NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicity Studies of Benzophenone (CAS No. 119-61-9) Administered in Feed to F344/N Rats and B6C3F$_1$ Mice

NTP TOX 61

APRIL 2000

National Toxicology Program
Toxicity Report Series
Number 61

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U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

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April 2000

NIH Publication No. 00-3943

U.S. Department of Health and Human Services
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PEER REVIEW

The draft report on the toxicity studies of benzophenone was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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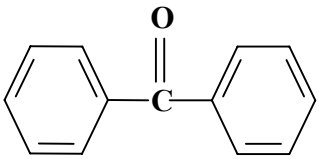
ABSTRACT



BENZOPHENONE

CAS No. 119-61-9

Chemical Formula: $C_{13}H_{10}O$ Molecular Weight: 182.22

Synonyms: Benzene, benzophenone (8CI); benzoyl; benzoylbenzene; benzoylbenzenephenyl; diphenyl ketone; diphenylmethanone; methanone, diphenyl-(9CI); α-oxodiphenylmethane; α-oxoditane; phenyl ketone

Benzophenone is used as a photoinitiator, a fragrance enhancer, an ultraviolet curing agent, and, occasionally, as a flavor ingredient; it is also used in the manufacture of insecticides, agricultural chemicals, and pharmaceuticals and is an additive for plastics, coatings, and adhesives. In 14-week studies conducted to determine the toxicity of benzophenone, groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were given 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm benzophenone in feed. These exposure concentrations resulted in the following average daily doses: 75, 150, 300, 700, or 850 mg benzophenone per kilogram body weight for male rats; 80, 160, 300, 700, or 1,000 mg/kg for female rats; 200, 400, 800, 1,600, or 3,300 mg/kg for male mice; and 270, 540, 1,000, 1,900, or 4,200 mg/kg for female mice. Animals were evaluated for clinical pathology, reproductive system effects, liver cytochrome P_{450} effects, and histopathology. Genetic toxicity studies were conducted in *Salmonella typhimurium* and mouse bone marrow polychromatic erythrocytes.

Benzophenone was unpalatable at 20,000 ppm. All 20,000 ppm rats had significant body weight loss and were terminated for humane reasons before the end of studies. All male mice and four female mice in the 20,000 ppm group died. There was no exposure-related mortality in the remaining groups. Significantly decreased body weights relative to the controls were observed in all exposed groups of female rats and all exposed groups of male rats except the 1,250 ppm group. Lower body weights were apparent in 10,000 ppm male mice and in 5,000 ppm or greater female mice.

In rats, the liver and kidney were identified as target organs of benzophenone toxicity. Treatment-related increases in liver weights were attributed to hypertrophy and/or cytoplasmic vacuolization of hepatocytes. Increased kidney weights were associated with a spectrum of renal changes in exposed males and females. Unique lesions observed in animals that died early as well as in survivors were well demarcated, wedge-shaped areas of prominent tubule dilatation. The lesion occurred in 2,500 ppm or greater males and in 10,000 and 20,000 ppm females. Foci of tubule regeneration were increased relative to the controls in exposed males and females.

In exposed mice, significant microscopic findings were limited to centrilobular hypertrophy in the liver that corresponded to increased liver weights. The severity of hepatocyte hypertrophy was exposure-concentration dependent, with marked severity in all 20,000 ppm animals.

Clinical chemistry analyses confirmed liver toxicity. In rats, increases in serum bile salt concentrations indicated cholestatic liver disease. On day 22, a 15-fold increase was evident in the 20,000 ppm groups, and at week 14, a twofold increase was seen in the 10,000 ppm groups. Increases in alanine aminotransferase and sorbitol dehydrogenase activities were mild in mice; however, more convincing of liver damage were increased alkaline phosphatase activities and serum bile salt concentrations, especially in 20,000 ppm females.

Biochemical data indicated that benzophenone was a relatively potent inducer of the phenobarbital-type (2B) cytochrome P_{450} enzymes. Overall, induction was greater in rats than in mice. The gross (increased organ weights) and microscopic (hepatocellular hypertrophy) liver changes associated with benzophenone administration in rats and mice accompanied benzophenone-induced increases in pentoxyresorufin dealkylase activity.

Benzophenone was not mutagenic in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without S9 activation, and it did not induce micronuclei in bone marrow erythrocytes of male mice administered benzophenone by intraperitoneal injection.

In conclusion, the liver is the primary target organ of benzophenone toxicity in rats and mice based on increases in liver weights, hepatocellular hypertrophy, clinical chemistry changes, and induction of liver microsomal cytochrome P_{450} 2B isomer. The kidney was also identified as a target organ of benzophenone toxicity in rats only, based on exposure concentration-related increases in kidney weights and microscopic changes. The no-observed-adverse-effect level for benzophenone was not achieved in these studies.

INTRODUCTION

PHYSICAL PROPERTIES

Benzophenone, a white crystal with a geranium- or rose-like odor, is an aryl ketone that is prepared in 66% yield by a Friedel-Crafts acylation using benzoyl chloride with an excess of benzene in the presence of anhydrous aluminum chloride (Furia and Bellanca, 1975; *Kirk-Othmer*, 1978; *Merck Index*, 1996). Some of the physical properties of benzophenone are given in Table 1. Benzophenone is photochemically reactive and is incompatible with strong oxidizing and reducing agents; it may attack some plastics. Decomposition of benzophenone produces toxic fumes of carbon monoxide and carbon dioxide (*Sigma-Aldrich*, 1988).

TABLE 1
Physical Properties of Benzophenone[a]

Molecular weight	182.22
Boiling point	305.4° C
Melting point	(α) = 49° C; (β) = 26° C
Flash point	greater than 110° C
Vapor pressure	1 mm Hg at 108.2° C
Specific gravity	d(α) = 1.0976 at 50°/50° C; d(β) = 1.108 at 23°/40° C
Refractive index	1.60
Log octanol/water partition coefficient	3.18
Solubility	Insoluble in water; soluble in organic solvents including alcohol, acetone, ether, acetic acid, chloroform, and benzene

[a] *Merck Index* (1996); *Hazardous Chemicals Desk Reference* (1997); Hansch and Leo (1979)

PRODUCTION, USE, AND HUMAN EXPOSURE

Benzophenone is used primarily as a photoinitiator and fragrance enhancer (*Am. Paint Coatings J.*, 1990; *Chem. Bus. Newsbase*, 1991). Production of benzophenone in the United States is estimated to range from 300,000 to 3,000,000 pounds per year (USEPA, 1991), with 100,000 pounds per year used in fragrances (Opdyke, 1973). Benzophenone is used in the manufacture of insecticides and agricultural chemicals and of hypnotics, antihistamines, and other pharmaceuticals; as an ultraviolet curing agent in sunglasses and ink; as an additive in plastics, coatings, and adhesive formulations; and, occasionally, as a flavor ingredient. Concentrations of benzophenone in food products range from 0.57 ppm in nonalcoholic beverages to 3.27 ppm

in frozen dairy products; it may also be an ingredient in baked goods, soft candy, gelatins, and puddings (NAS/NRC, 1979).

Because of its high octanol/water partition coefficient and its insolubility in water, benzophenone will partition in soil and sediment (USEPA, 1984); the adsorption of benzophenone to soil is proportional to the organic content of the soil (OHMTADS, 1991). Although benzophenone has been identified in the atmosphere, it is difficult to determine whether its presence is due to its being a direct product of combustion or a secondary product of atmospheric degradation (Helmig *et al.*, 1989). Leary *et al.* (1987) found that benzophenone is a component of emissions from a standard residential oil burner. It has also been detected in surface and ground water samples, primarily from the discharge of untreated sewage and wastewater into waterways.

Based on the use of benzophenone as an additive in fragrances, cosmetics, toiletries, pharmaceuticals, insecticides, and flavor ingredients, consumer exposure may be significant. Additionally, surveys by the National Institute for Occupational Safety and Health (1990) showed that 41,520 workers in the United States were potentially exposed to benzophenone between 1981 and 1983.

DISPOSITION AND METABOLISM

In rhesus monkeys, percutaneous absorption of benzophenone was found to be 44% and 69% for unoccluded and occluded sites, respectively (Bronaugh *et al.*, 1990). The primary pathway of benzophenone metabolism following dietary administration in rabbits was reported to be reduction of the keto group to yield benzhydrol, which was excreted at concentrations of 41% to 61% of the administered dose as a labile glucuronide in the urine (Robinson and Williams, 1957; Robinson, 1958). In male Sprague-Dawley rats that received benzophenone by gavage, 1% of the administered dose was detected as *p*-hydroxybenzophenone in enzyme-treated urine samples, but not in unhydrolyzed urine (Stocklinski *et al.*, 1979). No *p*-hydroxybenzophenone was detected in the feces.

TOXICITY

Experimental Animals

Median lethal oral, intraperitoneal, and dermal doses (LD_{50}) of benzophenone are given in Table 2; these data indicate that benzophenone is only slightly toxic.

TABLE 2
Summary of Selected Animal Toxicity Data for Benzophenone

Species	Route of Exposure	LD_{50} (mg/kg)	Reference
Rat	Oral	>10,000	Opdyke, 1973
Rat	Oral	1,900	Eastman Kodak Company, 1991
Mouse	Oral	2,895 (2,441-3,434)	Caprino *et al.*, 1976
Mouse	Intraperitoneal	727 (634-833)	Caprino *et al.*, 1976
Rabbit	Dermal	3,535 (2,007-6,226)	Opdyke, 1973

Groups of male rats (strain not specified) were fed diets containing 0.1% or 1.0% benzophenone for 10 consecutive days. Feed consumption and body weights were slightly reduced in the 1.0% group. Exposure concentration-dependent increases in absolute and relative liver weights and relative kidney weight were observed. Serum alanine aminotransferase activity of rats in the 1.0% group was increased compared to that of the controls. Mild degenerative effects were observed in the liver and bone marrow of rats in the 1.0% group, suggesting that the liver may be the primary target of the toxic effects of benzophenone and that the bone marrow may also be targeted (USEPA, 1984).

Benzophenone was administered in feed to Sprague-Dawley rats at concentrations of 20 mg/kg body weight per day for 90 days or 100 or 500 mg/kg per day for 28 days (Burdock *et al.*, 1991). Decreases in hematocrit values, erythrocyte counts, and hemoglobin concentrations were observed in females in the 100 and 500 mg/kg groups; a decrease in hemoglobin concentration was also evident in males in the 500 mg/kg group. Males in the 100 and 500 mg/kg groups had increased urea nitrogen concentrations; total bilirubin and protein were increased in males in the 500 mg/kg group and females in the 100 and 500 mg/kg groups. Males and females exposed to 100 or 500 mg/kg had increased albumin concentrations and absolute and relative liver and kidney weights. Histopathologic examination of the liver revealed hepatocellular enlargement with associated clumping of cytoplasmic basophilic material around the central vein in rats in the 100 and 500 mg/kg groups.

Slight skin irritation, evidenced by slight erythema and desquamation and slight to moderate edema, was observed in guinea pigs that received dermal applications of benzophenone on the abdomen for 24 hours, under an occlusive wrap, or on the back, uncovered, for 10 days (USEPA, 1984). Additional exposures to benzophenone failed to exacerbate the irritation, and no evidence of percutaneous absorption was reported. In a dermal study using the Draize method (Calas *et al.*, 1977), benzophenone was determined to have medium irritation potential, with a primary cutaneous irritation index of 2.0 in rabbits. Additional experiments were conducted in guinea pigs to determine skin irritation and contact hypersensitivity induced by benzophenone; in

the open epicutaneous test, the Draize test, the maximization test, and a test with Freund's complete adjuvant, benzophenone did not induce allergenicity in guinea pigs.

Acetophenone, which is structurally related to benzophenone, was tested for toxicity and tissue effects in rats fed diets containing up to 10,000 ppm for 17 weeks. No hematologic changes or gross or microscopic tissue changes were observed in exposed rats (IRIS, 1991).

Humans

No information on toxic effects of benzophenone in humans was found in a search of the literature (HSDB, 1997).

DEVELOPMENTAL AND REPRODUCTIVE EFFECTS

The developmental and teratogenic effects of benzophenone were studied in Japanese newts. Seven days after the forelimb was amputated at a position proximal to the elbow, benzophenone was inserted in the anterior part of the regeneration blastema. No retardation of regeneration was observed, and growth continued normally in the dosed group (Tsonis and Eguchi, 1982). Up to 10,000 ppm acetophenone administered in feed for 17 weeks caused no growth effects in rats (IRIS, 1991). No data were available in the literature on the reproductive toxicity of benzophenone.

CARCINOGENICITY

Experimental Animals

The carcinogenicity of benzophenone has been studied in female Swiss mice (Stenbäck and Shubik, 1974) and New Zealand white rabbits (Stenbäck, 1977). In lifetime studies, animals were administered 5%, 25%, or 50% benzophenone in acetone topically at dose volumes of 0.02 mL twice per week. Benzophenone was applied to a 1-inch square area on the dorsal skin between the flanks of mice; for rabbits, the dose was applied to the inside of the left ear. All mice died by week 110. The incidence of skin neoplasms in dosed mice was similar to that in the controls (Stenbäck and Shubik, 1974). Benzophenone had no effect on survival rates or on incidences of neoplasms or nonneoplastic lesions in rabbits after 160 weeks of treatment (Stenbäck, 1977).

Humans

No epidemiologic studies or case reports examining the relationship between exposure to benzophenone and human cancer were found in the literature (HSDB, 1997).

GENETIC TOXICITY

Benzophenone was not mutagenic in the standard Ames test using various strains of *Salmonella typhimurium* (Mortelmans *et al.*, 1986) or in the *Escherichia coli* pol A assay (Fluck *et al.*, 1976). In addition, negative results were reported for benzophenone in the mouse lymphoma cell mutagenicity test (CCRIS, 1991). All three of these *in vitro* assays were performed with and without liver S9 metabolic activation enzymes.

STUDY RATIONALE AND DESIGN

Benzophenone was nominated by the National Institute of Environmental Health Sciences for toxicity testing based on the potential for occupational and consumer exposure and the lack of chronic toxicity data. Assessment of cytochrome P_{450} content and activity in liver was included in the study design to further characterize the toxic effects of benzophenone. Feed was chosen as the route of exposure because this mimics exposure to humans consuming benzophenone as a flavoring agent. Endpoints evaluated during these 14-week studies included histopathology and clinical pathology in F344/N rats and B6C3F_1 mice. The effects of benzophenone on reproduction were assessed by the evaluation of testicular and spermatozoal parameters and by characterization of the estrous cycle. In addition, the genetic toxicity of benzophenone was assessed in studies in *S. typhimurium* and by determination of the induction of micronuclei in bone marrow cells of mice receiving intraperitoneal injections of benzophenone.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF BENZOPHENONE

A single lot of benzophenone (lot 06327AZ) was obtained from Aldrich Chemical Company (Milwaukee, WI). Information on identity and purity were provided by the supplier. The study laboratory confirmed the identity of the chemical, which consisted of off-white chips, with infrared spectroscopy; the spectrum was consistent with a literature reference (*Aldrich*, 1985). Gas chromatographic analyses conducted by the supplier indicated a purity greater than 99%.

Throughout the 14-week studies, benzophenone was stored in the original plastic jars. Periodic reanalyses performed by the study laboratory using gas chromatography indicated no degradation of the bulk chemical.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared 1 week before the exposures began and every 4 weeks thereafter. Benzophenone was ground and sieved to reduce particle size before being stirred manually with feed to prepare a premix. A blender was then used to combine the premix with the remaining feed. The dose formulations were stored in plastic bags inside plastic buckets, at room temperature, for up to 4 weeks. Homogeneity studies of the 1,250 and 20,000 ppm dose formulations and stability studies of the 1,250 ppm dose formulation were performed by the analytical chemistry laboratory using gas chromatography. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 5 weeks when stored at −20° C, 5° C, or room temperature, sealed and protected from ultraviolet light, or 7 days when stored sealed at room temperature, exposed to ultraviolet light.

Analyses of the dose formulations of benzophenone were conducted at the study laboratory with gas chromatography. The dose formulations and animal room samples were analyzed initially and after 8 weeks. All dose formulations analyzed were within 10% of the target concentrations. All but one animal room sample for rats and one for mice were within 10% of the target concentrations.

14-WEEK STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were approximately 5 to 6 weeks old. Animals were quarantined for 13 to 17 days and were approximately 8 to 9 weeks old on the first day of the studies. Before the studies began, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood was collected from five male and five female rats and mice at the beginning of the studies and again 4 weeks after the studies began. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b); all results were negative. Additional details concerning the study design are provided in Table 3.

The exposure concentrations for the 14-week studies were selected based on literature values. In a 28-day toxicity study in male and female Sprague-Dawley rats administered 0, 10, 100, or 500 mg benzophenone/kg body weight in feed, benzophenone was toxic at the two highest exposure levels (Burdock *et al.*, 1991). The exposure levels selected for the current studies took into consideration the absence of toxicity data in mice and possible strain differences in the expression of toxicity in rats. Therefore, groups of 10 male and 10 female rats and mice were fed diets containing 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm benzophenone for 14 weeks. Rats were housed five per cage and mice were housed individually. NIH-07 open formula meal (Zeigler Brothers, Inc., Gardners, PA), containing the appropriate concentrations of benzophenone, and water (Columbus municipal supply) were available *ad libitum*. Feed consumption by core study animals was recorded twice per week. Additional details on animal maintenance are provided in Table 3.

