National Toxicology Program
Toxicity Report Series
Number 70

# NTP Technical Report on the Toxicity Studies of

# *p-tert*-Butylcatechol

**(CAS No. 98-29-3)**

## Administered in Feed to F344/N Rats and B6C3F$_1$ Mice

## November 2002

**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.

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## Administered in Feed to F344/N Rats and B6C3F$_1$ Mice

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# PEER REVIEW

The draft report on the toxicity studies of *p-tert*-butylcatechol 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 this Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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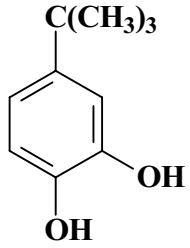
# ABSTRACT




## *p-tert*-BUTYLCATECHOL

CAS No. 98-29-3

Chemical Formula: $C_{10}H_{14}O_2$ Molecular Weight: 166.2

**Synonyms:** 1,2-Benzenediol, 4-(1,1-dimethylethyl-(9CI); 4-*tert*-butyl-1,2-benzenediol; 4-*tert*-butylcatechol; 4-*tert*-butyl-(8CI); 4-*tert*-butyl-1,2-dihydroxybenzene; 1,2-dihydroxy-4-*tert*-butylbenzene; PTBC; TBC; 4-TBC

*p-tert*-Butylcatechol is used as an antioxidant, stabilizer, and polymerization inhibitor for styrene, butadiene, neoprene, and other olefins and reactive monomers. *p-tert*-Butylcatechol was nominated by the National Cancer Institute and the U.S. Food and Drug Administration for testing based on reports of its increasing levels of production and use and to compare the toxicity of *p-tert*-butylcatechol with that of similar antioxidants, butylated hydroxyanisole and butylated hydroxytoluene, which are added to food. Male and female F344/N rats and B6C3$F_1$ mice were exposed to *p-tert*-butylcatechol (greater than 99% pure) in feed for 15 days or 14 weeks. Genetic toxicology studies were conducted in *Salmonella typhimurium*, rat bone marrow cells, and mouse peripheral blood erythrocytes.

In the 15-day studies, groups of five male and five female rats and mice were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm *p-tert*-butylcatechol (equivalent to average daily doses of approximately 290 to 2,470 mg *p-tert*-butylcatechol/kg body weight to rats and 590 to 8,200 mg/kg to mice). All animals in the 50,000 ppm groups were killed moribund on day 8 (rats) or by day 7 (mice). Mean body weights of all groups of rats exposed to 6,250 ppm or greater were significantly less than those of the controls. Mean body weights of male mice exposed to 12,500 or 25,000 ppm and of 25,000 ppm female mice were significantly less than those of the controls. Female rats, male and female mice in the 25,000 ppm groups, and 12,500 ppm male mice lost weight during

the studies. Feed consumption by exposed rats generally decreased with increasing exposure concentration; feed consumption by exposed mice was similar to that by the controls.

Thymus weights of 25,000 ppm rats and mice were significantly less than those of the controls. Gross findings noted at necropsy included thin carcasses for three male and all female rats in the 12,500 ppm groups and all male and female rats and mice in the 25,000 and 50,000 ppm groups. No exposure-related lesions were observed microscopically.

In the 14-week studies, groups of 10 male and 10 female rats and mice were fed diets containing 0, 781, 1,562, 3,125, 6,250, or 12,500 ppm *p-tert*-butylcatechol (equivalent to average daily doses of approximately 70 to 1,030 mg/kg to rats and 135 to 2,815 mg/kg to mice). All animals survived to the end of the studies. Mean body weights of male rats exposed to 1,562 ppm or greater, female rats exposed to 3,125 ppm or greater, male mice exposed to 12,500 ppm, and female mice exposed to 6,250 or 12,500 ppm were significantly less than those of the controls. Feed consumption by male and female rats in the 6,250 and 12,500 ppm groups at week 1 and the 12,500 ppm groups at week 14 was less than that by the controls; feed consumption by exposed and control mice was similar.

An erythrocytosis, indicated by increased hematocrit values, hemoglobin concentrations, and erythrocyte counts, was observed in 6,250 and 12,500 ppm rats on day 4 and in 12,500 ppm rats on day 22. At these time points, a transient hepatic effect was demonstrated by increases in alanine aminotransferase activities and bile salt concentrations in exposed rats.

In 12,500 ppm male rats, absolute left cauda epididymis, epididymis, and testis weights were decreased by 15%, 10%, and 9%, respectively, compared to the controls. The number of spermatid heads per testis and epididymal sperm motility of male rats in the 12,500 ppm group were significantly less than those of the controls. The numbers of cycling female rats and females with regular estrous cycles were decreased in the 6,250 and 12,500 ppm groups. Exposed groups of females had significantly fewer estrous cycles than did the controls. Estrous cycle length increased with increasing exposure concentration; female rats in the 6,250 and 12,500 ppm groups had significantly longer cycles and spent more time in diestrus and less time in proestrus, estrus, and metestrus than did the controls. Female mice in the 12,500 ppm group had a significantly longer estrous cycle than did the controls.

The incidences of hyperkeratosis of the forestomach epithelium were significantly increased in male and female rats in all exposed groups and in 12,500 ppm female mice. The incidences of hyperplasia of the forestomach epithelium were significantly increased in male and female rats exposed to 3,125 ppm or greater, male mice exposed to 12,500 ppm, and female mice exposed to 6,250 or 12,500 ppm. The severities of the forestomach lesions were

minimal to moderate in male rats and minimal to mild in female rats and in mice. All male rats exposed to 6,250 or 12,500 ppm had minimal cytoplasmic alteration in the liver.

The absorption, distribution, metabolism, and excretion of *p-tert*-butylcatechol following intravenous injection, gavage dosing, or dermal application were determined in male F344/N rats and B6C3F$_1$ mice. The absorption of [$^{14}$C]-*p-tert*-butylcatechol following gavage dosing or dermal application was high. The percent absorption following dermal application increased with increasing dose. Peak concentrations of [$^{14}$C]-*p-tert*-butylcatechol equivalents in plasma were reached 1 hour after gavage dosing (200 mg/kg) and 2 hours after dermal application (60 mg/kg); no parent compound was detected in the plasma extracts. Regardless of route of administration, *p-tert*-butylcatechol-derived radioactivity was readily excreted in the urine and was markedly nonpersistent in the tissues. *p-tert*-Butylcatechol was excreted as *p-tert*-butylcatechol sulfate and other polar metabolites that included predominately sulfate conjugates; it was not excreted as the parent compound. One metabolite was determined to be an *O*-methyl-*O′*-sulfate of *p-tert*-butylcatechol.

*p-tert*-Butylcatechol (10 to 1,000 µg/plate) was not mutagenic in any of several strains of *S. typhimurium* with or without rat or hamster liver S9. Bone marrow micronucleus tests in which 125 to 500 mg/kg *p-tert*-butylcatechol was administered three times by intraperitoneal injection to male rats gave negative results. No increases in the frequencies of micronucleated normochromatic erythrocytes were observed in the peripheral blood of male or female mice administered *p-tert*-butylcatechol in feed for 14 weeks. No significant alteration in the percentage of polychromatic erythrocytes in mouse bone marrow was observed.

In summary, the primary toxicity of *p-tert*-butylcatechol was to the forestomach of rats and mice. In the 14-week study in rats, forestomach toxicity was observed at all exposure concentrations, and the no-observed-adverse-effect level (NOAEL) was not reached for this effect. In the 14-week study in mice, the NOAEL for forestomach toxicity was 1,562 ppm.

# INTRODUCTION

## Chemical and Physical Properties

*p-tert*-Butylcatechol, a tertiary alcohol, is a colorless to white, crystalline or waxy solid with a boiling point of 285° C, a melting point ranging from 53° to 58° C, and a vapor pressure of 0.0028 mm at 25° C (Verschueren, 1983; Lewis, 1997; *Fluka Chemika-Biochemika*, 2001). It is soluble in ether, alcohol, acetone, and trifluoroacetic acid and slightly soluble in water (Verschueren, 1983; Lewis, 1997). *p-tert*-Butylcatechol has a flash point of 129° C; it is combustible when exposed to heat or flame and emits acrid, irritating fumes when heated to decomposition (Sax and Lewis, 1989).

## Production, Use, and Human Exposure

*p-tert*-Butylcatechol is manufactured by reacting isobutylene with catechol in the presence of ion exchange resins (*Kirk-Othmer*, 1980). It is sold as a powder or in a solution with 85% methanol or water. The only reported impurity is 3,5-di-*tert*-butylpyrocatechol (*Riedel-de Haën*, 1984). For 1975 through 1977, the Toxic Substance Control Act plant and production database indicated that approximately 0.1 million to 1 million pounds of *p-tert*-butylcatechol were imported and 10,000 to 100,000 pounds were produced by companies providing import and production volumes (TSCAPP, 1983). For 1989, consumption of *p-tert*-butylcatechol by industries in the United States was estimated to be 1.5 million pounds (*Chemical Marketing Reporter*, 1989).

*p-tert*-Butylcatechol is used as an antioxidant, stabilizer, and polymerization inhibitor for styrene, butadiene, neoprene, and other olefins and reactive monomers. Additionally, *p-tert*-butylcatechol is used as an activator for insecticides, a clay strengthener in building materials, a corrosion and radical inhibitor, an antiskinning additive, an emulsion breaker, a pour-point depressant, a chemical intermediate for organic syntheses, and a component of shoe adhesives (*Patty's*, 1981). Coconut shell charcoal coated with 10% *p-tert*-butylcatechol is used to analyze low concentrations of 1,3-butadiene in ambient air (Hendricks and Schultz, 1986). *p-tert*-Butylcatechol may be present in commercial products such as rubber gloves; polyester- or polyacrylic-based medical prostheses; duplicating, photoprocessing, and phototypesetting chemicals; fiberglass-reinforced polyester products; shoe adhesives; and melanogenesis-interrupting phenolic germicides and synthetic detergents (*Patty's*, 1981; Foussereau *et al.*, 1982; Fardal and Curphey, 1983; Gellin, 1983; Macfarlane *et al.*, 1990).

Surveys by the National Institute for Occupational Safety and Health (1990) indicated that approximately 27,460 workers in the United States were potentially exposed to *p-tert*-butylcatechol between 1981 and 1983. No threshold limit value has been recommended for *p-tert*-butylcatechol (ACGIH, 2001).

## ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

No metabolism studies of *p-tert*-butylcatechol were reported in the literature at the time the studies described in this report were designed. Hirose *et al.* (1989) cited results of *in vitro* studies in which derivatives of catechol were found to undergo perioxidative oxidation, which proceeds through oxidation-reduction cycling to produce the corresponding quinone metabolites or active oxygen species that interact with cellular macromolecules. Picardo *et al.* (1987) reported that *p-tert*-butylcatechol decomposed within 24 hours in an *in vitro* study. *p-tert*-Butylcatechol, present as an antioxidant and polymerization inhibitor, was microbially degraded before styrene in soil and enrichment cultures (Sielicki *et al.,* 1978). In a study by Marchesini *et al.* (1977), *p-tert*-butylcatechol was determined to yield the corresponding *o*-quinone via a secondary catecholoxidase activity of ascorbate oxidase, which is found in fruits and vegetables. The metabolism and disposition studies of *p-tert*-butylcatechol in mice and rats described in Appendix G have recently been published (Black and Mathews, 2000).

## TOXICITY

### *Experimental Animals*

Acute $LD_{50}$ values reported for *p-tert*-butylcatechol were 2,820 mg per kilogram body weight orally for rats, 32 mg/kg by intravenous injection for mice, and 630 mg/kg by dermal application for rabbits (Smyth *et al.*, 1954; Sax and Lewis, 1989). Guinea pigs exposed dermally to 1% *p-tert*-butylcatechol for 3 weeks had moderate irritation; guinea pigs exposed to 0.1% had mild irritation after three weeks (Smyth *et al.*, 1954; Sax and Lewis, 1989). In additional studies of *p-tert*-butylcatechol by the same authors, rabbits exhibited severe dermal irritation when exposed to 500 mg for 24 hours and severe eye irritation when exposed to 50 mg.

Yonemoto *et al.* (1983a) administered three successive topical applications (0.3 mL) of a solution of 1 M *p-tert*-butylcatechol in dimethyl sulfoxide (30:70) at 48-hour intervals to the ear skin of hairless mice. An increase in glutathione reductase activity was observed along with pheomelanogenesis (development of melanosomes with altered ultrastructure); these effects preceded melanocyte degeneration and pigment loss in the skin.

### *Humans*

As well as being a skin and eye irritant, *p-tert*-butylcatechol is moderately toxic when ingested or absorbed dermally. Systemic toxic effects similar to those induced by phenols might be expected to occur, as with the parent compound catechol (*Patty's*, 1981).

Polyvinylchloride chemical plant workers exposed to *p-tert*-butylcatechol had allergic dermatitis with depigmentation. Subsequently, one sensitized worker developed lesions after contact with free *p-tert*-butylcatechol present in polyvinylchloride in shoes (Laurell, 1984). A polyester resin plant worker who handled *p-tert*-butylcatechol powder developed leukoderma on the hands, arms, and face; the depigmentation that accompanied allergic dermatitis was still present, although reduced in size, 3 years after exposure (Horio *et al.*, 1977). In other studies, exposure to *p-tert*-butylcatechol from contact with rubber gloves, assembly lubricating oil, duplicating and phototypesetting papers, and other *p-tert*-butylcatechol-containing products has been associated with allergic dermatitis and depigmentation (Gellin *et al.*, 1970; McGuire and Hendee, 1971; Foussereau *et al.*, 1982; Hirosawa *et al.*, 1982; Fardal and Curphey, 1983; Gellin, 1983; Macfarlane *et al.*, 1990).

To determine substrate stability and oxidative mechanisms relative to their toxicity on different cell lines, Picardo *et al.* (1987) compared *p-tert*-butylcatechol and other diphenolic derivatives that act as tyrosinase substrates with analogs that do not. The *in vitro* study used Raji and K562 cell lines, which lack tyrosinase, and 1RE 1 and 1RE 2 human melanoma cell lines. Catechols such as *p-tert*-butylcatechol that are tyrosinase substrates were found to be equally toxic to melanoma and nonmelanoma cell lines. Usami *et al.* (1980) suggested that tyrosinase activity is inhibited by *p-tert*-butylcatechol at the second step of melanogenesis. A selective melanocytotoxic action on functional melanocytes has resulted in chemically induced leukoderma; a competitive inhibition of tyrosinase may be involved (Gellin and Maibach, 1983). In a study of the effects of *p-tert*-butylcatechol on enzyme activity and eumelanin and sulfur content in cultured human melanoma cells, Yonemoto *et al.* (1983a,b) concluded that the chemical alters the types of melanin formed by modulation of glutathione reductase and $\gamma$-glutamyl transpeptidase activity. A study in a tissue-cultured B16 melanoma cell line was performed to determine the mechanism of depigmentation; elevation of glutathione-metabolizing enzyme activity at a $10^{-4}$ M concentration of *p-tert*-butylcatechol was considered evidence that the chemical stimulates pheomelanogenesis in melanocytes (Kawashima *et al.*, 1984).

## Reproductive and Developmental Toxicity

No studies or reports of reproductive or developmental toxicity of *p-tert*-butylcatechol in animals or humans were found in a review of the literature.

## CARCINOGENICITY

### *Experimental Animals*

Results of a 1-year tumor promotion study indicate that *p-tert*-butylcatechol and other catechol derivatives may be weak, nongenotoxic stomach carcinogens with promoting activity (Hirose *et al.*, 1989). In this study, a group of 16 six-week-old F344 rats was administered a single intragastric dose of 150 mg/kg *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG) in dimethyl sulfoxide; from 1 week after the intragastric dose was administered until week 52, the rats were fed a powdered diet containing 0% or 1.5% *p-tert*-butylcatechol *ad libitum*. An additional group of 15 rats was maintained on the same diet as the pretreated rats but did not receive MNNG. Groups of 15 MNNG-pretreated rats and 10 untreated rats were maintained as controls. All 16 pretreated rats exposed to 1.5% *p-tert*-butylcatechol had hyperplasia of the forestomach; additionally, 15 had papilloma, four had carcinoma *in situ*, and 12 had squamous cell carcinoma. Forestomach hyperplasia also occurred in the rats that received *p-tert*-butylcatechol without the MNNG pretreatment. One rat administered *p-tert*-butylcatechol without MNNG pretreatment also had a papilloma. In additional groups of 10 to 16 F344 rats in the same studies, the analogs *p*-methylcatechol, hydroquinone, and resorcinol induced a less pronounced degree of hyperplasia (Hirose *et al.*, 1989). The analogs caffeic acid and butylated hydroxyanisole (BHA) were weak carcinogens in the rat forestomach epithelium; butylated hydroxytoluene (BHT) was not carcinogenic (Hirose *et al.*, 1988).

In the tumor promotion study by Hirose *et al.* (1989), the incidence of adenomatous hyperplasia in the pyloric region of the glandular stomach was significantly greater in rats pretreated with MNNG and then administered 1.5% *p-tert*-butylcatechol in the diet than in the MNNG-pretreated controls. Adenomatous hyperplasia occurred in five of 16 exposed rats, and three rats developed adenocarcinoma. No lesions were observed in the fundic region of the glandular stomach. Exposure to *p-tert*-butylcatechol without MNNG pretreatment did not result in lesions in either the fundic or the pyloric region of the glandular stomach. No lesions were observed in control rats.

The carcinogenicity of hydroquinone, resorcinol, and *t*-butylhydroquinone, chemicals structurally related to *p-tert*-butylcatechol, in rats and mice has been studied by the National Toxicology Program (1989, 1992, 1997). There was some evidence of carcinogenicity for hydroquinone administered by gavage for 2 years to male and female rats and female mice, based on increased incidences of renal tubule cell adenoma in male rats, mononuclear cell leukemia in female rats, and hepatocellular neoplasms in female mice. However, there was no evidence of carcinogenicity for resorcinol administered by gavage or *t*-butylhydroquinone administered in feed for 2 years to male or female rats or mice.

### *Humans*

No epidemiology studies or case reports associating *p-tert*-butylcatechol exposure with cancer risk in humans were found in the literature.

## Genetic Toxicity

Few mutagenicity studies of *p-tert*-butylcatechol have been published. Negative results were reported for *p-tert*-butylcatechol in a battery of short-term mutagenicity tests including bacterial gene mutation assays with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* strains $WP_2$ and $WP_2$ *uvrA*; a test for mitotic gene conversion in *Saccharomyces cerevisiae* JD1; and an assay for induced chromosomal aberrations in metabolically competent cultured rat liver cells (Dean *et al.*, 1985). In contrast to these negative results, significant dose-dependent increases in mutant frequencies were observed in L5178Y mouse lymphoma cells incubated with 0.08 to 5.0 µg/mL *p-tert*-butylcatechol in the absence of S9 activation enzymes (McGregor *et al.*, 1988).

## Study Rationale

*p-tert*-Butylcatechol was nominated by the National Cancer Institute and the U.S. Food and Drug Administration for testing based on reports of its increasing levels of production and use. The toxicity studies were conducted to compare the toxicity of *p-tert*-butylcatechol with that of similar antioxidants, BHA and BHT, which are added to food. *p-tert*-Butylcatechol was administered in feed to F344/N rats and $B6C3F_1$ mice in 15-day and 14-week studies. In addition, genetic toxicology studies were conducted in *S. typhimurium*, rat bone marrow cells, and mouse peripheral blood erythrocytes. Absorption, distribution, metabolism, and excretion studies were also conducted in male F344/N rats and $B6C3F_1$ mice.

# MATERIALS AND METHODS

## PROCUREMENT AND CHARACTERIZATION OF *P-TERT*-BUTYLCATECHOL

*p-tert*-Butylcatechol was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (03404 MF and 19115EN); lot 03404 MF was used during the 15-day studies and lot 19115EN was used during the 14-week studies. Identity, purity, and stability analyses were conducted by the study laboratories. Reports on analyses performed in support of the *p-tert*-butylcatechol studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a whitish, flaky solid, was identified as *p-tert*-butylcatechol by infrared spectroscopy and proton and carbon-13 nuclear magnetic resonance spectroscopy. The purity of each lot was determined by high-performance liquid chromatography (HPLC). Major peak comparisons of lot 03404 MF with lot 19115EN were also performed with HPLC. For lot 03404 MF, results of HPLC analyses indicated the major product peak and three impurities. One major impurity peak had an area of 0.3% relative to the major peak; two minor impurities had relative areas of less than 0.1%. For lot 19115EN, results of HPLC analyses indicated the major product peak and two impurity peaks with areas of 0.26% and 0.20% relative to the major peak area. The overall purity of each lot was determined to be greater than 99%. Major peak comparisons indicated nearly identical purity profiles and impurity concentrations between the two lots.

Accelerated stability studies of lot 03404 MF of the bulk chemical were conducted with HPLC. These studies indicated that *p-tert*-butylcatechol is stable as a bulk chemical for at least 14 days when stored under a nitrogen headspace, protected from strong oxidizers, at temperatures up to approximately 60° C. Based on the results of these studies and on information in the literature, the bulk chemical was stored under a nitrogen headspace (lot 03404 MF) in amber glass bottles, in the dark, at room temperature. No degradation of the bulk chemical was detected.

## PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared by mixing *p-tert*-butylcatechol with nonirradiated feed once (15-day studies) or with irradiated feed approximately every 4 weeks (14-week studies) (Table F2). Formulations were stored in plastic bags at –20° C for up to 3 weeks during the 15-day studies and in plastic bags inside buckets at 5° C for up to 42 days during the 14-week studies.

Homogeneity studies of 3,125 and 50,000 ppm dose formulations in nonirradiated feed (15-day studies) and 781 and 12,500 ppm dose formulations in irradiated feed (14-week studies) and stability studies of the 781 and 3,125 ppm dose formulations were performed by the study laboratories using HPLC. Homogeneity was confirmed. Stability of the 3,125 ppm dose formulation was confirmed for 35 days for samples stored at approximately –20° C. Stability of the 781 ppm dose formulation was confirmed for 42 days for samples stored in plastic bags at temperatures up to 5° C; samples subjected to animal room conditions showed declines of up to 37 percent in *p-tert*-butylcatechol concentrations with time.

Periodic analyses of the dose formulations of *p-tert*-butylcatechol were conducted by the study laboratories using HPLC. For the 15-day studies, dose formulations were analyzed once; all were within 10% of the target concentrations (Table F3). Animal room samples of these dose formulations were also analyzed; the concentrations of all samples were less than 90% of the target concentrations. For the 14-week studies, dose formulations were analyzed at the beginning, midpoint, and end of the studies; all dose formulations analyzed and used for dosing were within 10% of the target concentrations (Table F4). Animal room samples of these dose formulations were also analyzed; the concentrations of nine of 15 animal room samples for rats and 13 of 15 for mice were less than 90% of the target concentrations. The low concentrations of animal room samples were attributed to chemical degradation, oxidation, evaporation, or binding with feed.

## 15-Day Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were 5 weeks old. Animals were quarantined for 11 (rats) or 12 (mice) days and were 6 (rats) or 7 (mice) weeks old on the first day of the studies. Groups of five male and five female rats and mice were fed diets containing 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm *p-tert*-butylcatechol for 15 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage; male mice were housed individually. Feed consumption was recorded weekly by cage. The animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly and at the end 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. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all rats and mice. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Histopathologic examinations were performed on all rats and mice in the 0, 25,000, and 50,000 ppm groups. Table 1 lists the tissues and organs examined.

## 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 4 weeks old. Rats were quarantined for 11 (males) or 12 (females) days and were 6 weeks old on the first day of the studies; mice were quarantined for 14 (females) or 15 (males) days and were 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood samples were collected from five male and five female rats and mice at 4 weeks and at the end of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

Groups of 10 male and 10 female rats and mice were fed diets containing 0, 781, 1,562, 3,125, 6,250, or 12,500 ppm *p-tert*-butylcatechol 7 days per week for 14 weeks. Additional groups of 10 male and 10 female rats designated for clinical pathology testing were exposed to the same concentrations for 22 days. Feed and water were available *ad libitum*. Animals were given irradiated feed; the feed was irradiated to reduce potential microbial contamination. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings and feed consumption were recorded weekly for core study rats and mice. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 22 and from all core study rats and mice at the end of the studies for hematology and clinical chemistry (rats) analyses. The animals were anesthetized with a mixture of carbon dioxide and oxygen. Samples for hematology analysis were placed in micro-collection tubes (Sarstedt, Inc., Nümbrecht, Germany) coated with potassium EDTA and inverted by hand to prevent clotting; samples for clinical chemistry evaluations were placed in similar tubes devoid of anticoagulant and centrifuged for collection of serum. Hematocrit; hemoglobin concentration; erythrocyte, platelet, and leukocyte counts; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined with a Cell-Dyn® 3500 hematology analyzer (Abbott Diagnostics, Santa Clara, CA). Differential leukocyte counts and erythrocyte and platelet morphologies were determined microscopically from blood smears stained with a modified Wright-Giemsa stain. A Miller Disc was used to determine reticulocyte counts from smears prepared with blood stained with new methylene blue. For clinical chemistry analyses, serum samples were analyzed using a Hitachi 911® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN) using commercially available reagents. The parameters evaluated are listed in Table 1.

At the end of the 14-week studies, samples were collected for sperm count and motility and vaginal cytology evaluations of core study rats and mice exposed to 0, 3,125, 6,250, and 12,500 ppm. The parameters evaluated are

listed in Table 1. For 12 consecutive days prior to scheduled terminal 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 count and 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.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on rats and mice in the 0 and 12,500 ppm groups. The forestomach was identified as a target organ in rats and mice and was microscopically examined in all core study animals. The liver was examined in all groups of male rats. Table 1 lists the tissues and organs routinely examined.

