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Introduction

Groundwater is a primary source of drinking water for about 50 percent of the population in the U.S. This source of drinking water has been generally regarded as safe from contamination. Several papers indicate that numerous underground storage tanks containing petroleum products may be leaking and contaminating public water supply wells across the U.S.(Matis, 1971; Ferguson, 1979; Woodhull, 1981; Burmaster and Harris, 1982; Lehman, 1984; Dowd, 1984; OTA, 1984). A study conducted by the Utah Bureau of Solid and Hazardous Wastes in 1985 concluded that there are at least 2,314 underground steel tanks, most of which are used to store gasoline and diesel fuel, in Utah which are more than 20 years old and may be leaking.

Contamination of well water by petroleum products from leaking underground storage tanks (LUST) is a matter of increasing concern. LUST pose a serious threat to the groundwater and public health. Leaks of petroleum products from LUST at industrial plants, commercial establishments (e.g., automobile service stations), and other operations could be expected to increase the types and concentrations of petroleum products in groundwater used for drinking and exposure of humans to the toxic effects of these chemical compounds.

Petroleum products are persistent and highly mobile contaminants which are difficult to remove from groundwater. In addition, many of these chemicals are known or suspected carcinogens or mutagens which can pose undesirable human health risks (e.g., cancer, birth defects, and other chronic conditions) at 10 ppb and below (Council on Environmental Quality, 1980). There is a need for more research on the types and concentrations of petroleum products (e.g., benzene, toluene, ethylbenzene) found in public water supply wells used for drinking water and the immunotoxic and neurotoxic effects of these organic compounds.

The objectives of this research project were:

1. To characterize petroleum products in raw water from wells used for drinking water in selected areas (industrial, commercial, and other) of Utah.

2. To evaluate the toxicity of selected petroleum products in experimental animals, with emphasis on the following:

   a. Immunotoxic and hypersensitivity effects.

   b. Neurotoxic and behavioral effects.

Methodology

Characterization of Petroleum Contaminants in Well Water

Numerous well water sampling trips were conducted during the 1985-87 research project period. A total of 5 shallow USGS monitoring wells (depth to sediment: 12 to 14 feet) and 6 deep drinking water wells (140 to 380 feet deep) located over an area extending from Woods Cross, Utah, to Orem, Utah, were sampled for petroleum contaminants. All wells were located in the vicinity of petroleum refineries, gasoline stations, or other activities (e.g., steel manufacturing, explosives production, public works).

After purging each well by evacuating a minimum of three well casing volumes, water samples for volatile and semivolatile organic pollutant analyses were collected in duplicate or triplicate with a clean Teflon bailer or by hand, put into appropriate amber glass bottles with teflon septa and screw caps, and packed in ice coolers for transportation to the Utah Water Research Laboratory. At the laboratory, all samples were stored at $4^{\circ}C$ for later chemical analyses. Temperature, pH, and conductance of water samples collected in the field were measured and recorded. Temperature was measured with a YSI Model 33 S-C-T Meter, Orion SA 250 meter or a dry bulb thermometer. A Leeds and Northrup Model 7417 Meter or Orion SA 250 Meter were used to measure pH. Conductance was measured with a YSI Model 33 S-C-T Meter.

All sampling equipment that would be in contact with well water was cleaned with Micro laboratory cleaning solution, washed thoroughly with tap or distilled water, rinsed several times with acetone, and then washed with distilled water before use. For each well where a bailer was used to collect water, samples for volatile organic pollutants were obtained from the first bailer of well water. Water samples for semivolatile organic pollutants were obtained from subsequent bailer well water samples which had been thoroughly mixed in a large stainless steel bucket. For each well where water samples were collected by hand, the well water was allowed to flow for about 30 minutes before collection of water samples.

Water samples were analyzed for traces of volatile organic petroleum pollutants by EPA Purge and Trap Analysis Method 5030. Following extraction of the semivolatile organics in the water samples by solvent extraction (EPA Separatory Funnel Liquid-liquid Extraction Method 3510), the extractables (acids and base neutrals) were analyzed by EPA GC/MS Method 8250 for semivolatile organics--Packed Column Technique.

A concentrated effort was made to locate and sample shallow drinking water wells (wells capable of drawing surface water into them) in areas where Leaking Underground Storage Tanks (LUST) were known or suspected. This task was a diffi ult one. After consulting with water personnel at various agencies (e.g. North Davis, South Salt Lake City, and Provo County Health Departments, USGS Office in Salt Lake City, Bureau of Public Water Supplies in the State of Utah Department of Health, and Orem City's Engineering Office), it was apparent there are few, if any, shallow wells in the present study area that are used for drinking purposes. According to a Bureau of Public Water Supplies (Salt Lake City) engineer, there may be a few private shallow wells in the area we are studying that have not yet been inventoried and which are most likely used for agricultural irrigation or live-stock watering purposes. There is a possibility some of these wells may be serving as a source of drinking water.

Because of the above findings, we decided to collect water samples from shallow USGS monitoring wells and deep drinking water wells (wells that penetrate an impermeable strata and prevent surface water from being pumped or injected into them) in the study area where LUST were known or suspected. In future studies, more emphasis should be placed on obtaining water samples from shallow monitoring wells and nearby deep drinking water wells in those parts of a study area where LUST are known or suspected and then examining these samples for types and concentrations of petroleum contaminants.

Toxic Effects of Selected Petroleum Contaminants in Laboratory Animals

Toxicity studies were conducted from August 25, 1985, through June 30, 1987. For the 1985-86 project period, studies were performed to determine the immunotoxic and neurotoxic effects of selected petroleum contaminants, namely, benzene, toluene, and phenol in laboratory animals (mice)

following continuous ingestion of drinking water containing various concentrations of the test chemicals.

For the 1986-87 project period, studies were conducted primarily to determine the immunotoxic and neurotoxic effects resulting from the interaction of two petroleum contaminants, namely, benzene and toluene, in laboratory animals (mice) following continuous ingestion of drinking water containing combination concentrations of the two test chemicals.

Benzene-induced immunotoxicity and neurotoxicity in mice

Experiments were conducted to determine the effects of benzene on the immune and nervous systems of mice. Analytical reagent grade benzene (99.9% purity, JT Baker Chemical Co., Phillipsburg, NJ), was dissolved in normal tap water to provide nominal concentrations of 40, 200, and 1,000 mg/L. Benzene has a solubility of 1,780 mg/L in water at 25°C (USEPA, 1980). The benzene-treated water was administered to male, adult CD-1 mice (approximately 20-22 gm initial weight) continuously for 28 days via drinking water; the control group received untreated tap water. In addition to the benzene-treated tap water given as drinking water, all animals received lab chow. To minimize decomposition and to maintain the concentration of benzene, drinking water was provided in glass water bottles, shaken frequently during treatment and was changed every 3 days. Feed and water consumption was monitored continuously, and animals were weighed once each week. Benzene concentration in drinking water was confirmed on different days by gas chromatography (USEPA, 1982). The observed concentrations for benzene were 31, 166, and 790 mg/L.

Five mice per test group were housed in plastic cages on hardwood-chip bedding and maintained on a 12 hour light-dark cycle at an ambient room temperature of 21 ± 1°C during the experimental period. All animals in the study were individually identified with color bands on their tails.

To assess the general toxic effects of benzene in mice following continuous ingestion of drinking water containing various concentrations of benzene, food, water consumption, and individual body weight gain were monitored throughout the experimental period. Relative organ (kidney, spleen, liver, and thymus) weights of each animal were recorded at the time of animal sacrifice. In addition to the above observations, the following immunotoxic evaluations were performed on the test animals after 4 weeks of treatment to evaluate benzene-induced immunotoxicity: 1) Hematological tests [e.g. red blood cell (RBC) and white blood cell (WBC) counts] to determine benzene hematoxicity; 2) Mitogenesis Assay for inducing and measuring animal splenic lymphocyte proliferation in response to mitogens and antigens in vitro; 3) Mixed Lymphocyte Response (MLR) in which the mixing of two populations of allogeneic lymphoid cells (stimulator and responder cells) results in T cell proliferation; 4) Cell-mediated Cytolytic Response to evaluate splenic lymphocytes of test animals for cytotoxic cells; 5) Plaque Forming Assay (Cunningham and Szenberg, 1956) to evaluate the primary antibody response by mouse splenic lymphocytes toward sheep red blood cells (SRBC), a T-dependent antigen; and, 6) Enzyme Linked Immunosorbent Assay (ELISA) to determine specific hemolysin.

After 4 weeks of benzene treatment and for neurotoxic evaluations, five additional mice from the control and each of the test groups were killed by decapitation and their brains quickly dissected into six anatomic regions (hypothalamus, medulla oblongata, cerebellum, midbrain, corpus striatum, and cortex [Glowinsky and Iversen, 1966]). The midbrain included the hippocampus, thalamus, and subthalamus regions. Brain tissues were immediately placed into tared vials containing several volumes, in relation to tissue weight, of ice-cold 0.05 M $HC1O_4$ with 0.5% cysteine and then frozen at -80°C until analyzed. To minimize possible diurnal variations in regional brain neurotransmitter concentrations, all animals were sampled between 10:00 and 12:00 a.m. on the same day.

Samples from various brain regions were homogenized and centrifuged at 10,000 g for 60 minutes, and the supernatant fractions were passed through a 0.2 µm pore filter by gentle centrifugation for 3 minutes at 1000 g. These filtrates were analyzed for major catecholamines and indoleamines, namely dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT, serotonin), and their principal metabolites, i.e. dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxy-4-hydroxy-mandelic acid (VMA), and 5-hydroxyindoleacetic acid (5-HIAA) using high pressure liquid chromatography with a multiple electrode electrochemical detector (Mayer and Shoup, 1983) to determine possible changes in the levels of these selected neurotransmitters that control behavior.

The neurochemicals selected for evaluation are known to play an important role in physiology and behavior (Poirier and Bedard, 1984; Rogawski and Baker, 1985). Changes in concentrations of

their metabolites provide an appropriate indication of neural activity (Commissiong, 1985). Assays in various brain regions are essential to detect perturbations of a specific neurotransmitter(s).

The data were analyzed by one-way analysis of variance followed by the least significant difference test, if F ratios were significant. A $P<0.05$ was accepted as statistically significant.

Toluene-induced immunotoxicity and neurotoxicity in mice

Experiments were also conducted to determine the effects of toluene on the immune and nervous systems of mice. Chromatography grade toluene (99.7% purity, Burdick and Jackson Labs, Inc., Muskegon, MI) was dissolved in normal tap water to provide nominal concentrations of 20, 100, and 500 mg/L. Toluene has a solubility of 535 mg/L in water at 25°C (USEPA, 1980b). The toluene-treated water was also administered to the CD-1 mice continuously for 28 days via drinking water; the control group received untreated tap water. These animals also received lab chow. To minimize decomposition and to maintain the concentration of toluene, drinking water was provided in glass water bottles, shaken frequently during treatment and was changed every 3 days. Feed and water consumption was monitored continuously, and animals were weighed once each week. Toluene concentration in drinking water was also confirmed on different days by gas chromatography (USEPA, 1982). The observed concentrations for toluene were 17, 80, and 405 mg/L.

As with the benzene study, five mice per test group were housed in plastic cages on hardwood chip bedding and maintained on a 12 hour light-dark cycle at ambient room temperature during the experimental period. All animals received lab chow and were individually identified with color bands on their tails.

To assess the general toxic effects of toluene in mice following continuous ingestion of drinking water containing various concentrations of toluene, food and water consumption, and individual body weight gain were also monitored throughout the experimental period. The methods for evaluating toluene-induced immunotoxicity and neurotoxicity in mice were the same as those used for benzene.

Phenol-induced immunotoxicity and neurotoxicity in mice

Experiments were also conducted to determine the effects of phenol on the immune and nervous systems of mice. Analytical reagent grade phenol was dissolved in normal tap water to make the nominal concentrations of 5, 20, and 100 mg/L. Phenol has a solubility of approximately 67 g/L (Windholz, 1976). The phenol-treated water was administered to the CD-1 mice continuously for 28 days via drinking water; the control group received untreated tap water. In addition to the phenol-treated tap water given as drinking water, all animals received lab chow.

To minimize decomposition and maintain the concentration of phenol, drinking water was provided in glass water bottles, shaken frequently during treatment and changed every 3 days. Feed and water consumption was monitored continuously, and animals were weighed once each week. Phenol concentration in drinking water was confirmed on different days by the direct photometric method (APHA, 1980). Observed concentrations for phenol were approximately 95 percent of the nominal concentrations (4.75, 19, and 95 mg/L) used.

Similar to the benzene and toluene studies, five mice per test group were housed in plastic cages on hardwood chip bedding and maintained on a 12 hour light-dark cycle at ambient room temperature during the experimental period. All animals also received lab chow and were individually fitted with color bands on their tails.

The methods for assessing the general toxic effects of phenol in mice following continuous ingestion of drinking water containing various concentrations of phenol and for evaluating phenol-induced immunotoxicity and neurotoxicity in mice were the same as those used for the benzene and toluene studies.

Effect of benzene, and toluene, alone or combined on immunotoxicity and neurotoxicity in mice.

Experiments have been conducted to determine the effects of combinations of benzene and toluene on the immune and central nervous systems of mice. Male, adult, CD-1 mice were separ-ated into four groups (five mice per group): 1) control group exposed to untreated tap water; 2) benzene group exposed to benzene-treated tap water; 3) toluene group exposed to toluene-treated water ;and, 4) mixture group exposed to a mixture of benzene and toluene in tap water. Two experiments were performed.

In the first experiment, the animals were exposed to 200 mg/L benzene, 400 mg/L toluene, and 200 mg/L benzene + 400 mg/L toluene. In

the second experiment, the animals were exposed to 200 mg/L benzene, 100 mg/L toluene, and 200 mg/L benzene + 100 mg/L toluene. The animals were continuously fed tap water ad. lib., containing the various concentrations of benzene, toluene, and benzene + toluene for 4 weeks. The animals were housed, maintained, and identified with color bands on their tails as in the other toxicity studies.

To assess the general toxic effects in mice, food and water consumption were monitored continuously, and animals were weighed once a week. The methods for evaluating the effects of combinations of benzene and toluene on the immune and central nervous systems of mice were the same as those used for the benzene, toluene, and phenol toxicity studies.

Principal Findings and Significance

This portion of the final research project report has been chronologically divided into the following parts: 1) characterization of petroleum contaminants in well water; 2) benzene-induced immunotoxicity and neurotoxicity in mice; 3) toluene-induced immunotoxicity and neurotoxicity in mice; 4) phenol-induced immunotoxicity and neurotoxicity in mice; 5) immunotoxic effects of benzene (200 mg/L), toluene (400 mg/L), and a mixture of benzene (200 mg/L) + toluene (400 mg/L) in mice; 6) immunotoxic effects of benzene (200 mg/L), toluene (100 mg/L), and a mixture of benzene (200 mg/L) + toluene (100 mg/L) in mice; 7) neurotoxic effects of benzene (200 mg/L), toluene (400 mg/L), and a mixture of benzene (200 mg/L) + toluene (400 mg/L) in mice; and 8) neurotoxic effects of benzene (200 mg/L), toluene (100 mg/L), and a mixture of benzene (200 mg/L) + toluene (100 mg/L) in mice. In the interest of clarity and conciseness, the data are summarized and presented in tabular form (see Appendix A for tables) and results are discussed as they are presented.

Characterization of Petroleum Contaminants in Well Water

Table 1 shows the temperature, pH, and/or conductance values of well water samples collected from five USGS shallow monitoring wells during the 1985-1987 research project period. Water temperatures ranged from 6.8 to 18.0°C. The pH of the water samples ranged from 5.5 to 7.8. Conductance values (μmhos/cm) ranged from 1,339 to 5,105. Temperature and pH values of well water samples collected from six deep drinking water wells during the project period are presented in Table 2. Water temperatures for these samples ranged from 12.8 to 19.4°C. The pH of the water samples ranged from 6.2 to 7.4.

Results of volatile or semivolatile organic pollutant analyses of various shallow monitoring well water samples collected during the 1985-87 project period are presented in Table 3. Volatile organic compounds detected in the shallow well water samples by Purge and Trap Analysis (Table 3) were: benzene (0.81-196.83 μg/l), toluene (0.09-122.33 μg/l), ethylbenzene (0.96-101.27 μg/l), p-xylene (0.25-26.98 μg/l), m-xylene (0.29-8.36 μg/l), o-xylene (0.31-3.08 μg/l), 1,3,5-trimethylbenzene (5.36-62.45 μg/l), 1,2,3,4-tetramethylbenzene (5.83-9.49 μg/l), 1,2,4,5-tetramethylbenzene (1.27-10.46 μg/l), and naphthalene (4.14-6.71 μg/l). Volatile or semivolatile organic compounds detected in the shallow well water samples by Methylene Chloride Extraction (Table 3) were: Benzene (1.83-36.21 μg/l), toluene (1.13-17.39 μg/l), ethylbenzene (17.89-53.23 μg/l), p-xylene (0.48 μg/l), 1,2,3,4,-tetra-methylbenzene (9.57 μg/l), 1,3,5-trimethylbenzene (15.22-21.06 μg/l), and 1-methylnaphthalene (4.96 μg/l).