Clinical pathology studies were performed on rats designated for clinical pathology testing and on all core study rats and mice. Ten male and ten female rats and mice per group were evaluated. Blood for hematology and clinical chemistry evaluations was collected from clinical pathology study rats on days 4 and 22; blood was collected from core study rats and mice at the end of the studies. The animals were anesthetized with a mixture of carbon dioxide and oxygen, and blood was withdrawn from the retroorbital sinus. Samples for hematology analysis were placed in microcollection tubes (Sarstedt, Inc., Nümbrecht, Germany) containing potassium EDTA; samples for clinical chemistry evaluations were placed in tubes devoid of anticoagulant. The latter samples were allowed to clot and were then centrifuged, and the serum was removed.

Hematologic determinations were made on a Serono-Baker System 9000 hematology analyzer (Serono-Baker Diagnostics, Allentown, PA) with reagents obtained from the equipment manufacturer. The parameters that were evaluated are listed in Table 3. Differential leukocyte counts and morphologic evaluations of blood cells were conducted using light microscopy and blood smears stained with Wright-Giemsa.

Clinical chemistry variables were measured with a Hitachi 704® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN). The parameters that were evaluated are listed in Table 3. Reagents were obtained from the equipment manufacturer.

Vaginal cytology and sperm motility evaluations were performed on core study rats and mice at the end of the studies. Ten male and ten female rats from the 0, 1,250, 2,500, and 5,000 ppm groups and 10 male and 10 female mice from the 0, 2,500, 5,000, and 10,000 ppm groups were evaluated. The parameters that were evaluated are listed in Table 3. Methods used were those described in the NTP Statement of Work (NTP, 1991). For the 12 days prior to sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Complete necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all control animals, all animals in the highest exposure groups with at least 60% survival and all higher exposure groups, and all animals that died early. Table 3 lists the tissues and organs examined microscopically.

Upon completion of the laboratory pathologist's histopathologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working

Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

Because of the photoinitiating properties of benzophenone (Caprino *et al.*, 1976), special histopathology studies were conducted to evaluate the potential effects of benzophenone on the eyes of core study rats and mice. Five males and five females from the control, 10,000 ppm (rats and male mice), and 20,000 ppm (female mice) groups were randomly selected for evaluation at the end of the studies. The lens, retina, and other ocular structures were examined microscopically.

Residual liver tissue was collected from randomly selected core rats and mice (five males and five females per group) after liver sections for histopathologic analyses were prepared. Samples were analyzed for microsomal cytochrome P_{450} content and cytochrome P_{450}-mediated dealkylation of ethoxyresorufin and pentoxyresorufin.

Liver samples were divided into two equal portions and weighed. One portion was homogenized in ice-cold 1.15% potassium chloride in a 0.1 M Tris hydrochloride buffer (pH 7.5) and centrifuged. The homogenate was again centrifuged to remove nuclei and mitochondrial debris. The supernatant was centrifuged in a refrigerated ultracentrifuge, and the resulting microsomal pellet was resuspended in a microsomal dilution buffer. The suspensions were frozen at approximately –70° C for 1 week before being analyzed for alkoxyresorufin dealkylase activities and protein content.

Alkoxyresorufin dealkylase activities were determined with methods derived from Burke *et al.* (1985), Lubet *et al.* (1985), and Rutten *et al.* (1992). A mixture of Tris hydrochloride assay buffer (pH 7.8), an NADPH-generating system (NADP, glucose-6-phosphate, magnesium chloride hexahydrate, and glucose-6-phosphate dehydrogenase), and the microsomal suspension was prewarmed in a spectrofluorometer maintained at approximately 37° C. The amount of microsomal protein added to the mixture ranged from 0.082 to 1.42 mg. The enzymatic reaction was initiated with ethoxyresorufin or pentoxyresorufin and was analyzed for resorufin formation at an excitation wavelength of 530 nm and an emission wavelength of 585 nm. The reaction was scanned on the spectrofluorometer for approximately 2 to 10 minutes, until a linear reaction was observed. Enzyme-mediated increases in fluorescence were compared to mean fluorescence values from resorufin standard curves. Protein values were determined according to a modification of the method of Winsten (1965).

The remaining portion of liver was used to prepare microsomes for the determination of cytochrome P_{450} content. The liver was homogenized in ice-cold 1.15% potassium chloride in 0.1 M potassium phosphate buffer (pH 7.4) with 20% glycerol and centrifuged. The homogenate was again centrifuged to remove nuclei and mitochondrial debris. The supernatant was centrifuged in a refrigerated ultracentrifuge. The resulting

microsomal pellet was resuspended in 1.15% potassium chloride in 0.1 M potassium phosphate buffer (pH 7.4) with 20% glycerol and again centrifuged in an effort to remove residual hemoglobin; this step was repeated, and a portion of the resulting microsomal suspension was then used to determine cytochrome P_{450} content. The remaining suspension was stored frozen at approximately −70° C for protein determinations.

Cytochrome P_{450} content was determined with a modification of the carbon monoxide-difference spectrum method of Omura and Sato (1964). Sodium dithionite was mixed with the microsomal suspension and allowed to equilibrate for 3 to 5 minutes. Carbon monoxide was then bubbled through the sample (approximately 1 bubble/second) for 20 seconds, and the sample was again allowed to equilibrate for 3 to 5 minutes. The spectrum difference was then recorded between 400 and 510 nm on a spectrophotometer in the split-beam mode. The cytochrome P_{450} content was calculated from the optical density difference (approximately 450 to 480 nm) and the molar extinction coefficient of 91 $mM^{-1}cm^{-1}$.

TABLE 3
Experimental Design and Materials and Methods in the 14-Week Feed Studies of Benzophenone

Study Laboratory	Battelle Columbus Laboratories (Columbus, OH)
Strain and Species	F344/N rats $B6C3F_1$ mice
Animal Source	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies	Rats: 13 days (males) or 14 days (females) Mice: 16 days (males) or 17 days (females)
Average Age When Studies Began	Rats: 8 weeks (males and females) Mice: 8 weeks (males), 9 weeks (females)
Date of First Exposure	Rats: 4 January 1993 (males), 5 January 1993 (females) Mice: 7 January 1993 (males), 8 January 1993 (females)
Duration of Exposure	14 weeks (7 days per week)
Date of Last Exposure	Rats: 5 April 1993 (males), 6 April 1993 (females) Mice: 8 April 1993 (males), 9 April 1993 (females)
Necropsy Dates	Rats: 5 April 1993 (males), 6 April 1993 (females) Mice: 8 April 1993 (males), 9 April 1993 (females)
Average Age at Necropsy	Rats: 21 weeks (males), 21 or 22 weeks (females) Mice: 22 weeks
Size of Study Groups	10 males and 10 females
Method of Distribution	Animals were distributed randomly into groups of approximately equal initial mean body weights.
Animals per Cage	Rats: 5 Mice: 1
Method of Animal Identification	Tail tattoo
Diet	NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*
Water	Columbus Municipal Supply available *ad libitum*; automatic watering system (Edstrom Industries, Waterford, NJ)
Cages	Polycarbonate (Lab Products, Inc., Garfield, NJ)
Bedding	Sani-Chip® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ); rats changed twice weekly and mice changed once a week
Cage Filters	Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH)
Animal Room Environment	Temperature: 72° ± 3° F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Exposure Concentrations	0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm in feed, available *ad libitum*
Type and Frequency of Observation	Observed twice daily; animals were weighed initially, weekly, and at the end of the studies. Clinical findings were recorded weekly. Feed consumption was recorded two times per week by cage (rats) or by animal (mice).
Method of Sacrifice	Anesthetized with CO_2:O_2

TABLE 3
Experimental Design and Materials and Methods in the 14-Week Feed Studies of Benzophenone

Necropsy	A complete necropsy was performed on core study rats and mice. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology	Blood was collected from the retroorbital sinus of rats and mice. Rats in the clinical pathology study groups were evaluated on days 4 and 22. Core study animals were evaluated at the end of the studies. ***Hematology***: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet count; and total leukocyte count and differentials ***Clinical Chemistry***: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and total bile salts
Histopathology	A complete histopathologic evaluation was performed on male and female rats and male mice in the 0, 10,000, and 20,000 ppm groups; on female mice in the 0 and 20,000 ppm groups; and on all animals that died early. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone and marrow, brain (three sections), clitoral gland, esophagus, eye, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver (two sections), lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, stomach (forestomach and glandular stomach), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. Organs examined in the lower exposure groups included the liver, kidney, bone marrow, and testis of rats and the liver of mice.
Sperm Motility and Vaginal Cytology Evaluations	Sperm motility and vaginal cytology evaluations were performed on core study rats in the 0, 1,250, 2,500, and 5,000 ppm groups and mice in the 0, 2,500, 5,000, and 10,000 ppm groups at the end of the studies. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, epididymal spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percentage of cycle spent in the various estrous stages.
Ocular Studies	Special histopathologic studies were conducted to evaluate the potential effects of benzophenone on the eyes of core study rats and mice. At the end of the 14-week studies, the eye lens, retina, and other ocular structures of five animals per group from the control, 10,000 ppm (rats and male mice), and 20,000 ppm (female mice) groups were examined.
Cytochrome P_{450} Analyses	Liver samples were collected from core study rats and mice (five males and five females per group) and analyzed for cytochrome P_{450} content and for ethoxyresorufin deethylase and pentoxyresorufin dealkylase activities.

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, cytochrome P_{450}, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). If the P value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test. The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. Extreme values identified by the statistical test were reviewed by NTP personnel before being eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations.

QUALITY ASSURANCE

The 14-week studies were conducted in compliance with United States Food and Drug Administration Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance Unit of Battelle Columbus Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Mortelmans *et al.* (1986). Benzophenone was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX) and was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at

37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of five doses of benzophenone. The high dose was limited by toxicity. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by benzophenone exposure; the limiting factor was toxicity. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male B6C3F_1 mice were injected intraperitoneally three times at 24-hour intervals with benzophenone dissolved in corn oil; the total dosing volume was 0.4 mL. Solvent control mice were injected with 0.4 mL of corn oil only. The positive control mice received injections of 25 mg cyclophosphamide/kg. The mice were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five animals per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial was considered positive if the trend test P value was less than or equal to 0.025 or if the P value for any single dose group was less than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive

trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and differing results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Report represent a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

RATS

One female in the 20,000 ppm group died on day 12 of the study (Table 4). Due to the significantly lower mean body weight gains of males and females exposed to 20,000 ppm compared to those of the controls, these rats were removed from the study during week 6; all other rats survived to the end of the study. Body weights of male rats exposed to 2,500 ppm or greater and female rats in all exposed groups were significantly less than those of the controls (Table 4 and Figure 1). Clinical findings included thinness and lethargy in male and female rats in the 20,000 ppm groups and thinness in males in the 10,000 ppm group. Two males in the 20,000 ppm group also had prolapsed penises. Male and female rats exposed to 20,000 ppm consumed less feed than the controls (Table 4). Feed consumption by other exposed groups was generally similar to that by the controls; however, no attempts were made to estimate feed spillage.

TABLE 4
Survival, Body Weights, and Feed and Compound Consumption of Rats in the 14-Week Feed Study of Benzophenone

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Average Feed Consumption[c] (g/day)	Average Dose[c] (mg/kg/day)
		Initial	Final	Change			
Male							
0	10/10	185 ± 3	366 ± 7	181 ± 6		16.2	
1,250	10/10	185 ± 2	362 ± 6	177 ± 5	99	16.5	75
2,500	10/10	187 ± 2	339 ± 5**	153 ± 4**	93	16.3	155
5,000	10/10	186 ± 3	330 ± 5**	144 ± 4**	90	16.3	316
10,000	10/10	185 ± 2	268 ± 5**	83 ± 4**	73	15.8	698
20,000	0/10[d]	185 ± 2	—	—	—	6.0	839
Female							
0	10/10	131 ± 2	210 ± 3	79 ± 3		10.4	
1,250	10/10	128 ± 2	191 ± 2**	63 ± 2**	91	9.8	77
2,500	10/10	128 ± 2	185 ± 2**	57 ± 1**	88	10.0	160
5,000	10/10	128 ± 2	177 ± 2**	50 ± 2**	84	9.5	311
10,000	10/10	129 ± 1	176 ± 2**	47 ± 2**	84	10.8	708
20,000	0/10[e]	129 ± 1	—	—	—	5.5	982

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Average of individual consumption values for weeks 1 to 14 for all animals in the base study. For males and females in the 20,000 ppm groups, consumption values are given for weeks 1 to 6 only.
[d] Week of death: all died during week 6
[e] Week of death: one during week 2; nine during week 6

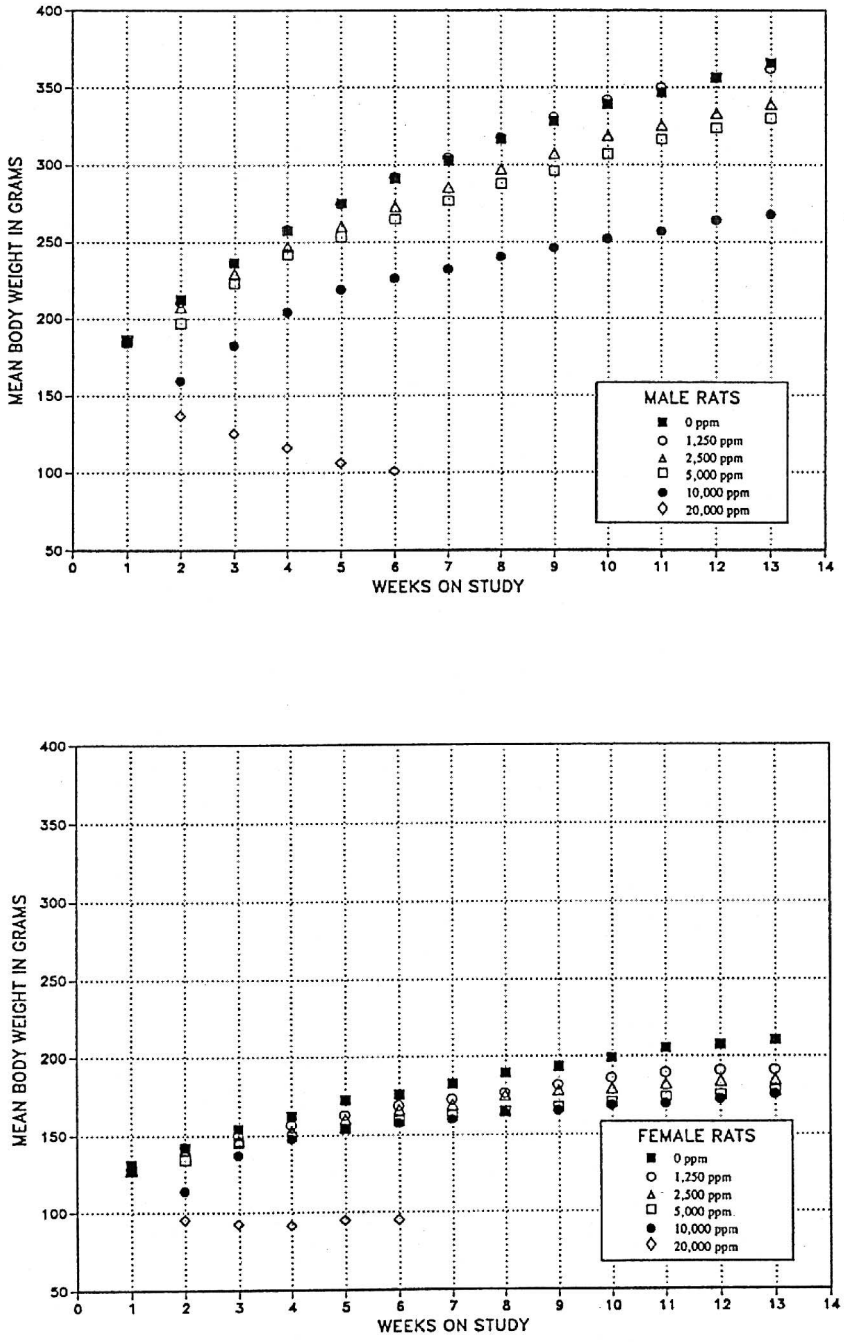


FIGURE 1
Body Weights of Rats Administered Benzophenone in Feed for 14 Weeks

The hematology data for rats are listed in Tables 5 and B1. Because of the mortality and early removal of 20,000 ppm animals, no hematology or clinical chemistry evaluations were performed on these rats at week 14. On day 4, an exposure concentration-related erythrocytosis, evidenced by increases in hematocrit values, hemoglobin concentrations, and erythrocyte counts, occurred in the 2,500 ppm or greater male and female rats. The erythrocytosis was transient and, by day 22, was replaced by evidence of a decreased erythron, as demonstrated by generally decreased hematocrit values, hemoglobin concentrations, and erythrocyte counts in the 2,500 ppm or greater groups; this erythron effect also was present at week 14. In exposed male rats, the anemia was accompanied by increases in reticulocyte counts, suggesting an erythropoietic response. Also, there were minimal to mild, exposure concentration-related increases in mean cell volume and significant, but minimal, decreases in mean cell hemoglobin concentration in males, indicating an erythrocytic macrocytosis and a tendency toward hypochromia. In exposed female rats, however, reticulocyte counts were generally unaffected and the erythrocytes demonstrated a tendency towards microcytosis and hypochromia, as evidenced by decreases in mean cell volumes, mean cell hemoglobin concentrations, and mean cell hemoglobin values. On day 4, minimal, exposure-related increases in platelet counts occurred in the 5,000 ppm or greater male and female rats. This early increase in platelet counts was transient and, by day 22, was replaced by minimal decreases; the platelet count decreases persisted through week 14 in 10,000 ppm males and 5,000 and 10,000 ppm females.