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. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

**TABLE 1**
**Experimental Design and Materials and Methods in the Feed Studies of *p-tert*-Butylcatechol**

| 15-Day Studies | 14-Week Studies |
|---|---|
| **Study Laboratory** | |
| Microbiological Associates, Inc. (Bethesda, MD) | Battelle Columbus Laboratories (Columbus, OH) |
| **Strain and Species** | |
| F344/N rats<br>$B6C3F_1$ mice | F344/N rats<br>$B6C3F_1$ mice |
| **Animal Source** | |
| Taconic Farms (Germantown, NY) | Taconic Laboratory Animals and Services (Germantown, NY) |
| **Time Held Before Studies** | |
| Rats: 11 days<br>Mice: 12 days | Rats: 11 (males) or 12 (females) days<br>Mice: 14 (females) or 15 (males) days |
| **Average Age When Studies Began** | |
| Rats: 6 weeks<br>Mice: 7 weeks | 6 weeks |
| **Date of First Exposure** | |
| Rats: March 20, 1995<br>Mice: March 21, 1995 | Rats: August 26 (males) or 27 (females), 1996<br>Mice: September 5 (females) or 6 (males), 1996 |
| **Duration of Exposure** | |
| 15 days | 14 weeks |
| **Date of Last Exposure** | |
| Rats: April 3, 1995<br>Mice: April 4, 1995 | Rats: November 25 (males) or 26 (females), 1996<br>Mice: December 5 (females) or 6 (males), 1996 |
| **Necropsy Dates** | |
| Rats: April 3, 1995<br>Mice: April 4, 1995 | Rats: November 25 (males) or 26 (females), 1996<br>Mice: December 5 (females) or 6 (males), 1996 |
| **Average Age at Necropsy** | |
| Rats: 8 weeks<br>Mice: 9 week | 19 weeks |
| **Size of Study Groups** | |
| 5 males and 5 females | 10 males and 10 females |
| **Method of Distribution** | |
| Animals were distributed randomly into groups of approximately equal initial mean body weights. | Same as 15-day studies |
| **Animals per Cage** | |
| Rats: 5<br>Mice: 1 (males) or 5 (females) | Rats: 5<br>Mice: 1 (males) or 5 (females) |
| **Method of Animal Identification** | |
| Tail tattoo | Tail tattoo |

**TABLE 1**
**Experimental Design and Materials and Methods in the Feed Studies of *p-tert*-Butylcatechol**

| 15-Day Studies | 14-Week Studies |
|---|---|
| **Diet** | |
| NTP-2000 mash feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum* | Same as 15-day studies, except feed was irradiated |
| **Water** | |
| Tap water (Washington Suburban Sanitary Commission) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum* | Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum* |
| **Cages** | |
| Polycarbonate (Lab Products, Inc., Maywood, NJ), changed once (male mice) or twice per week | Same as 15-day studies |
| **Bedding** | |
| Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed once (male mice) or twice per week | Irradiated Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed once (male mice) or twice per week |
| **Racks** | |
| Stainless steel, changed every 2 weeks | Same as 15-day studies |
| **Animal Room Environment** | |
| Temperature: 72° ± 3° F<br>Relative humidity: 50% ± 15%<br>Room fluorescent light: 12 hours/day<br>Room air changes: 10/hour | Temperature: 72° ± 3° F<br>Relative humidity: 50% ± 15%<br>Room fluorescent light: 12 hours/day<br>Room air changes: 10/hour |
| **Exposure Concentrations** | |
| 0, 3,125, 6,250, 12,500, 25,000, or 50,000 ppm in feed | 0, 781, 1,562, 3,125, 6,250, or 12,500 ppm in feed |
| **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 and at the end of the studies. Feed consumption was recorded weekly by cage. | Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies. Clinical findings and feed consumption were recorded weekly for core study animals. |
| **Method of Sacrifice** | |
| Carbon dioxide asphyxiation | Same as 15-day studies |
| **Necropsy** | |
| Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lungs, right testis, and thymus. | Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lungs, right testis, and thymus. |

**TABLE 1**
**Experimental Design and Materials and Methods in the Feed Studies of *p-tert*-Butylcatechol**

| 15-Day Studies | 14-Week Studies |
|---|---|
| **Clinical Pathology** | |
| None | Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 22 and from all core study rats and mice at the end of the studies for hematology and clinical chemistry (rats) determinations.<br>***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials<br>***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts |
| **Histopathology** | |
| In addition to gross lesions and tissue masses, the kidney, liver, and stomach (forestomach and glandular) were examined in all animals in the 0, 25,000, and 50,000 ppm groups. | Complete histopathology was performed on core study rats and mice in the 0 and 12,500 ppm groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart with aorta, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, 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), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The forestomach was also examined in the remaining core study groups; the liver was examined in all groups of male rats. |
| **Sperm Motility and Vaginal Cytology** | |
| None | At the end of the studies, sperm samples were collected from core study male animals in the 0, 3,125, 6,250, and 12,500 ppm groups for sperm count evaluations. The following parameters were evaluated: epididymal sperm motility and spermatid heads per testis, per gram testis, per cauda epididymis, and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for 12 consecutive days prior to the end of the studies from core study females exposed to 0, 3,125, 6,250, or 12,500 ppm for vaginal cytology evaluations. The number of cycling females, number of females with regular cycles, number of cycles, estrous cycle length, and percentage of time spent in the various estrous cycle stages were evaluated. |

## 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 (Gart *et al.*, 1979), a procedure based on the overall proportion of affected animals, was used to determine significance.

### 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 historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, 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). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). 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 METHODS

The 14-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Units of Microbiological Associates, Inc., and 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 Zeiger *et al.* (1992). *p-tert*-Butylcatechol was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *S. typhimurium* tester strains TA97, TA98, TA100, TA102, TA104, and TA1535 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 *p-tert*-butylcatechol. The high dose was limited by toxicity.

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.

## Rat Bone Marrow Micronucleus Test Protocol

The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male F344/N rats were injected intraperitoneally three times at 24-hour intervals with *p-tert*-butylcatechol dissolved in corn oil. Vehicle control animals were injected with corn oil only. The positive control rats received injections of 10 mg/kg cyclophosphamide. The rats 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 frequency of micronucleated cells in up to 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 with 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 is

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, reproducibility of any effects observed, and the magnitudes of those effects.

## Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 14-week study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with a chromatic-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983) and coded. Slides were scanned to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) in each of 10 animals per exposure group. The results for NCEs in mouse peripheral blood were tabulated as described for PCEs in the rat bone marrow micronucleus test protocol. In addition, the percentage of PCEs among the total erythrocyte population in the peripheral blood was scored for each dose group as a measure of toxicity.

## 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 different 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 Study Report represent a scientific judgement of the overall evidence for activity of the chemical in an assay.

# RESULTS

## RATS

### 15-DAY STUDY

All rats in the 50,000 ppm groups were killed moribund on day 8 (Table 2). The final mean body weights of all groups exposed to 6,250 ppm or greater and the mean body weight gains of all exposed groups were significantly less than those of the controls; females in the 25,000 ppm group lost weight during the study (Table 2). Feed consumption generally decreased with increasing exposure concentration. Exposure concentrations of 3,125, 6,250, 12,500, 25,000, and 50,000 ppm resulted in average daily doses of approximately 290, 525, 1,000, 1,650, and 1,700 mg *p-tert*-butylcatechol/kg body weight to males and 290, 520, 930, 1,440, and 2,470 mg/kg to females. All 50,000 ppm males and females exhibited abnormal posture, ataxia, ruffled fur, and thinness; one female exposed to 50,000 ppm was also lethargic. One 50,000 ppm female and one 25,000 ppm male had nasal/eye discharge.

Absolute and relative thymus weights of 25,000 ppm rats were significantly less than those of the controls, as was the absolute thymus weight of 12,500 ppm males (Table C1). Other organ weight differences in exposed rats reflected decreases in body weights.

Gross findings noted at necropsy included thin carcasses for three males and all females in the 12,500 ppm groups and all males and females in the 25,000 and 50,000 ppm groups. No lesions were observed microscopically in the groups examined (0, 25,000, and 50,000 ppm). Based on the body weight effects in the 25,000 ppm groups and mortality in the 50,000 ppm groups, the highest exposure concentration selected for the 14-week study was 12,500 ppm.

**TABLE 2**
**Survival, Body Weights, and Feed Consumption of Rats in the 15-Day Feed Study of *p-tert*-Butylcatechol**

| Concentration (ppm) | Survival[a] | Mean Body Weight[b] (g) | | | Final Weight Relative to Controls (%) | Feed Consumption[c] | |
|---|---|---|---|---|---|---|---|
| | | Initial | Final | Change | | Week 1 | Week 2 |
| **Male** | | | | | | | |
| 0 | 5/5 | 122 ± 6 | 198 ± 6 | 76 ± 2 | | 15.6 | 17.7 |
| 3,125 | 5/5 | 124 ± 4 | 190 ± 4 | 66 ± 1** | 96 | 15.4 | 16.5 |
| 6,250 | 5/5 | 125 ± 5 | 181 ± 5* | 56 ± 2** | 91 | 12.9 | 15.0 |
| 12,500 | 5/5 | 120 ± 6 | 154 ± 7** | 34 ± 2** | 78 | 9.5 | 13.0 |
| 25,000 | 5/5 | 122 ± 4 | 123 ± 4** | 1 ± 2** | 62 | 5.8 | 9.7 |
| 50,000 | 0/5[d] | 121 ± 4 | — | — | — | 2.9 | — |
| **Female** | | | | | | | |
| 0 | 5/5 | 104 ± 4 | 144 ± 5 | 41 ± 2 | | 11.9 | 14.1 |
| 3,125 | 5/5 | 104 ± 3 | 135 ± 4 | 31 ± 2** | 94 | 12.3 | 11.2 |
| 6,250 | 5/5 | 105 ± 4 | 133 ± 4* | 28 ± 1** | 92 | 10.0 | 10.7 |
| 12,500 | 5/5 | 105 ± 5 | 123 ± 4** | 18 ± 2** | 85 | 8.2 | 9.3 |
| 25,000 | 5/5 | 104 ± 3 | 100 ± 2** | –4 ± 2** | 69 | 3.9 | 7.2 |
| 50,000 | 0/5[d] | 105 ± 4 | — | — | — | 3.7 | — |

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights or weight changes were calculated for groups with 100% mortality.
[c] Feed consumption is expressed as grams per animal per day.
[d] Day of death: 8

## 14-Week Study

All rats survived to the end of the study (Table 3). Final mean body weights and body weight gains of male rats exposed to 1,562 ppm or greater and females exposed to 3,125 ppm or greater were significantly less than those of the controls (Table 3 and Figure 1). Feed consumption by 6,250 and 12,500 ppm rats at week 1 and by 12,500 ppm rats at week 14 was less than that by the controls (Table 3). Exposure concentrations of 781, 1,562, 3,125, 6,250, and 12,500 ppm resulted in average daily doses of approximately 70, 135, 270, 525, and 1,030 mg/kg to males and 70, 145, 265, 555, and 1,010 mg/kg to females. There were no clinical findings of toxicity.

**Table 3**
**Survival, Body Weights, and Feed Consumption of Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| Concentration (ppm) | Survival[a] | Mean Body Weight[b] (g) | | | Final Weight Relative to Controls (%) | Feed Consumption[c] | |
|---|---|---|---|---|---|---|---|
| | | Initial | Final | Change | | Week 1 | Week 14 |
| **Male** | | | | | | | |
| 0 | 10/10 | 79 ± 3 | 311 ± 4 | 232 ± 2 | | 14.1 | 17.4 |
| 781 | 10/10 | 79 ± 3 | 319 ± 5 | 240 ± 5 | 102 | 13.4 | 17.5 |
| 1,562 | 10/10 | 76 ± 2 | 297 ± 7* | 220 ± 6* | 95 | 12.3 | 17.2 |
| 3,125 | 10/10 | 78 ± 2 | 290 ± 4** | 212 ± 4** | 93 | 12.6 | 16.3 |
| 6,250 | 10/10 | 80 ± 3 | 273 ± 4** | 193 ± 3** | 88 | 9.3 | 15.4 |
| 12,500 | 10/10 | 76 ± 2 | 240 ± 3** | 164 ± 2** | 77 | 4.3 | 13.7 |
| **Female** | | | | | | | |
| 0 | 10/10 | 75 ± 1 | 178 ± 3 | 103 ± 2 | | 11.7 | 11.1 |
| 781 | 10/10 | 76 ± 2 | 183 ± 2 | 108 ± 2 | 103 | 11.7 | 11.5 |
| 1,562 | 10/10 | 74 ± 2 | 184 ± 3 | 110 ± 2 | 103 | 11.7 | 11.7 |
| 3,125 | 10/10 | 76 ± 2 | 170 ± 2* | 94 ± 2** | 96 | 11.0 | 10.1 |
| 6,250 | 10/10 | 75 ± 1 | 163 ± 3** | 88 ± 1** | 91 | 8.7 | 10.4 |
| 12,500 | 10/10 | 72 ± 2 | 162 ± 2** | 89 ± 2** | 91 | 4.5 | 9.3 |

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.

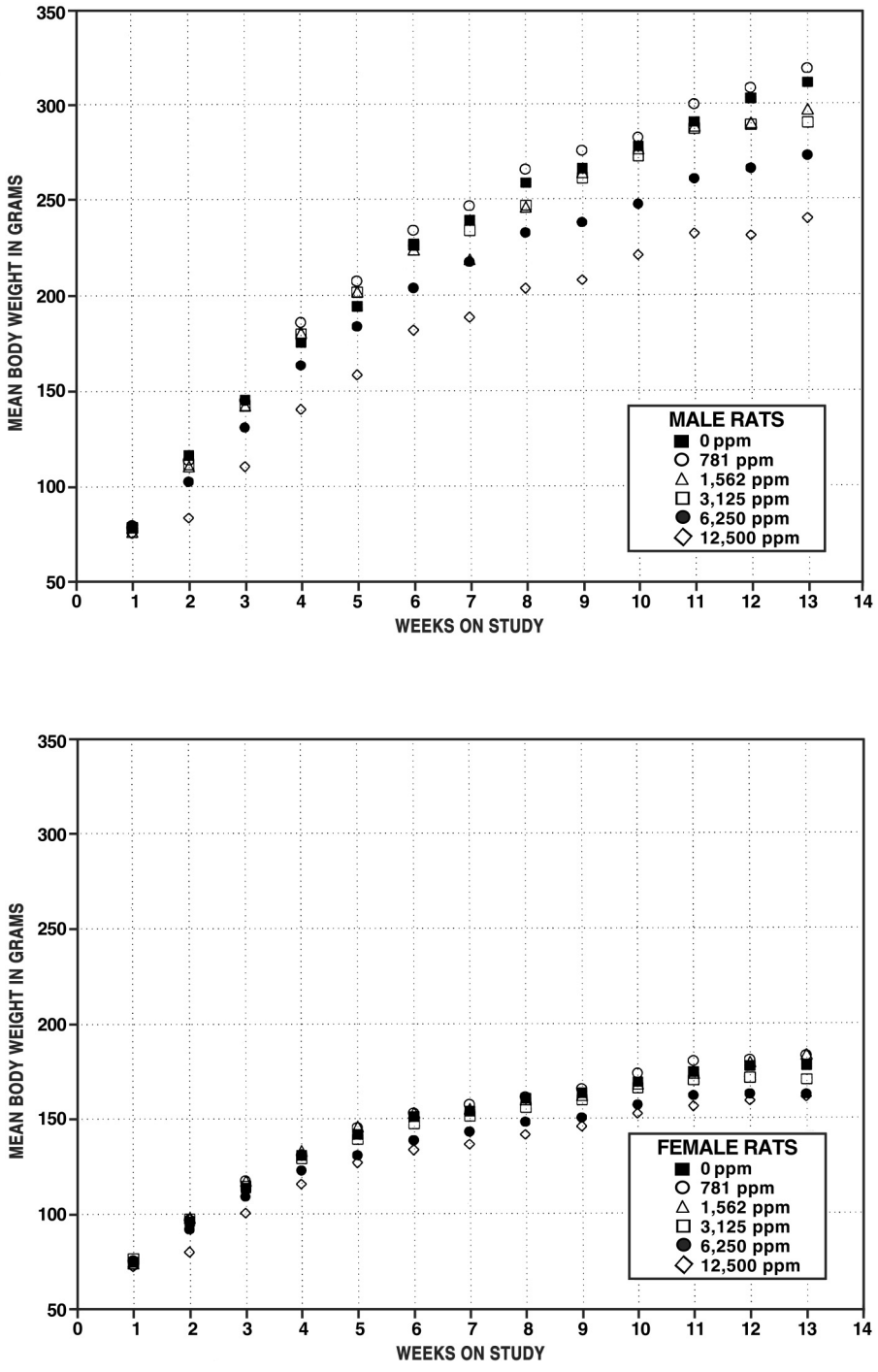



**FIGURE 1**
**Body Weights of Male and Female Rats Exposed to *p-tert*-Butylcatechol in Feed for 14 Weeks**

Hematology and clinical chemistry data are provided in Tables 4 and B1. After 4 days of exposure, hematocrit values, hemoglobin concentrations, and erythrocyte counts in 6,250 and 12,500 ppm males and females were increased, consistent with a minimal to mild erythrocytosis. This increase in the erythron was accompanied by unchanged to slightly decreased reticulocyte counts, suggesting that the erythrocytosis was related to an altered hydration status and resultant hemoconcentration; hemoconcentration was supported by decreased feed consumption (and, presumably, decreased water consumption) and transient increases in albumin, total protein, urea nitrogen, and creatinine concentrations in various exposed groups. The erythrocytosis was transient and occurred only in the 12,500 ppm groups on day 22; by the end of the study, no groups were affected. The erythrocytosis was accompanied by minimal decreases in mean cell volumes and mean cell hemoglobin values, suggesting that circulating red cells were slightly smaller than expected. This effect was also transient and, at week 14, had reversed, with minimally increased erythrocyte sizes in males in the 6,250 and 12,500 ppm groups. Platelet counts in 12,500 ppm rats also demonstrated a transient, minimal increase on day 4 that had abated by day 22.

On days 4 and 22, there was evidence of a transient hepatic effect, as indicated by increased serum alanine aminotransferase activities and bile salt concentrations in exposed males and females; these increases had abated by the end of the study. On day 4, alanine aminotransferase activities were minimally increased in 6,250 and 12,500 ppm males and all exposed groups of females. On day 22, alanine aminotransferase activities were minimally increased in 3,125 ppm males and in males and females exposed to 6,250 ppm or greater. The increases in activity were not exposure concentration related at either time point. While increases in alanine aminotransferase activities suggest increases in hepatocellular leakage, there were no significant increases in the activity of sorbitol dehydrogenase, another marker of hepatocellular leakage. Glucocorticoids have been shown to increase liver alanine aminotransferase activity (Rosen *et al.*, 1959a,b). Thus, if a drug or compound induced an increase in liver alanine aminotransferase activity or induced treatment-associated stress, an increase in serum alanine aminotransferase, but not sorbitol dehydrogenase, activity could occur. On day 4, bile salt concentrations were increased in males exposed to 1,562 ppm or greater and 12,500 ppm females; on day 22, only 12,500 ppm females were affected. Increases in bile salt concentrations are, in general, used as a marker of hepatic cholestasis. In the current study, however, alkaline phosphatase activities, another marker of cholestasis, were decreased (day 4) or unaffected; thus, increased bile salt concentrations and decreased alkaline phosphatase activities would appear to be incongruous. Serum bile salt concentration can be affected by mechanisms other than cholestasis; altered enterohepatic circulation, impaired liver function, and noncholestatic liver injury can result in increased circulating bile salt concentrations (Hofmann, 1988). Additionally, decreased alkaline phosphatase activity has been suggested to be related to altered feed intake (Travlos *et al*., 1996). Also, there was evidence early in the current study of decreased feed consumption by rats in the higher exposure groups; thus, the increases in alanine aminotransferase activities and bile salt concentrations could suggest a transient, minimal liver effect that was not expressed in the other markers of liver injury or cholestasis.

**TABLE 4**
**Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| Hematology | | | | | | |
| n | | | | | | |
| Day 4 | 9 | 10 | 10 | 10 | 10 | 9 |
| Day 22 | 10 | 9 | 9 | 8 | 9 | 10 |
| Week 14 | 10 | 10 | 9 | 10 | 10 | 9 |
| Hematocrit (%) | | | | | | |
| Day 4 | 35.7 ± 0.4 | 35.5 ± 0.2 | 35.7 ± 0.5 | 37.0 ± 0.7 | 39.1 ± 0.4** | 41.5 ± 0.4** |
| Day 22 | 40.4 ± 0.4 | 40.2 ± 0.4 | 45.4 ± 1.4** | 41.3 ± 0.6* | 41.9 ± 0.6* | 42.5 ± 0.5** |
| Week 14 | 45.8 ± 0.5 | 45.8 ± 0.3 | 44.3 ± 0.6 | 46.9 ± 0.4 | 46.8 ± 0.3 | 47.1 ± 0.3* |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 11.7 ± 0.2 | 11.8 ± 0.1 | 11.9 ± 0.2 | 12.2 ± 0.2* | 13.0 ± 0.2** | 13.8 ± 0.1** |
| Day 22 | 13.7 ± 0.1 | 13.6 ± 0.1 | 15.3 ± 0.5* | 13.9 ± 0.3 | 14.0 ± 0.2 | 14.3 ± 0.2 |
| Week 14 | 15.0 ± 0.2 | 14.9 ± 0.1 | 14.7 ± 0.1 | 15.3 ± 0.1 | 15.3 ± 0.1 | 15.3 ± 0.1* |
| Erythrocytes ($10^6$/μL) | | | | | | |
| Day 4 | 6.13 ± 0.05 | 6.10 ± 0.05 | 6.25 ± 0.08 | 6.42 ± 0.11 | 6.81 ± 0.06** | 7.28 ± 0.07** |
| Day 22 | 6.98 ± 0.09 | 6.96 ± 0.07 | 7.87 ± 0.27** | 7.12 ± 0.13 | 7.23 ± 0.12 | 7.52 ± 0.10** |
| Week 14 | 8.93 ± 0.12 | 8.83 ± 0.10 | 8.62 ± 0.13 | 9.03 ± 0.09 | 8.96 ± 0.09 | 8.96 ± 0.06 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 58.2 ± 0.4 | 58.2 ± 0.3 | 57.2 ± 0.5 | 57.6 ± 0.4 | 57.4 ± 0.4 | 57.0 ± 0.3* |
| Day 22 | 57.8 ± 0.3 | 57.7 ± 0.2 | 57.8 ± 0.3 | 58.1 ± 0.4 | 58.0 ± 0.3 | 56.5 ± 0.2* |
| Week 14 | 51.4 ± 0.2 | 51.9 ± 0.3* | 51.4 ± 0.2 | 51.9 ± 0.4* | 52.3 ± 0.4** | 52.6 ± 0.2** |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 19.2 ± 0.1 | 19.3 ± 0.1 | 19.0 ± 0.2 | 19.0 ± 0.2 | 19.0 ± 0.1 | 18.9 ± 0.1 |
| Day 22 | 19.6 ± 0.2 | 19.5 ± 0.1 | 19.5 ± 0.1 | 19.6 ± 0.1 | 19.4 ± 0.1 | 19.0 ± 0.1* |
| Week 14 | 16.8 ± 0.1 | 16.9 ± 0.1 | 17.1 ± 0.2 | 16.9 ± 0.1 | 17.1 ± 0.2* | 17.1 ± 0.0* |
| Platelets ($10^3$/μL) | | | | | | |
| Day 4 | 997.7 ± 15.4 | 977.3 ± 21.5 | 916.1 ± 36.1 | 955.8 ± 43.8 | 1,035.9 ± 18.0 | 1,116.8 ± 48.9* |
| Day 22 | 910.0 ± 35.4 | 1,002.1 ± 15.0 | 870.6 ± 73.1 | 869.5 ± 72.2 | 847.1 ± 62.1 | 886.5 ± 38.2 |
| Week 14 | 729.4 ± 7.1 | 723.4 ± 17.7 | 679.6 ± 12.9* | 743.3 ± 14.5 | 702.2 ± 14.5 | 719.1 ± 17.8 |
| Clinical Chemistry | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 4 | 11.7 ± 0.5 | 13.0 ± 0.5 | 13.4 ± 0.4* | 14.1 ± 0.5** | 14.3 ± 0.4** | 14.5 ± 0.4** |
| Day 22 | 13.8 ± 0.3 | 13.6 ± 0.6 | 15.5 ± 1.2 | 13.0 ± 0.4 | 13.8 ± 0.6 | 13.6 ± 0.4 |
| Week 14 | 16.6 ± 0.4 | 14.0 ± 0.3* | 15.2 ± 0.6 | 16.0 ± 0.6 | 17.6 ± 0.6 | 17.2 ± 0.5 |
| Creatinine (mg/dL) | | | | | | |
| Day 4 | 0.43 ± 0.02 | 0.47 ± 0.02 | 0.47 ± 0.02 | 0.48 ± 0.01 | 0.47 ± 0.02 | 0.49 ± 0.01* |
| Day 22 | 0.47 ± 0.02 | 0.46 ± 0.02 | 0.50 ± 0.02 | 0.56 ± 0.04 | 0.50 ± 0.00 | 0.49 ± 0.01 |
| Week 14 | 0.58 ± 0.01 | 0.61 ± 0.04 | 0.55 ± 0.02 | 0.57 ± 0.02 | 0.57 ± 0.02 | 0.59 ± 0.02 |
| Total protein (g/dL) | | | | | | |
| Day 4 | 5.1 ± 0.1 | 5.1 ± 0.1 | 5.2 ± 0.1 | 5.3 ± 0.1* | 5.4 ± 0.1** | 5.4 ± 0.1** |
| Day 22 | 6.3 ± 0.1 | 6.2 ± 0.0 | 6.9 ± 0.2* | 6.4 ± 0.1 | 6.3 ± 0.1 | 6.3 ± 0.1 |
| Week 14 | 6.6 ± 0.1 | 6.6 ± 0.0 | 6.5 ± 0.0 | 6.7 ± 0.1 | 6.7 ± 0.1 | 6.7 ± 0.1 |
| Albumin (g/dL) | | | | | | |
| Day 4 | 3.8 ± 0.0 | 3.9 ± 0.1 | 3.9 ± 0.0 | 4.0 ± 0.1** | 4.1 ± 0.0** | 4.1 ± 0.1** |
| Day 22 | 4.5 ± 0.1 | 4.5 ± 0.0 | 5.0 ± 0.1** | 4.6 ± 0.0 | 4.5 ± 0.1 | 4.7 ± 0.0 |
| Week 14 | 4.6 ± 0.1 | 4.6 ± 0.0 | 4.6 ± 0.0 | 4.7 ± 0.0* | 4.8 ± 0.0* | 4.8 ± 0.0* |

**TABLE 4**
**Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Male** (continued) | | | | | | |
| Clinical Chemistry (continued) | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 4 | 79 ± 2 | 85 ± 2 | 87 ± 5 | 86 ± 3 | 94 ± 5** | 94 ± 4** |
| Day 22 | 59 ± 2 | 62 ± 1 | 51 ± 4 | 68 ± 2* | 75 ± 3** | 79 ± 2** |
| Week 14 | 95 ± 5 | 79 ± 4* | 76 ± 2** | 85 ± 7* | 78 ± 6* | 78 ± 7** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 878 ± 37 | 815 ± 17 | 832 ± 21 | 821 ± 15 | 749 ± 18** | 671 ± 13** |
| Day 22 | 583 ± 16 | 558 ± 13 | 543 ± 30 | 591 ± 13 | 579 ± 13 | 574 ± 11 |
| Week 14 | 220 ± 14 | 238 ± 9 | 250 ± 8 | 249 ± 7 | 281 ± 8** | 275 ± 9** |
| Bile salts (μmol/L) | | | | | | |
| Day 4 | 25.1 ± 2.3 | 26.8 ± 3.1 | 41.7 ± 2.6** | 30.5 ± 3.5* | 34.1 ± 2.5* | 56.3 ± 3.8** |
| Day 22 | 39.7 ± 4.4 | 33.9 ± 6.0 | 29.2 ± 3.8 | 29.3 ± 1.5 | 34.2 ± 3.1 | 53.7 ± 4.2 |
| Week 14 | 20.1 ± 1.1 | 17.6 ± 1.3 | 19.1 ± 1.9 | 17.2 ± 1.4 | 15.7 ± 0.7 | 21.8 ± 2.1 |
| **Female** | | | | | | |
| Hematology | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 22 | 9 | 8 | 7 | 8 | 9 | 8 |
| Week 14 | 9 | 10 | 10 | 9 | 9 | 10 |
| Hematocrit (%) | | | | | | |
| Day 4 | 37.1 ± 0.3 | 37.4 ± 0.5 | 37.6 ± 0.4 | 38.2 ± 0.4 | 39.2 ± 0.3** | 42.2 ± 0.7** |
| Day 22 | 43.1 ± 0.4 | 43.0 ± 0.4 | 44.3 ± 0.7 | 43.8 ± 0.5 | 43.1 ± 0.6 | 44.4 ± 0.4 |
| Week 14 | 45.0 ± 0.4 | 45.8 ± 0.4 | 46.1 ± 0.6 | 44.7 ± 0.5 | 45.7 ± 0.4 | 45.9 ± 0.5 |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 12.2 ± 0.1 | 12.4 ± 0.1 | 12.4 ± 0.1 | 12.6 ± 0.1 | 13.1 ± 0.1** | 14.1 ± 0.2** |
| Day 22 | 14.6 ± 0.1 | 14.6 ± 0.1 | 14.9 ± 0.2 | 14.7 ± 0.2 | 14.7 ± 0.2 | 14.9 ± 0.1 |
| Week 14 | 14.7 ± 0.1 | 14.9 ± 0.2 | 15.0 ± 0.1 | 14.6 ± 0.1 | 14.8 ± 0.1 | 15.0 ± 0.1 |
| Erythrocytes ($10^6$/μL) | | | | | | |
| Day 4 | 6.33 ± 0.07 | 6.34 ± 0.08 | 6.38 ± 0.07 | 6.50 ± 0.07 | 6.74 ± 0.07** | 7.32 ± 0.11** |
| Day 22 | 7.34 ± 0.08 | 7.31 ± 0.07 | 7.65 ± 0.15 | 7.53 ± 0.09 | 7.48 ± 0.09 | 7.77 ± 0.07** |
| Week 14 | 8.17 ± 0.07 | 8.32 ± 0.05 | 8.37 ± 0.10 | 8.11 ± 0.08 | 8.27 ± 0.07 | 8.34 ± 0.08 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 58.6 ± 0.5 | 59.0 ± 0.1 | 59.0 ± 0.3 | 58.7 ± 0.3 | 58.2 ± 0.3 | 57.6 ± 0.2* |
| Day 22 | 58.7 ± 0.2 | 58.8 ± 0.3 | 57.9 ± 0.3 | 58.3 ± 0.3 | 57.6 ± 0.3* | 57.1 ± 0.2** |
| Week 14 | 55.1 ± 0.2 | 55.1 ± 0.2 | 55.0 ± 0.1 | 55.1 ± 0.2 | 55.2 ± 0.2 | 55.1 ± 0.1 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 19.3 ± 0.1 | 19.5 ± 0.1 | 19.5 ± 0.1 | 19.4 ± 0.1 | 19.4 ± 0.1 | 19.3 ± 0.1 |
| Day 22 | 19.9 ± 0.1 | 20.0 ± 0.1 | 19.5 ± 0.2 | 19.6 ± 0.1 | 19.7 ± 0.1 | 19.2 ± 0.1** |
| Week 14 | 18.1 ± 0.1 | 17.9 ± 0.2 | 17.9 ± 0.1 | 18.0 ± 0.1 | 17.8 ± 0.1 | 18.0 ± 0.1 |

