SUBCHRONIC INHALATION TOXICITY OF BENZENE IN RATS AND MICE

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SUBCHRONIC INHALATION TOXICITY OF BENZENE IN RATS AND MICE - Charles O. Ward (Gulf Oil Corporation); R. A. Kuna (Exxon Corporation); N. K. Snyder (American Petroleum Institute); Richard D. Alsaker and William B. Coate (Hazleton Laboratories America, Inc.). A subchronic inhalation toxicity study of benzene was conducted in CD-1 mice and Sprague-Dawley rats. Four groups of animals consisting of 150 mice and 50 rats per sex each were exposed to concentrations of 1, 10, 30, and 300 ppm benzene vapor, 6 hours per day, 5 days per week, for 13 weeks. Additional groups of mice and rats, of similar size, were exposed under similar conditions to filtered air and served as control groups. Sacrifices of 30 mice and 10 rats per sex occurred in each group after 7, 14, 28, 56, and 91 days of treatment. Criteria used to evaluate exposure-related effects included: behavior, body weights, organ weights, clinical pathology and gross and histopathology. 50 animals per sex of each species were also exposed concurrently for cytogenetic studies. In addition, blood serum was obtained for immunological assays. The results of these two studies will be reported separately. No consistent exposure-related trends were seen in the clinical observations and body weight data. Exposure-related clinical pathology changes were seen in the high-level (300 ppm) animals of both species. In the mice, these changes included decreases in hematocrit, total hemoglobin, erythrocyte count, leukocyte count, platelet count, myeloid/erythroid ratios, and percentage of lymphocytes. Mean cell volume, mean cell hemoglobin, glycerol lysis time, percentage of neutrophils and the incidence and severity of red cell morphologic changes were increased in the mice. In the rats, decreased leukocyte counts and decreased lymphocytic percentage were the only exposure-related clinical pathology alterations. Histopathologic changes were present in the thymus, bone marrow, lymph nodes, spleen, and testes of mice exposed to 300 ppm and in most cases the incidence and severity of the lesions were greater in the males. In rats, exposure-related lesions consisted of slightly decreased femoral marrow cellularity in the animals receiving 300 ppm.

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INTRODUCTION

The toxic effects of benzene on the hematopoietic systems of experimental animals, following inhalation exposure, have been studied extensively. Generally, the effects have been dose-dependent and consist of anemia, leukopenia, splenic hypoplasia and decreased cellularity of the bone marrow (Leong, 1970; Sandmeyer, 1981; Browning, 1965; Wolf et al., 1956). The hematologic responses to benzene are not consistent in all cases of exposure, especially at lower levels (Browning, 1965). Clotting disorders, followed by pancytopenia and aplastic anemia may be seen; at higher concentrations hypoplasia of the bone marrow and internal hemorrhaging may occur (Sandmeyer, 1981).

There appears to be some species variability in the toxic response to benzene, but the picture is clouded by the lack of comparable exposure conditions as well as dosage levels (Leong, 1977 and Sandmeyer, 1981). In a recent and excellent review, for example, the hematologic, immunologic, mutagenic, and teratogenic effects of benzene were reported in 7 different species in which benzene was administered by 6 different routes and an even larger number of dosage regimens (Leong, 1977). Generally, however, rodents appear to be the species of choice for studying the hematologic effects of benzene with mice, in some cases, appearing to be more sensitive than rats (Snyder, et al., 1978).

The effects of benzene on circulating blood cells in laboratory animals and man have been evaluated using a variety of techniques. Total and differential blood counts, measurement of several biochemical indicators in the blood, cytogenetic evaluation of bone marrow smears, and histopathologic evaluation of tissue slides are among the methods that have been employed in experimental studies (Deichmann, et al., 1963; Snyder et al., 1978; Kala, et al., 1978; and Leong, 1977). In evaluation of human exposures, a number of techniques have been investigated, including phenol or sulfate levels in the urine, hematologic evaluation of peripheral blood samples and cytologic examination of the bone marrow (Browning, 1965; Sandmeyer, 1981; and Proctor and Hughes, 1978). Commonly, however, occupational physicians will use the case history and a physical examination of the patient combined with a complete blood count (Proctor and Hughes, 1978).

It was the purpose of this study to evaluate a variety of available clinical techniques, as indicators of benzene toxicity, in an attempt to determine if some method would be a better early indicator of benzene toxicity than those currently employed.

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MATERIALS AND METHODS

Animals

Male or female Sprague-Dawley rats and CD-1 mice (approximately 6 weeks of age) were purchased from Charles River Breeding Laboratories, Kingston, New York. Animals were allowed to acclimate to laboratory conditions for 19 (mice) and 39 (rats) days prior to initiation of benzene exposures. During the acclimation period and throughout the study 160 mice per sex were housed 2 of the same sex per cage, and the remaining 40 mice per sex and all the rats were individually housed in stainless steel wire mesh cages which were rack-mounted in 6 M^3 glass and stainless steel exposure chambers. Food (Purina' Certified Lab Chow #5002) was available ad libitum during acclimation and non-exposure periods, while tap water (via an automatic watering system) was available at all times. Temperature and relative humidity ranged from 71 to 79°F and from 35 to 80%, respectively, in the control chambers during the daily exposure periods. An automatically-controlled 12-hour light/dark cycle was maintained. Prior to initiation of the exposures, 2000 healthy mice and 1000 healthy rats, equally divided by sex, were randomly assigned to 5 groups. Each group contained 100 rats and 200 mice per sex. Each group of animals was then divided into 5 squads of equal size. Exposures were initiated by species and clinical observations, body weights, sacrifices, necropsies and blood sampling were spaced 1 day apart for 5 days. Exposures of the mice were initiated 1 week ahead of the rats. Of these animals (both species) 50/sex/group were exclusively scheduled for cytogenetic evaluation, and are the subject of a separate report.

Test Material

A commercial grade of benzene was obtained, in 3 separate batches of the same lot, from Exxon Research and Engineering Company, Linden, New Jersey. Analyses of each batch of the test material showed a purity greater than 99.9%. Stability of the benzene used for this study was verified by repeated analyses during and following the 91-day exposure period.

Exposure Conditions

Each group of test animals (Groups 1-5) was exposed by species for 6 hours/day, 5 days/week, for a total of 13 weeks in 6 M^3 glass and stainless steel exposure chambers. Control animals (Group 1) were exposed to conditioned air filtered through HEPA and charcoal filters. Animals in Groups 2-5 were exposed to benzene vapor mixed with this air. Benzene vapor was generated into each chamber atmosphere by sweeping the head space of 500-ml (Groups 2-4) and 2000-ml (Group 5) Pyrex® bottles (1/chamber) containing 200-ml (Groups 2-4)

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and 1000-ml (Group 5) of liquid benzene. Filtered air was passed through Crescent® tubing into the generation flasks, and through Teflon® tubing from the flasks to the air-input ducts on top of the exposure chambers. The airflow through the generation system and exposure chamber was monitored. The airflow through the chambers was maintained at 1200 L/minute. All generation flasks were seated in water baths, agitated with air and maintained at room temperature (Groups 2-4) or heated at 30± 2°C (Group 5) with tank-type heaters.

Chamber Concentration Monitoring

The chamber concentrations of benzene were established using gas chromatography by the method outlined below. The primary method of within day monitoring of benzene concentrations utilized a Wilks-Miran® 801 infrared (IR) analyzer set to analyze at 3.22 and 5.22μ (benzene), 10.90μ (ammonia), and 7.28μ (water) with a path length of 20.25 meters. The IR analyzer was on-line via a multistation sampler and was calibrated by direct injections of known volumes of liquid benzene, ammonia, and distilled water into a 5.8 liter closed-loop on the instrument. By mean of an inversion matrix programmed into its microcomputer, the analyzer compensated for ammonia and water vapor absorbances at the 3.22 and 5.22μ analytical wavelengths used for benzene. IR analyses were performed on the 10 chambers (and the chamber room) at approximate 30-minute intervals throughout each daily exposure period.

Additional chamber benzene concentration analyses were performed during the study using a Hewlett Packard 5750 gas chromatograph (GC) with a 80/100 Carbopack® column. The GC samples were taken manually via a multistation sampling system. Four benzene peak height standard curves were utilized, one for each exposure level. Each standard curve was developed by injecting liquid benzene into evacuated glass flasks of known volumes, which were then equilibrated to atmospheric pressure. One-cc gas samples were taken via a rubber septum for injection into the GC column. These standard curves were prepared daily. GC column (oven), injection port, and detector temperatures were 155, 185, and 185°C, respectively, during all analyses. GC samples were obtained and analyzed by peak height at least once a day from each exposure chamber. On those occasional instances where the IR analyzer was malfunctioning, GC samples were obtained and analyzed hourly from each exposure chamber.