TABLE 5
Selected Hematology Data for Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n						
Day 4	10	10	9	10	10	10
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	44.3 ± 0.4	45.2 ± 0.4	47.9 ± 0.4**	50.0 ± 0.4**	50.7 ± 0.6**	51.4 ± 0.7 **
Day 22	48.1 ± 0.5	47.5 ± 0.4	47.3 ± 0.4	47.2 ± 0.5	47.2 ± 0.7	47.3 ± 0.4
Week 14	50.0 ± 0.6	49.2 ± 0.7	49.9 ± 0.6	49.3 ± 0.7	49.1 ± 0.6	
Hemoglobin (g/dL)						
Day 4	15.4 ± 0.1	15.6 ± 0.1	16.3 ± 0.1**	17.0 ± 0.1**	17.2 ± 0.1**	17.5 ± 0.2**
Day 22	16.5 ± 0.1	16.1 ± 0.1*	15.8 ± 0.2**	15.7 ± 0.2**	15.5 ± 0.3**	15.8 ± 0.1**
Week 14	16.9 ± 0.2	16.4 ± 0.2	16.4 ± 0.1*	16.1 ± 0.2*	15.8 ± 0.1**	
Erythrocytes ($10^6/\mu$L)						
Day 4	7.44 ± 0.07	7.60 ± 0.09	8.01 ± 0.08**	8.31 ± 0.08**	8.45 ± 0.11**	8.49 ± 0.12**
Day 22	8.50 ± 0.09	8.23 ± 0.08	8.12 ± 0.08*	8.00 ± 0.07**	7.97 ± 0.13**	8.35 ± 0.08
Week 14	9.29 ± 0.12	8.93 ± 0.10	8.98 ± 0.11	8.65 ± 0.14**	8.48 ± 0.11**	
Platelets ($10^3/\mu$L)						
Day 4	966.0 ± 11.1	922.3 ± 27.6	955.7 ± 22.0	1,069.5 ± 17.0**	1,087.1 ± 22.0**	1,050.8 ± 23.0**
Day 22	784.3 ± 14.7	827.6 ± 14.0	778.6 ± 11.4	793.0 ± 21.1	705.0 ± 15.3**	547.5 ± 15.7**
Week 14	750.9 ± 11.1	783.4 ± 18.4	827.0 ± 12.5*	796.5 ± 16.0	717.7 ± 11.5	
Female						
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	10	9	10
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	45.5 ± 0.4	46.2 ± 0.5	48.1 ± 0.5**	50.2 ± 0.4**	51.1 ± 0.3**	51.0 ± 0.5**
Day 22	47.5 ± 0.5	47.3 ± 0.4	47.7 ± 0.4	45.8 ± 0.4*	46.4 ± 0.6	44.9 ± 0.7**
Week 14	47.1 ± 0.5	47.7 ± 0.6	46.2 ± 0.7	46.2 ± 0.6	45.4 ± 0.4*	
Hemoglobin (g/dL)						
Day 4	15.6 ± 0.1	15.6 ± 0.2	16.3 ± 0.2**	16.9 ± 0.2**	17.3 ± 0.1**	17.2 ± 0.1**
Day 22	16.2 ± 0.1	15.7 ± 0.1*	15.6 ± 0.1**	15.2 ± 0.1**	15.2 ± 0.2**	15.1 ± 0.2**
Week 14	16.0 ± 0.2	16.1 ± 0.2	15.5 ± 0.2	15.3 ± 0.2*	14.5 ± 0.1**	
Erythrocytes ($10^6/\mu$L)						
Day 4	7.51 ± 0.07	7.50 ± 0.12	7.83 ± 0.10*	8.18 ± 0.09**	8.29 ± 0.08**	8.39 ± 0.07**
Day 22	7.83 ± 0.09	7.61 ± 0.06	7.76 ± 0.06	7.46 ± 0.07*	7.58 ± 0.11	7.74 ± 0.12
Week 14	8.07 ± 0.09	8.22 ± 0.12	8.03 ± 0.11	8.08 ± 0.12	7.95 ± 0.09	
Platelets ($10^3/\mu$L)						
Day 4	861.2 ± 13.0	898.3 ± 14.3	876.2 ± 12.1	943.0 ± 14.3**	996.0 ± 23.0**	1,006.2 ± 12.2**
Day 22	765.6 ± 21.6	764.4 ± 20.5	778.1 ± 11.5	795.4 ± 8.9	636.6 ± 23.1**	622.3 ± 14.0**
Week 14	780.2 ± 18.6	771.1 ± 15.8	788.8 ± 28.6	716.8 ± 13.0*	671.0 ± 11.8**	

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

There were several exposure-related alterations in the serum clinical chemistry evaluations for male and female rats (Tables 6 and B2). On day 4, alanine aminotransferase activities were minimally to mildly increased in all groups of exposed rats. By day 22 and week 14, this alteration ameliorated and alanine aminotransferase activity was increased only in the 10,000 ppm females and 20,000 ppm males and females. The activity of sorbitol dehydrogenase, another marker of hepatocellular leakage, was increased only in the 10,000 ppm females at week 14. The concentrations of bile salts, a marker of cholestasis or altered hepatic function, was minimally to markedly increased for all exposed groups at various time points. In contrast, activities of alkaline phosphatase, another marker of cholestasis, were minimally to mildly decreased for all exposed groups of animals at all time points. On day 4, total protein concentrations were minimally decreased in the 2,500 ppm or greater male and female rats. By day 22, the slight hypoproteinemia was replaced by a hyperproteinemia, demonstrated by increased total protein concentrations. The hyperproteinemia persisted at week 14 in all groups of exposed females. On day 22 and at week 14, the hyperproteinemia was accompanied by a hyperalbuminemia, evidenced by increased albumin concentrations. In animals, hyperalbuminemia has not been associated with increased albumin production but has been used as an indicator of dehydration (Kaneko, 1989); the hyperproteinemia would be consistent with the hyperalbuminemia. On day 22, there was evidence of a minimal azotemia, demonstrated by increased urea nitrogen concentrations, in the 10,000 ppm male and 20,000 ppm male and female rats. Considering the dehydration indicated by hyperalbuminemia, the increased urea nitrogen concentrations would be consistent with a prerenal azotemia (Finco, 1989; Ragan, 1989). In contrast, creatinine concentration, another marker of renal function, generally decreased minimally with increasing exposure concentration in the 5,000 ppm or greater male and female rats at all time points. It has been demonstrated that serum creatinine concentrations are related to muscle mass (Finco, 1989; Ragan, 1989). In this study, rats in the higher exposure groups weighed less than control animals; thus, the decreases in creatinine concentration would be consistent with muscle mass differences between the control and exposed animals.

TABLE 6
Selected Clinical Chemistry Data for Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	0
Male						
Alanine aminotransferase (IU/L)						
Day 4	43 ± 1	52 ± 4**	56 ± 3**	59 ± 3**	63 ± 2**	61 ± 3**
Day 22	50 ± 2	41 ± 1	40 ± 2	39 ± 1	50 ± 2	97 ± 4*
Week 14	71 ± 5	67 ± 5	55 ± 4	61 ± 4	90 ± 6	
Alkaline phosphatase (IU/L)						
Day 4	1,307 ± 26	1,237 ± 27	1,141 ± 32**	912 ± 18**	847 ± 35**	799 ± 28**
Day 22	951 ± 17	798 ± 19**	715 ± 11**	647 ± 14**	725 ± 27**	664 ± 25**
Week 14	574 ± 17	473 ± 14**	458 ± 8**	404 ± 11**	485 ± 15**	
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	21 ± 2	23 ± 2*	18 ± 1	18 ± 1	15 ± 1
Day 22	19 ± 1	16 ± 1*	16 ± 1*	16 ± 1	16 ± 1	10 ± 1**
Week 14	30 ± 4	29 ± 3	25 ± 2	27 ± 3	34 ± 3	
Bile salts (μmol/L)						
Day 4	31.3 ± 2.9	32.2 ± 4.1	32.7 ± 3.6	42.5 ± 3.5	33.1 ± 2.1	29.8 ± 1.7
Day 22	24.1 ± 2.1	28.3 ± 2.5	33.3 ± 3.2*[b]	34.2 ± 2.7**	70.4 ± 11.0**	330.4 ± 26.0**
Week 14	21.5 ± 2.1	24.7 ± 0.9*	27.2 ± 2.7*	29.0 ± 1.4**	52.1 ± 4.2**	
Female						
Alanine aminotransferase (IU/L)						
Day 4	36 ± 2	42 ± 2*	48 ± 3**	54 ± 3**	53 ± 3**	59 ± 9**
Day 22	37 ± 1	35 ± 1	36 ± 1	35 ± 1	47 ± 1**	95 ± 4**
Week 14	48 ± 3	49 ± 2	43 ± 2	47 ± 3	89 ± 11**	
Alkaline phosphatase (IU/L)						
Day 4	1,048 ± 25	925 ± 36**	824 ± 37**	680 ± 22**	599 ± 13**	596 ± 12**
Day 22	766 ± 24	585 ± 19**	516 ± 12**	531 ± 15**	635 ± 22	698 ± 35
Week 14	495 ± 15	369 ± 12**	331 ± 11**	333 ± 18**	400 ± 21**	
Sorbitol dehydrogenase (IU/L)						
Day 4	18 ± 1	17 ± 1	15 ± 1	17 ± 1	17 ± 1	24 ± 5
Day 22	20 ± 2	16 ± 1	16 ± 1	15 ± 1	18 ± 2	13 ± 1**
Week 14	21 ± 2	22 ± 1	18 ± 1	21 ± 1	43 ± 6**	
Bile salts (μmol/L)						
Day 4	33.0 ± 5.2	53.9 ± 4.9*	58.0 ± 5.9*	49.6 ± 4.5	46.8 ± 6.8	40.3 ± 3.5
Day 22	28.5 ± 3.4	38.9 ± 3.4*	44.8 ± 4.2**	54.1 ± 3.9**	82.5 ± 12.5**	435.4 ± 22.8**
Week 14	41.7 ± 4.9	38.5 ± 3.7	44.2 ± 4.0	39.7 ± 2.6	71.0 ± 3.7**	

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

The kidney and liver weights of all exposed groups were significantly greater than those of the controls, except for the absolute right kidney weight of females in the 1,250 ppm group (Tables 7 and C1). The absolute heart and thymus weights of males in the 10,000 ppm group and the absolute thymus weights of females in the 5,000 and 10,000 ppm groups were significantly less than those of the controls (Table C1). Other differences in the relative organ weights of exposed males and females generally reflected differences in mean body weights.

At necropsy, the only gross findings considered related to benzophenone exposure were small seminal vesicles in three 20,000 ppm males. Microscopically, no specific changes were seen other than overall decreased size.

TABLE 7
Selected Organ Weight Data for Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10
Male					
Necropsy body wt	379 ± 7	374 ± 5	352 ± 5**	340 ± 5**	276 ± 5**
R. Kidney					
Absolute	1.308 ± 0.027	1.516 ± 0.041**	1.615 ± 0.039**	2.056 ± 0.091**	1.782 ± 0.051**
Relative	3.46 ± 0.05	4.06 ± 0.08**	4.59 ± 0.09**	6.03 ± 0.20**	6.46 ± 0.17**
Liver					
Absolute	13.647 ± 0.261	17.338 ± 0.354**	19.463 ± 0.548**	21.629 ± 0.740**	19.379 ± 0.387**
Relative	36.10 ± 0.56	46.40 ± 0.51**	55.29 ± 0.96**	63.52 ± 1.39**	70.23 ± 1.33**
Female					
Necropsy body wt	219 ± 3	197 ± 2**	192 ± 3**	182 ± 2**	180 ± 2**
R. Kidney					
Absolute	0.744 ± 0.014	0.792 ± 0.013	0.832 ± 0.014**	0.821 ± 0.015**	0.960 ± 0.033**
Relative	3.40 ± 0.06	4.03 ± 0.07**	4.34 ± 0.05**	4.51 ± 0.07**	5.33 ± 0.15**
Liver					
Absolute	7.566 ± 0.179	8.446 ± 0.136**	9.653 ± 0.284**	10.432 ± 0.175**	12.643 ± 0.308**
Relative	34.52 ± 0.54	42.99 ± 0.82**	50.30 ± 1.02**	57.31 ± 0.80**	70.21 ± 1.19**

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). All 20,000 ppm rats died before the end of the study.

Increased kidney weights were associated with a spectrum of renal changes in exposed rats (Tables 8, A1, and A2). One change found predominantly in 20,000 ppm animals, which died early, was papillary necrosis characterized by acute coagulative necrosis of the distal tips of the renal papillae. Unique lesions seen in rats that died early as well as in survivors were well-demarcated, wedge-shaped areas of prominent tubule dilatation. These areas were based at the capsular surface and extended deep into the medulla. Within these areas, tubules were dilated and usually empty, although some contained fine, granular eosinophilic material. The dilated tubules were lined by epithelial cells with various tinctorial alterations. In male rats, this change was present at exposure concentrations of 2,500 ppm and higher, while in females it occurred only at 10,000 and 20,000 ppm. Increased incidences and/or severities of focal tubule regeneration was observed in all exposed groups. Foci of tubule regeneration may be seen as a component of spontaneous chronic nephropathy in control rats in the 14-week studies. These foci consist of small clusters of tubules with more basophilic cytoplasm and slightly enlarged and vesicular nuclei. In exposed males and females, the numbers of these foci were increased relative to controls. Tubules containing eosinophilic protein casts were found in most male rats surviving to the end of the study and less commonly in females. Based on these findings, a no-effect level for kidney changes was not reached in rats.

Exposure-related increases in liver weights were attributed to hypertrophy and/or cytoplasmic vacuolization of hepatocytes. Hypertrophy was characterized by slight increases in the size of centrilobular hepatocytes and was present in all exposed groups of females. Vacuolization occurred in all exposed groups of males and consisted of randomly scattered hepatocytes with uniformly sized vacuoles in the cytoplasm imparting a "bubbly" appearance. These changes were of minimal severity. A change present only in 20,000 ppm males was minimal hyperplasia of immature bile ductules from portal areas into adjacent sinusoids.

Two lesions were seen primarily in 20,000 ppm rats, which died early, and were considered secondary to reduced body weight gain and inanition. These were hypocellularity of the bone marrow in males and females and poorly developed seminiferous tubules in males.

No changes were observed in the microscopic evaluation of the lens, retina, and other ocular structures of the control or 10,000 ppm rats.

TABLE 8
Incidence of Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
Bone Marrow[a]	10	10	10	10	10	10
Atrophy[b]	0	0	0	0	0	10** (3.7)[c]
Kidney	10	10	10	10	10	10
Mineralization	0	0	0	5* (1.0)	10** (1.1)	0
Papilla, Necrosis	0	0	0	0	2 (1.0)	6** (1.2)
Renal Tubule, Protein Casts	0	8** (1.0)	8** (1.0)	9** (1.2)	10** (1.3)	0
Renal Tubule, Dilatation	0	0	6** (1.0)	8** (1.0)	9** (1.3)	8** (1.8)
Renal Tubule, Regeneration	10 (1.0)	10 (2.0)	10 (1.5)	10 (2.0)	10 (2.0)	8 (1.6)
Liver	10	10	10	10	10	10
Bile Duct, Hyperplasia	0	0	0	0	0	9** (1.1)
Hepatocyte, Hypertrophy	0	0	0	0	5* (1.2)	7** (1.0)
Hepatocyte, Vacuolization Cytoplasmic	1 (1.0)	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.4)	10** (1.2)
Female						
Bone Marrow	10	10	10	10	10	10
Atrophy	0	0	0	0	2 (1.0)	10** (3.8)
Kidney	10	10	10	10	10	9
Mineralization	10 (1.5)	10 (1.6)	10 (1.6)	10 (1.1)	10 (1.2)	9 (1.2)
Papilla, Necrosis	0	0	0	0	0	3 (1.0)
Renal Tubule, Protein Casts	0	0	2 (1.0)	0	4* (1.0)	0
Renal Tubule, Dilatation	0	0	0	0	3 (1.0)	5* (1.6)
Renal Tubule, Regeneration	3 (1.0)	8* (1.0)	6 (1.0)	6 (1.0)	9** (1.2)	7 (1.6)
Liver	10	10	10	10	10	9
Hepatocyte, Hypertrophy	0	2 (1.0)	8** (1.0)	10** (1.1)	10** (1.0)	7** (1.0)
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	9** (1.1)	10** (1.0)	7** (1.1)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

There were no significant differences in sperm motility or vaginal cytology parameters between exposed and control males or females (Tables D1 and D2).

Males and females exposed to 2,500 or 5,000 ppm and females in the 1,250 ppm group had significantly greater cytochrome P_{450} concentrations than the controls (Table E1). Pentoxyresorufin dealkylase activities (expressed as pmol/min per mg protein or per nmol cytochrome P_{450}) were generally significantly greater in exposed rats than in the controls.