**TABLE 4**
**Selected Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Female** (continued) | | | | | | |
| Hematology (continued) | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 22 | 9 | 8 | 7 | 8 | 9 | 8 |
| Week 14 | 9 | 10 | 10 | 9 | 9 | 10 |
| Platelets ($10^3/\mu L$) | | | | | | |
| Day 4 | 925.3 ± 6.7 | 912.4 ± 19.1 | 950.3 ± 18.2 | 923.7 ± 20.1 | 972.2 ± 17.8 | 1,119.7 ± 27.2** |
| Day 22 | 869.6 ± 20.3 | 869.1 ± 16.1 | 806.0 ± 50.7 | 846.4 ± 28.8 | 907.3 ± 16.0 | 901.5 ± 23.7 |
| Week 14 | 709.7 ± 12.1 | 707.9 ± 14.8 | 725.3 ± 8.7 | 674.3 ± 10.2 | 688.2 ± 13.9 | 716.5 ± 12.7 |
| Clinical Chemistry | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 10 | 9 | 10 | 10 | 10 |
| Day 22 | 10 | 10 | 10 | 10 | 10 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 9 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 4 | 10.0 ± 0.3 | 11.1 ± 0.5 | 12.8 ± 0.7** | 11.9 ± 0.4** | 12.1 ± 0.4** | 12.4 ± 0.3** |
| Day 22 | 13.5 ± 0.6 | 15.9 ± 1.1 | 15.3 ± 0.7 | 14.1 ± 0.3 | 14.9 ± 0.8 | 15.0 ± 0.6 |
| Week 14 | 16.5 ± 0.3 | 17.1 ± 0.6 | 18.0 ± 0.5 | 14.7 ± 0.6 | 16.7 ± 0.5 | 17.9 ± 0.9 |
| Creatinine (mg/dL) | | | | | | |
| Day 4 | 0.40 ± 0.00 | 0.40 ± 0.00 | 0.42 ± 0.01[b] | 0.41 ± 0.01 | 0.40 ± 0.00 | 0.41 ± 0.01 |
| Day 22 | 0.49 ± 0.01 | 0.49 ± 0.02 | 0.45 ± 0.02 | 0.47 ± 0.02 | 0.47 ± 0.02 | 0.51 ± 0.01 |
| Week 14 | 0.59 ± 0.01 | 0.62 ± 0.01 | 0.59 ± 0.01 | 0.59 ± 0.01 | 0.57 ± 0.02 | 0.59 ± 0.01 |
| Total protein (g/dL) | | | | | | |
| Day 4 | 5.3 ± 0.1 | 5.4 ± 0.1 | 5.4 ± 0.1 | 5.3 ± 0.1 | 5.3 ± 0.1 | 5.1 ± 0.1 |
| Day 22 | 5.9 ± 0.1 | 6.0 ± 0.0 | 6.1 ± 0.1 | 5.9 ± 0.1 | 6.0 ± 0.1 | 5.9 ± 0.1 |
| Week 14 | 6.4 ± 0.1 | 7.1 ± 0.1 | 7.0 ± 0.1 | 6.3 ± 0.1 | 6.2 ± 0.1 | 6.3 ± 0.1 |
| Albumin (g/dL) | | | | | | |
| Day 4 | 4.0 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.1 | 4.1 ± 0.0 | 4.0 ± 0.1 |
| Day 22 | 4.5 ± 0.0 | 4.5 ± 0.0 | 4.6 ± 0.0 | 4.5 ± 0.1 | 4.5 ± 0.0 | 4.5 ± 0.1 |
| Week 14 | 4.8 ± 0.0 | 5.2 ± 0.1 | 5.1 ± 0.1 | 4.7 ± 0.1 | 4.5 ± 0.1 | 4.6 ± 0.0 |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 4 | 64 ± 1 | 71 ± 2** | 71 ± 1** | 77 ± 3** | 80 ± 3** | 85 ± 4** |
| Day 22 | 47 ± 2 | 53 ± 3 | 55 ± 4 | 51 ± 2 | 55 ± 2* | 63 ± 3** |
| Week 14 | 86 ± 6 | 69 ± 5 | 69 ± 3 | 72 ± 2 | 72 ± 2 | 70 ± 3 |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 688 ± 9 | 702 ± 20 | 683 ± 12 | 689 ± 12 | 658 ± 13 | 573 ± 9** |
| Day 22 | 454 ± 5 | 463 ± 8 | 448 ± 8 | 446 ± 6 | 466 ± 8 | 466 ± 11 |
| Week 14 | 230 ± 4 | 215 ± 7 | 232 ± 4 | 244 ± 7 | 251 ± 6* | 240 ± 4 |
| Bile salts (μmol/L) | | | | | | |
| Day 4 | 32.8 ± 3.2 | 32.2 ± 4.3 | 26.8 ± 3.9 | 29.7 ± 1.4 | 37.0 ± 2.2 | 51.5 ± 4.1** |
| Day 22 | 28.9 ± 2.7 | 23.3 ± 2.6 | 22.3 ± 2.6 | 33.7 ± 2.7 | 35.5 ± 2.6 | 48.4 ± 3.6** |
| Week 14 | 28.8 ± 1.7 | 25.9 ± 2.6 | 27.4 ± 1.8 | 24.3 ± 1.8 | 25.3 ± 2.9 | 36.2 ± 3.1 |

* 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=10

Sporadic differences occurred in other clinical chemistry parameters at varying time points; these differences, which generally did not demonstrate a treatment relationship and/or were inconsistent between males and females, were not considered to be toxicologically relevant.

Organ weight differences in exposed rats reflected decreases in body weights (Table C2). The absolute left cauda epididymis, epididymis, and testis weights, number of spermatid heads per testis, and epididymal sperm motility of males in the 12,500 ppm group were significantly less than those of the controls (Table D1). The numbers of cycling females and females with regular estrous cycles were decreased in the 6,250 and 12,500 ppm groups (Table D2). Exposed groups of females had significantly fewer estrous cycles than did the controls. Estrous cycle length increased with increasing exposure concentration; females in the 6,250 and 12,500 ppm groups had significantly longer cycles and spent more time in diestrus and less time in proestrus, estrus, and metestrus than did the controls.

There were no exposure-related gross lesions. The incidences of hyperkeratosis of the forestomach epithelium were significantly increased in males and females in all exposed groups (Tables 5, A1, and A2). Forestomach epithelial hyperplasia also occurred in males exposed to 1,562 ppm or greater and females exposed to 3,125 ppm or greater, and the incidences in these groups were significantly increased except for the 1,562 ppm group. The severity of the forestomach lesions generally increased with increasing exposure concentration. Morphologically, forestomach hyperplasia consisted of thickening of the epithelium due to increased numbers of cell layers. Hyperkeratosis was a thickening of the keratin layer overlying the epithelium (Plates 1 and 2). All 6,250 and 12,500 ppm males had minimal cytoplasmic alteration in the liver. Cytoplasmic alteration of the liver was a tinctorial change characterized by pallor of centrilobular hepatocytes.

**TABLE 5**
**Incidences of Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| Forestomach[a] | 10 | 10 | 10 | 10 | 10 | 10 |
| Epithelium, Hyperkeratosis[b] | 0 | 4* (1.0)[c] | 8** (2.0) | 9** (1.8) | 10** (2.2) | 10** (3.1) |
| Epithelium, Hyperplasia | 0 | 0 | 2 (1.0) | 8** (1.8) | 8** (2.0) | 10** (2.6) |
| Liver | 10 | 10 | 10 | 10 | 10 | 10 |
| Cytoplasmic Alteration | 0 | 0 | 0 | 0 | 10** (1.0) | 10** (1.0) |
| **Female** | | | | | | |
| Forestomach | 10 | 10 | 10 | 10 | 10 | 10 |
| Epithelium, Hyperkeratosis | 0 | 6** (1.0) | 7** (1.4) | 9** (1.6) | 10** (2.0) | 10** (2.2) |
| Epithelium, Hyperplasia | 0 | 0 | 0 | 5* (1.6) | 8** (1.8) | 10** (2.2) |

* 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 grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

# MICE

## 15-DAY STUDY

All 50,000 ppm mice were killed moribund by day 7 (Table 6). Final mean body weights and body weight gains of males exposed to 12,500 or 25,000 ppm and females exposed to 25,000 ppm were significantly less than those of the controls; these groups of mice lost weight during the study (Table 6). Feed consumption by exposed groups was similar to that by the controls (Table 6); feed spillage was noted for all groups. Exposure concentrations of 3,125, 6,250, 12,500, and 25,000 ppm resulted in average daily doses of approximately 690, 1,640, 3,480, and 8,200 mg/kg to males and 590, 1,345, 3,010, and 7,860 mg/kg to females; no average daily doses were calculated for mice in the 50,000 ppm groups. Mice in the 50,000 ppm groups exhibited abnormal posture, lethargy, thinness, and ruffled fur; males in this group also had abnormal breathing, and one male had ataxia. Males and females exposed to 25,000 ppm were also thin, and females in this group exhibited abnormal posture and lethargy.

**TABLE 6**
**Survival, Body Weights, and Feed Consumption of Mice in the 15-Day Feed Study of *p-tert*-Butylcatechol**

| Concentration (ppm) | Survival[a] | Mean Body Weight[b] (g) | | | Final Weight Relative to Controls (%) | Feed Consumption[c] | |
|---|---|---|---|---|---|---|---|
| | | Initial | Final | Change | | Week 1 | Week 2 |
| **Male** | | | | | | | |
| 0 | 5/5 | 21.5 ± 1.0 | 23.4 ± 1.1 | 2.0 ± 0.2 | | 5.5 | 5.7 |
| 3,125 | 5/5 | 21.2 ± 0.5 | 23.3 ± 0.4 | 2.0 ± 0.3 | 99 | 5.1 | 4.9 |
| 6,250 | 5/5 | 20.9 ± 0.3 | 22.9 ± 0.5 | 2.0 ± 0.4 | 98 | 4.1 | 7.7 |
| 12,500 | 5/5 | 21.0 ± 0.3 | 20.4 ± 0.6** | –0.6 ± 0.7** | 87 | 4.3 | 7.1 |
| 25,000 | 5/5 | 21.6 ± 0.8 | 16.6 ± 0.5** | –5.0 ± 0.5** | 71 | 4.8 | 6.0 |
| 50,000 | 0/5[d] | 21.4 ± 0.6 | — | — | — | — | — |
| **Female** | | | | | | | |
| 0 | 5/5 | 18.1 ± 0.5 | 20.6 ± 0.3 | 2.5 ± 0.3 | | 3.3 | 3.8 |
| 3,125 | 5/5 | 17.0 ± 0.6 | 20.1 ± 0.6 | 3.1 ± 0.3 | 98 | 3.7 | 3.8 |
| 6,250 | 5/5 | 18.0 ± 0.6 | 20.1 ± 0.6 | 2.1 ± 0.2 | 98 | 3.8 | 4.7 |
| 12,500 | 5/5 | 16.5 ± 0.5 | 18.9 ± 0.6 | 2.4 ± 0.2 | 92 | 4.2 | 4.7 |
| 25,000 | 5/5 | 18.5 ± 0.6 | 14.1 ± 0.6** | –4.4 ± 0.2** | 69 | 4.1 | 5.0 |
| 50,000 | 0/5[d] | 17.7 ± 0.2 | — | — | — | — | — |

** Significantly different (P≤0.01) from the control group by Williams' test
[a] Number of animals surviving at 15 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. No final mean body weights or weight changes were calculated for groups with 100% mortality.
[c] Feed consumption is expressed as grams per animal per day.
[d] Day of death: 4, 5, 6, 6, 7

The absolute and relative thymus weights of 25,000 ppm male and female mice were significantly less than those of the controls (Table C3). Other organ weight changes reflected the decreased body weights of exposed mice.

At necropsy, all mice in the 25,000 and 50,000 ppm groups were noted to have thin carcasses. No exposure-related lesions were observed microscopically in the groups examined (0, 25,000, and 50,000 ppm). Based on the body weight effects in the 25,000 ppm groups and mortality in the 50,000 ppm groups, the highest exposure concentration selected for the 14-week study was 12,500 ppm.

## 14-Week Study

All mice survived to the end of the study (Table 7). Final mean body weights and body weight gains of 12,500 ppm males and 6,250 and 12,500 ppm females, as well as the mean body weight gains of 3,125 and 6,250 ppm males, were significantly less than those of the controls (Table 7 and Figure 2). Feed consumption by exposed groups was similar to that by the controls (Table 7). Exposure concentrations of 781, 1,562, 3,125, 6,250, and 12,500 ppm resulted in average daily doses of approximately 150, 300, 635, 1,300, and 2,815 mg/kg to males and 135, 300, 610, 1,400, and 2,440 mg/kg to females. There were no clinical findings of toxicity.

No alterations in hematology data for mice were attributed to exposure to *p-tert*-butylcatechol (Table B2). Organ weight differences in exposed mice reflected decreases in body weights (Table C4).

**Table 7**
**Survival, Body Weights, and Feed Consumption of Mice in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| Concentration (ppm) | Survival[a] | Mean Body Weight[b] (g) | | | Final Weight Relative to Controls (%) | Feed Consumption[c] | |
|---|---|---|---|---|---|---|---|
| | | Initial | Final | Change | | Week 1 | Week 14 |
| **Male** | | | | | | | |
| 0 | 10/10 | 21.3 ± 0.3 | 33.0 ± 0.5 | 11.7 ± 0.4 | | 3.7 | 5.8 |
| 781 | 10/10 | 21.3 ± 0.4 | 32.2 ± 0.8 | 10.9 ± 0.6 | 98 | 5.1 | 6.0 |
| 1,562 | 10/10 | 21.8 ± 0.5 | 33.7 ± 0.9 | 11.9 ± 0.6 | 102 | 4.1 | 5.6 |
| 3,125 | 10/10 | 21.4 ± 0.3 | 30.6 ± 1.1 | 9.1 ± 1.1** | 93 | 3.6 | 6.1 |
| 6,250 | 10/10 | 22.1 ± 0.4 | 31.1 ± 0.6 | 9.0 ± 0.3** | 94 | 4.2 | 6.1 |
| 12,500 | 10/10 | 21.3 ± 0.4 | 26.6 ± 0.5** | 5.3 ± 0.3** | 81 | 4.2 | 7.7 |
| **Female** | | | | | | | |
| 0 | 10/10 | 17.2 ± 0.4 | 25.3 ± 0.7 | 8.1 ± 0.4 | | 2.0 | 5.4 |
| 781 | 10/10 | 17.7 ± 0.2 | 27.3 ± 0.5 | 9.6 ± 0.5 | 108 | 2.9 | 4.9 |
| 1,562 | 10/10 | 17.0 ± 0.3 | 25.9 ± 0.5 | 8.8 ± 0.4 | 102 | 2.8 | 6.4 |
| 3,125 | 10/10 | 17.2 ± 0.3 | 24.2 ± 0.4 | 7.0 ± 0.3 | 96 | 2.8 | 5.0 |
| 6,250 | 10/10 | 17.3 ± 0.3 | 23.6 ± 0.4* | 6.4 ± 0.3** | 94 | 3.0 | 6.3 |
| 12,500 | 10/10 | 17.7 ± 0.4 | 19.7 ± 0.2** | 2.0 ± 0.3** | 78 | 2.0 | 5.6 |

* Significantly different (P≤0.05) from the control group by Williams' test
** P≤0.01
[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error
[c] Feed consumption is expressed as grams per animal per day.

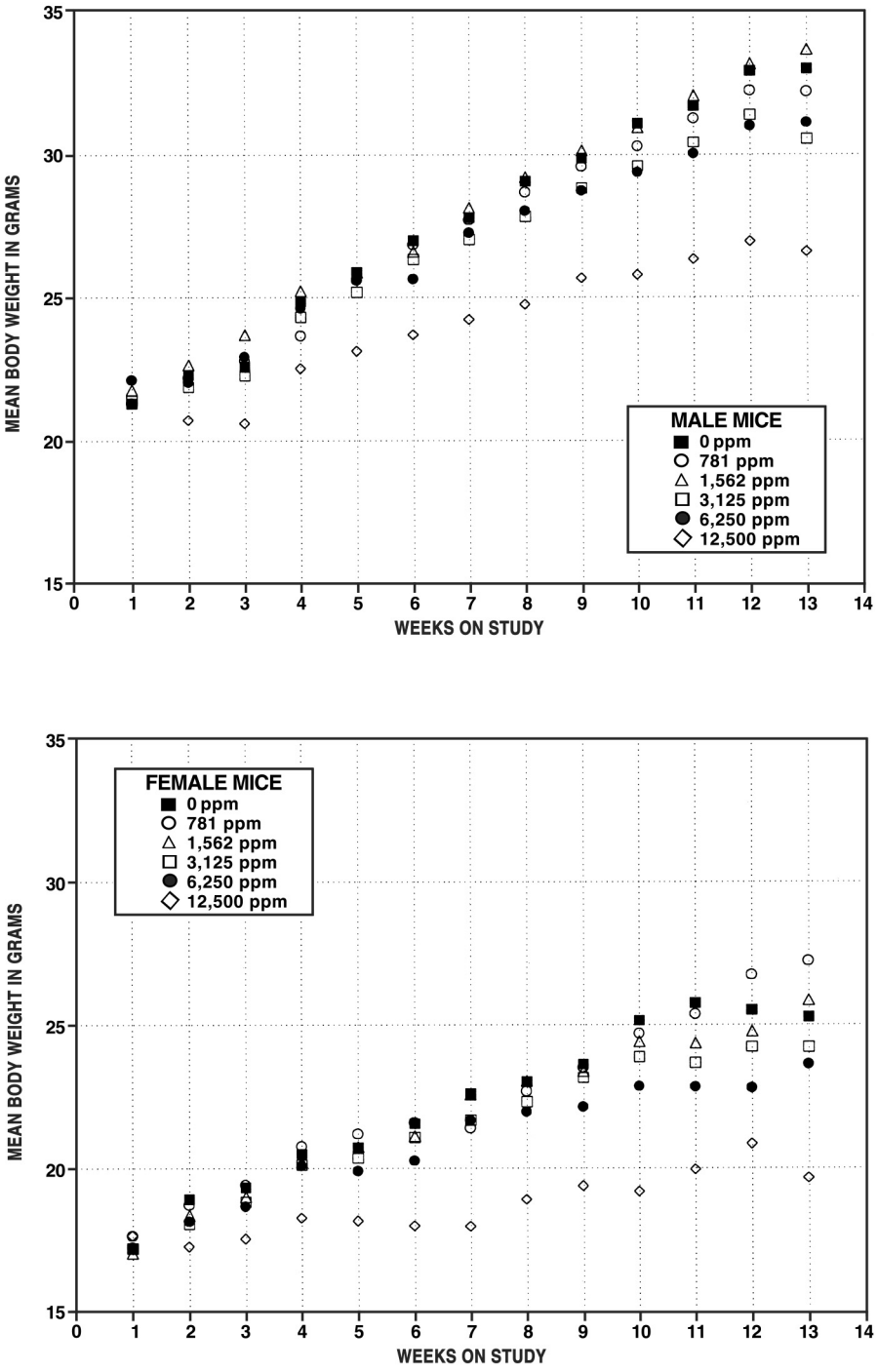



**FIGURE 2**
**Body Weights of Male and Female Mice Exposed to *p-tert*-Butylcatechol in Feed for 14 Weeks**

There were no biologically significant differences in reproductive tissue parameters between exposed and control male mice (Table D3). Females in the 12,500 ppm group had a significantly longer estrous cycle than did the controls (Table D4).

There were no exposure-related gross lesions. Forestomach epithelial hyperplasia occurred in males and females exposed to 3,125 ppm or greater, and the incidences in 12,500 ppm males and females and females in the 6,250 ppm group were significantly greater than those in the controls (Tables 8, A3, and A4). The incidence of hyperkeratosis of the forestomach epithelium was significantly increased in 12,500 ppm females; hyperkeratosis was also observed in males in the 12,500 ppm group and females in the 6,250 ppm group. The severity of the forestomach lesions was minimal to mild. Histologically, forestomach hyperplasia and hyperkeratosis in mice was seen as an increased thickness of the epithelium, similar to the lesions in rats (Plates 3 and 4).

**TABLE 8**
**Incidences of Selected Nonneoplastic Lesions in Mice in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| Forestomach[a] | 10 | 10 | 10 | 10 | 10 | 10 |
| Epithelium, Hyperplasia[b] | 0 | 0 | 0 | 3 (1.0)[c] | 3 (1.0) | 6** (1.3) |
| Epithelium, Hyperkeratosis | 0 | 0 | 0 | 0 | 0 | 3 (1.3) |
| **Female** | | | | | | |
| Forestomach | 10 | 10 | 9 | 10 | 10 | 10 |
| Epithelium, Hyperplasia | 0 | 0 | 0 | 2 (1.0) | 4* (1.3) | 7** (1.7) |
| Epithelium, Hyperkeratosis | 0 | 0 | 0 | 0 | 2 (1.5) | 6** (1.3) |

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

## ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION STUDIES

The absorption, distribution, metabolism, and excretion of *p-tert*-butylcatechol following intravenous injection, gavage dosing, or dermal application were determined in male F344/N rats and $B6C3F_1$ mice (Appendix G). The absorption of [$^{14}$C]-*p-tert*-butylcatechol following gavage dosing or dermal application was high. The percent absorption following dermal application increased with increasing dose. Peak concentrations of [$^{14}$C]-*p-tert*-butylcatechol equivalents in plasma were reached 1 hour after gavage dosing (200 mg/kg) and 2 hours after dermal application (60 mg/kg); no parent compound was detected in the plasma extracts. Regardless of route of administration, *p-tert*-butylcatechol-derived radioactivity was readily excreted in the urine and was markedly nonpersistent in the tissues. *p-tert*-Butylcatechol was excreted as *p-tert*-butylcatechol sulfate and other polar metabolites that included predominately sulfate conjugates; it was not excreted as the parent compound. One metabolite was determined to be an *O*-methyl-*O′*-sulfate of *p-tert*-butylcatechol.

## GENETIC TOXICOLOGY

*p-tert*-Butylcatechol (10 to 1,000 µg/plate) was tested for mutagenicity in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, and TA1535, with and without rat and hamster liver S9 metabolic activation enzymes; no mutagenicity was detected in any strain under any of the test conditions (Table E1). *p-tert*-Butylcatechol induced micronuclei in bone marrow cells of rats in a trial with doses of 125 and 250 mg/kg (the highest dose, 500 mg/kg, was lethal) administered by intraperitoneal injection; however, results of a second trial with doses of 125 to 300 mg/kg were negative (Table E2). Due to the lack of reproducibility of the initial response, the overall results of this test were judged to be negative. *p-tert*-Butylcatechol administered in feed at concentrations up to 12,500 ppm for 14 weeks did not increase the frequency of micronucleated normochromatic erythrocytes in the peripheral blood of male or female mice (Table E3). In addition, no significant alteration in the percentage of polychromatic erythrocytes was seen in dosed mice, indicating a lack of bone marrow toxicity.

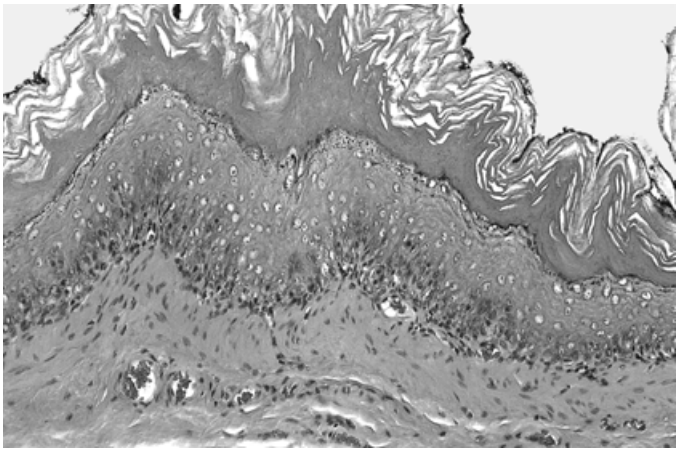


**PLATE 1**
Forestomach of a male F344/N rat administered 12,500 ppm *p-tert*-butylcatechol in feed for 14 weeks. The epithelium is moderately thickened due to increased numbers of cellular layers (hyperplasia) and an increased amount of superficial keratin (hyperkeratosis). Compare to normal rat forestomach at the same magnification in Plate 2.

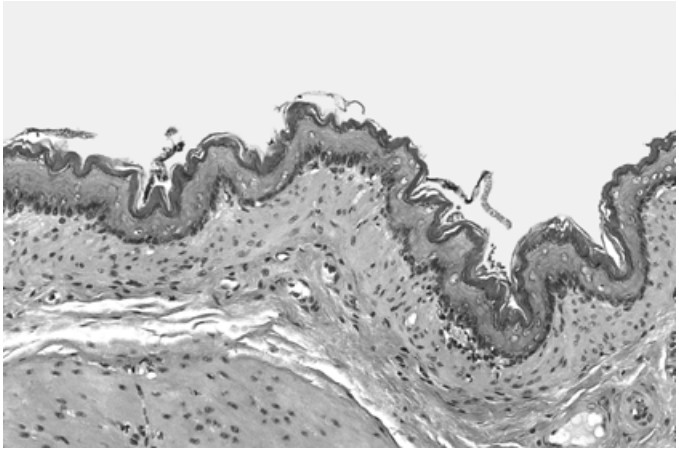


**PLATE 2**
Forestomach of a male F344/N control rat.

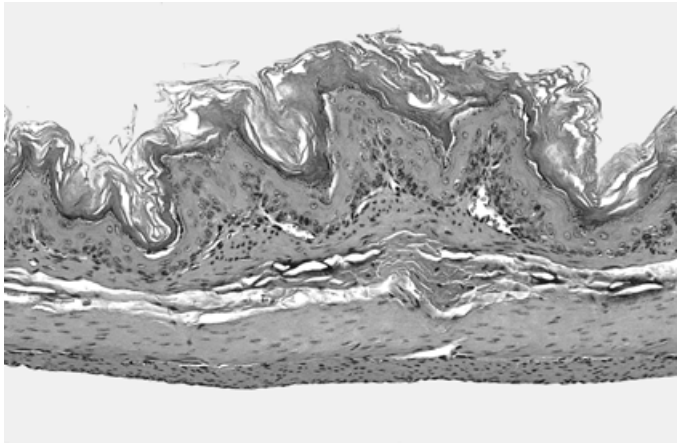


**PLATE 3**
Forestomach of a female $B6C3F_1$ mouse administered 12,500 ppm *p-tert*-butylcatechol in feed for 14 weeks. There is mild hyperplasia and hyperkeratosis of the epithelium similar to that seen in the rat. Compare to normal mouse forestomach at the same magnification in Plate 4.

