4.18	92.2 ± 7.53*	80.5 ± 3.21	81.6 ± 2.41	99.3 ± 4.40*	83.1 ± 4.12
Triglycerides	134 ± 13.4	151 ± 15.1	144 ± 10.6	185 ± 13.9*	114 ± 7.57	126 ± 7.22	107 ± 4.26	147 ± 6.25*
Blood urea nitrogen	20.5 ± 0.79	20.3 ± 0.97	20.1 ± 0.62	36.2 ± 4.43*	25.1 ± 1.41	24.7 ± 0.98	25.1 ± 1.02	31.9 ± 3.32*
Glucose	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	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	49.9 ± 3.41	50.3 ± 4.24	37.4 ± 1.63	126 ± 33.79*	90.7 ± 13.0	64.2 ± 8.09	55.2 ± 5.58	49.1 ± 8.83*
LDH	1128 ± 90	1233 ± 99	1070 ± 116	422 ± 70*	789 ± 84	743 ± 20	909 ± 108	326 ± 55*

*Significantly different from control mean ($P < 0.05$)

TABLE 8. Lesions (Number of Lesions/Number Examined) for Rats Sacrificed after 5 Weeks Inhalation Exposure to SRC-II HD

Measurement	Control		High		Middle		Low	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver								
Hepatopathy	0/10	0/10	9/10*	10/10*	2/10	0/10	0/10	NE**
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*	0/10	0/10	0/10	NE	NE
Thymus								
Atrophy	0/10	0/7	8/8*	2/3	0/8	0/10	NE	NE
Ovary								
Decreased luteal tissue		0/10		5/10*		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*	10/10*	10/10*	9/10*	10/10*	10/10*
Spleen								
Decrease in megakaryocytes	0/10	0/10	6/10*	7/10*	0/10	0/10	NE	NE
Cecum								
Epithelial plasia	0/9	1/10	4/10*	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*	3/9	0/10	0/10	NE	NE

*Significantly different from control ($P < 0.05$)
**Not examined

TABLE 9. Lesions (Number of Lesions/Number Examined) for Rats Sacrificed after 13 Weeks Inhalation Exposure to SRC-II HD

Measurement	Control		High		Middle		Low	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver								
Hepatopathy	0/10	0/10	10/10*	9/10*	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*	10/10*	0/10	0/10	0/10	NE**
Pelvic epithelial hyperplasia	0/8	0/10	6/10*	8/10*	6/9*	0/10	0/10	NE
Thymus								
Atrophy	0/10	0/10	6/6*	8/8*	0/8	0/8	NE	NE
Ovary								
Decreased luteal tissue		0/10		5/10*		0/10		NE
Bone Marrow								
General hypocellularity	0/10	0/10	6/10*	4/10*	0/8	0/9	NE	NE
Decrease in megakaryocytes	0/10	0/10	8/10*	5/10*	0/8	0/9	NE	NE
Nasal Sections (most anterior section)								
Squamous metaplasia	0/10	0/10	2/10	7/10*	1/10	2/10	0/10	0/10
Suppurative inflammation	0/10	0/10	6/10*	4/10*	0/10	1/10	0/10	0/10
Lung								
Histiocytosis	0/10	0/10	10/10*	10/10*	8/10*	10/10*	7/10*	7/10*
Spleen								
Decrease in megakaryocytes	2/10	0/10	10/10*	10/10*	0/10	2/10	NE	NE
Cecum								
Epithelial plasia	0/10	0/10	8/10*	6/10*	0/10	0/10	NE	NE
Ulcer	0/10	0/10	7/10*	3/10	0/10	0/10	NE	NE
Chronic active inflammation	0/10	0/10	8/10*	7/10	0/10	0/10	NE	NE

*Significantly different from control ($P < 0.05$)
**Not examined

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 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 (Figure 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 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 (Figure 3). These hyperplastic lesions were present in both sexs 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 brown pigmentation occurred in the cortical renal tubular epithelium of male and female rats from the high-dose group after 13 weeks 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 control/medullary architecture. This was apparent only in the high-dose male and female animals after 5 or 13 weeks 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

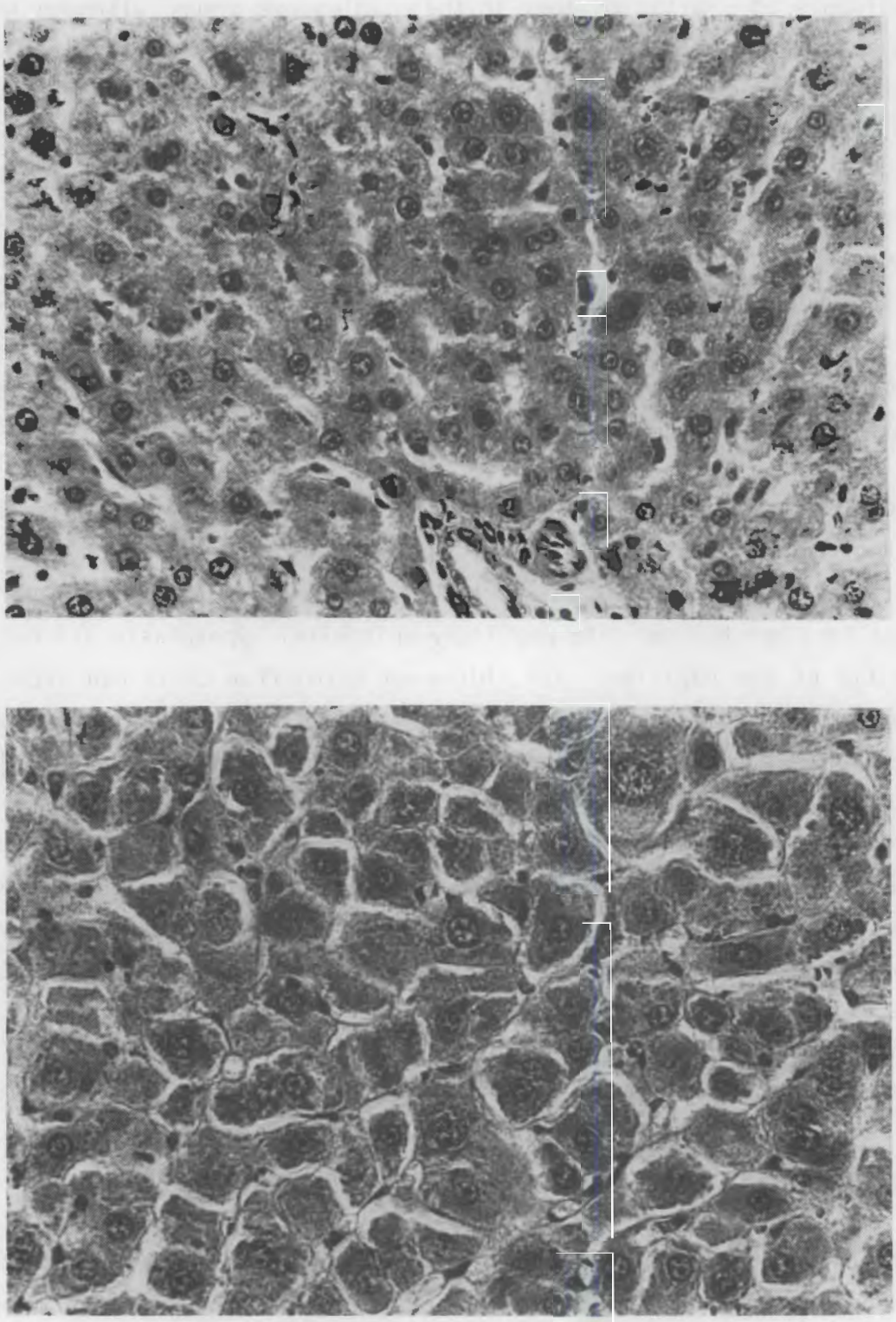

FIGURE 2. Photomicrographs of Liver Sections from a F-344 Rat, Demonstrating an Increase in Variability of Hepatocyte and Nucleus Size, Hepatomegalocytes, and Loss of Cording Pattern of the Liver. A) Control; B) Section from a rat exposed by inhalation to 0.69 mg/L of HD for 13 weeks. (H&E stain; original magnification, 250X)