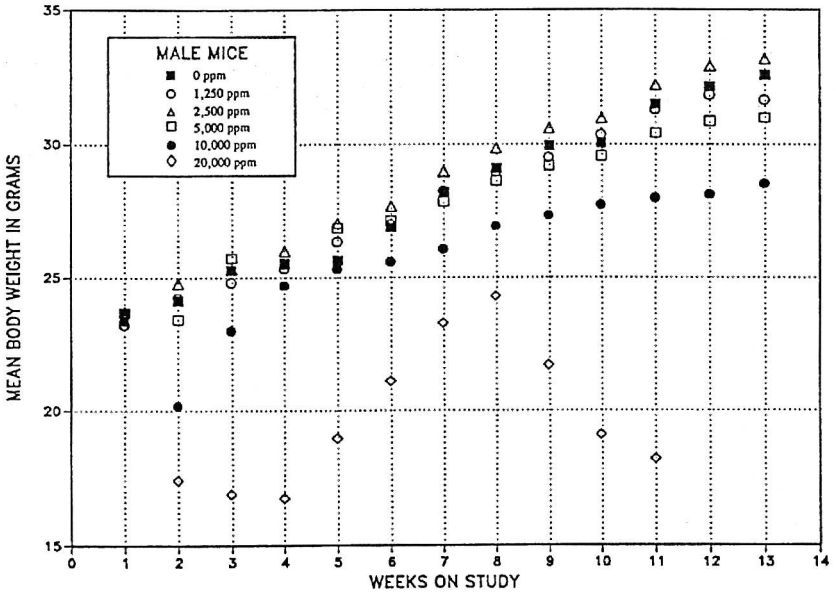
MICE

One male in the 1,250 ppm group was accidentally killed on day 26 of the study (Table 9). Four males exposed to 20,000 ppm died during week 1; one male and one female exposed to 20,000 ppm died during week 2. Due to the significantly lower mean body weight gains of some males and females exposed to 20,000 ppm compared to those of the controls, two males were removed from the study during week 10, three males were removed during week 11, and three females were removed during week 12. Body weights of male mice exposed to 10,000 ppm and female mice exposed to 5,000 ppm or greater were significantly less than those of the controls (Table 9 and Figure 2). Surviving females in the 20,000 ppm group lost weight during the study. Clinical findings included thinness and lethargy in male and female mice in the 20,000 ppm groups. Male and female mice exposed to 20,000 ppm consumed less feed than the controls (Table 9). Spilled feed was observed in the cages of control and exposed mice, particularly in females. In the two highest exposure groups, spillage might have been due to impalatibility of the diet.

TABLE 9
Survival, Body Weights, and Feed and Compound Consumption of Mice in the 14-Week Feed Study of Benzophenone

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Average Feed Consumption[c] (g/day)	Average Dose[c] (mg/kg/day)
		Initial	Final	Change			
Male							
0	10/10	23.6 ± 0.3	32.6 ± 0.7	8.9 ± 0.6		4.4	
1,250	9/10[d]	23.2 ± 0.2	31.7 ± 1.2	8.6 ± 1.0	97	4.4	200
2,500	10/10	23.7 ± 0.2	33.2 ± 0.8	9.5 ± 0.7	102	4.6	404
5,000	10/10	23.7 ± 0.4	31.0 ± 0.6	7.3 ± 0.4	95	4.3	786
10,000	10/10	23.3 ± 0.2	28.5 ± 0.5**	5.2 ± 0.3**	88	4.1	1,583
20,000	0/10[e]	23.2 ± 0.3	—	—	—	3.4	3,285
Female							
0	10/10	19.6 ± 0.1	26.7 ± 0.5	7.1 ± 0.5		5.1	
1,250	10/10	19.5 ± 0.2	26.5 ± 0.3	7.1 ± 0.3	99	4.9	266
2,500	10/10	19.6 ± 0.2	26.8 ± 0.4	7.2 ± 0.4	100	5.0	539
5,000	10/10	19.9 ± 0.2	24.8 ± 0.3**	4.9 ± 0.3**	93	4.6	1,029
10,000	10/10	19.6 ± 0.2	23.6 ± 0.2**	4.0 ± 0.1**	88	4.1	1,898
20,000	6/10[f]	19.7 ± 0.2	16.5 ± 0.3**	−3.3 ± 0.4**	62	3.8	4,199

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Average of individual consumption values for weeks 1 to 14 for all animals in the base study. For males in the 20,000 ppm group, consumption values are given for weeks 1 to 11 only.
[d] Week of death: 4 (accidental death)
[e] Week of death: 1, 1, 1, 1, 2, 10, 10, 11, 11, 11
[f] Week of death: 2, 12, 12, 12



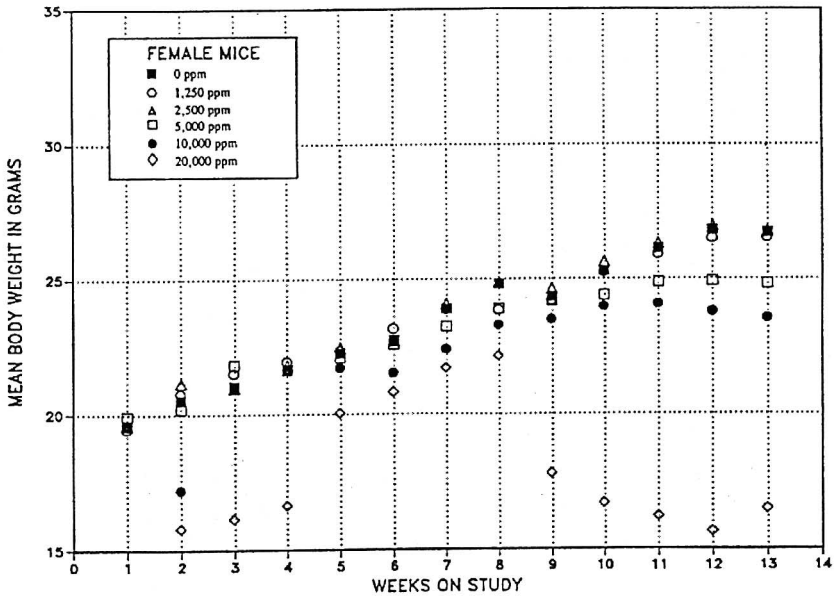


FIGURE 2
Body Weights of Mice Administered Benzophenone in Feed for 14 Weeks

The hematology and clinical chemistry data for mice are listed in Tables B3 and B4. Because of the mortality and early removal of the 20,000 ppm male mice, no hematology or clinical chemistry evaluations were performed at week 14 for this group. Similar to the rat study, male mice at week 14 showed evidence of an anemia in the 5,000 and 10,000 ppm groups, demonstrated by minimal decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. In contrast, female mice in the 5,000, 10,000, and 20,000 ppm groups showed evidence of a minimal erythrocytosis, indicated by increases in hematocrit values, hemoglobin concentrations, and/or erythrocyte counts. The erythrocytosis would be consistent with hemoconcentration caused by dehydration and would be supported by the minimal increases in albumin and total protein concentrations that occurred in various groups of exposed females. Also similar to the rat study, there was evidence of a hepatic effect in mice. This was evidenced by increases in total bile salt concentrations and sorbitol dehydrogenase activities in the 2,500 ppm or greater male and all exposed female groups. Alkaline phosphatase activity also was increased in the 20,000 ppm females.

The kidney weights of males exposed to 2,500 ppm or greater and the liver weights of all groups of exposed males were significantly greater than those of the controls (Tables 10 and C2). The absolute and relative liver weights of exposed females in all groups except the 20,000 ppm group were also significantly greater than those of the controls; however, the absolute liver weight of females in the 20,000 ppm group was significantly less than that of the controls. Exposed females in all groups except the 20,000 ppm group had slightly greater absolute kidney weights than the controls, and this difference was significant in the 2,500 and 10,000 ppm groups; females exposed to 2,500 ppm or greater had significantly greater relative kidney weights than the controls. The absolute and relative thymus weights of females exposed to 20,000 ppm and the absolute thymus weights of females exposed to 5,000 or 10,000 ppm were significantly less than those of the controls. Other differences in organ weights between exposed and control mice were considered to reflect the lower body weights of exposed mice.

TABLE 10
Selected Organ Weight Data for Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n	10	9	10	10	10	0
Necropsy body wt	33.3 ± 0.8	33.2 ± 0.8	33.7 ± 0.8	31.8 ± 0.6	28.6 ± 0.5**	
R. Kidney						
Absolute	0.272 ± 0.007	0.270 ± 0.006	0.301 ± 0.005**	0.312 ± 0.007**	0.284 ± 0.008**	
Relative	8.19 ± 0.18	8.17 ± 0.20	8.96 ± 0.18**	9.80 ± 0.15**	9.92 ± 0.18**	
Liver						
Absolute	1.590 ± 0.056	2.157 ± 0.099**	2.467 ± 0.061**	2.762 ± 0.090**	2.822 ± 0.052**	
Relative	47.62 ± 0.64	65.18 ± 3.33**	73.15 ± 0.72**	86.64 ± 1.43**	98.60 ± 1.44**	
Female						
n	10	10	10	10	10	6
Necropsy body wt	27.5 ± 0.5	27.7 ± 0.3	27.5 ± 0.4	25.6 ± 0.4**	24.7 ± 0.3**	16.8 ± 0.3**
R. Kidney						
Absolute	0.179 ± 0.002	0.183 ± 0.008	0.199 ± 0.005*	0.191 ± 0.002	0.199 ± 0.003*	0.175 ± 0.004
Relative	6.54 ± 0.12	6.61 ± 0.26	7.25 ± 0.17**	7.45 ± 0.07**	8.09 ± 0.06**	10.41 ± 0.09**
Liver						
Absolute	1.343 ± 0.028	1.850 ± 0.039**	2.091 ± 0.046**	2.260 ± 0.057**	2.422 ± 0.037**	1.315 ± 0.034**
Relative	48.88 ± 0.91	66.80 ± 1.03**	76.09 ± 0.85**	88.10 ± 1.14**	98.28 ± 1.39**	78.32 ± 1.34**
Thymus						
Absolute	0.055 ± 0.004	0.052 ± 0.002	0.049 ± 0.002	0.046 ± 0.002*	0.045 ± 0.002**	0.015 ± 0.002**
Relative	2.02 ± 0.13	1.89 ± 0.09	1.79 ± 0.09	1.80 ± 0.06	1.82 ± 0.06	0.90 ± 0.12**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

No exposure-related lesions were observed grossly at necropsy. Significant microscopic findings were limited to centrilobular hypertrophy in the liver which corresponded to increased liver weights. This change was characterized by an increase in the size of the cytoplasm and nucleus of centrilobular cells. Severity of hepatocyte hypertrophy was exposure concentration dependent, with marked severity in all 20,000 ppm mice except those that died after only 1 to 2 weeks of exposure to benzophenone (Tables 11, A3, and A4). Because there was an increased incidence of minimal hypertrophy relative to control groups at 1,250 ppm, a no-effect level for the liver was not reached. Cytoplasmic vacuolization of randomly scattered hepatocytes was another liver effect seen in three 20,000 ppm males and in females exposed to 5,000 ppm or greater.

TABLE 11
Incidence of Liver Lesions in Mice in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Inflammation, Chronic Active[a]	5 (1.0)[b]	4 (1.0)	8 (1.0)	8 (1.0)	5 (1.0)	1 (1.0)
Centrilobular, Hypertrophy	3 (1.0)	8* (1.0)	10** (2.0)	10** (3.0)	10** (3.0)	10** (3.2)
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	0	0	3 (2.0)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Inflammation, Chronic Active	8 (1.0)	9 (1.1)	9 (1.0)	9 (1.0)	9 (1.0)	3 (1.0)
Centrilobular, Hypertrophy	3 (1.0)	9** (1.0)	10** (2.0)	10** (3.0)	10** (3.0)	10** (4.0)
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	2 (1.0)	9** (2.4)	1 (1.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

Depletion of lymphoid cells in the thymus and spleen was observed only in 20,000 ppm mice and was considered secondary to reduced body weight gain and inanition. Unlike the observations in rats, no microscopic effects that would account for increased kidney weights were seen in mice.

The testis and epididymis weights of male mice in the 10,000 ppm group were significantly less than those of the controls. There were no other significant differences in sperm motility or vaginal cytology parameters between exposed and control males or females (Tables D3 and D4).

Males in all exposed groups except the 10,000 ppm group had significantly greater cytochrome P_{450} concentrations than the controls (Table E2). Ethoxyresorufin deethylase activities (expressed as pmol/min per mg protein) and pentoxyresorufin dealkylase activities (expressed as pmol/min per mg protein or per nmol cytochrome P_{450}) were significantly greater in all groups of exposed male mice than in the controls. For females, both measurements of ethoxyresorufin deethylase activity were significantly greater in all exposed groups than in the controls. Pentoxyresorufin dealkylase activities were generally greater in females in all exposed groups than in the controls; however, the difference for each measurement in the 20,000 ppm group was not significant.

No changes were observed in the microscopic evaluation of the lens, retina, and other ocular structures of the control mice, 10,000 ppm males, or 20,000 ppm females.

GENETIC TOXICOLOGY

Benzophenone showed no evidence of mutagenicity *in vitro* or *in vivo*. Benzophenone (1 to 1,000 μg/plate) did not induce mutations in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without induced liver S9 metabolic activation enzymes (Table F1; Mortelmans *et al.*, 1986). Intraperitoneal injections of 200 to 500 mg benzophenone/kg body weight (three injections at 24-hour intervals) did not induce micronuclei in bone marrow polychromatic erythrocytes of male B6C3F$_1$ mice (Table F2). A small increase in the frequency of micronucleated polychromatic erythrocytes was noted in the 400 mg/kg group, but this was not statistically significant.

DISCUSSION

Benzophenone is used to manufacture insecticides, agricultural chemicals, and hypnotics, antihistamines, and other pharmaceuticals; as an ultraviolet curing agent in sunglasses and ink; as an additive in plastics, coatings, and adhesive formulations; and, occasionally, as a flavor ingredient. Concentrations of benzophenone in food products range from 0.57 ppm in nonalcoholic beverages to 3.27 ppm in frozen dairy products; it may also be an ingredient in baked goods, soft candy, gelatins, and puddings (NAS/NRC, 1979).

The liver, kidney, and hematopoietic system were identified as targets of benzophenone toxicity in rats exposed to benzophenone in feed for 10 to 90 days (USEPA, 1984; Burdock *et al.*, 1991). In those studies, exposure concentration-dependent increases in absolute and relative liver weights and relative kidney weight were observed. Mild degenerative effects were observed in the liver and bone marrow of rats, suggesting that the liver may be the primary target of the toxic effects of benzophenone and that the bone marrow may also be targeted. The no-observed-adverse-effect level (NOAEL) was reached at 20 mg/kg (0.05%) in 13-week studies.

In the current studies, benzophenone was unpalatable at 20,000 ppm. All 20,000 ppm rats had significant body weight loss and were terminated for humane reasons before the end of studies. All male mice and four female mice in the 20,000 ppm group died. There was no exposure-related mortality in the remaining groups. Significantly decreased body weights were observed in all exposed groups of male (except 1,250 ppm) and female rats. Lower body weights were apparent in 10,000 ppm male mice and in 5,000 ppm or greater female mice.

In rats, pathologic changes in the liver and kidney were discovered upon microscopic observation. Increased kidney weights were associated with a spectrum of renal changes in exposed male and female rats. One change found predominantly in 20,000 ppm animals, which died early, was papillary necrosis characterized by acute coagulative necrosis of the distal tips of the renal papillae. Unique lesions seen in rats that died early as well as in survivors were well-demarcated, wedge-shaped areas of prominent tubule dilatation. In male rats, this change was present at exposure concentrations of 2,500 ppm and greater, while in females it occurred only at 10,000 and 20,000 ppm. Foci of tubule regeneration were increased relative to the controls in exposed males and females. Based on these findings, a NOAEL for kidney changes was not reached in either males or females. Exposure-related increases in liver weights were attributed to hypertrophy and/or cytoplasmic vacuolization of hepatocytes.

In mice, significant microscopic findings were limited to centrilobular hypertrophy in the liver, which corresponded to increased liver weights. The severity of hepatocyte hypertrophy was exposure concentration dependent, with marked severity occurring in 20,000 ppm mice. Because there were increased incidences of minimal hypertrophy relative to the control groups at 1,250 ppm, a NOAEL for benzophenone in mice was not reached.

Hematology results indicated that exposure of rats and mice to benzophenone affected the circulating erythroid mass. There was evidence of a transient erythrocytosis in exposed rats on day 4. This alteration would be consistent with hemoconcentration of dehydration. Dehydration as a possible mechanism would be supported by the early and dramatic body weight decreases in the 10,000 ppm and 20,000 ppm groups. It is generally thought that rats that do not eat, do not drink. Because there was a marked reduction in body weight gains in the higher exposure groups, it could be theorized that the exposed animals were not eating properly and, thus, not drinking properly. The minimal to mild increases in albumin, total protein, and urea nitrogen concentrations which occurred in exposed animals in various groups on day 22 and at week 14 also would be consistent with dehydration.

There was evidence of an anemia on day 22 and at week 14 for the rats and at week 14 for the male mice. In a separate study, Sprague-Dawley rats given 100 and 500 mg benzophenone per kilogram body weight in feed for 28 days developed decreases of the circulating erythron (Burdock *et al.*, 1991). Also, an anemia has been described for Wistar rats given a structurally related compound, 2-hydroxy-4-methoxybenzophenone, at concentrations of 5,000 and 10,000 ppm in the diet for 28 days (Lewerenz *et al.*, 1972). Anemia did not occur, however, in F344/N rats administered up to 50,000 ppm 2-hydroxy-4-methoxybenzophenone in feed for 13 weeks (NTP, 1992). In the present studies, the evidence indicated that the anemia was of minimal severity and, though this evidence indicated a biological effect, it would not be considered clinically relevant; the possibility, however, that dehydration masked the severity of the anemia must be considered. The mechanism for the anemia is not known. There was evidence of a bone marrow response to the anemia for the male rats but not for the female rats or the mice. However, the lack of a strong erythroid response could be related to the minimal severity of the erythron change and, possibly, an erythropoietic suppression related to an altered nutritional status because of decreased feed intake.