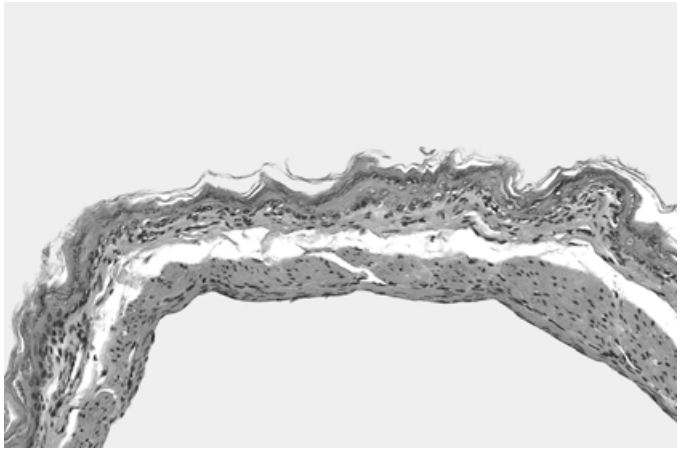
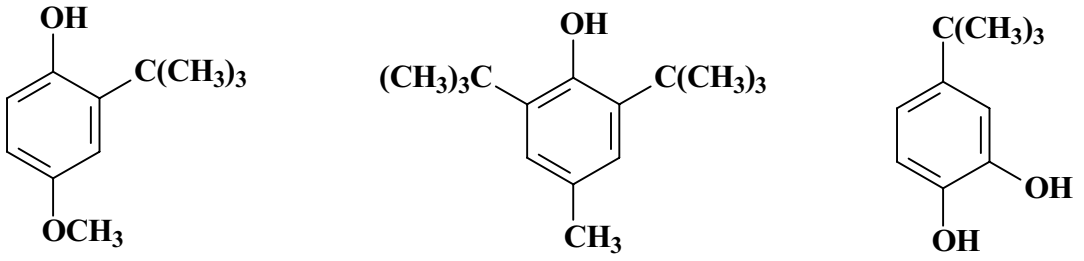


**PLATE 4**
Forestomach of a female $B6C3F_1$ control mouse.

# DISCUSSION

The catechol compounds have been used as antioxidants in food. *p-tert*-Butylcatechol is used as an antioxidant, stabilizer, and polymerization inhibitor for styrene, butadiene, neoprene, and other olefins and reactive monomers. *p-tert*-Butylcatechol was nominated for study, in part, because the U.S. Food and Drug Administration (FDA) was considering its use as a replacement antioxidant for the catechols butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in foods (Figure 3).




Butylated Hydroxyanisole | Butylated Hydroxytoluene | *p-tert*-Butylcatechol

**Figure 3**
**Structures of Butylated Hydroxyanisole, Butylated Hydroxytoluene, and *p-tert*-Butylcatechol**

The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives established acceptable daily intake guidelines for antioxidants (up to 0.5 mg/kg body weight for BHA; up to 0.125 mg/kg for BHT). Maximum BHA and BHT usage levels permitted by the FDA for a variety of foods, such as breakfast cereals, potato shreds, and poultry products, range from 50 to 1,000 ppm (Shahidi and Wanasundara, 1992).

In a 2-year study in which F344 rats were exposed to BHA in feed at concentrations of 0.125%, 0.25%, 0.5%, 1%, or 2% (1,250 to 20,000 ppm, estimated daily dose 55 to 1,322 mg/kg), proliferative lesions of the forestomach were observed in groups exposed to 0.25% or greater (Ito *et al.*, 1986). Forestomach papillomas occurred in the 1% and

2% groups, and squamous cell carcinoma of the forestomach occurred in the 2% group. $B6C3F_1$ mice exposed to 0.5% or 1% BHA for 2 years had hyperplasia of the forestomach but no significantly increased incidences of forestomach papilloma or carcinoma; forestomach papilloma did occur in one 1% mouse (Ito *et al.*, 1986). BHA had no toxicity in the glandular stomach.

Catechol has also been found to induce forestomach papillomas and glandular stomach adenocarcinomas (Tanaka *et al.*, 1995). BHT administered in feed for 2 years was not carcinogenic in Wistar rats (Hirose *et al.*, 1981) or $B6C3F_1$ mice (Shirai *et al.*, 1982). The relevance of the forestomach tumors in the BHA rodent studies to humans has not been established. The FDA continues to maintain BHA and BHT on the Generally Recognized As Safe list of chemicals (21 CFR 182.3169, 182.3173).

The current studies were performed to determine the toxicity of *p-tert*-butylcatechol in F344/N rats and $B6C3F_1$ mice. The chemical was administered in feed to compare its toxicity to that of similar antioxidants, for which the primary target organ of toxicity after administration in feed is the rodent forestomach. Absorption, distribution, metabolism, and excretion studies determined that *p-tert*-butylcatechol was readily absorbed when administered orally or dermally. *p-tert*-Butylcatechol is not expected to bioaccumulate; only approximately 0.2% of the administered dose remained in the tissues 3 days after dosing. Metabolism led to conjugation and methylation products as would be expected for a catechol derivative. There was no evidence in the metabolites for the formation of reactive intermediates.

In the 15-day studies, rats and mice received *p-tert*-butylcatechol in the feed at concentrations of 3,125, 6,250, 12,500, 25,000, or 50,000 ppm. All 50,000 ppm rats and mice died before the end of the studies. The final mean body weights of 25,000 ppm rats and mice were 29% to 38% less than those of the controls. No exposure-related microscopic lesions were observed in the rat or mouse groups examined (0, 25,000, and 50,000 ppm). The decreased body weights or mortalities in the 25,000 and 50,000 ppm groups were the primary factors in selecting exposure concentrations for the 14-week feed studies.

In the 14-week studies, rats and mice received 0, 781, 1,562, 3,125, 6,250, or 12,500 ppm *p-tert*-butylcatechol in feed. The forestomach was examined histologically at all exposure concentrations. Hyperkeratosis of the forestomach occurred in all exposed groups of rats, and rats exposed to 3,125 ppm or greater also had significantly increased incidences of forestomach hyperplasia. In mice, forestomach hyperkeratosis occurred in the 12,500 ppm males and females and in 6,250 ppm females, and forestomach hyperplasia occurred in groups exposed to 3,125 ppm or greater. The forestomach lesions were more severe in rats than in mice.

Forestomach hyperplasia and hyperkeratosis are common and nonspecific treatment-related lesions in rodents. A basic distinction is whether the response is regenerative (reparative) following epithelial damage or mitogenic. The absence of associated ulceration and inflammation would suggest a primary proliferative effect as opposed to an irritant effect. This would be consistent with the known hyperplasiogenic effect of analogs such as BHA and BHT (Ito *et al.*, 1986). The hyperplasia in rats and mice exposed to *p-tert*-butylcatechol had no atypical histologic features that were suggestive of a preneoplastic lesion.

In the 14-week studies, complete histopathologic examinations were conducted only on the 0 and 12,500 ppm groups; the only other exposure-related lesion observed was cytoplasmic alteration in the liver of male rats in the 12,500 ppm group. The livers of male rats in the lower exposure groups were also examined; the no-observed-adverse-effect level for cytoplasmic alteration was 3,125 ppm.

In rats in the 14-week study, hematocrit values, hemoglobin concentrations, and bile salt concentrations were generally elevated in the 6,250 and 12,500 ppm groups on day 4 and in the 12,500 ppm groups on day 22 but were not affected at week 14. In mice, there were no treatment-related changes in hematology parameters.

Sperm motility and vaginal cytology evaluations were conducted in rats and mice in the 0, 3,125, 6,250, and 12,500 ppm groups at the end of the 14-week studies. In 12,500 ppm male rats, absolute left cauda epididymis, epididymis, and testis weights were decreased by 15%, 10%, and 9%, respectively, compared to the controls. The epididymal sperm motility and number of spermatid heads per testis were also decreased by 7% and 17%, respectively. Although necropsy body weights were decreased in the exposed groups (7%, 13%, and 23%), these decreases were no greater than those in Sprague-Dawley rats in feed restriction studies in which reproductive tissue indices were largely unchanged (Chapin *et al.*, 1993a). These results suggest that *p-tert*-butylcatechol may have a mild effect on sperm parameters in the male rat. *p-tert*-Butylcatechol had no adverse effects on sperm motility of mice in the 14-week study.

In the 14-week study, female rats exposed to 6,250 or 12,500 ppm spent more time in diestrus and less time in proestrus, estrus, and metestrus than did the control females; the cycle lengths of these two groups were increased 31% and 47%, respectively, relative to the controls. The numbers of estrous cycles were decreased by 28%, 44%, and 44% in the 3,125, 6,250, and 12,500 ppm groups, respectively, and the numbers of cycling females were decreased by 50% in the 6,250 ppm group and 60% in the 12,500 ppm group. Decreases in body weights of the exposed groups (5%, 8%, and 12%) relative to the controls may explain some of these differences in reproductive parameters; however, the changes are greater than those which would be expected from decreased body weights alone

(Chapin *et al.*, 1993a,b). In mice, the estrous cycle length of the 12,500 ppm females was significantly longer than that of the controls.

BHA and BHT are known to modify the effects of mammary gland tumors induced by *N*-nitrosomethylurea (NMU) or 7,12-dimethylbenz(*a*)anthracene (DMBA). Results of recent studies suggest that BHA may decrease the hormonal or carcinogenic actions of other chemicals, including DMBA and NMU, by increasing the activities of liver microsomal enzymes that catalyze uridine 5′-diphosphoglucuronic acid-dependent glucuronidation and NADPH-dependent oxidation of estradiol and estrone (Zhu *et al.*, 1997). It is not known if *p-tert*-butylcatechol affects estrogen levels in the female F344 rat; however, such an action would be one explanation for the effects of *p-tert*-butylcatechol on the estrous cycle of rats in the current study.

At the end of the 14-week study, the blood of mice was examined for evidence of an exposure-related increase in the frequency of micronucleated erythrocytes; none was found. *p-tert*-Butylcatechol also showed no evidence of genotoxicity in tests with *Salmonella typhimurium*. BHA, BHT, and other antioxidants used in food also are not genotoxic in the *S. typhimurium* assay (Mortelmans *et al.*, 1986; Zeiger *et al.*, 1992).

In conclusion, *p-tert*-butylcatechol is rapidly absorbed after oral exposure and is excreted in urine as *p-tert*-butylcatechol sulfate and other polar metabolites including sulfate conjugates. It caused forestomach lesions in rats and mice including hyperkeratosis of the forestomach in rats exposed to 781 ppm or greater and in 6,250 (females) and 12,500 ppm mice and forestomach hyperplasia in rats and mice exposed to 3,125 ppm or greater. Forestomach lesions were more severe in rats than in mice.

In previous 2-year feed studies of BHA (Ito *et al.*, 1986), forestomach hyperplasia was observed in F344 rats at exposure concentrations of 2,500 ppm or greater; forestomach hyperplasia at 2,500 ppm or greater; forestomach papilloma at 10,000 and 20,000 ppm; and squamous cell carcinoma of the forestomach at 20,000 ppm. B6C3$F_1$ mice exposed to 5,000 or 10,000 ppm had hyperplasia of the forestomach but no significantly increased incidences of forestomach papilloma or carcinoma. In the present studies, *p-tert*-butylcatechol caused forestomach hyperplasia in rats and mice at lower exposure concentrations (rats, 1,562 ppm; mice, 3,125 ppm) than did BHA (Ito *et al.*, 1986).

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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 *p-tert*-Butylcatechol[a]**

| | 0 ppm | | 781 ppm | | 1,562 ppm | | 3,125 ppm | | 6,250 ppm | | 12,500 ppm | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Disposition Summary** | | | | | | | | | | | | |
| Animals initially in study | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| Survivors | | | | | | | | | | | | |
| Terminal sacrifice | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| Animals examined microscopically | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **Alimentary System** | | | | | | | | | | | | |
| Intestine large, rectum | (10) | | | | | | | | | | (10) | |
| Parasite metazoan | 2 | (20%) | | | | | | | | | | |
| Liver | (10) | | (10) | | (10) | | (10) | | (10) | | (10) | |
| Basophilic focus | 1 | (10%) | | | | | | | | | | |
| Cytoplasmic alteration | | | | | | | | | 10 | (100%)` | 10 | (100%) |
| Hepatodiaphragmatic nodule | 1 | (10%) | 1 | (10%) | 1 | (10%) | 2 | (20%) | 2 | (20%) | | |
| Inflammation, chronic active | 5 | (50%) | 5 | (50%) | 3 | (30%) | 4 | (40%) | 3 | (30%) | 5 | (50%) |
| Stomach, forestomach | (10) | | (10) | | (10) | | (10) | | (10) | | (10) | |
| Epithelium, hyperkeratosis | | | 4 | (40%) | 8 | (80%) | 9 | (90%) | 10 | (100%) | 10 | (100%) |
| Epithelium, hyperplasia | | | | | 2 | (20%) | 8 | (80%) | 8 | (80%) | 10 | (100%) |
| **Cardiovascular System** | | | | | | | | | | | | |
| Heart | (10) | | | | | | | | | | (10) | |
| Cardiomyopathy | 7 | (70%) | | | | | | | | | 5 | (50%) |
| **Endocrine System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **General Body System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Genital System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Hematopoietic System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Integumentary System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Musculoskeletal System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Nervous System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |

**TABLE A1**
**Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Respiratory System** | | | | | | |
| Lung | (10) | | | | | (10) |
| Inflammation, chronic active | | | | | | 4 (40%) |
| Alveolar epithelium, hyperplasia | 1 (10%) | | | | | |
| **Special Senses System** | | | | | | |
| None | | | | | | |
| **Urinary System** | | | | | | |
| Kidney | (10) | | | | | (10) |
| Nephropathy | 7 (70%) | | | | | 4 (40%) |

[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 *p-tert*-Butylcatechol[a]**

| | 0 ppm | | 781 ppm | | 1,562 ppm | | 3,125 ppm | | 6,250 ppm | | 12,500 ppm | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Disposition Summary** | | | | | | | | | | | | |
| Animals initially in study | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| Survivors | | | | | | | | | | | | |
| Terminal sacrifice | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| Animals examined microscopically | 10 | | 10 | | 10 | | 10 | | 10 | | 10 | |
| **Alimentary System** | | | | | | | | | | | | |
| Liver | (10) | | (5) | | (1) | | (1) | | (2) | | (10) | |
| Hepatodiaphragmatic nodule | 3 | (30%) | 5 | (100%) | 1 | (100%) | 1 | (100%) | 2 | (100%) | | |
| Inflammation, chronic active | 10 | (100%) | | | | | | | | | 4 | (40%) |
| Stomach, forestomach | (10) | | (10) | | (10) | | (10) | | (10) | | (10) | |
| Epithelium, hyperkeratosis | | | 6 | (60%) | 7 | (70%) | 9 | (90%) | 10 | (100%) | 10 | (100%) |
| Epithelium, hyperplasia | | | | | | | 5 | (50%) | 8 | (80%) | 10 | (100%) |
| **Cardiovascular System** | | | | | | | | | | | | |
| Heart | (10) | | | | | | | | | | (10) | |
| Cardiomyopathy | 1 | (10%) | | | | | | | | | 3 | (30%) |
| **Endocrine System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **General Body System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Genital System** | | | | | | | | | | | | |
| Clitoral gland | (10) | | | | | | | | | | (10) | |
| Cyst | 1 | (10%) | | | | | | | | | | |
| Ovary | (10) | | | | | | | | | | (10) | |
| Cyst | | | | | | | | | | | 1 | (10%) |
| **Hematopoietic System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Integumentary System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Musculoskeletal System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Nervous System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |

**TABLE A2**
**Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | | 781 ppm | | 1,562 ppm | | 3,125 ppm | | 6,250 ppm | | 12,500 ppm | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Respiratory System** | | | | | | | | | | | | |
| Lung | (10) | | | | | | | | | | (10) | |
| Inflammation, chronic active | | | | | | | | | | | 2 | (20%) |
| Alveolar epithelium, inflammation, chronic active | 1 | (10%) | | | | | | | | | | |
| **Special Senses System** | | | | | | | | | | | | |
| None | | | | | | | | | | | | |
| **Urinary System** | | | | | | | | | | | | |
| Kidney | (10) | | | | | | | | | | (10) | |
| Mineralization | 3 | (30%) | | | | | | | | | | |
| Nephropathy | 1 | (10%) | | | | | | | | | 4 | (40%) |

[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 *p-tert*-Butylcatechol[a]**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Disposition Summary** | | | | | | |
| Animals initially in study | 10 | 10 | 10 | 10 | 10 | 10 |
| Survivors | | | | | | |
| Terminal sacrifice | 10 | 10 | 10 | 10 | 10 | 10 |
| Animals examined microscopically | 10 | 10 | 10 | 10 | 10 | 10 |
| **Alimentary System** | | | | | | |
| Liver | (10) | | | | | (10) |
| Inflammation, chronic active | 1 (10%) | | | | | |
| Stomach, forestomach | (10) | (10) | (10) | (10) | (10) | (10) |
| Epithelium, hyperkeratosis | | | | | | 3 (30%) |
| Epithelium, hyperplasia | | | | 3 (30%) | 3 (30%) | 6 (60%) |
| **Cardiovascular System** | | | | | | |
| None | | | | | | |
| **Endocrine System** | | | | | | |
| Adrenal cortex | (10) | | | | | (10) |
| Accessory adrenal cortical nodule | | | | | | 1 (10%) |
| Subcapsular, hyperplasia | | | | | | 2 (20%) |
| **General Body System** | | | | | | |
| None | | | | | | |
| **Genital System** | | | | | | |
| Preputial gland | (10) | | | | | (10) |
| Cyst | 5 (50%) | | | | | 6 (60%) |
| **Hematopoietic System** | | | | | | |
| None | | | | | | |
| **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 *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Respiratory System** | | | | | | |
| None | | | | | | |
| **Special Senses System** | | | | | | |
| None | | | | | | |
| **Urinary System** | | | | | | |
| Urinary bladder | (10) | | | | | (10) |
| Transitional epithelium, hyperplasia | 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 *p-tert*-Butylcatechol[a]**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Disposition Summary** | | | | | | |
| Animals initially in study | 10 | 10 | 10 | 10 | 10 | 10 |
| Survivors | | | | | | |
| Terminal sacrifice | 10 | 10 | 10 | 10 | 10 | 10 |
| Animals examined microscopically | 10 | 10 | 9 | 10 | 10 | 10 |
| **Alimentary System** | | | | | | |
| Intestine large, cecum | (10) | | | | | (10) |
| Inflammation, chronic active | | | | | | 1 (10%) |
| Liver | (10) | | | | | (10) |
| Inflammation, chronic active | 1 (10%) | | | | | |
| Stomach, forestomach | (10) | (10) | (9) | (10) | (10) | (10) |
| Epithelium, hyperkeratosis | | | | | 2 (20%) | 6 (60%) |
| Epithelium, hyperplasia | | | | 2 (20%) | 4 (40%) | 7 (70%) |
| **Cardiovascular System** | | | | | | |
| None | | | | | | |
| **Endocrine System** | | | | | | |
| Adrenal cortex | (10) | | | | | (10) |
| Subcapsular, hyperplasia | 5 (50%) | | | | | 5 (50%) |
| **General Body System** | | | | | | |
| None | | | | | | |
| **Genital System** | | | | | | |
| None | | | | | | |
| **Hematopoietic System** | | | | | | |
| None | | | | | | |
| **Integumentary System** | | | | | | |
| None | | | | | | |
| **Musculoskeletal System** | | | | | | |
| None | | | | | | |
| **Nervous System** | | | | | | |
| None | | | | | | |

**TABLE A4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Respiratory System** | | | | | | |
| None | | | | | | |
| **Special Senses System** | | | | | | |
| None | | | | | | |
| **Urinary System** | | | | | | |
| Urinary bladder | (10) | | | | | (10) |
| Inflammation, chronic active | 3 (30%) | | | | | |

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

# APPENDIX B
# CLINICAL PATHOLOGY RESULTS

**TABLE B1**
**Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| Hematology | | | | | | |
| n | | | | | | |
| Day 4 | 9 | 10 | 10 | 10 | 10 | 9 |
| Day 22 | 10 | 9 | 9 | 8 | 9 | 10 |
| Week 14 | 10 | 10 | 9 | 10 | 10 | 9 |
| Hematocrit (%) | | | | | | |
| Day 4 | 35.7 ± 0.4 | 35.5 ± 0.2 | 35.7 ± 0.5 | 37.0 ± 0.7 | 39.1 ± 0.4** | 41.5 ± 0.4** |
| Day 22 | 40.4 ± 0.4 | 40.2 ± 0.4 | 45.4 ± 1.4** | 41.3 ± 0.6* | 41.9 ± 0.6* | 42.5 ± 0.5** |
| Week 14 | 45.8 ± 0.5 | 45.8 ± 0.3 | 44.3 ± 0.6 | 46.9 ± 0.4 | 46.8 ± 0.3 | 47.1 ± 0.3* |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 11.7 ± 0.2 | 11.8 ± 0.1 | 11.9 ± 0.2 | 12.2 ± 0.2* | 13.0 ± 0.2** | 13.8 ± 0.1** |
| Day 22 | 13.7 ± 0.1 | 13.6 ± 0.1 | 15.3 ± 0.5* | 13.9 ± 0.3 | 14.0 ± 0.2 | 14.3 ± 0.2 |
| Week 14 | 15.0 ± 0.2 | 14.9 ± 0.1 | 14.7 ± 0.1 | 15.3 ± 0.1 | 15.3 ± 0.1 | 15.3 ± 0.1* |
| Erythrocytes ($10^6$/μL) | | | | | | |
| Day 4 | 6.13 ± 0.05 | 6.10 ± 0.05 | 6.25 ± 0.08 | 6.42 ± 0.11 | 6.81 ± 0.06** | 7.28 ± 0.07** |
| Day 22 | 6.98 ± 0.09 | 6.96 ± 0.07 | 7.87 ± 0.27** | 7.12 ± 0.13 | 7.23 ± 0.12 | 7.52 ± 0.10** |
| Week 14 | 8.93 ± 0.12 | 8.83 ± 0.10 | 8.62 ± 0.13 | 9.03 ± 0.09 | 8.96 ± 0.09 | 8.96 ± 0.06 |
| Reticulocytes ($10^6$/μL) | | | | | | |
| Day 4 | 0.40 ± 0.04 | 0.39 ± 0.02 | 0.41 ± 0.04 | 0.38 ± 0.03 | 0.42 ± 0.03 | 0.34 ± 0.04 |
| Day 22 | 0.22 ± 0.02 | 0.21 ± 0.02 | 0.19 ± 0.02 | 0.24 ± 0.02 | 0.22 ± 0.01 | 0.24 ± 0.02 |
| Week 14 | 0.12 ± 0.01 | 0.13 ± 0.01 | 0.11 ± 0.01 | 0.11 ± 0.01 | 0.13 ± 0.02 | 0.10 ± 0.01 |
| Nucleated erythrocytes ($10^3$/μL) | | | | | | |
| Day 4 | 0.02 ± 0.01 | 0.05 ± 0.01 | 0.05 ± 0.02 | 0.11 ± 0.03* | 0.06 ± 0.02 | 0.02 ± 0.01 |
| Day 22 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Week 14 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 58.2 ± 0.4 | 58.2 ± 0.3 | 57.2 ± 0.5 | 57.6 ± 0.4 | 57.4 ± 0.4 | 57.0 ± 0.3* |
| Day 22 | 57.8 ± 0.3 | 57.7 ± 0.2 | 57.8 ± 0.3 | 58.1 ± 0.4 | 58.0 ± 0.3 | 56.5 ± 0.2* |
| Week 14 | 51.4 ± 0.2 | 51.9 ± 0.3* | 51.4 ± 0.2 | 51.9 ± 0.4* | 52.3 ± 0.4** | 52.6 ± 0.2** |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 19.2 ± 0.1 | 19.3 ± 0.1 | 19.0 ± 0.2 | 19.0 ± 0.2 | 19.0 ± 0.1 | 18.9 ± 0.1 |
| Day 22 | 19.6 ± 0.2 | 19.5 ± 0.1 | 19.5 ± 0.1 | 19.6 ± 0.1 | 19.4 ± 0.1 | 19.0 ± 0.1* |
| Week 14 | 16.8 ± 0.1 | 16.9 ± 0.1 | 17.1 ± 0.2 | 16.9 ± 0.1 | 17.1 ± 0.2* | 17.1 ± 0.0* |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 4 | 32.9 ± 0.1 | 33.2 ± 0.1 | 33.3 ± 0.1 | 33.0 ± 0.2 | 33.1 ± 0.1 | 33.2 ± 0.1 |
| Day 22 | 33.8 ± 0.1 | 33.8 ± 0.2 | 33.7 ± 0.1 | 33.7 ± 0.2 | 33.5 ± 0.2 | 33.6 ± 0.1 |
| Week 14 | 32.8 ± 0.1 | 32.5 ± 0.1 | 33.3 ± 0.5 | 32.6 ± 0.1 | 32.6 ± 0.2 | 32.6 ± 0.1 |
| Platelets ($10^3$/μL) | | | | | | |
| Day 4 | 997.7 ± 15.4 | 977.3 ± 21.5 | 916.1 ± 36.1 | 955.8 ± 43.8 | 1,035.9 ± 18.0 | 1,116.8 ± 48.9* |
| Day 22 | 910.0 ± 35.4 | 1,002.1 ± 15.0 | 870.6 ± 73.1 | 869.5 ± 72.2 | 847.1 ± 62.1 | 886.5 ± 38.2 |
| Week 14 | 729.4 ± 7.1 | 723.4 ± 17.7 | 679.6 ± 12.9* | 743.3 ± 14.5 | 702.2 ± 14.5 | 719.1 ± 17.8 |
| Leukocytes ($10^3$/μL) | | | | | | |
| Day 4 | 7.63 ± 0.43 | 7.50 ± 0.38 | 7.75 ± 0.37 | 7.08 ± 0.41 | 8.64 ± 0.45 | 8.03 ± 0.45 |
| Day 22 | 8.86 ± 0.74 | 9.62 ± 0.35 | 9.52 ± 0.72 | 9.60 ± 0.59 | 8.91 ± 0.32 | 9.45 ± 0.55 |
| Week 14 | 7.80 ± 0.49 | 7.68 ± 0.51 | 7.28 ± 0.37 | 6.69 ± 0.14 | 8.20 ± 0.47 | 6.87 ± 0.48 |
| Segmented neutrophils ($10^3$/μL) | | | | | | |
| Day 4 | 1.15 ± 0.12 | 0.91 ± 0.14 | 0.99 ± 0.09 | 0.98 ± 0.05 | 1.13 ± 0.09 | 1.12 ± 0.08 |
| Day 22 | 0.95 ± 0.15 | 0.98 ± 0.07 | 0.95 ± 0.11 | 1.05 ± 0.11 | 0.91 ± 0.17 | 0.88 ± 0.09 |
| Week 14 | 1.57 ± 0.25 | 1.31 ± 0.08 | 1.24 ± 0.10 | 1.04 ± 0.05** | 1.26 ± 0.10 | 0.97 ± 0.14** |