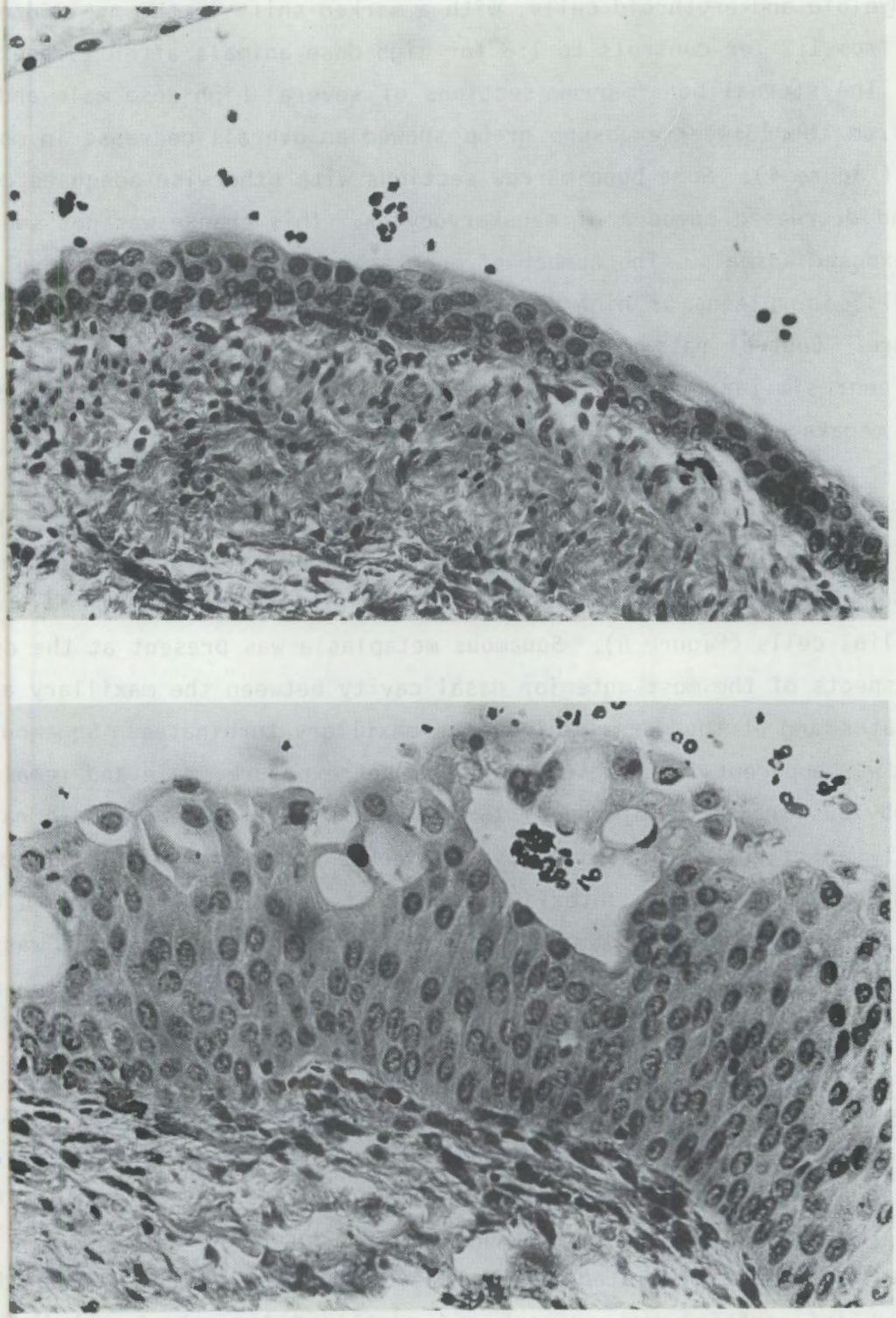

FIGURE 3. Photomicrographs of the Renal Pelvic Epithelium of a F-344 Rat, Demonstrating an Increase in the Number of Layers of Cells and Vacuolar Degenerative Changes. Both sections are from the same plane of sectioning and same location of pelvic epithelium. A) Control; B) Section from a rat exposed by inhalation to 0.69 mg/L of HD for 13 weeks. (H&E stain; original magnification, 250X)

both myeloid and erythroid cells, with a marked shift in the myeloid/erythroid 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 (Figure 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 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 (Figure 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 prevalent in the high- and middle-dose groups of the 13-week-exposure male and 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

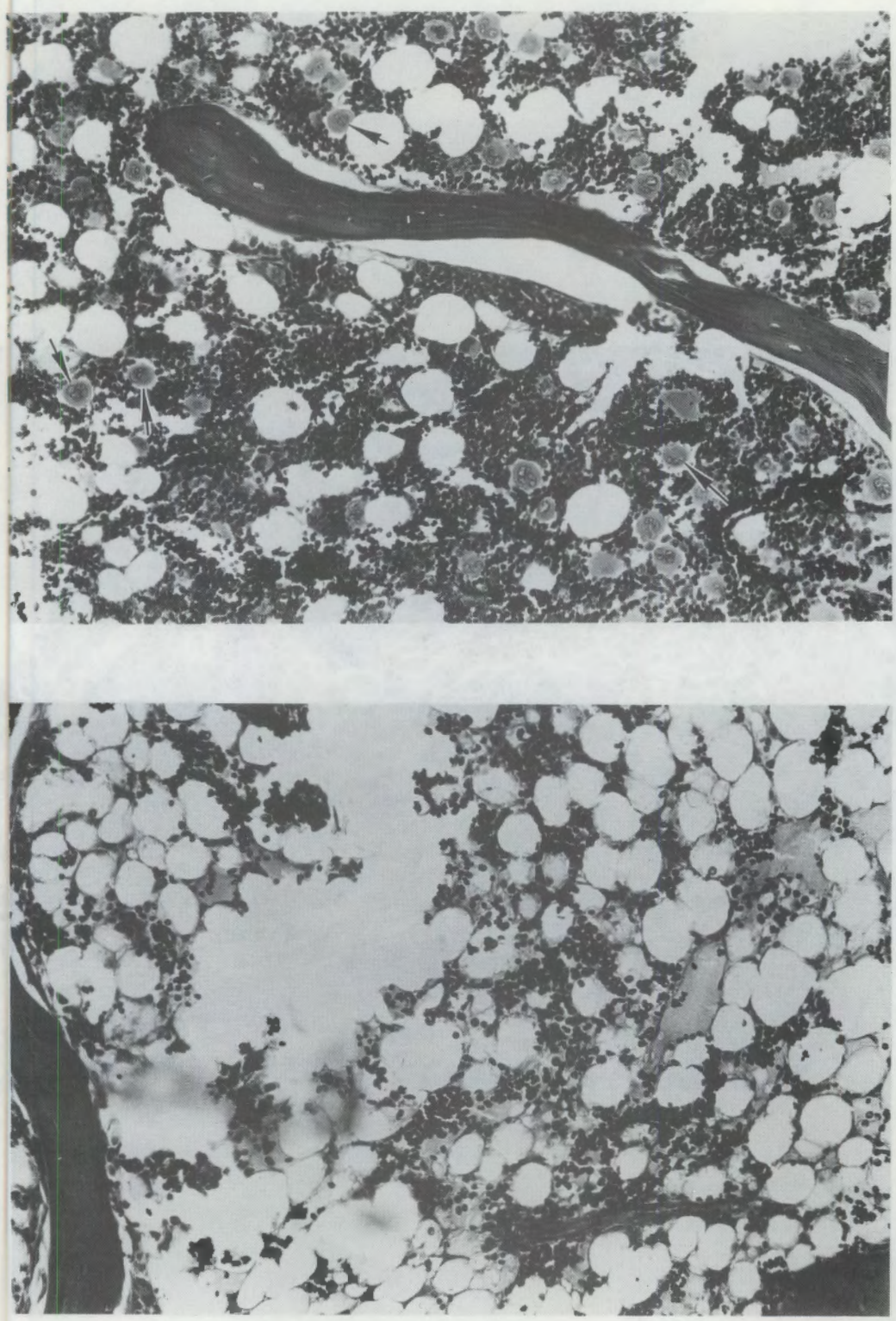

FIGURE 4. Photomicrographs of the Sternal Bone Marrow from an F-344 Rat, Demonstrating an Overall Decrease in Cellularity and Lack of Megakaryocytes. A) Control animal with abundant megakaryocytes (arrows); B) Section from an F-344 rat exposed by inhalation to 0.69 mg/L of HD for 13 weeks. (H&E stain; original magnification, 125X)

