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Number 21

NTP Technical Report
on Toxicity Studies of

2-Hydroxy-4-methoxybenzophenone

(CAS Number: 131-57-7)

Adminstered Topically and in Dosed Feed
to F344/N Rats and B6C3F$_1$ Mice

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The NTP Report on the Toxicity Studies of 2-Hydroxy-4-methoxybenzophenone is based primarily on 2- and 13-week studies that began in June, 1985, and concluded in July, 1988, at EG&G Mason Research Institute, Worcester, MA.

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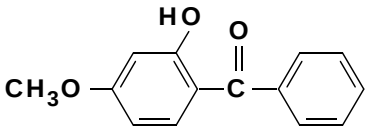
2-Hydroxy-4-methoxybenzophenone



CAS Number: 131-57-7

Molecular Weight: 228.26

Synonyms: Oxybenzone; 4-Methoxy-2-hydroxy-benzophenone; Cyasorb UV; Uvinul M 40; (2-hydroxy-4-methoxyphenyl)phenyl-methanone; NSC-7778; Spectra-sorb UV; Syntase 62; UF 3; USAF CY-9; NCI-C60957

ABSTRACT

2-Hydroxy-4-methoxybenzophenone (HMB) occurs naturally in flower pigments and is synthesized for use in sunscreens, as a UV stabilizer in various cosmetic products, and in plastic surface coatings and polymers. Toxicity studies of HMB were performed in F344/N rats and B6C3F$_1$ mice, by administering HMB in feed and by topical application, in studies of 2 weeks' (5 animals/sex, dose and species) and 13 weeks' (10 animals/sex, dose and species) duration. Assessments included hematology, clinical chemistry, urinalysis, reproductive toxicity, and histopathologic evaluations.

In both 2- and 13-week dosed feed studies, rats received diets containing 0, 3125, 6250, 12500, 25000, or 50000 ppm HMB. One high-dose female rat died during the 2-week study. Body weight gains of high-dose male and female rats were reduced in the 13-week study. Liver and kidney weights were increased in dosed rats in both studies. In the 2-week studies, enlarged livers were associated with a marked hepatocyte cytoplasmic vacuolization in rats receiving diets containing concentrations of 6250 ppm HMB or higher; renal lesions, consisting of dilated tubules and regeneration of tubular epithelial cells, were found primarily in high-dose rats. In the 13-week studies, kidney lesions progressed to include papillary degeneration, or necrosis, and inflammation, while the liver lesion appeared to regress; liver enzymes in serum remained elevated. Rats receiving a diet with 50000 ppm HMB showed markedly lower epididymal sperm density and an increase in the length of the estrous cycle at the end of the 13-week studies.

In 2-week dermal studies, rats received topical applications of 1.25 to 20 mg of HMB in an acetone or lotion vehicle. The only effects noted were small and variable increases in liver and kidney weights, reaching statistical significance primarily in the higher dose groups. In 13-

week studies, rats received topical doses from 12.5 to 200 mg/kg HMB in acetone. Kidney weights were elevated in dosed groups of female rats. No other findings were attributed to HMB treatment.

In 2- and 13-week dosed feed studies, mice received feed containing 0, 3125, 6250, 12500, 25000, or 50000 ppm HMB. A dose-related increase in liver weight associated with hepatocyte cytoplasmic vacuolization was the only finding in mice in the 2-week studies. Decreased body weight gains were dose-related in mice in the 13-week studies; mild increases in liver weights were seen in dosed mice of both sexes. Kidney weights were increased variably in dosed females. Microscopic lesions were noted only in the kidneys of males receiving 50000 ppm HMB; these included eosinophilic protein casts in dilated renal tubules and a mild inflammation associated with the dilated tubules. Mice in the highest dose group exhibited a decrease in epididymal sperm density and an increase in length of the estrous cycle.

In 2-week dermal studies, mice received topical applications from 0.5 to 8 mg HMB in an acetone or lotion vehicle. The only effects noted were minimal, variable increases in liver and kidney weights, primarily in the higher dose groups. In 13-week studies, mice received topical doses of 22.75 to 364 mg/kg in acetone. Kidney weights were increased variably in dosed male mice. Epididymal sperm density was decreased at all 3 dose levels evaluated (22.75, 91, and 200 mg/kg).

The genetic toxicity of HMB also was evaluated in mutagenicity studies with *Salmonella typhimurium*, in cytogenetic studies with Chinese hamster ovary (CHO) cells, and by evaluation of micronucleated erythrocytes in peripheral blood smears from mice in the 13-week studies. HMB was weakly mutagenic in *Salmonella* with metabolic activation, and induced sister-chromatid exchanges and chromosomal aberrations in CHO cells in the presence of a metabolic activation system. There was no increase in the frequency of micronucleated erythrocytes in the blood of mice receiving HMB.

In summary, HMB produced generally similar effects following topical and oral administration to rats and mice. Consistent findings included decreases in epididymal sperm density, lengthened estrous cycle, and increased liver and kidney weights. Mice in the dosed feed studies exhibited microscopic changes in the kidneys, comprising tubular dilatation with eosinophilic protein casts. Dilatation, tubular regeneration, papillary degeneration, and inflammation were noted in the kidneys of rats; and liver lesions consisting of an apparently reversible hepatocyte cytoplasmic vacuolization occurred in both rats and mice. A no-observed-adverse-effect level (NOAEL) for microscopic lesions was 6250 ppm HMB in the diet for rats and mice. A NOAEL was not reached for decreased epididymal sperm density in the 13-week dermal study in mice (<23 mg/kg/day).

PEER REVIEW

Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies on 2-hydroxy-4-methoxybenzophenone on November 21, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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Summary of Peer Review Comments

Dr. J.E. French, NIEHS, introduced the short-term toxicity studies of 2-hydroxy-4-methoxy-benzophenone (HMB) by reviewing the natural occurrences and uses of HMB, experimental design, and results. Review of unpublished proprietary information as well as FDA files led to the decision to use a sunscreen lotion base as a dose vehicle and to use both oral and dermal routes of exposure. Additionally, liver, kidney, and male and female reproductive organs were identified as target organs; only the kidney had been indicated in published literature.

Dr. Carlson, a principal reviewer, remarked that this report dealt with 8 studies and that a good job was done of handling a lot of data. He suggested that use of the term, "topical application," was more common and would be more correct than "dermal application." Dr. French commented that the NTP historically has used "dermal" as specific to skin, while "topical" could apply to other sites, e.g., the eye. Dr. Carlson noted that liver function was not determined as stated in the abstract, and that the enzyme changes were measures of damage.

Dr. Goodman, a second principal reviewer, commented that the report was well-written and the results clearly presented. He asked that a clearer rationale be given as to why the study was performed in view of the mention in the report that both an FDA panel and the Cosmetic Ingredient Panel concluded that HMB was safe with regard to its current uses. Dr. French responded that HMB was selected from a review of the ether chemical class study and was nominated primarily on the basis of human exposure and as a representative benzophenone derivative used as a UV screen and UV stabilizer. Dr. Goodman said it would be useful to indicate how the doses that produced toxicity compared with the dose one might anticipate from the "safe" human use of HMB. Dr. French remarked that this would be difficult to do; however, at least in the 2-week dermal studies, a lotion vehicle was used with HMB concentrations which represented the maximum amount to be applied in a sunscreen lotion to human skin.

Dr. Carlson commented that there appeared to be too much emphasis placed on the lack of a NOAEL for decreased epididymal sperm density in the 13-week dermal study in mice. Dr. Richard Davis, American Cyanamid, suggested adding other measures of male reproductive function, such as spermatid counts, in addition to sperm density counts. He said that this would improve the consistency in reporting, noting that for the 4 studies being reviewed there was a three-fold range for control groups alone in sperm density. Dr. B. Schwetz, NIEHS, reported that, since the time the HMB studies were conducted, spermatid head counts were being collected as a reflection of the activity of the spermatogenesis process. Dr. French responded that comparisons between reproductive endpoints collected in different laboratories are made, but that primary emphasis is placed on the concurrent control for interpretation and conclusion. [Subsequent studies of the effect of topically applied HMB on sperm production and characteristics in B6C3F_1 mice, sponsored by the Cosmetic Toiletry and Fragrance Association, failed to show statistically significant decreases in epididymal sperm density or other effects on the reproductive system (Daston, G.P. Gettings, S.D., Carlton, B.D. *et al.*,

(1992) Assessment of the reproductive toxic potential of dermally applied 2-hydroxy-4-methoxybenzophenone to male B6C3F_1 mice, Fundam. Appl. Toxicol., in press].

Seeing no objections, Dr. Klaassen accepted the report with the suggested editorial and other changes on behalf of the panel.

INTRODUCTION

Physical Properties, Production, Uses, and Exposure

2-Hydroxy-4-methoxybenzophenone (HMB), an extended phenyl ring ether, is a pale cream-colored powder (technical grade) with a melting point of 66°C and low volatility.

HMB occurs naturally and has been extracted from flower pigments (Stecher, 1958). Commercial use HMB is prepared by the Friedel-Crafts reaction of benzoyl chloride with 3-hydroxyanisole. The product is isolated by organic extraction and re-crystallized from water/methanol and dried (Cosmetic Ingredient Review, 1983a). The 1979 EPA-TSCA inventory of commercial chemicals listed 6 U.S. companies as manufacturers/importers of HMB., with production ranges varying from 0 - 1000 to 100,000 - 1,000,000 pounds per year. The U.S. International Trade Commission (1979) reported 1976 and 1977 production values at 2.4×10^5 kg and 3.6×10^5 kg, respectively.

HMB is used as a UV stabilizer in cosmetic, pharmaceutical, and plastic products. In 1979, the Cosmetic, Toiletry, and Fragrance Association (CTFA) identified 62 different cosmetic products containing HMB; the largest product lines identified were nail polish and enamel (Cosmetic Ingredient Review, 1983a). HMB is used in skin moisturizing products and sunscreen lotions, usually in conjunction with 2, 2'-dihydroxy-4-methoxy-benzophenone (Reynolds, 1982). In plastics manufacturing, HMB is used in surface coatings, and in the following polymers: ABS resins, cellulosic esters, polyesters, polystyrenes, rubber, flexible (plasticized and semi-rigid) and rigid vinyl, and vinylidene chloride (Abramoff, 1978-79).

Exposure to HMB occurs through both occupational and consumer routes. The National Occupational Health Survey conducted by NIOSH in 1972-1974 estimated 549 workers were exposed to the subject chemical during that period. Annual dermal exposure through cosmetic and sunscreen products was estimated in the NCI/SRI data base to be between 1.06×10^3 and 3.6×10^3 kg (NCI/SRI, 1977). HMB is an approved FDA OTC (over-the-counter) category I product. These products are considered to be a deterrent to ultraviolet radiation-induced skin cancer (U.S. FDA, 1978; Klingman *et al.,* 1980; Sayre, 1981; Cripps and de Dennis, 1981; Girard *et al.*, 1982; Folsom *et al.*, 1983).

HMB has been approved for over-the-counter use in sunscreen and other cosmetic preparations (U.S. FDA, 1978). The FDA Panel on Review of Topical Analgesics has proposed that HMB is safe and effective (Category I) at concentrations of 2-6% in over-the-counter use. The FDA has approved HMB for use as an indirect food additive; i.e., it is permitted in the formulation of rigid acrylic and modified acrylic plastics which are components of single- and repeated-use food contact surfaces (Code of Federal Regulations 21 CFR 177.1010, 1978). HMB may be present in concentrations between 0.01% and 0.05% in olefinic polymers as a UV stabilizer or as an antioxidant. The Cosmetic Ingredient Review Expert Panel, established by the Cosmetic, Toiletries, and Fragrance Association, concluded that HMB is safe for topical application to humans in the present practices of use and concentration in cosmetics (Cosmetic Ingredient Review, 1983a).

Human Toxicity

The Cosmetic Ingredient Review (1983a) cites several unpublished studies relevant to HMB under the heading of clinical assessment of safety. These clinical tests were designed to determine skin irritation and sensitivity under conditions of human use and were not in-depth toxicity studies. It was the opinion of the Review's expert panel that the subject chemical and other benzophenones were safe and effective under the conditions of current cosmetic use. Contact dermatitis, with topical photosensitivity reactions and eczematous eruptions, has been reported in one clinical case (Hölzel and Plewig, 1982).

Animal Toxicity

The acute toxicity of HMB is low. For rats, the oral LD_{50} is greater than 12.8 g/kg (Lewerenz *et al.*, 1972a); the LD_{50} in rabbits (dermal) is greater than 16.0 g/kg (Cosmetic Ingredient Review, 1983a).

In 13-week toxicity studies (Lewerenz *et al.* 1972b), Wistar rats were fed HMB in the diet at concentrations of 0, 0.02, 0.1, 0.5, and 1.0%. Rats fed the chemical at 0.5 and 1.0% of the diet exhibited depressed growth, leukocytosis, anemia, reduced organ weights, and renal toxicity characterized by tubule dilatation.