Observations and Records

All animals were observed twice daily, before and after exposures and on non-exposure days, for mortality and moribundity throughout the study. At weekly intervals the individually-caged 40 mice/sex and rats/sex were observed for signs of toxicity, weighed, and individual body weights recorded.

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Clinical Pathology Studies

Prior to initiation and on study days 7, 14, 28, 56, and 91, blood samples were obtained at necropsy, by exsanguination from the abdominal aorta, from randomly-selected animals. Ten rats and 30 mice, per sex per group, were used in these studies. Fewer animals were used for pre-study samples. At each scheduled interval the following parameters were determined using standard laboratory procedures: hematocrit; total hemoglobin; erythrocyte count; mean cell volume; mean cell hemoglobin; total and differential leukocyte count; platelet count; reticulocyte count (only on days 28, 56, and 91); myeloid/erythroid (M/E) ratio (from bone marrow smears); leukocyte alkaline phosphatase (from peripheral blood smears, rats only); total protein; alkaline phosphatase; total bilirubin; creatinine; serum glutamic oxaloacetic transaminase; serum glutamic pyruvic transaminase; and erythrocyte glycerol lysis time. In addition, serum samples were frozen immediately and sent to another laboratory for immunological investigation. These studies will be the subject of a separate report.

Interim and Terminal Sacrifices

On days 0, 7, 14, 28, 56, and 91, 10 rats/sex/group and 10 mice/sex/group (10 of the 20 mice/sex/group used to obtain clinical pathology samples) were randomly selected, fasted (rats overnight and mice for 2 hours), weighed, and sacrificed by exsanguination under sodium pentabarbital anesthesia. Complete necropsies were performed on all these animals and on all animals found dead or sacrificed in a moribund condition during the study. Mice utilized for clinical pathology and immunology blood sampling, but not designated for interval necropsies, were sacrificed in the same manner and discarded without necropsy.

Organ Weights

The following organs from each animal that was necropsied at each interval were trimmed of fat and contiguous tissues, then weighed, and organ/terminal body weight ratios determined: brain, pituitary (rats only), adrenals, thyroids (rats only), lungs, heart, liver, spleen, kidneys, and testes.

Histopathology

The following tissues from each animal necropsied at each interval sacrifice were preserved in 10% neutral buffered formalin: heart, lungs (all lobes), bronchi, trachea, thyroids/parathyroids (rats only), cervical lymph nodes, salivary gland, esophagus, stomach, small intestine (3 sections), cecum, colon, bone marrow (femur; section and smear fixed in alcohol), adrenals, testes/ovaries,

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prostate/uterus, mesenteric lymph nodes, urinary bladder, mammary gland, brain, pituitary (rats only), spinal cord (2 sections), sciatic nerve/muscle, thymus, spleen, liver, pancreas, kidneys, gallbladder (mice only), bone, eye (fixed in Bouin's solution), Harderian gland, nasopharynx, and Zymbal's gland. Sections from these tissues, from the control and high-level groups of both species at each sacrifice period, were embedded in Paraphast®, sectioned at approximately 5μ, mounted on glass slides, stained with hematoxylin and eosin, and examined by a board-certified veterinary pathologist. In addition, sections of the lungs, trachea, cervical lymph nodes, thymus, spleen, bone marrow (femur), mesenteric lymph nodes, and nasal turbinates from all remaining necropsied animals of both species at each interval and at the terminal sacrifice were prepared and examined in the same manner.

Statistical Analyses

Body weight data at each weekly interval were analyzed by one-way classification analysis of covariance (ANCOVA) (Snedocor and Cochran, 1967) using pre-exposure (day 0) weights as the covariate. If the overall ANCOVA was significant, F-test comparisons for significance between pairs of group means were performed. Clinical pathology data, organ weight and organ/body weight ratio data of the control group (by species) at each interval were compared statistically to the data of the treated groups of the same sex by Bartlett's test for homogeneity of variance (Bartlett, 1937). This analysis was followed by a one-way classification analysis of variance (ANOVA) (Snedecor and Cochran, 1967) if the variances proved to be homogeneous. If the variances proved to be heterogeneous a $\log_{10}$ transformation was performed, which was followed by Bartlett's test. If the $\log_{10}$ transformation was ineffective in removing variance heterogeneity, a $\log_e$ transformation of the original data was performed which was followed by Bartlett's test. If homogeneity could not be achieved by transformation, ANOVA of the nontransformed data was completed. If ANOVA of homogeneous data was significant, Scheffe's (1953) multiple pairwise comparison procedure was used to compare the group mean values. If ANOVA of heterogeneous data was significant, Games and Howell's (1976) multiple pairwise comparison procedure was used to compare the group mean values. If ANOVA was not significant, no pairwise comparisons were made. The null hypothesis was rejected only if $P < 0.05$ (one-tailed) in all cases (i.e., the 5% level of significance was chosen).

RESULTS

Chamber Concentration Analyses

A summary of representative weekly gas chromatographic (GC) and infrared (IR) analysis results is presented in Table I. Results from GC and IR analyses slightly exceeded the target concentrations.

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Overall mean GC analyses (for Groups 2-5) varied from the target concentrations by 25.0, 7.5, 0.2, and -1.0% (respectively) in the mouse exposure chambers and by 15.0, 17.1, 1.3, and 2.3% (respectively) in the rat exposure chambers. Overall mean IR analyses (for Groups 2-5) varied from the target concentrations by 11.0, 3.5, 1.2, and -5.9% (respectively) in the mouse exposure chambers and by 9.0, 3.3, 1.7, and -8.3% (respectively) in the rat exposure chambers.

Mortality

Thirty-four male and 12 female mice were found dead or were sacrificed in a moribund condition during the course of the study. Two male rats died during the study. No exposure-related mortality was seen in either species.

Clinical Observations/Body Weights

No consistent exposure-related trends were apparent in the clinical observations or mean body weight data in either species. Sporadic statistically significant differences in mean body weight data between controls and treated groups were seen, however.

Clinical Pathology Studies

Mean clinical pathology values for both species are summarized in Tables II, III, VII and VIII (hematology) and Tables IV and V (chemistry). Exposure-related hematology changes, which occurred only in Group 5 (300 ppm) animals, are summarized in Table VI. These generally consisted, in the mice, of decreases in RBC, WBC, platelets, hemoglobin, myeloid/erythroid ratios and hematocrit, as well as increases in mean cell volume, mean cell hemoglobin and glycerol lysis time. In contrast, the rats were considerably less sensitive and significant decreases were noted only in the white cell counts. In addition, effects on differential leukocyte counts were apparent in the male and female mice and the male rats in the treated groups, when compared to controls, as follows: Mice - From day 14 on, there was a trend toward a decreased percentage of lymphocytes and an increased percentage of neutrophils, especially in the Group 5 males (Figures I and II, Table VII). In addition, an increased incidence and severity of red cell morphologic changes was noted in the Group 5 mice, which was more pronounced in the males. These changes included: anisocytosis, poikilocytosis, acanthocytosis, hypochromasia, nucleated red blood cells, Howell-Jolly bodies, polychromasia, echinocytosis and basophilic stippling. Rats - As early as day 14, a trend toward a decreased percentage of lymphocytes and an increased percentage of neutrophils was seen in the Group 5 animals (Figures III and IV, Table VIII).

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Sporadic statistically significant differences in the clinical pathology results, not considered to be related to benzene exposure, included decreased platelet count (Group 3 male mice, day 28), decreased total protein (Group 5 female mice, day 14) and decreased serum creatinine (Group 5 male rats, day 56). All other clinical pathology data were comparable between treated and control animals of both species.

Gross Pathology

Exposure-related trends apparent in the gross pathology observations in mice included: a slight increase in incidence of small thymuses observed in Group 5 males at the day 56 and 91 sacrifices; an increased incidence of small spleens in Group 5 males; enlarged lymph nodes in all groups of treated males and females at the day 56 sacrifice; and an increased incidence of cystic ovaries in the Group 5 females on day 91.

Exposure-related observations noted in all treated rat groups included: a slightly increased incidence of discolorations and irregularities of the lung surfaces observed at the day 56 sacrifice and a high incidence of enlarged lymph nodes (especially tracheobronchial) observed at the day 56 and day 91 sacrifices.