In the rats, transient exposure concentration-related increases in platelet counts occurred in males and females on day 4. Transient increases in platelet counts could be consistent with a physiologic response resulting from mobilization of platelets from splenic and/or nonsplenic storage pools. The spleen acts as a reservoir for a significant portion of the total intravascular platelet mass, and altered or decreased splenic function could also

cause an increased release of platelets from the splenic pool to the circulation (Jain, 1986). Increased platelet counts have been described for F344/N rats given up to 50,000 ppm 2-hydroxy-4-methoxybenzophenone in feed for 13 weeks (NTP, 1992). Platelet counts were decreased on day 22 and at week 14 in the present study. Decreased platelet counts can be related to decreased platelet production, increased platelet consumption, excessive blood loss, or abnormal distribution (Jain, 1986); the mechanism for the decrease in this study is unknown. The decrease in platelet counts indicated a biologic effect but was of a mild severity that would not have been expected to lead to a clinical hemorrhagic diathesis. Thus, it does not appear that hemorrhage related to decreased platelet counts contributed to the anemia that was observed.

In these rat and mouse studies, treatment-related increases in alanine aminotransferase and/or sorbitol dehydrogenase activities and bile salt concentrations would indicate a hepatic effect and were consistent with the histopathologic liver alterations and increases in liver weights. In general, increases in serum activities of alanine aminotransferase and sorbitol dehydrogenase, considered liver-specific enzymes in rodents, are used as markers of hepatocellular necrosis or increased cell membrane permeability (Boyd, 1983; Clampitt and Hart, 1978). At the interim time points for rats, however, increases in alanine aminotransferase activity occurred without concomitant increases in serum sorbitol dehydrogenase activity. Certain compounds can induce increases in liver alanine aminotransferase activity (Rosen *et al.*, 1959a,b). Thus, compound-induced increases in liver alanine aminotransferase activity may help explain the increased serum alanine aminotransferse, but not sorbitol dehydrogenase, activity at the early time points. In rats, similar responses for alanine aminotransferase and sorbitol dehydrogenase activity also occurred in the 13-week feed study of 2-hydroxy-4-methoxybenzophenone (NTP, 1992).

Increases in serum bile salt concentration and alkaline phosphatase activity are used as markers of cholestasis. In the rat study, increased bile salt concentrations occurred, but alkaline phosphatase activity was decreased for all exposed rats at all time points. Though these findings would appear to be incongruous, similar bile salt concentration and alkaline phosphatase activity responses have been observed in other subchronic toxicity studies (Travlos *et al.*, 1996). Bile salt concentrations can be affected by mechanisms other than cholestasis. For example, altered enterohepatic circulation and impaired hepatocellular function or hepatocellular injury can elevate circulating bile salt concentrations (Hofmann, 1988). Additionally, circulating alkaline phosphatase in normal rats is thought to be primarily of intestinal and bone origin (Bianchi-Bosisio Righetti and Kaplan, 1971), and fasting or feed restriction causes decreases in serum alkaline phosphatase activity (Jenkins and Robinson, 1975). Substantial, treatment-related decreases in mean body weights and body weight gains suggested that exposed rats did not eat well, which may, in part, explain the decreases in the serum alkaline phosphatase activity. In the mouse study, treatment-related increases in bile salt concentrations were accompanied by

increased alkaline phosphatase activity in the 20,000 ppm female mice; this is consistent with a cholestatic effect. In the 13-week feed study of 2-hydroxy-4-methoxybenzophenone, evidence of cholestasis was demonstrated by increases of serum γ-glutamyltransferase activity in rats (NTP, 1992).

The results of the current studies are similar in target organs and/or morphology to those seen in the studies of 2-hydroxy-4-methoxybenzophenone (NTP, 1992). However, the effects were more prominent at lower exposure concentrations in the benzophenone studies, suggesting quantitative differences in toxicity between the congeners. A NOAEL of 6,250 ppm in feed was determined for 2-hydroxy-4-methoxybenzophenone for rats and mice, while a NOAEL was not established for rats or mice in these benzophenone studies.

The toxication-detoxication of a large number of chemicals is mediated by a group of enzymes referred to as cytochrome P_{450}-dependent, mixed-function oxidases (Parke and Ioannides, 1990). In rats, 14 weeks of exposure to benzophenone resulted in increases in cytochrome P_{450} concentrations in all exposed male and female groups with survivors. This increase was not exposure concentration related and ranged from 18% to 51% for males and 27% to 42% for females. In mice, the increases in cytochrome P_{450} concentrations (25% to 28% in males, 15% to 19% in females) occurred only in the 1,250, 2,500, and 5,000 ppm groups of males and the 1,250 and 2,500 ppm groups of females. There was induction of a form of cytochrome P_{450} characteristic of a phenobarbital-type inducer that preferentially increases the concentration of the 2B form of the enzyme. The weak induction response observed with ethoxyresorufin deethylase activity suggested that benzophenone was not a polycyclic aromatic hydrocarbon-type inducer of cytochrome P_{450}. The marked increase in pentoxyresorufin dealkylase activity provided evidence that benzophenone was a relatively potent inducer of the phenobarbital-type (2B) cytochrome P_{450}. The induction was not as great in mice as it was in rats. On a per mg protein basis, the increase in pentoxyresorufin dealkylase activity was on the order of 57- to 99-fold for rats and 9- to 21-fold for mice.

Results of the current studies identified the liver as the primary target organ of benzophenone toxicity. In rats, liver changes were observed at exposure concentrations greater than or equal to 5,000 ppm, while in mice, microscopic changes in the liver were observed in all but the 1,250 ppm group. The gross (increased organ weights) and microscopic (hepatocellular hypertrophy) liver changes associated with benzophenone administration in males and females were accompanied by benzophenone-induced increases in the activity of pentoxyresorufin dealkylase, an enzyme linked to the cytochrome P_{450} 2B isomer. Hypertrophy (increases in cell size) is often attributed in part to induction of metabolic enzymes. Many chemical agents inducing liver hypertrophy are mainly metabolized by hepatic drug-metabolizing enzymes; stimulation of these enzymes is usually associated with liver enlargement. These effects are considered to be an overall adaptive response of

the liver to xenobiotic compounds or hyperfunctional liver enlargement (Schulte-Hermann, 1974, 1985; Schulte-Hermann *et al.*, 1983). Hepatocarcinogens also induce changes such as hypertrophy and enzyme induction (Schulte-Hermann, 1974). According to Parke and Ioannides (1990), compounds inducing cytochrome P_{450} enzymes that may react with the compound repeatedly produce oxygen radicals in a process called *futile cycling*. Oxygen radicals and oxidative stress have been implicated in all stages of carcinogenesis, that is, initiation, promotion, and progression (Trush and Kensler, 1991). Furthermore, it has been shown that a linear correlation exists between a compound's ability to induce cytochrome P_{450} 2B and its ability to function as a hepatic tumor promoter in mice. This correlation exists for a number of hepatocarcinogens such as phenobarbital, lindane, and DDT (Klaunig and Kolaja, 1998). Based on the above information, it may be speculated that the liver is a likely target site for benzophenone carcinogenicity. However, carcinogenicity studies are needed to establish benzophenone as a hepatocarcinogen.

In the current studies, the kidney was identified as an additional target organ of benzophenone toxicity in rats. While the liver is the major organ involved in drug metabolism, the kidneys are also capable of carrying out extensive oxidation, reduction, hydrolysis, and conjugation reactions (Lash, 1994). In the current studies, cytochrome P_{450} was not determined in the kidneys of animals administered benzophenone.

Some notable species and sex differences were observed in the current toxicity studies. The toxicity data indicated that rats were somewhat more sensitive to the effects of benzophenone exposure than mice. Mice required higher doses on a mg/kg body weight basis to display benzophenone toxicity. No microscopic effects similar to those in the kidney of exposed rats were present in mice to account for increased kidney weights. A NOAEL for liver toxicity was achieved in rats but not in mice. These species differences could be due to the differences in disposition and metabolism of benzophenone between rats and mice.

The testis and epididymis weights of male mice in the 10,000 ppm group were significantly less than those of the controls. There were no other significant differences in sperm motility or vaginal cytology parameters between exposed and control male or female rats or mice. No treatment-related changes of the lens, retina, or other ocular structures of rats or mice were observed.

In conclusion, the liver is a primary target organ of benzophenone toxicity in rats and mice based on increases in liver weights, hepatocellular hypertrophy, clinical chemistry changes, and induction of the liver microsomal cytochrome P_{450} 2B isomer. The kidney was also identified as a target organ of benzophenone toxicity in rats only, based on exposure concentration-related increases in kidney weights and microscopic changes. The NOAEL for benzophenone was not achieved in these studies. Benzophenone is not mutagenic in *Salmonella*

typhimurium with or without metabolic activation. The toxicologic profile of benzophenone is similar to a number of known nongenotoxic hepatocarcinogens in mice, suggesting that benzophenone is a potential liver carcinogen.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS AND MICE

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund						10
Survivors						
Terminal sacrifice	10	10	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)				(10)	(10)
Epithelium, hyperkeratosis						1 (10%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Cyst						1 (10%)
Hematopoietic cell proliferation					1 (10%)	
Hepatodiaphragmatic nodule	1 (10%)	2 (20%)	3 (30%)	2 (20%)	1 (10%)	1 (10%)
Inflammation, chronic active					1 (10%)	
Necrosis				1 (10%)		
Bile duct, hyperplasia						9 (90%)
Bile duct, inflammation, chronic active		1 (10%)				
Hepatocyte, hypertrophy					5 (50%)	7 (70%)
Hepatocyte, vacuolization cytoplasmic	1 (10%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Stomach, glandular	(10)				(10)	(10)
Epithelium, erosion					1 (10%)	
Cardiovascular System						
Heart	(10)				(10)	(10)
Myocardium, inflammation, chronic active	6 (60%)				3 (30%)	
Endocrine System						
Adrenal cortex	(10)			(3)	(10)	(10)
Accessory adrenal cortical nodule					1 (10%)	
General Body System						
None						
Genital System						
Preputial gland	(10)				(10)	(10)
Inflammation, chronic active	2 (20%)				6 (60%)	4 (40%)
Inflammation, granulomatous	1 (10%)					
Prostate	(10)				(10)	(9)
Inflammation, chronic active					1 (10%)	1 (11%)
Seminal vesicle	(10)				(10)	(9)
Atrophy						3 (33%)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization					1 (10%)	
Germinal epithelium, degeneration						10 (100%)

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy						10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)		(2)	(3)	(10)	(10)
Inflammation, chronic active	1 (10%)		2 (100%)	3 (100%)	1 (10%)	5 (50%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active		2 (20%)	3 (30%)	4 (40%)	2 (20%)	
Mineralization				5 (50%)	10 (100%)	
Papilla, necrosis					2 (20%)	6 (60%)
Renal tubule, accumulation, hyaline droplet		1 (10%)				
Renal tubule, casts protein		8 (80%)	8 (80%)	9 (90%)	10 (100%)	
Renal tubule, dilatation			6 (60%)	8 (80%)	9 (90%)	8 (80%)
Renal tubule, regeneration	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	8 (80%)
Renal tubule, vacuolization cytoplasmic			1 (10%)			
Ureter						(2)
Transitional epithelium, necrosis						2 (100%)
Urinary bladder	(10)				(10)	(9)
Transitional epithelium, hyperplasia					1 (10%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund						9
Natural death						1
Survivors						
Terminal sacrifice	10	10	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, cecum	(9)				(10)	(9)
Inflammation, chronic active	1 (11%)					
Liver	(10)	(10)	(10)	(10)	(10)	(9)
Hepatodiaphragmatic nodule	1 (10%)	4 (40%)	2 (20%)	3 (30%)	1 (10%)	2 (22%)
Inflammation, chronic active	3 (30%)					
Inflammation, granulomatous	2 (20%)	4 (40%)				
Hepatocyte, hypertrophy		2 (20%)	8 (80%)	10 (100%)	10 (100%)	7 (78%)
Hepatocyte, vacuolization cytoplasmic				9 (90%)	10 (100%)	7 (78%)
Mesentery	(1)		(1)			(1)
Inflammation, granulomatous			1 (100%)			
Necrosis			1 (100%)			
Fat, inflammation, chronic active	1 (100%)					
Cardiovascular System						
Heart	(10)				(10)	(9)
Myocardium, inflammation, chronic active					1 (10%)	1 (11%)
Endocrine System						
Adrenal cortex	(10)				(10)	(9)
Accessory adrenal cortical nodule	1 (10%)					
Inflammation	1 (10%)					
Thyroid gland	(10)				(10)	(9)
Ultimobranchial cyst					2 (20%)	
General Body System						
None						
Genital System						
Clitoral gland	(10)				(10)	(9)
Inflammation, chronic active	4 (40%)				4 (40%)	
Ovary	(10)	(2)	(1)		(10)	(9)
Periovarian tissue, cyst	3 (30%)	2 (100%)	1 (100%)			
Periovarian tissue, inflammation, chronic active						1 (11%)

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy					2 (20%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)		(5)	(10)	(9)
Inflammation, chronic active		1 (100%)		5 (100%)	3 (30%)	6 (67%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(9)
Inflammation, chronic active	2 (20%)	2 (20%)				
Mineralization	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (100%)
Papilla, necrosis						3 (33%)
Pelvis, inflammation, suppurative					1 (10%)	
Renal tubule, casts protein			2 (20%)		4 (40%)	
Renal tubule, dilatation					3 (30%)	5 (56%)
Renal tubule, regeneration	3 (30%)	8 (80%)	6 (60%)	6 (60%)	9 (90%)	7 (78%)
Urinary bladder	(10)				(10)	(9)
Transitional epithelium, hyperplasia					1 (10%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death		1				
Moribund						6
Natural deaths						4
Survivors						
Terminal sacrifice	10	9	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	5 (50%)	4 (40%)	8 (80%)	8 (80%)	5 (50%)	1 (10%)
Centrilobular, hypertrophy	3 (30%)	8 (80%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Hepatocyte, vacuolization cytoplasmic						3 (30%)
Cardiovascular System						
Heart	(10)	(1)			(10)	(10)
Myocardium, mineralization						2 (20%)
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Spleen	(10)	(1)			(10)	(10)
Lymphoid follicle, depletion cellular						5 (50%)
Thymus	(8)	(1)			(10)	(8)
Atrophy						7 (88%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Respiratory System						
Lung	(10)	(1)			(10)	(10)
Alveolus, hemorrhage, acute		1 (100%)				
Arteriole, embolus		1 (100%)				
Nose	(10)	(1)			(10)	(10)
Inflammation, chronic active						1 (10%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(1)			(10)	(10)
Inflammation, chronic active	1 (10%)					
Renal tubule, regeneration					1 (10%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund						3
Natural death						1
Survivors						
Terminal sacrifice	10	10	10	10	10	6
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	8 (80%)	9 (90%)	9 (90%)	9 (90%)	9 (90%)	3 (30%)
Centrilobular, hypertrophy	3 (30%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Hepatocyte, vacuolization cytoplasmic				2 (20%)	9 (90%)	1 (10%)
Salivary glands	(10)					(10)
Infiltration cellular, mononuclear cell	1 (10%)					
Cardiovascular System						
Heart	(10)					(10)
Myocardium, mineralization						2 (20%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Ovary	(10)			(1)		(10)
Cyst				1 (100%)		
Hematopoietic System						
Spleen	(10)					(10)
Lymphoid follicle, depletion cellular						1 (10%)
Thymus	(10)					(7)
Atrophy						5 (71%)
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Nervous System						
Brain	(10)					(10)
Cyst epithelial inclusion	1 (10%)					
Respiratory System						
Lung	(10)				(1)	(10)
Arteriole, vasculitis, acute					1 (100%)	
Nose	(10)					(10)
Inflammation, chronic active						5 (50%)
Special Senses System						
None						
Urinary System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