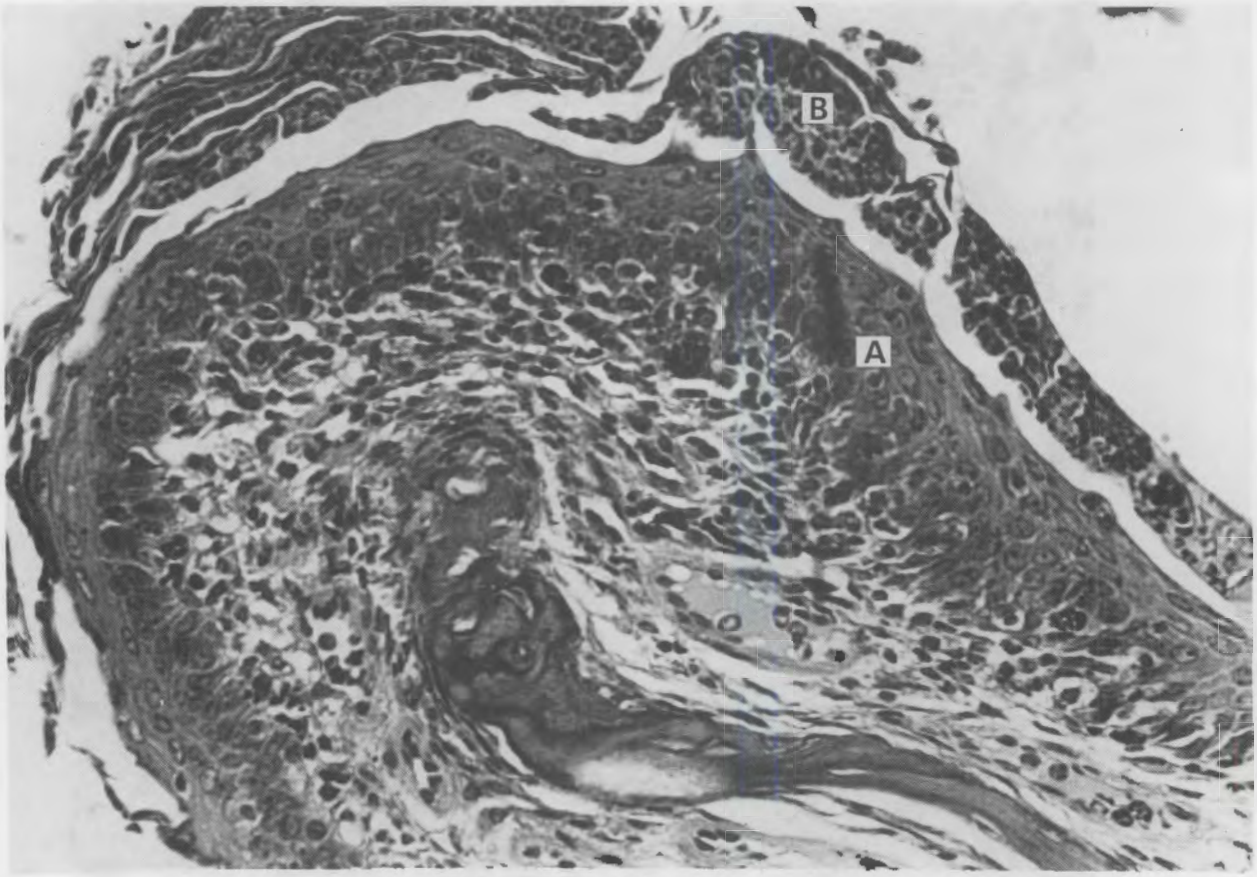

FIGURE 5. Photomicrograph of a Nasal Section from the Dorsal Tip of the Maxilloturbinate of an F-344 Rat Exposed by Inhalation to 0.69 mg/L of HD for 13 Weeks. Demonstrates prominent squamous metaplasia of respiratory epithelium (A) and exudate containing neutrophils (B) on the surface of the metaplastic epithelium. (H&E stain; original magnification, 250X)

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 (Figure 6). Frequently, the proliferating epitheium was seen beneath the muscularis mucosa (Figure 7). 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 weeks exposure and, to a lesser degree, in males from the high-dose group after 5 weeks exposure. Females from the 5-week-exposure, high-dose group had evidence of only the chronic inflammatory infiltrate.

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 sacrifice were negative for Kilham rat virus, H-1, rat coronavirus/sialodacryoadenitis virus, and M. pulmonis; however, titers for Sendai virus were positive.