Organ Weights

A statistically significant exposure-related decrease was apparent in mean Group 5 mouse testes weights at the day 56 and 91 sacrifices and decreases in mean testes/body weight ratios on days 28, 56 and 91. In addition, the mean Group 5 female mouse liver/body weight ratio and the mean Group 3 female rat thyroid weight and thyroid/body weight ratios were significantly increased compared to the controls at the day 14 sacrifice.

All other organ weight and organ/body weight ratio data for both species and sexes were comparable between treatment and control groups.

Histopathology

To facilitate reporting of the histopathology data, each species and sacrifice interval is considered separately below. The following comments describe those lesions in which a change in incidence and/or severity was indicated by exposure to 300 ppm benzene vapors: Mice - At the day 7 sacrifice, a slight to moderate thymic atrophy was found in five males and one female. Slight to moderately severe myeloid hypoplasia in the femoral marrow was noted in 3 males

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and 1 female. At the day 14 sacrifice, a slight to moderate thymic atrophy was seen in 5 males and 2 females, while slight to moderate myeloid hypoplasia was found in 4 males and 1 female. In addition, minimal to moderately severe splenic periarteriolar lymphoid sheath depletion was detected in 6 males and 1 female. Slight to moderate lymphoid depletion in the mesenteric lymph nodes occurred in 2 males. An increase (above basal levels) in the incidence and severity of splenic extramedullary hematopoiesis was found in both sexes.

At the day 28 sacrifice, a slight to moderately severe thymic atrophy was seen in 8 males and 1 female. Minimal to severe myeloid hypoplasia was noted in the femoral marrow of 6 males. Slight to moderately severe splenic periarteriolar lymphoid sheath depletion was noted in 8 males. Increased splenic extramedullary hematopoiesis was found in all 10 males and 8 females. Six males exhibited slight to moderately severe lymphoid depletion in the mesenteric lymph nodes. Slight to severe plasma cell infiltration was seen in the mandibular lymph nodes in 7 males.

At the day 56 sacrifice, 7 males exhibited minimal to severe thymic atrophy. Moderate to severe myeloid hypoplasia was seen in 3 males. Moderate to severe splenic periarteriolar lymphoid sheath depletion was found in all of the males, while 10 males and 9 females showed an increase in extramedullary hematopoiesis. Nine males were found to have moderate to moderately severe lymphoid depletion in the mesenteric lymph nodes. In the mandibular lymph nodes, 4 males exhibited slight to moderately severe lymphoid depletion, while 5 males had minimal to moderately severe plasma cell infiltration.

The only neoplasm that occurred in the mouse study was a malignant lymphoma, lymphoblastic type, which was seen in the thymus of a male sacrificed at day 56. It could not be determined whether the test material contributed to the development of the tumor.

During the day 91 sacrifice, 9 males and 7 females exhibited minimal to severe thymic atrophy. Seven males were found to have moderate to severe femoral myeloid hypoplasia, with 5 of the 7 showing a significant degree of fatty replacement. Minimal to severe periarteriolar lymphoid sheath depletion was noted in the spleens of 9 males. Also, the males exhibited an increase in the incidence and severity of extramedullary hematopoiesis in the spleen. Minimal to moderate hypertrophy of centrilobular hepatocytes was found in the livers of 3 males. The significance of this finding is uncertain. In the mandibular lymph nodes, lymphoid depletion was seen in 3 males and an increase in plasma cell infiltration was noted in 7 males. Nine males exhibited slight to moderately severe lymphoid depletion in the mesenteric lymph nodes. Six males were found to have minimal to moderately severe testicular atrophy and decreased spermatozoa in the epididymal ducts. Ovarian cysts were present in 5 females.

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In general, the most consistently-observed treatment-related lesions in mice occurring at the 300 ppm level tended to increase in incidence and severity with time. It must be noted that, in a few instances, lesions resembling those seen in the high-level mice were detected in lower level groups. For example, thymic atrophy was noted in 2 Group 3 and 1 Group 4 males. Also, some animals in Groups 3 and 4 exhibited increased extramedullary hematopoiesis in the spleen, plasma cell infiltration in the mandibular lymph nodes and sinus histiocytosis in the mesenteric lymph nodes. In addition to the changes specifically noted above, some of the animals in the high-level group tended to have decreased lymphoid (mononuclear cell, lymphohistiocytic) infiltrates in many tissues, including the trachea, lung, kidneys, testes, prostate, urinary bladder and nasal turbinates. Also, it is readily apparent that the male mice were much more susceptible to the effects of benzene than the females.

Rats - As early as day 7, a slight trend toward decreased femoral bone marrow cellularity was evident at 300 ppm. No pattern was discernible in the marrow cellularity at the day 14 sacrifice. At days 28, 56, and 91, the treated groups also tended to have lower marrow cellularity estimates than controls. A malignant lymphoma, poorly differentiated lymphocytic type, was diagnosed in a high-level male rat at the day 14 sacrifice. No further neoplasms were encountered in the study and it is not known whether the test material contributed to the development of this tumor.

DISCUSSION

The results clearly demonstrated the hematological effects of high concentrations of benzene vapor in rats and mice. In mice, the principal hematological effects, which were significant only at 300 ppm, were leukopenia, anemia and thrombocytopenia. There were also associated increases in mean cell volume, mean cell hemoglobin and glycerol lysis time. These hematologic changes have been reported by others (Green et al., 1981; Snyder et al., 1978; and Svirbely et al., 1944), following inhalation of benzene vapors. However, Green et al., (1981) reported granulocytopenia and lymphopenia in CD-1 male mice exposed to 103 ppm for 5 days; these effects were seen, but were not significant, in this study until 14 days of exposure to 300 ppm of benzene. In rats, the only significant hematological effect was leukopenia, which began at 14 days in the 300 ppm group. There was a tendency for the incidence and severity to be greater and the time of onset earlier for the clinical pathology and histological findings in the male mice. Deichmann et al., (1963) found the opposite to be true in rats in that females exhibited a greater leukopenia than males when exposed to 831 ppm of benzene. Leong (1977) also suggests that female rats are more sensitive than the males.

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The toxic effects of benzene in both species appeared to be dose-related. Significant changes in the hematological and clinical chemistry data were seen only in the high-level animals (300 ppm). The same was generally true for histopathologic findings, although occasionally, observations such as thymic atrophy and increased extramedullary hematopoiesis in the spleen were recorded in mice exposed to 10 and 30 ppm. Others (Green et al., 1981; Dhasmana et al., 1977; and Wolf et al., 1956) have shown both hematological and pathological effects following exposure to benzene vapor at levels below 300 ppm. These differences may be due to strain variability and/or differences in exposure conditions. In this study the toxic effects of benzene were generally seen, in rats, only at the 300 ppm level.

This study also confirmed the findings of others (Snyder et al., 1978) that mice are more susceptible to the hematologic effects of benzene vapors than rats. In this study, a variety of the hematologic effects, including leukopenia, anemia and thrombocytopenia, were observed in mice while only leukopenia was observed in rats. A significant decrease in the myeloid/erythroid ratio, for example, was seen only in mice on day 91. Histopathological changes, such as myeloid hypoplasia of the femoral bone marrow, were generally more pronounced in mice and were seen at earlier time intervals. Snyder et al., (1978) suggested that these species-related differences in response may be related to differences in clearance rates of inhaled benzene between rats and mice.

Generally, the clinical chemistry findings in both mice and rats were unremarkable and tended to confirm the findings of others. Sporadic decreases in total protein and serum creatinine were seen. Other data including alkaline phosphatase, bilirubin, SGOT and SGPT were similar between treated and control groups. Others have reported changes in these parameters following benzene exposure. Dhasmana et al., (1977) reported a slight decrease in serum alkaline phosphatase after intraperitoneal adminstration of benzene to rats. Ikeda (1964) found no effects on either SGOT or SGPT in rats.

The hematological effects seen in this study generally paralleled those reported by others, except that they were seen only at the 300 ppm level in both species. The decreased myeloid/erythroid ratio, which was seen only in mice on day 91, was reported by Johnston et al., (1979) in rats exposed to 500 ppm for 3 weeks. At this higher level, the response occurred within 72 hours. The changes in red cell morphology reported here were also seen by Green et al., (1981). They found polychromasia, anisocytosis, poikilocytosis, stippling, and numerous Howell-Jolly bodies in mice exposed to 302 ppm of benzene vapor for 26 weeks. The hematological effects in this study were generally present in both mice and rats at the day 7 sampling period but did not become statistically significant until day 14 or later. This early onset of the hematologic effects of benzene has also been reported by others.

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Green et al., (1981) reported hematological changes in CD-1 mice after 5 days of exposure to 103 ppm. Novikov (1956) reported leukopenia in rats after 2-4 week exposures to benzene at varying dose levels.