Benzene (0.81-196.83 μg/l), toluene (0.09-122.33 μg/l), ethylbenzene (0.96-101.29 μg/l), and o-xylene (0.31-3.08 μg/l) were detected in well water samples from three shallow monitoring wells (Well numbers 3, 4, and 5) which were located near gasoline pumps. A level up to 6.4 mg/L toluene has been detected in drinking water (Tardiff and Youngren, 1986). Benzene, toluene, ethylbenzene and o-xylene are known chemical constituents of gasoline.

Well number 3 was located at the Sandy Public Works Department (8775 South 700 West, Sandy, Utah). Three gasoline pumps were located 90 to 100 feet east of the monitoring well. Well number 4 was located in a parking lot approximately 105 feet north of a Sinclair gas station at 7000 South 2000 East, Salt Lake City, Utah. Well number 5 was located in an apartment complex at 450 South, 945 East, Salt Lake City, Utah. A GC/MS analysis of water samples from well number 3 indicated the presence of numerous other organic compounds as shown in Table 4. The following organic compounds were also detected in water from well number 3: p-xylene (0.25-26.99 μg/l), 1,3,5-trimethylbenzene (12,23-62.45 μg/l), 1,2,3,4- tetramethylbenzene (5.83-9.49 μg/l), 1,2,4,5-tetramethylbenzene (1.27-13.96 μg/l), m-xylene (0.29 μg/l), and naphthalene (4.14-5.18 μg/l). In water from well number, 4 other organic compounds detected were: p-xylene (6.50 μg/l), m-xylene (2.49 μg/l), 1,3,5-trimethylbenzene (2.68 μg/l), 1,2,4,5- tetramethylbenzene (2.66 μg/l), and naphthalene (3.36 μg/l). In water from well number 5 other organic compounds detected were: p-xylene (14.54 μg/l), m-xylene (8.36 μg/l), 1,3,5-trimethylbenzene (6.70 μg/l), 1,2,4,5-tetramethylbenzene (6.90 μg/l), and naphthalene (4.81 μg/l). Surprisingly, although not detected by Purge and Trap Analysis during the first sampling trip (9/09/85), toluene (1.13 μg/l), 1,2,3,4-tetramethylbenzene (9.57 μg/l), and 1-

methylnaphthalene (4.96 µg/l) were detected by Methylene Chloride Extraction in water from well number 2 during the second sampling trip (02/06/86). The shallow monitoring well from which this water sample was collected is located adjacent to a private residence and about one and one-half blocks southwest of a public gasoline station. Benzene (17.98 µg/l) was detected by Purge and Trap Analysis in water from well number 2 during a later sampling trip (04/24/87).

No volatile or semivolatile organic compounds were detected in any of the deep well water samples collected during the entire research project period. Table 5 shows the organic compounds detected by GC/MS analysis of all shallow monitoring well water samples analyzed during the study.

Benzene-induced Immunotoxicity and Neurotoxicity in Mice

Benzene-induced immunotoxicity in mice

Continuous exposure of adult CD-1 mice to various concentrations of benzene (0, 40, 200, 1000 mg/L) via drinking water over a 4 week period did not cause mortality or induce any clinical toxicity signs in the animals. Overall, there was no change in total feed and water consumption; however, water consumption by mice in the 1000 mg/L treatment group was slightly decreased. No gross lesions were observed on any of the organs of mice in all treatment groups at the time of sacrifice. There was also no significant change in body weight gain (Table 6).

Table 7 shows the organ and body weights of mice following 4 weeks of benzene exposure. Spleen and thymus decreased in weight at all the benzene concentrations. Spleen showed a significant decrease in weight at the 1000 mg/L level. Kidney increased in weight at all the concentrations and showed a significant increase in weight at the 1000 mg/L level.

The effects of 4 weeks of benzene exposure on spleen cellularity and selected blood parameters are presented in Table 8. Total spleen cellularity decreased significantly at the 1000 mg/L level. It can be seen from the table that WBC and RBC counts decreased significantly with increased dose. PCV also decreased significantly at the 200 and 1000 mg/L concentrations. Benzene is a proven hemotoxin (Fishbein, 1984). In man, it is causally related to pancytopenia (USEPA, 1980).

From the differential WBC counts shown in Table 8, it can be seen that there was a significant depression of lymphocytes at 40 and 1000 mg/L, and a significant stimulation of neutrophils at these two concentrations. While all blood cell types are targets for benzene poisoning, lymphocytes are particularly sensitive (Rosenthal and Snyder, 1985).

Table 9 shows the effects of benzene exposure on the uptake of [^{3}H]-thymidine by mouse spleen cells in culture. Spleen cells are evaluated for their ability to proliferate after mitogen stimulation. Following four weeks of exposure, the proliferative response of mitogen-treated spleen cells is biphasic, significantly elevated in low dose (40 mg/L) group and significantly decreased in higher dose (200, 1000 mg/L) groups. The results of the mixed lymphocyte response to allogeneic cells (Table 10) are the same as the mitogenesis results (Table 9), and there are also significant differences.

The killing of target cells by sensitized T-lymphocytes is an important indicator of cell-mediated immunity. The effect of benzene exposure on cell-mediated cytolytic response is shown in Table 11. The results of ^{51}Cr-release assay show that cell-mediated cytolytic response against tumor cells (target cells) is affected by benzene exposure. The response is also biphasic, elevated in low dose (40 mg/L) group and decreased in high dose (200, 1000 mg/L) groups. There is a significant depression in response at the 200 and 1000 mg/L levels in the 50:1 effector-to-target cell ratio. The results for the 25:1 ratio also showed significant stimulation in the 40 mg/L group, but significant suppression only at the 1000 mg/L level.

After 4 weeks of benzene exposure via drinking water, animals were injected with sheep red blood cells (SRBC) and their anti-SRBC antibodies evaluated by enumeration of plaque-forming cells (PFC). The effect of 4 weeks of benzene exposure on the antibody plaque-forming cells (PFC) is presented in Table 12. The results of primary antibody response show that there is a significant depression of plaque formation by cells collected from animals exposed to 200 and 1000 mg/L benzene in drinking water and no significant stimulation in the 40 mg/L group. The titer of α-SRBC antibodies corresponds with the changes in numbers of PFC. According to the ELISA tests, a significant inhibition was found at the highest dose (1000 mg/L) group (Table 12).

Benzene-induced neurotoxicity in mice

The regional concentrations of catecholamines and indoleamines in different brain regions are presented in Tables 13, 14, 15 and 16. Ingestion of benzene in drinking water induced significant increases in catecholamine neurotransmitters in several brain regions at all three benzene-treated levels when compared with control animals (Tables 13 and 14).

The significant benzene-induced increases of concentrations of NE relative to controls were found in the hypothalamus (200, 1000 mg/L), medulla oblongata (200 mg/L), cerebellum (1000 mg/L), midbrain (40, 200, and 1000 mg/L) and cortex (40, 200 and 1000 mg/L). The concentration of VMA, a metabolite of NE, also showed a significant increase in the cerebellum, corpus striatum and cortex (40, 200 and 1000 mg/L). DA, the parent neurotransmitter of dopaminergic neuronal systems, exhibited a moderate increase in various brain regions but this finding was not significant.

Significant increases in the DOPAC concentration, one metabolite of DA, were found in the hypothalamus (200 mg/L), and in other brain regions such as the medulla oblongata (40, 200 mg/L), midbrain (40, 200, and 1000 mg/L), corpus striatum (1000 mg/L), and cortex (40, 200 mg/L). HVA, the last step metabolite of DA, was also significantly increased in the midbrain (40, 200, and 1000 mg/L). Likewise, there were significant increases of HVA in the corpus striatum (1000 mg/L) and in the cortex (40, 200 mg/L). No significant increases of HVA concentrations were found in the other brain regions (hypothalamus, medulla oblongata, and cerebellum).

Benzene ingestion also induced significant increases in indoleamine neurotransmitters in several brain regions (Tables 15 and 16). The levels of 5-HT were significantly increased in midbrain (all treatment groups), cortex (40, 200 mg/L), and medulla oblongata (40 mg/L), the regions of the brain containing relatively high amounts of this amine. Concommitant with the observed increases of 5-HT concentrations, levels of its metabolite (5-HIAA) were likewise significantly increased in the same brain regions and the same dose groups. These findings of alterations in brain neurotransmitter concentrations may support the reported clinical and behavioral effects associated with benzene exposure (Haley, 1977; Brief et al., 1980; Evans et al., 1981; Dempster et al., 1984).

Toluene-induced Immunotoxicity and Neurotoxicity in Mice

Toluene induced immunotoxicity in mice

Continuous exposure of CD-1 male mice to various concentrations of toluene (0, 20, 100, 500 mg/L) via drinking water over a 4 week period did not induce mortality or overt signs of toxic stress. Overall, a dose-related slight decrease of total food consumption was observed; however, there was no change in total water consumption. No gross lesions were found on any of the organs of mice in all treatments groups at the time of sacrifice. There was also no change in body weight gain (Table 17).

Table 18 shows the organ and body weights of mice following 4 weeks of toluene exposures. The weight of liver was significantly increased in the two higher dosed groups (100, 500 mg/L). Thymus, the primary lymphatic tissue, showed a significant decrease in weight at the 500 mg/L dose level. Spleen and kidney showed no change in weight during the experiment.

The effects of 4 weeks of toluene exposure on spleen cellularity and selected blood parameters are given in Table 19. Total spleen cellularity did not show significant decrease in any of the treatment groups. The hematological data demonstrated a significant decrease in peripheral leukocytes at the highest dose level (500 mg/L) and a slight increase in the other two toluene-treated groups (20, 100 mg/L). Hobara et al., (1984), observed significant leukocyte decreases in mature cross-breed dogs exposed by inhalation to more than 500 ppm toluene.

From the differential WBC counts shown in Table 19, it can be seen that there was no significant depression of lymphocytes with increased dose and only a slight increase in neutrophils. No change of monocytes was found in all the treated groups when compared to the control group. There is general agreement that toluene does not have the hematotoxic properties of benzene (Fishbein, 1985, U.S. EPA, 1980b).

Table 20 shows the effects of toluene exposure on the uptake of [^{3}H]-thymidine by either mitogen stimulated or nonmitogen stimulated mouse splenic cells in cultures after 4 weeks of treatment. A significant decrease in [^{3}H]-thymidine incorporation in non-mitogen stimulated cultures was observed at all dose levels in the in vivo toluene-treated splenic cells. There also appeared to be a depressing mitogenesis response to all mitogens, i.e. LPS, PWM, ConA, and PHA.

Statistical analysis revealed significant differences from controls at 20, 100, and 500 mg/L toluene dose levels. From these findings, toluene was shown to have a significant depressing effect on the mitogenic response to both T cell mitogens (ConA, PHA) and B cell mitogens (LPS, PWM).

Table 21 demonstrates the lymphocyte-transformation response to allogeneic cells. Similar to mitogenesis, a significant reduction of [^{3}H]-thymidine incorporation occurred in non-stimulated and allogeneic cell cultures in all toluene-treated mice splenic cells. These results show that toluene depressed T cell function in response to allogeneic cells.

The effects of toluene exposure on cell-mediated cytolytic response are presented in Table 22. The results of ^{51}Cr-release assay show that cell-mediated cytolytic response against tumor cells (target cells) is moderately affected by toluene exposure. The decreased responses are a dose-related effect. A significant reduction was only seen at the highest dose levels (500 mg/L) in the 50:1 effector-to-target cell ratio.

After 4 weeks of toluene exposure via drinking water, our studies demonstrated that toluene causes an immunosuppressive effect by decreasing the development of SRBC-specific plaque-forming cells (PFC) (Table 23). This immunosuppression was a dose-related effect but the significant suppressions were only found in the 500 mg/L toluene-treated group. The results of ELISA test showed that the titer of α-SRBC antibodies did not decrease significantly (Table 23).

Toluene-induced neurotoxicity in mice

The levels of toluene used in this study did not cause the animals to display noticeable clinical behavioral effects during the course of the experiment. After 4 weeks of toluene treatment via drinking water, toluene ingestion induced significant increases in catecholamine neurotransmitters in several brain regions. The effects were often maximal at 100 mg/L with no further increase apparent at the highest dose (Tables 24 and 25).

Toluene exerts its greatest effect on NE. In the hypothalamus, a region especially rich in this neurochemical, significant toluene-induced increases were detected relative to the control group in all three treatment groups. The three doses of toluene also caused significant increases in concentrations of NE in the midbrain, one major region containing relatively high amounts of this amine. Likewise, a significant increase in NE concentrations was seen in the medulla oblongata in the 100 mg/L group (Table 24).

The apparent increases of NE in the other three brain compartments (cerebellum, corpus striatum, and cortex), the regions with lower amounts of NE, were not significant as determined by analysis of variance. The concentrations of VMA, a major metabolite of NE, were also influenced by toluene treatment. It can be seen that there were significant increases of VMA in midbrain and corpus striatum at the two highest doses (100, 500 mg/L), medulla oblongata at the 100 mg/L, and cortex in the 500 mg/L group (Table 25).

Levels of DA and its principal metabolites, namely, DOPAC and HVA, showed significant increases in several brain regions following 4 weeks of toluene treatment. For example, in the corpus striatum, a region with the highest concentrations of these three neurotransmitters, there were significant increases of DA in the two higher dosed groups and of HVA in the highest dosed group. Concentrations of DA were also significantly increased in the hypothalamus at all three toluene doses. DOPAC showed a significant increase in the medulla oblongata and HVA in the cerebellum at the 100 mg/L dose. The medulla oblongata and the cerebellum are two brain regions containing relatively low concentrations of DOPAC, DA, and HVA. DOPAC also showed a significant increase in the hypothalamus at the 20 and 100 mg/L doses.

Toluene ingestion also induced significant increases in indoleamine neurotransmitters, 5-HT and its major metabolite 5-HIAA (Tables 26 and 27). Remarkable changes were observed in 5-HT concentrations. There were significant increases of 5-HT in all brain regions except the cerebellum where 5-HT occurs in small amounts. Significant increases of 5-HT were found in hypothalamus (100 mg/L), medulla oblongata (100, 500 mg/L), midbrain (20, 100, and 500 mg/L), corpus striatum (100, 500 mg/L), and cortex (20, 100, and 500 mg/L). Concommitant with the observed increases of 5-HT concentrations, concentrations of 5-HIAA also increased, primarily in the 100 mg/L dose groups such that significant increases of this chemical occurred in the hypothalamus, medulla oblongata, and cortex regions of the brain.

The neurotoxic properties of toluene represent the main health hazards (Fishbein, 1985). The study results suggest that toluene has an effect on adrenergic, dopaminergic, and serotonergic pathways. These observations support the hypothesis that physio-chemical properties of toluene may lead to changes in membrane fluidity.

Phenol-induced Immunotoxicity and Neurotoxicity in Mice

Phenol-induced immunotoxicity in mice

Continuous exposure of adult, CD-1 mice to various concentrations of phenol (0, 5, 20, 100 mg/L) via drinking water for 4 weeks had no effect on total food and water consumption or on body weight gain (Table 28). However, 4 weeks of exposure did result in non-significantly decreased spleen, thymus, and liver weights and increased kidney weight (Table 29). No gross lesions were observed on any of the organs of mice in all treatment groups at the time of sacrifice.

The effects of 4 weeks of phenol exposure on spleen cellularity and selected blood parameters are given in Table 30. Total spleen cellularity did not show a significant decrease in any of the treatment groups. Phenol exposure resulted in a dose-dependent decrease in peripheral red blood cell counts and the change was significantly different in all treatment groups when compared to controls. A similar decrease was seen in leucocyte counts, but the decrease was not significant. The change in packed cell volume (PCV) exhibited a significant decrease in the highest dose group (100 mg/L) only. From the differential WBC counts shown in Table 30, it can be seen that there was no treatment-related effect on lymphocytes, neutrophils, and monocytes when compared to the control group.