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 (288-454°C) with essen-

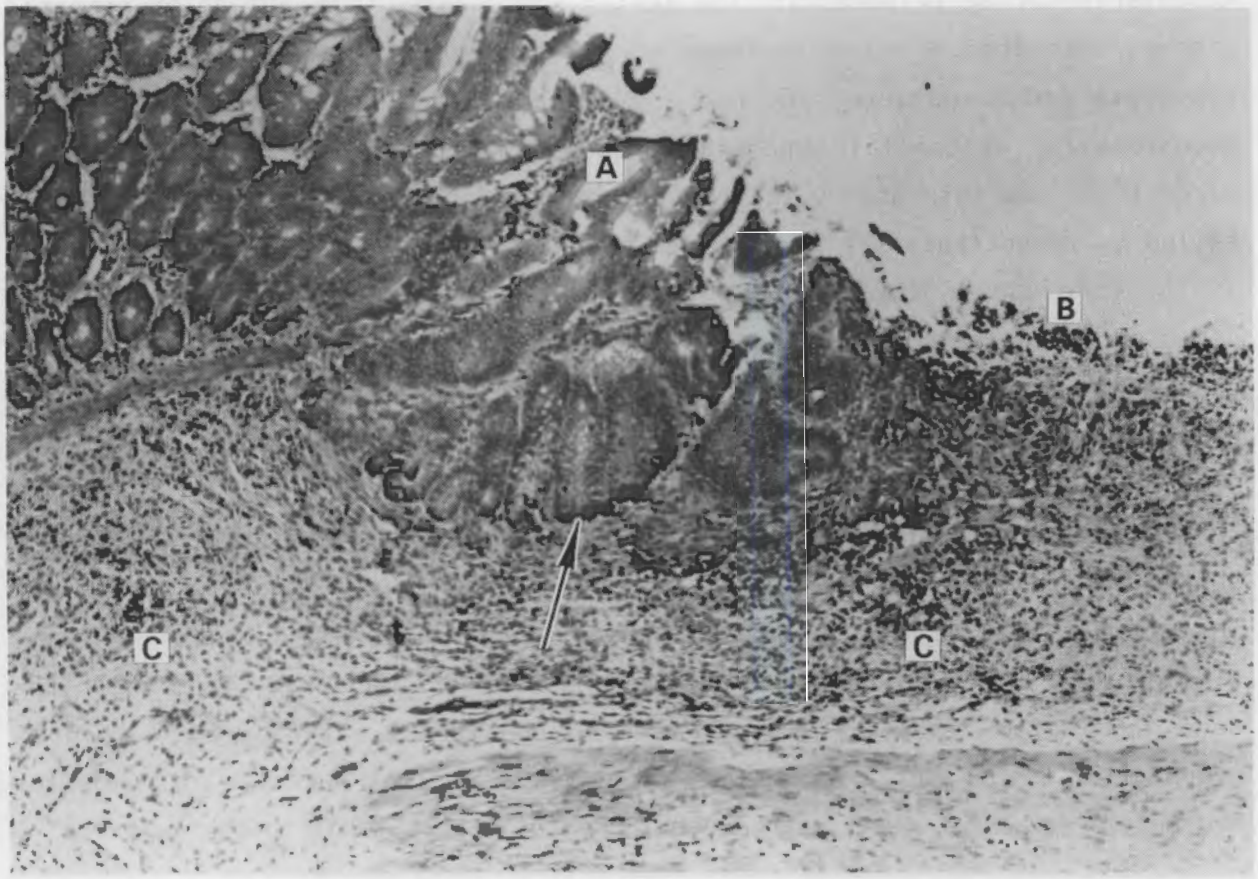

FIGURE 6. Photomicrograph of a Section of Cecum from an F-344 Rat Exposed by Inhalation to 0.69 mg/L of HD for 13 Weeks, Demonstrating Hyperplastic Epithelium (A) Next to an Ulcer (B). Hyperplastic epithelium is also seen below the muscularis mucosa (arrow), and prominent chronic inflammation of the Submucosa (C) is evident. (H&E stain; original magnification, 50X)

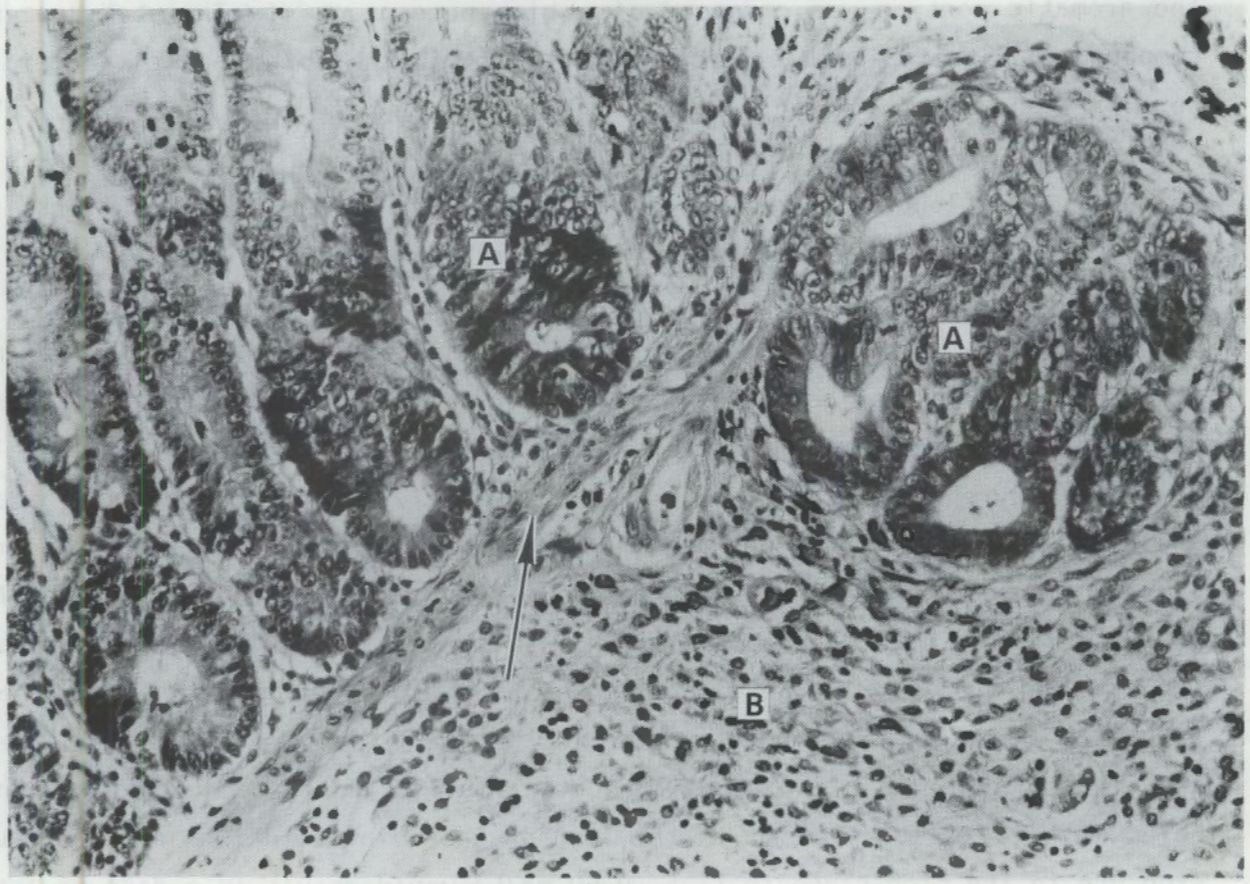

FIGURE 7. Photomicrograph of a Section of Cecum from an F-344 Rat Exposed by Inhalation to 0.69 mg/L of HD for 13 Weeks, Demonstrating Hyperplastic Epithelium (A) Above and Below the Muscularis Mucosa (Arrow). Chronic inflammation of the submucosa (B) is prominent. (H&E stain; original magnification, 125X)

tially all components of the aerosol present in the liquid state in the oil droplets (Schirmer et al., in press). 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/L. The materials boiled from 150-500°F, which was considerably lower than the boiling range for HD. Those petroleum-derived hydrocarbons were of lower molecular weight than ours 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-hour exposure to the high concentration (0.69 mg/L) would result in a dose of about 60 mg/kg body weight/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. Increased amounts of endoplasmic reticulum are known to be accompanied by increased amounts of mixed-function oxidase enzymes. Changes in the liver following exposure to toxicants have been demonstrated to reflect an attempt by the animal to increase metabolism and excretion rates of foreign compounds (Neal, 1980); presumably the pattern of changes in liver size and structure in our animals following exposure to HD reflects similar adaptations.

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 (1982) 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 (1982) reported that Stoddard solvent produced (in male rats only) symptoms similar to those reported for JP-5. The types of kidney lesions observed by those and other workers (Carpenter et al. 1975; Gaworski et al., 1982; Halder et al., 1984, Phillips and Egan, 1984; Phillips and Cockrell, 1984) were produced by hydrocarbon mix-

tures boiling between 60-220°C; the effect appears to be due to branched chain aliphatics and cycloparaffinic compounds.

The origin of the nasal lesions (submucosal mononuclear cell inflammation and respiratory epithelial hyperplasia) observed in our animals was unclear. Although serum samples from rats sacrificed after 13 weeks of exposure were positive for Sendai virus, it is unlikely that the infection occurred during the exposure period, since mice housed in the chambers during the entire exposure period had negative titers for the virus. This observation suggests that the infection in the rats occurred prior to the beginning of the exposure. Furthermore, the lack of a dose-response relationship for the lesions suggests that they were not treatment-related (Burek et al., 1977; Castleman, 1983). The second nasal lesion (suppurative inflammation and respiratory epithelial squamous metaplasia) observed for these rats was similar to that 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 the effect was mild for high-dose animals, distinguishable changes were also present in tissue sections from the middle- and low-dose groups, and the data demonstrate a clear dose-response relationship. 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 lung response in our animals was less pronounced than and qualitatively different from those observed in guinea pigs exposed for a shorter period of time (3 weeks) to a similar boiling-range material obtained from the SRC-I process (Loscutoff et al., 1983). This may represent a species difference in lung response or a difference in toxicity due to differences in chemical composition of the two coal liquids.