The gross and histopathologic findings generally paralleled the expected effects of high levels of benzene on the hemic system. Necropsy revealed decreased size of the thymus and spleen and enlarged lymph nodes. These observations were more prevalent in the mice and were generally confirmed by histopathologic examination of the tissues. Thymic atrophy, myeloid hypoplasia of the femoral marrow, lymphoid depletion in the spleen and lymph nodes and increased extramedullary hematopoiesis in the spleen were among the more significant histopathological findings in mice. The gonads were examined only in the high level and control animals at day 91; testicular atrophy and ovarian cysts were seen in the mice exposed to 300 ppm. Similar findings of a toxic effect of benzene on the gonads of mice were reported by Hett and Maak (1938). Following exposures to unspecified levels of benzene by inhalation or subcutaneous injection in olive oil, degeneration of the testicular epithelium and atresia of the ovarian follicles were among the effects attributed to benzene. Preliminary studies, however, could not produce sterility in either sex following treatment with benzene.

The only treatment-related histopathological findings in rats, in this study, was a decreased cellularity in the femoral bone marrow seen in the high dose group as early as day 7. Similar findings have been previously reported. Green et al., (1981) reported decreased cellularity of the bone marrow and spleen at 103 ppm and increased splenic weight and cellularity at lower (9.6 ppm) levels. The decreased cellularity was primarily the result of a reduction in lymphocytes. Matsushita (1966) reported decreased weights in the thymus, spleen and testes after 1 mg/kg of benzene administered subcutaneously, for 14 days. Histopathological examination showed atrophy of these tissues as well. On the other hand, both Jenkins et al., (1970) and Wolf (1956) found no histopathological changes following exposures to varying concentrations of benzene vapor. It seems clear, however, that the histological changes in the tissues of the hemic system are among the earliest signs of benzene toxicity. In keeping with this observation, Leong (1977) has suggested that the myeloid tissue is more sensitive than the erythroid.

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In summary, rats and mice exposed to benzene vapors, at concentrations of 1, 10, 30 and 300 ppm for 6 hours per day, 5 days per week for 13 weeks, showed the expected hematological and histopathological changes at the highest exposure level. Leukopenia, anemia, thrombocytopenia and atrophy of the thymus and periarteriolar lymphoid sheaths of the spleen were among the effects observed. No consistent effects on mortality, body weight or clinical observations were reported. There was a tendency towards decreased cellularity in the femoral bone marrow in both species. Mice, however, appeared to be more sensitive than rats to the toxic effects of benzene with the male mice being more sensitive than the females. No parameter measured was found to be more predictive of the toxic effects of benzene than hematological evaluation of the peripheral blood.

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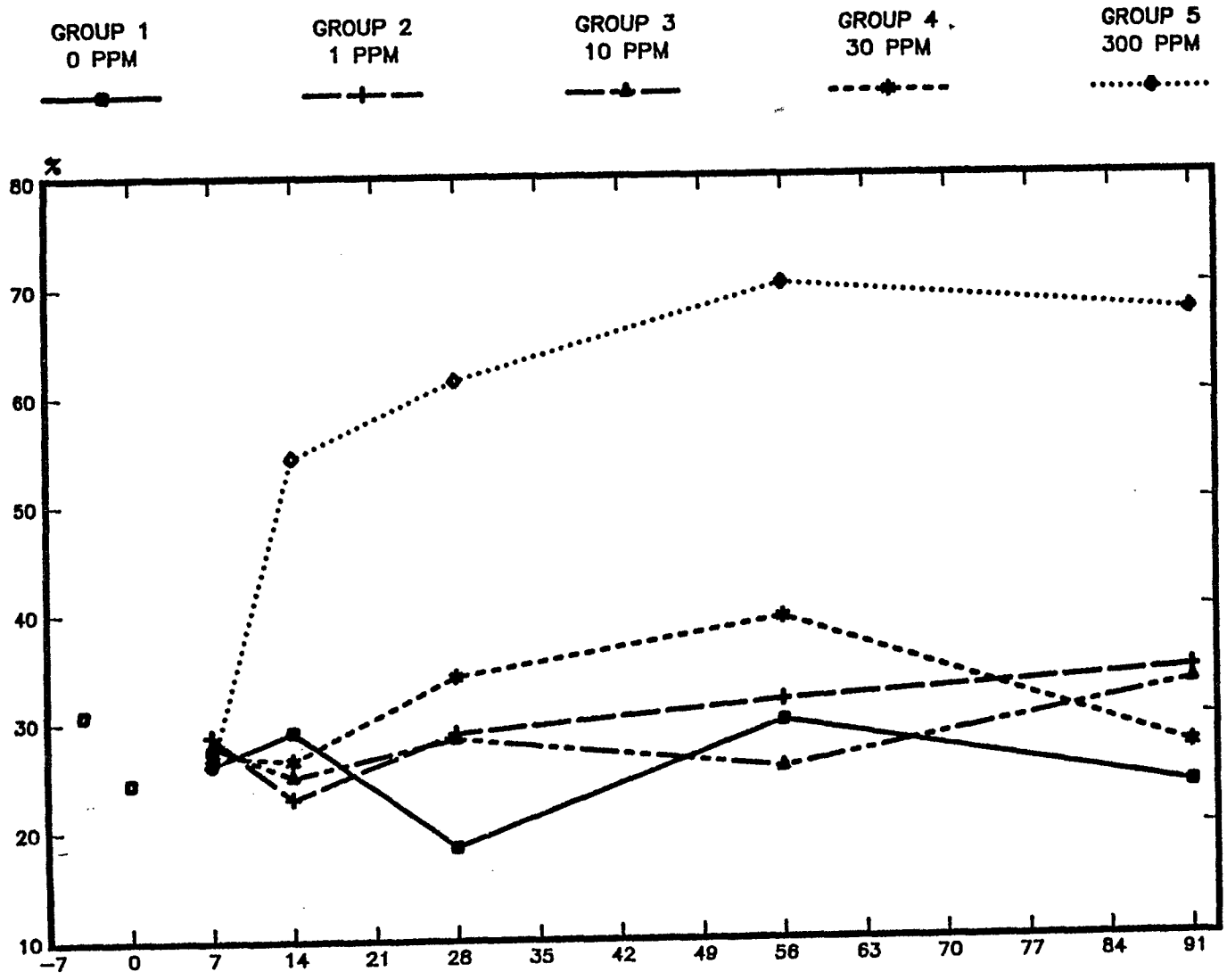
DBZ0029119

FIGURE I

PERCENT SEGMENTED NEUTROPHILS

MALE MICE



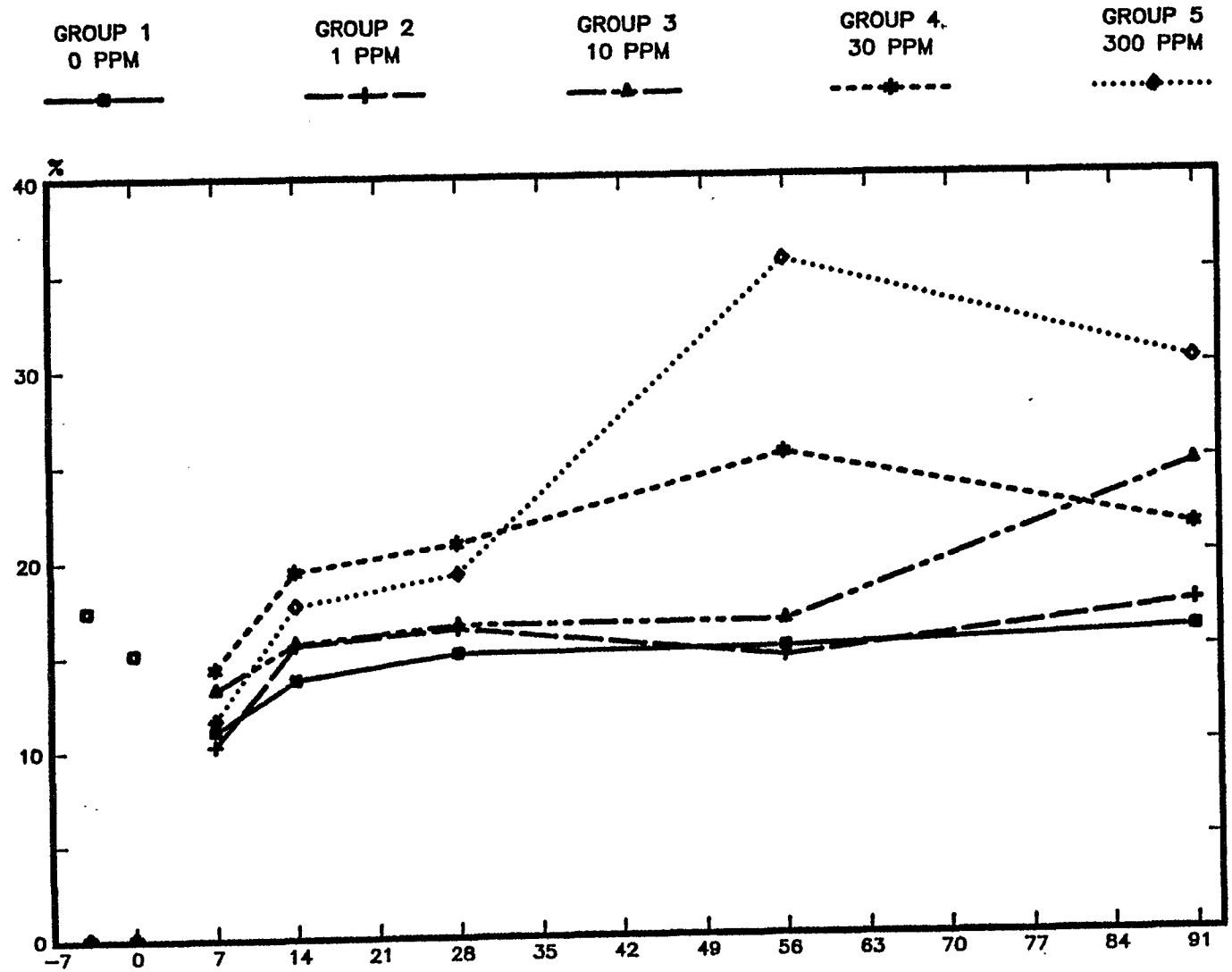
PRE-EXPOSURE DATA AT DAYS -4 AND 0.