Table 31 shows the effect of a 4-week phenol exposure on the uptake of [^{3}H]-thymidine by mouse splenic cells. Exposure of mice to phenol resulted in a significant depression of lymphocyte blastogenesis in splenic cell cultures without mitogen at all dose levels tested. There also appeared to be a depressing mitogenesis response to all selected mitogens i.e., LPS (a murine B-cell mitogen), Con A (a T-cell mitogen), PWM (a T- and B-cell mitogen) and PHA (a T-cell mitogen). From the uptake of [^{3}H]-thymidine by mice splenic lymphocyte cultures as shown in Table 31, phenol exposure resulted in a significant suppression of B lymphocyte blastogenesis in all dose groups (LPS) and T lymphocyte blastogenesis in the 20 and 100 mg/L groups (PHA) and in the 5 and 20 mg/L (Con A) groups. A similar trend was also observed with PWM mitogens-stimulated lymphoblastogenesis of splenic lymphocytes in all dose groups.

The effects of various concentrations of phenol exposure via drinking water on T-cell proliferation of mice splenic lymphocytes in response to mixed lymphocyte culture are shown in Table 32. A significant reduction of [^{3}H]-thymidine incorporation occurred in allogeneic cell cultures in all phenol-treated mice splenic cells. Similar responses were found in non-stimulated cultures but there was no significant difference from control. These findings show that phenol depresses T-cell function in response to allogeneic cells.

Results of ^{51}Cr release assays suggest that cell-mediated cytolytic response against tumor cells was not affected by phenol exposure (Table 33).

After 4 weeks of phenol exposure via drinking water, the studies demonstrated that phenol causes a statistically significant immunosuppressive effect at the two highest doses (20, 100 mg/L) by decreasing the development of SRBC (sheep red blood cell)-specific antibody plaque forming cells (PFC) (Table 34). From the ELISA study, the titer of α-SRBC antibodies was decreased significantly in the 20 and 100 mg/L dosed groups and corresponded quite well with the reduction of antibody production assessed by the number of plaque-forming cells. This α-SRBC response is a primary immune response arbitrated by the IgM class antibody.

Phenol-induced neurotoxicity in mice

At the doses administered, mice showed no apparent clinical behavioral effects during the period of these studies. After 4 weeks of phenol exposure via drinking water, the levels of catecholamine and indoleamine neurotransmitters and their major metabolites were measured in six different brain regions. In most cases, phenol ingestion caused a dose-related decreased response (Tables 35, 36, 37 and 38). Significant changes in these selected neurotransmitters occurred in several brain regions. However, the effects were not often exhibited in the lowest dose group (5 mg/L).

The greatest effect of phenol on the major biogenic brain amines was in the hypothalamic region. The concentration of norepinephrine (NE) showed a significant decrease at the two higher dose levels (20, 100 mg/L). VMA, a major metabolite of NE, was influenced moderately by phenol exposure (Table 35). The indoleamine, 5-HT, and its metabolite 5-HIAA were decreased significantly in the high exposure groups [20, 100 mg/L] (Table 37). The hypothalamic region is rich in these four biogenic amines. Significant changes of dopamine and its metabolite, DOPAC, in the hypothalamus were not induced by phenol ingestion (Table 35).

The effects of phenol treatment were also obvious in the corpus striatum region. The levels of DA in this region were decreased significantly in

all treatment groups. The corpus striatum is a brain region rich in DA (Table 36). The 5-HT levels were significantly decreased in the highest treatment dose (Table 38). The concentration of VMA was also significantly decreased in the 100 mg/L group but its parent chemical, NE, was not significantly decreased at this level (Table 36).

The concentration of 5-HT was also significantly influenced by phenol exposure via drinking water in the midbrain and medulla oblongata, the regions containing relatively high amounts of this amine (Table 37, 38). In other brain regions, the levels of these seven selected neurotransmitters were not significantly decreased, except for the concentration of VMA in cortex, and showed a dose-dependent response. The levels of HVA, the last metabolite of DA, were not detected or low in each group in the hypothalamus, medulla oblongata and cerebellum due to the fact that these three brain regions contain relatively low amounts of this chemical.

The results of these studies indicated that phenol exposure via drinking water can induce a decrease in the levels of all these selected neurotransmitters, and the concentration alteration may be responsible for the reported encephalopathic effect of phenol exposure (Windus-Podehl et al., 1983).

Immunotoxic Effects of Benzene (200 mg/L), Toluene (400 mg/L) and a Mixture of Benzene (200 mg/L) + Toluene (400 mg/L) in Mice

Continuous exposure of adult male CD-1 mice to benzene (200 mg/L), toluene (400 mg/L) and a mixture of benzene (200 mg/L) + toluene (400 mg/L) via drinking water for 4 weeks did not produce overt clinical symptoms of toxicity in the experimental animals. Overall, there was no change in food and water consumption. At the termination of the study, no significant increase in body weight gain was observed in the benzene (200 mg/L) only or mixture (200 mg/L benzene + 400 mg/L toluene) treatment groups when compared to controls. However, there was a significant increase in body weight gain in the toluene (400 mg/L) only treatment group when compared to untreated controls. The weight gain in this group was significantly different from that in the mixture group (Table 39). In addition, the exposures resulted in decreased spleen and thymus weights and increased liver and kidney weights in all treatment groups. Thymus weight showed a significant decrease in the benzene (200 mg/L) only treatment group when compared to controls (Table 40).

The effects on selected hematological parameters of exposures to benzene, toluene and a mixture of benzene + toluene are given in Table 41. Erythrocytes decreased significantly in the benzene (200 mg/L) group when compared to controls. In contrast, there was little change in erythrocytes in the toluene (400 mg/L) and mixture (200 mg/L benzene + 400 mg/L toluene) groups when compared to controls. When compared to the mixture group (Table 41), the decrease in erythrocytes in the benzene only treatment group was antagonized by toluene.

Erythrocytes increased significantly in the mixture group when compared to the benzene only treatment group. The blood leukocyte counts and leukocyte differentials also exhibited similar antagonized effects in the above treatment groups. Exposures to mixtures of chemically related substances are frequently encountered. In such cases the balance between different pathways of metabolism for chemicals could be radically changed (Sipes and Gandolfi, 1986).

Toluene has been shown to alleviate benzene-induced hematotoxicity (Andrews et al., 1977). One possible explanation for this phenomenon is that toluene competitively inhibits the biotransformation of benzene (Ikeda et al., 1972; Andrews et al, 1977; Gilmour et al., 1986). However, the toluene only treatment did not have a noticeable effect on the selected blood parameters when compared to control animals.

Table 42 shows the effect of a 4-week exposure on total spleen cellularity and the mitogen-induced splenic lymphocytic proliferations. Following 4 weeks of treatment, the proliferative responses of either mitogen stimulated or non-mitogen stimulated splenic lymphocytes were depressed in the benzene (200 mg/L) and toluene (400 mg/L) groups and increased in the mixture group, but there was no statistically significant difference between the toluene only treatment group when compared to controls. Total spleen cellularity revealed a similar phenomenon, but did not change significantly in any of the treatment groups.

The effects on T-cell proliferation of mice splenic lymphocytes in response to mixed lymphocyte culture (MLC) are shown in Table 43. Inhibition of [^{3}H]-thymidine uptake occurred in allogeneic cell cultures in the benzene (200 mg/L) and toluene (400 mg/L) treatment groups. In contrast, [^{3}H]-thymidine uptake was elevated in the mixture group. This response was also observed in the "responder only" (non-stimulated) culture.

The results presented in Table 44 demonstrate that exposure of mice to benzene (200 mg/L) significantly impaired the capacity of the cytotoxic-T lymphocyte to lyse target cells (YAC-1 tumor cells) after 4 weeks of oral ingestion. However, this response was antagonized by toluene and showed a significant increase in the mixture group. There were no significant depressions of cytotoxic T-lymphocyte activity in the toluene (400 mg/L) group when compared to the control group.

The antibody production response to sheep red blood cell (SRBC) assessed by enumeration of the SRBC-specific plaque-forming cells (PFC) and the alterations of α-SRBC antibody titers are presented in Table 45. As shown in Table 45, there was a marked change in the ability of treated animals to produce SRBC-specific antibody. The numbers of PFC showed a significant decrease in benzene (200 mg/L) and toluene (400 mg/L) treatment groups and a significant increase in the benzene (200 mg/L) plus toluene (400 mg/L) group. The α-SRBC antibody titer corresponds to changes in numbers of PFC with a significant increase in the benzene (200 mg/L) + toluene (400 mg/L) group.

Immunotoxic Effects of Benzene (200 mg/L), Toluene (100 mg/L) and a Mixture of Benzene (200 mg/L) + Toluene (100 mg/L) in Mice

Continuous exposure of adult CD-1 mice to benzene (200 mg/L), toluene (100 mg/L) and a mixture of benzene (200 mg/L) + toluene (100 mg/L) via drinking water for 28 days was performed to evaluate the immunotoxic effects in CD-1 mice.

There was no significant change in body weight gain during the exposure period in all the treatment groups when compared to the control group (Table 46). Total food and water consumption was not affected in all treatment groups. The relative organ weights for spleen and thymus decreased in the benzene only and mixture treatment groups when compared to control animals. Thymus weight showed statistically significant changes in these treatment groups compared to controls (Table 47). Depression of spleen and thymus weights was not observed in mice exposed to 100 mg/L toluene. No changes in liver and kidney weights were found in all experimental groups (Table 47).

Red blood cell counts were significantly depressed in the benzene (200 mg/L) group when compared to untreated controls. In contrast, a significant increase in RBC counts was found in the mixture group when compared to the benzene only treatment group (Table 48). No differences in spleen cellularities were detected except in the group treated with 200 mg/L benzene, but this depression was not statistically different from the control group (Table 48).

Table 49 shows the effects of exposures to benzene (200 mg/L), toluene (100 mg/L) and a mixture of benzene (200 mg/L) + toluene (100 mg/L) on splenic lymphocyte proliferations to the various mitogens. When compared to the untreated controls, the DNA synthesis (cell proliferations) of splenic cells was decreased in either non-mitogen stimulation or mitogen stimulation in all treatment groups and showed a significant depression in the 200 mg/L benzene (LPS, Con A), 100 mg/L toluene (LPS), and the mixture groups (LPS, Con A). However, no interaction effects on mitogenic responses were found between the mixture and benzene or toluene treatment only groups.

The results of mixed lymphocyte responses are shown in Table 50. A significant depression in mixed lymphocyte responses was observed only in the studies of responder-to-stimulator ratio at 2:1 and 4:1 in all treatment groups when compared to controls. There was also no interaction effects observed in the mixture group when compared to the benzene or toluene only treatment groups.

In conclusion, our immunotoxic studies of benzene and toluene interactions in CD-1 mice have shown that a high dose of toluene (400 mg/L) has remarkable alteration effects on the immunotoxicity of benzene at a 200 mg/L concentration when administered in combination to adult, male CD-1 mice via continuous ingestion of drinking water. The same alteration effects were not observed with the low dose of toluene (100 mg/L) in combination with benzene (200 mg/L).

Neurotoxic Effects of Benzene (200 mg/L), Toluene (400 mg/L) and a Mixture of Benzene (200 mg/L) + Toluene (400 mg/L) in Mice

After 4 weeks of oral exposure via drinking water at the doses employed, no clinical behavioral signs were observed in any of the treatment groups including those animals exposed to a mixture of benzene (200 mg/l) and toluene (400 mg/L).

In mice exposed to either benzene (200 mg/L), toluene (400 mg/L), or a mixture of benzene (200 mg/L) + toluene (400 mg/L), increased levels of all selected neurotransmitters and their major metabolites were detected in all brain regions of every treatment group when compared to the untreated controls. In most cases statistically significant increases of the major biogenic amines

studied were more often found in the various brain regions analyzed than their metabolites (Tables 51, 52, 53 and 54).

NE levels showed a significant increase in hypothalamus and medulla oblongata in all treatment groups, cerebral cortex (benzene 200 mg/L), midbrain (benzene 200 mg/L) and cerebellum (benzene 200 mg/L + toluene 400 mg/L). Significant increases of DA concentrations occurred in hypothalamus (benzene 200 mg/l, benzene 200 mg/L + toluene 400 mg/L), cerebral cortex (toluene 400 mg/L) and corpus striatum (benzene 200 mg/L).

Significant increases of 5-HT levels were observed in hypothalamus in all treatment groups, cerebral cortex (benzene 200 mg/L, toluene 400 mg/L), medulla oblongata (benzene 200 mg/L), corpus striatum (benzene 200 mg/L), and midbrain (benzene 200 mg/L, toluene 400 mg/L).

Concommitantly with the increases of these parent neurochemicals, levels of each of their major metabolites also increased significantly in several brain regions. For example, there was a significant increase of VMA in medulla oblongata (toluene 400 mg/L, benzene 200 mg/L + toluene 400 mg/L), DOPAC in corpus striatum (benzene 200 mg/L + toluene 400 mg/L), and HVA in hypothalamus (benzene 200 mg/L, benzene 200 mg/L + toluene 400 mg/L), corpus striatum (benzene 200 mg/L, benzene 200 mg/L + toluene 400 mg/L), midbrain (benzene 200 mg/L) while the levels of 5-HIAA were found significantly increased in hypothalamus in all treatment groups and in midbrain (benzene 200 mg/L, benzene 200 mg/L + toluene 400 mg/L).

When compared to the benzene (200 mg/L) or toluene (400 mg/L) only treatment groups, significant differences in levels of selected neurotransmitters were not often found in the mixture (200 mg/L benzene + 400 mg/L toluene) treatment group in the present studies. However, levels of VMA were significantly different from those in benzene (200 mg/L) only treatment in medulla oblongata and in midbrain. DOPAC levels also showed significant differences from those in benzene (200 mg/L) only treatment in corpus striatum.

Neurotoxic Effects of Benzene (200 mg/L), Toluene (100 mg/L), and a Mixture of Benzene (200 mg/L) + Toluene (100 mg/L) in Mice

Benzene (200 mg/L), toluene (100 mg/L) and a mixture of benzene (200 mg/L) + toluene (100 mg/L) was continuously administered to adult CD-1 male mice via drinking water for 28 days. The catecholamines, indoleamine and their major metabolites in six brain regions were analyzed by HPLC to evaluate the neurotoxic effects.

Similar to the exposure studies of benzene (200 mg/L) + toluene (400 mg/L), the mice exposed to either benzene (200 mg/L), toluene (100 mg/L) or a mixture of benzene (200 mg/L) + toluene (100 mg/L) showed increased levels of brain biogenic amines and their major metabolites in all brain regions studied when compared to the untreated controls (Tables 55, 56, 57, and 58).

NE levels showed a significant increase in hypothalamus (toluene 100 mg/L), medulla oblongata (benzene 200 mg/L), corpus striatum (toluene 100 mg/L, benzene 200 mg/L + toluene 100 mg/L) and midbrain (benzene 200 mg/L). Significant increases of DA were observed in hypothalamus (toluene 100 mg/L) and in corpus striatum (benzene 200 mg/L + toluene 100 mg/L). The 5-HT levels in medulla oblongata (benzene 200 mg/L, toluene 100 mg/L) and in midbrain (benzene 200 mg/L) also increased significantly.

The major metabolites of selected neurotransmitters increased in several brain regions: 5-HIAA in hypothalamus (benzene 200 mg/L), cerebral cortex (toluene 100 mg/L, benzene 200 mg/L + toluene 100 mg/L) and medulla oblongata (toluene 100 mg/L); DOPAC in cerebral cortex (toluene 100 mg/L), medulla oblongata (benzene 200 mg/L + toluene 100 mg/L), and corpus striatum (toluene 100 mg/L, benzene 200 mg/L + toluene 100 mg/L); and HVA in cerebral cortex (toluene 100 mg/L, benzene 200 mg/L + toluene 100 mg/L), medulla oblongata (all treatment groups) and corpus striatum (all treatment groups).

Significant differences between benzene or toluene treatment alone and the mixture exposure group, as with the results of interaction studies of benzene (200 mg/L) + toluene (400 mg/L), were not often detected. Only the concentration of 5-HIAA in hypothalamus and HVA in corpus striatum of mixture group were found significantly different from the benzene only treatment.

The neurotoxic studies of benzene and toluene interactions in CD-1 mice have shown that neither a high dose of toluene (400 mg/L) nor a low dose of toluene (100 mg/L) significantly alters brain regional biogenic amines induced by benzene at 200 mg/L.

Summary and Conclusions

Groundwater is an important source of drinking water for approximately one-half of the U.S. population. Numerous underground storage tanks containing petroleum products may be leaking and contaminating public water supply wells across the U.S. Contamination of well water by petroleum products from LUST is a matter of great importance. LUST pose a serious threat to the groundwater and public health. Many petroleum products are known or suspected carcinogens or mutagens which can cause adverse health effects. More research on the types and concentrations of petroleum products occurring in public drinking water well supplies, and the immunotoxic and neurotoxic effects of these organic compounds is needed.