The potential of HMB to cause acute irritation was tested on intact and abraded skin of albino rabbits. HMB was reported to be nonirritating to skin at concentrations from 4% to 100% (Hölzle and Plewig, 1982). Phototoxicity and photosensitization of HMB also were studied in albino rabbits. Approximately 24 mg of HMB was applied to the shaved skin. The application site was irradiated with UV light, 5 times per week for 2 weeks (10 applications total). Mild erythema, mild edema, and desquamation were noted, but no phototoxicity was reported. The sensitization potential of HMB was tested in guinea pigs by intradermal application of 2.5 mg HMB with Freund's adjuvant, followed 2 weeks later by a topical application of 2.5 mg in petrolatum at a previously untreated site. No indication of HMB-induced skin sensitization was observed in this limited test.

Absorption, Metabolism, and Distribution

An NTP-sponsored study of ^{14}C HMB absorption, distribution, and clearance following oral, intravenous, or topical administration demonstrated that this compound is readily absorbed from the gastrointestinal tract and excreted primarily in urine (El Dareer *et al.*, 1986; Appendix E). Absorption from the gastrointestinal tract was nearly complete at all doses administered across a range of approximately 3 mg/kg to 2.5 g/kg. Similarly, metabolism and clearance were unaffected by dose. Only a trace of the parent compound was excreted unmetabolized; HMB was converted to at least five metabolites and excreted in bile and urine. The major metabolites were identified as glucuronide conjugates of the parent compound and 2,4-dihydroxybenzophenone, and a sulfate ester of a hydroxylated derivative of the parent compound. Excretion was rapid following oral or i.v. administration and was nearly complete within 48 hours after administration. Approximately two-thirds of the dose, whether given intravenously or by oral gavage, was excreted in urine. An examination of residues in all major

tissues indicated some retention of HMB-derived material in liver and kidney, but the levels were relatively low, even in these tissues, accounting for less than 0.1% of the dose within 72 hours after exposure (El Dareer *et al.*, 1986).

Dermal absorption was studied by applying HMB, in either ethanol or a lotion formula, to an area of 1 cm^2. All dermal doses were shielded to prevent removal by grooming. Dermal absorption of low doses applied in ethanol or lotion was appreciable and accounted for approximately 58% of a dose of 0.2 mg/cm^2, but decreased significantly as the dose increased, accounting for only 20% (approximately) of a dose of 0.8 mg/cm^2.

Mutagenicity and Structural Activity Relationships

HMB was negative according to an unpublished *Salmonella* mutagenesis assay reviewed by the Cosmetic Ingredient Review Expert Panel (Cosmetic Ingredient Review, 1983b), in tests screening for mutagenic agents in dental materials (Jonsen *et al.*, 1980), and in tests screening for mutagenic agents in sunscreens (Morita *et al.*, 1981; Bonin *et al.*, 1982). The parent compound, benzophenone, also was negative in an NTP *Salmonella* mutagenesis assay (Mortelmans *et al.*, 1986), but both 2,2'-dihydroxy-4-methoxybenzophenone and 4,4'-bis(dimethylamino) benzophenone (Michler's Ketone) were mutagenic. Michler's ketone also was positive in the mouse lymphoma (L5178Y) mutation assay, in *in vitro* cell transformation, in a Rauscher leukemia virus/rat embryo assay, and in an assay for unscheduled DNA synthesis. Michler's ketone caused hepatocellular carcinomas in male and female rats and female mice, and hemangiosarcomas in male mice in 2-year studies (National Cancer Institute, 1979).

Study Rationale and Design

HMB was selected from a review of the ether chemical class study and was nominated, primarily, on the basis of human exposure and as a representative benzophenone derivative used as a UV screen (suntan lotions) and UV stabilizer in cosmetics and plastics. Because the toxicity and potential carcinogenicity of this chemical class is incompletely documented in the literature, or is unknown, the NTP conducted disposition studies of HMB by the dermal, oral, and i.v. routes (El Dareer *et al.*, 1986; Appendix E), and performed 2- and 13-week toxicity studies in F344 rats and $B6C3F_1$ mice by the dermal and oral routes, including assessments of hematology, clinical chemistry, urinalysis, and the reproductive system. The genetic toxicity of HMB was evaluated in *Salmonella* mutagenicity assays, in Chinese hamster ovary cell cytogenetics assays, and by examination of peripheral blood smears from mice in the 13-week studies for the presence of micronucleated erythrocytes.

MATERIALS AND METHODS

Procurement and Characterization of 2-Hydroxy-4-methoxybenzophenone

The HMB used in these studies was obtained from American Cyanamid Co. (Bridgewater, NJ), and samples were analyzed at Midwest Research Institute (Kansas City, MO). The infrared, ultraviolet/visible, and nuclear magnetic resonance spectra were consistent with the structure of HMB and with available literature references. Elemental analysis results for carbon were slightly high but agreed with theoretical values for hydrogen. Karl Fischer analysis for water indicated less than 0.04%. Analysis by two thin-layer chromatography systems indicated a single spot; no impurities with relative peak areas of greater than 0.1% of the major peak were detected by gas chromatography.

Dose formulation stability studies indicated that acetone solutions of HMB were stable for at least 3 weeks in the dark in sealed vials at room temperature, as were solutions stored for 3 hours open to light and air. HMB also was stable under similar conditions when formulated as part of a lotion comprised of lanolin oil (5.4%), white petrolatum (2.6%), stearic acid (4.3%), propyl paraben (0.05%), propylene glycol (5.4%), methyl paraben triethanolamine (0.1%), disodium EDTA (0.05%), and deionized water (80%). Stability studies conducted on a feed blend indicated a small but significant loss (~2%) after 3 weeks' storage in the dark in sealed containers at room temperature. No significant losses were observed with similar blends kept at 5°C or -20°C. Feed blends stored open to air and light for 3 days in a rat cage exhibited losses (~2%).

Study Design

Male and female F344/N rats and B6C3F$_1$ mice used in the 2-week studies were obtained from the Frederick Cancer Research Facility (Frederick, MD). Rats and mice used in the 13-week studies were obtained from Taconic Farms (Germantown, NY). Rats were housed 5/cage for the feed study and were housed individually for the dermal studies; mice were housed individually during all studies. Animals received NIH-07 diet (Zeigler Bros., Gardners, PA) and water *ad libitum* throughout the studies. Blood samples were collected, and the sera analyzed for viral titers from 5 animals per sex and species at study start and at termination in the 13-week studies. No positive antibody titers were detected in 5 viral screens performed in rats and 12 viral screens performed in mice (Boorman *et al.*, 1986; Rao *et al.*, 1989a, 1989b). Viral serology procedures for the male mice in the 13-week dermal studies were inadequately documented, and no results were available for males in those studies.

In 2-week dosed feed studies, groups of 5 rats and 5 mice of each sex received diets containing 0, 3125, 6250, 12500, 25000, and 50000 ppm HMB. In dermal studies, the same numbers of rats and mice of each sex received applications of HMB 5 days per week in acetone, while similar groups received applications of the chemical in the lotion. The dermal vehicles were formulated to concentrations of 0, 5, 10, 20, 40, or 80 mg/ml. A constant volume of 0.25 ml for rats and 0.1 ml for mice was applied over a fixed standard area (10%) of the interscapular

region. The area was clipped 24 hours prior to the initial application, and weekly thereafter, with an electric clipper. Complete necropsies were performed on all animals at termination; organ weights were recorded for the brain, liver, right kidney, thymus, heart, lung, and right testicle. Tissues examined microscopically are listed in Tables 1 and 2.

In 13-week studies, groups of 10 rats and 10 mice of each sex received diets containing 0, 3125, 6250, 12500, 25000, and 50000 ppm HMB, or received topical applications of HMB, 5 days per week in acetone, at doses of 0, 12.5, 25.0, 50.0, 100.0, and 200.0 mg/kg body weight for rats, and 0, 22.8, 45.5, 91.0, 182.0, and 364.0 mg/kg body weight for mice. Topical doses were given in a volume equal to 300 µl/120 g body weight for rats and 100 µl/120 g body weight for mice. Dosing volume was adjusted weekly based on changes in group mean body weight. When the dosing volume exceeded 300 µl for rats or 100 µl for mice, the volume was applied in two equal doses.

Male and female rats were anesthetized with CO_2 and bled from the retroorbital sinus at day 3, day 15, and after week 12 of treatment. Blood samples were collected with EDTA (~0.50 ml) and without EDTA (~0.75 ml) for the analysis of hematologic and biochemical variables, respectively. Prior to blood collection, rats were housed individually overnight (16 hours) in metabolism cages for the collection of urine; animals were provided water, but not food, and collection tubes were immersed in ice/water baths. Urine samples were measured or evaluated for volume, pH, specific gravity, appearance, and microscopic features.

Samples for hematologic determinations were analyzed using a Baker 7000 analyzer (Baker Instruments Corp., Allentown, PA). Platelet counts were measured using a Baker 810 platelet analyzer. Automated measurements and calculations were red blood cell count (RBC), hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), white blood cell count (WBC), and platelet count. WBC differential counts (absolute) were calculated from relative values determined from microscopic evaluation of Wright's stained blood smears. Reticulocyte counts (absolute) were calculated from relative values determined from the microscopic evaluation of blood smears prepared from samples incubated with equal volumes of blood and new methylene blue.

Biochemical analyses were performed on serum using a Gemini chemistry analyzer (Electro-Nucleonics, Inc., Fairfield, NJ). Except for sorbitol dehydrogenase (SDH), reagents and applications for all assays were obtained from the manufacturer. Reagents for SDH were obtained from Sigma Chemical Co. (St. Louis, MO), and the assay was adapted for the automated analyzer. The remaining analyses were alanine aminotransferase (ALT), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGT), urea nitrogen (UN), and creatinine.

Sperm Morphology and Vaginal Cytology evaluations (SMVCE) were performed for rats and mice given diets containing 0, 3125, 12500, and 50000 ppm in the 13-week dosed feed studies. For the dermal studies, evaluations were performed for rats administered 0, 12.5, 50.0, and 200.0 mg/kg and mice administered 0, 22.8, 91.0, and 364.0 mg/kg HMB. Procedures described by Morrissey *et al.* (1988) were used. For the 12 days prior to sacrifice, females were subject to vaginal lavage with saline. The aspirated cells were air-dried onto slides, stained with

Toluidine Blue O, and cover slipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrous cycle.

Sperm motility was evaluated at necropsy as follows: the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, and a small cut was made in the distal cauda epididymis. The sperm that extruded from the epididymis were dispersed throughout the solution, cover slipped, and the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field were counted. After sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS) and minced; the solution was mixed gently, and heat-fixed at 65°C. Sperm density was subsequently determined using a hemocytometer.

To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis, and per gram of testis.

Complete necropsies were performed on all animals at termination; organ weights were recorded for the brain, liver, right kidney, thymus, heart, lung, and right testicle. In the dosed feed studies, all tissues from the control and 50000 ppm animals were evaluated microscopically; in the dermal studies, all tissues from the control and high-dose animals were evaluated microscopically. Gross lesions from all dose levels received microscopic evaluation in both studies. Tissues were preserved in 10% neutral buffered formalin and were routinely processed for preparation of histologic sections for microscopic examination. Tissues and groups examined for rats and mice are listed in Tables 1 and 2.

Upon completion of the histologic evaluation by the laboratory pathologist, 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; the 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).

Genetic Toxicity

Mutagenicity Studies

Mutagenicity studies of HMB in *Salmonella typhimurium* were conducted as described in Zeiger *et al.* (1988). Briefly, HMB was tested for mutagenicity in *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537, using a preincubation assay in both the absence or presence of Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. HMB was tested at doses up to 1000 µg/plate. Higher concentrations were toxic to the cells. A positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal

response was defined as an increase in revertants which was not dose-related, not reproducible, or was of insufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies has observed following chemical treatment.

Chinese Hamster Ovary Cytogenetics Assays

Testing was performed as reported by Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells (CHO) were incubated with HMB or solvent (dimethylsulfoxide) for induction of sister-chromatid exchanges (SCE) and chromosomal aberrations (ABS) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Additional details are provided in Appendix D.

Mouse Peripheral Blood Micronucleus Assays

At the termination of the 13-week study, blood smears were prepared from peripheral blood samples obtained from the retroorbital sinus of all dosed and control mice. The slides were stained with Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983). Ten thousand normochromatic erythrocytes from each animal were scored for micronuclei.

Statistical Methods

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose-response (Dunnett, Dunn). 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.

Analysis of Vaginal Cytology Data

Since these data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a

multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

Analysis of CHO Cytogenetics Assays

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response (Margolin *et al.*, 1986). The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. A single increased dose was considered weak evidence of a positive response (+W); two increased doses were sufficient to evaluate the trial as positive (+). Chromosomal aberration data are presented as percentage of cells with aberrations. Both the dose-response and individual dose points were statistically analyzed. For a single trial, a statistically significant ($P<0.05$) increase for one dose point and a significant trend ($P<0.015$) was considered weak evidence for a positive response (W+); significant increases for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

Analysis of Micronucleus Data

Statistical analyses for micronuclei were performed using linear trend tests on log-transformed data for normochromatic erythrocytes. The frequency of micronuclei in the dosed groups was compared with the frequency determined for the concurrent untreated control animals using Student's t-test.