DBZ0029120

FIGURE II

PERCENT SEGMENTED NEUTROPHILS

FEMALE MICE



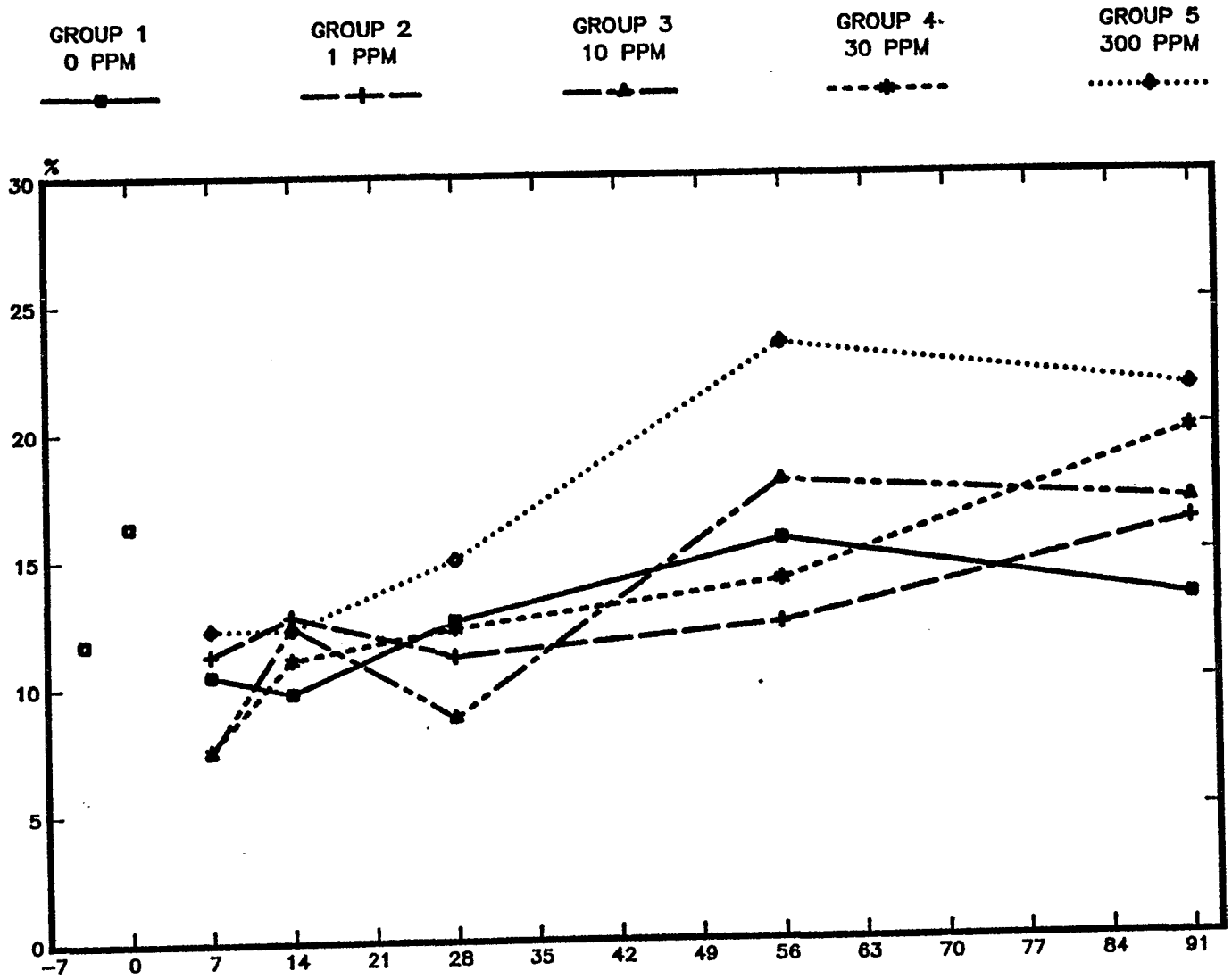
PRE-EXPOSURE DATA AT DAYS -4 AND 0.

DBZ0029121

FIGURE III

PERCENT SEGMENTED NEUTROPHILS

MALE RATS



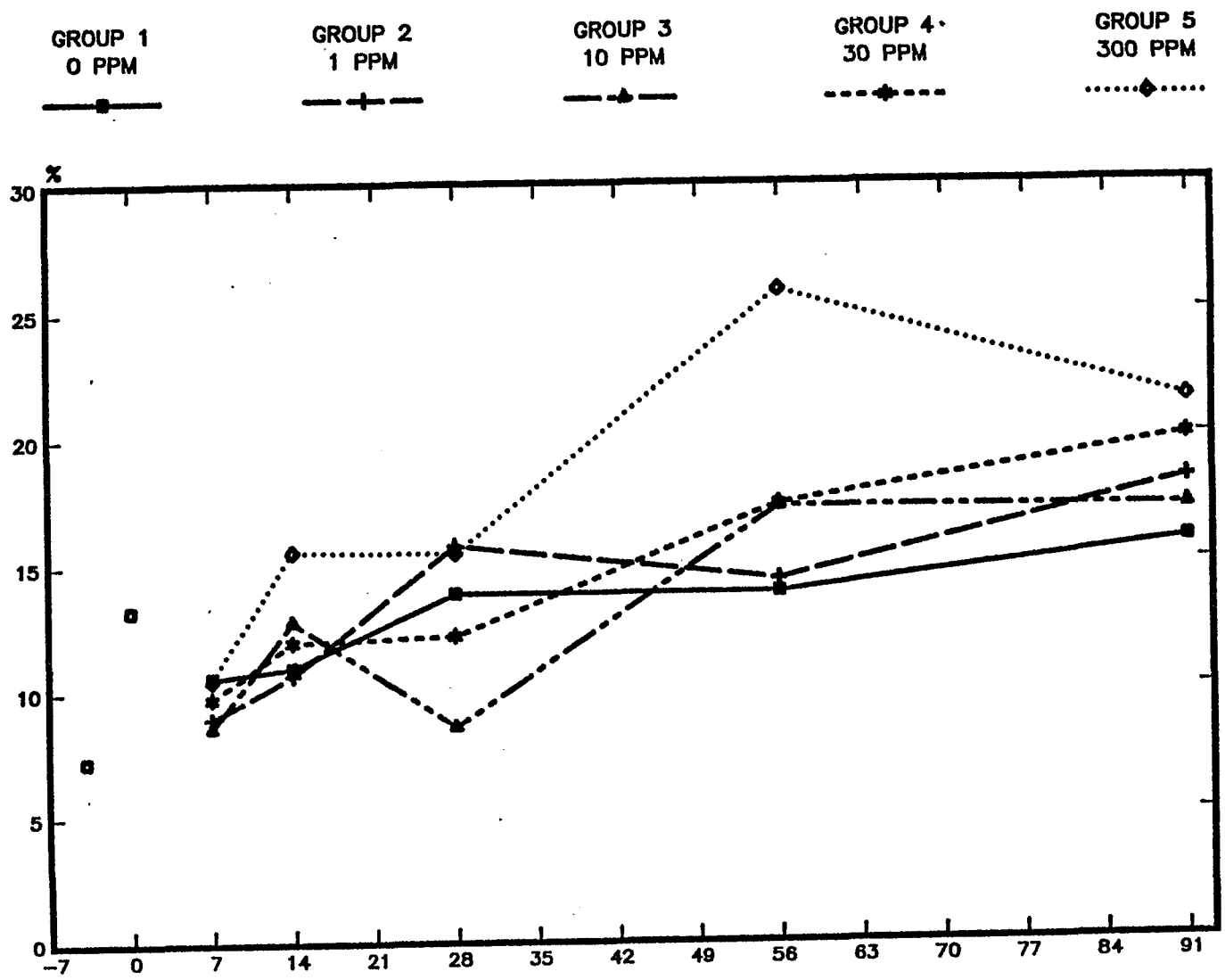
PRE-EXPOSURE DATA AT DAYS -4 AND 0.