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 similiar organ-

isms. This lesion appears to be related to exposure and future long-term studies may provide information as to its significance.

The changes in ovarian weights for females exposed to HD were characterized by an apparent decrease in the amounts of luteal tissue. This effect was different from that reported by Mattison and Thorgeirsson (1979) following intraperitoneal administration of BaP (and other PAH) to rats at doses of 80-100 mg/kg body weight; ovaries in that case had decreased numbers of primordial oocytes. Further evaluation is needed to determine the consequences of HD exposure with regard to female reproductive function.

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 the level for 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 levels. This apparent recovery was supported by the return of eye color to normal appearance within a few weeks after the exposure ended.

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 levels 6 months after exposure ended, suggesting that effects on these stem cells were less reversible.

Decreased number of leukocyte after 13 weeks of exposure might be the result of reduced lymphopoiesis; however, a contributing cause may be reductions mediated by increased levels 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.

After 13 weeks of exposure, there were changes in many clinical chemistry parameters in animals from the high-exposure group. Since these changes were not present at lower doses, they may be the result of a direct toxic effect or the result of stress due to the exposure. Failure of animals in the high-exposure group to gain weight also indicates that they were heavily stressed. Since the levels of serum components are a composite result of both toxic effects and nutritional influences, it is possible that the nutritional influences in severely stressed animals contribute substantially to levels of these parameters. These confounding effects contribute to the uncertainty associated with interpretation of these data.

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/L indicate that these doses must be considered in setting exposure limits for humans. For comparison, 0.03 mg/L 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).

The biological significance of changes in organ weights and peripheral blood parameters are not always readily apparent following a subchronic study. Because of this, we exposed animals in a manner similar to that for the subchronic experiment and have followed these animals throughout their lives for the development of adverse effects such as reduced longevity and the appearance of tumors. Results from this study will be available for mice in Fiscal Year 1985 and for rats in Fiscal Year 1986.

III. 4-WEEK EXPOSURE TO MD

METHODS

The coal liquid used, SRC-II MD (boiling range, 176-288°C), was obtained on September 23, 1980 from the pilot plant operated by the P&M Co. located at Fort Lewis, WA. It was stored at 4°C under nitrogen in teflon-lined drums until May 1983, when this experiment was started. Analysis of this material for stability indicated that its chemical composition and biological activity was constant for at least 2 years (Wright and Weimer, 1984).

The inhalation exposure system used for the study was described previously (Springer et al., 1982a). Aerosol was produced with a Solo-sphere nebulizer housed in a 20°C water bath. Pressure to the generator was provided by compressed nitrogen gas; this procedure eliminated the possibility of an explosion within the generating system but did not substantially alter the nitrogen concentration within the exposure chamber.

The orifice-controlled manifold was prepared from 7.61 cm (3-in.) stainless-steel pipe and fittings; aerosol was transferred from the manifold to the chamber through flexible polyethylene tubing. Appropriate amounts of the aerosol were mixed with HEPA-filtered room air at the top of each chamber to give the appropriate concentration in each chamber. The exposure chambers were operated at flow rates of 283 L/min. Control animals received HEPA-filtered room air.

Aerosol concentration in each chamber was determined by collecting material from the chamber onto a 0.45-μm pore-size Metricel filter, eluting the MD into chloroform and determining the UV absorption at 275 nm; absorbance for MD standards increased in a linear manner between 0.5 and 50.0 μg/L. Samples of the aerosol were collected at 1, 3, and 5 hours of exposure to give a daily time-weighted average concentration for each chamber. Animals were exposed for 6 hours/day, 5 days/week for 4 weeks. Target aerosol concentrations for the study were 0.70 mg/L for the high-dose group with a factor of 3-4 between each of the lower exposure groups; concentrations were similar to those used for HD in a previous study (Springer et al., 1982b). Mean (±SEM) concentra-

tions for the high, middle, low and control groups during the 4-week exposure were 0.73 ± 0.01, 0.20 ± 0.01, 0.04 ± 0.004 or 0.0 mg/L, respectively. Mercer cascade impactor samples were collected twice weekly from each chamber for particle-size determination. At the end of exposure, the weight of material that collected on the same stages of the impactor were summed to give a composite weight for that stage over the exposure period; these weights were used to calculate a composite particle size for each chamber. Values for the particle sizes were 2.1, 1.8 and 1.4 μm MMAD for the high, middle and low chambers, respectively; GSD ranged from 2.4 to 2.9.

Animals were continuously housed in the exposure chambers. Food (Wayne Lab Blox) was removed each day during the exposure period; water was continuously available. At the end of each exposure, Deotized animal cage board was placed on the catch pans to maintain the concentrations of ammonia to acceptable levels (less than 5 ppm). The cage board was removed the next morning prior to exposure. Chamber temperatures, which were continuously monitored, averaged 76°F (range 71-79°F) during the 4-week exposure.

F-344 rats and CD-1 mice were obtained from Charles River and quarantined for 3 weeks prior to exposure. Animals were individually identified by attaching a numbered metal tag in the skin between the shoulder blades. Prior to exposure, 20 males and 20 females of each species were randomly assigned, by weight, to the control or one of the three exposure groups. Ten animals from each group were randomly selected for sacrifice immediately following the exposure; all remaining animals were sacrificed 5 weeks after exposure. Animals were observed twice daily and weighed weekly, beginning 2 weeks prior to the exposure.

After 4 weeks of exposure, blood samples were obtained from both sexes of rats for hematologic evaluation. These samples were collected from free-flowing tail-vein blood of unanesthetized animals into tubes containing EDTA. After blood samples were obtained, the animals were killed with CO_2 and immediately necropsied. Major organs, including liver, thymus, spleen, kidney, adrenals, gonads, heart and brain were weighed, and bone marrow smears were obtained from the femur. Animals found dead were necropsied as soon after death as possible; moribund animals were killed and necropsied immediately.

Major organs were preserved in NBF for histopathological examination. Currently, we are evaluating these specimens.

RESULTS

One hundred percent of the rats and 55% of the mice survived the exposure; all animals that survived the exposure lived until scheduled sacrifice. Both rats and mice from the high-dose group lost weight during the first week of the exposure, failed to gain weight during the remaining exposure period, then gained weight so that they were not different from controls by 5 weeks after exposure (Figures 8 and 9). Animals in the middle-dose group (except male rats) gained less weight during exposure than controls but returned to control levels by 5 weeks after exposure. Body weights for animals in the low-dose group were unaffected by exposure.

Examination of organ weights for rats killed immediately after exposure demonstrated effects attributable to the exposure. Absolute liver weights increased in a dose-dependent manner for males but were unchanged for females; however, on a relative-weight basis, significant increases were observed for males and females from the middle- and high-dose groups (Table 10). Significant dose-related changes were observed for thymus weights from females; changes for male rats were not dose-related. Spleen weights, on a relative basis, increased for both males and females. Absolute kidney weights increased for males from all three dose groups and for females from the high-dose group; on a relative-weight basis, these increases were significant for high-dose males and middle- and high-dose females. Relative adrenal weights increased for both males and females from the high-dose group. Ovary weights were lower, on both an absolute- and relative-weight basis, for animals from the middle- and high-dose groups. Absolute brain weights were also significantly lower for middle- and high-dose females (but not males); however, relative brain weights increased for animals from the high-dose group.