Quality Assurance

The studies of HMB were performed in compliance with FDA Good Laboratory Practices regulations (Code of Federal Regulations 21 CFR 58). The Quality Assurance Unit of EG&G Mason performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The operations of the Quality Assurance Unit were monitored by the NTP.

TABLE 1 Materials and Methods and Experimental Design in the 2-Week and 13-Week Dosed Feed and Dermal Studies of 2-Hydroxy-4-methoxybenzophenone

ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N rats B6C3F$_1$ mice
Animal Source	2-Week Studies: Frederick Cancer Research Facilities, Frederick, MD 13-Week Studies: Taconic Farms, Germantown, NY
Study Laboratory	EG&G Mason Research Institute, Worcester, MA
Time Held Before Study	2-Week Studies, Dosed Feed: 10-12 days
	13-Week Studies, Dosed Feed: Male rats – 11-12 days; Female rats--18-19 days; Mice--13-14 days
	2-Week Studies, Dermal: Male rats--12-14 days; Female Rats--13 -15 days; Male mice--11-12 days: Female mice--13 days
	13-Week Studies, Dermal: Male rats--11-12 days; Female Rats--18-19 days; \ Mice--13-14 days
Age When Placed on Study	2-Week Studies: 6 wks
	13-Week Studies: Male rats--6 wks; Female rats--7 wks; Mice--44-45 days
Age When Killed	2-Week Studies: 8 wks
	13-Week Studies: Male rats -- 19 wks; Female rats -- 20 wks; Mice--19 wks
Method of Animal Distribution	Animals assigned to groups using a stratified weight method and then assigned to study groups in random order
Diet	NIH-07 Open Formula, *ad libitum* Zeigler Bros., Gardners, PA
Animal Room Environment	Temp--72 ± 3°F; relative humidity--50 ± 15%; fluorescent light 12 h/d; 10 air changes/h.
EXPERIMENTAL DESIGN	
Size of Study Groups	2-Week Studies, Dosed Feed: 5/sex/group of each species. Rats were housed 5 per cage, and mice were individually housed.
	13-Week Studies, Dosed Feed: 10/sex/group of each species. Rats were housed 5 per cage, and mice were individually housed.
	2-Week Studies, Dermal: 5/sex/group of each species. Rats and mice were individually housed.
	13-Week Studies, Dermal: 10/sex/group of each species. Rats and mice were individually housed.
Doses/Duration of Dosing	2-Week Studies, Dosed Feed: 0, 3125, 6250, 12500, 25000, and 50000 ppm in the feed
	13-Week Studies, Dosed Feed: 0, 3125, 6250, 12500, 25000, and 50000 ppm in the feed
	2-Week Studies, Dermal: Doses administered dermally in an acetone or lotion vehicle in a constant volume of 0.25 ml for rats and 0.1 ml for mice: Rats--0, 1.25, 2.5, 5, 10, or 20 mg, Mice--0, 0.5, 1.0, 2, 4, or 8 mg
	13-Week Studies, Dermal: Doses administered dermally in an acetone vehicle in a constant volume of 0.25 ml for rats and 0.1 ml for mice: Rats--0, 12.5, 25.0, 50.0, 100.0, and 200.0 mg/kg body weight; Mice--0, 22.8, 45.5, 191.0, 182, and 364 mg/kg body weight.

TABLE 1 Materials and Methods and Experimental Design in the 2-Week and 13-Week Dosed Feed and Dermal Studies of 2-Hydroxy-4-methoxybenzophenone (continued)

Type and Frequency of Observation	2-Week Studies, Dosed Feed: Observed 2 x d for mortality/moribundity; 1 x wk for clinical signs of toxicity; food consumption measured weekly; weighed initially, after first week, at termination of dosing period, and at necropsy. 13-Week Studies, Dosed Feed: Observed 2 x d for mortality/moribundity; 1 x wk for clinical signs of toxicity; food consumption measured weekly; weighed initially, weekly, and at necropsy. 2-Week Studies, Dermal: Observed 2 x d for mortality/moribundity; 1 x wk for clinical signs of toxicity; site of application examined weekly; weighed initially, after the first week, and at necropsy. 13-Week Studies, Dermal: Observed 2 x d for mortality/moribundity; 1 x wk for clinical signs of toxicity; site of application examined weekly; weighed initially, weekly, and at necropsy.
Necropsy and Histologic Examinations	Necropsy performed on all animals; the following tissues were examined microscopically from all high dose and controls: 2-Week Studies Gross lesions, adrenal gland, brain, esophagus, femur with bone marrow, gall bladder (mice), heart, intestine (duodenum, jejunum, ileum, cecum, colon, rectum), kidney, liver, lungs and bronchi, mandibular and mesenteric lymph nodes, mammary gland with adjacent skin, nasal cavity and turbinates, ovary, pancreas, parathyroid, pituitary, preputial or clitoral gland (rats only) prostate, salivary gland, seminal vesicle, spleen, stomach (forestomach and glandular), testis and epididymis, thymus, thyroid, trachea, urinary bladder, uterus. Other tissues examined: skin (application site)(dermal studies,only) 13-Week Studies Gross lesions, adrenal gland, brain, esophagus, eyes (if grossly abnormal), femur with bone marrow, gall bladder (mice), heart, intestine (duodenum, jejunum, ileum, cecum, colon, rectum), kidney, liver, lungs and bronchi, mandibular and mediastinal lymph nodes, nasal cavity with turbinates, ovary, pancreas, parathyroid, pituitary, preputial or clitoral gland (rats only), prostate, salivary gland, seminal vesicle, spinal cord (if neurologic signs present), spleen, stomach (forestomach and glandular), testis and epididymis, thymus, thyroid, trachea, urinary bladder, uterus. In addition to all gross lesions, the kidney was examined in all dose groups. Organ weights obtained from all core study animals include: liver, thymus, right kidney, right testis, heart, and lungs.
Supplemental Evaluations	Hematology, Clinical Chemistry, and Urinalysis: Hematology, clinical chemistry, and urinalysis were evaluated in rats, only, on day 3, day 15, and week 12. Sperm Morphology/Vaginal Cytology: Dosed Feed: sperm morphology and vaginal cytology were evaluated in rats and mice exposed to 0, 3125, 12500, and 50000 ppm HMB. Dermal: sperm morphology and vaginal cytology were evaluated in rats exposed to 0, 12.5, 50.0, and 200.0 mg/kg HMB and in mice exposed to 0, 22.8, 91.0, and 364.0 mg/kg HMB.

RESULTS

2-Week Dosed Feed Studies in Rats

One female rat in the 50000 ppm dose group died on day 8; the cause of death could not be determined. No clinical observations or gross pathological changes were noted that were considered related to chemical exposure. Feed consumption of male and female rats given diet containing 50000 ppm HMB was reduced compared to controls, and body weight gains of male rats also were decreased (Table 2).

TABLE 2 Survival, Weight Gain, and Feed Consumption of F344/N Rats in the 2-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
MALE							
0	5/5	123.6	203.9	80.3		15.8	
3125	5/5	122.7	208.6	85.9	102	15.8	295
6250	5/5	122.1	208.7	86.6	102	15.8	589
12500	5/5	122.5	209.3	86.8	103	15.5	1159
25000	5/5	120.8	200.2	79.4	98	14.7	2259
50000	5/5	125.1	178.2	53.1	87	12.9	4210
FEMALE							
0	5/5	100.5	135.6	35.1		10.9	
3125	5/5	104.8	133.8	29.0	99	12.0	311
6250	5/5	100.8	131.2	30.4	97	10.6	564
12500	5/5	102.1	139.9	37.8	103	10.8	1104
25000	5/5	103.1	141.4	38.3	104	11.0	2218
50000	4/5	103.1	139.7	36.6	103	8.7	3527

[a] Number surviving to study termination / number of animals per group.
[b] Mean weight change of the animals in each dose group.
[c] [Dosed group mean / control group mean] x 100.
[d] Food consumption is given in grams/animal/day.
[e] Time-weighted compound consumption = average compound consumed during the 2-week study (mg/kg body weight/day).

Marked increases in liver weights were seen in dosed male and female rats; kidney weights were increased in dosed male rats (Table 3). Increased liver weights were associated with the presence of cytoplasmic vacuolization of hepatocytes in males and females in the 6250 ppm and higher dose groups. This change was characterized by an irregular perinuclear vacuolization of the cytoplasm, resembling that typically observed after cytoplasmic glycogen deposits are dissolved during tissue processing for histopathologic examination. However, this vacuolization was much more extensive than that which occurred in the control and low dose groups. Special stains for fat and glycogen did not reveal a difference between livers from dosed and control animals.

Chemically-related microscopic lesions in the kidney were limited to male rats given feed containing 50000 ppm HMB. Focal dilatation of renal tubules in the cortex and/or medulla (Plate 1) was present in 4/5 male rats. Minimal to mild regeneration of the renal tubular epithelium, associated with these foci, was present in 3 of these rats; a focal area of necrosis in the renal papilla was present in 1 rat (Plate 2).

TABLE 3 Kidney and Liver Weights and Organ-Weight-to-Body-Weight Ratios of F344/N Rats in the 2-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone[a]

Dose	0 ppm	3125 ppm	6250 ppm	12500 ppm	25000 ppm	50000 ppm
MALE						
n	5	5	5	5	5	5
Necropsy body wt	203.9 ± 2.4	208.6 ± 5.2	208.7 ± 2.9	208.6 ± 5.8	200.2 ± 3.5	178.2 ± 3.3
R. Kidney						
Absolute	0.955 ± 0.017	1.058 ± 0.047	1.067 ± 0.014**	1.092 ± 0.039*	1.006 ± 0.030	0.996 ± 0.021
Relative	4.686 ± 0.055	5.073 ± 0.017	5.118 ± 0.040**	5.229 ± 0.050**	5.024 ± 0.135*	5.590 ± 0.105**
Liver						
Absolute	10.22 ± 0.39	12.65 ± 0.30**	13.74 ± 0.43**	16.15 ± 0.69**	16.84 ± 0.68**	16.61 ± 0.74**
Relative	50.1 ± 1.4	60.7 ± 0.8**	65.8 ± 1.3**	77.3 ± 2.0**	84.0 ± 2.0**	93.2 ± 3.4**
FEMALE						
n	5	5	5	5	5	4
Necropsy body wt	135.6 ± 1.6	133.8 ± 2.4	131.2 ± 2.2	139.9 ± 1.7	141.4 ± 2.1	139.7 ± 2.8
R. Kidney						
Absolute	0.688 ± 0.011	0.733 ± 0.082	0.685 ± 0.0423	0.729 ± 0.020	0.735 ± 0.030	0.748 ± 0.049
Relative	4.099 ± 0.150	4.460 ± 0.387	4.152 ± 0.349	4.430 ± 0.191	4.452 ± 0.238	4.570 ± 0.409
Liver						
Absolute	6.60 ± 0.21	6.54 ± 0.32	7.72 ± 0.27*	9.14 ± 0.20**	10.28 ± 0.21**	11.36 ± 0.47**
Relative	48.7 ± 1.6	48.8 ± 1.7	58.8 ± 1.9**	65.4 ± 1.4**	72.8 ± 2.2**	81.2 ± 1.9**

[a] Organ weights and body weights are given in grams; organ-weight-to-body weight ratios are given as mg organ weight/g body weight (mean ± standard error).
* Significantly different from the control group by Student's t-test. (P 0.05).
** Significantly different from the control group by Student's t-test. (P 0.01).

13-Week Dosed Feed Studies in Rats

One low dose male rat was killed accidentally during the first week; there were no other deaths during the study (Table 4). Dose-related decreases in growth (Figure 1) and in final body weights were noted in male and female rats (Table 4). Chemically-related clinical signs were limited to urine-stained fur in the perineal area and a dark yellow to opaque-greenish urine.

At necropsy, chemically-related gross lesions were observed in the two highest dose groups of male rats and in the highest dose group of female rats. The kidneys were enlarged and had an abnormal shape and granular surface. In some animals both kidneys were affected, while in others, the changes were limited to one kidney. Absolute and relative right kidney weights were increased in males in the 50000 ppm group and in females in the 25000 and 50000 ppm groups (Table 5; Appendix A3).