DBZ0029122

FIGURE IV

PERCENT SEGMENTED NEUTROPHILS

FEMALE RATS



PRE-EXPOSURE DATA AT DAYS -4 AND 0.

DBZ0029123

TABLE I

REPRESENTATIVE WEEKLY CHAMBER CONCENTRATIONS OF BENZENE (PPM)[1]

Week of Study	Species	EXPOSURE LEVEL (PPM)							
		1		10		30		300	
		GC[2]	IR[3]	GC	IR	GC	IR	GC	IR
1	Mice	1.35 0.140	1.08 0.154	11.54 1.403	10.36 0.206	29.23 3.237	31.08 0.256	283.0 44.3	301.6 3.66
4	"	1.18 0.092	1.14 0.080	11.60 1.003	10.42 0.227	30.66 0.868	30.52 0.328	297.0 13.8	286.7 4.97
8	"	1.20 0.159	1.08 0.125	9.56 0.680	10.34 0.022	31.56 1.488	30.12 0.124	307.0 12.2	278.5 7.49
13	"	1.87 0.147	1.24 0.244	9.47 0.557	10.28 0.131	30.65 1.215	30.54 0.183	308.0 10.3	279.0 10.9
TOTAL[4]	"	1.25 0.207	1.11 0.059	10.75 1.114	10.35 0.106	30.08 0.950	30.37 0.330	297.0 12.7	282.3 10.9
1	Rats	1.10 0.223	1.05 0.105	13.06 1.445	9.96 4.263	32.3 1.94	30.56 0.313	292.0 22.9	268.1 26.99
4	"	1.38 0.132	1.12 0.050	13.27 1.176	10.22 0.149	29.6 2.74	30.25 0.281	311.0 8.1	274.2 6.33
8	"	0.99 0.184	1.04 0.128	9.89 0.756	10.37 0.133	28.6 1.77	30.44 0.355	294.0 32.5	284.0 0.51
13	"	1.11 0.138	1.08 0.060	11.59 0.671	10.48 0.076	31.2 1.97	30.69 0.365	323.0 23.2	255.9 4.61
TOTAL[4]	"	1.15 0.139	1.09 0.048	11.71 1.176	10.33 0.444	30.4 2.11	30.52 0.182	307.0 13.2	275.1 11.98

(1) Weekly Mean ± S.D. (2) n = 4-19 (3) n = 7-15

(4) Mean for 13 Weeks ± S.D.

DBZ0029124

TABLE II

CLINICAL HEMATOLOGY VALUES[1] FOLLOWING BENZENE EXPOSURE IN MICE

Exp. Day	Dose Level (ppm)	HCT%		HGB (gm/dl)		RBC (mi/ul)		WBC (th/ul)		MCV (fl)		MCH (pg)		Plat. (th/ul)		M/E Ratio		Retic (% RBC)	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0	0	39.1 2.24	42.3 2.78	14.1 0.76	15.3 0.82	7.91 0.396	8.26 0.549	2.7 1.25	1.6 0.84	49.5 1.73	51.1 1.57	17.8 0.76	18.5 0.54	697.0 452.3	587.0 488.5	1.40 0.270	1.39 0.236	ND	ND
14	0	41.2 5.56	44.7 2.35	14.5 1.87	15.9 0.77	8.18 0.946	8.81 0.528	2.7 1.08	2.2 1.10	50.3 1.76	50.8 1.35	17.7 0.43	18.1 0.43	1207.0 137.7	1063.0 123.2	1.54 0.428	1.26 0.464	ND	ND
	1	40.7 1.85	44.4 2.23	14.4 0.64	15.9 0.95	8.06 0.367	8.82 0.351	2.8 0.75	2.0 0.74	50.5 1.25	50.3 1.67	17.9 0.49	18.0 0.75	1111.0 182.8	1022.0 94.4	1.31 0.278	1.28 0.217	ND	ND
	10	40.4 2.73	43.9 2.40	14.4 0.94	15.6 0.76	8.09 0.620	8.64 0.621	2.9 1.39	2.9 1.01	50.0 1.58	50.8 1.31	17.8 0.54	18.0 0.56	1265.0 158.9	977.0 97.7	1.58 0.570	1.33 0.536	ND	ND
	30	42.2 2.49	42.2 3.08	15.0 0.86	15.0 1.07	8.41 0.591	8.17 0.522	2.5 1.39	2.4 1.0	50.2 1.41	51.6 1.25	17.8 0.54	18.3 0.47	1211.0 111.8	951.0 100.8	1.58 0.732	1.12 0.494	ND	ND
	300	31.7* 2.79	38.1* 2.37	11.4* 0.95	13.9* 0.80	6.19* 0.571	7.46* 0.515	1.8 1.68	0.9* 0.40	51.3 1.87	51.2 1.80	18.6* 0.78	18.6 0.61	1212.0 207.4	966.0 106.1	1.51 0.640	1.25 0.349	ND	ND
91	0	41.1 1.61	41.6 1.37	14.8 0.70	15.3 0.46	8.39 0.432	8.39 0.357	1.9 0.63	1.7 0.44	49.0 1.58	49.6 2.53	17.6 0.46	18.3 0.69	1400.0 166.8	1290.0 189.6	2.21 1.142	3.05 1.364	0.9 0.34	1.0 0.59
	1	38.4 3.93	43.2 1.76	13.9 1.38	15.7 0.59	7.58 1.177	8.65 0.311	1.4 0.56	1.6 0.72	51.2 4.79	49.9 0.68	18.6 1.82	18.1 0.34	1363.0 76.7	1269.0 144.3	1.93 0.737	2.33 1.309	1.3 0.97	1.4 0.61
	10	40.8 3.22	42.7 1.94	14.7 1.22	15.5 0.74	8.22 0.568	8.65 0.398	2.2 1.55	1.6 0.87	49.6 1.96	49.3 1.10	17.9 0.63	18.0 0.36	1335.0 153.2	1232.0 139.9	2.32 0.936	1.92 0.506	1.4 0.49	1.2 0.38
	30	38.4 5.65	43.6 2.17	13.9 1.93	15.8 0.89	7.62 1.359	8.52 0.457	2.0 2.05	1.5 0.94	50.8 3.57	51.1 1.86	18.4 1.50	18.5 0.62	1383.0 128.3	1164.0 137.5	2.37 1.171	1.86 0.742	1.8 1.59	1.0 0.47
	300	27.9* 4.75	39.1 3.45	10.3* 1.70	14.2 1.17	4.49* 9.60	7.20* 0.986	0.7* 0.36	0.8 0.39	62.6* 3.81	54.8* 4.10	23.1* 1.57	19.9* 1.48	1024.0* 242.0	1140.0 133.6	1.19 0.479	1.61* 0.928	2.5 2.40	1.4 0.58

(1) Means ± S.D.