Well water samples from five U. S. Geological Survey shallow monitoring wells and six deep drinking water wells in the vicinity of petroleum refineries, gasoline stations, or other activities (e.g., public works, steel manufacturing), were collected during this project (9/9/85 to 5/7/87). The volatile or semivolatile organic compounds detected in the shallow well water samples by GC or GC/MS analysis varied in concentration, µg/L (Tables 3 and 4). In addition to benzene, toluene, ethylbenzene, and o-xylene which are chemical constituents of gasoline, numerous other organic compounds were detected by GC/MS analysis (Table 5).

Benzene, toluene, and phenol are major aromatic hydrocarbon compounds with environmental and occupational significance (Hsieh, 1988). The basic immunotoxic and neurotoxic effects of these chemicals were evaluated in this study by utilizing various immunological and neurological endocrine-biochemical parameters in CD-1 mice continuously exposed to nominal concentrations of 0, 40, 200, and 1,000 mg/L benzene, 0, 20, 100, and 500 mg/L toluene and 0, 5, 20, and 100 mg/L phenol in drinking water for 4 weeks. The observed concentrations for benzene and toluene as determined by gas chromatography were 0, 31, 166, and 790 mg/L, and 0, 17, 80, and 405 mg/L, respectively. The observed concentrations for phenol were approximately 95 percent of the nominal concentrations (0, 4.75, 19, and 95 mg/L) used.

Benzene exposure caused a dose-dependent reduction in numbers of peripheral blood leukocytes, lymphocytes, erythrocytes and resulted in a severe macrocytic anemia. Splenic lymphocyte proliferation to both B cell and T cell mitogens (lipopolysaccharide, pokeweed mitogen, concanavalin A, and phytohemagglutinin) was followed by a dose-related biphasic responsiveness, enhanced at the lowest dose (40 mg/L) and depressed in the higher dosage groups (200 and 1000 mg/L). Cell-mediated immunity as measured by mixed-lymphocyte culture response to allogeneic cells and cytotoxic lymphocyte activity to YAC-1 tumor cells exhibited similar biphasic phenomenon. Antibody production as assessed by enumeration of sheep red blood cell (SRBC)-specific plaque-forming cells (PFC) indicated a significant suppression of PFC in animals exposed to 200 and 1000 mg/L benzene. A decrease in anti-SRBC antibody titer corresponded to the numbers of PFC. The findings indicate that oral ingestion of benzene produced a biologically significant alteration in both humoral and cellular immune responses.

Benzene also induced both synthesis and catabolism of regional brain monoamine neurotransmitters. In the hypothalamus, the brain region richest in NE, concentrations of NE increased by 50, 58, and 61 percent when mice received doses of 40, 200, and 1000 mg/L benzene, respectively. Significant increases of NE were also observed in the medulla oblongata and cerebellum. Dopamine concentrations increased significantly in the hypothalamus and corpus striatum. Increases of catecholamine metabolites were seen in a number of brain regions: midbrain (DOPAC), corpus striatum (VMA, DOPAC, HVA), cerebral cortex (VMA) and cerebellum (VMA). Benzene treatment significantly increased 5-HT concentrations in the hypothalamus, corpus striatum, midbrain, cerebral cortex and medulla oblongata. Concommitant with increases of 5-HT, 5-HIAA increased in hypothalamus, corpus striatum, midbrain, cerebral cortex and medulla oblongata.

Toluene is not as strong an immunotoxicant as its analogue benzene. No effects on hematological parameters, including erythrocytes, leukocytes and their differentials were noticed. Splenocyte lymphoproliferations to alloantigens decreased at the 500 mg/L toluene dose only. Numbers of SRBC-specific PFC decreased in the 500 mg/L dosed animals, however, no significant change was observed in anti-SRBC antibody level. Toluene (500 mg/L) also adversely affected IL- 2 synthesis. It appeared that suppression of immune

functions of mice ingesting toluene was generally evident at relatively high doses, except for splenic lymphocyte responses to selected mitogens.

The maximum toluene-induced increases in brain biogenic amines and their metabolites occurred at a toluene concentration of 100 mg/L. In the hypothalamus, the concentrations of NE significantly increased by 51, 53, and 34 percent in groups dosed with 20, 100, and 500 mg/L toluene, respectively. Increases of NE were also observed in the medulla oblongata and midbrain. Correspondingly, concentrations of VMA increased in various brain regions.

Concentrations of DA were higher in the corpus striatum and hypothalamus. Alterations in levels of the DA metabolites, DOPAC and HVA, were marginal. Toluene increased 5-HT in all dissected brain regions except cerebellum, and 5-HIAA levels in the hypothalamus, corpus striatum and cerebral cortex. Noradrenergic and serotonergic neurons appeared to be highly vulnerable to toluene while dopaminergic neurons were rather resistant.

The concentrations of hypothalamic NE and VMA, plasma ACTH and serum corticosterone were increased following both chemical exposures.

Comparisons of benzene-treated mice with appropriate-time-controls revealed that corticosterone levels were significantly higher in mice after 7 days (200 and 1000 mg/L benzene) and at 28 days (1000 mg/L of benzene). Toluene elevated corticosterone levels at 14 and 28 days at the 500 mg/L exposure. IL-2 production was suppressed in the two higher benzene-treated groups, while toluene decreased IL-2 synthesis at the 500 mg/L concentration only.

Results indicated that both benzene and toluene ingestion stimulated the hypothalamic-pituitary-adrenocortical (HPA) axis, resulting in elevation of corticosterone which has been reported to inhibit IL-2 production and impair immunocompetence.

Phenol exposure, like the benzene exposure, caused a dose-dependent decrease in peripheral erythrocytes and leucocytes. Splenic lymphocyte proliferation to both B cell and T cell mitogens (LPS, PHA, Con A and PWM) also resulted in a significant suppression in the higher dosage groups (20 and 100 mg/L). Cell-mediated immunity as measured by mixed-lymphocyte culture response to allogeneic cells exhibited a biphasic response enhanced at the lowest dose (5 mg/L) and depressed in the higher dosage groups (20 and 100 mg/L). Phenol depresses T-cell function in response to allogeneic cells. Antibody production as assessed by enumeration of sheep red blood cell (SRBC)-specific antibody plaque-forming cells (PFC) showed a significant immunosuppression of PFC in animals exposed to 20 and 100 mg/L phenol.

Phenol caused a dose-related decrease in regional brain monoamine neurotransmitters in several brain regions. The greatest effect of phenol on the major biogenic brain amines was in the hypothalamic region. NE showed a significant decrease at the two highest dose levels (20 and 100 mg/L). Likewise, the indoleamine, 5-HT, and its metabolite 5-HIAA were significantly decreased in the high exposure groups.

In the corpus striatum, a brain region which is rich in DA, levels of this neurotransmitter were significantly decreased in all treatment groups as were 5-HT and VMA levels in the highest treatment group (100 mg/L). Phenol exposure via drinking water can induce a decrease in the levels of selected neurotransmitters and the concentration alteration may be responsible for the reported encephalopathic effect of phenol exposure.

The interactions of benzene and toluene were also evaluated in this study by utilizing various immunological and neuroendocrino-biochemical parameters in CD-1 mice continuously exposed to 0, 40, 200, and 1000 mg/L benzene and 0, 20, 100, and 500 mg/L toluene, respectively, in drinking water for 4 weeks. The toxicity of environmental pollutants such as benzene and toluene may be expressed as combined effects of the chemicals. Benzene frequently occurs in a co-contaminated environment with toluene, a competitive inhibitor of the biotransformation of benzene. When co-administered with benzene (200 mg/L), toluene (400 mg) completely inhibited benzene-induced immunotoxicity, i.e., involution of thymic mass and suppressions of both B- and T-cell mitogeneses, mixed lymphocyte culture response to alloantigens, the ability of cytotoxic lymphocytes to lyse tumor cells, antibody production response to SRBC, and IL-2 secretion by Con A-stimulated mouse T-cells. However, the low dose of toluene (100 mg/L) did not protect against benzene-induced depressions of immune function. The results demonstrate that toluene, in sufficient amounts, has an antagonistic effect on benzene immunotoxicity. When compared to the untreated controls, mice given the combined treatments of benzene and toluene exhibited elevated regional concentrations of brain amines and their metabolites in several brain regions. Increased concentrations of biogenic amine metabolites in a few brain regions were greater in the combined exposures of

benzene and toluene than when either chemical was used alone; there was a marginal additive effect between these two types of neurotoxicants.

The present study demonstrates that benzene, toluene, and phenol possess the potential for inducing immunotoxic and neurotoxic effects in CD-1 mice following 4 weeks of continuous exposure via drinking water. Of particular importance was the fact that neurotoxic effects were evident at the lowest dose of benzene or toluene treatment, and a no-effect level could not be established. Further studies involving lower levels of exposure of environmental pollutants are necessary to fully ascertain the safety of these compounds in drinking water. The results of combination studies suggest that benzene immunotoxicity is mainly due to action of the reactive metabolites, whereas benzene neurotoxicity results from benzene itself. In addition, both benzene and toluene have, at least partially, an additive negative effect on immune function via the activated HPA axis.

Application of Research Results

Since this research project was approved and initiated on June 25, 1985, several agencies have expressed an interest in receiving data on the characterization and concentrations of organic chemicals detected in well water. The Davis County and South Salt Lake City Health Departments and the Bureau of Public Water Supplies, Division of Environmental Health, State of Utah Department of Health, would like to use the results to assist them in development of groundwater quality standards, to help pinpoint leaking underground storage tanks and to support efforts for protection of groundwater drinking water supplies and public health. Others who could make use of data produced by this project include the USGS, USEPA, Utah Bureau of Solid and Hazardous Waste, petroleum refining industries, and universities, among others.

Although there is a large amount of information available detailing the effects of benzene on the hematopoietic system, studies concerning the immunotoxic evaluations of this chemical are scant. Also, little information is available regarding the effects of toluene exposure on immune systems. In addition, despite several published studies about the positive effects of benzene and toluene on behavioral changes, research conducted on the neurotoxic potential, (i.e., alterations in brain neurochemicals), is still limited. Little research has been done on the chronic effects of long-term exposure to trace levels of organic chemicals detected in drinking water (Varma et al., 1976). The research results will provide toxicologists and other researchers with more information for better understanding the mechanisms and rates at which petroleum compounds impact human health, especially long-term health effects (i.e., immunotoxic and neurotoxic) of exposure to low levels of these organic compounds in drinking water obtained from contaminated public well water supplies.

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Appendix A

TABLE 1. Temperature, pH, and Conductance Values Of Well Water Collected from USGS Shallow Monitoring Wells During the September 9, 1985 to April 24, 1987 Sampling Period.

Shallow Well Water Samples†	Sampling Dates												
	1985				1986				1987				
	9-9	12-10	12-16	12-17	2-6*	3-3	6-10	10-31	2-3	2-14	2-20	3-16	4-24
Sample 1													
Temperature(°C)††	17.0	10.0			6.8	17.0	12.8						
pH*	7.8	7.8			6.6	7.8	7.4						
Conductance** (µmhos/cm)	4717	5105			-	4104	-						
Sample 2													
Temperature(°C)††	18.0		13.0		10.6	10.5	15.7						16.0
pH*	7.0		6.6		6.6	6.9	6.9						7.23
Conductance** (µmhos/cm)	1546		2306		-	2277	-						-
Sample 3													
Temperature(°C)††	16.0			11.0	9.7	12.0	13.3	13.9	10.4		10.0	10.6	
pH*	6.8			7.4	6.5	6.6	6.9	5.5	6.9		7.0	7.0	
Conductance** (µmhos/cm)	1339			1662	-	1567	-	-	-		-	-	
Sample 4													
Temperature(°C)††								16.7	10.4	8.5		10.7	
pH*								5.5	7.1	6.8		7.2	
Conductance** (µmhos/cm)								-	-	-		-	
Sample 5													
Temperature(°C)††										11.6			
pH*										7.6***			
Conductance** (µmhos/cm)										-			

† Well water sample number: Sample 1 collected at a private residence at 3138 N. 2450 W., North Salt Lake City, Utah; Sample 2 at a private residence at 5549 South Redwood Road near Murray, Utah; Sample 3 at the Sandy Public Works Dept. at 8775 S. 700 W., Sandy, Utah; Sample 4 at 7000 S. 2000 E., Salt Lake City, Utah, just north of a Sinclair gas station; and, Sample 5 at the La Manns Apartments at 4500 S. 945 E., Salt Lake City, Utah.

†† Temperature measured with a dry bulb thermometer, or an Orion SA 250 meter.

* pH measured with Hydrion paper or an Orion SA 250 meter

** Conductance measured with YSI Model 33 S-C-T meter and corrected for condition at 25° C.

*** Sediment masked color development.

TABLE 2. Temperature, pH, and Conductance Values Of Well Water Collected from Deep Wells During the November 8, 1985 to May 7, 1987 Sampling Period.

Deep Well Water Samples†	Sampling Dates									
	1985	1986							1987	
	11-8	2-1	2-2	2-6	5-31	6-1	6-5	6-10	4-24	5-7
Sample 6										
Temperature(oC)††	15.0		13.5			19.4				
pH*	6.6		6.5			6.7				
Conductance** (µmhos/cm)	–		–			–				
Sample 7										
Temperature(°C)††	15.6		13.4			19.2				
pH*	6.5		6.5			6.6				
Conductance** (µmhos/cm)	–		–			–				
Sample 8										
Temperature(°C)††		13.7			16.1					
pH*		6.5			6.6					
Conductance** (µmhos/cm)		–			–					
Sample 9										
Temperature(°C)††				12.8		14.4			17.2	
pH*				6.2		7.4			7.1	
Conductance** (µmhos/cm)				–		–			1127	
Sample 10										
Temperature(°C)††				13.6			14.2			17.0
pH*				6.5			6.9			7.3
Conductance** (µmhos/cm)				–		–				1179
Sample 11										
Temperature(°C)††				16.7				19.4		
pH*				6.2				6.6		
Conductance** (µmhos/cm)				–				–		

† Well water sample number: Sample 6 and 7 were collected at private residences at Pelican Point, Utah, west side of Utah Lake; Sample 8 at dairy farm, 17 Vineyard, S. of U.S. Steel--Geneva Works, Orem, Utah; Samples 9 and 10 from pasture west of Crysen Oil Refinery, 2355 S. 1100 W., Woods Cross, Utah; and Sample 11 at a private residence at 3215 N. 2450 W., North Salt Lake City, Utah.

†† Temperature measured with a dry bulb thermometer, or an Orion SA 250 meter.

* pH measured with Hydrion paper or an Orion SA 250 meter.

** Conductance measured with YSI Model 33 S-C-T meter and corrected for condition at 25°C.

TABLE 3. Volatile Organic Compounds Detected by GC Analysis in Water Collected From Shallow Monitoring Wells Numer 1,2,3,4 and 5†.

	COMPOUND CONCENTRATION (µg/L)									
	Well Number 1		Well Number 2							
Compound	9/9/85 P&T	9/9/85 MCE	9/9/85 P&T	9/9/85 MCE	2/6/86 P&T	2/6/86 MCE	6/10/86 P&T	6/10/86 MCE	4/24/87 P&T	4/24/87 MCE
Benzene	0.05	5.95							17.98	
Toulene						1.13			0.32	
Ethylbenzene										
p-Xylene										
1,3,5-Trimethylbenzene										
1,2,3,4-Tetramethylbenzene						9.57				
1,2,4,5-Tetramethylbenzene									0.90	
1-Methylnaphthalene						4.96				

	COMPOUND CONCENTRATION (µg/L)													
	Well Number 3													
Compound	9/9/85 P&T	9/9/85 MCE	2/6/86 P&T	2/6/86 MCE	6/10/86 P&T	6/10/87 MCE	2/3/87 P&T	2/3/87 MCE	2/20/87 P&T	2/20/87 MCE	3/16/87 P&T	3/16/87 MCE	5/7/87 P&T	5/7/87 MCE
Benzene	34.00	13.70	31.01	4.82	93.29		112.27		122.01	1.83	71.43	8.87	0.81	
Toulene			4.19	1.87			8.67		23.47		4.01	8.70		
Ethylbenzene			53.17	53.23	101.27	17.89	96.43	36.21	74.36	20.98	83.15	44.93		
p-Xylene			7.79	0.48			25.26		26.99		13.67			
m-Xylene									0.29					
o-Xylene									0.31		2.58			
1,3,5-Trimethylbenzene			12.23						55.55	15.22	62.45	19.34		
1,2,3,4-Tetramethylbenzene			5.83				7.03		8.90		9.49			
1,2,4,5-Tetramethylbenzene	8.92	1.94			1.27		5.48		13.96		10.46			
1-Methylnaphthalene														
Naphthalene							5.18		4.14					

TABLE 3. Volatile Organic Compounds Detected GC Analysis in Water Collected from Shallow Monitoring Wells Number 1,2,3,4 and 5† (cont.).