Although less pronounced, the pattern of changes in organ weights for mice were similar to those for rats (Table 11). Both absolute and relative liver weights increased for female mice (but not males) from the high-dose group. Thymus weights were less, on both an absolute and relative-weight

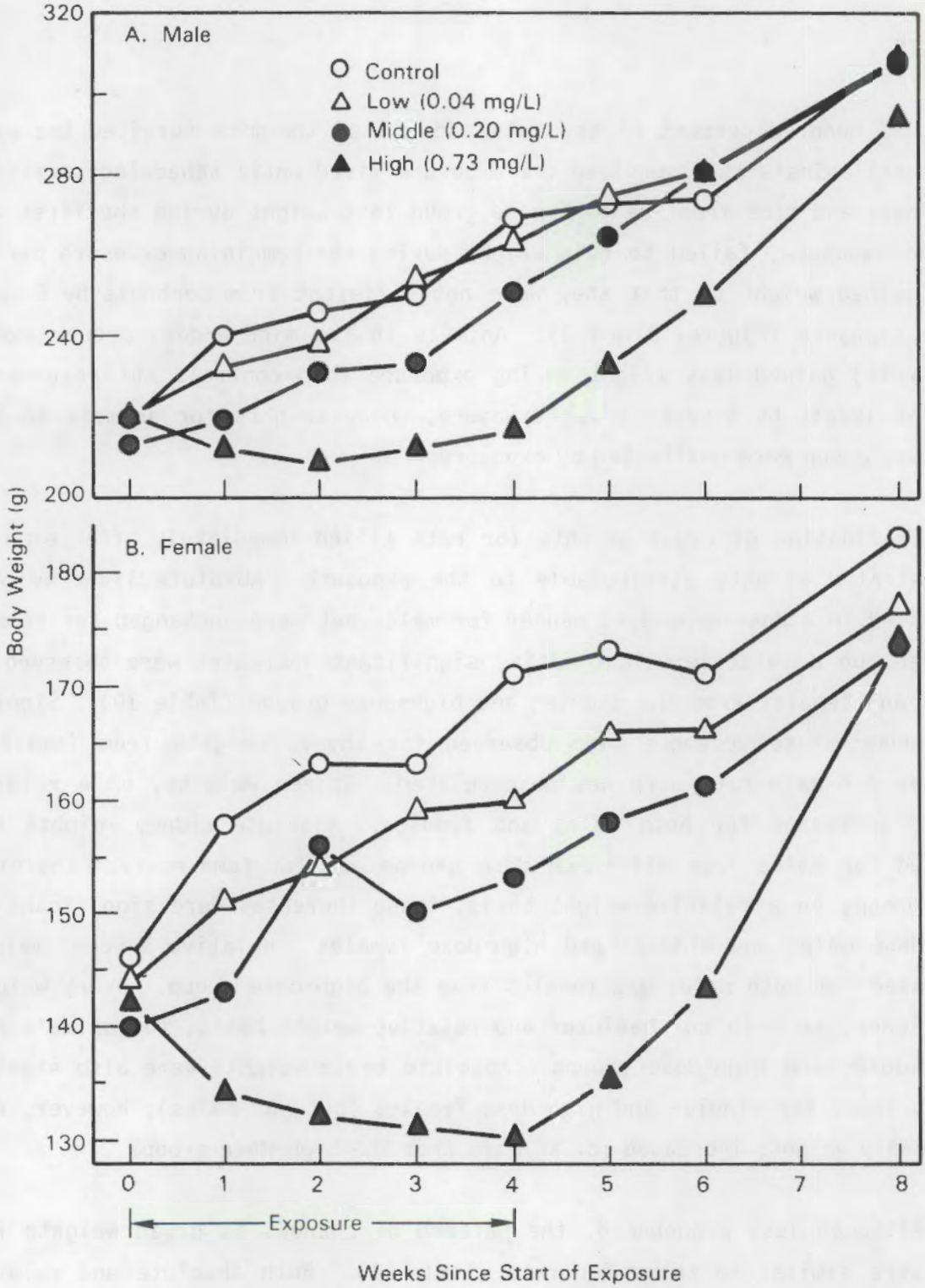

FIGURE 8. Body Weights for Male and Female Rats During and After 4-Week Inhalation Exposure to SRC-II MD: A) Male Rats; B) Female Rats

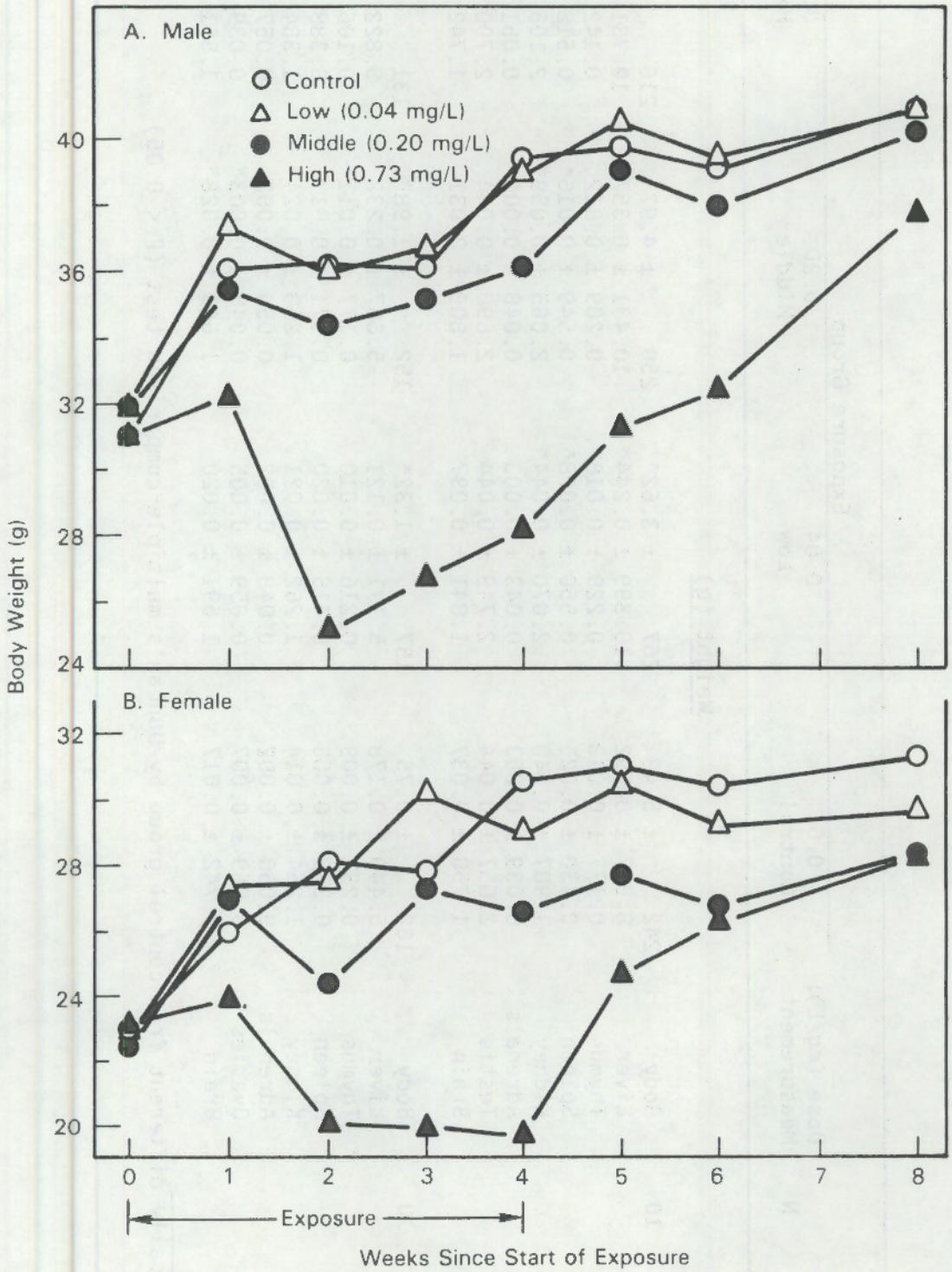

FIGURE 9. Body Weights for Male and Female Mice During and After 4-Week Inhalation Exposure to SRC-II MD: A) Male Mice; B) Female Mice

TABLE 10. Summary of Weight Data ($\bar{x}$ ± SEM) for Rats Sacrificed After 4 Weeks of Inhalation Exposure to SRC-II MD