**TABLE B1**
**Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Male** (continued) | | | | | | |
| Hematology (continued) | | | | | | |
| n | | | | | | |
| Day 4 | 9 | 10 | 10 | 10 | 10 | 9 |
| Day 22 | 10 | 9 | 9 | 8 | 9 | 10 |
| Week 14 | 10 | 10 | 9 | 10 | 10 | 9 |
| Bands ($10^3/\mu L$) | | | | | | |
| 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 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Week 14 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Lymphocytes ($10^3/\mu L$) | | | | | | |
| Day 4 | 6.31 ± 0.37 | 6.42 ± 0.30 | 6.59 ± 0.32 | 5.95 ± 0.41 | 7.28 ± 0.44 | 6.69 ± 0.45 |
| Day 22 | 7.75 ± 0.64 | 8.41 ± 0.34 | 8.32 ± 0.67 | 8.26 ± 0.49 | 7.75 ± 0.27 | 8.36 ± 0.50 |
| Week 14 | 6.13 ± 0.41 | 6.19 ± 0.45 | 5.91 ± 0.29 | 5.52 ± 0.16 | 6.78 ± 0.40 | 5.74 ± 0.42 |
| Monocytes ($10^3/\mu L$) | | | | | | |
| Day 4 | 0.15 ± 0.03 | 0.12 ± 0.01 | 0.15 ± 0.03 | 0.13 ± 0.03 | 0.22 ± 0.04 | 0.17 ± 0.04 |
| Day 22 | 0.14 ± 0.05 | 0.22 ± 0.04 | 0.19 ± 0.04 | 0.24 ± 0.07 | 0.20 ± 0.05 | 0.16 ± 0.04 |
| Week 14 | 0.10 ± 0.02 | 0.12 ± 0.02 | 0.10 ± 0.02 | 0.09 ± 0.02 | 0.11 ± 0.02 | 0.11 ± 0.02 |
| Basophils ($10^3/\mu L$) | | | | | | |
| Day 4 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Day 22 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Week 14 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Eosinophils ($10^3/\mu L$) | | | | | | |
| Day 4 | 0.02 ± 0.01 | 0.05 ± 0.02 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.05 ± 0.02 |
| Day 22 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.07 ± 0.03 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.05 ± 0.02 |
| Week 14 | 0.03 ± 0.02 | 0.06 ± 0.02 | 0.02 ± 0.01 | 0.04 ± 0.02 | 0.06 ± 0.02 | 0.05 ± 0.02 |
| Clinical Chemistry | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 4 | 11.7 ± 0.5 | 13.0 ± 0.5 | 13.4 ± 0.4* | 14.1 ± 0.5** | 14.3 ± 0.4** | 14.5 ± 0.4** |
| Day 22 | 13.8 ± 0.3 | 13.6 ± 0.6 | 15.5 ± 1.2 | 13.0 ± 0.4 | 13.8 ± 0.6 | 13.6 ± 0.4 |
| Week 14 | 16.6 ± 0.4 | 14.0 ± 0.3* | 15.2 ± 0.6 | 16.0 ± 0.6 | 17.6 ± 0.6 | 17.2 ± 0.5 |
| Creatinine (mg/dL) | | | | | | |
| Day 4 | 0.43 ± 0.02 | 0.47 ± 0.02 | 0.47 ± 0.02 | 0.48 ± 0.01 | 0.47 ± 0.02 | 0.49 ± 0.01* |
| Day 22 | 0.47 ± 0.02 | 0.46 ± 0.02 | 0.50 ± 0.02 | 0.56 ± 0.04 | 0.50 ± 0.00 | 0.49 ± 0.01 |
| Week 14 | 0.58 ± 0.01 | 0.61 ± 0.04 | 0.55 ± 0.02 | 0.57 ± 0.02 | 0.57 ± 0.02 | 0.59 ± 0.02 |
| Total protein (g/dL) | | | | | | |
| Day 4 | 5.1 ± 0.1 | 5.1 ± 0.1 | 5.2 ± 0.1 | 5.3 ± 0.1* | 5.4 ± 0.1** | 5.4 ± 0.1** |
| Day 22 | 6.3 ± 0.1 | 6.2 ± 0.0 | 6.9 ± 0.2* | 6.4 ± 0.1 | 6.3 ± 0.1 | 6.3 ± 0.1 |
| Week 14 | 6.6 ± 0.1 | 6.6 ± 0.0 | 6.5 ± 0.0 | 6.7 ± 0.1 | 6.7 ± 0.1 | 6.7 ± 0.1 |
| Albumin (g/dL) | | | | | | |
| Day 4 | 3.8 ± 0.0 | 3.9 ± 0.1 | 3.9 ± 0.0 | 4.0 ± 0.1** | 4.1 ± 0.0** | 4.1 ± 0.1** |
| Day 22 | 4.5 ± 0.1 | 4.5 ± 0.0 | 5.0 ± 0.1** | 4.6 ± 0.0 | 4.5 ± 0.1 | 4.7 ± 0.0 |
| Week 14 | 4.6 ± 0.1 | 4.6 ± 0.0 | 4.6 ± 0.0 | 4.7 ± 0.0* | 4.8 ± 0.0* | 4.8 ± 0.0* |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 4 | 79 ± 2 | 85 ± 2 | 87 ± 5 | 86 ± 3 | 94 ± 5** | 94 ± 4** |
| Day 22 | 59 ± 2 | 62 ± 1 | 51 ± 4 | 68 ± 2* | 75 ± 3** | 79 ± 2** |
| Week 14 | 95 ± 5 | 79 ± 4* | 76 ± 2** | 85 ± 7* | 78 ± 6* | 78 ± 7** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 878 ± 37 | 815 ± 17 | 832 ± 21 | 821 ± 15 | 749 ± 18** | 671 ± 13** |
| Day 22 | 583 ± 16 | 558 ± 13 | 543 ± 30 | 591 ± 13 | 579 ± 13 | 574 ± 11 |
| Week 14 | 220 ± 14 | 238 ± 9 | 250 ± 8 | 249 ± 7 | 281 ± 8** | 275 ± 9** |

**TABLE B1**
**Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Male** (continued) | | | | | | |
| Clinical Chemistry (continued) | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Creatine kinase (IU/L) | | | | | | |
| Day 4 | 432 ± 70 | 388 ± 62 | 401 ± 59 | 446 ± 46[b] | 325 ± 34 | 441 ± 82[b] |
| Day 22 | 409 ± 79 | 346 ± 50 | 498 ± 108 | 404 ± 46 | 400 ± 102 | 275 ± 35[b] |
| Week 14 | 251 ± 40 | 271 ± 46 | 263 ± 45 | 223 ± 22 | 208 ± 59 | 278 ± 41 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 4 | 18 ± 2 | 17 ± 1 | 23 ± 5 | 18 ± 1 | 17 ± 1 | 16 ± 1 |
| Day 22 | 18 ± 1 | 17 ± 1 | 19 ± 1 | 18 ± 1 | 17 ± 1 | 19 ± 1 |
| Week 14 | 28 ± 3 | 25 ± 3 | 19 ± 1** | 25 ± 4* | 23 ± 5* | 25 ± 6* |
| Bile salts (μmol/L) | | | | | | |
| Day 4 | 25.1 ± 2.3 | 26.8 ± 3.1 | 41.7 ± 2.6** | 30.5 ± 3.5* | 34.1 ± 2.5* | 56.3 ± 3.8** |
| Day 22 | 39.7 ± 4.4 | 33.9 ± 6.0 | 29.2 ± 3.8 | 29.3 ± 1.5 | 34.2 ± 3.1 | 53.7 ± 4.2 |
| Week 14 | 20.1 ± 1.1 | 17.6 ± 1.3 | 19.1 ± 1.9 | 17.2 ± 1.4 | 15.7 ± 0.7 | 21.8 ± 2.1 |
| **Female** | | | | | | |
| Hematology | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 22 | 9 | 8 | 7 | 8 | 9 | 8 |
| Week 14 | 9 | 10 | 10 | 9 | 9 | 10 |
| Hematocrit (%) | | | | | | |
| Day 4 | 37.1 ± 0.3 | 37.4 ± 0.5 | 37.6 ± 0.4 | 38.2 ± 0.4 | 39.2 ± 0.3** | 42.2 ± 0.7** |
| Day 22 | 43.1 ± 0.4 | 43.0 ± 0.4 | 44.3 ± 0.7 | 43.8 ± 0.5 | 43.1 ± 0.6 | 44.4 ± 0.4 |
| Week 14 | 45.0 ± 0.4 | 45.8 ± 0.4 | 46.1 ± 0.6 | 44.7 ± 0.5 | 45.7 ± 0.4 | 45.9 ± 0.5 |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 12.2 ± 0.1 | 12.4 ± 0.1 | 12.4 ± 0.1 | 12.6 ± 0.1 | 13.1 ± 0.1** | 14.1 ± 0.2** |
| Day 22 | 14.6 ± 0.1 | 14.6 ± 0.1 | 14.9 ± 0.2 | 14.7 ± 0.2 | 14.7 ± 0.2 | 14.9 ± 0.1 |
| Week 14 | 14.7 ± 0.1 | 14.9 ± 0.2 | 15.0 ± 0.1 | 14.6 ± 0.1 | 14.8 ± 0.1 | 15.0 ± 0.1 |
| Erythrocytes ($10^6$/μL) | | | | | | |
| Day 4 | 6.33 ± 0.07 | 6.34 ± 0.08 | 6.38 ± 0.07 | 6.50 ± 0.07 | 6.74 ± 0.07** | 7.32 ± 0.11** |
| Day 22 | 7.34 ± 0.08 | 7.31 ± 0.07 | 7.65 ± 0.15 | 7.53 ± 0.09 | 7.48 ± 0.09 | 7.77 ± 0.07** |
| Week 14 | 8.17 ± 0.07 | 8.32 ± 0.05 | 8.37 ± 0.10 | 8.11 ± 0.08 | 8.27 ± 0.07 | 8.34 ± 0.08 |
| Reticulocytes ($10^6$/μL) | | | | | | |
| Day 4 | 0.40 ± 0.02 | 0.36 ± 0.03 | 0.33 ± 0.02 | 0.39 ± 0.02 | 0.34 ± 0.02 | 0.30 ± 0.02* |
| Day 22 | 0.08 ± 0.01 | 0.09 ± 0.01 | 0.07 ± 0.01 | 0.10 ± 0.02 | 0.10 ± 0.01 | 0.10 ± 0.02 |
| Week 14 | 0.09 ± 0.01 | 0.11 ± 0.01 | 0.09 ± 0.01 | 0.12 ± 0.01 | 0.11 ± 0.02 | 0.12 ± 0.02 |
| Nucleated erythrocytes ($10^3$/μL) | | | | | | |
| Day 4 | 0.02 ± 0.02 | 0.03 ± 0.02 | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.01 ± 0.01 |
| Day 22 | 0.08 ± 0.03 | 0.00 ± 0.00 | 0.03 ± 0.02 | 0.01 ± 0.01 | 0.02 ± 0.02 | 0.01 ± 0.01 |
| Week 14 | 0.02 ± 0.01 | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.03 ± 0.03 | 0.00 ± 0.00 | 0.06 ± 0.02 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 58.6 ± 0.5 | 59.0 ± 0.1 | 59.0 ± 0.3 | 58.7 ± 0.3 | 58.2 ± 0.3 | 57.6 ± 0.2* |
| Day 22 | 58.7 ± 0.2 | 58.8 ± 0.3 | 57.9 ± 0.3 | 58.3 ± 0.3 | 57.6 ± 0.3* | 57.1 ± 0.2** |
| Week 14 | 55.1 ± 0.2 | 55.1 ± 0.2 | 55.0 ± 0.1 | 55.1 ± 0.2 | 55.2 ± 0.2 | 55.1 ± 0.1 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 19.3 ± 0.1 | 19.5 ± 0.1 | 19.5 ± 0.1 | 19.4 ± 0.1 | 19.4 ± 0.1 | 19.3 ± 0.1 |
| Day 22 | 19.9 ± 0.1 | 20.0 ± 0.1 | 19.5 ± 0.2 | 19.6 ± 0.1 | 19.7 ± 0.1 | 19.2 ± 0.1** |
| Week 14 | 18.1 ± 0.1 | 17.9 ± 0.2 | 17.9 ± 0.1 | 18.0 ± 0.1 | 17.8 ± 0.1 | 18.0 ± 0.1 |

**TABLE B1**
**Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Female** (continued) | | | | | | |
| Hematology (continued) | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 22 | 9 | 8 | 7 | 8 | 9 | 8 |
| Week 14 | 9 | 10 | 10 | 9 | 9 | 10 |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 4 | 32.9 ± 0.1 | 33.1 ± 0.1 | 33.1 ± 0.2 | 33.0 ± 0.1 | 33.3 ± 0.1* | 33.4 ± 0.1** |
| Day 22 | 33.9 ± 0.1 | 34.0 ± 0.1 | 33.6 ± 0.2 | 33.5 ± 0.2 | 34.1 ± 0.1 | 33.5 ± 0.2 |
| Week 14 | 32.7 ± 0.1 | 32.5 ± 0.2 | 32.6 ± 0.1 | 32.6 ± 0.1 | 32.3 ± 0.1 | 32.6 ± 0.2 |
| Platelets ($10^3$/μL) | | | | | | |
| Day 4 | 925.3 ± 6.7 | 912.4 ± 19.1 | 950.3 ± 18.2 | 923.7 ± 20.1 | 972.2 ± 17.8 | 1,119.7 ± 27.2** |
| Day 22 | 869.6 ± 20.3 | 869.1 ± 16.1 | 806.0 ± 50.7 | 846.4 ± 28.8 | 907.3 ± 16.0 | 901.5 ± 23.7 |
| Week 14 | 709.7 ± 12.1 | 707.9 ± 14.8 | 725.3 ± 8.7 | 674.3 ± 10.2 | 688.2 ± 13.9 | 716.5 ± 12.7 |
| Leukocytes ($10^3$/μL) | | | | | | |
| Day 4 | 8.13 ± 0.39 | 7.63 ± 0.48 | 7.46 ± 0.51 | 8.23 ± 0.46 | 7.55 ± 0.39 | 9.88 ± 0.82 |
| Day 22 | 11.69 ± 0.22 | 10.13 ± 0.35* | 11.10 ± 0.35 | 10.84 ± 0.54 | 10.73 ± 0.43 | 10.54 ± 0.52 |
| Week 14 | 9.32 ± 0.35 | 8.32 ± 0.40 | 8.73 ± 0.35 | 11.19 ± 0.70 | 10.07 ± 0.32 | 9.41 ± 0.45 |
| Segmented neutrophils ($10^3$/μL) | | | | | | |
| Day 4 | 1.02 ± 0.06 | 1.10 ± 0.16 | 0.83 ± 0.08 | 0.96 ± 0.06 | 0.96 ± 0.10 | 1.28 ± 0.10 |
| Day 22 | 1.06 ± 0.11 | 0.88 ± 0.11 | 0.97 ± 0.14 | 0.91 ± 0.16 | 0.78 ± 0.07 | 0.79 ± 0.11 |
| Week 14 | 1.57 ± 0.12 | 1.42 ± 0.09 | 1.41 ± 0.08 | 1.93 ± 0.20 | 1.40 ± 0.11 | 1.29 ± 0.09 |
| Bands ($10^3$/μL) | | | | | | |
| Day 4 | 0.00 ± 0.00 | 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 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Week 14 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Lymphocytes ($10^3$/μL) | | | | | | |
| Day 4 | 6.96 ± 0.37 | 6.37 ± 0.42 | 6.46 ± 0.49 | 7.14 ± 0.42 | 6.41 ± 0.31 | 8.42 ± 0.71 |
| Day 22 | 10.20 ± 0.27 | 9.01 ± 0.31 | 9.78 ± 0.32 | 9.67 ± 0.50 | 9.59 ± 0.45 | 9.45 ± 0.47 |
| Week 14 | 7.57 ± 0.39 | 6.73 ± 0.33 | 7.11 ± 0.34 | 9.11 ± 0.55 | 8.47 ± 0.29 | 7.95 ± 0.37 |
| Monocytes ($10^3$/μL) | | | | | | |
| Day 4 | 0.09 ± 0.02 | 0.14 ± 0.03 | 0.13 ± 0.04 | 0.09 ± 0.02 | 0.15 ± 0.02 | 0.16 ± 0.04 |
| Day 22 | 0.34 ± 0.05 | 0.21 ± 0.08 | 0.24 ± 0.06 | 0.15 ± 0.05 | 0.30 ± 0.04 | 0.28 ± 0.06 |
| Week 14 | 0.15 ± 0.04 | 0.12 ± 0.03 | 0.13 ± 0.02 | 0.14 ± 0.03 | 0.13 ± 0.02 | 0.12 ± 0.03 |
| Basophils ($10^3$/μL) | | | | | | |
| Day 4 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Day 22 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Week 14 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Eosinophils ($10^3$/μL) | | | | | | |
| Day 4 | 0.06 ± 0.03 | 0.02 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.02 | 0.02 ± 0.01 | 0.01 ± 0.01 |
| Day 22 | 0.09 ± 0.04 | 0.03 ± 0.02 | 0.11 ± 0.04 | 0.12 ± 0.06 | 0.07 ± 0.02 | 0.03 ± 0.03 |
| Week 14 | 0.03 ± 0.02 | 0.05 ± 0.02 | 0.07 ± 0.02 | 0.01 ± 0.01 | 0.07 ± 0.02 | 0.05 ± 0.02 |

**TABLE B1**
**Hematology and Clinical Chemistry Data for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Female** (continued) | | | | | | |
| Clinical Chemistry | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 10 | 9 | 10 | 10 | 10 |
| Day 22 | 10 | 10 | 10 | 10 | 10 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 9 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 4 | 10.0 ± 0.3 | 11.1 ± 0.5 | 12.8 ± 0.7** | 11.9 ± 0.4** | 12.1 ± 0.4** | 12.4 ± 0.3** |
| Day 22 | 13.5 ± 0.6 | 15.9 ± 1.1 | 15.3 ± 0.7 | 14.1 ± 0.3 | 14.9 ± 0.8 | 15.0 ± 0.6 |
| Week 14 | 16.5 ± 0.3 | 17.1 ± 0.6 | 18.0 ± 0.5 | 14.7 ± 0.6 | 16.7 ± 0.5 | 17.9 ± 0.9 |
| Creatinine (mg/dL) | | | | | | |
| Day 4 | 0.40 ± 0.00 | 0.40 ± 0.00 | 0.42 ± 0.01[c] | 0.41 ± 0.01 | 0.40 ± 0.00 | 0.41 ± 0.01 |
| Day 22 | 0.49 ± 0.01 | 0.49 ± 0.02 | 0.45 ± 0.02 | 0.47 ± 0.02 | 0.47 ± 0.02 | 0.51 ± 0.01 |
| Week 14 | 0.59 ± 0.01 | 0.62 ± 0.01 | 0.59 ± 0.01 | 0.59 ± 0.01 | 0.57 ± 0.02 | 0.59 ± 0.01 |
| Total protein (g/dL) | | | | | | |
| Day 4 | 5.3 ± 0.1 | 5.4 ± 0.1 | 5.4 ± 0.1 | 5.3 ± 0.1 | 5.3 ± 0.1 | 5.1 ± 0.1 |
| Day 22 | 5.9 ± 0.1 | 6.0 ± 0.0 | 6.1 ± 0.1 | 5.9 ± 0.1 | 6.0 ± 0.1 | 5.9 ± 0.1 |
| Week 14 | 6.4 ± 0.1 | 7.1 ± 0.1 | 7.0 ± 0.1 | 6.3 ± 0.1 | 6.2 ± 0.1 | 6.3 ± 0.1 |
| Albumin (g/dL) | | | | | | |
| Day 4 | 4.0 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.0 | 4.1 ± 0.1 | 4.1 ± 0.0 | 4.0 ± 0.1 |
| Day 22 | 4.5 ± 0.0 | 4.5 ± 0.0 | 4.6 ± 0.0 | 4.5 ± 0.1 | 4.5 ± 0.0 | 4.5 ± 0.1 |
| Week 14 | 4.8 ± 0.0 | 5.2 ± 0.1 | 5.1 ± 0.1 | 4.7 ± 0.1 | 4.5 ± 0.1 | 4.6 ± 0.0 |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 4 | 64 ± 1 | 71 ± 2** | 71 ± 1** | 77 ± 3** | 80 ± 3** | 85 ± 4** |
| Day 22 | 47 ± 2 | 53 ± 3 | 55 ± 4 | 51 ± 2 | 55 ± 2* | 63 ± 3** |
| Week 14 | 86 ± 6 | 69 ± 5 | 69 ± 3 | 72 ± 2 | 72 ± 2 | 70 ± 3 |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 688 ± 9 | 702 ± 20 | 683 ± 12 | 689 ± 12 | 658 ± 13 | 573 ± 9** |
| Day 22 | 454 ± 5 | 463 ± 8 | 448 ± 8 | 446 ± 6 | 466 ± 8 | 466 ± 11 |
| Week 14 | 230 ± 4 | 215 ± 7 | 232 ± 4 | 244 ± 7 | 251 ± 6* | 240 ± 4 |
| Creatine kinase (IU/L) | | | | | | |
| Day 4 | 358 ± 24[b] | 392 ± 42 | 327 ± 30[b] | 402 ± 61 | 344 ± 40 | 402 ± 60 |
| Day 22 | 332 ± 59[b] | 385 ± 34 | 323 ± 38[b] | 383 ± 46 | 356 ± 36 | 297 ± 38 |
| Week 14 | 170 ± 20 | 178 ± 34 | 213 ± 49 | 148 ± 16 | 135 ± 12 | 218 ± 42 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 4 | 13 ± 0 | 14 ± 1 | 13 ± 1 | 13 ± 0 | 13 ± 1 | 12 ± 1 |
| Day 22 | 17 ± 1 | 18 ± 1 | 18 ± 1 | 17 ± 1 | 18 ± 0 | 19 ± 1 |
| Week 14 | 24 ± 2 | 19 ± 2 | 16 ± 1** | 17 ± 1** | 13 ± 1** | 14 ± 1** |
| Bile salts (μmol/L) | | | | | | |
| Day 4 | 32.8 ± 3.2 | 32.2 ± 4.3 | 26.8 ± 3.9 | 29.7 ± 1.4 | 37.0 ± 2.2 | 51.5 ± 4.1** |
| Day 22 | 28.9 ± 2.7 | 23.3 ± 2.6 | 22.3 ± 2.6 | 33.7 ± 2.7 | 35.5 ± 2.6 | 48.4 ± 3.6** |
| Week 14 | 28.8 ± 1.7 | 25.9 ± 2.6 | 27.4 ± 1.8 | 24.3 ± 1.8 | 25.3 ± 2.9 | 36.2 ± 3.1 |

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=10

**TABLE B2**
**Hematology Data for Mice in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| **Male** | | | | | | |
| n | 10 | 9 | 10 | 10 | 10 | 9 |
| Hematocrit (%) | 47.8 ± 0.4 | 48.9 ± 0.8[b] | 48.0 ± 0.5 | 48.6 ± 0.7 | 48.7 ± 0.8 | 48.5 ± 0.8 |
| Hemoglobin (g/dL) | 16.1 ± 0.2 | 16.4 ± 0.2[b] | 15.9 ± 0.2 | 16.2 ± 0.2 | 16.1 ± 0.2 | 16.3 ± 0.3 |
| Erythrocytes ($10^6$/μL) | 10.53 ± 0.11 | 10.88 ± 0.17[b] | 10.58 ± 0.13 | 10.72 ± 0.16 | 10.80 ± 0.18 | 10.83 ± 0.20 |
| Reticulocytes ($10^6$/μL) | 0.06 ± 0.01 | 0.08 ± 0.01 | 0.08 ± 0.02 | 0.06 ± 0.01 | 0.09 ± 0.01 | 0.08 ± 0.01 |
| Nucleated erythrocytes ($10^3$/μL) | 0.00 ± 0.00 | 0.00 ± 0.00[b] | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Mean cell volume (fL) | 45.4 ± 0.2 | 45.0 ± 0.2[b] | 45.3 ± 0.2 | 45.4 ± 0.1 | 45.1 ± 0.2 | 44.9 ± 0.1 |
| Mean cell hemoglobin (pg) | 15.3 ± 0.1 | 15.1 ± 0.1[b] | 15.0 ± 0.1 | 15.1 ± 0.1 | 14.9 ± 0.1* | 15.1 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | 33.6 ± 0.1 | 33.6 ± 0.2[b] | 33.2 ± 0.1 | 33.4 ± 0.1 | 33.1 ± 0.2 | 33.5 ± 0.1 |
| Platelets ($10^3$/μL) | 749.7 ± 42.7 | 753.9 ± 52.1[b] | 791.0 ± 49.7 | 728.3 ± 41.5 | 749.7 ± 42.3 | 835.8 ± 44.0 |
| Leukocytes ($10^3$/μL) | 6.52 ± 0.42 | 5.97 ± 0.39 | 6.48 ± 0.46 | 6.09 ± 0.50 | 5.36 ± 0.61 | 4.81 ± 0.61 |
| Segmented neutrophils ($10^3$/μL) | 0.89 ± 0.08 | 0.83 ± 0.07 | 0.80 ± 0.08 | 0.74 ± 0.10 | 0.85 ± 0.10 | 0.70 ± 0.09 |
| Bands ($10^3$/μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Lymphocytes ($10^3$/μL) | 5.51 ± 0.40 | 5.01 ± 0.36 | 5.54 ± 0.40 | 5.21 ± 0.41 | 4.40 ± 0.54 | 3.97 ± 0.54 |
| Monocytes ($10^3$/μL) | 0.06 ± 0.02 | 0.08 ± 0.03 | 0.09 ± 0.04 | 0.09 ± 0.02 | 0.07 ± 0.01 | 0.09 ± 0.02 |
| Basophils ($10^3$/μL) | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Eosinophils ($10^3$/μL) | 0.06 ± 0.02 | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.05 ± 0.02 | 0.05 ± 0.02 | 0.06 ± 0.02 |
| **Female** | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematocrit (%) | 47.5 ± 0.6 | 46.0 ± 0.4 | 45.4 ± 0.4 | 46.7 ± 0.2 | 46.8 ± 0.4 | 47.6 ± 0.6 |
| Hemoglobin (g/dL) | 15.9 ± 0.2 | 15.6 ± 0.1 | 15.3 ± 0.2 | 15.8 ± 0.1 | 15.7 ± 0.2 | 16.0 ± 0.2 |
| Erythrocytes ($10^6$/μL) | 10.33 ± 0.14 | 10.00 ± 0.10 | 9.86 ± 0.07* | 10.14 ± 0.07 | 10.15 ± 0.15 | 10.50 ± 0.12 |
| Reticulocytes ($10^6$/μL) | 0.11 ± 0.01 | 0.12 ± 0.02 | 0.11 ± 0.01 | 0.12 ± 0.01 | 0.10 ± 0.01 | 0.10 ± 0.01 |
| Nucleated erythrocytes ($10^3$/μL) | 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) | 46.0 ± 0.2 | 46.1 ± 0.1 | 46.0 ± 0.2 | 46.1 ± 0.2 | 46.1 ± 0.3 | 45.4 ± 0.2* |
| Mean cell hemoglobin (pg) | 15.4 ± 0.1 | 15.7 ± 0.1 | 15.5 ± 0.1 | 15.6 ± 0.1 | 15.5 ± 0.1 | 15.2 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | 33.5 ± 0.2 | 34.0 ± 0.1 | 33.8 ± 0.1 | 33.8 ± 0.1 | 33.6 ± 0.1 | 33.5 ± 0.1 |
| Platelets ($10^3$/μL) | 855.5 ± 46.4 | 838.8 ± 51.4 | 925.5 ± 33.8 | 811.0 ± 35.1 | 841.8 ± 53.3 | 819.1 ± 47.4 |
| Leukocytes ($10^3$/μL) | 5.60 ± 0.57 | 5.11 ± 0.26 | 5.53 ± 0.33 | 6.36 ± 0.43 | 5.43 ± 0.59 | 4.39 ± 0.43 |
| Segmented neutrophils ($10^3$/μL) | 0.85 ± 0.09 | 0.91 ± 0.11 | 0.96 ± 0.09 | 1.09 ± 0.10 | 0.94 ± 0.12 | 0.71 ± 0.07 |
| Bands ($10^3$/μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Lymphocytes ($10^3$/μL) | 4.64 ± 0.49 | 4.12 ± 0.17 | 4.45 ± 0.27 | 5.16 ± 0.37 | 4.33 ± 0.46 | 3.62 ± 0.40 |
| Monocytes ($10^3$/μL) | 0.03 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.04 ± 0.01 | 0.05 ± 0.02 | 0.02 ± 0.01 |
| Basophils ($10^3$/μL) | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Eosinophils ($10^3$/μL) | 0.09 ± 0.03 | 0.06 ± 0.01 | 0.10 ± 0.03 | 0.08 ± 0.02 | 0.11 ± 0.03 | 0.03 ± 0.01 |