* = Statistically significant at P< 0.05

ND = Parameter not evaluated at this interval

DBZ0029125

TABLE III

CLINICAL HEMATOLOGY VALUES(1) FOLLOWING BENZENE EXPOSURE IN RATS

Exp. Day	Dose Level (ppm)	HCT%		HGB (gm/dl)		RBC (mi/ul)		WBC (th/ul)		MCV (fl)		MCH (pg)		Plat. (th/ul)		M/E Ratio		Retic (%RBC)		LAP	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
0	0	40.4 2.03	40.9 2.31	14.9 0.70	15.1 0.77	7.19 0.319	7.41 0.505	6.8 1.89	4.7 1.59	56.2 1.92	55.3 1.47	20.8 0.51	20.5 0.61	1217.0 195.9	1166.0 147.2	1.06 0.269	1.06 0.338	ND	ND	165.0 50.9	159.0 57.4
14	0	43.9 1.78	41.1 1.44	16.1 0.57	15.2 0.50	8.30 0.315	7.73 0.294	8.4 1.74	4.6 1.26	52.9 1.61	53.1 1.73	19.4 0.57	19.7 0.61	860.0 103.8	928.0 102.3	1.15 0.377	1.28 0.409	ND	ND	155.0 34.6	178.0 46
	1	42.4 1.64	40.6 0.96	15.6 0.55	15.0 0.41	7.88 0.492	7.46 0.137	6.5 1.50	6.0 1.60	53.9 2.20	54.4 1.40	19.9 1.02	20.2 0.61	900.0 58.7	952.0 84.4	1.38 0.575	1.41 0.488	ND	ND	177.0 49.7	188.0 46.8
	10	42.9 4.13	41.3 1.99	16.0 1.40	15.3 0.65	8.06 0.791	7.58 0.380	7.6 1.22	5.2 1.22	53.2 1.29	54.4 1.17	19.8 0.55	20.3 0.34	987.0 139	957.0 73.6	1.31 0.832	1.33 0.545	ND	ND	171.0 38.3	191.0 53.8
	30	44.1 2.19	40.1 0.86	16.2 0.80	14.9 0.25	8.19 0.379	7.54 0.216	7.3 1.85	5.1 1.05	53.9 1.16	53.2 1.03	19.8 0.45	19.8 0.38	892.0 89.8	896.0 82.7	1.33 0.287	1.36 0.623	ND	ND	212.0 31.4	178.0 51.8
	300	41.9 2.64	40.5 1.44	15.6 0.85	15.1 0.42	7.85 0.599	7.45 0.313	5.6* 1.35	3.8 1.38	53.5 2.63	54.4 1.02	19.9 0.96	20.3 0.42	847.0 184.8	896.0 130.1	1.37 0.539	1.05 0.361	ND	ND	184.0 70.8	173.0 60.8
91	0	42.2 1.34	42.0 0.53	15.6 0.54	15.7 0.20	8.03 0.271	7.60 1.82	6.4 1.75	4.4 1.31	52.5 1.19	55.2 1.02	19.5 0.46	20.6 0.40	1045.0 136.5	989.0 97.5	1.18 0.342	1.14 0.318	0.6 0.44	0.5 0.33	207.0 28.2	211.0 51.7
	1	43.0 2.20	41.6 1.67	15.9 0.83	15.5 0.56	8.28 0.474	7.72 0.408	6.3 1.34	4.1 1.17	51.9 1.59	53.9 1.36	19.3 0.57	20.1 0.58	1034.0 137.7	1028.0 127.6	1.45 0.601	1.28 0.301	0.7 0.58	0.5 0.48	204.0 47.8	195.0 45.1
	10	41.7 3.67	42.2 1.46	15.5 1.36	15.7 0.43	8.05 0.850	7.73 0.324	6.3 1.70	5.2 2.16	51.8 1.86	54.6 1.58	19.3 0.75	20.4 0.57	1134.0 135.6	997.0 105.5	1.52 0.755	1.71 1.002	0.6 1.18	0.2 0.20	197.0 58.8	201.0 59.2
	30	42.1 1.24	40.8 2.27	15.6 0.44	15.5 0.64	7.98 0.375	7.56 0.476	5.7 1.74	4.3 1.18	52.7 1.53	49.1 15.93	19.6 0.64	20.6 1.0	987.0 100.8	1008.0 136.6	1.38 0.414	1.30 0.385	0.5 0.41	0.4 0.29	199.0 48.1	200.0 52.6
	300	42.4 1.62	41.8 1.18	15.8 0.54	15.7 0.36	8.08 0.308	7.70 0.224	4.3 1.58	2.5* 0.52	52.4 2.19	54.2 1.53	19.5 0.78	20.4 0.63	1028.0 139.8	987.0 110.2	1.11 0.284	1.28 0.429	0.3 0.32	0.3 0.33	225.0 58.2	214.0 42.8

(1) Means ± S.D. * = Statistically significant at P< 0.05 N.D. = Parameter not evaluated at this interval.

DBZ0029126

TABLE IV

CLINICAL CHEMISTRY VALUES[1] FOLLOWING BENZENE EXPOSURE IN MICE

Exp. Day	Dose Level (ppm)	T. PROT.(gm/dl)		A.P. (IU/l)		T. BIL. (mg/dl)		CREAT. (mg/dl)		SGPT (IU/l)		SGOT (IU/l)		GLT (sec)	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
0	0	4.3 0.32	4.4 0.14	48.0 14.8	66.0 19.6	0.44 0.305	1.04 0.822	0.37 0.220	0.25 0.374	27.0 25.0	22.0 12.4	ND	ND	14.0 2.61	16.5 2.08
14	0	4.5 0.13	4.8 0.25	36.0 5.8	55.0 15.5	0.27 0.070	0.44 0.333	0.37 0.077	0.31 0.112	22.0 7.8	19.0 4.0	ND	ND	18.4 3.24	21.0 2.76
	1	4.5 0.26	4.5 0.12	38.0 8.3	59.0 10.7	0.32 0.140	0.42 0.197	0.27 0.115	0.29 0.072	25.0 17.8	19.0 8.0	ND	ND	18.3 3.33	21.6 2.38
	10	4.4 0.16	4.5 0.19	42.0 13.9	52.0 9.7	0.43 0.325	0.50 0.451	0.32 0.223	0.22 0.105	22.0 4.6	21.0 10.9	ND	ND	19.9 4.98	20.8 3.59
	30	4.4 0.27	4.6 0.22	40.0 10.0	49.0 15.3	0.37 0.223	0.65 0.744	0.29 0.062	0.22 0.111	27.0 10.9	18.0 5.4	ND	ND	19.1 5.63	22.1 3.26
	300	4.4 0.22	4.4* 0.14	31.0 6.2	49.0 10.6	0.37 0.177	0.37 0.263	0.38 0.397	0.30 0.085	24.0 12.4	15.0 2.9	ND	ND	22.6 3.45	25.3 5.09
91	0	4.5 0.13	4.6 0.14	25.0 6.1	38.0 7.9	0.51 0.537	0.45 0.390	0.38 0.151	0.35 0.132	28.0 10.1	15.0 2.6	54.0 14.0	50.0 5.9	21.6 2.68	24.7 2.11
	1	4.8 0.28	4.5 0.18	23.0 6.9	33.0 6.7	0.75 0.617	0.63 0.523	0.22 0.198	0.25 0.155	26.0 6.9	18.0 5.4	53.0 9.9	53.0 11.0	21.9 3.69	26.6 3.38
	10	4.8 0.25	4.5 0.18	26.0 6.6	32.0 6.7	0.66 0.876	0.78 0.858	0.33 0.177	0.26 0.208	27.0 12.1	19.0 7.2	54.0 13.9	62.0 20.2	22.9 3.41	23.2 3.54
	30	4.6 0.25	4.7 0.27	25.0 5.6	39.0 10.0	0.83 0.641	0.74 0.966	0.27 0.245	0.38 0.341	25.0 7.0	17.0 9.7	57.0 10.6	54.0 25.4	23.4 2.57	28.2 4.62
	300	4.5 0.26	4.5 0.26	27.0 5.7	44.0 12.1	0.47 0.446	0.73 0.559	0.30 0.121	0.24 0.186	25.0 7.0	15.0 3.9	50.0 12.7	54.0 23.3	44.8* 6.41	34.5* 6.42

(1) Mean ± S.D * = Statistically significant $P < 0.05$ ND = Parameter not measured at this interval