Histopathologic lesions in the kidney of male and female rats were similar, consisting of dilatation of renal tubules, regeneration of tubule epithelial cells, papillary degeneration or necrosis, and inflammation (Table 6). The most prominent lesion was dilatation of the renal tubules (Plate 3), the severity of which was greater in males than in females, occurring in

TABLE 4 Survival, Weight Gain, and Feed Consumption of F344/N Rats in the 13-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
MALE							
0	10/10	116	333	217		16.4	
3125	9/10	115	335	220	101	16.9	213
6250	10/10	115	321	206	96	16.5	429
12500	10/10	114	318	204	95	16.6	875
25000	10/10	116	295	179	89	16.5	1805
50000	10/10	114	228	114	68	14.0	3656
FEMALE							
0	10/10	130	200	70		11.2	
3125	10/10	130	196	66	98	10.8	196
6250	10/10	130	190	60	95	10.6	393
12500	10/10	128	178	50	89	10.3	780
25000	10/10	129	179	50	90	10.4	1599
50000	10/10	131	171	40	86	10.2	3261

[a] Number surviving to study termination / number of animals per group.
[b] Mean weight change of the animals in each dose group.
[c] [Dosed group mean / control group mean] x 100.
[d] Food consumption is given in grams/animal/day.
[e] Time-weighted compound consumption = average compound consumed during the 13-week study (mg/kg body weight/day).

TABLE 5 Kidney and Liver Weights and Organ-Weight-to-Body-Weight Ratios of F344/N Rats in the 13-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone[a]

Dose	0 ppm	3125 ppm	6250 ppm	12500 ppm	25000 ppm	50000 ppm
MALE						
n	10	9	10	10	10	10
Necropsy body wt	334 ± 5	333 ± 6	327 ± 6	321 ± 6	299 ± 7**	230 ± 9**
R. Kidney						
Absolute	1.26 ± 0.03	1.39 ± 0.03	1.42 ± 0.04	1.44 ± 0.03	1.48 ± 0.04	2.11 ± 0.32**
Relative	3.76 ± 0.07	4.19 ± 0.09	4.33 ± 0.05	4.49 ± 0.07	4.96 ± 0.09	9.73 ± 1.84**
Liver						
Absolute	14.41 ± 0.42	16.38 ± 0.32*	17.64 ± 0.56**	18.11 ± 0.46**	17.55 ± 0.80**	15.68 ± 0.71**
Relative	43.1 ± 0.97	49.2 ± 0.74**	53.9 ± 1.0**	56.5 ± 0.86**	58.5 ± 1.95**	68.3 ± 1.42**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	186 ± 6	187 ± 2	188 ± 3	175 ± 3	181± 3	165 ± 3**
R. Kidney						
Absolute	0.781 ± 0.018	0.759 ± 0.011	0.851 ± 0.016	0.777 ± 0.010	0.857 ± 0.012*	0.842 ± 0.029 *
Relative	4.23 ± 0.12	4.05 ± 0.07	4.52 ± 0.06	4.44 ± 0.06	4.74 ± 0.04**	5.10 ± 0.16**
Liver						
Absolute	6.97 ± 0.17	7.76 ± 0.18**	8.12 ± 0.07**	7.91 ± 0.21**	9.29 ± 0.21**	9.10 ± 0.24**
Relative	37.7 ± 1.22	41.4 ± 0.84**	43.2 ± 0.73**	45.1 ± 0.79**	51.3 ± 0.87**	55.00 ± 0.97**

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

TABLE 6 Histopathologic Lesions in F344/N Rats in the 13-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone[a]

Dose (ppm)	0	3125	6250	12500	25000	50000
MALE						
Kidney						
Papilla						
necrosis	0	0	0	0	0	4 (2.3)
Interstitium						
inflammation	0	0	0	0	0	10 (2.2)
Renal tubule						
dilatation	0	0	0	3 (1.0)	10(1.2)	10 (2.8)
regeneration	9 (1.0)	9 (1.2)	10 (1.1)	10 (2.0)	10 (2.0)	10 (2.0)
FEMALE						
Kidney						
Papilla						
necrosis	0	0	0	0	0	5 (2.0)
degeneration	0	0	0	0	1 (1.0)	0
Interstitium						
inflammation	0	0	0	0	0	2 (1.5)
Renal tubule						
dilatation	0	0	0	0	0	9 (2.1)
regeneration	0	0	0	0	0	5 (2.0)

[a] Incidence and severity score ( ) based on a scale of 1 to 4; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.Severity scores are averages based on the number of animals with lesions from groups of 10.

at all exposure levels. The entire length of the nephron was generally affected, with dilated tubules present in the cortex, outer, and inner medulla. In kidneys with minimal to mild dilatation, there generally was a focal distribution of the lesion in which much of the kidney appeared relatively unaffected (Plates 3 and 5). The majority of the tubules from some of the more severely affected kidneys in the 50000 ppm groups of male and female rats were dilated and often contained protein casts and cell debris; more extensively dilated tubules were lined by a flattened epithelium. Renal tubule epithelial cell regeneration increased in severity and/or incidence in groups of rats with tubule dilatation. At the highest dose, particularly in male rats, there was mild to moderate inflammation with fibrosis in the renal interstitium. The cellular infiltrate was a mixture of neutrophils, lymphocytes, and macrophages. Necrosis of the tip of the renal papilla was also present in highest dose males and females (Plate 4). Adjacent to the area of papillary necrosis, there was minimal hyperplasia of the cuboidal epithelium on the surface of the renal papilla or the transitional epithelium of the renal pelvis. There was no evidence of inflammation or hyperplasia of the transitional epithelium of the urinary bladder in male or female rats.

Both absolute and relative liver weights increased markedly in a dose-related fashion in males and females (Table 5, Appendix A3). There were no histopathologic changes associated with the weight increase, but changes in activities of hepatic enzymes in serum occurred (described below).

In hematologic evaluations, male rats given 25000 or 50000 ppm HMB exhibited significant increases in platelet counts beginning at day 3 of the study and persisting at day 15 and week 12. Similar responses occurred in the 12500 ppm dose groups at day 15 and week 12 as well as in the 2 lower dose groups at day 15 (Appendix B). Sporadic increases occurred during the study in counts of segmented neutrophils and reticulocytes. There were increases in serum

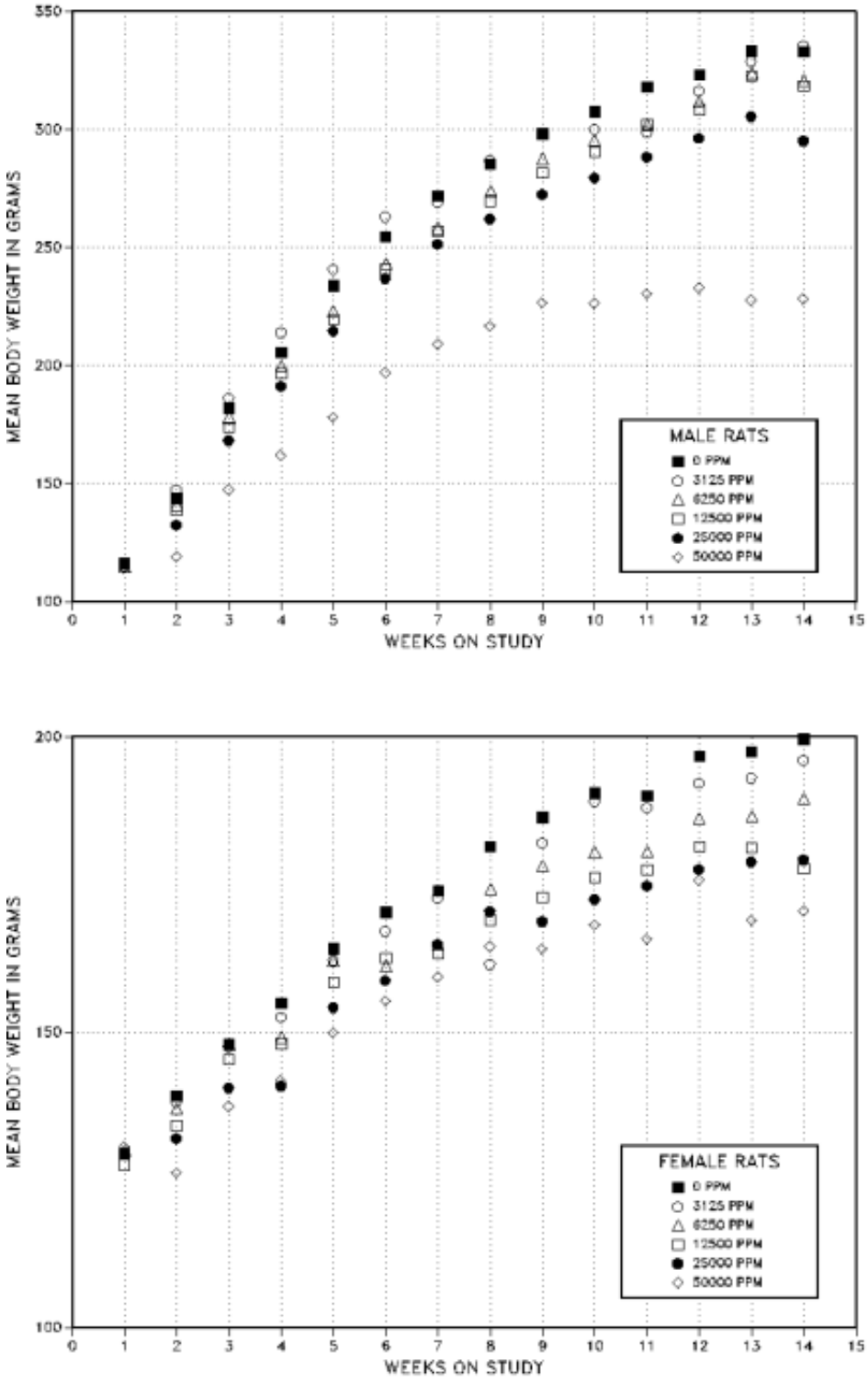


Figure 1 Body Weights of F344/N Rats Exposed to 2-Hydroxy-4-methoxybenzophenone by Dosed Feed for 13 Weeks

concentrations of UN at 3 and 15 days (50000 ppm dose groups), and increases in activities of serum ALT at 3 days (6250 to 50000 ppm groups) and in serum GGT at all time points (50000 ppm and lower dose groups at 15 days). Urine volume in male rats was increased in animals at 15 days and 12 weeks (50000 ppm group), and specific gravity was increased at 3 days and decreased at 12 weeks (50000 ppm) (Appendix B).

In female rats, there were increases in HCT, HGB concentration, MCHC, and RBC count at 3 days in animals treated with 6250 to 50000 ppm HMB (25000 and 50000 ppm for MCHC). Other hematologic findings included decreases in HGB concentration at 15 days and decreases in MCV at 3 days and 12 weeks. Significant changes in serum enzyme activities in treated female rats included minimal to mild increases in ALT at 3 days (25000 and 50000 ppm) and in SDH at 12 weeks (all treatment groups). GGT increased in female rats at 15 days (25000 and 50000 ppm) and 12 weeks (all treatment groups). Urine volume was increased in female rats at 15 days and 12 weeks (50000 ppm), and specific gravity was increased at 3 days (25000 and 50000 ppm).

In reproductive system evaluations, a significant decrease in epididymal sperm density and a non-significant increase in the percentage of abnormal sperm were observed in male rats. An increase in the length of the estrous cycle was seen in female rats receiving the highest concentration of HMB in the diet (Appendix C).

2-Week Dermal Studies in Rats, with Acetone and Lotion Vehicles

All animals survived to the end of the studies. There were no changes observed in body weight gains, food consumption, clinical observations, necropsy findings, or by histologic examination of all tissues including skin samples from the site of application for male or female rats with HMB applied to the skin in either the acetone or lotion vehicle. Liver weights were slightly increased in female rats given HMB in acetone and in lotion at the 3 highest dose levels (Tables 8, 9). Smaller increases also were noted in the liver weights of male rats given the higher doses of HMB in lotion. Kidney weights were minimally increased in male rats given the highest dose of HMB in lotion and in female rats given the highest dose in acetone. There were no discernible histopathologic changes associated with the increases in liver or kidney weights.