Sex	N	Dose (mg/L): Measurement	Exposure Group 0.0 Control	0.04 Low	0.20 Middle	0.73 High
			Weight (g)			
Male	10	Body	242 ± 5.43	267 ± 3.62*	250 ± 4.97	216 ± 3.42*
		Liver	8.637 ± 0.592	10.399 ± 0.244*	10.431 ± 0.357*	10.731 ± 0.512*
		Thymus	0.177 ± 0.013	0.229 ± 0.018*	0.209 ± 0.012	0.142 ± 0.011
		Spleen	0.430 ± 0.025	0.550 ± 0.008*	0.549 ± 0.016*	0.518 ± 0.016*
		Kidney	1.907 ± 0.040	2.070 ± 0.044*	2.065 ± 0.059*	2.105 ± 0.031*
		Adrenals	0.039 ± 0.003	0.043 ± 0.003	0.048 ± 0.004	0.051 ± 0.003
		Testis	2.657 ± 0.044	2.749 ± 0.044	2.690 ± 0.044	2.700 ± 0.039
		Brain	1.750 ± 0.037	1.841 ± 0.037	1.809 ± 0.031	1.742 ± 0.039
Female	10	Body	162 ± 0.75	157 ± 1.32*	152 ± 1.98*	131 ± 1.15*
		Liver	5.440 ± 0.178	5.171 ± 0.121	5.667 ± 0.232	5.822 ± 0.140
		Thymus	0.231 ± 0.009	0.216 ± 0.010	0.177 ± 0.011*	0.106 ± 0.009*
		Spleen	0.379 ± 0.006	0.416 ± 0.020	0.371 ± 0.013	0.388 ± 0.007
		Kidney	1.264 ± 0.014	1.262 ± 0.021	1.258 ± 0.024	1.384 ± 0.025*
		Adrenals	0.050 ± 0.002	0.048 ± 0.002	0.050 ± 0.002	0.057 ± 0.002
		Ovaries	0.069 ± 0.007	0.059 ± 0.005	0.048 ± 0.003*	0.039 ± 0.003*
		Brain	1.742 ± 0.017	1.691 ± 0.020	1.640 ± 0.028*	1.551 ± 0.059*

*Statistically different from control group by Duncan's multiple-comparison test ($P < 0.05$)

TABLE 10. (Continued)

Sex	N	Dose (mg/L): Measurement	Exposure Group 0.0 Control	0.04 Low	0.20 Middle	0.73 High
			Organ/Body Weight Ratio (%)			
Male	10	Liver	3.53 ± 0.177	3.89 ± 0.076	4.16 ± 0.096*	4.95 ± 0.188*
		Thymus	0.072 ± 0.004	0.086 ± 0.007*	0.084 ± 0.005*	0.066 ± 0.004
		Spleen	0.176 ± 0.007	0.206 ± 0.003*	0.220 ± 0.005*	0.240 ± 0.007*
		Kidney	0.788 ± 0.013	0.774 ± 0.011	0.824 ± 0.013	0.975 ± 0.014*
		Adrenals	0.016 ± 0.001	0.016 ± 0.001	0.019 ± 0.002	0.024 ± 0.001*
		Testis	1.099 ± 0.021	1.030 ± 0.019	1.078 ± 0.018	1.252 ± 0.022
		Brain	0.724 ± 0.020	0.689 ± 0.013	0.725 ± 0.016	0.809 ± 0.025*
Female	10	Liver	3.36 ± 0.105	3.29 ± 0.066	3.74 ± 0.135*	4.44 ± 0.108*
		Thymus	0.142 ± 0.005	0.137 ± 0.006	0.116 ± 0.006*	0.081 ± 0.006*
		Spleen	0.234 ± 0.004	0.264 ± 0.012	0.244 ± 0.007	0.296 ± 0.005*
		Kidney	0.780 ± 0.009	0.804 ± 0.014	0.830 ± 0.011*	1.056 ± 0.020*
		Adrenals	0.031 ± 0.001	0.031 ± 0.001	0.033 ± 0.002	0.043 ± 0.002*
		Ovaries	0.042 ± 0.004	0.038 ± 0.003	0.032 ± 0.001*	0.029 ± 0.002*
		Brain	1.075 ± 0.009	1.077 ± 0.011	1.083 ± 0.022	1.181 ± 0.041*

*Statistically different from control group by Duncan's multiple-comparison test ($P < 0.05$)

TABLE 11. Summary of Weight Data ($\bar{x}$ ± SEM) for Mice Sacrificed After 4 Weeks of Inhalation Exposure to SRC-II MD

Sex	N	Oose (mg/L): Measurement	Exposure Group 0.0 Control	0.04 Low	0.20 Middle	0.73 High
			Weight (g)			
Male	10	Body	37.3 ± 0.74	35.6 ± 0.63	33.9 ± 0.55*	28.3 ± 1.43¶
		Liver	2.565 ± 0.114	2.222 ± 0.084	2.145 ± 0.106	2.198 ± 0.172
		Thymus	0.040 ± 0.003	0.039 ± 0.005	0.042 ± 0.007	0.018 ± 0.004*
		Spleen	0.102 ± 0.008	0.088 ± 0.005	0.083 ± 0.007	0.083 ± 0.006
		Kidney	0.694 ± 0.026	0.659 ± 0.021	0.667 ± 0.023	0.607 ± 0.015
		Adrenals	0.006 ± 0.001	0.007 ± 0.001	0.006 ± 0.001	0.007 ± 0.001
		Testis	0.239 ± 0.009	0.238 ± 0.009	0.230 ± 0.013	0.195 ± 0.009*
		Brain	0.518 ± 0.009	0.521 ± 0.032	0.509 ± 0.009	0.461 ± 0.010
Female	10	Body	26.8 ± 0.70	27.6 ± 0.74	24.9 ± 0.53	21.9 ± 0.75*¶
		Liver	1.566 ± 0.055	1.743 ± 0.088	1.484 ± 0.041	1.840 ± 0.094*
		Thymus	0.050 ± 0.004	0.057 ± 0.003	0.063 ± 0.006	0.026 ± 0.007*
		Spleen	0.101 ± 0.007	0.104 ± 0.004	0.086 ± 0.006	0.103 ± 0.011
		Kidney	0.441 ± 0.014	0.439 ± 0.014	0.418 ± 0.014	0.428 ± 0.012
		Adrenals	0.009 ± 0.001	0.012 ± 0.002	0.010 ± 0.002	0.007 ± 0.001
		Ovaries	0.016 ± 0.002	0.016 ± 0.002	0.015 ± 0.003	0.009 ± 0.002
		Brain	0.467 ± 0.018	0.471 ± 0.012	0.483 ± 0.019	0.436 ± 0.010

*Statistically different from control group by Duncan's multiple-comparison test ($P < 0.05$)

¶Due to mortality in this group, only five mice were sacrificed

TABLE 11. (Continued)

Sex	N	Dose (mg/L): Measurement	Exposure Group 0.0 Control	0.04 Low	0.20 Middle	0.73 High
			Organ/Body Weight Ratio (%)			
Male	10	Liver	6.857 ± 0.226	6.237 ± 0.178	6.317 ± 0.237	7.724 ± 0.262
		Thymus	0.106 ± 0.007	0.109 ± 0.013	0.125 ± 0.021	0.062 ± 0.011*
		Spleen	0.274 ± 0.025	0.247 ± 0.012	0.243 ± 0.018	0.293 ± 0.017
		Kidney	1.858 ± 0.050	1.856 ± 0.062	1.967 ± 0.051	2.151 ± 0.050*
		Adrenals	0.017 ± 0.003	0.020 ± 0.004	0.017 ± 0.003	0.026 ± 0.004
		Testis	0.643 ± 0.027	0.668 ± 0.020	0.680 ± 0.040	0.691 ± 0.033
		Brain	1.390 ± 0.019	1.466 ± 0.090	1.506 ± 0.035	1.642 ± 0.064*
Female	10	Liver	5.828 ± 0.104	6.296 ± 0.235	5.964 ± 0.123	8.482 ± 0.603*
		Thymus	0.188 ± 0.017	0.208 ± 0.018	0.254 ± 0.023*	0.119 ± 0.031*
		Spleen	0.373 ± 0.022	0.376 ± 0.012	0.347 ± 0.023	0.464 ± 0.041*
		Kidney	1.649 ± 0.060	1.592 ± 0.040	1.679 ± 0.033	1.957 ± 0.016*
		Adrenals	0.034 ± 0.002	0.044 ± 0.007*	0.040 ± 0.006*	0.032 ± 0.004
		Ovaries	0.059 ± 0.007	0.057 ± 0.009	0.060 ± 0.010	0.043 ± 0.011
		Brain	1.737 ± 0.051	1.712 ± 0.046	1.944 ± 0.073*	1.999 ± 0.049*