Compound	COMPOUND CONCENTRATION (µg/L)							
	Well Number 4						Well Number 5	
	2/3/87 P&T	2/3/87 MCE	2/14/87 P&T	2/14/87 MCE	3/16/87 P&T	3/16/87 MCE	2/14/87 P&T	2/14/87 MCE
Benzene			97.58		18.23		196.83	
Toulene			57.70		3.53		122.33	
Ethylbenzene			2.13				5.55	
p-Xylene			6.50				14.54	
o-Xylene			0.81				3.08	
m-Xylene			2.49				8.36	
1,3,5-Trimethylbenzene			2.68				6.70	
1,2,3,4-Tetramethylbenzene								
1,2,4,5-Tetramethylbenzene			2.66				6.90	
1-Methylnaphthalene								
Naphthalene			3.36				4.81	

† Well water sample number: Well number 1 water sample collected at a private residence at 3138 N. 2450 W., North Salt Lake City, Utah; Well number 2 water sample collected at a private residence at 5549 South Redwood Road near Murray, Utah; Well number 3 water sample collected at the Sandy Public Works Dept. at 8775 S. 700 W., Sandy, Utah; Well number 4 water sample collected at 7000 S. 2000 E., Salt Lake City, Utah, just north of a Sinclair gas station; and Well number 5 water sample collected at the La Manns Apartments at 4500 S. 945 E., Salt Lake City, Utah.

TABLE 4. Organic Compounds Quantified by GC/MS Analysis in Water Collected from Shallow Monitoring Wells Number 3 and 4.

	COMPOUND CONCENTRATION (µg/L)							
	Well Number 3							Well Number 4
Compound	2/3/87 MCE	2/3/87 MCE	2/20/87 MCE	3/16/87 MCE	3/16/87 MCE	5/7/87 P&T	5/7/87 P&T	2/3/87 MCE
1,2,3,4-Tetramethylbenzene	11.58	12.68					29.84	
1,2,3,5-Tetramethylbenzene	15.32	16.91						
1,2,3-Trimethylbenzene							5.78	
1,2,4-Trimethylbenzene							15.45	
1-Ethyl-2-methylbenzene	3.67	6.26				0.33		
1-Ethyl-4-methylbenzene							0.33	
1-Ethylnaphthalene						0.01		
1-Methylnaphthalene	2.31	3.28				4.58		
2,3-Dihydro-1H-indene	1.79		45.01	39.73	42.23	1.20	1.47	
Benzene						119.5	34.86	
Diethylbenzene	0.06	0.48	0.58				5.13	
Ethylbenzene	10.69	12.37	161.70	273.70	21.63		1.19	
Ethylmethylbenzene			56.24	43.21	39.89			
Naphthalene	13.61	16.43				8.20		
Propylbenzene	0.12	0.13	1.04	1.40	1.05	0.01	0.11	
Toluene	2.89	3.56					0.38	1.87
Trimethylbenzene	51.69	55.02	494.40	676.10	738.80			0.83
Xylene			33.12				1.02	

† Well water sample number: Well number 3 water sample collected at the Sandy Public Works Dpt. at 8775 S. 700 W., Sandy, Utah; and Well number 4 water sample collected at 7000 S. 2000 E., Salt Lake City, Utah, just north of a Sinclair gas station.

TABLE 5. Compounds Detected by GC/MS analysis of Shallow Monitoring Well Water Samples Analyzed in the Study.

Extraction/Concentration Methods	
Purge and Trap	Methylene Chloride Extract
(1-Methylethyl)benzene	(1,1-Dimethyl-2-propenyl)benzene
1,1,1-Trichloroethane	(2-Methyl-2-propenyl)benzene
1,2,3,4-Tetrahydronaphthalene	(2-Propenyloxy)-benzene
1,2,3,4-Tetramethylbenzene	1,3,5-Trimethylbenzene
1,2,3,5-Tetramethylbenzene	1-Ethenyl-2-methylbenzene
1,2,3-Trimethylbenzene	1-Ethenyl-3,5-dimethylbenzene
1,2,4-Trimethylbenzene	1-Ethenyl-3-ethylbenzene
1,3-Diethylbenzene	1-Methyl-2-(2-propenyl)benzene
1-Ethyl-2-methylbenzene	1-Methylnaphthalene
1-Ethyl-4-(1-methylethyl)benzene	1-Ethyl-2-methylbenzene
1-Ethyl-4-methylbenzene	1H-Imidizole-2-carboxaldehyde
1-Ethylidene-1H-indene	2-Chlorocyclohexanol
1-Ethylnaphthalene	2,3-Dihydro-1,1,3-trimethyl-3-phenyl-1H-indene
1-Methyl-2-propyl-benzene	2,3-Dihydro-1-methyl-1H-indene
1-Methyl-4-(1-methylethyl)benzene	2,3-Dihydro-1H-indene-1-one
1-Methyl-4-propylbenzene	2,3-Dihydro-1H-indene
1-Methylnaphthalene	2,3-Dihydro-2-methyl-1H-indene
1-Pentylcyclohexane	2-Ethyl-1,4-dimethylbenzene
2,3-Dihydro-1,3-dimethyl-1H-indene	2-[(Phenylmethyl)amino]ethanol
2,3-Dihydro-1-methyl-1H-indene	3-Methylundecene
2,3-Dihydro-1H-indene	Benzo[B]thiophene
2,3-Dihydro-4,6-dimethyl-1H-indene	Bis(2-ethylhexyl)phthalate
2-Chloro-1-phenylethanone	Dibutylphthalate
2-Ethyl-1,3-dimethylbenzene	Diethylbenzene
2-Ethyl-1,4-dimethylbenzene	Dimethylethylbenzene
3,4-Dimethyl-1-hexane	Ethylbenzene
5-Methyl,(Z)-4-undecene	Ethylmethylbenzene
Benzaldehyde	Iso-propylbenzene
Benzene	Methylpropylbenzene
Dichloromethane	Methylundecene
Diethylbenzene	Naphthalene
Ethylbenzene	Propylbenzene
Methylenepropanedinitrile	Sulfur dioxide
Naphthalene	Sulfur
Oxybis[dichloro]methane	Tetramethylbenzene
Propylbenzene	Toluene
Toluene	Trimethylbenzene
Trichloromethane	Xylene
Xylene	

TABLE 6. Effect of Benzene Exposure on Body Weight Gain

Concentration in water/mg/l		Dose (mg/kg/day)	Body Weight Gain (gm)[a]
Nominal	Observed		
0	0	0 (control)	7.82± 0.91[a]
40	31	8	7.26 ± 1.68
200	166	40	9.04 ± 1.10
1000	790	180	9.12 ± 1.66

[a]Values are given as mean ± S.D. (n=5).

TABLE 7. Organ and Body Weight of CD-1 Mice Following 4 Weeks of Benzene Exposure[a]

Concentration in water (mg/L)		Doses mg/kg/day	Body Weight (g)[b]		Organ weight (g/100g body weight)[b]			
Nominal	Observed		Day 0	Day 28	Spleen	Liver	Kidney	Thymus
0	0	0	23.20±0.37	31.22±0.62	0.33±0.02	5.57±0.13	1.55±0.06	0.16±0.02
40	31	8	22.68±0.33	29.94±0.98	0.30±0.01	5.46±0.29	1.58±0.08	0.12±0.01
200	166	40	23.74±0.22	32.98±0.65	0.28±0.01	5.86±0.70	1.65±0.10	0.12±0.01
1000	790	180	23.14±0.29	32.26±0.86	0.26±0.01*	5.98±0.20	1.87±0.05*	0.11±0.01

[a]Benzene was administered continuously via drinking water for four weeks.

[b]Values are given as mean ± S.E. (n = 5).

*Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 8. Effect of Benzene Exposure on Spleen Cellularity and Selected Blood Parameters[a].

Dose* (mg/L)	Spleen Cell No. ($x10^{-7}$)	Erythrocytes ($10^{-6}/mm^3$)	Hematocrit (%)	MCV** (fl)	Leukocytes ($10^{-3}/mm^3$)	Leukocyte absolute differentials		
						Lymphocytes ($10^{-2}/mm^3$)	Neutrophils ($10^{-2}/mm^3$)	Others*** ($10^{-2}/mm^3$)
0	7.51±0.40	7.01±0.65	50.30±0.58	74.01±6.03	5.96±0.60	43.75±5.23	10.97±1.33	4.49±0.78
40	7.91±0.62	4.57±0.28[b]	48.80±0.46	108.30± 6.49[b]	4.23±0.07[b]	29.94±0.70[b]	8.49±1.03	3.77±0.54
200	7.50±0.41	3.68±0.65[b]	46.20±0.46[b]	125.18±15.68[b]	4.22±0.57[b]	23.48±3.04[b]	14.60±2.42	3.97±0.92
1000	5.17±0.64[b]	3.43±0.39[b]	45.00±1.22[b]	136.76±12.57[b]	2.87±0.16[b]	16.06±2.02[b]	10.16±0.74	2.47±0.93

[a] Values are given as mean ± S.E. (n = 5).

[b] Significantly ($p < 0.05$) different from control (0 mg/L) values.

* Benzene was administered continuously to CD-1 mice in drinking water for 4 weeks.

**Mean corpuscular volume.

***Including monocytes, eosinophils and basophils.,

TABLE 9. Effect of Benzene Exposure on Splenic Lymphocyte Proliferative Response to Mitogens.

Dose* (mg/L)	Mitogenic responses**				
	None	LPS	PWM	ConA	PHA
0	1.68±0.31[a]	102.04±23.66	7.45±1.90	154.69± 49.33	68.02±18.57
40	3.55±0.89[b]	261.23±25.62[b]	12.92±2.19[b]	384.90±108.61[b]	258.81±88.01[b]
200	1.13±0.13	22.43±3.48[b]	3.25±0.71	29.30± 7.69[b]	16.43±3.94[b]
1000	0.91±0.19	25.44±6.20[b]	3.65±0.72	14.61± 2.66[b]	8.87±1.83[b]

* Benzene was administered continuously to CD-1 mice in drinking water for 4 weeks.

** dpm/10^6 splenic cells ($x10^{-3}$): response evaluated by incorporation of [methyl-^{3}H]-thymidine into day 2 splenocyte cultures for 6 hours pulsing.

[a] Values are given as mean ± S.E. (n=5).

[b] Significantly ($p<0.05$) different from control (0 mg/L) values.

TABLE 10. Effect of Benzene Exposure on Mixed Lymphocyte Response.

Doses* (mg/L)	DPM/10^6 cells ($x10^{-3}$)**	
	Responders	Responders + Simulators
0 (Control)	2.77±0.30	31.49±2.77
40	3.59±0.87	56.24±1.59[a]
200	1.71±0.42	18.77±2.36[a]
1000	1.58±0.18	9.30±0.88[a]

* Benzene was administered continuously to CD-1 mice via drinking water for 4 weeks.

** Responder cells were the syngeneic splenic cells of CD-1 mice and stimulator cells were the allogeneic Yac-1 cells. A ratio of two stimulators to one responder was used, and the stimulator cells were treated with mitomycin-C before addition to the culture. MLC evaluated by incorporation of [methyl-^{3}H] - thymidine into day 3 MLC cultures for 6 hours pulsing. Values are given as mean ±S.E. (n = 5).

[a] Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 11. Effect of Benzene Exposure on Cytotoxic T-Lymphocytes (CTL) Response.

Dose* (mg/L)	% Cytotoxicity[a]	
	50:1**	25:1
0 (Control)	15.72±1.16	15.98±2.98
40	17.94±1.61	23.32±2.05[b]
200	8.77±1.87[b]	11.09±1.71
1000	5.82±0.94[b]	9.17±1.53[b]

* Benzene was administered continuously to CD-1 mice in drinking water for 4 weeks.

** Effector (day 5 MLC lymphocytes) -to - target (^{51}Cr labelled Yac-1 cells) cell ratio.

[a] Values are given as mean ± S.E. (n = 5).

[b] Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 12. Effect of Benzene Exposure on the Antibody Responses to Thymic-Dependent Antigen Sheep Erythrocyte (SRBC)*.

Dose** (mg/Kg/day)	Spleen cell No ($x10^6$)	PFC/10^6 Splenic cells	PFC/totalSpleen cells ($x10^6$)	α-SRBC Antibody Titer
0	22.59±3.02	1,254±17[a]	295.72±74.51	0.44±0.06
8	16.80±1.59[a]	1,576±65	268.80±36.10	0.57±0.11
40	15.36±0.65[a]	643±49[a]	99.76±10.32[a]	0.31±0.05
180	12.70±1.51[a]	229±40[a]	31.41± 9.52[a]	0.21±0.01[a]

* Mice were sensitized with SRBC 4 days before the end of the benzene exposure. Splenic lymphocytes were analyzed for antibody forming cells (plaque-forming cell, PFC) and sera were detected for antibody titer (α-SRBC) determined in sera. Values are given as mean ± S.E. (n = 5).

** Benzene was administered continuously to CD-1 mice in drinking water for four weeks.

[a] Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 13. Effect of Benzene on Regional Brain Catecholamines*.

Catecholamine[b]	Doses[c] (mg/L)	Brain Region[a]		
		Hypothalamus	Medulla Oblongata	Cerebellum
NE	1000	1.63±0.15*	0.71±0.01	0.38±0.03*
	200	1.60±0.22*	0.78±0.03*	0.33±0.02
	40	1.27±0.11	0.69±0.05	0.29±0.03
	0	0.96±0.08	0.61±0.03	0.26±0.05
VMA	1000	0.40±0.03	0.24±0.01	0.28±0.01*
	200	0.37±0.05	0.24±0.01	0.23±0.01*
	40	0.30±0.04	0.24±0.01	0.24±0.02*
	0	0.26±0.02	0.19±0.03	0.19±0.01
DA	1000	0.45±0.05	0.04±0.00	0.03±0.01
	200	0.49±0.11	0.05±0.01	0.03±0.01
	40	0.41±0.05	0.08±0.02	0.04±0.02
	0	0.30±0.05	0.05±0.01	0.04±0.01
DOPAC	1000	0.13±0.02	0.07±0.00	0.07±0.01
	200	0.16±0.02*	0.08±0.01*	0.06±0.01
	40	0.08±0.01	0.08±0.01*	0.05±0.01
	0	0.10±0.02	0.05±0.01	0.04±0.01
HVA	1000	0.07±0.03	0.05±0.00	0.03±0.00
	200	0.11±0.02	0.06±0.01	0.03±0.00
	40	0.13±0.02	0.07±0.01	0.05±0.02
	0	0.09±0.03	0.05±0.01	0.02±0.01

[a] µg brain biogenic amines/g wet tissue (mean ± S.E.)

[b] NE: Norepinephrine; VMA: Vanillylmandelic acid; DA: dopamine; DOPAC: dihydroxyphenylacetic acid; HVA: homovanillic acid.

[c] Doses were administered continuously to CD-1 mice in drinking water daily for 4 weeks.

* Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 14. Effect of Benzene on Regional Brain Catecholamines.

Catecholamine	Doses[c] (mg/L)	Brain Region[a]		
		Midbrain	Corpus Stratium	Cortex
NE	1000	0.72±0.03*	0.34±0.04	0.52±0.02
	200	0.62±0.03*	0.36±0.03	0.72±0.08*
	40	0.65±0.02*	0.27±0.02	0.83±0.20
	0	0.48±0.05	0.33±0.07	0.34±0.05
VMA	1000	0.27±0.03	0.30±0.04*	0.29±0.01*
	200	0.25±0.02	0.29±0.01*	0.35±0.04*
	40	0.29±0.02	0.28±0.03*	0.28±0.03*
	0	0.23±0.03	0.20±0.03	0.17±0.02
DA	1000	0.25±0.01	8.88±0.66	1.11±0.06
	200	0.21±0.02	8.15±0.72	1.64±0.23
	40	0.23±0.03	7.25±0.35	1.62±0.44
	0	0.19±0.01	6.59±0.81	0.87±0.09
DOPAC	1000	0.15±0.01*	1.04±0.09*	0.21±0.01
	200	0.15±0.01*	0.84±0.06	0.30±0.03*
	40	0.15±0.01*	0.71±0.02	0.28±0.05*
	0	0.11±0.01	0.71±0.09	0.14±0.02
HVA	1000	0.18±0.01*	1.04±0.10*	0.22±0.01
	200	0.14±0.01*	0.97±0.09	0.30±0.04*
	40	0.19±0.01*	0.87±0.06	0.29±0.06*
	0	0.11±0.01	0.73±0.08	0.14±0.01

[a] µg brain biogenic amines/g wet tissue (mean ± S.E.).