APPENDIX B
CLINICAL PATHOLOGY RESULTS

TABLE B1
Hematology Data for Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n						
Day 4	10	10	9	10	10	10
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	44.3 ± 0.4	45.2 ± 0.4	47.9 ± 0.4**	50.0 ± 0.4**	50.7 ± 0.6**	51.4 ± 0.7**
Day 22	48.1 ± 0.5	47.5 ± 0.4	47.3 ± 0.4	47.2 ± 0.5	47.2 ± 0.7	47.3 ± 0.4
Week 14	50.0 ± 0.6	49.2 ± 0.7	49.9 ± 0.6	49.3 ± 0.7	49.1 ± 0.6	
Hemoglobin (g/dL)						
Day 4	15.4 ± 0.1	15.6 ± 0.1	16.3 ± 0.1**	17.0 ± 0.1**	17.2 ± 0.1**	17.5 ± 0.2**
Day 22	16.5 ± 0.1	16.1 ± 0.1*	15.8 ± 0.2**	15.7 ± 0.2**	15.5 ± 0.3**	15.8 ± 0.1**
Week 14	16.9 ± 0.2	16.4 ± 0.2	16.4 ± 0.1*	16.1 ± 0.2*	15.8 ± 0.1**	
Erythrocytes ($10^6/\mu L$)						
Day 4	7.44 ± 0.07	7.60 ± 0.09	8.01 ± 0.08**	8.31 ± 0.08**	8.45 ± 0.11**	8.49 ± 0.12**
Day 22	8.50 ± 0.09	8.23 ± 0.08	8.12 ± 0.08*	8.00 ± 0.07**	7.97 ± 0.13**	8.35 ± 0.08
Week 14	9.29 ± 0.12	8.93 ± 0.10	8.98 ± 0.11	8.65 ± 0.14**	8.48 ± 0.11**	
Reticulocytes ($10^6/\mu L$)						
Day 4	0.28 ± 0.02	0.24 ± 0.01	0.22 ± 0.02	0.21 ± 0.01*	0.18 ± 0.01**	0.17 ± 0.01**
Day 22	0.13 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.19 ± 0.01*	0.07 ± 0.01
Week 14	0.14 ± 0.01	0.16 ± 0.01*	0.18 ± 0.01**	0.21 ± 0.01**	0.19 ± 0.01**	
Nucleated erythrocytes ($10^3/\mu L$)						
Day 4	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01
Week 14	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	
Mean cell volume (fL)						
Day 4	59.6 ± 0.2	59.5 ± 0.2	59.8 ± 0.2	60.1 ± 0.3	60.0 ± 0.2	60.6 ± 0.3*
Day 22	56.5 ± 0.1	57.7 ± 0.1**	58.3 ± 0.2**	59.0 ± 0.2**	59.2 ± 0.2**	56.7 ± 0.2**
Week 14	53.9 ± 0.2	55.1 ± 0.1**	55.6 ± 0.1**	57.0 ± 0.3**	57.9 ± 0.2**	
Mean cell hemoglobin (pg)						
Day 4	20.6 ± 0.1	20.5 ± 0.2	20.4 ± 0.1	20.4 ± 0.1	20.4 ± 0.2	20.6 ± 0.2
Day 22	19.4 ± 0.1	19.6 ± 0.1	19.5 ± 0.1	19.7 ± 0.1	19.4 ± 0.1	19.0 ± 0.1
Week 14	18.2 ± 0.1	18.3 ± 0.1	18.3 ± 0.1	18.6 ± 0.1	18.6 ± 0.2	
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.7 ± 0.2	34.5 ± 0.2	34.1 ± 0.2	34.0 ± 0.2	34.0 ± 0.2	34.0 ± 0.3
Day 22	34.4 ± 0.1	33.9 ± 0.2*	33.5 ± 0.2**	33.4 ± 0.2**	32.8 ± 0.2**	33.5 ± 0.1**
Week 14	33.8 ± 0.2	33.3 ± 0.2*	32.9 ± 0.3**	32.6 ± 0.2**	32.1 ± 0.3**	
Platelets ($10^3/\mu L$)						
Day 4	966.0 ± 11.1	922.3 ± 27.6	955.7 ± 22.0	1,069.5 ± 17.0**	1,087.1 ± 22.0**	1,050.8 ± 23.0**
Day 22	784.3 ± 14.7	827.6 ± 14.0	778.6 ± 11.4	793.0 ± 21.1	705.0 ± 15.3**	547.5 ± 15.7**
Week 14	750.9 ± 11.1	783.4 ± 18.4	827.0 ± 12.5*	796.5 ± 16.0	717.7 ± 11.5	
Leukocytes ($10^3/\mu L$)						
Day 4	8.56 ± 0.43	7.92 ± 0.42	9.09 ± 0.58	9.53 ± 0.69	9.18 ± 0.41	9.79 ± 0.62
Day 22	7.50 ± 0.55	7.62 ± 0.46	8.08 ± 0.30	7.98 ± 0.42	7.58 ± 0.33	9.51 ± 0.72
Week 14	12.98 ± 0.74	11.43 ± 0.59	10.17 ± 0.57*	11.31 ± 0.59	13.12 ± 0.97	
Segmented neutrophils ($10^3/\mu L$)						
Day 4	1.24 ± 0.14	1.00 ± 0.08	0.93 ± 0.12	1.16 ± 0.13	1.32 ± 0.11	1.31 ± 0.16
Day 22	1.17 ± 0.09	1.17 ± 0.08	1.27 ± 0.16	1.45 ± 0.22	1.43 ± 0.17	1.72 ± 0.24
Week 14	2.05 ± 0.25	1.61 ± 0.13	1.62 ± 0.38*	1.48 ± 0.14	1.83 ± 0.21	
Lymphocytes ($10^3/\mu L$)						
Day 4	7.19 ± 0.35	6.77 ± 0.43	8.09 ± 0.50	8.14 ± 0.59	7.70 ± 0.42	8.35 ± 0.52
Day 22	6.17 ± 0.49	6.29 ± 0.41	6.64 ± 0.33	6.41 ± 0.31	5.96 ± 0.23	7.61 ± 0.63
Week 14	10.64 ± 0.58	9.69 ± 0.54	8.39 ± 0.51	9.57 ± 0.55	10.98 ± 0.83	

TABLE B1
Hematology Data for Rats in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male (continued)						
n						
Day 4	10	10	9	10	10	10
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	0
Monocytes ($10^3/\mu L$)						
Day 4	0.12 ± 0.03	0.11 ± 0.02	0.06 ± 0.03	0.14 ± 0.05	0.10 ± 0.03	0.10 ± 0.03
Day 22	0.11 ± 0.02	0.09 ± 0.02	0.11 ± 0.02	0.06 ± 0.02	0.13 ± 0.03	0.14 ± 0.03
Week 14	0.21 ± 0.04	0.12 ± 0.03	0.11 ± 0.04	0.20 ± 0.06	0.24 ± 0.03	
Eosinophils ($10^3/\mu L$)						
Day 4	0.01 ± 0.01	0.05 ± 0.02	0.01 ± 0.01	0.09 ± 0.02**	0.06 ± 0.04	0.03 ± 0.02
Day 22	0.04 ± 0.02	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.03	0.06 ± 0.03	0.04 ± 0.02
Week 14	0.08 ± 0.04	0.01 ± 0.01	0.05 ± 0.03	0.07 ± 0.02	0.08 ± 0.03	
Female						
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	10	9	10
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	45.5 ± 0.4	46.2 ± 0.5	48.1 ± 0.5**	50.2 ± 0.4**	51.1 ± 0.3**	51.0 ± 0.5**
Day 22	47.5 ± 0.5	47.3 ± 0.4	47.7 ± 0.4	45.8 ± 0.4*	46.4 ± 0.6	44.9 ± 0.7**
Week 14	47.1 ± 0.5	47.7 ± 0.6	46.2 ± 0.7	46.2 ± 0.6	45.4 ± 0.4*	
Hemoglobin (g/dL)						
Day 4	15.6 ± 0.1	15.6 ± 0.2	16.3 ± 0.2**	16.9 ± 0.2**	17.3 ± 0.1**	17.2 ± 0.1**
Day 22	16.2 ± 0.1	15.7 ± 0.1*	15.6 ± 0.1**	15.2 ± 0.1**	15.2 ± 0.2**	15.1 ± 0.2**
Week 14	16.0 ± 0.2	16.1 ± 0.2	15.5 ± 0.2	15.3 ± 0.2*	14.5 ± 0.1**	
Erythrocytes ($10^6/\mu L$)						
Day 4	7.51 ± 0.07	7.50 ± 0.12	7.83 ± 0.10*	8.18 ± 0.09**	8.29 ± 0.08**	8.39 ± 0.07**
Day 22	7.83 ± 0.09	7.61 ± 0.06	7.76 ± 0.06	7.46 ± 0.07*	7.58 ± 0.11	7.74 ± 0.12
Week 14	8.07 ± 0.09	8.22 ± 0.12	8.03 ± 0.11	8.08 ± 0.12	7.95 ± 0.09	
Reticulocytes ($10^6/\mu L$)						
Day 4	0.17 ± 0.02	0.14 ± 0.01	0.13 ± 0.01*	0.09 ± 0.01**	0.13 ± 0.01**	0.11 ± 0.01**
Day 22	0.13 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.17 ± 0.02	0.13 ± 0.01
Week 14	0.18 ± 0.02	0.18 ± 0.02	0.17 ± 0.02	0.18 ± 0.01	0.20 ± 0.02	
Nucleated erythrocytes ($10^3/\mu L$)						
Day 4	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Mean cell volume (fL)						
Day 4	60.6 ± 0.2	61.6 ± 0.4	61.5 ± 0.4	61.4 ± 0.2	61.6 ± 0.2*	60.8 ± 0.4
Day 22	60.7 ± 0.2	62.2 ± 0.3	61.5 ± 0.2	61.5 ± 0.1	61.3 ± 0.3	58.0 ± 0.3*
Week 14	58.4 ± 0.3	58.0 ± 0.2	57.5 ± 0.1*	57.2 ± 0.1**	57.1 ± 0.3**	
Mean cell hemoglobin (pg)						
Day 4	20.7 ± 0.1	20.9 ± 0.1	20.9 ± 0.2	20.7 ± 0.1	20.9 ± 0.1	20.5 ± 0.1
Day 22	20.6 ± 0.1	20.7 ± 0.1	20.1 ± 0.1**	20.4 ± 0.1*	20.0 ± 0.2**	19.4 ± 0.1**
Week 14	19.9 ± 0.1	19.6 ± 0.1	19.4 ± 0.2**	19.0 ± 0.1**	18.3 ± 0.1**	
Mean cell hemoglobin concentration (g/dL)						
Day 4	34.2 ± 0.2	33.9 ± 0.2	34.0 ± 0.2	33.7 ± 0.1	33.9 ± 0.2	33.7 ± 0.2
Day 22	34.0 ± 0.2	33.3 ± 0.2	32.6 ± 0.1**	33.1 ± 0.1	32.7 ± 0.2**	33.5 ± 0.2
Week 14	34.1 ± 0.2	33.8 ± 0.2	33.7 ± 0.3	33.2 ± 0.2*	32.0 ± 0.1**	

TABLE B1
Hematology Data for Rats in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Female (continued)						
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	10	9	10
Week 14	10	10	10	10	10	0
Platelets ($10^3/\mu$L)						
Day 4	861.2 ± 13.0	898.3 ± 14.3	876.2 ± 12.1	943.0 ± 14.3**	996.0 ± 23.0**	1,006.2 ± 12.2**
Day 22	765.6 ± 21.6	764.4 ± 20.5	778.1 ± 11.5	795.4 ± 8.9	636.6 ± 23.1**	622.3 ± 14.0**
Week 14	780.2 ± 18.6	771.1 ± 15.8	788.8 ± 28.6	716.8 ± 13.0*	671.0 ± 11.8**	
Leukocytes ($10^3/\mu$L)						
Day 4	7.89 ± 0.57	8.22 ± 0.63	9.39 ± 0.58	9.22 ± 0.43	7.80 ± 0.45	8.39 ± 0.71
Day 22	6.74 ± 0.52	6.44 ± 0.41	6.50 ± 0.36	6.62 ± 0.36	5.47 ± 0.34	7.74 ± 0.51
Week 14	10.33 ± 0.87	9.47 ± 0.59	9.24 ± 0.60	8.31 ± 0.27	8.31 ± 0.24	
Segmented neutrophils ($10^3/\mu$L)						
Day 4	1.05 ± 0.12	1.15 ± 0.12	1.15 ± 0.13	1.13 ± 0.13	1.07 ± 0.11	0.97 ± 0.12
Day 22	0.83 ± 0.11	0.89 ± 0.08	0.88 ± 0.07	0.97 ± 0.15	0.84 ± 0.10	1.56 ± 0.12**
Week 14	1.89 ± 0.30	1.40 ± 0.12	1.39 ± 0.14	1.21 ± 0.14	1.05 ± 0.07	
Lymphocytes ($10^3/\mu$L)						
Day 4	6.70 ± 0.46	6.92 ± 0.66	8.09 ± 0.52	7.94 ± 0.45	6.63 ± 0.38	7.28 ± 0.61
Day 22	5.78 ± 0.53	5.45 ± 0.35	5.50 ± 0.30	5.50 ± 0.28	4.52 ± 0.27	6.02 ± 0.51
Week 14	8.26 ± 0.65	7.93 ± 0.55	7.62 ± 0.58	6.99 ± 0.28	7.13 ± 0.24	
Monocytes ($10^3/\mu$L)						
Day 4	0.10 ± 0.03	0.12 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.06 ± 0.02
Day 22	0.08 ± 0.02	0.08 ± 0.02	0.09 ± 0.02	0.10 ± 0.03	0.07 ± 0.01	0.13 ± 0.03
Week 14	0.15 ± 0.03	0.11 ± 0.02	0.18 ± 0.04	0.05 ± 0.02	0.08 ± 0.03	
Eosinophils ($10^3/\mu$L)						
Day 4	0.05 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.08 ± 0.02	0.02 ± 0.01	0.09 ± 0.04
Day 22	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.04 ± 0.01	0.03 ± 0.02
Week 14	0.03 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE B2
Clinical Chemistry Data for Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Day 4	20.3 ± 0.6	20.6 ± 0.6	21.7 ± 0.3	21.9 ± 0.4	22.3 ± 0.6*	22.2 ± 0.5*
Day 22	20.5 ± 0.5	20.6 ± 0.4	22.0 ± 0.9	21.5 ± 0.2	24.2 ± 0.6**	31.5 ± 1.5**
Week 14	17.9 ± 0.3	17.1 ± 0.3	17.5 ± 0.2	18.2 ± 0.5	18.9 ± 0.6	
Creatinine (mg/dL)						
Day 4	0.60 ± 0.00	0.60 ± 0.00	0.62 ± 0.01	0.58 ± 0.01	0.57 ± 0.02	0.54 ± 0.02**[b]
Day 22	0.69 ± 0.01	0.66 ± 0.02	0.64 ± 0.02*	0.61 ± 0.01**	0.61 ± 0.01**	0.56 ± 0.02**
Week 14	0.70 ± 0.01	0.72 ± 0.01	0.71 ± 0.01	0.66 ± 0.02	0.59 ± 0.01**	
Total protein (g/dL)						
Day 4	6.2 ± 0.0	6.1 ± 0.1	5.8 ± 0.0**	5.9 ± 0.1**	6.1 ± 0.0	6.0 ± 0.1
Day 22	6.9 ± 0.1	7.0 ± 0.1	7.4 ± 0.1*	7.4 ± 0.1*	7.3 ± 0.1*	5.7 ± 0.1
Week 14	7.7 ± 0.1	8.1 ± 0.1	8.4 ± 0.1*	8.1 ± 0.2	7.1 ± 0.1	
Albumin (g/dL)						
Day 4	4.5 ± 0.0	4.4 ± 0.0	4.3 ± 0.0	4.4 ± 0.1	4.6 ± 0.1	4.5 ± 0.1
Day 22	4.9 ± 0.1	4.9 ± 0.0	5.2 ± 0.1*	5.2 ± 0.0*	5.2 ± 0.1*	4.0 ± 0.1
Week 14	5.2 ± 0.0	5.4 ± 0.1	5.6 ± 0.1	5.2 ± 0.1	4.7 ± 0.1*	
Alanine aminotransferase (IU/L)						
Day 4	43 ± 1	52 ± 4**	56 ± 3**	59 ± 3**	63 ± 2**	61 ± 3**
Day 22	50 ± 2	41 ± 1	40 ± 2	39 ± 1	50 ± 2	97 ± 4*
Week 14	71 ± 5	67 ± 5	55 ± 4	61 ± 4	90 ± 6	
Alkaline phosphatase (IU/L)						
Day 4	1,307 ± 26	1,237 ± 27	1,141 ± 32**	912 ± 18**	847 ± 35**	799 ± 28**
Day 22	951 ± 17	798 ± 19**	715 ± 11**	647 ± 14**	725 ± 27**	664 ± 25**
Week 14	574 ± 17	473 ± 14**	458 ± 8**	404 ± 11**	485 ± 15**	
Creatine kinase (IU/L)						
Day 4	332 ± 40	480 ± 90	373 ± 93	273 ± 35	390 ± 39	355 ± 72[b]
Day 22	291 ± 43[b]	244 ± 34[b]	253 ± 30	241 ± 47	215 ± 22	187 ± 16
Week 14	147 ± 21	153 ± 25	158 ± 13	112 ± 10	137 ± 14	
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	21 ± 2	23 ± 2*	18 ± 1	18 ± 1	15 ± 1
Day 22	19 ± 1	16 ± 1*	16 ± 1*	16 ± 1	16 ± 1	10 ± 1**
Week 14	30 ± 4	29 ± 3	25 ± 2	27 ± 3	34 ± 3	
Bile salts (μmol/L)						
Day 4	31.3 ± 2.9	32.2 ± 4.1	32.7 ± 3.6	42.5 ± 3.5	33.1 ± 2.1	29.8 ± 1.7
Day 22	24.1 ± 2.1	28.3 ± 2.5	33.3 ± 3.2*[b]	34.2 ± 2.7**	70.4 ± 11.0**	330.4 ± 26.0**
Week 14	21.5 ± 2.1	24.7 ± 0.9*	27.2 ± 2.7*	29.0 ± 1.4**	52.1 ± 4.2**	

TABLE B2
Clinical Chemistry Data for Rats in the 14-Week Feed Study of Benzophenone