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10

# 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 15-Day Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm | 25,000 ppm | 50,000 ppm |
|---|---|---|---|---|---|---|
| n | 5 | 5 | 5 | 5 | 5 | 0[b] |
| **Male** | | | | | | |
| Necropsy body wt | 198 ± 6 | 190 ± 4 | 181 ± 5* | 154 ± 7** | 123 ± 4** | |
| Heart | | | | | | |
| Absolute | 0.700 ± 0.025 | 0.643 ± 0.021* | 0.630 ± 0.011* | 0.519 ± 0.023** | 0.420 ± 0.010** | |
| Relative | 3.541 ± 0.078 | 3.390 ± 0.114 | 3.489 ± 0.102 | 3.383 ± 0.072 | 3.425 ± 0.135 | |
| R. Kidney | | | | | | |
| Absolute | 0.834 ± 0.035 | 0.828 ± 0.009 | 0.768 ± 0.015 | 0.680 ± 0.032** | 0.555 ± 0.013** | |
| Relative | 4.218 ± 0.157 | 4.370 ± 0.109 | 4.246 ± 0.034 | 4.433 ± 0.072 | 4.524 ± 0.155 | |
| Liver | | | | | | |
| Absolute | 9.410 ± 0.226 | 9.601 ± 0.259 | 9.069 ± 0.238 | 7.651 ± 0.339** | 6.314 ± 0.076** | |
| Relative | 47.723 ± 1.747 | 50.585 ± 0.899 | 50.144 ± 1.132 | 49.866 ± 0.676 | 51.395 ± 1.012 | |
| Lung | | | | | | |
| Absolute | 1.120 ± 0.082 | 1.126 ± 0.114 | 1.014 ± 0.059 | 0.821 ± 0.077* | 0.749 ± 0.031** | |
| Relative | 5.686 ± 0.470 | 5.963 ± 0.667 | 5.639 ± 0.463 | 5.335 ± 0.384 | 6.124 ± 0.418 | |
| R. Testis | | | | | | |
| Absolute | 1.102 ± 0.043 | 1.165 ± 0.026 | 1.113 ± 0.034 | 1.067 ± 0.050 | 0.942 ± 0.020* | |
| Relative | 5.581 ± 0.204 | 6.138 ± 0.093* | 6.150 ± 0.117* | 6.959 ± 0.196** | 7.665 ± 0.170** | |
| Thymus | | | | | | |
| Absolute | 0.447 ± 0.024 | 0.404 ± 0.009 | 0.399 ± 0.017 | 0.309 ± 0.014** | 0.188 ± 0.012** | |
| Relative | 2.276 ± 0.171 | 2.130 ± 0.063 | 2.210 ± 0.105 | 2.019 ± 0.057 | 1.522 ± 0.073** | |
| **Female** | | | | | | |
| Necropsy body wt | 144 ± 5 | 135 ± 4 | 133 ± 4* | 123 ± 4** | 100 ± 2** | |
| Heart | | | | | | |
| Absolute | 0.506 ± 0.019 | 0.482 ± 0.015 | 0.473 ± 0.010 | 0.444 ± 0.013** | 0.370 ± 0.011** | |
| Relative | 3.503 ± 0.045 | 3.571 ± 0.137 | 3.577 ± 0.113 | 3.596 ± 0.067 | 3.702 ± 0.090 | |
| R. Kidney | | | | | | |
| Absolute | 0.620 ± 0.023 | 0.589 ± 0.020 | 0.577 ± 0.023 | 0.574 ± 0.042 | 0.456 ± 0.011** | |
| Relative | 4.290 ± 0.101 | 4.349 ± 0.069 | 4.346 ± 0.111 | 4.658 ± 0.318 | 4.573 ± 0.126 | |
| Liver | | | | | | |
| Absolute | 6.028 ± 0.237 | 6.130 ± 0.090[c] | 5.883 ± 0.162 | 5.766 ± 0.127 | 4.897 ± 0.145**[c] | |
| Relative | 41.745 ± 1.131 | 45.218 ± 1.032[c] | 44.400 ± 1.024 | 46.805 ± 1.090** | 48.867 ± 0.812**[c] | |
| Lung | | | | | | |
| Absolute | 0.851 ± 0.055 | 0.809 ± 0.054 | 0.760 ± 0.049 | 0.813 ± 0.070 | 0.624 ± 0.022** | |
| Relative | 5.879 ± 0.261 | 5.999 ± 0.450 | 5.723 ± 0.302 | 6.614 ± 0.613 | 6.258 ± 0.210 | |
| Thymus | | | | | | |
| Absolute | 0.359 ± 0.020 | 0.331 ± 0.020 | 0.345 ± 0.014 | 0.331 ± 0.014 | 0.176 ± 0.020** | |
| Relative | 2.505 ± 0.198 | 2.441 ± 0.096 | 2.608 ± 0.123 | 2.690 ± 0.128 | 1.770 ± 0.221* | |

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** Significantly different (P≤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).
[b] No data were available for the 50,000 ppm groups due to 100% mortality.
[c] n=4

**TABLE C2**
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | |
| Necropsy body wt | 320 ± 3 | 328 ± 5 | 307 ± 7 | 296 ± 4** | 279 ± 5** | 247 ± 4** |
| Heart | | | | | | |
| Absolute | 0.944 ± 0.030 | 0.963 ± 0.020 | 0.936 ± 0.025 | 0.919 ± 0.019 | 0.850 ± 0.019** | 0.766 ± 0.013** |
| Relative | 2.945 ± 0.076 | 2.938 ± 0.038 | 3.043 ± 0.032 | 3.106 ± 0.046 | 3.045 ± 0.055 | 3.105 ± 0.070 |
| R. Kidney | | | | | | |
| Absolute | 1.076 ± 0.031 | 1.083 ± 0.022 | 1.057 ± 0.025 | 1.064 ± 0.020 | 0.973 ± 0.028** | 0.883 ± 0.015** |
| Relative | 3.358 ± 0.075 | 3.306 ± 0.046 | 3.440 ± 0.032 | 3.597 ± 0.054* | 3.481 ± 0.055* | 3.573 ± 0.056** |
| Liver | | | | | | |
| Absolute | 10.774 ± 0.232 | 11.804 ± 0.254 | 11.345 ± 0.382 | 11.523 ± 0.297 | 10.746 ± 0.269 | 9.640 ± 0.195** |
| Relative | 33.672 ± 0.722 | 36.070 ± 0.775* | 36.883 ± 0.751** | 38.974 ± 0.911** | 38.474 ± 0.627** | 38.992 ± 0.556** |
| Lung | | | | | | |
| Absolute | 1.552 ± 0.060 | 1.742 ± 0.044 | 1.759 ± 0.062 | 1.680 ± 0.096 | 1.528 ± 0.070 | 1.434 ± 0.069 |
| Relative | 4.856 ± 0.199 | 5.329 ± 0.165 | 5.723 ± 0.141* | 5.657 ± 0.260 | 5.474 ± 0.231 | 5.819 ± 0.317* |
| R. Testis | | | | | | |
| Absolute | 1.450 ± 0.016 | 1.445 ± 0.027 | 1.456 ± 0.028 | 1.482 ± 0.017 | 1.413 ± 0.020 | 1.367 ± 0.023* |
| Relative | 4.532 ± 0.047 | 4.411 ± 0.061 | 4.743 ± 0.050 | 5.014 ± 0.040** | 5.076 ± 0.126** | 5.535 ± 0.085** |
| Thymus | | | | | | |
| Absolute | 0.370 ± 0.013 | 0.360 ± 0.009 | 0.332 ± 0.011* | 0.315 ± 0.008** | 0.294 ± 0.012** | 0.276 ± 0.011** |
| Relative | 1.158 ± 0.047 | 1.098 ± 0.024 | 1.080 ± 0.030 | 1.070 ± 0.039 | 1.053 ± 0.041 | 1.121 ± 0.055 |
| **Female** | | | | | | |
| Necropsy body wt | 184 ± 3 | 185 ± 2 | 186 ± 3 | 175 ± 2* | 170 ± 3** | 162 ± 3** |
| Heart | | | | | | |
| Absolute | 0.634 ± 0.018 | 0.634 ± 0.022 | 0.666 ± 0.014 | 0.598 ± 0.015 | 0.592 ± 0.012 | 0.563 ± 0.012** |
| Relative | 3.451 ± 0.091 | 3.430 ± 0.110 | 3.581 ± 0.062 | 3.414 ± 0.062 | 3.484 ± 0.059 | 3.481 ± 0.091 |
| R. Kidney | | | | | | |
| Absolute | 0.644 ± 0.016 | 0.658 ± 0.013 | 0.673 ± 0.013 | 0.606 ± 0.011 | 0.592 ± 0.016** | 0.558 ± 0.011** |
| Relative | 3.499 ± 0.050 | 3.561 ± 0.068 | 3.618 ± 0.049 | 3.458 ± 0.051 | 3.482 ± 0.066 | 3.449 ± 0.053 |
| Liver | | | | | | |
| Absolute | 6.171 ± 0.130 | 6.383 ± 0.121 | 6.816 ± 0.187 | 6.099 ± 0.112 | 5.908 ± 0.150 | 5.757 ± 0.080* |
| Relative | 33.554 ± 0.376 | 34.551 ± 0.519 | 36.599 ± 0.568** | 34.813 ± 0.386 | 34.778 ± 0.737 | 35.578 ± 0.390* |
| Lung | | | | | | |
| Absolute | 1.242 ± 0.046 | 1.187 ± 0.050 | 1.375 ± 0.050 | 1.200 ± 0.047 | 1.193 ± 0.042 | 1.163 ± 0.046 |
| Relative | 6.743 ± 0.193 | 6.429 ± 0.278 | 7.384 ± 0.215 | 6.850 ± 0.249 | 7.008 ± 0.171 | 7.206 ± 0.339 |
| Thymus | | | | | | |
| Absolute | 0.274 ± 0.008 | 0.271 ± 0.010 | 0.255 ± 0.011 | 0.237 ± 0.004** | 0.238 ± 0.005** | 0.228 ± 0.006** |
| Relative | 1.495 ± 0.049 | 1.466 ± 0.048 | 1.369 ± 0.049 | 1.354 ± 0.026 | 1.405 ± 0.036 | 1.413 ± 0.049 |

* 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).

**TABLE C3**
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 15-Day Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm | 25,000 ppm | 50,000 ppm |
|---|---|---|---|---|---|---|
| n | 5 | 5 | 5 | 5 | 5 | 0[b] |
| **Male** | | | | | | |
| Necropsy body wt | 23.4 ± 1.1 | 23.3 ± 0.4 | 22.9 ± 0.5 | 20.4 ± 0.6** | 16.6 ± 0.5** | |
| Heart | | | | | | |
| Absolute | 0.121 ± 0.006 | 0.118 ± 0.003 | 0.115 ± 0.003 | 0.105 ± 0.001* | 0.096 ± 0.006** | |
| Relative | 5.161 ± 0.095 | 5.083 ± 0.132 | 5.049 ± 0.130 | 5.146 ± 0.118 | 5.768 ± 0.253* | |
| R. Kidney | | | | | | |
| Absolute | 0.228 ± 0.011 | 0.225 ± 0.008 | 0.210 ± 0.009 | 0.185 ± 0.005** | 0.148 ± 0.009** | |
| Relative | 9.742 ± 0.222 | 9.651 ± 0.306 | 9.175 ± 0.297 | 9.100 ± 0.292 | 8.897 ± 0.327 | |
| Liver | | | | | | |
| Absolute | 1.208 ± 0.060 | 1.316 ± 0.033 | 1.396 ± 0.018* | 1.303 ± 0.056 | 1.134 ± 0.058 | |
| Relative | 51.493 ± 0.319 | 56.497 ± 0.502** | 61.063 ± 0.819** | 63.844 ± 0.946** | 68.076 ± 2.388** | |
| Lung | | | | | | |
| Absolute | 0.166 ± 0.010 | 0.154 ± 0.005 | 0.161 ± 0.004 | 0.163 ± 0.009 | 0.136 ± 0.007* | |
| Relative | 7.065 ± 0.130 | 6.613 ± 0.172 | 7.051 ± 0.253 | 8.000 ± 0.320* | 8.140 ± 0.278** | |
| R. Testis | | | | | | |
| Absolute | 0.100 ± 0.004 | 0.100 ± 0.003 | 0.097 ± 0.002 | 0.098 ± 0.001 | 0.091 ± 0.002 | |
| Relative | 4.303 ± 0.188 | 4.312 ± 0.074 | 4.241 ± 0.074 | 4.828 ± 0.165* | 5.476 ± 0.119** | |
| Thymus | | | | | | |
| Absolute | 0.046 ± 0.003 | 0.046 ± 0.003 | 0.051 ± 0.002 | 0.035 ± 0.004* | 0.012 ± 0.002** | |
| Relative | 1.992 ± 0.141 | 1.985 ± 0.125 | 2.240 ± 0.087 | 1.695 ± 0.173 | 0.719 ± 0.131** | |
| **Female** | | | | | | |
| Necropsy body wt | 20.6 ± 0.3 | 20.1 ± 0.6 | 20.1 ± 0.6 | 18.9 ± 0.6 | 14.1 ± 0.6** | |
| Heart | | | | | | |
| Absolute | 0.105 ± 0.002 | 0.100 ± 0.003 | 0.100 ± 0.003 | 0.090 ± 0.003** | 0.086 ± 0.005** | |
| Relative | 5.108 ± 0.064 | 4.987 ± 0.074 | 4.996 ± 0.140 | 4.783 ± 0.092 | 6.082 ± 0.262** | |
| R. Kidney | | | | | | |
| Absolute | 0.152 ± 0.003 | 0.148 ± 0.003 | 0.143 ± 0.004 | 0.134 ± 0.006** | 0.114 ± 0.005** | |
| Relative | 7.402 ± 0.071 | 7.368 ± 0.132 | 7.111 ± 0.158 | 7.084 ± 0.175 | 8.078 ± 0.214* | |
| Liver | | | | | | |
| Absolute | 0.975 ± 0.043 | 1.003 ± 0.044 | 1.076 ± 0.033 | 1.048 ± 0.033 | 0.885 ± 0.040 | |
| Relative | 47.357 ± 1.640 | 49.936 ± 1.094 | 53.634 ± 1.210** | 55.560 ± 1.015** | 62.675 ± 0.721** | |
| Lung | | | | | | |
| Absolute | 0.153 ± 0.005 | 0.143 ± 0.005 | 0.148 ± 0.004 | 0.164 ± 0.021 | 0.137 ± 0.005 | |
| Relative | 7.434 ± 0.213 | 7.146 ± 0.295 | 7.394 ± 0.326 | 8.680 ± 1.013 | 9.806 ± 0.641* | |
| Thymus | | | | | | |
| Absolute | 0.070 ± 0.003 | 0.068 ± 0.004 | 0.062 ± 0.004 | 0.073 ± 0.002 | 0.012 ± 0.002** | |
| Relative | 3.394 ± 0.182 | 3.412 ± 0.210 | 3.085 ± 0.128 | 3.856 ± 0.109 | 0.809 ± 0.102** | |

* 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] No data were available for the 50,000 ppm groups due to 100% mortality.

**TABLE C4**
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 781 ppm | 1,562 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | |
| Necropsy body wt | 33.8 ± 0.5 | 33.1 ± 0.8 | 34.3 ± 0.9 | 32.3 ± 0.6 | 31.6 ± 0.7* | 26.9 ± 0.7** |
| Heart | | | | | | |
| Absolute | 0.160 ± 0.003 | 0.159 ± 0.005 | 0.160 ± 0.002 | 0.156 ± 0.005 | 0.153 ± 0.003 | 0.137 ± 0.005** |
| Relative | 4.734 ± 0.089 | 4.817 ± 0.161 | 4.684 ± 0.106 | 4.834 ± 0.174 | 4.869 ± 0.133 | 5.103 ± 0.115 |
| R. Kidney | | | | | | |
| Absolute | 0.298 ± 0.008 | 0.293 ± 0.007 | 0.296 ± 0.006 | 0.281 ± 0.002 | 0.265 ± 0.006** | 0.234 ± 0.007** |
| Relative | 8.792 ± 0.174 | 8.835 ± 0.123 | 8.665 ± 0.162 | 8.711 ± 0.137 | 8.392 ± 0.171 | 8.724 ± 0.138 |
| Liver | | | | | | |
| Absolute | 1.519 ± 0.029 | 1.558 ± 0.038 | 1.577 ± 0.031 | 1.593 ± 0.027 | 1.674 ± 0.033 | 1.563 ± 0.078 |
| Relative | 44.881 ± 0.509 | 47.063 ± 0.699 | 46.092 ± 0.421 | 49.453 ± 0.910** | 53.083 ± 0.624** | 57.869 ± 1.807** |
| Lung | | | | | | |
| Absolute | 0.284 ± 0.017 | 0.272 ± 0.016 | 0.258 ± 0.016 | 0.288 ± 0.014 | 0.272 ± 0.011 | 0.250 ± 0.020 |
| Relative | 8.439 ± 0.542 | 8.257 ± 0.550 | 7.549 ± 0.462 | 8.897 ± 0.381 | 8.614 ± 0.324 | 9.283 ± 0.641 |
| R. Testis | | | | | | |
| Absolute | 0.114 ± 0.004 | 0.115 ± 0.002 | 0.110 ± 0.003 | 0.113 ± 0.003 | 0.113 ± 0.004 | 0.110 ± 0.004 |
| Relative | 3.380 ± 0.120 | 3.482 ± 0.070 | 3.232 ± 0.116 | 3.495 ± 0.070 | 3.582 ± 0.150 | 4.107 ± 0.128** |
| Thymus | | | | | | |
| Absolute | 0.044 ± 0.002 | 0.041 ± 0.002 | 0.041 ± 0.002 | 0.043 ± 0.003 | 0.041 ± 0.002 | 0.037 ± 0.003 |
| Relative | 1.303 ± 0.045 | 1.238 ± 0.042 | 1.199 ± 0.062 | 1.343 ± 0.087 | 1.292 ± 0.080 | 1.363 ± 0.085 |
| **Female** | | | | | | |
| Necropsy body wt | 26.5 ± 0.7 | 28.3 ± 0.6 | 26.0 ± 0.5 | 24.7 ± 0.5* | 24.0 ± 0.6** | 20.7 ± 0.3** |
| Heart | | | | | | |
| Absolute | 0.126 ± 0.003 | 0.124 ± 0.004 | 0.122 ± 0.002 | 0.119 ± 0.003 | 0.119 ± 0.005 | 0.096 ± 0.001** |
| Relative | 4.782 ± 0.122 | 4.383 ± 0.130 | 4.720 ± 0.077 | 4.816 ± 0.119 | 4.969 ± 0.157 | 4.638 ± 0.080 |
| R. Kidney | | | | | | |
| Absolute | 0.180 ± 0.006 | 0.180 ± 0.002 | 0.178 ± 0.004 | 0.173 ± 0.004 | 0.165 ± 0.005* | 0.133 ± 0.004** |
| Relative | 6.815 ± 0.133 | 6.368 ± 0.130 | 6.871 ± 0.133 | 7.041 ± 0.187 | 6.906 ± 0.104 | 6.421 ± 0.154 |
| Liver | | | | | | |
| Absolute | 1.185 ± 0.029 | 1.223 ± 0.031 | 1.200 ± 0.036 | 1.159 ± 0.024 | 1.201 ± 0.054 | 1.047 ± 0.026** |
| Relative | 44.848 ± 0.625 | 43.275 ± 0.732 | 46.143 ± 0.800 | 47.037 ± 0.940 | 50.014 ± 1.320** | 50.584 ± 1.059** |
| Lung | | | | | | |
| Absolute | 0.227 ± 0.016 | 0.226 ± 0.011 | 0.200 ± 0.006 | 0.221 ± 0.015 | 0.217 ± 0.013 | 0.200 ± 0.011 |
| Relative | 8.633 ± 0.619 | 8.030 ± 0.474 | 7.727 ± 0.285 | 8.905 ± 0.507 | 9.049 ± 0.499 | 9.627 ± 0.438 |
| Thymus | | | | | | |
| Absolute | 0.051 ± 0.002 | 0.054 ± 0.003 | 0.045 ± 0.002 | 0.048 ± 0.003 | 0.050 ± 0.002 | 0.029 ± 0.002** |
| Relative | 1.921 ± 0.061 | 1.915 ± 0.076 | 1.719 ± 0.075 | 1.929 ± 0.085 | 2.094 ± 0.085 | 1.407 ± 0.081** |

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's 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).

# APPENDIX D
# REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

**TABLE D1**
**Summary of Reproductive Tissue Evaluations for Male Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 |
| Weights (g) | | | | |
| Necropsy body wt | 320 ± 3 | 296 ± 4** | 279 ± 5** | 247 ± 4** |
| L. Cauda epididymis | | | | |
| Absolute | 0.1611 ± 0.0054 | 0.1593 ± 0.0057 | 0.1613 ± 0.0063 | 0.1364 ± 0.0044** |
| Relative | 0.50 ± 0.02 | 0.54 ± 0.02 | 0.58 ± 0.02* | 0.55 ± 0.02 |
| L. Epididymis | | | | |
| Absolute | 0.4706 ± 0.0061 | 0.4713 ± 0.0116 | 0.4634 ± 0.0109 | 0.4221 ± 0.0052** |
| Relative | 1.47 ± 0.02 | 1.59 ± 0.03 | 1.66 ± 0.04 | 1.71 ± 0.03 |
| L. Testis | | | | |
| Absolute | 1.5258 ± 0.0202 | 1.5280 ± 0.0207 | 1.5006 ± 0.0160 | 1.3843 ± 0.0799* |
| Relative | 4.77 ± 0.06 | 5.17 ± 0.06 | 5.39 ± 0.12 | 5.59 ± 0.32 |
| Spermatid and sperm measurements | | | | |
| Spermatid heads ($10^7$/g testis) | 159.84 ± 9.90 | 149.00 ± 9.12 | 165.01 ± 6.75 | 179.29 ± 6.25 |
| Spermatid heads ($10^7$/testis) | 181.88 ± 8.40 | 157.13 ± 9.76* | 168.69 ± 5.36 | 150.31 ± 3.26** |
| Sperm heads ($10^7$/g cauda epididymis) | 449.84 ± 21.42 | 482.92 ± 30.52 | 434.79 ± 16.93 | 505.94 ± 19.88 |
| Sperm heads ($10^7$/cauda epididymis) | 71.88 ± 3.07 | 76.37 ± 4.97 | 69.87 ± 3.50 | 68.76 ± 3.07 |
| Epididymal sperm motility (%) | 72.15 ± 0.96 | 70.87 ± 1.03 | 70.76 ± 0.78 | 67.08 ± 1.22** |

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test (tissue weights) or by Dunn's test (spermatid heads per testis)

** Significantly different ($P \leq 0.01$) from the control group by Williams' test (body and tissue weights), Dunn's test (spermatid heads per testis), or Shirley's test (motility)

[a] Data are presented as mean ± standard error. 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. Differences from the control group for spermatid heads per g testis, sperm heads per g cauda epididymis, and sperm heads per cauda epididymis are not significant by Dunn's test.

**TABLE D2**
**Estrous Cycle Characterization for Female Rats in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt (g) | 184 ± 3 | 175 ± 2* | 170 ± 3** | 162 ± 3** |
| Number of cycling females[b] | 10 | 10 | 5* | 4* |
| Number of females with regular estrous cycles | 10 | 8 | 2* | 2* |
| Number of estrous cycles | 1.8 ± 0.1 | 1.3 ± 0.2* | 1.0 ± 0.0* | 1.0 ± 0.0* |
| Estrous cycle length (days) | 4.750 ± 0.201 | 5.500 ± 0.316 | 6.200 ± 0.663* | 7.000 ± 0.577** |
| Estrous stages[c] (% of cycle) | | | | |
| Diestrus | 47.1 | 57.5 | 78.3 | 77.1 |
| Proestrus | 15.1 | 14.2 | 4.2 | 5.9 |
| Estrus | 23.5 | 17.5 | 11.7 | 10.2 |
| Metestrus | 14.3 | 10.8 | 5.8 | 6.8 |

* Significantly different ($P \leq 0.05$) from the control group by Williams' test (body weights), chi-square test (numbers of cycling females and of females with regular cycles), or Shirley's test (number of estrous cycles and estrous cycle length)
** $P \leq 0.01$
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error.
[b] Does not include animals with estrous cycles that were longer than 12 days or unclear
[c] Evidence shows that females exposed to 6,250 or 12,500 ppm differ significantly (Wilk's Criterion: 6,250 ppm, $P \leq 0.05$; 12,500 ppm, $P \leq 0.01$) from the control females in the relative length of time spent in the estrous stages. Exposed females spent more time in diestrus and less time in proestrus, estrus, and metestrus than did the control females.