[b] NE: norepinephrine; VMA: Vanillylmandelic acid; DA: dopamine; DOPAC: dihydroxyphenylacetic acid; HVA: homovanillic acid.

[c] Doses were administered continuously to CD-1 mice in drinking water daily for 4 weeks.

* Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 15. Effect of Benzene on Regional Brain Indoleamines.

Indoleamine[b]	Doses[c] (mg/L)	Brain Region[a]		
		Hypothalamus	Medulla Oblongata	Cerebellum
5-HT	1000	0.81±0.12	0.84±0.03	0.31±0.04
	200	1.03±0.15	0.82±0.03	0.23±0.02
	40	0.88±0.12	0.94±0.05*	0.27±0.03
	0 (Control)	0.63±0.10	0.76±0.05	0.23±0.03
5-HIAA	1000	0.44±0.09	0.34±0.02	0.12±0.02
	200	0.40±0.07	0.29±0.02	0.08±0.00
	40	0.45±0.10	0.35±0.02	0.10±0.02
	0 (Control)	0.26±0.03	0.30±0.02	0.09±0.01

[a] µg brain biogenic amines/g wet tissue (mean ± S.E.).

[b] 5-HT : 5-hydroxytryptamine (serotonin); 5-HIAA: 5-hydroxyindoleacetic acid.

[c] Doses were administered in drinking water daily for 4 weeks.

* Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 16. Effect of Benzene on Regional Brain Indoleamines.

Indoleamine[b]	Doses[c] (mg/L)	Brain Region[a]		
		Midbrain	Corpus Stratium	Cortex
5-HT	1000	1.07±0.02*	0.70±0.06	0.89±0.05
	200	1.04±0.03*	0.80±0.03	1.31±0.17*
	40	1.05±0.06*	0.70±0.04	1.34±0.28*
	0 (Control)	0.81±0.08	0.61±0.08	0.57±0.06
5-HIAA	1000	0.52±0.02*	0.39±0.03	0.26±0.02*
	200	0.40±0.01*	0.40±0.02	0.31±0.04*
	40	0.51±0.03*	0.32±0.03	0.33±0.05*
	0 (Control)	0.33±0.03	0.30±0.05	0.14±0.02

[a] µg brain biogenic amines/g wet tissue (mean ± S.E.).

[b] 5-HT : 5-hydroxytryptamine (serotonin); 5-HIAA: 5-hydroxyindoleacetic acid.

[c] Doses were administered in drinking water daily for 4 weeks.

* Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 17. Effect of Toluene Exposure on Body Weight Gain in CD-1 Mice.

Concentration in water, mg/L		Dose (mg/kg/day)	Body Weight Gain (gm)[a]
Nominal	Observed		
0	0	0 (Control)	14.24 ± 2.52
20	17	5	13.70 ± 1.42
100	80	22	13.02 ± 1.05
500	405	105	15.14 ± 1.59

[a] Values are given as mean ± S.D. (n = 5).

TABLE 18. Organ and Body Weight of Mice Following Toluene Exposure

Dose[a] (mg/L)	Body Weight (g)[b]		Organ Weight (g/100 g body weight)[b]			
	Day 0	Day 28	Spleen	Liver	Kidney	Thymus
0	20.62±0.13	34.86±1.18	0.34±0.03	5.67±0.07	1.62±0.05	0.19±0.02
20	20.56±0.02	34.26±0.64	0.31±0.01	6.09±0.17	1.72±0.06	0.18±0.01
100	20.16±0.31	33.18±0.21	0.33±0.01	6.32±0.17	1.68±0.03	0.18±0.02
500	20.00±0.23	35.12±0.72	0.28±0.01	6.73±0.14*	1.68±0.05	0.13±0.02*

[a] Toluene was administered continuously to CD-1 mice in drinking water for 4 weeks.

[b] Values are given as mean ± S.E. (n = 5).

* Significantly different from control values at $p < 0.05$.

TABLE 19. Effect of Toluene Exposure on Spleen Cellularity and Selected Blood Parameters[a].

Dose[b] (mg/L)	Total Splenocyte (x 10^{-7})	Erythrocyte (10^6/mm^3)	Leukocyte (10^3/mm^3)	Leukocyte absolute differentials		
				Lymphocyte (10^3/mm^3)	Neutrophil (10^3/mm^3)	Other[c] (10^3/mm^3)
0	7.71±0.27[c]	9.30±0.26	5.96±1.02	4.40±0.62	1.13±0.38	0.41±0.08
20	6.18±0.52	9.87±0.49	6.34±0.57	4.46±0.38	1.46±0.39	0.42±0.09
100	6.57±0.91	8.79±0.85	6.43±0.57	4.50±0.78	1.42±0.48	0.50±0.07
500	6.58±0.47	9.38±0.38	3.90±0.36	2.81±0.32	0.82±0.16	0.36±0.05

[a] Values are given as mean ± S.E. (n = 5).

[b] Toluene was administered continously to CD-1 mice in drinking water for 4 weeks.

[c] Including monocytes, eosinophils and basophils.

TABLE 20. Effect of Toluene Exposure on Splenic Lymphocyte Proliferative Response to Mitogens.

Dose* (mg/L)	Mitogenic responses**				
	None	LPS	PWM	ConA	PHA
0	4.30±1.86	369.88±76.57	28.98±13.23	707.93±170.38	141.62± 2.92
20	1.80±0.81[a]	228.33±133.25	9.76± 5.75[a]	268.67±234.72	59.01±42.67
100	1.82±0.89[a]	182.70± 52.44[a]	7.79± 3.87[a]	143.43±109.34[a]	43.31±22.48
b					
500	1.41±0.52[a]	170.33± 67.67[a]	7.34± 1.37[a]	179.04±105.21[a]	39.97±24.05[a]

* Toluene was administered continuously to CD-1 mice in drinking water for 4 weeks.

** dpm/2x10^6 splenic cells (x10^{-3}): response evaluated by incorporation of [methyl- ^{3}H] - thymidine into day 2 splenocyte cultures for 6 hours pulsing. Values are given as mean ± S.D. (n = 5).

[a] Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 21. Effect of Toluene Exposure on Mixed Lymphocyte Response.

Dose* (mg/L)	DPM/10^6 cells (x10^{-3})**	
	Responders	Responders + Stimulators
0	5.20±0.67	28.99± 9.30
20	4.62±0.92	29.18±11.26
100	4.72±2.20	23.30± 7.80
500	2.47±0.42[a]	14.87± 4.79[a]

** Responder cells were the syngeneic splenic cells of CD-1 mice and stimulator cells were the allogeneic Yac-1 cells. A ratio of two stimulators to one responder was used, and the stimulator cells were treated with mitomycin-C before addition to the culture. MLC evaluated by incorporation of [methyl-^{3}H] - thymidine into day 3 MLC cultures for 6 hours pulsing. Values are given as mean ± S.D. (n = 5).

* Toluene was administered continuously to CD-1 mice in drinking water for 4 weeks.

[a] Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 22. Effect of Toluene Exposure on Cytotoxic T-Lymphocytes (CTL) Response.

Dose (mg/L)	%Cytotoxicity[a]	
	50:1[b]	25:1
0	13.74±1.47[b]	16.49±7.45
20	12.09±0.75	13.29±2.47
100	11.19±0.82	12.34±1.31
500	8.67±1.60*	10.30±1.40

[a] Values are given as mean ± S.D. (n = 5).

[b] Effector-to-target cell ratio.

* Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 23. Effect of Toluene Exposure on Antibody Plaque Forming Cells (PFC) and Antibody Titer (α-SRBC)*

Dose** (mg/kg/day)	Total Splenocytes ($x10^{-7}$)	PFC/10^6 Splenocytes	PFC/Total Spleen Cells	α-SRBC Antibody Titer
0	20.16±2.75	1,184± 90	238,545±37,429	0.44±0.04
5	16.68±1.19	985±105	166,015±30,231	0.55±0.06
22	21.78±3.05	973±116	217,891±47,845	0.43±0.05
105	13.66±1.79	631± 27[a]	89,936±13,269[a]	0.32±0.03

* Mice were sensitized with thymic-dependent antigen sheep erythrocyte (SRBC) four days before the end of toluene exposure. Splenocytes were analyzed for antibody plague forming cell and antibody titer (α-SRBC) determined in Sera. Values are given as mean ± S.E. (n = 5).

** Toluene was administered continuously to CD-1 mice in drinking water for four weeks.

[a] Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 24. Effect of Toluene On Regional Brain Catecholamines

Catecholamine[b]	Dose[c] (mg/L)	Brain Region[a] Hypothalamus	Medulla Oblongata	Cerebellum
NE	500	1.76±0.30*	0.78±0.07	0.38±0.04
	100	2.13±0.37*	0.95±0.21*	0.42±0.05
	20	1.98±0.08*	0.70±0.10	0.34±0.03
	0	1.31±0.15	0.66±0.06	0.33±0.09
VMA	500	0.60±0.47	0.30±0.04	0.24±0.02
	100	0.39±0.10	0.36±0.09*	0.27±0.04
	20	0.42±0.03	0.30±0.03	0.29±0.02
	0	0.24±0.04	0.25±0.01	0.25±0.03
)				
DOPAC	500	0.13±0.05	0.03±0.01	0.03±0.01
	100	0.23±0.08*	0.07±0.02*	0.03±0.01
	20	0.18±0.03*	0.04±0.01	0.02±0.00
	0	0.09±0.02	0.04±0.02	0.03±0.01
DA	500	0.68±0.21*	0.10±0.02	0.05±0.01
	100	0.74±0.19*	0.10±0.04	0.04±0.02
	20	0.66±0.14*	0.07±0.03	0.04±0.01
	0	0.40±0.10	0.10±0.01	0.04±0.01
HVA	500	0.11±0.16	0.03±0.04	0.02±0.02*
	100	0.22±0.06	0.10±0.05	0.04±0.01
	20	0.09±0.13	0.03±0.02	0.01±0.02
	0	0.11±0.11	0.06±0.02	0.01±0.02

[a] µg: brain biogenic amines/g wet brain tissue (means ± S.D., n = 5).

[b] NE: norepinephrine, VMA: Vanillylmandelic acid, DOPAC: dihydroxyphenylacetic acid, DA: dopamine, HVA: homovanillic acid.

[c] Doses were administered continously to CD-1 mice in drinking water for 4 weeks.

* Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 25. Effect of Benzene on Regional Brain Catecholamines

Catecholamine[b]	Dose[c] (mg/L)	Brain Region[a] Midbrain	Corpus Striatum	Cortex
NE	500	0.69±0.04*	0.36±0.11	0.43±0.05
	100	0.67±0.06*	0.35±0.03	0.46±0.06
	20	0.66±0.07*	0.37±0.09	0.41±0.06
	0	0.58±0.03	0.32±0.10	0.36±0.04
VMA	500	0.38±0.02*	0.57±0.07*	0.35±0.01*
	100	0.36±0.03*	0.41±0.04*	0.25±0.03
	20	0.32±0.02	0.32±0.04	0.27±0.01
	0	0.30±0.05	0.31±0.02	0.25±0.04
DOPAC	500	0.12±0.02	1.39±0.17	0.18±0.03
	100	0.13±0.04	1.06±0.12	0.19±0.03
	20	0.19±0.11	1.06±0.47	0.16±0.04
	0	0.14±0.02	1.06±0.46	0.19±0.02
DA	500	0.39±0.14	11.65±1.11*	1.13±0.19
	100	0.31±0.09	8.28±1.07*	1.24±0.36
	20	0.78±0.68	7.34±2.09	1.12±0.29
	0	0.27±0.06	6.41±0.76	1.20±0.21
HVA	500	0.13±0.02	1.24±0.17*	0.17±0.04
	100	0.12±0.02	0.95±0.14	0.18±0.05
	20	0.16±0.07	0.79±0.35	0.14±0.03
	0	0.13±0.05	0.83±0.20	0.18±0.04

[a] μg: brain biogenic amines/g wet brain tissue (mean ± S.D., n = 5).

[b] NE: norepinephrine, VMA: Vanillylmandelic acid, DOPAC: dihydroxyphenylacetic acid, DA: dopamine, HVA: homovanillic acid.

[c] Doxes were administered continuously to CD-1 mice in drinking water for 4 weeks.

*Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 26. Effect of Toluene on Regional Brain Indoleamines.

Indoleamine[b]	Doses[c] (mg/L)	Brain Region[a] Hypothalamus	Medulla Oblongata	Cerebellum
5-HT	500	1.00±0.26	0.90±0.10*	0.19±0.06
	100	1.44±0.43*	1.07±0.15*	0.27±0.06
	20	0.94±0.20	0.79±0.07	0.19±0.06
	0	0.70±0.16	0.76±0.05	0.22±0.12
5-HIAA	500	0.49±0.17	0.31±0.07	0.06±0.01
	100	0.86±0.28*	0.43±0.10*	0.11±0.04
	20	0.65±0.15	0.33±0.04	0.08±0.03
	0	0.37±0.07	0.32±0.03	0.09±0.05

[a] µg: brain biogenic amines/g wet brain tissue (mean ± S.D., n = 5).

[b] 5-HT: 5-hydroxytryptamine (serotonin), 5-HIAA: 5-hydroxyindoleacetic acid.

[c] Doses were administered continuously to CD-1 mice in drinking water daily for 4 weeks.

* Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 27. Effect of Toluene on Regional Brain Indoleamines

Indoleamine[b]	Doses[c] (mg/L)	Brain Region[a] Midbrain	Corpus Striatum	Cortex
5-HT	500	0.98±0.04*	0.72±0.08*	0.65±0.01*
	100	1.10±0.16*	0.76±0.09*	0.79±0.11*
	20	1.02±1.10*	0.64±0.11	0.66±0.02*
	0	0.76±0.04	0.53±0.08	0.53±0.03
5-HIAA	500	0.32±0.02	0.40±0.07	0.18±0.02
	100	0.42±0.14	0.42±0.03	0.23±0.06*
	20	0.42±0.05	0.39±0.08	0.19±0.03
	0	0.37±0.01	0.35±0.06	0.17±0.02

[a] µg: brain biogenic amines/g wet brain tissue (mean ± S.D., n = 5).

[b] 5-HT: 5-hydroxytryptamine (serotonin), 5-HIAA: 5-hydroxyindoleacetic acid.

[c] Doses were administered in drinking water daily for 4 weeks.

* Significantly ($p < 0.05$) different from control (0 mg/L) values.

TABLE 28. Effect of Phenol Exposure on Body Weight Gain in CD-1 Mice.

Concentration in water, mg/L[a]			
Nominal	Observed	Dose (mg/kg/day)	Body Weight Gain (gm)[b]
0	0	0 (Control)	9.58 ± 1.04
5	4.75	1.36	10.26 ± 1.46
20	19.0	5.35	9.78 ± 2.03
100	95.0	26.55	10.92 ± 1.84

[a] The observed concentrations for phenol were approximately 95 percent of the nominal concentrations.
[b] Values are given as mean ± S.D. (n = 5).

TABLE 29. Organ and Body Weight of CD-1 Mice Following Phenol Exposure.

		Organ Weight Relative to Body Weight (gm/100gm)[b]			
Dose[a] (mg/L)	Body Weight (gm)	Spleen	Liver	Kidney	Thymus
0	33.66±1.23	0.35±0.04	6.48±0.59	1.56±0.20	0.15±0.01
5	33.54±1.57	0.32±0.07	6.25±0.44	1.76±0.11	0.14±0.04
20	33.68±1.73	0.33±0.04	6.10±0.79	1.68±0.19	0.14±0.03
100	34.86±2.20	0.29±0.03	6.01±0.07	1.71±0.25	0.12±0.02

[a] Phenol was administered continuously to CD-1 mice in drinking water for four weeks.
[b] Values are given as mean ± S.D. (n = 5).

TABLE 30. Effect of Phenol Exposure on Spleen Cellularity and Selected Blood Parameters[a].

Dose[b] (mg/L)	Total spleen Cellularity ($x10^{-7}$)	WBC[c] ($x10^{-3}$)	RBC[c] ($x10^{-3}$)	PCV[d] %	Expressed as % of WBC's		
					Lymphocytes	Neutrophils	Monocytes
0	8.59±0.75	6.06±0.38	7.17±1.24	48.00±1.17	74.20±4.09	17.00±2.24	4.60±1.14
5	7.94±0.44	5.82±1.34	4.90±1.20*	49.10±1.52	71.80±4.60	19.40±1.67	4.80±2.28
20	7.31±0.90	5.05±1.19	4.64±1.69*	48.20±2.77	69.20±7.26	21.80±5.36	4.60±1.82
100	7.26±1.23	5.68±1.54	3.23±1.51*	44.10±1.82*	73.60±5.18	17.00±3.46	6.20±2.59

[a]Values are given as mean ± S.D. (n = 5).
[b]Phenol was administered continuously to CD-1 mice in drinking water for four weeks.
[c]Cell/cu mm.
[d]Packed cell volume
*Significantly ($p<0.05$) different from control (0 mg/L) values

TABLE 31. Effect of Phenol Exposure on the Uptake of [^{3}H]-thymidine by Mice Spleen Cells in Culture.