TABLE 7 Kidney and Liver Weights and Organ-Weight-to-Body-Weight Ratios of F344/N Rats in the 2-Week Dermal Studies of 2-Hydroxy-4-methoxybenzophenone in Acetone[a]

Dose (mg)	0	1.25	2.50	5.00	10.00	20.00
MALE (n=5)						
Necropsy body wt	213 ± 4	207 ± 3	218 ± 4	210 ± 1	214 ± 3	213 ± 4
R. Kidney						
Absolute	1.08 ± 0.03	1.05 ± 0.04	1.08 ± 0.05	1.02 ± 0.02	1.06 ± 0.08	1.10 ± 0.03
Relative	5.06 ± 0.05	5.06 ± 0.13	4.93 ± 0.16	4.87 ± 0.10	5.01 ± 0.08	5.17 ± 0.07
Liver						
Absolute	12.75 ± 0.32	12.16 ± 0.17	13.20 ± 0.47	11.65 ± 0.70	12.71 ± 0.15	13.84 ± 0.69
Relative	59.82 ± 0.76	58.79 ± 0.13	60.34 ± 1.17	55.45 ± 3.25	59.22 ± 0.57	64.76 ± 2.14
FEMALE (n=5)						
Necropsy body wt	145 ± 3	150 ± 1	150 ± 3	151 ± 3	150 ± 3	151 ± 2
R. Kidney						
Absolute	0.73 ± 0.019	0.75 ± 0.02	0.77 ± 0.018	0.76 ± 0.03	0.77 ± 0.023	0.80 ± 0.015
Relative	5.06 ± 0.10	4.96 ± 0.13	5.16 ± 0.12	5.06 ± 0.09	5.11 ± 0.09	5.30 ± 0.04*
Liver						
Absolute	6.68 ± 0.27	7.06 ± 0.10	7.14 ± 0.18	7.71 ± 0.30*	7.89 ± 0.29*	7.75 ± 0.19*
Relative	46.06 ± 1.50	47.01 ± 0.36	47.75 ± 1.32	51.10 ± 0.98*	53.26 ± 1.66*	51.27 ± 0.73*

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
* Significantly different from the control group by Student's t-test (P 0.05).
** Significantly different from the control group by Student's t-test (P 0.01).

TABLE 8 Kidney and Liver Weights and Organ-Weight-to-Body-Weight Ratios of F344/N Rats in the 2-Week Dermal Studies of 2-Hydroxy-4-methoxybenzophenone in Lotion[a]

Dose (mg)	0	1.25	2.50	5.00	10.00	20.00
MALE (n=5)						
Necropsy body wt	202 ± 4	212 ± 3	204 ± 3	210 ± 5	206 ± 5	211 ± 3
Kidney						
Absolute	1.05 ± 0.04	1.11 ± 0.02	1.08 ± 0.02	1.07 ± 0.04	1.09 ± 0.02	1.14 ± 0.02*
Relative	5.18 ± 0.12	5.22 ± 0.06	5.28 ± 0.08	5.09 ± 0.07	5.28 ± 0.07	5.43 ± 0.07
Liver						
Absolute	12.26 ± 0.16	13.40 ± 0.33*	12.41 ± 0.28	13.88 ± 0.36**	13.16 ± 0.62	13.80 ± 0.34**
Relative	61.30 ± 0.65	63.06 ± 0.91	60.76 ± 0.48	66.08 ± 0.63**	63.89 ± 0.18	65.41 ± 0.78**
FEMALE (n=5)						
Necropsy body wt	141 ± 2	138 ±2	141 ± 2	143 ± 3	142 ± 1	139 ± 2
R. Kidney						
Absolute	0.75 ± 0.01	0.72 ± 0.01*	0.78 ± 0.01	0.78 ± 0.02	0.77 ± 0.01	0.76 ± 0.02
Relative	5.30 ± 0.07	5.18 ± 0.01	5.50 ± 0.07	5.48 ± 0.11	5.42 ± 0.08	5.48 ± 0.10
Liver						
Absolute	7.19 ± 0.16	7.24 ± 0.03	7.50 ± 0.21	7.70 ± 0.31	7.87 ± 0.19*	7.93 ± 0.15**
Relative	51.18 ± 1.01	52.41 ± 0.48	53.20 ± 1.58	53.93 ± 1.30	55.40 ± 0.99*	56.91 ± 0.54**

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
* Significantly different from the control group by Student's t-test (P 0.05).
** Significantly different from the control group by Student's t-test (P 0.01).

13-Week Dermal Studies in Rats, with the Acetone Vehicle

All animals survived to the end of the studies. No chemically-related changes were observed in body weight gains (Figure 2), food consumption, clinical observations, reproductive system evaluations (Appendix C), necropsy findings, or by histologic examination of skin samples from the site of application. Relative kidney weights were increased in a non-dose-related manner in female rats treated topically with 25 mg/kg or larger doses of HMB (Table 9; Appendix A1).

In male rats, there were no changes that were considered chemically-related in serum hematologic, biochemical, or urinary variables at any time point (Appendix B). In female rats, there were decreases in reticulocyte counts in animals in all dose groups at 12 weeks, increases in platelet counts in animals in the 50, 100, and 200 mg/kg dose groups at 15 days, and an increase in WBC count produced by a lymphocytosis in the 200 mg/kg group at 12 weeks. There were no relevant changes in biochemical or urinalysis variables in female rats at any time point.

TABLE 9 Kidney and Organ-Weight-to-Body-Weight Ratios of F344/N Rats in the 13-Week Dermal Studies of 2-Hydroxy-4-methoxybenzophenone[a]

Dose (mg/kg)	0.0	12.5	25.0	50.0	100.0	200.0
MALE (n=10)						
Necropsy body wt.	313 ± 7	303 ± 7	310 ±8	313 ± 7	312 ± 9	314 ± 5
R. Kidney						
Absolute	1.23 ± 0.03	1.19 ± 0.04	1.34 ± 0.04	1.22 ± 0.04	1.34 ± 0.02	1.22 ± 0.03
Relative	3.93 ± 0.06	3.92 ± 0.10	4.34 ± 0.07**	3.90 ± 0.09	4.30 ± 0.09*	3.89 ± 0.07
FEMALE (n=10)						
Necropsy body wt.	193 ± 4	181 ± 3	183 ± 3	188 ± 4	186 ± 4	182 ± 4
R. Kidney						
Absolute	0.781 ± 0.029	0.832 ± 0.017	0.862 ± 0.012*	0.817 ± 0.023	0.892 ± 0.013**	0.797 ± 0.01
Relative	4.04 ± 0.12	4.61 ± 0.10	4.72 ± 0.09**	4.35 ± 0.06**	4.80 ± 0.07**	4.38 ± 0.09 *

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error.

* Statistically significant from the control group by Williams' or Dunnett's test (P 0.05).

** Statistically significant from the control group by Williams' or Dunnett's test (P 0.01).

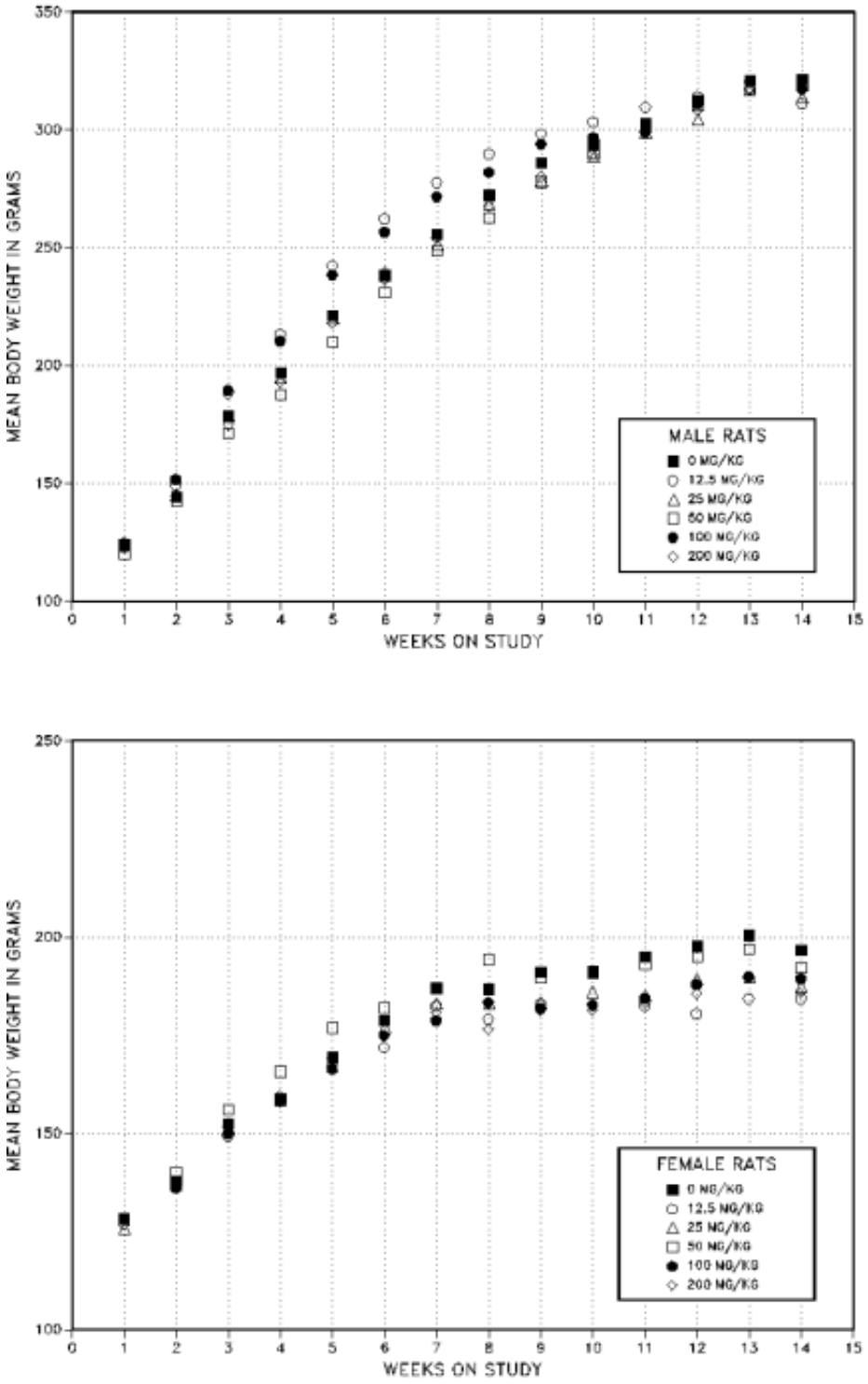


Figure 2 Body Weights of F344/N Rats Exposed Dermally to 2-Hydroxy-4-methoxybenzophenone for 13 Weeks

2-Week Dosed Feed Studies in Mice

All animals survived to the end of the studies (Table 10). No clinical signs were noted that could be clearly related to chemical administration. Body weight gains were variable, but only in female mice receiving feed containing 50000 ppm HMB did weight gains clearly appear to be decreased because of the chemical. Feed consumption was increased somewhat at higher HMB concentrations, but the data were not corrected for scattering of feed. A dose-related increase was seen in liver weights in male and female mice; a decrease was seen in right kidney weights in male mice in the top 2 dose groups (Table 11).

TABLE 10 Survival, Weight Gain, and Feed Consumption of B6C3F$_1$ Mice in the 2-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone

Dose (ppm) In Feed	Survival(a)	Mean Body Weight (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent) (c)	Average Feed Consumption (d)	Estimated Chemical Consumed (e)
MALE							
0	5/5	19.2	21.6	2.4		5.0	
3125	5/5	20.0	22.1	2.1	102	6.8	992
6250	5/5	19.7	22.3	2.6	103	6.0	1752
12500	5/5	19.4	22.0	2.6	102	6.6	3947
25000	5/5	19.5	22.1	2.6	102	6.3	7438
50000	5/5	19.3	22.7	3.4	105	7.9	18624
FEMALE							
0	5/5	16.5	18.7	2.2		5.2	
3125	5/5	16.9	18.9	2.0	101	6.1	1050
6250	5/5	16.7	19.6	2.9	105	6.9	2330
12500	5/5	16.1	18.9	2.8	101	7.0	4914
25000	5/5	16.8	18.5	1.7	99	7.1	9859
50000	5/5	16.7	18.0	1.3	96	8.1	22968

a Number surviving to study termination / number of animals per group.
b Mean weight change of the animals in each dose group.
c [Dosed group mean / control group mean] x 100.
d Food consumption is given in grams/animal/day.
e Time-weighted compound consumption = average compound consumed during the 2-Week study (mg/kg body weight/day).

Increased liver weights were associated with the presence of cytoplasmic vacuolization of hepatocytes in males and females in the 6250 ppm and higher dose groups; this change was also present in one female mouse in the 3125 ppm group. The morphologic appearance of the hepatocyte vacuolization was the same as that described for the rat in 2-week feed studies in that it was characterized by an irregular perinuclear vacuolization of the cytoplasm which was centrilobular to diffuse in distribution, and likely represented areas of glycogen deposition that had been dissolved during tissue processing. Special stains for glycogen did not demonstrate differences between the groups. There were no microscopic lesions associated with the increased kidney weights.

Plates

Plate 1. Kidney from male rat exposed to 50000 ppm 2-hydroxy-4-methoxybenzophenone in the diet for two weeks. A focal area of tubular dilatation (outlined by arrows) extends from the outer stripe of the outer medulla to the tip of the papilla. Detail of area in **[ ]** (lower right of photomicrograph) is shown in Plate 2. H&E 10X.

Plate 2. Higher magnification of papilla from kidney in Plate 1 shows focal necrosis (N) at the tip of the papilla and hyperplasia of the cuboidal epithelium (arrows) at the margin of the area of necrosis. H&E 110X.