**TABLE D3**
**Summary of Reproductive Tissue Evaluations for Male Mice in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 |
| Weights (g) | | | | |
| Necropsy body wt | 33.8 ± 0.5 | 32.3 ± 0.5 | 31.6 ± 0.7* | 26.9 ± 0.7** |
| L. Cauda epididymis | | | | |
| Absolute | 0.0146 ± 0.0004 | 0.0138 ± 0.0004 | 0.0151 ± 0.0004 | 0.0132 ± 0.0006 |
| Relative | 0.43 ± 0.01 | 0.43 ± 0.01 | 0.48 ± 0.02* | 0.49 ± 0.01** |
| L. Epididymis | | | | |
| Absolute | 0.0511 ± 0.0017 | 0.0477 ± 0.0015 | 0.0488 ± 0.0019 | 0.0466 ± 0.0020 |
| Relative | 1.52 ± 0.06 | 1.48 ± 0.05 | 1.55 ± 0.06 | 1.74 ± 0.05* |
| L. Testis | | | | |
| Absolute | 0.1107 ± 0.0023 | 0.1074 ± 0.0035[b] | 0.1088 ± 0.0041 | 0.1045 ± 0.0040 |
| Relative | 3.28 ± 0.08 | 3.32 ± 0.10[b] | 3.46 ± 0.14 | 3.90 ± 0.11** |
| Spermatid and sperm measurements | | | | |
| Spermatid heads ($10^7$/g testis) | 507.65 ± 112.51 | 309.17 ± 32.70 | 302.56 ± 22.08 | 310.68 ± 12.60[b] |
| Spermatid heads ($10^7$/testis) | 21.23 ± 1.56 | 22.55 ± 1.39 | 22.92 ± 1.33 | 20.27 ± 1.45[b] |
| Sperm heads ($10^7$/g cauda epididymis) | 958.64 ± 41.79 | 1,130.09 ± 63.98 | 871.20 ± 102.15 | 1,029.41 ± 86.14 |
| Sperm heads ($10^7$/cauda epididymis) | 13.99 ± 0.66 | 15.65 ± 1.03 | 13.14 ± 1.57 | 13.44 ± 1.24 |
| Epididymal sperm motility (%) | 69.10 ± 0.43 | 69.40 ± 0.97 | 68.97 ± 1.81 | 68.54 ± 0.58 |

* Significantly different ($P \le 0.05$) from the control group by Williams' test (body weight) or Dunnett's test (cauda epidiymis weight)
** Significantly different ($P \le 0.01$) from the control group by Williams' test
[a] Data are presented as mean ± standard error. 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. Differences from the control group for spermatid measurements and epididymal sperm motility are not significant by Dunn's test.
[b] n=9

**TABLE D4**
**Estrous Cycle Characterization for Female Mice in the 14-Week Feed Study of *p-tert*-Butylcatechol[a]**

| | 0 ppm | 3,125 ppm | 6,250 ppm | 12,500 ppm |
|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt (g) | 26.5 ± 0.7 | 24.7 ± 0.5* | 24.0 ± 0.6** | 20.7 ± 0.3** |
| Number of cycling females[b] | 10 | 8 | 10 | 9 |
| Number of females with regular estrous cycles | 8 | 8 | 8 | 6 |
| Number of estrous cycles | 1.7 ± 0.2 | 2.3 ± 0.2 | 1.8 ± 0.2 | 1.1 ± 0.1 |
| Estrous cycle length (days) | 4.400 ± 0.314 | 4.238 ± 0.163 | 4.070 ± 0.131 | 5.222 ± 0.278* |
| Estrous stages (% of cycle) | | | | |
| Diestrus | 47.1 | 43.3 | 37.5 | 50.4 |
| Proestrus | 3.4 | 8.3 | 0.8 | 5.9 |
| Estrus | 30.3 | 29.2 | 40.0 | 27.7 |
| Metestrus | 19.3 | 19.2 | 21.7 | 16.0 |

* Significantly different ($P \leq 0.05$) from the control group by Williams' test (body weight) or Dunn's test (estrous cycle length)
** Significantly different ($P \leq 0.01$) from the control group by Williams' test (body weight)
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. 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] Does not include animals with estrous cycles that were longer than 12 days or unclear

# APPENDIX E
# GENETIC TOXICOLOGY

**TABLE E1**
**Mutagenicity of *p-tert*-Butylcatechol in *Salmonella typhimurium*[a]**

| Strain | Dose (µg/plate) | Revertants/Plate[b] –S9 | +hamster S9 10% | +hamster S9 30% | +rat S9 10% | +rat S9 30% |
|---|---|---|---|---|---|---|
| **TA102** | 0 | 221 ± 2.5 | 350 ± 3.5 | 312 ± 5.0 | 330 ± 3.2 | 297 ± 3.5 |
| | 10 | 220 ± 3.1 | 354 ± 3.3 | 313 ± 3.8 | 327 ± 3.8 | 296 ± 6.7 |
| | 33 | 219 ± 2.9 | 353 ± 5.2 | 312 ± 4.9 | 331 ± 4.5 | 291 ± 3.0 |
| | 100 | 217 ± 4.1 | 339 ± 5.0 | 296 ± 6.7 | 331 ± 1.9 | 302 ± 2.6 |
| | 333 | 213 ± 4.0 | 322 ± 2.2 | 313 ± 4.8 | 328 ± 3.2 | 314 ± 5.2 |
| | 1,000 | 174 ± 4.9[c] | 303 ± 4.1 | 296 ± 2.5 | 316 ± 3.8 | 294 ± 4.9 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative |
| Positive control[d] | | 1,046 ± 22.4 | 1,085 ± 6.7 | 991 ± 10.8 | 1,057 ± 22.0 | 932 ± 6.8 |
| **TA104** | 0 | 349 ± 3.8 | 372 ± 6.2 | 240 ± 2.8 | 384 ± 3.2 | 259 ± 4.3 |
| | 10 | 338 ± 2.4 | 371 ± 3.1 | 254 ± 5.0 | 369 ± 3.5 | 249 ± 2.6 |
| | 33 | 337 ± 3.5 | 375 ± 3.5 | 234 ± 4.0 | 375 ± 4.9 | 268 ± 7.5 |
| | 100 | 349 ± 4.1 | 374 ± 4.2 | 247 ± 4.6 | 384 ± 3.5 | 293 ± 4.9 |
| | 333 | 355 ± 3.2 | 381 ± 3.5 | 223 ± 7.6 | 376 ± 4.3 | 284 ± 8.1 |
| | 1,000 | 305 ± 4.0 | 370 ± 2.3 | 255 ± 5.2 | 379 ± 1.9 | 256 ± 4.1 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 1,074 ± 5.4 | 1,031 ± 9.6 | 968 ± 13.6 | 1,065 ± 11.3 | 936 ± 15.0 |
| **TA100** | 0 | 131 ± 3.5 | 143 ± 3.0 | 145 ± 3.3 | 128 ± 3.5 | 147 ± 3.6 |
| | 10 | 129 ± 3.5 | 145 ± 2.0 | 142 ± 3.3 | 130 ± 2.3 | 138 ± 2.7 |
| | 33 | 134 ± 2.0 | 149 ± 2.6 | 133 ± 3.8 | 131 ± 3.3 | 142 ± 3.8 |
| | 100 | 128 ± 3.2 | 145 ± 2.7 | 136 ± 3.2 | 127 ± 2.4 | 139 ± 2.6 |
| | 333 | 131 ± 2.1 | 137 ± 2.4 | 133 ± 3.8 | 138 ± 2.6 | 145 ± 0.9 |
| | 1,000 | 131 ± 3.8 | 137 ± 3.3 | 139 ± 3.5 | 131 ± 2.3 | 140 ± 3.8 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 625 ± 9.0 | 714 ± 16.3 | 751 ± 19.8 | 616 ± 6.7 | 582 ± 10.7 |
| **TA1535** | 0 | 22 ± 1.7 | 20 ± 1.2 | 20 ± 1.8 | 20 ± 3.5 | 15 ± 1.3 |
| | 10 | 26 ± 3.0 | 17 ± 1.2 | 19 ± 1.9 | 19 ± 1.8 | 19 ± 0.9 |
| | 33 | 22 ± 3.2 | 20 ± 1.9 | 18 ± 1.2 | 17 ± 1.5 | 21 ± 1.2 |
| | 100 | 30 ± 0.9 | 17 ± 2.4 | 21 ± 2.2 | 17 ± 1.2 | 18 ± 1.3 |
| | 333 | 23 ± 3.8 | 19 ± 2.9 | 21 ± 2.6 | 21 ± 1.5 | 17 ± 1.9 |
| | 1,000 | 24 ± 0.3 | 19 ± 1.5 | 20 ± 0.9 | 16 ± 0.7 | 13 ± 2.5 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 430 ± 13.5 | 300 ± 10.7 | 255 ± 4.7 | 271 ± 7.9 | 240 ± 5.5 |
| **TA97** | 0 | 127 ± 4.1 | 139 ± 2.6 | 123 ± 1.5 | 134 ± 2.7 | 133 ± 3.8 |
| | 10 | 127 ± 3.2 | 133 ± 2.6 | 135 ± 3.5 | 133 ± 2.3 | 139 ± 2.6 |
| | 33 | 135 ± 4.4 | 138 ± 3.1 | 140 ± 0.6 | 131 ± 2.7 | 144 ± 2.9 |
| | 100 | 125 ± 3.2 | 133 ± 4.3 | 129 ± 4.4 | 133 ± 2.9 | 147 ± 2.9 |
| | 333 | 126 ± 3.5 | 141 ± 2.3 | 111 ± 3.1 | 133 ± 3.5 | 132 ± 3.9 |
| | 1,000 | 132 ± 2.4 | 135 ± 3.8 | 135 ± 3.3 | 131 ± 2.6 | 132 ± 2.5 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 329 ± 5.6 | 541 ± 6.1 | 816 ± 9.3 | 703 ± 26.2 | 318 ± 9.5 |

**TABLE E1**
**Mutagenicity of *p-tert*-Butylcatechol in *Salmonella typhimurium***

| Strain | Dose (µg/plate) | Revertants/Plate –S9 | +hamster S9 10% | +hamster S9 30% | +rat S9 10% | +rat S9 30% |
|---|---|---|---|---|---|---|
| **TA98** | 0 | 37 ± 2.5 | 35 ± 2.3 | 43 ± 2.6 | 32 ± 2.2 | 40 ± 2.3 |
| | 10 | 29 ± 2.2 | 48 ± 4.4 | 39 ± 3.2 | 38 ± 3.6 | 49 ± 2.6 |
| | 33 | 31 ± 2.6 | 39 ± 4.6 | 41 ± 2.8 | 41 ± 2.6 | 44 ± 3.1 |
| | 100 | 27 ± 2.1 | 43 ± 2.1 | 41 ± 3.0 | 34 ± 2.6 | 42 ± 4.8 |
| | 333 | 23 ± 0.9 | 38 ± 4.8 | 40 ± 3.8 | 26 ± 2.3 | 42 ± 2.6 |
| | 1,000 | 21 ± 1.5 | 40 ± 4.0 | 41 ± 2.7 | 20 ± 1.5 | 37 ± 3.2 |
| Trial summary | | Negative | Negative | Negative | Negative | Negative |
| Positive control | | 304 ± 5.2 | 971 ± 16.3 | 882 ± 26.0 | 347 ± 6.2 | 261 ± 10.7 |

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Zeiger *et al*. (1992).
[b] 0 µg/plate was the solvent control.
[c] Revertants are presented as mean ± standard error from three plates.
[d] Slight toxicity
The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), 4-nitro-*o*-phenylenediamine (TA98), mytomycin-C (TA102), and methyl methanesulfonate (TA104). The positive control for metabolic activation with all strains was 2-aminoanthracene, and 2-aminoanthracene or sterigmatocystin was used for TA102.

**TABLE E2**
**Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with *p-tert*-Butylcatechol by Intraperitoneal Injection[a]**

| Compound | Dose (mg/kg) | Number of Rats with Erythrocytes Scored | Micronucleated PCEs/ 1,000 PCEs[b] | P Value[c] |
|---|---|---|---|---|
| **Trial 1** | | | | |
| Corn oil[d] | | 5 | 1.00 ± 0.16 | |
| *p-tert*-Butylcatechol | 125 | 5 | 1.80 ± 0.66 | 0.1225 |
| | 250 | 5 | 2.90 ± 0.83 | 0.0050 |
| | 500 | 1 | 2.00[e] | |
| | | | P=0.010[f] | |
| Cyclophosphamide[g] | 10 | 5 | 17.80 ± 2.41 | 0.0000 |
| **Trial 2** | | | | |
| Corn oil | | 5 | 1.80 ± 0.34 | |
| *p-tert*-Butylcatechol | 125 | 5 | 3.00 ± 0.69 | 0.0414 |
| | 250 | 3 | 1.33 ± 0.17 | 0.7610 |
| | 300 | 4 | 2.13 ± 0.24 | 0.3114 |
| | | | P=0.582 | |
| Cyclophosphamide | 10 | 5 | 11.70 ± 2.08 | 0.0000 |

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al*. (1993).
[b] PCE=polychromatic erythrocyte
[c] Mean ± standard error
[d] Pairwise comparison with the vehicle control. Dosed group values are significant at P≤0.013 (Trial 1) or P≤0.008 (Trial 2); positive control values are significant at P≤0.05 (ILS, 1990).
[e] Vehicle control
[f] Due to excess lethality at this dose, data were not included in statistical analyses.
[g] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
Positive control

**TABLE E3**
**Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of *p-tert*-Butylcatechol in Feed for 14 Weeks[a]**

| Concentration (ppm) | Number of Mice with Erythrocytes Scored | Micronucleated NCEs/ 1,000 NCEs[b] | P Value[c] | PCEs (%) |
|---|---|---|---|---|
| **Male** | | | | |
| 0 | 10 | 0.40 ± 0.12 | | 2.0 |
| 781 | 10 | 0.40 ± 0.10 | 0.5000 | 1.8 |
| 1,562 | 10 | 0.60 ± 0.20 | 0.1855 | 1.7 |
| 3,125 | 10 | 0.55 ± 0.16 | 0.2456 | 1.6 |
| 6,250 | 10 | 0.70 ± 0.08 | 0.1004 | 1.7 |
| 12,500 | 10 | 0.60 ± 0.15 | 0.1855 | 1.9 |
| | | P=0.159[d] | | |
| **Female** | | | | |
| 0 | 10 | 0.70 ± 0.15 | | 1.7 |
| 781 | 10 | 0.55 ± 0.12 | 0.7258 | 1.7 |
| 1,562 | 10 | 0.50 ± 0.11 | 0.7930 | 1.7 |
| 3,125 | 10 | 0.45 ± 0.14 | 0.8515 | 2.1 |
| 6,250 | 10 | 0.70 ± 0.17 | 0.5000 | 1.8 |
| 12,500 | 10 | 0.75 ± 0.11 | 0.4263 | 1.7 |
| | | P=0.186 | | |

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al*. (1990).
NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the controls, significant at P≤0.005 (ILS, 1990)
[d] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

# APPENDIX F
# CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

# CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

## PROCUREMENT AND CHARACTERIZATION OF *P-TERT*-BUTYLCATECHOL

*p-tert*-Butylcatechol was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (03404 MF and 19115EN); lot 03404 MF was used during the 15-day studies and lot 19115EN was used during the 14-week studies. Identity, purity, and stability analyses were conducted by the study laboratories. Reports on analyses performed in support of the *p-tert*-butylcatechol studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a whitish, flaky solid, was identified as *p-tert*-butylcatechol by infrared spectroscopy (lots 03404 MF and 19115EN) and proton and carbon-13 nuclear magnetic resonance spectroscopy (lot 19115EN). All spectra were consistent with the literature spectra (*Aldrich*, 1981) or the structure of *p-tert*-butylcatechol. The infrared and nuclear magnetic resonance spectra are presented in Figures F1 through F3.

The purity of each lot was determined by high-performance liquid chromatography (HPLC) by system A (lot 03404 MF) or system B (lot 19115EN) (Table F1). Major peak comparisons of lot 03404 MF with lot 19115EN were performed with HPLC by system B.

For lot 03404 MF, results of HPLC analyses indicated the major product peak and three impurities. One major impurity peak had an area of 0.3% relative to the major peak; two minor impurities had relative areas of less than 0.1%. For lot 19115EN, results of HPLC analyses indicated the major product peak and two impurity peaks with areas of 0.26% and 0.20% relative to the major peak area. The overall purity of each lot was determined to be greater than 99%. Major peak comparisons indicated nearly identical purity profiles and impurity concentrations between the two lots.

Accelerated stability studies of lot 03404 MF of the bulk chemical were conducted with HPLC by system A. These studies indicated that *p-tert*-butylcatechol is stable as a bulk chemical for at least 14 days when stored under a nitrogen headspace, protected from strong oxidizers, at temperatures up to approximately 60° C. Based on the results of these studies and on information in the literature, the bulk chemical was stored under a nitrogen headspace (lot 03404 MF) in amber glass bottles, in the dark, at room temperature. No degradation of the bulk chemical was detected.

## PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared by mixing *p-tert*-butylcatechol with nonirradiated feed once (15-day studies) or with irradiated feed approximately every 4 weeks (14-week studies) (Table F2). A premix of ground *p-tert*-butylcatechol and feed was prepared by hand and then blended with additional feed in a twin-shell blender for 15 minutes. Formulations were stored in plastic bags at –20° C for up to 3 weeks during the 15-day studies and in plastic bags inside buckets at 5° C for up to 42 days during the 14-week studies.

Homogeneity studies of 3,125 and 50,000 ppm dose formulations in nonirradiated feed (15-day studies) and 781 and 12,500 ppm dose formulations in irradiated feed (14-week studies) and stability studies of the 781 and 3,125 ppm dose formulations were performed by the study laboratories. The 3,125 and 50,000 ppm dose formulations were analyzed with HPLC by system B; the 781 and 12,500 ppm dose formulations were extracted with a modified process employing a Soxhlet apparatus and analyzed with HPLC by system C. Homogeneity was confirmed. Stability of the 3,125 ppm dose formulation was confirmed for 35 days for samples stored at approximately –20° C. Stability of the 781 ppm dose formulation was confirmed for 42 days for samples stored in

plastic bags at temperatures up to 5° C; samples subjected to animal room conditions showed declines in *p-tert*-butylcatechol concentrations with time.

Periodic analyses of the dose formulations of *p-tert*-butylcatechol were conducted by the study laboratories using HPLC by system A (15-day studies) or system C (14-week studies). For the 15-day studies, dose formulations were analyzed once; all five were within 10% of the target concentrations, with no value greater than 105% of the target concentration (Table F3). Animal room samples of these dose formulations were also analyzed; the concentrations of all samples were 59% to 81% of the target concentrations. For the 14-week studies, dose formulations were analyzed at the beginning, midpoint, and end of the studies; 14 of 15 were within 10% of the target concentrations with no value greater than 105% of the target concentration (Table F4). The single dose formulation that was not within specifications was remixed and analyzed and was found to be within 10% of the target concentration. Animal room samples of these dose formulations were also analyzed; the concentrations of 9 of 15 animal room samples for rats and 13 of 15 for mice were less than 90% of the target concentrations. The low concentrations of animal room samples were attributed to chemical degradation, oxidation, evaporation, or binding with feed.

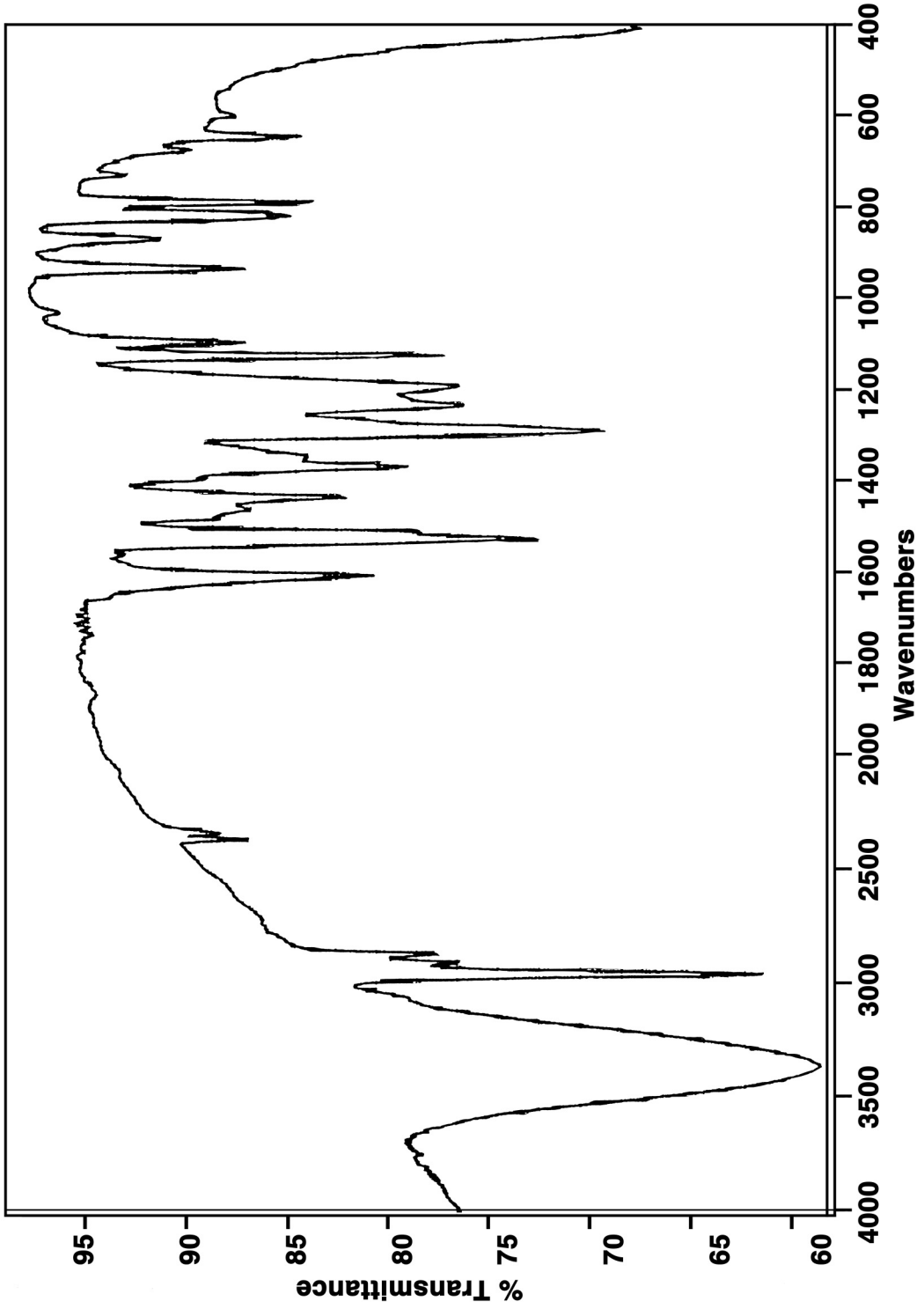


**Figure F1**
**Infrared Absorption Spectrum of *p-tert*-Butylcatechol**

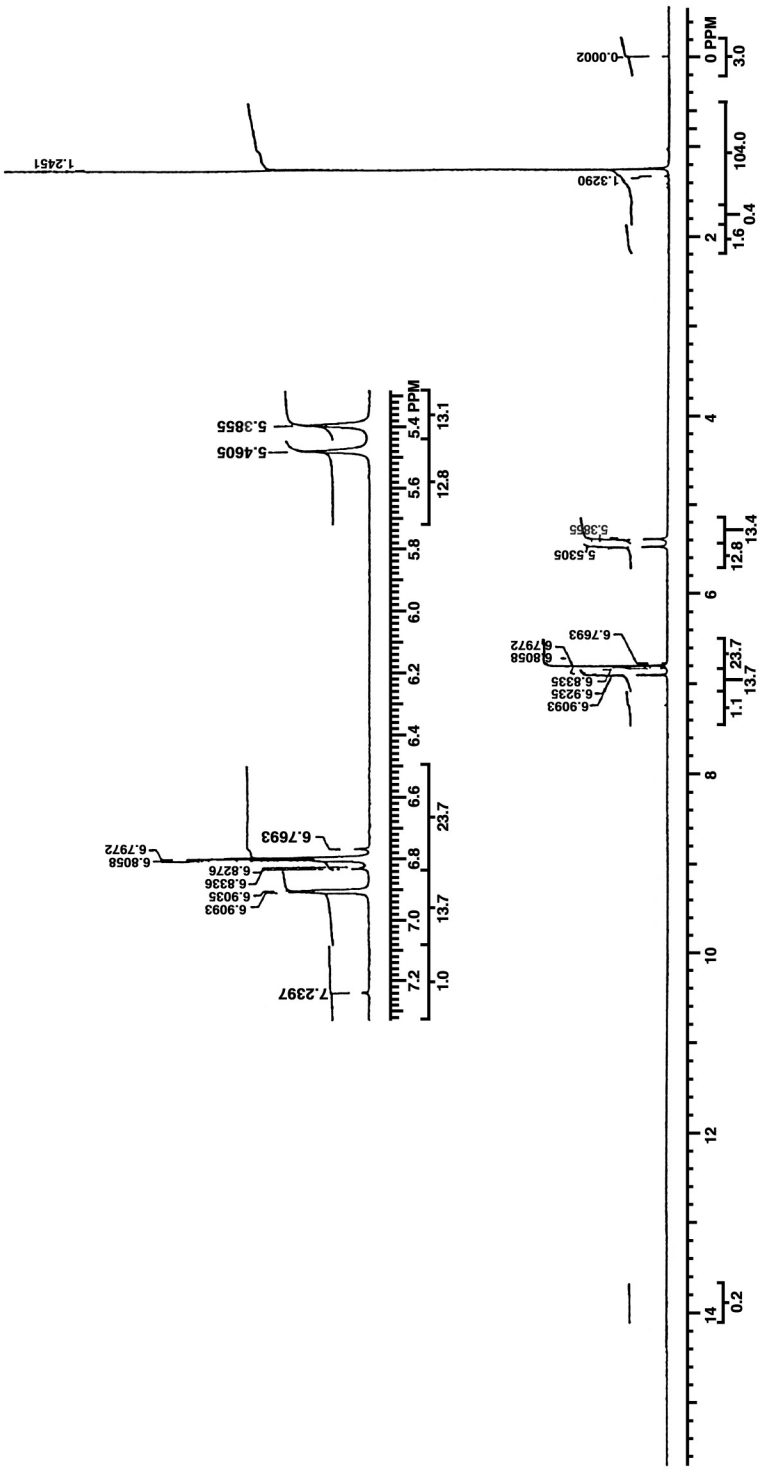


**Figure F2**
**Proton Nuclear Magnetic Resonance Spectrum of *p-tert*-Butylcatechol**

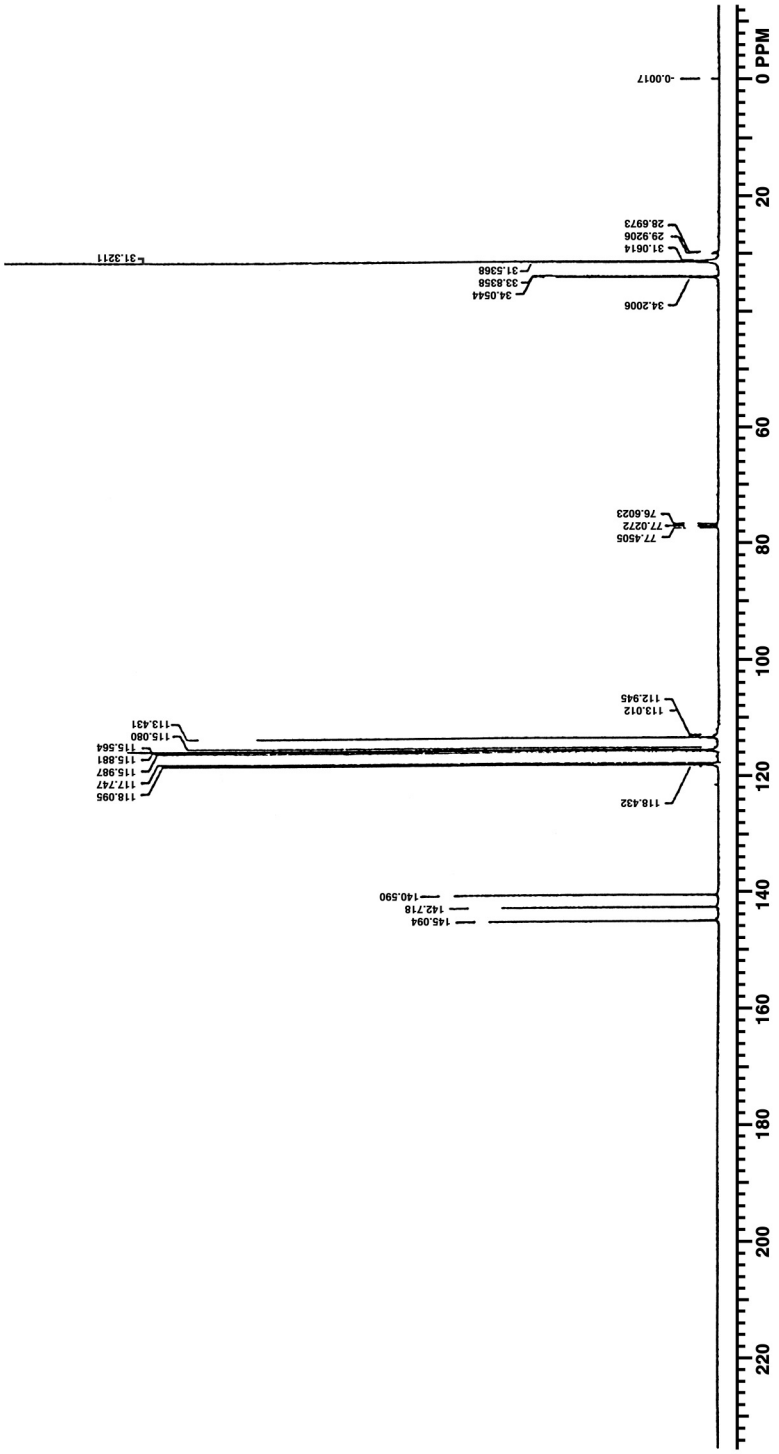


**Figure F3**
**$^{13}$C-Nuclear Magnetic Resonance Spectrum of *p-tert*-Butylcatechol**

**TABLE F1**
**High-Performance Liquid Chromatography Systems Used in the Feed Studies of *p-tert*-Butylcatechol**

| Detection System | Column | Solvent System |
|---|---|---|
| **System A** | | |
| Ultraviolet (278 nm) | Ultrasphere ODS, 150 mm × 4 mm, 5-µm particle size (Beckman, Fullerton, CA) | 1% aqueous glacial acetic acid:acetonitrile (60:40) at a flow rate of 1.0 or 1.5 mL/minute |
| **System B** | | |
| Ultraviolet (282 nm) | Inertsil ODS-2, 150 mm × 4.6 mm, 5-µm particle size (Phenomenex, Torrance, CA) | 1% acetic acid:acetonitrile; 65:35 for 15 minutes, then 65:35 to 10:90 in 20 minutes, held for 10 minutes |
| **System C** | | |
| Ultraviolet (278 nm) | Prodigy 5 ODS-3, 150 mm × 4.6 mm, 5-µm particle size (Phenomenex) | 1% acetic acid:acetonitrile (60:40), isocratic; flow rate 1.5 mL/minute |