Dose[a] (mg/L)	DPM/1x10^6 Splenic cells ($x10^{-3}$)[b]				
	Unstimulated Cultures	LPS	PWM	Con A	PHA
0	5.98±2.18	166.26±53.74	23.59±10.44	174.33±120.17	146.16 ± 76.44
5	5.40±1.80	173.08±53.92	22.00 ± 6.06	118.94± 91.72	150.66±100.72
20	5.62±2.48	148.78±78.82	16.06 ± 8.94	79.00 ± 46.10	106.60 ± 31.34
100	3.13±0.80*	89.74±61.58*	8.16 ± 2.99*	95.39 ± 35.96	62.25 ± 42.28*

[a]Phenol was administered continuously to CD-1 mice in drinking water for four weeks.
[b]Values are given as mean ± S.D. (n=5).
*Significant ($p<0.05$) difference from control (0 mg/L) values

TABLE 32. Effect of Phenol Exposure on Mixed Lymphocyte Response.

Dose* (mg/L)	DPM/10^6 cells ($x10^{-3}$)**	
	Responders	Responders + Stimulators
0	7.02±1.96[a]	22.04±6.71
5	4.68±2.29	24.87±5.26
20	3.99±2.89	10.68±0.84[a]
100	4.16±1.01	11.46±3.94[a]

* Phenol was administered continuously to CD-1 mice in drinking water for four weeks.

** Responder cells were the syngeneic splenic cells of CD-1 mice and stimulator cells were the allogeneic Yac-1 cells. A ratio of two stimulators to one responder was used, and the stimulator cells were treated with mitomycin-C before addition to the culture. MLC evaluated by incorporation of [methyl-^{3}H]-thymidine into day 3 MLC cultures for 6 hours pulsing. Values are given as mean ± S. D. (n=5)

[a] Significant ($p<0.05$) difference from control (0 mg/L) values

TABLE 33. Effect of Phenol Exposure on Cell-Mediated Cytolytic Response.

Dose (mg/L)	% Cytoxicity[a]	
	50:1[b]	25:1
0	12.59±1.04	13.84±0.75
5	12.03±2.55	12.30±2.30
20	11.80±1.67	12.20±2.94
100	10.99±1.74	11.23±2.45

[b]Values are given as mean ± S.D. (n=5)

[a]Effector-target cell ratio

TABLE 34. Effect of Phenol Exposure on Antibody Plaque Forming Cells (PFC) and Antibody Titer (α-SRBC)[a].

Dose[b] (mg/L)	PFC/10^6 Splenic Cells	PFC/Total Splenic Cells	α-SRBC Antibody Titer
0	1,123±221	265,947±118,731	0.446±0.086
5	896±135	241,678± 39,129	0.392±0.152
20	795±110*	207,659± 42,184	0.325±0.042*
100	616±186*	130,185± 40,699*	0.263±0.082*

[a]Values are given as mean ± S.D. (n=5)

[b]Phenol was administered continuously to CD-1 mice in drinking water for four weeks.

*Significantly ($p<0.05$) different from control (0 mg/L) values.

TABLE 35. Effect of Phenol on Regional Brain Catecholamines.

Catecholamine[b]	Dose[c] (mg/L)	Brain Regions[a]		
		Hypothalamus	Medulla Oblangata	Cerebellum
NE	100	1.272±0.416*	0.599±0.089	0.287±0.021
	20	1.470±0.403*	0.628±0.085	0.325±0.033
	5	1.878±0.166	0.681±0.065	0.348±0.066
	0	2.089±0.282	0.767±0.147	0.376±0.066
VMA	100	0.351±0.117	0.305±0.029	0.330±0.018
	20	0.402±0.058	0.324±0.047	0.384±0.051
	5	0.420±0.065	0.366±0.053	0.382±0.038
	0	0.492±0.067	0.400±0.074	0.377±0.052
DOPAC	100	0.216±0.081	0.079±0.007	0.047±0.006*
	20	0.255±0.042	0.094±0.027	0.058±0.008
	5	0.268±0.066	0.085±0.019	0.057±0.007
	0	0.310±0.079	0.081±0.010	0.057±0.003
DA	100	0.412±0.158	0.042±0.020	0.020±0.006
	20	0.490±0.154	0.037±0.013	0.024±0.003
	5	0.523±0.124	0.045±0.015	0.027±0.006
	0	0.569±0.098	0.047±0.006	0.032±0.015

[a] µg: Brain biogenic amines/g wet brain tissue (mean ± S.D., n=5). Amounts of HVA were not detected.

[b] NE: norepinephrine, VMA: Vanillylmandelic acid, DOPAC: dihydroxyphenylacetic acid,, DA: dopamine.

[c] Doses were administered continuously to CD-1 mice in drinking water for 4 weeks.

* Significantly ($p<0.05$) different from control (0 ppm) values.

TABLE 36. Effect of Phenol on Regional Brain Catecholamines.

Catecholamine[b]	Dose[c] (mg/L)	Brain Regions[a]		
		Midbrain	Corpus Striatum	Cortex
NE	100	0.560±0.025	0.308±0.068	0.405±0.029
	20	0.570±0.094	0.379±0.045	0.370±0.068
	5	0.656±0.058	0.414±0.088	0.473±0.142
	0	0.608±0.093	0.379±0.127	0.424±0.031
VMA	100	0.318±0.011*	0.298±0.030*	0.326±0.022*
	20	0.352±0.042	0.383±0.056	0.329±0.028*
	5	0.379±0.028	0.414±0.034	0.371±0.012
	0	0.402±0.050	0.398±0.032	0.372±0.007
DOPAC	100	0.161±0.024	0.947±0.153*	0.221±0.008
	20	0.174±0.012	1.148±0.377	0.239±0.023
	5	0.209±0.058	1.000±0.153	0.277±0.059
	0	0.219±0.040	1.326±0.203	0.261±0.018
DA	100	0.275±0.061	6.529±0.950*	1.173±0.180
	20	0.275±0.046	7.257±0.849*	1.310±0.115
	5	0.463±0.205	7.749±1.166*	1.685±0.405
	0	0.320±0.092	9.797±1.887	1.517±0.317
HVA	100	0.105±0.013	0.774±0.146	0.176±0.015
	20	0.109±0.009	0.805±0.049	0.173±0.017
	5	0.131±0.038	0.752±0.128	0.201±0.060
	0	0.136±0.022	0.952±0.142	0.214±0.029

[a] µg: Brain biogenic amines/g wet brain tissue (mean ± S.D., n=5).

[b] NE: norepinephrine, VMA: Vanillylmandelic acid, DOPAC: dihydroxyphenylacetic acid, DA: dopamine, HVA: homovanillic acid.

[c] Doses were administered continuously to CD-1 mice in drinking water for four weeks.

* Significant ($p<0.05$) difference from control (0 mg/L) values.

TABLE 37. Effect of Phenol on Regional Brain Indoleamines.

Indoleamine[b]	Dose[c] (mg/L)	Brain Regions[a]		
		Hypothalamus	Medulla Oblangata	Cerebellum
5-HT	100	0.422±0.119*	0.531±0.079*	0.161±0.050
	20	0.656±0.236*	0.613±0.105	0.193±0.035
	5	0.859±0.114	0.705±0.045	0.207±0.063
	0	0.967±0.087	0.752±0.163	0.243±0.068
5-HIAA	100	0.237±0.061*	0.207±0.031	0.067±0.021
	20	0.342±0.108*	0.247±0.024	0.073±0.013
	5	0.476±0.081	0.258±0.026	0.081±0.021
	0	0.502±0.097	0.272±0.057	0.088±0.026

[a] µg: Brain biogenic amines/g wet brain tissue (mean ± S.D., n=5).

[b] 5-HT: 5-hydroxytryptamine (serotonin), 5-HIAA: 5-hydroxyindoleacetic acid.

[c] Doses were administered continuously to CD-1 mice in drinking water for four weeks.

* Significantly ($p<0.05$) different from control (0 mg/L) values.

TABLE 38. Effect of Phenol on Regional Brain Indoleamines.

Indoleamine[b]	Dose[c] (mg/L)	Brain Regions[a]		
		Midbrain	Corpus Striatum	Cortex
5-HT	100	0.722±0.059*	0.457±0.054*	0.591±0.050
	20	0.824±0.154	0.627±0.082	0.611±0.052
	5	0.994±0.085	0.735±0.140	0.797±0.186
	0	0.890±0.037	0.669±0.052	0.707±0.039
5-HIAA	100	0.320±0.060	0.257±0.036	0.185±0.020
	20	0.332±0.069	0.358±0.045	0.167±0.015
	5	0.411±0.055	0.390±0.097	0.213±0.067
	0	0.384±0.023	0.344±0.066	0.197±0.016

[a] µg: Brain biogenic amines/g wet brain tissue (mean ± S.D., n=5).

[b] 5-HT: hydroxytryptamine (serotonin, 5-HIAA: 5-hydroxyindoleacetic acid.

[c] Doses were administered continuously to CD-1 mice in drinking water for four weeks

* Significantly ($p<0.05$) different from control (0 mg/L) values.

TABLE 39. Body Weights of Mice Following 4 Weeks of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), alone or Combined.

Concentration in Water (mg/L)	Body Weight (g)[a]		
	Day 0	Day 28	Gain
Control (0)	27.02 ± 0.73	34.36 ± 0.78	7.34 ± 0.27
Benzene (200)	27.02 ± 0.56	35.70 ± 1.01	8.68 ± 0.61
Toluene (400)	27.46 ± 0.48	37.32 ± 1.34	9.86 ± 0.90[b]
Benzene (200) + Toluene (400)	26.78 ± 0.37	34.22 ± 0.39	7.44 ±0.43[c]

[a] Values are given as mean ± S.E. (n=5)

[b] Significantly different from untreated controls ($p<0.05$)

[c] Significantly different from toluene treatment alone ($p<0.05$)

TABLE 40. Organ Weights of Mice Following 4 Weeks of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), alone or Combined.

Concentration in Water (mg/L)	Organ Weights (g/100g body weight)[a]			
	Spleen	Kidney	Liver	Thymus
Control (0)	0.31 ± 0.01	1.46 ± 0.05	5.77 ± 0.16	0.14 ± 0.01
Benzene (200)	0.28 ± 0.02	1.59 ± 0.08	6.28 ± 0.17	0.09 ± 0.02[b]
Toluene (400)	0.29 ± 0.02	1.54 ± 0.04	5.92 ± 0.17	0.11 ± 0.01
Benzene (200) + Toluene (400)	0.28 ± 0.01	1.56 ± 0.05	5.77 ± 0.16	0.11 ± 0.01

[a] Values are given as mean ± S.E. (n=5)

[b] Significantly different from untreated controls ($p<0.05$)

TABLE 41. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Selected Blood Parameters[a].

Concentration (mg/L)	Erythrocyte (10^6/mm^3)	Leukocyte (10^3/mm^3)	Leukocyte absolute differentials (10^3/mm^3)		
			Lymphocyte	Neutrophil	Other[b]
Control (0)	9.73 ± 0.18	6.90 ± 1.08	5.19 ± 1.03	1.17 ± 0.11	0.54 ± 0.09
Benzene (200)	7.09 ± 0.25[c]	4.44 ± 0.16[c]	2.43 ± 0.16[c]	1.60 ± 0.11[c]	0.31 ± 0.04
Toluene (400)	9.78 ± 0.23	6.46 ± 0.61	4.67 ± 0.54	1.24 ± 0.05	0.55 ± 0.08
Benzene (200) + Toluene (400)	9.45 ± 0.38[d]	5.62 ± 0.35	3.60 ± 0.31[c,d]	1.57 ± 0.16[c]	0.44 ± 0.07

[a] Values are given as mean ± S.E. (n=5).

[b] Including monocytes, eosinophils and basophils.

[c] Significantly different from untreated controls ($p<0.05$).

[d] Significantly different from benzene treatment alone ($p<0.05$).

TABLE 42. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Splenic Lymphocyte Proliferative Response to Mitogens.

Concentration (mg/L)	Splenocytes[a] ($x10^{-7}$)	Mitogenic responses[a,b]				
		None	LPS	PWM	ConA	PHA
Control (0)	8.94 ± 1.90	2.02 ± 0.48	79.36 ± 20.14	8.99 ± 0.43	85.45 ± 39.75	103.40 ± 34.64
Benzene (200)	7.90 ± 1.67	1.07 ± 0.29	15.01 ± 3.22[c]	2.53 ± 0.83[c]	13.79 ± 3.48[c]	14.18 ± 2.15[c]
Toluene (400)	8.61 ± 1.16	1.59 ± 0.52	32.14 ± 5.24	7.72 ± 2.11	34.87 ± 7.37	43.53 ± 13.55
Benzene (200) + Toluene (400)	9.29 ± 0.75	3.17 ± 0.59[d,e]	125.41 ± 23.93[d,e]	10.66 ± 1.31[d]	174.51 ± 93.59[d]	119.57 ± 2.50[d]

[a] Values are given as mean ± S.E. (n=5).

[b] dpm x $10^{-3}/10^{6}$ splenic cells: response evaluated by incorporation of [methyl-^{3}H]-thymidine. into day 2 splenocyte cultures for 6 hours pulsing.

[c] Significantly different from untreated controls (p.0.05).

[d] Significantly different from benzene treatment alone (p.0.05).

[e] Significantly different from toluene treatment alone (p.0.05).

TABLE 43. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Mixed Lymphocyte Response.

Concentration (mg/L)	Responder-to-stimulator cell ratio[a]			
	Responders alone	1:1	2:1	4:1
Control (0)	1.91 ± 0.36	6.58 ± 0.88	17.69 ± 2.70	17.25 ± 2.59
Benzene (200)	0.88 ± 0.22[b]	4.03 ± 0.53	5.69 ± 1.64[b]	6.50 ± 1.17[b]
Toluene (400)	1.09 ± 0.14	3.93 ± 0.60	8.67 ± 2.24[b]	9.00 ± 0.95[b]
Benzene (200) + Toluene (400)	1.99 ± 0.33[c,d]	7.80 ± 2.46	22.85 ± 4.62[c,d]	21.84 ± 3.83[c,d]

[a] Responder cells were the splenocytes of CD-1 mice and stimulator cells were the allogeneic YAC-1 lymphoma cells (H-2[a]) of A/Sn origin. The stimulator cells were treated with mytomycin-C before addition to the culture. MLR was evaluated by incorporation (dpm x 10^{-3}) of [methyl-^{3}H]-thymidine into day 3 MLR cultures for 6 hours pulsing. Values are given as mean ± S.E. (n=5).

[b] Significantly different from untreated controls ($p<0.05$).

[c] Significantly different from benzene treatment alone ($p<0.05$).

[d] Significantly different from toluene treatment alone ($p<0.05$).

TABLE 44. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Cytotoxic T- Lymphocyte Response.

Concentration (mg/L)	% Cytotoxicity[a]			
	50:1[b]	25:1	12.5:1	6.25:1
Control (0)	18.74 ± 1.79	18.66 ± 1.39	13.23 ± 1.65	7.88 ± 1.66
Benzene (200)	13.29 ± 1.07	14.43 ± 3.78	7.20 ± 0.81[c]	4.67 ± 0.59
Toluene (400)	17.33 ± 1.85	16.85 ± 2.39	9.43 ± 0.71	6.34 ± 0.86
Benzene (200) + Toluene (400)	24.11 ± 1.83[c,d,e]	23.74 ± 1.06[d,e]	18.11 ± 2.69[d,e]	9.83 ± 1.02[d]

[a] Values are given as mean ± S.E. (n=5).

[b] Effector (day 5 mixed lymphocyte culture lymphocytes)-to-target (^{51}Cr-labeled YAC-1 cells) cell ratio.

[c] Significantly different from untreated controls ($p<0.05$).

[d] Significantly different from benzene treatment alone ($p<0.05$).

[e] Significantly different from toluene treatment alone ($p<0.05$).

TABLE 45. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Antibody Responses to T-Dependent Antigen Sheep Erythrocyte (SRBC)[a].