*Statistically different from control group by Duncan's multiple-comparison test ($P < 0.05$)

¶Due to mortality in this group, only five mice were sacrificed

basis, for animals from the high-dose group, and on a relative-weight basis for middle-dose females. Absolute testis weight was lower for high-dose animals. Relative weights for kidney increased for both males and females from the high-dose group. Relative weights of adrenals increased for females from the low- and middle-dose groups but not for females from the high-dose group. Relative brain weights increased for both sexes from the high-exposure group.

For rats killed 5 weeks after exposure, body weights for the high-dose group were significantly less than those of controls. Relative organ weights for heart, brain and adrenals for high-dose female rats were significantly increased relative to controls, but this effect was not apparent when the data were evaluated on an absolute-weight basis. In addition, absolute kidney weights (but not relative weight) for high-dose female rats were significantly lower than those for controls. For male rats of the high-dose group, the absolute thymus weight was significantly less than that for controls, and relative weights for kidney, brain and testis were significantly elevated.

For blood samples collected from rats immediately after exposure, numbers of RBC, VPRC and Hgb concentrations significantly decreased for the middle- and high-dose animals (Table 12); these effects were dose-related. Numbers of reticulocytes increased, in a dose-related manner, relative to those of controls. Significant increases in total WBC for female rats (but not males) were dose-related; numbers for neutrophils were significantly increased in the middle- and high-dose groups for male rats and in all three exposure groups for females.

Five weeks after exposure, the only significant change in peripheral blood parameters examined (Table 13) was the decrease in the numbers of reticulocytes, relative to the control groups, for high-dose animals.

DISCUSSION

Results from this study suggest that mice are more sensitive to the effects of MD than rats in terms of mortality and body-weight changes. Conversely, changes in organ weights for rats occurred more frequently, and these changes were present in lower-dose groups, than for mice. Results from this

TABLE 12. Mean RBC and W8C Parameters ($\bar{x}$ ± SEM) for Rats Sacrificed Immediately After 4 Weeks of Inhalation Exposure to SRC-II MD

Sex	N	Dose (mg/L): Measurement	Exposure Group 0.0 Control	0.04 Low	0.20 Middle	0.73 High
Male	10	RBC (10^6/µl)	9.98 ± 0.29	8.96 ± 0.06*	8.58 ± 0.07*	8.43 ± 0.14*
		VPRC (ml/dl)	51.33 ± 1.50	46.29 ± 0.32*	44.94 ± 0.24*	44.69 ± 0.73*
		Hgb (g/dl)	18.7 ± 0.46	17.0 ± 0.11	16.3 ± 0.12*	16.04 ± 0.26*
		Reticulocytes (10^3/µl)	167 ± 35.4	252 ± 29.6	315 ± 33.4*	437 ± 32.1*
		Nucleated RBC (10^3/µl)	0.00 ± 0.00	0.017 ± 0.009	0.008 ± 0.008	0.013 ± 0.013
		Total WBC**	7.67 ± 0.30	7.04 ± 0.25	7.72 ± 0.18	8.24 ± 0.40
		Lymphocytes	5.91 ± 0.32	5.24 ± 0.23	4.87 ± 0.41	5.07 ± 0.40
		Neutrophils	1.60 ± 0.09	1.74 ± 0.12	2.71 ± 0.35*	2.97 ± 0.27*
		Eosinophils	0.09 ± 0.03	0.04 ± 0.02	0.05 ± 0.02	0.08 ± 0.03
		Monocytes	0.06 ± 0.02	0.03 ± 0.02	0.09 ± 0.02	0.12 ± 0.02
Female	10	RBC(10^6/µl)	8.59 ± 0.07	8.29 ± 0.08	8.15 ± 0.16*	7.75 ± 0.08*
		VPRC (ml/dl)	46.6 ± 0.30	45.1 ± 0.39	44.6 ± 0.80*	43.7 ± 0.48*
		Hgb (g/dl)	17.1 ± 0.11	16.6 ± 0.15*	16.7 ± 0.19	15.6 ± 0.18*
		Reticulocytes (10^3/µl)	149 ± 34.38	268 ± 45.78	176 ± 41.5	467 ± 59.34*
		Nucleated RBC (10^3/µl)	0.041 ± 0.02	0.056 ± 0.03	0.031 ± 0.02	0.088 ± 0.03
		Total WBC	7.55 ± 0.28	7.70 ± 0.35	8.83 ± 0.33*	8.69 ± 0.42*
		Lymphocytes	6.02 ± 0.27	5.66 ± 0.30	6.65 ± 0.34	6.11 ± 0.39
		Neutrophils	1.41 ± 0.18	1.98 ± 0.20*	1.94 ± 0.15*	2.41 ± 0.16*
		Eosinophils	0.06 ± 0.02	0.03 ± 0.01	0.07 ± 0.02	0.05 ± 0.02
		Monocytes	0.06 ± 0.02	0.04 ± 0.02	0.18 ± 0.03	0.12 ± 0.03

*Statistically different from control group by Duncan's multiple-comparison test ($P < 0.05$)

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

TABLE 13. RBC and WBC Parameters ($\bar{x}$ ± SEM) for Rats 5 Weeks After Completion of 4 Weeks of Inhalation Exposure to SRC-II MD

Sex	N	Dose (mg/L): Measurement	Exposure Group 0.0 Control	Exposure Group 0.73 High
Male	10	RBC ($10^6/\mu l$)	9.20 ± 0.14	9.26 ± 0.06
		VPRC (ml/dl)	46.4 ± 0.33	46.4 ± 0.33
		Hgb (g/dl)	17.9 ± 0.14	17.8 ± 0.12
		Reticulocytes ($10^3/\mu l$)	258 ± 25.5	168 ± 24.6*
		Nucleated RBC ($10^3/\mu l$)	0.039 ± 0.02	0.004 ± 0.004
		Total WBC**	8.10 ± 0.32	7.88 ± 0.26
		Lymphocytes	5.47 ± 0.36	5.74 ± 0.27
		Neutrophils	2.49 ± 0.22	2.00 ± 0.13
		Eosinophils	0.092 ± 0.02	0.076 ± 0.02
		Monocytes	0.051 ± 0.01	0.064 ± 0.03

*Statistically different from control group by Duncan's multiple-comparison test ($P < 0.05$)

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

study also demonstrated significant changes in both RBC and WBC. Several weeks after exposure, body-weight data indicated that both rats and mice were recovering, so that by 5 weeks the weight differences between treated and control animals were small but statistically significant. None of the treated animals died during the postexposure recovery period. Weights for most organs returned to control levels during the recovery period, and differences attributable to the effects of the exposure were infrequent and less severe.

Effects observed after exposure to MD were very similar to those observed in previous studies with a high-boiling coal liquid (HD) including lower body, thymus, and ovary weights and numbers of RBC, and higher liver weight and numbers of reticulocytes. Dissimilarities included a lower survival rate for mice (but not rats) following exposure to MD; few rats died after exposure to HD. Furthermore, following exposure to MD, numbers of WBC increased; after HD exposure they decreased. These similarities and differences were observed at nearly equivalent doses of the two materials.

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