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Female						
n						
Day 4	10	10	10	10	10	10
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Day 4	22.0 ± 0.6	20.5 ± 0.7	20.2 ± 1.5*	17.0 ± 0.7**	15.6 ± 0.6**	17.0 ± 0.7**
Day 22	20.4 ± 0.8	19.2 ± 0.6	19.4 ± 0.6	21.7 ± 0.5	21.5 ± 0.5	29.8 ± 0.9**
Week 14	20.0 ± 0.5	18.5 ± 0.7	19.7 ± 0.7	19.1 ± 0.4	19.6 ± 0.6	
Creatinine (mg/dL)						
Day 4	0.62 ± 0.01	0.61 ± 0.01	0.59 ± 0.01[b]	0.58 ± 0.01*	0.57 ± 0.02*	0.54 ± 0.02**
Day 22	0.68 ± 0.01	0.63 ± 0.02*	0.62 ± 0.01**	0.62 ± 0.01**	0.61 ± 0.01**	0.55 ± 0.02**
Week 14	0.73 ± 0.03	0.70 ± 0.02	0.69 ± 0.02	0.68 ± 0.02	0.62 ± 0.01**	
Total protein (g/dL)						
Day 4	6.1 ± 0.1	6.0 ± 0.0	5.8 ± 0.1*	5.7 ± 0.0**	5.8 ± 0.1**	5.8 ± 0.1**
Day 22	6.4 ± 0.1	6.8 ± 0.1	7.1 ± 0.1*	7.4 ± 0.1**	7.5 ± 0.1**	5.8 ± 0.1
Week 14	7.3 ± 0.2	8.1 ± 0.1**	8.4 ± 0.1**	8.6 ± 0.1**	8.0 ± 0.2**	
Albumin (g/dL)						
Day 4	4.5 ± 0.1	4.4 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.0	4.4 ± 0.1
Day 22	4.7 ± 0.1	5.0 ± 0.1	5.2 ± 0.1*	5.4 ± 0.1**	5.5 ± 0.1**	4.3 ± 0.1
Week 14	5.1 ± 0.1	5.6 ± 0.1**	5.9 ± 0.1**	6.1 ± 0.1**	5.7 ± 0.1**	
Alanine aminotransferase (IU/L)						
Day 4	36 ± 2	42 ± 2*	48 ± 3**	54 ± 3**	53 ± 3**	59 ± 9**
Day 22	37 ± 1	35 ± 1	36 ± 1	35 ± 1	47 ± 1**	95 ± 4**
Week 14	48 ± 3	49 ± 2	43 ± 2	47 ± 3	89 ± 11**	
Alkaline phosphatase (IU/L)						
Day 4	1,048 ± 25	925 ± 36**	824 ± 37**	680 ± 22**	599 ± 13**	596 ± 12**
Day 22	766 ± 24	585 ± 19**	516 ± 12**	531 ± 15**	635 ± 22	698 ± 35
Week 14	495 ± 15	369 ± 12**	331 ± 11**	333 ± 18**	400 ± 21**	
Creatine kinase (IU/L)						
Day 4	342 ± 60[b]	391 ± 81	303 ± 43	392 ± 40[b]	389 ± 37	445 ± 75
Day 22	220 ± 31	194 ± 38	191 ± 28	160 ± 23	208 ± 32	178 ± 19
Week 14	196 ± 38	198 ± 39	171 ± 23	125 ± 15	157 ± 22	
Sorbitol dehydrogenase (IU/L)						
Day 4	18 ± 1	17 ± 1	15 ± 1	17 ± 1	17 ± 1	24 ± 5
Day 22	20 ± 2	16 ± 1	16 ± 1	15 ± 1	18 ± 2	13 ± 1**
Week 14	21 ± 2	22 ± 1	18 ± 1	21 ± 1	43 ± 6**	
Bile salts (μmol/L)						
Day 4	33.0 ± 5.2	53.9 ± 4.9*	58.0 ± 5.9*	49.6 ± 4.5	46.8 ± 6.8	40.3 ± 3.5
Day 22	28.5 ± 3.4	38.9 ± 3.4*	44.8 ± 4.2**	54.1 ± 3.9**	82.5 ± 12.5**	435.4 ± 22.8**
Week 14	41.7 ± 4.9	38.5 ± 3.7	44.2 ± 4.0	39.7 ± 2.6	71.0 ± 3.7**	

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

TABLE B3
Hematology Data for Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n	10	9	10	10	10	0
Hematocrit (%)	49.5 ± 0.4	48.6 ± 0.6	48.9 ± 0.7	47.1 ± 0.5**	46.7 ± 0.6**	
Hemoglobin (g/dL)	17.2 ± 0.2	17.1 ± 0.2	17.2 ± 0.2	16.6 ± 0.1*	16.5 ± 0.1*	
Erythrocytes ($10^6/\mu L$)	10.59 ± 0.09	10.40 ± 0.16	10.40 ± 0.16	9.95 ± 0.10**	10.02 ± 0.12**	
Reticulocytes ($10^6/\mu L$)	0.10 ± 0.01	0.14 ± 0.01*	0.12 ± 0.00	0.13 ± 0.01	0.11 ± 0.01	
Nucleated erythrocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	
Mean cell volume (fL)	46.7 ± 0.2	46.7 ± 0.3	47.0 ± 0.2	47.4 ± 0.3	46.7 ± 0.2	
Mean cell hemoglobin (pg)	16.2 ± 0.1	16.5 ± 0.2	16.5 ± 0.1	16.7 ± 0.1*	16.5 ± 0.1	
Mean cell hemoglobin concentration (g/dL)	34.7 ± 0.1	35.2 ± 0.3	35.1 ± 0.2	35.2 ± 0.3	35.4 ± 0.2	
Platelets ($10^3/\mu L$)	967.4 ± 37.9	1,084.3 ± 42.6	1,053.8 ± 42.2	1,111.8 ± 29.4*	1,016.4 ± 19.1	
Leukocytes ($10^3/\mu L$)	8.80 ± 0.40	6.80 ± 0.55	7.52 ± 0.56	9.81 ± 0.60	9.37 ± 0.62	
Segmented neutrophils ($10^3/\mu L$)	1.59 ± 0.15	1.22 ± 0.21	1.50 ± 0.22	2.13 ± 0.25	1.78 ± 0.21	
Lymphocytes ($10^3/\mu L$)	7.03 ± 0.34	5.52 ± 0.50	5.95 ± 0.47	7.58 ± 0.54	7.52 ± 0.48	
Monocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	
Eosinophils ($10^3/\mu L$)	0.18 ± 0.04	0.06 ± 0.03	0.07 ± 0.03	0.11 ± 0.04	0.06 ± 0.02	
Female						
n	10	10	10	10	10	6
Hematocrit (%)	46.8 ± 0.6	47.2 ± 0.5	47.5 ± 0.5	48.3 ± 0.6	48.0 ± 0.5	49.6 ± 0.6**
Hemoglobin (g/dL)	16.2 ± 0.1	16.5 ± 0.2	16.5 ± 0.1	16.7 ± 0.2*	16.5 ± 0.1	16.7 ± 0.1
Erythrocytes ($10^6/\mu L$)	9.86 ± 0.14	9.92 ± 0.11	9.97 ± 0.13	10.24 ± 0.13*	10.23 ± 0.09*	10.81 ± 0.15**
Reticulocytes ($10^6/\mu L$)	0.14 ± 0.01	0.14 ± 0.02	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.5 ± 0.3	47.5 ± 0.2	47.6 ± 0.2	47.1 ± 0.1	46.9 ± 0.2	45.9 ± 0.3**
Mean cell hemoglobin (pg)	16.5 ± 0.1	16.6 ± 0.2	16.6 ± 0.1	16.4 ± 0.1	16.2 ± 0.1	15.5 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	34.6 ± 0.2	35.0 ± 0.3	34.8 ± 0.2	34.7 ± 0.2	34.5 ± 0.2	33.7 ± 0.4
Platelets ($10^3/\mu L$)	871.4 ± 28.4	933.7 ± 27.0	929.9 ± 25.1	979.3 ± 28.4*	933.6 ± 48.0	881.8 ± 8.9
Leukocytes ($10^3/\mu L$)	8.21 ± 0.86	7.94 ± 0.51	8.87 ± 0.69	8.64 ± 0.82	7.77 ± 0.53	7.82 ± 0.82
Segmented neutrophils ($10^3/\mu L$)	1.31 ± 0.26	1.05 ± 0.13	1.06 ± 0.12	1.15 ± 0.26	0.89 ± 0.15	2.32 ± 0.16
Lymphocytes ($10^3/\mu L$)	6.77 ± 0.67	6.71 ± 0.46	7.67 ± 0.59	7.37 ± 0.65	6.76 ± 0.49	5.48 ± 0.70
Monocytes ($10^3/\mu L$)	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Eosinophils ($10^3/\mu L$)	0.12 ± 0.02	0.16 ± 0.05	0.14 ± 0.04	0.12 ± 0.04	0.12 ± 0.03	0.02 ± 0.02

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

TABLE B4
Clinical Chemistry Data for Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n	8	6	10	10	10	0
Urea nitrogen (mg/dL)	30.9 ± 1.2	32.2 ± 1.2	29.8 ± 2.0	28.9 ± 3.6	26.0 ± 1.1*	
Creatinine (mg/dL)	0.45 ± 0.02	0.47 ± 0.03	0.45 ± 0.02	0.50 ± 0.06	0.45 ± 0.02	
Total protein (g/dL)	5.6 ± 0.1	6.0 ± 0.1*	6.1 ± 0.1**[b]	6.0 ± 0.1*	6.2 ± 0.1**	
Albumin (g/dL)	4.0 ± 0.1	4.2 ± 0.1	4.2 ± 0.1[b]	4.2 ± 0.1	4.6 ± 0.1**	
Alanine aminotransferase (IU/L)	94 ± 26[b]	98 ± 26[c]	76 ± 9	98 ± 23[b]	107 ± 12	
Alkaline phosphatase (IU/L)	179 ± 5[b]	174 ± 5[c]	159 ± 3	169 ± 7	204 ± 8	
Creatine kinase (IU/L)	1,171 ± 297	674 ± 345	757 ± 207	645 ± 142[c]	1,045 ± 214	
Sorbitol dehydrogenase (IU/L)	64 ± 2[d]	88 ± 14[b]	90 ± 8**	102 ± 10**	124 ± 9**	
Bile salts (μmol/L)	17.0 ± 1.2	19.3 ± 3.1[e]	17.9 ± 1.2[f]	24.5 ± 2.4*	26.9 ± 1.4**	
Female						
n	10	10	9	10	10	6
Urea nitrogen (mg/dL)	24.9 ± 1.5	23.6 ± 1.0	21.4 ± 1.6	21.7 ± 1.5	22.0 ± 0.5	15.8 ± 0.8**
Creatinine (mg/dL)	0.47 ± 0.02	0.48 ± 0.01[b]	0.46 ± 0.02	0.47 ± 0.03[b]	0.45 ± 0.02	0.38 ± 0.02*
Total protein (g/dL)	5.7 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	6.3 ± 0.1*	6.1 ± 0.1	5.0 ± 0.1
Albumin (g/dL)	4.3 ± 0.0	4.6 ± 0.1	4.5 ± 0.0	4.8 ± 0.1**	4.6 ± 0.1	3.7 ± 0.1
Alanine aminotransferase (IU/L)	57 ± 9	96 ± 15	75 ± 13	80 ± 13	59 ± 5	88 ± 6
Alkaline phosphatase (IU/L)	251 ± 4	260 ± 7	256 ± 10[d]	257 ± 11	242 ± 8	428 ± 21**
Creatine kinase (IU/L)	774 ± 128	1,269 ± 205[b]	910 ± 145	801 ± 111[f]	826 ± 186	584 ± 117
Sorbitol dehydrogenase (IU/L)	52 ± 2	66 ± 3**	70 ± 3**[d]	78 ± 4**	84 ± 5**	77 ± 4**
Bile salts (μmol/L)	13.1 ± 0.8[f]	16.6 ± 2.9[c]	19.6 ± 0.8**	17.5 ± 1.8*[f]	21.0 ± 0.9**[f]	35.3 ± 15.2*[g]

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=7
[d] n=10
[e] n=5
[f] n=8
[g] n=3

APPENDIX C
ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE C1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10
Male					
Necropsy body wt	379 ± 7	374 ± 5	352 ± 5**	340 ± 5**	276 ± 5**
Heart					
Absolute	1.103 ± 0.029	1.125 ± 0.025	1.049 ± 0.022	1.104 ± 0.023	0.893 ± 0.019**
Relative	2.91 ± 0.04	3.01 ± 0.04	2.98 ± 0.05	3.25 ± 0.07**	3.24 ± 0.08**
R. Kidney					
Absolute	1.308 ± 0.027	1.516 ± 0.041**	1.615 ± 0.039**	2.056 ± 0.091**	1.782 ± 0.051**
Relative	3.46 ± 0.05	4.06 ± 0.08**	4.59 ± 0.09**	6.03 ± 0.20**	6.46 ± 0.17**
Liver					
Absolute	13.647 ± 0.261	17.338 ± 0.354**	19.463 ± 0.548**	21.629 ± 0.740**	19.379 ± 0.387**
Relative	36.10 ± 0.56	46.40 ± 0.51**	55.29 ± 0.96**	63.52 ± 1.39**	70.23 ± 1.33**
Lung					
Absolute	1.849 ± 0.074	1.694 ± 0.072	1.636 ± 0.062*	1.569 ± 0.044**	1.358 ± 0.023**
Relative	4.90 ± 0.21	4.53 ± 0.17	4.65 ± 0.14	4.62 ± 0.11	4.92 ± 0.10
R. Testis					
Absolute	1.410 ± 0.037	1.445 ± 0.023	1.443 ± 0.039	1.464 ± 0.017	1.405 ± 0.026
Relative	3.73 ± 0.09	3.87 ± 0.05	4.10 ± 0.09**	4.32 ± 0.09**	5.09 ± 0.09**
Thymus					
Absolute	0.345 ± 0.021	0.335 ± 0.015	0.317 ± 0.013	0.323 ± 0.016	0.232 ± 0.008**
Relative	0.91 ± 0.05	0.90 ± 0.05	0.90 ± 0.03	0.95 ± 0.05	0.84 ± 0.03
Female					
Necropsy body wt	219 ± 3	197 ± 2**	192 ± 3**	182 ± 2**	180 ± 2**
Heart					
Absolute	0.698 ± 0.021	0.704 ± 0.016	0.666 ± 0.018	0.675 ± 0.017	0.665 ± 0.016
Relative	3.18 ± 0.07	3.58 ± 0.09**	3.47 ± 0.06**	3.72 ± 0.11**	3.70 ± 0.08**
R. Kidney					
Absolute	0.744 ± 0.014	0.792 ± 0.013	0.832 ± 0.014**	0.821 ± 0.015**	0.960 ± 0.033**
Relative	3.40 ± 0.06	4.03 ± 0.07**	4.34 ± 0.05**	4.51 ± 0.07**	5.33 ± 0.15**
Liver					
Absolute	7.566 ± 0.179	8.446 ± 0.136**	9.653 ± 0.284**	10.432 ± 0.175**	12.643 ± 0.308**
Relative	34.52 ± 0.54	42.99 ± 0.82**	50.30 ± 1.02**	57.31 ± 0.80**	70.21 ± 1.19**
Lung					
Absolute	1.350 ± 0.067	1.106 ± 0.036**	1.149 ± 0.033**	1.040 ± 0.027**	1.102 ± 0.046**
Relative	6.17 ± 0.30	5.62 ± 0.17	5.99 ± 0.16	5.72 ± 0.15	6.13 ± 0.24
Thymus					
Absolute	0.284 ± 0.009	0.250 ± 0.006	0.274 ± 0.015	0.239 ± 0.007**	0.241 ± 0.006**
Relative	1.30 ± 0.04	1.27 ± 0.03	1.43 ± 0.08	1.31 ± 0.04	1.34 ± 0.04

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). All 20,000 ppm rats died before the end of the study.