Plate 3. Kidney from male rat exposed to 25000 ppm 2-hydroxy-4-methoxybenzophenone in the diet for 13 weeks. Focal wedge-shaped area of tubular dilatation (outlined by arrows) extends from the capsular surface to the inner medulla. Papillary degeneration or necrosis was not present in this rat. H&E 10X.

Plate 4. Kidney from female rat exposed to 50000 ppm 2-hydroxy-4-methyoxybenzophenone in the diet for 13 weeks. There is necrosis of the entire tip (arrows) of renal papilla and minimal hyperplasia of the transitional epithelium of the renal pelvis at the top right. H&E 60X.

Plate 5. Kidney from female rat exposed to 50000 ppm 2-hydroxy-4-methoxybenzophenone in the diet for 13 weeks. Detail of junction between normal (N) tubules and focal area of dilatation consisting of distended tubules lined by flattened epithelium. H&E 135X.

Plate 6. Kidney from male mouse exposed to 50000 ppm 2-hydroxy-4-methoxybenzophenone in the diet for 13 weeks. Protein casts fill lumen of several tubules of the outer medulla. Note mild increase in inflammatory cells in the renal interstitium adjacent to these dilated tubules. H&E 165X.

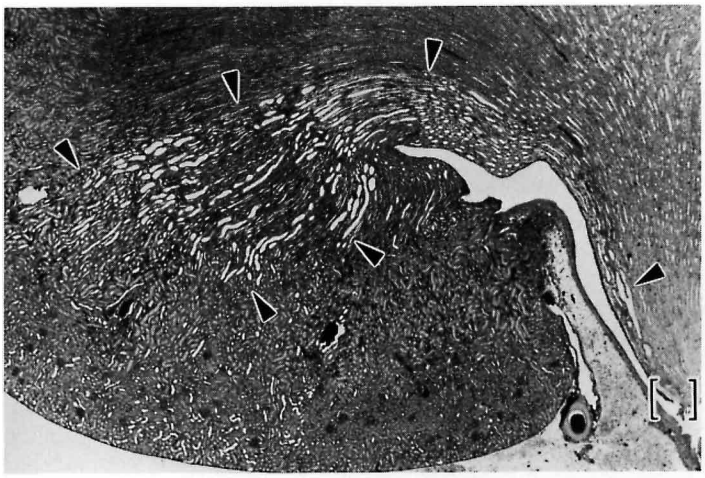


Plate 1

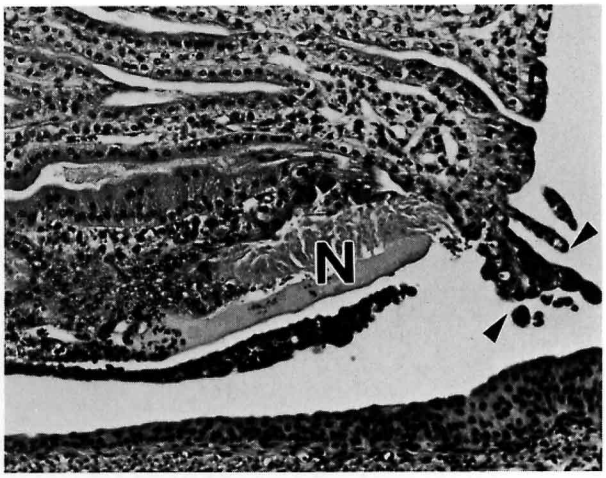


Plate 2

Plate 3

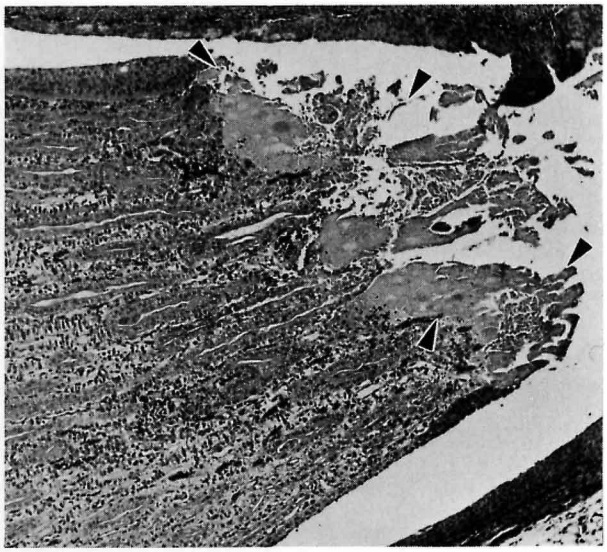
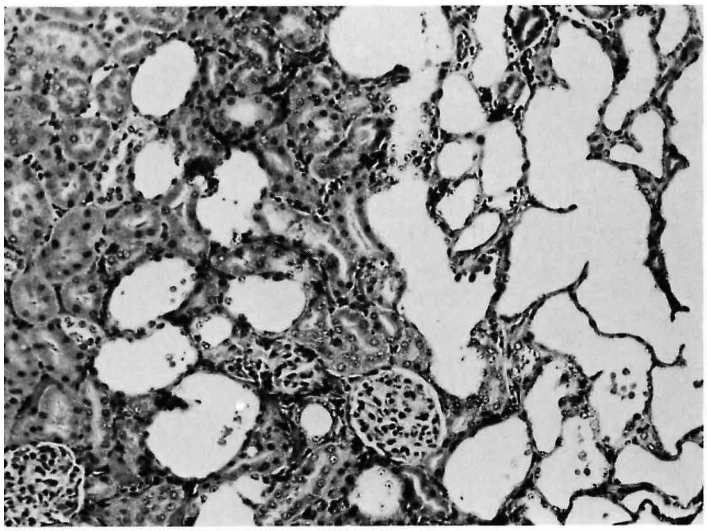


Plate 4



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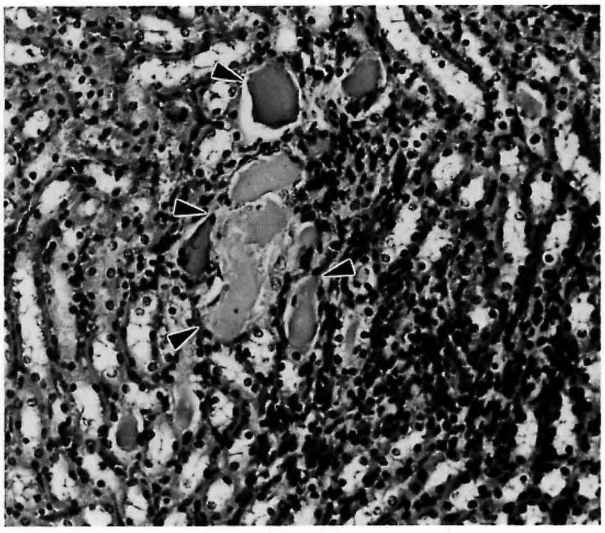

Plate 6

TABLE 11 Kidney and Liver Weights and Organ-Weight-to-Body-Weight Ratios of B6C3F$_1$ Mice in the 2-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone[a]

Dose (ppm)	0	3125	6250	12500	25000	50000
MALE (n=5)						
Necropsy body wt	22.7 ± 0.6	22.1 ± 0.2	22.3 ± 0.5	22.0 ± 0.4	22.1 ± 0.3	21.6 ± 0.4
R. Kidney						
Absolute	0.242 ± 0.011	0.221 ± 0.009	0.227 ± 0.008	0.226 ± 0.008	0.215 ± 0.005	0.210 ± 0.007
Relative	10.6 ± 0.29	9.99 ± 0.36	10.17 ± 0.30	10.27 ± 0.20	9.76 ± 0.23*	9.73 ± 0.20*
Liver						
Absolute	1.378 ± 0.037	1.405 ± 0.029	1.548 ± 0.047*	1.646 ± 0.105*	1.920 ± 0.088**	2.078 ± 0.135
Relative	60.8 ± 1.0	63.5 ± 0.9	69.3 ± 1.7	74.6 ± 3.5	86.9 ± 3.8	96.2 ± 5.5
FEMALE (n=5)						
Necropsy body wt	18.0 ± 0.72	18.9 ± 0.4	19.6 ± 0.1	18.8 ± 0.3	18.5 ± 0.3	18.7 ± 0.7
R. Kidney						
Absolute	0.162 ± 0.007	0.165 ± 0.004	0.173 ± 0.005	0.173 ± 0.003	0.161 ± 0.003	0.171 ± 0.005
Relative	9.03 ± 0.21	8.71 ± 0.24	8.84 ± 0.21	9.24 ± 0.17	8.67 ± 0.05	9.19 ± 0.23
Liver						
Absolute	0.95 ± 0.08	1.23 ± 0.06*	1.37 ± 0.02**	1.37 ± 0.06**	1.61 ± 0.06**	1.83 ± 0.09**
Relative	52.6 ± 2.9	64.9 ± 2.2**	69.7 ± 1.0**	73.0 ± 2.4**	87.1 ± 2.8**	98.2 ± 3.4**

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error). All animals survived to the end of the study.
* Significantly different from the control group by Student's t-test (P 0.05).
** Significantly different from the control group by Student's t-test (P 0.01).

13-Week Dosed Feed Studies in Mice

One female mouse in the 50000 ppm group was accidentally killed during week 11 of the study (Table 12). A female mouse in the 3125 ppm group was killed in a moribund condition during week 1. There were no other unscheduled deaths of mice. Body weight gains were decreased in both males and females fed diets with the higher doses of HMB (Figure 3). Final body weights of males and females in the top 2 dose groups were significantly less than controls. Food consumption appeared to increase with increasing HMB concentration, but the data were not corrected for scattering of feed by the animals.

There were no clinical signs attributed to consumption of the HMB diets. At necropsy, there were no gross lesions related to the chemical. Consistent organ weight changes were seen in the liver of male and female mice and in the kidney of females; a moderate, dose-related increase in both absolute and relative weights was seen in liver (Table 13). The increase in relative kidney weight was not dose-related. Chemically-related histopathologic changes were present in the kidney of male mice fed a diet containing 50000 ppm HMB. Seven mice from this dose group exhibited a minimal lesion consisting of several homogeneous eosinophilic protein casts in renal tubules located within the inner stripe of the outer medulla or in collecting ducts of the inner medulla (Plate 6). Tubules containing these casts were slightly dilated; in a few of these mice there was a mild inflammatory cell infiltrate in the renal interstitium adjacent to the dilated tubules. No renal lesions were observed in female mice or in the lower dose groups of male mice.

TABLE 12 Survival, Weight Gain, and Feed Consumption of B6C3F$_1$ Mice in the 13-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change [b]	Final Weight Relative to Controls (percent) [c]	Average Feed Consumption [d]	Estimated Chemical Consumed [e]
MALE							
0	10/10	19.9	30.5	10.6		4.0	
3125	10/10	20.5	30.5	10.0	100	4.0	480
6250	10/10	19.9	30.2	10.3	99	4.5	1068
12500	10/10	19.8	28.3	8.5	93	5.0	2487
25000	10/10	19.9	26.2	6.3	86	5.5	5981
50000	10/10	19.9	25.7	5.8	84	6.3	13937
FEMALE							
0	10/10	16.7	25.0	8.3		4.8	
3125	9/10	17.1	24.7	7.6	99	4.5	629
6250	10/10	17.5	25.3	7.8	101	5.0	1425
12500	10/10	17.8	24.4	6.6	98	5.5	3232
25000	10/10	17.2	23.0	5.8	92	6.2	7579
50000	9/10	16.9	22.0	5.1	88	7.2	18539

[a] Number of animals surviving at 13 weeks/number/dose group.
[b] Mean weight change of the animals in each dose group.
[c] (Dosed group mean/Control group mean) x 100.
[d] Food consumption is given in grams/animal/day.
[e] Time weighted compound consumption = average compound consumed during the 13-week study (mg/kg body weight/day).