Concentration (mg/L)	Total splenocytes ($\times 10^{-7}$)	PFC/10^6 splenocytes	PFC/total spleen cells ($\times 10^{-3}$)	α -SRBC antibody titer
Control (0)	22.01 ± 2.29	1485 ± 66	337.36 ± 44.82	0.78 ± 0.07
Benzene (200)	17.81 ± 1.18	833 ± 89[b]	151.41 ± 21.53[b]	0.47 ± 0.05[b]
Toluene (400)	19.02 ± 1.03	1190 ± 109[b]	229.51 ± 32.30	0.37 ± 0.03
Benzene (200) + Toluene (400)	22.93 ± 1.61	1972 ± 159[b,c,d]	454.90 ± 42.48[c,d]	1.24 ± 0.10[b,c,d]

[a] Mice were sensitized with SRBC 4 days before the end of exposure. Splenic cells were analyzed for antibody forming cells (plaque-forming cell, PFC) and antibody titer (α -SRBC) determined in Sera. Values are given as mean ± S.E. (n=5).

[b] Significantly different from untreated controls ($p<0.05$).

[c] Significantly different from benzene treatment alone ($p<0.05$).

[d] Significantly different from toluene treatment alone ($p<0.05$).

TABLE 46. Body Weights of CD-1 Mice Following 4 Weeks of Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined.

Concentration in Water (mg/L)	Body Weight (g)[a]		
	Day 0	Day 28	Gain
Control (0)	23.38 ± 0.79	31.12 ± 1.02	7.73 ± 0.41
Benzene (200)	22.50 ± 0.72	30.44 ± 0.75	7.94 ± 0.54
Toluene (100)	25.38 ± 0.75	34.72 ± 1.20	9.34 ± 0.56
Benzene (200) + Toluene (100)	25.42 ± 0.75	34.14 ± 1.25	8.72 ±0.84

[a] Values are given as mean ± S.E. (n=5).

TABLE 47. Organ Weights of Mice Following 4 Weeks Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined.

Concentration in Water (mg/L)	Organ Weights (g/100g body weight)[a]			
	Spleen	Kidney	Liver	Thymus
Control (0)	0.29 ± 0.01	1.79 ± 0.10	6.19 ± 0.13	0.17 ± 0.01
Benzene (200)	0.24 ± 0.92	1.73 ± 0.11	6.29 ± 0.23	0.14 ± 0.01[b]
Toluene (100)	0.29 ± 0.03	1.74 ± 0.05	6.02 ± 0.17	0.15 ± 0.01
Benzene (200) + Toluene (100)	0.26 ± 0.01	1.73 ± 0.08	6.38 ± 0.17	0.14 ± 0.01[b]

[a] Values are given as mean ± S.E. (n=5).

[b] Significantly different from untreated controls ($p<0.05$).

TABLE 48. Effect of Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined, on Splenocyte and Erythrocyte Cellularities.

Concentration (mg/L)	Splenocyte[a] ($x10^{-7}$)	Erythrocyte[a] ($10^6/mm^3$)
Control (0)	10.80 ± 1.14	9.36 ± 0.18
Benzene (200)	7.8 ± 0.05	6.77 ± 0.35[b]
Toluene (100)	11.38 ± 1.87	10.42 ± 0.34
Benzene (200) + Toluene (100)	10.74 ± 0.70	9.28 ± 0.25[c,d]

[a] Values are given as mean ± S.E. (n=5).

[b] Significantly different from untreated controls ($p<0.05$).

[c] Significantly different from benzene treatment alone ($p<0.05$).

[d] Significantly different from toluene treatment alone ($p<0.05$).

TABLE 49. Effect of Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined, on Splenic Lymphocyte Proliferative Response to Mitogens.

Concentration (mg/L)	Mitogenic responses[a]				
	None	LPS	PWM	ConA	PHA
Control (0)	2.10 ± 0.71	55.52 ± 10.24	6.72 ± 1.83	72.03 ± 13.33	ND[c]
Benzene (200)	0.52 ± 0.23	4.07 ± 1.10[b]	3.31 ± 1.32	10.55 ± 2.82[b]	ND
Toluene (100)	1.49 ± 0.28	27.13 ± 9.92	4.49 ± 0.53	35.72 ± 8.77	ND
Benzene (200) + Toluene (100)	2.05 ± 0.72	6.02 ± 1.68[b]	3.79 ± 1.06	23.55 ± 2.82[b]	ND

[a] dpm x $10^{-3}/10^{6}$ splenic cells: response evaluated by incorporation of [methyl-^{3}H]-thymidine into day 2 splenocyte cultures for 6 hours pulsing. Values are given as mean ± S.E. (n=5).

[b] Significantly different from untreated controls ($p<0.05$).

[c] ND, not determined.

TABLE 50. Effect of Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined, on Mixed Lymphocyte Responses.

Concentration (mg/L)	Responder-to-stimulator cell ratio[a]			
	Responders Alone	1:1	2:1	4:1
Control (0)	2.13 ± 0.59	7.98 ± 1.84	18.73 ± 2.16	16.47 ± 0.89
Benzene (200)	1.00 ± 0.13	7.05 ± 1.49	8.62 ± 1.60[b]	5.76 ± 0.69[b]
Toluene (100)	1.43 ± 0.63	6.29 ± 0.64	14.25 ± 1.51	11.29 ± 3.37
Benzene (200) + Toluene (100)	1.54 ± 0.37	7.36 ± 1.36	8.54 ± 1.16[b,c]	8.16 ± 1.60[b]

[a] Responder cells were the splenocytes of CD-1 mice and stimulator cells were the allogeneic YAC-1 lymphoma cells (H-2[a]) of A/Sn origin. The stimulator cells were treated with mytomycin-C before addition to the culture. MLR was evaluated by incorporation (dpm x 10^{-3}) of [methyl-^{3}H]-thymidine into day 3 MLR cultures for 6 hours pulsing. Values are given as mean ± S.E. (n=5).

[b] Significantly different from untreated controls ($p<0.05$).

[c] Significantly different from toluene treatment alone ($p<0.05$).

TABLE 51. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Brain Biogenic Amines and Their Major Metabolites in Hypothalamus.

Doses (mg/L)	Concentrations (µg/g Wet Tissue)[a]					
	NE	DA	5-HT	DOPAC	HVA	5-HIAA
Control (0)	1.87±0.14	0.49±0.08	0.37±0.05	0.22±0.04	0.16±0.02	0.32±0.07
Benzene (200)	2.77±0.26[b]	0.74±0.07[b]	0.71±0.04[b]	0.37±0.07	0.26±0.03[b]	0.69±0.04[b]
Toluene (400)	2.90±0.18[b]	0.62±0.07	0.76±0.07[b]	0.35±0.05	0.24±0.03	0.68±0.05[b]
Benzene (200) + Toluene (400)	3.19±0.30[b]	0.82±0.06[b]	0.71±0.08[b]	0.42±0.05	0.29±0.03[b]	0.76±0.06[b]

[a] Values are given as mean ± S.E. (n=5).

[b] Significantly different from untreated controls ($p<0.05$).

TABLE 52. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Brain Biogenic Amines and Their Major Metabolities in Cerebral Cortex and Medulla Oblongata of CD-1 Mice.

Brain Region	Doses (mg/L)	Concentrations (µg/g Wet Tissue)[a]						
		NE	DA	5-HT	VMA	DOPAC	HVA	5-HIAA
Cerebral Cortex	Control (0)	0.42±0.03	0.76±0.07	0.56±0.05	0.11±0.02	0.21±0.01	0.16±0.01	0.28±0.02
	Benzene (200)	0.53±0.04[b]	1.16±0.13	0.77±0.02[b]	0.15±0.02	0.23±0.03	0.22±0.05	0.36±0.02
	Toluene (400)	0.48±0.01	1.40±0.19[b]	0.74±0.02[b]	0.13±0.01	0.27±0.03	0.23±0.04	0.34±0.02
	Benzene (200) + Toluene (400)	0.41±0.01	1.16±0.12	0.68±0.04	0.13±0.01	0.28±0.03	0.21±0.02	0.34±0.02
Medulla Oblongata	Control (0)	0.61±0.02	0.04±0.02	0.57±0.02	0.11±0.01	0.03±0.02	0.05±0.01	0.37±0.34
	Benzene (200)	0.83±0.04[b]	0.05±0.00	0.76±0.04[b]	0.12±0.00	0.07±0.01	0.06±0.01	0.46±0.03
	Toluene (400)	0.80±0.03[b]	0.05±0.01	0.70±0.06	0.17±0.03[b]	0.04±0.01	0.05±0.01	0.43±0.03
	Benzene (200) + Toluene (400)	0.81±0.04[b]	0.05±0.01	0.73±0.04[b]	0.21±0.01[b,c]	0.07±0.01	0.06±0.01	0.49±0.03

[a] Values are given as mean ± S.E. (n=5).

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

[c] Significantly different from benzene treatment alone ($p<0.05$).

TABLE 53. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Brain Biogenic Amines and Their Major Metabolites in Corpus Striatum and Midbrain of CD-1 Mice.

Brain Region	Doses (mg/L)	Biogenic Amines Concentrations (µg/g Wet Tissue)[a]						
		NE	DA	5-HT	VMA	DOPAC	HVA	5-HIAA
Corpus Striatum	Control (0)	0.31±0.06	7.27±0.55	0.36±0.05	0.12±0.02	1.21±0.07	0.93±0.07	0.55±0.07
	Benzene (200)	0.43±0.06	10.10±0.62[b]	0.64±0.05[b]	0.16±0.01	1.43±0.11	1.28±0.09[b]	0.67±0.06
	Toluene (400)	0.44±0.07	9.01±0.48	0.61±0.09[b]	0.13±0.01	1.46±0.13	1.09±0.05	0.58±0.05
	Benzene (200) + Toluene (400)	0.45±0.03	9.25±0.59[b]	0.60±0.03[b]	0.11±0.01	1.92±0.16[b,c,d]	1.27±0.05[b]	0.72±0.04
Midbrain	Control (0)	0.66±0.03	0.27±0.05	0.68±0.03	0.09±0.02	0.21±0.03	0.13±0.01	0.55±0.01
	Benzene (200)	0.84±0.03[b]	0.39±0.04	1.10±0.02[b]	0.08±0.00	0.18±0.01	0.19±0.01[b]	0.71±0.04[b]
	Toluene (400)	0.73±0.02	0.44±0.18	0.95±0.04[b]	0.10±0.01	0.22±0.04	0.18±0.02	0.63±0.03
	Benzene (200) + Toluene (400)	0.67±0.05[c]	0.38±0.04	0.89±0.09	0.13±0.01[b,c]	0.18±0.01	0.16±0.01	0.71±0.03[b]

[a] Values are given as mean ± S.E. (n=5).

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

[c] Significantly different from benzene treatment alone ($p<0.05$).

[d] Significantly different from toluene treatment alone ($p<0.05$).

TABLE 54. Effect of Exposure to Benzene (200 mg/L) and Toluene (400 mg/L), Alone or Combined, on Brain Biogenic Amines and Their Major Metabolites in Cerebellum of CD-1 Mice.

Doses (mg/L)	Concentrations (µg/g Wet Tissue)[a]			
	NE	5-HT	VMA	5-HIAA
Control (0)	0.24 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.17 ± 0.01
Benzene (200)	0.30 ± 0.01	0.19 ± 0.01	0.10 ± 0.02	0.17 ± 0.00
Toluene (400)	0.31 ± 0.02	0.13 ± 0.02	0.14 ± 0.05	0.16 ± 0.02
Benzene (200) + Toluene (400)	0.34 ± 0.03[b]	0.16 ± 0.01	0.15 ± 0.03	0.18 ± 0.01

[a] Values are given as mean ± S.E. (n=5).
[b] Significantly different from controls ($p<0.05$).

TABLE 55. Effect of Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined, on Brain Biogenic Amines and Their Major Metabolites in Hypothalamus.

Doses (mg/L)	Concentrations (µg/g Wet Tissue)[a]					
	NE	DA	5-HT	DOPAC	VMA	5-HIAA
Control (0)	1.87±0.24	0.51±0.06	0.76±0.09	0.21±0.03	0.16±0.02	0.60±0.06
Benzene (200)	2.73±0.17	0.66±0.05	0.98±0.07	0.34±0.05	0.44±0.14	1.18±0.18[b]
Toluene (100)	2.96±0.42[b]	0.81±0.09[b]	1.05±0.12	0.28±0.02	0.26±0.04	0.87±0.11
Benzene (200) + Toluene (400)	2.47±0.29	0.63±0.08	0.75±0.07	0.26±0.02	0.26±0.03	0.71±0.08[c]

[a] Values are given as mean ± S.E. (n=5).
[b] Significantly different from controls ($p<0.05$).
[c] Significantly different from benzene treatment alone ($p<0.05$).

TABLE 56. Effect of Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined, on Brain Biogenic Amines and Their Major Metabolites in Cerebral Cortex and Medulla Oblongata of CD-1 Mice.

Brain Region	Doses (mg/L)	Concentrations (µg/g Wet Tissue)[a]					
		NE	DA	5-HT	DOPAC	HVA	5-HIAA
Cerebral Cortex	Control (0)	0.38±0.02	0.81±0.03	0.63±0.02	0.14±0.01	0.16±0.01	0.25±0.01
	Benzene (200)	0.42±0.01	1.02±0.16	0.79±0.13	0.17±0.01	0.22±0.01	0.26±0.01
	Toluene (100)	0.46±0.03	1.15±0.10[b]	0.70±0.04	0.20±0.02[b]	0.26±0.02[b]	0.32±0.02[b]
	Benzene (200) + Toluene (100)	0.48±0.02	1.97±0.18	0.65±0.02	0.19±0.02	0.26±0.02[b]	0.31±0.01[b]
Medulla Oblongata	Control (0)	0.66±0.04	0.04±0.00	0.68±0.05	0.03±0.00	0.04±0.01	0.39±0.03
	Benzene (200)	0.79±0.03[b]	0.05±0.01	0.84±0.03[b]	0.05±0.01	0.06±0.01[b]	0.48±0.02
	Toluene (100)	0.77±0.03[b]	0.04±0.00	0.82±0.03[b]	0.04±0.00	0.06±0.00[b]	0.51±0.04[b]
	Benzene (200) + Toluene (100)	0.70±0.02	0.05±0.01	0.73±0.02	0.06±0.01[b]	0.06±0.00[b]	0.45±0.01

[a] Values are given as mean ± S.E. (n=5).

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

TABLE 57. Effect of Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined, on Brain Biogenic Amines and Their Major Metabolites in Corpus Striatum and Midbrain of CD-1 Mice.

Brain Region	Doses (mg/L)	Biogenic Amines Concentrations (µg/g Wet Tissue)[a]					
		NE	DA	5-HT	DOPAC	HVA	5-HIAA
Corpus Striatum	Control (0)	0.33±0.04[b]	7.14±0.63	0.64±0.04	0.82±0.08	0.87±0.11	0.41±0.04
	Benzene (200)	0.43±0.04	8.68±0.21	0.67±0.05	1.07±0.05	1.21±0.04[b]	0.49±0.06
	Toluene (100)	0.54±0.04[b]	8.02±0.46	0.67±0.06	1.16±0.06[b]	1.28±0.04[b]	0.54±0.05
	Benzene (200) + Toluene (100)	0.54±0.05[b]	9.23±0.36[b]	0.77±0.08	1.25±0.10[b]	1.51±0.07[b,c,d]	0.56±0.08
Midbrain	Control (0)	0.55±0.05	0.19±0.01	0.80±0.09	0.13±0.01	0.16±0.02	0.49±0.02
	Benzene (200)	0.74±0.02[b]	0.33±0.08	1.13±0.04[b]	0.13±0.01	0.19±0.01	0.66±0.04[b]
	Toluene (100)	0.66±0.04	0.22±0.02	0.96±0.07	0.11±0.02	0.15±0.01	0.67±0.08[b]
	Benzene (200) + Toluene (100)	0.65±0.04	0.34±0.07	0.97±0.07	0.17±0.03	0.22±0.03	0.67±0.08[b]

[a] Values are given as mean ± S.E. (n=5).

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

[c] Significantly different from benzene treatment alone ($p<0.05$).

[d] Significantly different from toluene treatment alone ($p<0.05$).

TABLE 58. Effect of Exposure to Benzene (200 mg/L) and Toluene (100 mg/L), Alone or Combined, on Brain Biogenic Amines and Their Major Metabolites in Cerebellum of CD-1 Mice.

Doses (mg/L)	Concentrations (μg/g Wet Tissue)[a]		
	NE	5-HT	5-HIAA
Control (0)	0.24 ± 0.03	0.14 ± 0.03	0.14 ± 0.02
Benzene (200)	0.26 ± 0.01	0.15 ± 0.02	0.14 ± 0.01
Toluene (100)	0.28 ± 0.03	0.18 ± 0.02	0.16 ± 0.01
Benzene (200) + Toluene (100)	0.23 ± 0.01	0.14 ± 0.01	0.15 ± 0.02

[a] Values are given as mean ± S.E. (n=5). Amounts of other amines or their metabolites were not detected.