TABLE C2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n	10	9	10	10	10	0
Necropsy body wt	33.3 ± 0.8	33.2 ± 0.8	33.7 ± 0.8	31.8 ± 0.6	28.6 ± 0.5**	
Heart						
Absolute	0.152 ± 0.003	0.155 ± 0.003	0.165 ± 0.004	0.165 ± 0.006	0.140 ± 0.004	
Relative	4.59 ± 0.12	4.70 ± 0.13	4.90 ± 0.14	5.19 ± 0.14**	4.90 ± 0.11	
R. Kidney						
Absolute	0.272 ± 0.007	0.270 ± 0.006	0.301 ± 0.005**	0.312 ± 0.007**	0.284 ± 0.008**	
Relative	8.19 ± 0.18	8.17 ± 0.20	8.96 ± 0.18**	9.80 ± 0.15**	9.92 ± 0.18**	
Liver						
Absolute	1.590 ± 0.056	2.157 ± 0.099**	2.467 ± 0.061**	2.762 ± 0.090**	2.822 ± 0.052**	
Relative	47.62 ± 0.64	65.18 ± 3.33**	73.15 ± 0.72**	86.64 ± 1.43**	98.60 ± 1.44**	
Lung						
Absolute	0.242 ± 0.026	0.231 ± 0.023	0.228 ± 0.014	0.191 ± 0.009	0.195 ± 0.005	
Relative	7.22 ± 0.68	7.04 ± 0.80	6.80 ± 0.48	6.01 ± 0.25	6.84 ± 0.22	
R. Testis						
Absolute	0.121 ± 0.002	0.114 ± 0.004	0.124 ± 0.002[b]	0.122 ± 0.003	0.115 ± 0.002	
Relative	3.64 ± 0.07	3.44 ± 0.10	3.66 ± 0.09[b]	3.84 ± 0.09	4.03 ± 0.08**	
Thymus						
Absolute	0.046 ± 0.003	0.046 ± 0.004	0.042 ± 0.002	0.037 ± 0.002	0.040 ± 0.001	
Relative	1.38 ± 0.07	1.39 ± 0.12	1.26 ± 0.06	1.16 ± 0.05	1.39 ± 0.05	
Female						
n	10	10	10	10	10	6
Necropsy body wt	27.5 ± 0.5	27.7 ± 0.3	27.5 ± 0.4	25.6 ± 0.4**	24.7 ± 0.3**	16.8 ± 0.3**
Heart						
Absolute	0.124 ± 0.004	0.126 ± 0.003	0.130 ± 0.005	0.129 ± 0.004	0.123 ± 0.004	0.098 ± 0.006**
Relative	4.50 ± 0.14	4.57 ± 0.10	4.73 ± 0.19	5.02 ± 0.15*	5.00 ± 0.19*	5.87 ± 0.35**
R. Kidney						
Absolute	0.179 ± 0.002	0.183 ± 0.008	0.199 ± 0.005*	0.191 ± 0.002	0.199 ± 0.003*	0.175 ± 0.004
Relative	6.54 ± 0.12	6.61 ± 0.26	7.25 ± 0.17**	7.45 ± 0.07**	8.09 ± 0.06**	10.41 ± 0.09**
Liver						
Absolute	1.343 ± 0.028	1.850 ± 0.039**	2.091 ± 0.046**	2.260 ± 0.057**	2.422 ± 0.037**	1.315 ± 0.034**
Relative	48.88 ± 0.91	66.80 ± 1.03**	76.09 ± 0.85**	88.10 ± 1.14**	98.28 ± 1.39**	78.32 ± 1.34**
Lung						
Absolute	0.197 ± 0.006	0.195 ± 0.007	0.202 ± 0.012	0.183 ± 0.010	0.180 ± 0.007	0.134 ± 0.004**
Relative	7.15 ± 0.18	7.07 ± 0.28	7.38 ± 0.51	7.15 ± 0.38	7.32 ± 0.32	7.96 ± 0.17
Thymus						
Absolute	0.055 ± 0.004	0.052 ± 0.002	0.049 ± 0.002	0.046 ± 0.002*	0.045 ± 0.002**	0.015 ± 0.002**
Relative	2.02 ± 0.13	1.89 ± 0.09	1.79 ± 0.09	1.80 ± 0.06	1.82 ± 0.06	0.90 ± 0.12**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

APPENDIX D
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE D1
Summary of Reproductive Tissue Evaluations in Male Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	379 ± 7	374 ± 5	352 ± 5**	340 ± 5**
Left cauda epididymis	0.1393 ± 0.0039	0.1327 ± 0.0055	0.1339 ± 0.0037	0.1373 ± 0.0032
Left epididymis	0.4626 ± 0.0060	0.4459 ± 0.0039	0.4484 ± 0.0064	0.4453 ± 0.0038
Left testis	1.4755 ± 0.0266	1.5253 ± 0.0237	1.5054 ± 0.0326	1.5477 ± 0.0191
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.63 ± 0.16	9.18 ± 0.19	9.46 ± 0.15	9.37 ± 0.16
Spermatid heads (10^7/testis)	14.21 ± 0.36	13.98 ± 0.23	14.21 ± 0.25	14.50 ± 0.28
Spermatid count (mean/10^{-4} mL suspension)	71.05 ± 1.80	69.88 ± 1.16	71.05 ± 1.27	72.48 ± 1.41
Epididymal spermatozoal measurements				
Motility (%)	66.97 ± 0.76	68.85 ± 1.27	67.80 ± 1.12	66.49 ± 1.22
Concentration (10^6/g cauda epididymal tissue)	507 ± 29	544 ± 34	529 ± 27	461 ± 20

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE D2
Summary of Estrous Cycle Characterization in Female Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
n	10	10	10	10
Necropsy body wt (g)	219 ± 3	197 ± 2**	192 ± 3**	182 ± 2**
Estrous cycle length (days)	5.00 ± 0.14[b]	5.40 ± 0.41	5.50 ± 0.30	6.11 ± 0.48[b]
Estrous stages (% of cycle)				
Diestrus	40.0	47.5	44.2	49.2
Proestrus	15.8	16.7	15.0	17.5
Estrus	24.2	18.3	20.0	15.8
Metestrus	19.2	17.5	20.8	17.5
Uncertain diagnoses	0.8	0.0	0.0	0.0

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

TABLE D3
Summary of Reproductive Tissue Evaluations in Male Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	33.3 ± 0.8	33.7 ± 0.8	31.8 ± 0.6	28.6 ± 0.5**
Left cauda epididymis	0.0147 ± 0.0007	0.0142 ± 0.0009	0.0134 ± 0.0008	0.0124 ± 0.0010
Left epididymis	0.0429 ± 0.0017	0.0440 ± 0.0010	0.0413 ± 0.0013	0.0381 ± 0.0010*
Left testis	0.1174 ± 0.0012	0.1207 ± 0.0019	0.1190 ± 0.0020	0.1098 ± 0.0024*
Spermatid measurements				
Spermatid heads (10^7/g testis)	20.02 ± 0.50	19.96 ± 0.48	19.50 ± 0.31	21.34 ± 0.64
Spermatid heads (10^7/testis)	2.35 ± 0.06	2.40 ± 0.04	2.32 ± 0.06	2.34 ± 0.07
Spermatid count (mean/10^{-4} mL suspension)	73.38 ± 1.72	75.08 ± 1.30	72.58 ± 1.95	73.10 ± 2.15
Epididymal spermatozoal measurements				
Motility (%)	67.35 ± 0.90	65.23 ± 1.28	67.74 ± 0.87	67.10 ± 0.87
Concentration (10^6/g cauda epididymal tissue)	832 ± 71	1,049 ± 94	1,070 ± 92	934 ± 76

* Significantly different (P≤0.05) from the control group by Dunnett's test
** Significantly different (P≤0.01) from the control group by Williams' test
[a] Data are presented as mean ± standard error. Differences from the control group for spermatid and epididymal spermatozoal measurements are not significant by Dunn's test.

TABLE D4
Summary of Estrous Cycle Characterization in Female Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Necropsy body wt (g)	27.5 ± 0.5	27.5 ± 0.4	25.6 ± 0.4**	24.7 ± 0.3**
Estrous cycle length (days)	4.10 ± 0.10	4.00 ± 0.00	4.00 ± 0.00	4.15 ± 0.11
Estrous stages (% of cycle)				
Diestrus	30.0	28.3	28.3	24.2
Proestrus	20.8	20.8	20.8	22.5
Estrus	26.7	26.7	27.5	29.2
Metestrus	22.5	24.2	23.3	24.2

** Significantly different (P≤0.01) from the control group by Williams' test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.

APPENDIX E
CYTOCHROME P_{450} ANALYSIS RESULTS

TABLE E1
Liver Cytochrome P_{450} Concentrations and Enzyme Activities for Rats in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	5	5	5		5
Male					
Cytochrome P_{450} (nmol/mg protein)	0.704 ± 0.023	0.940 ± 0.024	1.064 ± 0.034**	0.992 ± 0.032**	0.830 ± 0.035
Ethoxyresorufin deethylase (pmol/min/mg protein)	49.2 ± 3.9	134.5 ± 4.9**	125.3 ± 11.2**	68.8 ± 5.3	66.5 ± 4.3
Ethoxyresorufin deethylase (pmol/min/nmol cytochrome P_{450})	70.1 ± 5.5	143.7 ± 7.6**	118.7 ± 12.5*	69.1 ± 4.0	80.2 ± 4.4
Pentoxyresorufin dealkylase (pmol/min/mg protein)	8.8 ± 0.8	628.0 ± 48.9	769.7 ± 32.5**	612.9 ± 110.2*	574.8 ± 108.1
Pentoxyresorufin dealkylase (pmol/min/nmol cytochrome P_{450})	12.7 ± 1.5	667.5 ± 48.3**	729.4 ± 50.3**	629.5 ± 119.8**	702.9 ± 134.5**
Female					
Cytochrome P_{450} (nmol/mg protein)	0.703 ± 0.025	0.956 ± 0.056*	0.995 ± 0.059**	0.928 ± 0.034*	0.892 ± 0.036
Ethoxyresorufin deethylase (pmol/min/mg protein)	56.5 ± 2.9	92.9 ± 6.1**	85.0 ± 2.6	79.3 ± 7.0	64.3 ± 7.0
Ethoxyresorufin deethylase (pmol/min/nmol cytochrome P_{450})	80.8 ± 5.3	99.8 ± 11.5	86.5 ± 5.4	85.8 ± 8.4	72.3 ± 8.2
Pentoxyresorufin dealkylase (pmol/min/mg protein)	5.4 ± 0.5	307.0 ± 23.0**	383.9 ± 36.5**	534.1 ± 39.3**	515.2 ± 34.3**
Pentoxyresorufin dealkylase (pmol/min/nmol cytochrome P_{450})	7.7 ± 0.8	324.1 ± 27.9**	395.5 ± 53.7**	576.0 ± 40.4**	579.2 ± 38.1**

* Significantly different ($P \le 0.05$) from the control group by Dunn's test
** Significantly different ($P \le 0.01$) from the control group by Dunn's or Shirley's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data. All 20,000 ppm rats died before the end of the study.

TABLE E2
Liver Cytochrome P_{450} Concentrations and Enzyme Activities for Mice in the 14-Week Feed Study of Benzophenone[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n	5	5	5	5	5	0
Cytochrome P_{450} (nmol/mg protein)	1.082 ± 0.050	1.347 ± 0.040*	1.385 ± 0.042**	1.361 ± 0.057*	1.145 ± 0.039	
Ethoxyresorufin deethylase (pmol/min/mg protein)	300.7 ± 13.3	378.8 ± 23.9*	448.5 ± 32.4**	422.3 ± 31.1**	449.8 ± 50.4**	
Ethoxyresorufin deethylase (pmol/min/nmol cytochrome P_{450})	278.9 ± 12.5	281.2 ± 15.8	326.7 ± 29.5	314.1 ± 32.3	399.9 ± 56.9	
Pentoxyresorufin dealkylase (pmol/min/mg protein)	25.5 ± 3.6	352.3 ± 10.0**	454.0 ± 23.0**	503.8 ± 29.4**	524.0 ± 39.4**	
Pentoxyresorufin dealkylase (pmol/min/nmol cytochrome P_{450})	24.0 ± 3.7	262.3 ± 9.6**	330.8 ± 25.9**	370.7 ± 17.9**	458.8 ± 33.9**	
Female						
n	5	5	5	5	5	5
Cytochrome P_{450} (nmol/mg protein)	0.924 ± 0.041	1.101 ± 0.006	1.063 ± 0.016	1.014 ± 0.069	0.862 ± 0.062	0.501 ± 0.056
Ethoxyresorufin deethylase (pmol/min/mg protein)	209.7 ± 12.4	415.0 ± 20.3**	378.9 ± 36.3**	347.0 ± 20.1**	383.1 ± 24.2**	434.7 ± 21.2**
Ethoxyresorufin deethylase (pmol/min/nmol cytochrome P_{450})	227.7 ± 13.8	376.8 ± 18.4**	356.2 ± 32.1**	358.2 ± 51.2**	454.9 ± 45.1**	895.2 ± 75.6**
Pentoxyresorufin dealkylase (pmol/min/mg protein)	43.5 ± 3.4	385.8 ± 8.5*	451.4 ± 29.5**	481.8 ± 18.2**	349.2 ± 11.9	144.1 ± 15.3
Pentoxyresorufin dealkylase (pmol/min/nmol cytochrome P_{450})	47.1 ± 2.8	350.3 ± 7.4	425.2 ± 28.6**	486.9 ± 32.5**	418.2 ± 47.4*	303.6 ± 48.0

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

APPENDIX F
GENETIC TOXICOLOGY

TABLE F1
Mutagenicity of Benzophenone in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	118 ± 12.3	118 ± 11.7	111 ± 1.8	133 ± 7.2	105 ± 8.5	146 ± 4.4
	1	113 ± 7.4					
	3	107 ± 8.1	125 ± 2.2	95 ± 4.6	130 ± 3.8		
	10	110 ± 10.3	132 ± 7.7	102 ± 7.3	136 ± 4.1	90 ± 6.6	131 ± 4.3
	33	100 ± 6.4	123 ± 2.8	84 ± 3.9	128 ± 10.7	96 ± 4.2	112 ± 11.7
	100	110 ± 4.7	114 ± 9.8	78 ± 6.3	154 ± 7.0	99 ± 7.5	124 ± 1.9
	166		52 ± 7.5[c]				
	333			80 ± 4.1	117 ± 8.7	86 ± 7.0	90 ± 6.6
	1,000					50 ± 6.1[c]	35 ± 10.9[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		383 ± 14.9	297 ± 16.9	1,784 ± 26.1	2,174 ± 37.4	922 ± 112.2	1,638 ± 60.4
TA1535	0	36 ± 1.9	32 ± 2.3	11 ± 2.1	16 ± 1.8	13 ± 3.5	6 ± 1.2
	1	33 ± 2.5					
	3	37 ± 0.7	30 ± 3.2	9 ± 1.7	9 ± 1.7		
	10	31 ± 0.7	30 ± 1.2	9 ± 1.8	10 ± 2.2	11 ± 2.7	12 ± 3.0
	33	26 ± 5.2	27 ± 2.0	10 ± 2.7	10 ± 1.5	8 ± 0.3	6 ± 3.7
	100	32 ± 3.8	22 ± 5.4	7 ± 0.6	11 ± 3.0	10 ± 2.7	8 ± 3.4
	166		0 ± 0.0[c]				
	333			6 ± 1.5	8 ± 0.9	8 ± 2.7	5 ± 0.3
	1,000					4 ± 1.0	1 ± 0.9[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		395 ± 21.7	404 ± 28.2	492 ± 17.2	691 ± 15.2	211 ± 18.1	535 ± 23.0
TA1537	0	4 ± 0.9	7 ± 0.3	9 ± 0.9	7 ± 0.6	7 ± 0.3	6 ± 1.2
	1	6 ± 2.1					
	3	5 ± 0.7	5 ± 1.8	8 ± 2.3	7 ± 2.4		
	10	4 ± 0.9	7 ± 0.6	5 ± 1.2	8 ± 2.6	6 ± 1.2	5 ± 0.7
	33	6 ± 1.7	6 ± 1.2	7 ± 1.5	8 ± 2.3	6 ± 1.2	13 ± 2.0
	100	4 ± 0.3	5 ± 1.8	7 ± 1.8	8 ± 2.7	8 ± 0.6	8 ± 0.6
	166		2 ± 0.3[c]				
	333			3 ± 1.5	5 ± 1.5	7 ± 0.9	5 ± 1.5
	1,000					4 ± 1.8	3 ± 0.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		186 ± 19.4	443 ± 51.6	408 ± 11.7	125 ± 7.3	132 ± 20.3	509 ± 19.9

TABLE F1
Mutagenicity of Benzophenone in *Salmonella typhimurium*

Strain	Dose (μg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0	98 ± 78.0	13 ± 2.6	36 ± 2.5	32 ± 0.0	23 ± 2.3	31 ± 3.3
	1	19 ± 0.3					
	3	19 ± 3.8	13 ± 4.8	34 ± 3.3	39 ± 1.5		
	10	19 ± 1.9	10 ± 2.4	30 ± 2.8	34 ± 4.5	33 ± 1.3	30 ± 0.7
	33	20 ± 2.3	17 ± 0.9	31 ± 2.7	36 ± 4.2	21 ± 2.4	27 ± 7.5
	100	14 ± 1.9	12 ± 2.2	30 ± 3.2	33 ± 4.4	28 ± 5.5	27 ± 1.2
	166		0 ± 0.0[c]				
	333			23 ± 1.0	15 ± 1.2	25 ± 4.5	14 ± 3.2
	1,000					15 ± 2.1	6 ± 0.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		475 ± 5.4	431 ± 38.4	1,629 ± 25.7	1,901 ± 39.4	867 ± 11.9	1,221 ± 9.9

[a] Study was performed at SRI International. The detailed protocol and these data are presented by Mortelmans *et al*. (1986). 0 μg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE F2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with Benzophenone by Intraperitoneal Injection[a]

Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	Pairwise P Value[c]
Corn Oil[d]			
	5	1.2 ± 0.4	
Cyclophosphamide[e]			
25	5	22.4 ± 1.9	0.000
Benzophenone			
200	5	1.5 ± 0.3	0.282
300	5	1.5 ± 0.4	0.282
400	5	2.2 ± 0.7	0.043
500	5	1.7 ± 0.4	0.176
		P=0.085[f]	

[a] Study was performed at Environmental Health Research and Testing, Inc. The protocol is presented by Shelby *et al*. (1993).
[b] Mean ± standard error. PCE=polychromatic erythrocyte
[c] Pairwise comparison of treated group to control group; significant at P=0.006 (ILS, 1990)
[d] Solvent control
[e] Positive control
[f] Significance of micronucleated PCEs/1,000 PCEs was tested by a one-tailed trend test; significant at P≤0.025 (ILS, 1990).

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ISSN 2378-8992