TABLE 13 Kidney and Liver Weights and Organ-Weight-to-Body-Weight Ratios of B6C3F$_1$ Mice in the 13-Week Dosed Feed Studies of 2-Hydroxy-4-methoxybenzophenone[a]

Dose (ppm)	0	3125	6250	12500	25000	50000
MALE						
n	10	10	10	10	10	10
Necropsy body wt	28.0 ± 0.1	27.9 ± 1.0	28.8 ± 0.6	25.6 ± 0.4**	25.1 ± 0.4**	23.6 ± 0.3**
R. Kidney						
Absolute	0.255 ± 0.008	0.243 ± 0.005	0.294 ± 0.008	0.238 ± 0.005	0.262 ± 0.005	0.209 ± 0.007**
Relative	9.13 ± 0.29	8.81 ± 0.25	10.21 ± 0.23**	9.31 ± 0.12	10.47 ± 0.14**	8.84 ± 0.19
Liver						
Absolute	1.38 ± 0.03	1.44 ± 0.06	1.58 ± 0.04**	1.54 ± 0.03**	1.61 ± 0.03**	1.69 ± 0.03**
Relative	49.4 ± 1.1	51.5 ± 1.0	55.0 ± 1.3**	60.1 ± 0.7**	64.3 ± 1.6**	71.7 ± 1.4**
FEMALE						
n	10	9	10	10	10	9
Necropsy body wt	23.4 ± 0.9	23.1 ± 0.6	23.6 ± 0.4	22.4 ± 0.4	21.9 ± 0.3	20.3 ± 0.2**
R. Kidney						
Absolute	0.177 ± 0.005	0.179 ± 0.005	0.213 ± 0.003**	0.174 ± 0.004	0.194 ± 0.005*	0.163 ± 0.002
Relative	7.64 ± 0.16	7.81 ± 0.21	9.02 ± 0.13**	7.79 ± 0.16**	8.88 ± 0.15**	8.03 ± 0.11**
Liver						
Absolute	1.16 ± 0.02	1.27 ± 0.03	1.35 ± 0.04**	1.40 ± 0.03**	1.47 ± 0.05**	1.41 ± 0.04**
Relative	50.1 ± 1.1	54.9 ± 1.0*	57.1 ± 1.1**	62.4 ± 1.2**	66.8 ± 1.6**	69.5 ± 1.9**

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

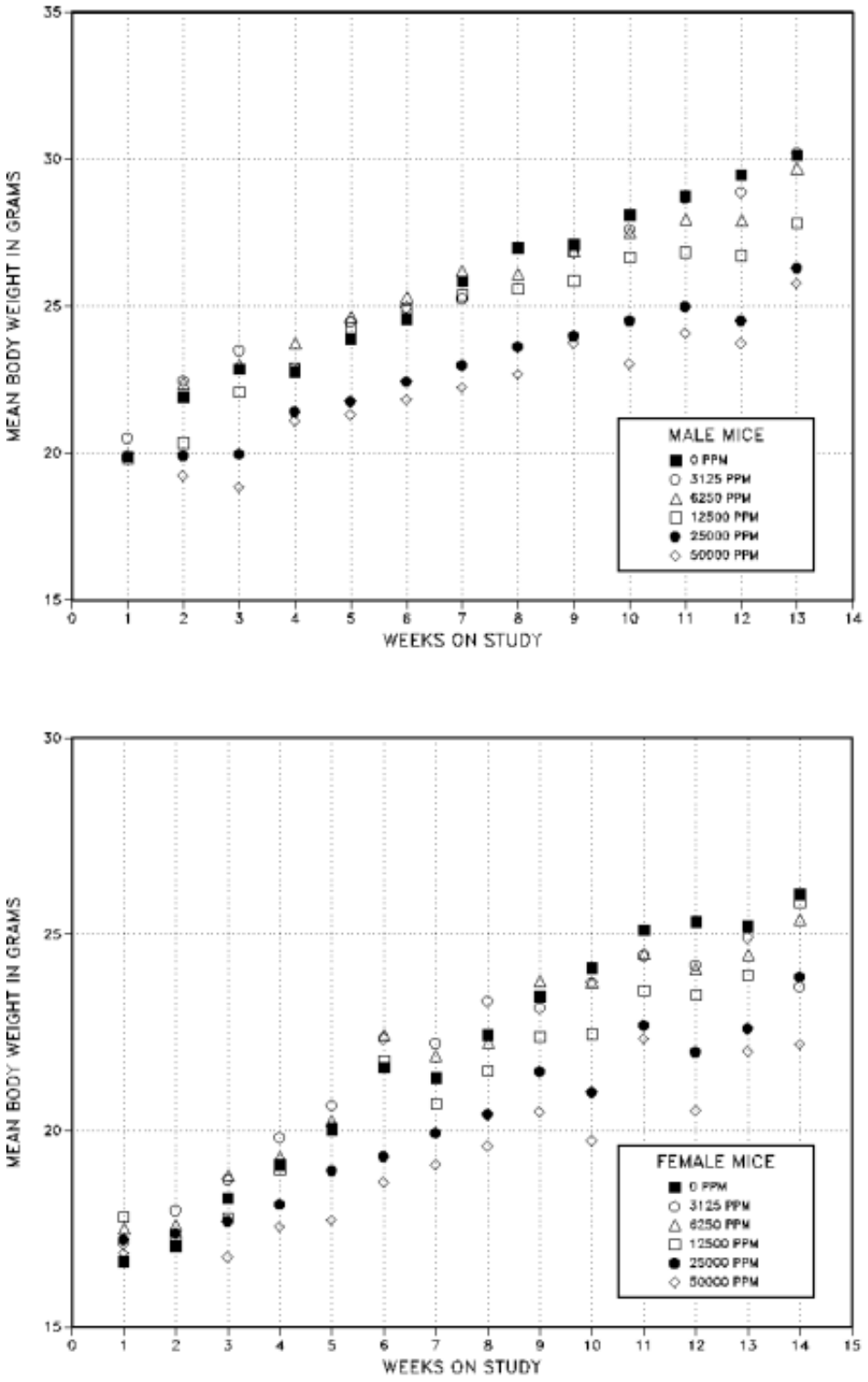

Figure 3 Body Weights of Mice Exposed to 2-Hydroxy-4-methoxybenzophenone by Dosed Feed for 13 Weeks

Although liver weights were mildly increased at 6250 ppm and higher doses, microscopic changes were limited to the 25000 and 50000 ppm groups. Four males and 6 females in the top concentration group had minimal cytoplasmic vacuolization of hepatocytes. This change was also present in 3 male and 2 female mice from the 25000 ppm group.

A decrease in sperm density as well as an increase in abnormal sperm was noted in mice given 50000 ppm HMB in the feed. Female mice in the 50000 ppm dose group exhibited an increase in the length of the estrous cycle (Appendix C).

2-Week Dermal Studies in Mice, with Acetone and Lotion Vehicles

All animals survived to the end of the studies. No chemically-related changes were observed in body weight gain, food consumption, clinical observations, necropsy findings or by histologic examination of skin samples from the site of application in male or female mice receiving HMB in the acetone or lotion vehicles. Relative kidney weights were variably increased in male mice receiving HMB in the acetone vehicle (Table 14). Relative liver weights were increased in male and female mice given the higher doses of HMB in acetone or lotion (Tables 14, 15).

TABLE 14 Kidney and Liver Weights and Organ-Weight-to-Body-Weight Ratios of B6C3F$_1$ Mice in the 2-Week Dermal Studies of 2-Hydroxy-4-methoxybenzophenone in Acetone[a]

Dose (mg)	0	0.5	1.0	2.0	4.0	8.0
MALE (n=5)						
Necropsy body wt	20.9 ± 0.4	21.7 ± 0.9	21.9 ± 0.8	21.8 ± 0.4	21.6 ± 0.3	22.6 ± 0.5*
Kidney						
Absolute	0.22 ± 0.01	0.23 ± 0.01	0.25 ± 0.02	0.23 ± 0.01	0.24 ± 0.01	0.25 ± 0.04**
Relative	10.37 ± 0.01	10.57 ± 0.02	11.24 ± 0.05	10.77 ± 0.04	11.11 ± 0.01**	11.20 ± 0.01**
Liver						
Absolute	1.38 ± 0.05	1.32 ± 0.07	1.46 ± 0.07	1.46 ± 0.02	1.53 ± 0.03*	1.62 ± 0.03**
Relative	64.56 ± 1.22	60.81 ± 2.43	66.94 ± 2.74	67.74 ± 0.55	70.86 ± 0.69**	71.86 ± 0.55**
FEMALE (n=5)						
Necropsy body wt	17.5 ± 0.41	18.6 ± 0.59	19.4 ± 0.19**	18.1 ± 0.39	19.1 ± 0.47*	18.6 ± 0.67
R. Kidney						
Absolute	0.17 ± 0.00	0.17 ± 0.01	0.18 ± 0.00	0.17 ± 0.00	0.18 ± 0.00	0.17 ± 0.01
Relative	9.84 ± 0.17	9.33 ± 0.25	9.42 ± 0.18	9.60 ± 0.15	9.64 ± 0.19	9.33 ± 0.13
Liver						
Absolute	1.09 ± 0.42	1.20 ± 0.23	1.27 ± 0.02**	1.20 ± 0.06	1.35 ± 0.03**	1.28 ± 0.04*
Relative	61.99 ± 1.59	64.31 ± 2.18	65.40 ± 0.91	66.31 ± 2.16	70.45 ± 0.89**	68.38 ± 1.42*

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
* Significantly different from the control group by Student's t-test (P 0.05).
** Significantly different from the control group by Student's t-test (P 0.01).

TABLE 15 Kidney and Liver Weights and Organ-Weight-to-Body-Weight Ratios of B6C3F$_1$ Mice in the 2-Week Dermal Studies of 2-Hydroxy-4-methoxybenzophenone in Lotion[a]

Dose (mg)	0	0.5	1.0	2.0	4.0	8.0
MALE (n=5)						
Necropsy body wt	22.0 ± 0.38	22.6 ± 0.21	22.2 ± 0.43	22.5 ± 0.25	23.2 ± 0.42	23.5 ± 0.55*
Kidney						
Absolute	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.28 ± 0.01
Relative	11.87 ± 0.45	11.52 ± 0.34	11.47 ± 0.19	11.52 ± 0.29	11.26 ± 0.28	11.95 ± 0.33
Liver						
Absolute	1.35 ± 0.05	1.46 ± 0.03	1.44 ± 0.06	1.46 ± 0.03	1.62 ± 0.05**	1.62 ± 0.02**
Relative	61.23 ± 1.55.	64.48 ± 1.43	64.84 ± 2.16	64.76 ± 1.30	69.84 ± 1.13**	69.09 ± 0.90**
FEMALE (n=5)						
Necropsy body wt	19.2 ± 0.3	20.2 ± 0.5	19.7 ± 0.3	20.2 ± 0.3*	19.2 ± 0.7	20.0 ± 0.3
R. Kidney						
Absolute	0.18 ± 0.00	0.19 ± 0.01	0.19 ± 0.00	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01
Relative	9.21 ± 0.11	9.25 ± 0.35	9.42 ± 0.19	9.56 ± 0.26	9.37 ± 0.11	9.33 ± 0.31
Liver						
Absolute	1.20 ± 0.05	1.33 ± 0.06	1.31 ± 0.05	1.36 ± 0.06	1.26 ± 0.08	1.47 ± 0.04**
Relative	62.41 ± 1.75	65.86 ± 1.56	66.73 ± 1.62	67.55 ± 2.47	65.63 ± 2.79	73.30 ± 1.18**

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
* Significantly different from the control group by Student's t-test (P 0.05).
** Significantly different from the control group by Student's t-test (P 0.01).

13-Week Dermal Studies in Mice, with Acetone Vehicle

All mice survived to the end of the studies. No chemically-related changes were observed in body weight gains (Figure 4), food consumption, clinical observations, necropsy findings (including organ weights) (Appendix A2) or by histologic examination of skin samples from the site of application. A mild increase in relative kidney weights in dosed male mice was possibly significant; the increases were not dose related, however, and no abnormal histopathologic findings were seen in this tissue. A significant dose-related decrease in epididymal sperm density was seen in mice at all doses studied (22.75, 91.0, and 364.0 mg/kg) (Appendix C).