DBZ0029127

TABLE V

CLINICAL CHEMISTRY VALUES(1) FOLLOWING BENZENE EXPOSURE IN RATS

Exp. Day	Dose Level (ppm)	T. PROT. (gm/dl)		A.P. (IU/l)		T. BIL. (mg/dl)		CREAT. (mg/dl)		SGPT (IU/l)		SGOT (IU/l)		GLT (sec)	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
0	0	5.9 0.18	5.8 0.31	93.0 27.3	65.0 15.9	0.24 0.055	0.34 0.165	0.62 0.057	0.53 0.105	20.0 3.8	23.0 6.2	81.0 18.3	112.0 35.8	13.5 2.06	11.5 1.95
14	0	6.0 0.17	6.2 0.37	89.0 25.7	54.0 6.4	0.27 0.037	0.36 0.107	0.65 0.064	0.71 0.175	19.0 4.1	18.0 5.7	91.0 23.9	86.0 12.6	16.1 5.29	11.8 4.04
	1	5.9 0.24	6.2 0.30	75.0 11.2	50.0 10.1	0.35 0.302	0.32 0.126	0.69 0.127	0.65 0.106	18.0 3.7	18.0 4.5	83.0 20.4	92.0 26.4	15.9 5.28	12.9 5.08
	10	6.0 0.33	6.1 0.18	90.0 15.3	57.0 23.0	0.23 0.046	0.28 0.099	0.59 0.063	0.62 0.072	17.0 5.7	15.0 3.4	74.0 12.5	76.0 13.9	16.2 6.98	12.6 2.97
	30	6.1 0.20	6.2 0.29	84.0 16.9	51.0 13.1	0.27 0.077	0.31 0.135	0.60 0.058	0.60 0.088	18.0 5.8	18.0 5.3	84.0 18.9	73.0 17.8	15.8 6.09	12.8 4.52
	300	6.0 0.18	6.1 0.32	73.0 22.0	54.0 12.9	0.25 0.095	0.37 0.083	0.61 0.085	0.62 0.065	18.0 4.6	20.0 9.5	74.0 18.8	85.0 23.5	14.9 5.10	12.1 4.53
91	0	6.3 0.20	6.5 0.23	58.0 13.8	30.0 8.4	0.25 0.053	0.39 0.056	0.72 0.055	0.69 0.094	24.0 9.5	25.0 7.0	89.0 15.9	92.0 25.0	12.3 1.74	11.6 1.36
	1	6.2 0.24	6.5 0.22	60.0 12.9	37.0 5.1	0.26 0.031	0.38 0.118	0.64 0.077	0.67 0.117	23.0 11.1	49.0 53.7	82.0 16.3	127.0 84.6	12.4 1.47	12.6 1.68
	10	6.2 0.38	6.6 0.34	45.0 9.1	36.0 16.5	0.23 0.060	0.35 0.121	0.64 0.090	0.65 0.116	26.0 10.4	35.0 19.7	88.0 28.5	106.0 26.8	13.5 1.48	12.1 1.50
	30	6.2 0.26	6.5 0.38	55.0 10.3	35.0 14.9	0.25 0.060	0.41 0.162	0.66 0.080	0.65 0.126	21.0 3.1	28.0 13.6	83.0 16.0	103.0 36.3	12.1 1.68	13.0 1.79
	300	6.1 0.37	6.4 0.37	52.0 15.2	33.0 8.5	0.26 0.104	0.48 0.128	0.71 0.119	0.63 0.096	25.0 13.1	25.0 4.1	81.0 20.9	110.0 12.6	14.3 3.75	11.7 1.37

(1) Mean ± S.D. * = Statistically significant at P< 0.05 ND = Parameter not measured at this interval.

DBZ0029128

TABLE VI

MAJOR HEMATOLOGICAL EFFECTS OF BENZENE VAPOR

(300 PPM) IN RATS AND MICE

SPECIES	PARAMETER	SEX	RESULTS	DAY OF STUDY
Mice	Hematocrit	M	Decrease	7
			Decrease*	14, 28, 56, 91
		F	Decrease*	14
	Hemoglobin	M	Decrease	7
			Decrease*	14, 28, 56, 91
		F	Decrease*	14
	Erythrocyte Count	M	Decrease	7
			Decrease*	14, 28, 56, 91
		F	Decrease*	14, 28, 56, 91
	Leukocyte Count	M	Decrease	7, 14
			Decrease*	28, 56, 91
		F	Decrease	28, 91
			Decrease*	14, 56
	Platelet Count	M	Decrease*	28, 56, 91
	Mean Cell Volume	M	Increase*	14, 28, 56, 91
			Increase*	28, 56, 91
	Mean Cell Hemoglobin	M	Increase*	28, 56, 91
		F	Increase*	28, 56, 91
	Glycerol Lysis Time	M	Increase*	56, 91
		F	Increase*	56, 91
	M/E Ratio	M	Decrease	91
		F	Decrease*	91
Rat	Leukocyte Count	M	Decrease*	14
			Decrease	28, 56, 91
		F	Decrease	7, 14, 28, 56
			Decrease*	91

* = Statistically Significant $P < 0.05$.

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TABLE VIII

SUMMARY OF MEAN NEUTROPHILS (%)
SUBCHRONIC INHALATION STUDY OF BENZENE IN RATS

Group and Exposure Level (ppm)		Day of Study:	Mean Segmented Neutrophils (%) -4	0	7	14	28	56	91	Mean Segmented Neutroophils (%) -4	0	7	14	28	56	91
			MALES							FEMALES						
1 (0)	N		10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean		11.7	16.3	10.4	9.7	12.5	15.6	13.2	7.2	13.2	10.5	10.9	13.8	13.8	15.8
	S.D.		6.00	5.98	5.21	3.77	5.64	3.60	6.43	12.44	3.12	6.12	8.08	3.97	5.03	6.61
2 (1)	N				10	10	10	10	10			10	10	10	10	10
	Mean				11.2	12.7	11.1	12.3	16.2			8.9	10.6	15.7	14.3	18.2
	S.D.				6.94	10.11	3.76	4.76	5.75			5.90	3.47	10.86	6.55	6.93
3 (10)	N				10	10	10	10	9			10	10	10	10	10
	Mean				7.4	12.3	8.7	17.9	17.0			8.5	12.7	8.5	17.2	17.1
	S.D.				2.72	3.68	4.57	7.16	5.45			7.03	5.87	4.50	7.73	6.89
4 (30)	N				10	10	10	10	10			9	10	10	10	10
	Mean				7.5	11.0	12.2	14.0	19.8			9.7	11.9	12.1	17.3	19.9
	S.D.				3.27	3.65	5.47	7.59	9.32			4.09	7.52	7.08	6.73	3.81
5 (300)	N				10	10	10	10	10			10	10	10	10	10
	Mean				12.2	12.2	14.9	23.3	21.5			10.4	15.5	15.4	25.8	21.4
	S.D.				5.92	4.26	4.33	6.02	5.08			3.69	7.84	5.30	12.65	6.75

[a]All pre-exposure values were based on a randomly selected group of 10 rats/sex.

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TABLE VII

SUMMARY OF MEAN NEUTROPHILS (%)
SUBCHRONIC INHALATION STUDY OF BENZENE IN MICE

Group and Exposure Level (ppm)		Day of Study:	Mean Segmented Neutrophils (%) -4	0	7	14	28	56	91	Mean Segmented Neutroophils (%) -4	0	7	14	28	56	91
			MALES							FEMALES						
1 (0)	N		10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean		30.7	24.4	26.1	29.0	18.4	29.8	23.7	17.4	15.1	11.0	13.7	15.0	15.2	16.0
	S.D.		17.24	11.06	15.86	13.75	6.50	14.82	14.46	3.60	9.05	5.50	5.85	3.92	5.41	6.48
2 (1)	N				10	10	10	10	10			10	10	10	10	10
	Mean				28.7	22.9	28.8	31.7	34.3			10.2	15.5	16.3	14.7	17.4
	S.D.				18.60	19.04	17.27	19.93	20.47			4.69	8.11	6.95	6.57	8.25
3 (10)	N				9	10	10	10	10			10	10	10	10	10
	Mean				27.8	24.9	28.3	25.5	33.1			13.2	15.6	16.5	16.6	24.7
	S.D.				26.39	12.62	10.28	11.54	11.72			3.29	7.62	6.15	6.43	10.70
4 (30)	N				8	10	9	10	10			10	10	10	10	10
	Mean				26.9	26.4	34.0	39.3	27.3			14.3	19.4	20.8	25.4	21.4
	S.D.				17.90	9.90	14.52	21.74	10.29			7.01	9.62	4.57	15.20	7.32
5 (300)	N				10	10	10	10	10			9	10	10	10	10
	Mean				26.1	54.3	61.4^{S+}	70.2^{S+}	67.5^{S+}			11.6	17.6	19.2	35.6^{S+}	30.2
	S.D.				13.10	20.82	28.28	23.93	27.58			5.83	7.99	9.27	14.46	12.83

a = pre-exposure values were based on a randomly selected group of 10 mice/sex.

S+ = Statistically significantly greater than mean control ($P \leq 0.05$).

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