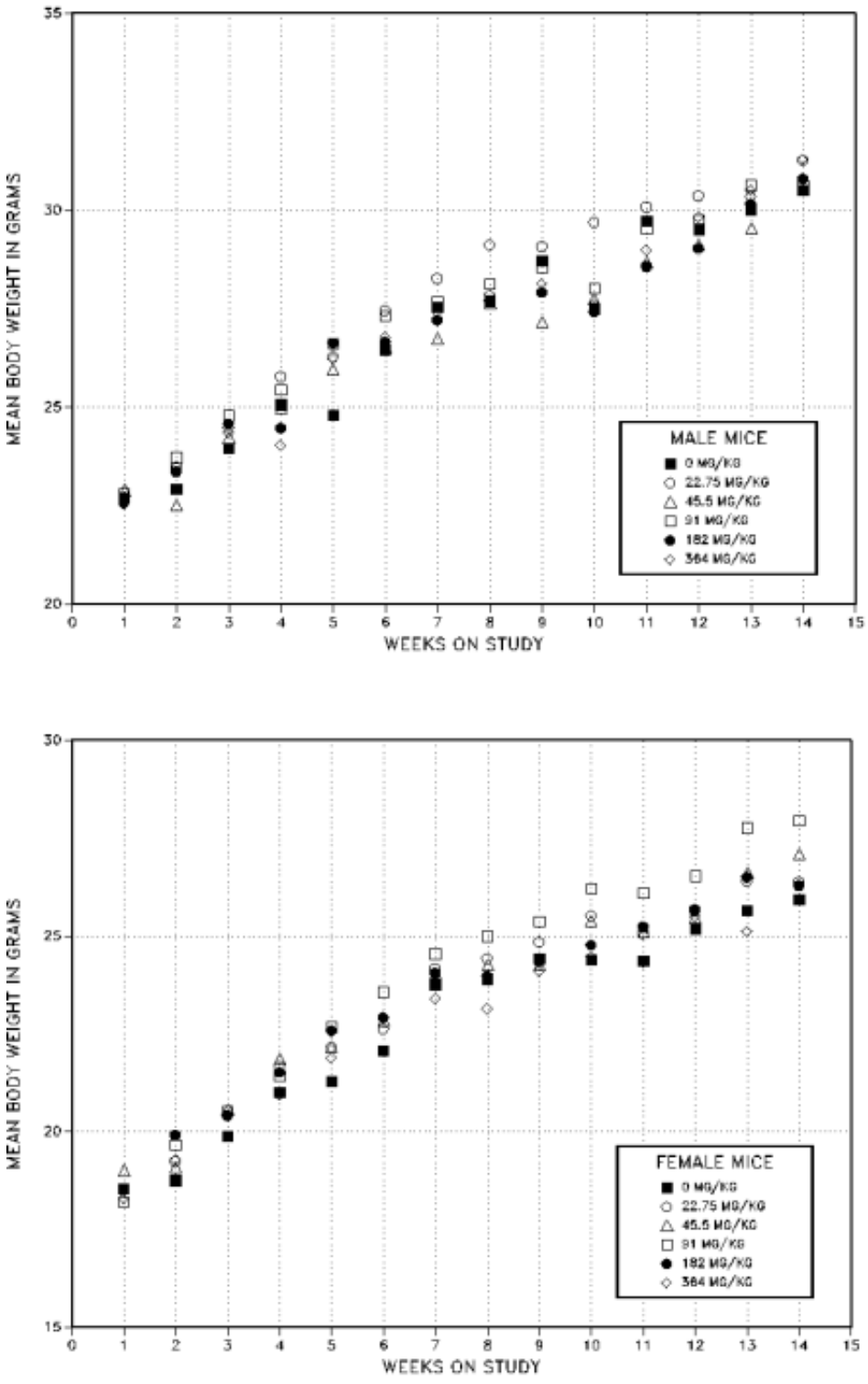

Figure 4 Body Weights of B6C3F$_1$ Mice Exposed Dermally to 2-Hydroxy-4-methoxybenzophenone for 13 Weeks

Genetic Toxicology

HMB was tested using a preincubation protocol in 2 independent studies for induction of gene mutations in *Salmonella typhimurium*. In the first study, strains TA100, TA1535, TA97, TA1537, and TA98 were used with 10% liver S9 from rats and hamsters. No mutagenicity was seen in any of the strains under these conditions (Appendix D, Table D1; Zeiger *et al.*, 1987).

The second study, performed at a later date, used TA97 in place of TA1537, and both 10% and 30% rat and hamster S9. In this study, HMB was weakly mutagenic only in strain TA97 with 30% hamster S9. These conditions were not used in the first study. On the basis of this finding, it is concluded that HMB is weakly mutagenic in *Salmonella* (Appendix D, Table D2).

In cytogenetic tests with Chinese hamster ovary cells, HMB induced sister-chromatid exchanges (effective dose range 5-50 µg/ml) and chromosomal aberrations (20-45 µg/ml) in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver S9. In the ABS test with S9, the lack of a dose response in Trial 2 may have been due to cytotoxic delay or to precipitation of the test chemical at the higher doses. In the single SCE trial without S9, a questionable response was obtained; a dose-related increase in SCE was observed, but no single dose level was significantly elevated over the control frequency. The ABS test without S9 was negative (Appendix D, Tables D3 and D4).

Peripheral blood smears from the mice used in the 13-week toxicity studies were analyzed for frequency of micronucleated normochromatic erythrocytes; no increases were noted in either male or female mice treated with up to 50000 ppm HMB in feed (Appendix D, Table D5).

DISCUSSION

Because of its widespread use in sunscreens and cosmetics, comparative toxicity studies by the oral and dermal routes were performed with 2-hydroxy-4-methoxybenzophenone. When HMB was administered at 3125 to 50000 ppm in the diet of F344/N rats and B6C3F$_1$ mice (approximately 200 to 4200 mg/kg/day for rats and 500 to 23000 mg/kg/day for mice), gross and microscopic injury to the liver and kidney and adverse effects upon body weight gain and the reproductive system were noted. When administered on the skin at up to 200 mg/kg to F344/N rats or 384 mg/kg to B6C3F$_1$ mice, there were fewer adverse effects, although decreased sperm density was seen in mice receiving as little as 23 mg/kg/day. The only effect on the kidney was an increase in weight in male mice, which was not related to dose.

HMB is excreted primarily through the kidney after oral or topical exposure (El Dareer *et al.*, 1986). The absence of microscopic liver and kidney lesions in the dermal studies is likely due to lower systemic exposure levels, because the maximum dose which could be administered dermally was similar to the lowest dose administered orally, which also produced little systemic toxicity.

The renal toxicity produced by feeding diets containing HMB was more severe in rats than in mice. A spectrum of morphologic lesions including tubule dilatation with regeneration of tubular epithelial cells, interstitial inflammation, and papillary necrosis was present in both sexes of rats, but was generally more severe in males.

The occurrence of both papillary necrosis and renal tubule dilatation has been reported in several studies with rats; however, the relationship between the two lesions is not clear. Renal cortical lesions may develop secondary to total papillary necrosis and obstruction, or occlusion, of the tubules at the tip of the papilla with cell debris or hyperplastic uroepithelium (Nanra *et al.*, 1986; Elliot, 1986; Black, 1986); however, when rats were administered phenylbutazone, dilatation of renal tubules occurred in some rats without evidence of papillary necrosis, while other rats had papillary necrosis without dilatation of tubules (Arnold *et al.* 1976). In studies with phenacetin, cortical lesions, including dilatation, inflammation, and atrophy, were seen only in rats also exhibiting papillary necrosis (Molland, 1978). A number of mechanisms have been proposed for the development of drug-induced renal papillary necrosis (Sabatini, 1989). These mechanisms are interrelated and include such factors as redistribution of renal blood flow, free radical formation with direct chemical injury to the papillary cells, and inhibition of prostaglandin synthesis. It also has been demonstrated that decreased urinary flow and increased urinary solute concentration contribute to the development of papillary necrosis (Sabatini *et al.*, 1983).

While it was is not clear which mechanism(s) might apply to HMB, the urinalysis results suggested that if an increased urinary solute concentration was involved, the injury must have occurred very early. There was a transient increase in urine specific gravity in highest dose male and female rats at 3 days, likely due to dehydration. However, in highest dose male rats, changes at 15 days (increase in urine volume) and especially at 12 weeks (increase in urine

volume and decrease in specific gravity) were consistent with a decrease in urine concentrating ability. Although other causes of increases in volume and decreases in specific gravity cannot be excluded (e.g. polydipsia, osmotic diuresis, decrease in renal interstitial osmolality, diabetes insipidus, etc.), the findings are consistent with histopathologic evidence of renal papillary necrosis at 13 weeks.

Based upon the results in the 2- and 13-week studies of HMB, it appears that tubule dilatation and epithelial cell regeneration preceded the development of papillary necrosis and inflammation in the kidney of rats. The tubule dilatation in rats occurred often in the absence of papillary necrosis in both male and female rats, and in lower dose groups. Studies of HMB (10000 ppm in the diet) in Wistar rats showed tubule dilatation in the absence of papillary necrosis (Lewerenz *et al.*, 1972). It is possible that the tubule dilatation was of sufficient severity to restrict regional blood flow to the papilla, contributing to the development of papillary necrosis, although a number of xenobiotics, including diphenylthiazole and diphenylamine, have been demonstrated to cause tubular dilatation without affecting the renal papilla (Carone *et al.*, 1974; McCormack *et al.*, 1981; Kanwar *et al.*, 1984).

Kidney lesions in mice were minimal and occurred only in males at the highest exposure concentration. The predominant feature of the renal lesion in mice were protein casts in tubules in the inner stripe of the outer medulla or in collecting ducts of the inner medula. There were no similarities in the kidney lesions between mice and rats. It is not unusual in short-term toxicity studies for a chemical to cause dissimilar lesions in the kidney of different species (Elwell *et al.*, 1989).

In the dosed feed studies, the only relevant hematologic changes were mild to moderate, persistent increases in platelet counts in dosed male rats at most timepoints and in female rats at 12 weeks. Thrombocytosis can be produced by increased production of platelets in the bone marrow and by decreased sequestration in the vasculature of tissues (for example, lung and spleen) produced by physiologic mechanisms (exercise, epinephrine release) or splenic atrophy or dysfunction. Primary increases in platelet production are associated with myeloproliferative diseases, regenerative processes, infectious and inflammatory disorders, certain neoplasms, and a variety of miscellaneous causes including nephrotic syndrome and other forms of chronic renal disorders (Wintrobe, 1981). In the current study, the development of renal lesions in rats provides a possible explanation for the thrombocytosis. However, this and other mechanisms would have to be specifically examined before a clear association could be established.

Decreases in activity of AP, as occurred in male and female rats at 15 days and in male rats at 12 weeks, are generally associated with decreased intake of food and not from direct, chemically-related effects. The mild increase in activity of ALT at 3 days in male and female rats does indicate release of the cytosolic enzyme from damaged or necrotic hepatocytes. Additionally, the significant increase in SDH at 12 weeks in female rats is consistent with minimal hepatocellular damage. Moderate increases in activity of GGT at all time points in male rats and at 15 days and 12 weeks in female rats are consistent with impairment in bile flow and release of the enzyme from canalicular membranes. Together, the increases in ALT and GGT provide biochemical evidence of early damage to the hepatobiliary system, evidence of

minimal hepatocellular damage in female rats at 12 weeks, and persistent, increasing cholestasis in animals of both sexes. In some cases, these cellular biochemical responses were associated with markedly enlarged livers and hepatocyte vacuolization which appeared much more severe in comparable rat and mouse dose groups in the 2-week than in the 13-week studies, suggesting that the lesion was reversible. Characteristic rarefaction and "clumping" of the stainable cytoplasm has previously been described as a potentially reversible, treatment-related effect (Newberne, 1982; Gopinath *et al.*, 1987). Although the appearance of the livers returned to near normal in the 13-week study, the persistent cholestasis noted in rats at the top dose indicated that hepatobiliary function remained impaired.

Topical application of HMB resulted in no relevant changes in hematologic, biochemical, or urinary variables in male rats. In female rats, sporadic changes in HGB concentration and in RBC, platelet, leukocyte, and lymphocyte counts were not considered biologically important. Although reticulocyte counts were decreased in female rats in all dermal treatment groups at 12 weeks, lack of additional hematologic evidence consistent with decreased regeneration of RBC lessened the importance of this finding. In female rats, there were no significant changes in urine variables, and serum biochemical changes were minimal in magnitude and were not considered biologically meaningful.

Reproductive system toxicity was associated with both oral and topical administration in rats and mice. The most common finding was a decrease in epididymal sperm density, and, in some studies, the number of abnormal sperm increased. Female rats and mice were observed to have an increased estrous cycle length. These findings, which were consistent across species and sex, prompted further evaluation of HMB in a continuous breeding study. Parental generations of CD-1 mice were given up to 50000 ppm HMB in feed and were maintained on these diets for sufficient time to produce 5 litters. In contrast to the results with the B6C3F_1 mice, there were no observed changes in CD-1 mice for sperm density or abnormal sperm, or for estrous cycle length. The CD-1 mice were observed to have dose-related decreases in dam weights and total number of litters produced. The number of live pups born per litter also decreased in a dose-related manner. There was no evidence for a dominant lethal effect reflected in the sex of the pups. There was, however, a dose-related decrease in pup survival in litters with dams receiving 25000 and 50000 ppm HMB in the diet. The first 3 F_1 generation litters in the 2 highest dose groups were observed to have an increased number of days to parturition. These data demonstrate moderate reproductive toxicity of HMB in CD-1 mice at high dietary levels (National Toxicology Program, 1991). Whether the B6C3F_1 mouse would prove more sensitive to the reproductive toxicity of HMB in continuous breeding studies remains to be determined. On the whole, increases in estrous cycle length in B6C3F_1 mice and time to parturition in CD-1 mice suggest possible effects on hormonal mechanisms in females.

In summary, administration of HMB was associated with effects on the liver, kidney, and reproductive organs of rats and mice. Although these effects were observed primarily in dietary studies at concentrations that also affected body weight gain, these effects are considered to be tissue specific and dose-responsive. A no-observed-adverse-effect level (NOAEL) for microscopic kidney lesions was 25000 ppm HMB in the feed for mice and 6250 ppm for rats. An apparently reversible enlargement and cytoplasmic vacuolization of the livers of rats and mice

was noted at dietary concentrations of 6250 ppm and above. The dermal studies were limited by the reduced systemic exposure achievable by this route, but indicated that rats and mice are generally similarly affected by oral and dermal exposures. Consistent findings included decreases in epididymal sperm density, lengthened estrous cycle, and increased liver and kidney weights. A NOAEL was not reached for decreased epididymal sperm density in the 13-week dermal study in mice (<23 mg/kg/day).

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