**TABLE F2**
**Preparation and Storage of Dose Formulations in the Feed Studies of *p-tert*-Butylcatechol**

| 15-Day Studies | 14-Week Studies |
|---|---|
| **Preparation** | |
| *p-tert*-Butylcatechol was ground in a mortar and passed through a No. 20 sieve. A premix of feed and *p-tert*-butylcatechol was then prepared and layered with the remaining feed in a twin-shell blender and blended with the intensifier bar on for 5 minutes and off for 10 minutes. Dose formulations were prepared once, at the beginning of the studies. | *p-tert*-Butylcatechol was ground in a mortar to a semifine powder. A premix of feed and *p-tert*-butylcatechol was then prepared and and ground in a mortar; the premix was passed through sieves (781 ppm, 20- and 40-mesh; 12,500 ppm, 14-mesh; other premixes, 14- and 20-mesh). The premix was layered with the remaining feed in a Patterson-Kelly twin-shell blender and mixed for approximately 15 minutes. Dose formulations were prepared approximately every 4 weeks. |
| **Chemical Lot Number** | |
| 03404 MF | 19115EN |
| **Maximum Storage Time** | |
| 3 weeks | 42 days |
| **Storage Conditions** | |
| Stored in sealed, double plastic bags at –20° C | Stored in plastic bags inside buckets at approximately 5° C |
| **Study Laboratory** | |
| Microbiological Associates, Inc. (Bethesda, MD) | Battelle Columbus Laboratories (Columbus, OH) |

**TABLE F3**
**Results of Analyses of Dose Formulations Administered to Rats and Mice in the 15-Day Feed Studies of *p-tert*-Butylcatechol**

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration[a] (ppm) | Difference from Target (%) |
|---|---|---|---|---|
| **Rats** | | | | |
| March 13, 1995 | March 13, 1995 | 3,125 | 2,870 | –8 |
| | | 6,250 | 6,350[b] | +2 |
| | | 12,500 | 13,100[b] | +5 |
| | | 25,000 | 24,700 | –1 |
| | | 50,000 | 47,100 | –6 |
| | April 6, 1995[c] | 3,125 | 2,060 | –34 |
| | | 6,250 | 4,340 | –31 |
| | | 12,500 | 9,390 | –25 |
| | | 25,000 | 20,200 | –19 |
| **Mice** | | | | |
| March 13, 1995 | March 13, 1995 | 3,125 | 2,870 | –8 |
| | | 6,250 | 6,350[b] | +2 |
| | | 12,500 | 13,100[b] | +5 |
| | | 25,000 | 24,700 | –1 |
| | | 50,000 | 47,100 | –6 |
| | April 6, 1995[c] | 3,125 | 1,830[b] | –41 |
| | | 6,250 | 3,820 | –39 |
| | | 12,500 | 8,370 | –33 |
| | | 25,000 | 18,800 | –25 |

[a] Results of duplicate analyses
[b] Results of triplicate analyses
[c] Animal room samples

**TABLE F4**
**Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of *p-tert*-Butylcatechol**

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration[a] (ppm) | Difference from Target (%) |
|---|---|---|---|---|
| **Rats** | | | | |
| August 20, 1996 | August 22, 1996 | 781 | 822 | +5 |
| | | 1,562 | 1,509 | –3 |
| | | 3,125 | 3,142 | +1 |
| | | 6,250 | 6,494 | +4 |
| | | 12,500 | 11,342 | –9 |
| | September 26, 1996[b] | 781 | 670 | –14 |
| | | 1,562 | 1,444 | –8 |
| | | 3,125 | 2,990 | –4 |
| | | 6,250 | 6,116 | –2 |
| | | 12,500 | 11,591 | –7 |
| September 17, 1996 | September 20, 23, and 24, 1996 | 781 | 768 | –2 |
| | | 1,562 | 1,462 | –6 |
| | | 3,125 | 3,049 | –2 |
| | | 6,250 | 5,745 | –8 |
| | | 12,500 | 11,831 | –5 |
| | October 25, 1996[b] | 781 | 602 | –23 |
| | | 1,562 | 1,283 | –18 |
| | | 3,125 | 2,516 | –19 |
| | | 6,250 | 4,442 | –29 |
| | | 12,500 | 9,158 | –27 |
| November 12, 1996 | November 18, 1996 | 781 | 781 | 0 |
| | | 1,562 | 1,149[c] | –26 |
| | | 3,125 | 2,827 | –10 |
| | | 6,250 | 5,685 | –9 |
| | | 12,500 | 11,546 | –8 |
| November 19, 1996 | November 21, 1996 | 1,562 | 1,480[d] | –5 |
| | December 12 and 13, 1996[b] | 781 | 582 | –25 |
| | | 1,562 | 1,266 | –19 |
| | | 3,125 | 2,897 | –7 |
| | | 6,250 | 5,352 | –14 |
| | | 12,500 | 11,612 | –7 |

**TABLE F4**
**Results of Analyses of Dose Formulations Administered to Rats and Mice in the 14-Week Feed Studies of *p-tert*-Butylcatechol**

| Date Prepared | Date Analyzed | Target Concentration (ppm) | Determined Concentration (ppm) | Difference from Target (%) |
|---|---|---|---|---|
| **Mice** | | | | |
| August 20, 1996 | August 22, 1996 | 781 | 822 | +5 |
| | | 1,562 | 1,509 | –3 |
| | | 3,125 | 3,142 | +1 |
| | | 6,250 | 6,494 | +4 |
| | | 12,500 | 11,342 | –9 |
| | September 26, 1996[b] | 781 | 571 | –27 |
| | | 1,562 | 1,204 | –23 |
| | | 3,125 | 2,679 | –14 |
| | | 6,250 | 4,688 | –25 |
| | | 12,500 | 10,302 | –18 |
| September 17, 1996 | September 20, 23, and 24, 1996 | 781 | 768 | –2 |
| | | 1,562 | 1,462 | –6 |
| | | 3,125 | 3,049 | –2 |
| | | 6,250 | 5,745 | –8 |
| | | 12,500 | 11,831 | –5 |
| | October 25, 1996[b] | 781 | 608 | –22 |
| | | 1,562 | 1,275 | –18 |
| | | 3,125 | 2,769 | –11 |
| | | 6,250 | 4,549 | –27 |
| | | 12,500 | 9,355 | –25 |
| November 12, 1996 | November 18, 1996 | 781 | 781 | 0 |
| | | 1,562 | 1,149[c] | –26 |
| | | 3,125 | 2,827 | –10 |
| | | 6,250 | 5,685 | –9 |
| | | 12,500 | 11,546 | –8 |
| November 19, 1996 | November 21, 1996 | 1,562 | 1,480[d] | –5 |
| | December 12 and 13, 1996[b] | 781 | 672 | –14 |
| | | 1,562 | 1,425 | –9 |
| | | 3,125 | 2,798 | –10 |
| | | 6,250 | 5,271 | –16 |
| | | 12,500 | 10,036 | –20 |

[a] Results of duplicate analyses
[b] Animal room samples
[c] Remixed; not used in study
[d] Results of remix

# APPENDIX G
# ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION STUDIES

# ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION STUDIES

## INTRODUCTION

Studies were conducted in adult male F344/N rats and B6C3F$_1$ mice to determine the absorption, distribution, metabolism, and excretion of *p-tert*-butylcatechol following intravenous injection, gavage dosing, or dermal application. These studies were conducted by Research Triangle Institute (Research Triangle Park, NC).

## MATERIALS AND METHODS

[$^{14}$C]-*p-tert*-Butylcatechol (15.0 mCi/mmol; lot 940207), labeled on the methine carbon, was obtained from Wizard Laboratories (West Sacramento, CA); the radiochemical purity was determined to be approximately 94% by high-performance liquid chromatography (HPLC) with a DuPont Zorbax Rx-C$_{18}$ column, a mobile phase of methanol:water (55:45) at a flow rate of 1 mL/minute, and a Ramona-5-LS radioactivity detector with a 0.5-mL solid scintillate flow cell. Radioactivity eluting in each fraction was measured by liquid scintillation spectrometry (LSS). Nonradiolabeled *p-tert*-butylcatechol (lot DF04501LZ) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI); the chemical was identified as *p-tert*-butylcatechol by proton nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry.

Male F344/N rats and B6C3F$_1$ mice were obtained from Charles River Laboratories, Inc. (Raleigh, NC, and Portage, MI). Animals were quarantined for at least 1 week; rats were 13 to 15 weeks old and mice were 9 to 10 weeks old when the studies began. Animals received certified Purina Rodent Chow No. 5002 and tap water *ad libitum*. Rats and mice were housed in polycarbonate cages; the day before dosing, animals were transferred to individual glass metabolism chambers that allowed for separate collection of urine and feces.

Rats designated for plasma *p-tert*-butylcatechol concentration analyses were anesthetized with an intraperitoneal dose of 60 mg/kg ketamine:xylazine (7:1) and then implanted with indwelling jugular cannulae to facilitate the collection of blood samples. The design of the cannula was similar to that of Harms and Ojeda (1974) as modified by McKenna and Bieri (1984). The rats were allowed to recover for 1 day prior to dosing.

Approximately 24 hours before dermal doses were applied, animals were anesthetized with intramuscular injections of 60 mg/kg ketamine:xylazine (7:1) (rats) or intraperitoneal injections of 60 mg/kg sodium pentobarbital (mice). The fur on the back of each animal was clipped, wiped with acetone, dried, and examined; animals with nicks in the clipped area were excluded from the study. The doses were applied to 4 cm$^2$ (rats) or 1 cm$^2$ (mice) areas of skin; the dosing areas were outlined with a permanent, felt-tip marker. Prior to dosing, protective foam appliances were glued onto the rats' backs with Hollister's medical adhesive; doses were administered with a ball-tipped gavage needle and syringe. A nonocclusive cloth cover was attached to the foam appliance, and a protective metal mesh cover was secured over the appliance with elastoplast adhesive bandage after dosing. For mice, a metal tissue capsule was glued over the dosing site with Quicktite™ Super Glue Gel; doses were administered with a 50 µL Wiretrol (Drummond Scientific Co., Broomall, PA).

Doses for intravenous injection contained 6.3 to 23 µCi [$^{14}$C]-radiolabel and an appropriate amount of unlabeled *p-tert*-butylcatechol. The doses for rats were formulated in 10% Emulphor in F344 rat plasma at a dosing volume of 1 mL/kg. The initial set of doses for mice were formulated in isotonic saline; doses for a repeat study were formulated in 10% Emulphor in B6C3F$_1$ mouse plasma. The dosing volume for mice was 2 mL/kg. The doses were injected into a lateral tail vein. The gavage dose formulations contained 16.7 to 19.3 µCi [$^{14}$C]-radiolabel and an appropriate amount of unlabeled *p-tert*-butylcatechol. The 2 mg/kg dose was formulated in water, the 200 and 300 mg/kg doses were formulated in 20% Emulphor in water, and the 1,000 mg/kg dose was formulated in 80%

Emulphor in water. The gavage dosing volume was 5 mL/kg for rats and mice. Doses for dermal application were formulated in acetone and contained 13.5 to 23.1 µCi (rats) or 2.5 to 22.2 µCi (mice) [$^{14}$C]-radiolabel and an appropriate amount of unlabeled *p-tert*-butylcatechol; the dosing volume was approximately 200 µL for rats and 25 µL for mice. The concentration of [$^{14}$C]-*p-tert*-butylcatechol in each dose formulation was measured in weighed aliquots taken before, during, and after dosing each series of animals. To accurately measure the individual animal doses, the dosing apparatuses for all routes were weighed before and after each dose was administered. To measure residual *p-tert*-butylcatechol left on the dosing apparatuses after dosing, each needle was wiped clean with a Kimwipe® which was placed in a vial containing 2 mL methanol; the radiolabel extracted from the Kimwipe® was analyzed by LSS. [$^{3}$H]-Methionine doses were formulated in isotonic saline and were administered to rats by intraperitoneal injection at a rate of 300 µCi (approximately 3.75 nmol) per rat.

## Determination of Excretion, Urinary Metabolites, and Tissue Distribution of [$^{14}$C]-*p-tert*-Butylcatechol in Rats and Mice

Groups of four rats were administered single intravenous injections of 3 mg *p-tert*-butylcatechol per kilogram body weight, single gavage doses of 2, 200, or 1,000 mg/kg, or single dermal applications of 0.6, 6, or 60 mg/kg. Groups of four mice (initial study) or two mice (repeat study) were administered single 3 mg/kg intravenous injections; additional groups of four mice received gavage doses of 300 mg/kg or dermal applications of 0.04 or 4 mg per animal. The dermal doses for mice were selected to provide approximately equal concentrations of *p-tert*-butylcatechol per square centimeter of skin as the 0.6 and 60 mg/kg doses administered to rats. Radioactivity was measured in urine collected 6, 24, 48, and 72 hours after dosing and feces collected 24, 48, and 72 hours after dosing. Urine and feces were collected separately into round-bottom flasks cooled with dry ice and were stored in the dark at -20° C until analysis.

At the end of the excreta collection period, rats were anesthetized with an intramuscular injection of 60 mg/kg ketamine and 8.6 mg/kg xylazine and mice with an intraperitoneal injection of 60 mg/kg sodium pentobarbital. Blood was withdrawn by cardiac puncture with a syringe containing heparin. Rats were then killed by an intracardiac injection of 300 mg/kg sodium pentobarbital and mice by cervical dislocation. For all animals administered intravenous or gavage doses of *p-tert*-butylcatechol (except rats administered 1,000 mg/kg by gavage), the adrenal gland, blood, brain, kidney, liver, lung, prostate gland, spleen, and testis with seminal vesicle as well as three samples each of adipose tissue, muscle, and skin were analyzed for carbon-14 content. For some animals, the cecum, large and small intestine, and stomach were also analyzed. For rats administered *p-tert*-butylcatechol dermally, the skin at the site of application (without muscle or adipose tissue) was excised with the foam appliance still attached; the elastoplast was removed. The appliance (including the cloth cover) was removed from the skin and divided among 10 scintillation vials for analysis by LSS; for rats in the 6 mg/kg group, the cloth cover was removed from the appliance and analyzed separately. For mice administered *p-tert*-butylcatechol dermally, the metal appliance was removed from the skin by dissolving the adhesive with acetone; the site of application was rinsed with ethanol, washed with cotton gauzes soaked in soapy water, and then rinsed with gauzes soaked with water. Rinses were collected in amber glass bottles; the gauzes were placed in individual scintillation vials for analysis by LSS. The skin from the site of application and the carcass were digested in 2 N ethanolic sodium hydroxide.

For determinations of total radioactivity, aliquots of urine, skin wash, and digests of application site skin and carcass were added directly to vials containing scintillation cocktail (Ultima Gold™; Packard Instrument Company, Inc., Meriden, CT). Samples of other tissues, feces, and blood (0.1-0.3 g) were digested in 2 mL Soluene®-350 (Packard Instrument Company, Inc.). After digestion, samples requiring bleaching were decolorized with perchloric acid/hydrogen peroxide prior to addition of scintillation cocktail. Cecum, large and small intestine, and stomach samples were digested in 2 N ethanolic sodium hydroxide, and aliquots were added to scintillation cocktail for analysis. Scintillation cocktail and either water or ethanol were added to dermal appliance and skin gauze samples before analysis by LSS.

Urine samples were filtered through a 0.45-µm Millex®-HV filter (Millipore Corp., Bedford, MA) and analyzed by HPLC (using the system described for chemical purity analyses) for unchanged *p-tert*-butylcatechol and its metabolites. Selected urine samples were incubated with β-glucuronidase/sulfatase, purified β-glucuronidase, or sulfatase obtained from Sigma Chemical Company (St. Louis, MO) before HPLC analysis. Solutions of β-glucuronidase/sulfatase were prepared from *Helix pomatia* and contained 100,000 units glucuronidase and 1,000 to 5,000 units sulfatase per mL; 20 µL were added to 450 µL urine and 50 µL 1 M ammonium acetate. The mixture was heated for 2 hours at 37° C before HPLC analysis. Purified β-glucuronidase was prepared from *Escherichia coli*; 1,000 U were added to 200 to 400 µL urine, and the mixture was incubated for 4 to 18 hours at 37° C before analysis. Sulfatase was prepared from *Aerobacter aerogenes* and contained 14.5 units sulfatase and no glucuronidase per mL of solution; 10 µL sulfatase was added to 300 µL of 0.05 M TRIZMA buffer (pH 7.5; Sigma Chemical Company) and 100 µL urine. The mixture was incubated at 37° C for 2 to 4 hours before analysis.

### Characterization of Urinary Metabolites in Rats

Urine collected during the first 24 hours after dosing from rats administered 60 mg/kg dermally was analyzed for urinary metabolites. Solid-phase extraction was performed with a Bond Elut $C_{18}$ SPE cartridge containing 500 mg packing material in a 6-mL syringe barrel (Varian, Inc., Palo Alto, CA); the cartridge was prewetted with 1 mL methanol followed by 2 mL water. The cartridge was loaded with 1 mL urine and then rinsed with 2 mL water followed by 2 mL 5% aqueous methanol to remove salts. Metabolites were eluted with a rinse of 2 mL methanol, which was evaporated under a stream of nitrogen at 37° C. β-Glucuronidase/sulfatase (20-30 µL) was added, and the mixture was incubated overnight at 37° C. The sample was then centrifuged and the supernatant was aspirated and collected; 1 mL methanol was added to the pellet, and the sample was vortexed and centrifuged. The original and methanol supernatants were analyzed by HPLC as described above. Both supernatants contained undeconjugated metabolites, *p-tert*-butylcatechol, and a late-eluting, deconjugated metabolite; the methanol supernatant, which contained the larger portion of the deconjugated metabolite, was analyzed by HPLC and the fractions containing this deconjugated metabolite were collected. This eluant was concentrated to approximately 50% of its original volume under a stream of nitrogen at 37° C. Deconjugated *p-tert*-butylcatechol metabolites were characterized by gas chromatography/mass spectrometry with electron impact ionization and NMR spectroscopy.

### Determination of Plasma Concentrations of [$^{14}$C]-*p-tert*-Butylcatechol in Rats

Groups of four rats were administered single gavage doses of 200 mg/kg or single dermal applications of 60 mg/kg. Blood samples (0.3 mL) were collected before dosing and at 15 and 30 minutes and 1, 2, 4, 8, and 24 hours after dosing. The samples were centrifuged to separate the plasma, which was analyzed by liquid scintillation spectrometry for total radioactivity. Plasma samples were extracted twice with 400 µL methyl alcohol; the supernatants were combined, evaporated to dryness, and reconstituted in 100 µL of the mobile phase before analysis by HPLC as described above. Rats were asphyxiated with carbon dioxide. No necropsies were performed on these groups of rats. For rats administered *p-tert*-butylcatechol dermally, the foam appliance was analyzed for carbon-14 content as described for the tissue distribution studies; the cloth cover was removed from the appliance and analyzed separately.

## Results and Discussion

### Excretion, Urinary Metabolites, and Tissue Distribution of [$^{14}$C]-*p-tert*-Butylcatechol in Rats and Mice

The excretion of [$^{14}$C]-*p-tert*-butylcatechol administered to rats by intravenous injection is shown in Table G1; distribution in tissues is shown in Table G2. After intravenous injection, the radioactivity was rapidly excreted in urine, with 54% of the administered radioactivity recovered in the first 6 hours after dosing. After 72 hours, approximately 90% of the dose had been recovered in the urine and 6% in the feces; only 0.3% of the dose

remained in the tissues. No tissues developed high tissue/blood ratios of *p-tert*-butylcatechol equivalents; this finding is consistent with the production of very polar metabolites.

The excretion of [$^{14}$C]-*p-tert*-butylcatechol administered to rats by gavage is shown in Table G3; distribution of the 2 and 200 mg/kg doses in tissues is shown in Table G4. The overall rates and routes of excretion were similar between the 2 and 200 mg/kg doses 6 hours after dosing. The doses were excreted primarily in the urine and approximately 90% of the radiolabel was recovered, indicating a very high rate of oral absorption. For the 1,000 mg/kg dose, the rate of excretion was slower than for the lower doses, and a greater percentage of the radioactivity (25%) was excreted in the feces. Similar to rats dosed intravenously, the tissues of rats in the 2 and 200 mg/kg groups did not develop significant concentrations of *p-tert*-butylcatechol-derived residues; also, very little radioactivity remained in the gastrointestinal tract tissues or contents. The 0-to-6-hour urinary metabolite profile for the 2 mg/kg gavage dose is shown in Figure G1a. This profile is representative of those obtained for each route of administration and for both species. *p-tert*-Butylcatechol was excreted primarily as conjugates and other polar metabolites. Incubation with β-glucuronidase or sulfatase hydrolyzed the conjugates to give parent and a less polar analyte (retention time 23.5 minutes), as shown in Figure G1b. HPLC analyses of urine samples treated with purified β-glucuronidase or purified sulfatase demonstrated that sulfatase liberated a greater proportion of the polar metabolites, indicating that sulfate conjugates comprise the majority of the polar metabolites (data not shown).

The excretion of [$^{14}$C]-*p-tert*-butylcatechol applied dermally to rats is shown in Table G5; distribution of radioactivity 72 hours after dosing is shown in Table G6. Absorption increased with increasing dose. Approximately two-thirds of the 60 mg/kg dose was absorbed by 24 hours after application and more than 80% by 72 hours. The 0.6 mg/kg dose was far less well absorbed. The severity of local inflammation at the site of application increased with increasing dose; this increase in blood flow to the area may have been responsible for the dose-related increase in absorption. Alternatively, *p-tert*-butylcatechol may have had a solvent effect on the permeability of the skin, or saturation of the covalent binding of dermal sulfhydryls with the Michael-accepting oxidative products of *p-tert*-butylcatechol may have occurred at the higher doses. The percentage of the dose recovered in the foam appliance was greatest for the 0.6 mg/kg dose and decreased with increasing dose. The cloth appliance cover in the 6 mg/kg group contained 1.5% to 5.4% of the dose (data not shown). HPLC analyses of urine collected from the 0.6 and 60 mg/kg groups from 0 to 6 hours after dosing indicated only polar metabolites (data not shown); no parent compound was detected in the urine.

The excretion of [$^{14}$C]-*p-tert*-butylcatechol administered to mice by intravenous injection is shown in Table G7; distribution in tissues is shown in Table G8. Mice excreted the radioactivity more slowly than did rats, with greater concentrations excreted in the feces (28% for mice versus 6% for rats). However, the mouse feces samples were often contaminated with urine-soaked feed pellet solids. As for rats, no tissues developed high concentrations of *p-tert*-butylcatechol residues. Because the tissue-to-blood ratio favored lung over liver by a factor of 10 for mice but was approximately equal for rats, a repeat study was conducted for mice with the dose formulated in a mixture of Emulphor in plasma as for rats. The percentage of dose excreted was similar between vehicles, and the tissue-to-blood ratio in the repeat study was only slightly greater for lung than for liver (Tables G7 and G8). Therefore, it was speculated that the dose for the initial study (formulated in isotonic saline) contained particles of *p-tert*-butylcatechol large enough to lodge in the lung following injection; the dose for the repeat study contained no particles of this size.

The excretion of [$^{14}$C]-*p-tert*-butylcatechol administered to mice by gavage is shown in Table G9; distribution in tissues is shown in Table G10. Approximately half of the administered dose was excreted by 24 hours and 90% by 72 hours, mostly in urine. The relatively high concentration of radioactivity recovered in feces may have been due to urine-soaked feed particles and feces in the fecal samples. No tissues developed high concentrations of *p-tert*-butylcatechol residues.

The excretion of [$^{14}$C]-*p-tert*-butylcatechol applied dermally to mice is shown in Table G11; distribution of radioactivity 72 hours after dosing is shown in Table G12. Most of the doses were absorbed by 72 hours (72% of

the 0.04 mg dose and 86% of the 4 mg dose). The absorbed dose was excreted rapidly, with 40% of the 0.04 mg dose and 57% of the 4 mg dose excreted in the first 24 hours after dosing. Mice administered 4 mg had local inflammation at the site of application.

## Characterization of Urinary Metabolites in Rats

After intravenous injection, gavage dosing, or dermal application of *p-tert*-butylcatechol to rats, polar metabolites were excreted in urine. Incubation of the urine with sulfatase-free β-glucuronidase caused little change in the metabolite profile; however, incubation of the urine with sulfatase caused significant changes. The polar metabolite peak was greatly reduced, with the concomitant appearance of a peak coeluting with *p-tert*-butylcatechol and a later-eluting peak with a retention time of 23.5 minutes (Figure G1b). A small polar peak with a retention time of 5 minutes also appeared. The late-eluting peak was collected and analyzed by gas chromatography with mass spectrometry (Figure G2). The mass spectrum displayed ions at 180 (M+) and 165 (M-$CH_3$) consistent with mono-*O*-methylated *p-tert*-butylcatechol; the spectrum matched a literature reference (91% probability) for *t*-butyl-2-methoxyphenol (Wiley/NBS Mass Spectral Database). The proton NMR spectrum of the isolated metabolite is shown in Figure G3. Resonances from the methyl protons of the *t*-butyl group appear as a singlet at 1.22 ppm. The phenyl protons appear in the pattern of a 1,3,4-trisubstituted benzene at 6.79 to 6.99 ppm, and the protons of the *O*-methyl appear as a singlet at 3.81 ppm. The data were not sufficient to distinguish between the two possible *O*-methylated metabolites. *p-tert*-Butylcatechol is probably *O*-methylated by catechol *O*-methyltransferase. *In vitro*, catechol *O*-methyltransferase catalyzes the conversion of catechols to both *o*- and *p*-methylated products. Creveling *et al.* (1972) showed that the *meta*/*para* (*m*/*p*) ratio is dependent on the nature of the ring substituents, and less polar substrates such as 4-methyl and 4-ethyl catechol gave ratios near unity. However, the authors apparently were unable to separate the isomers of *O*-methylated *p-tert*-butylcatechol and, therefore, did not report a ratio for this compound. Katz and Jacobson (1972) correlated substrate kinetic parameters with steric parameters of the substituents of the substrate and calculated a predicted *m*/*p* ratio of 0.13 for the *O*-methylation of *p-tert*-butylcatechol. This value suggests that the late-eluting isolated metabolite is the *p-O*-methylated isomer. However, Daly *et al.* (1960) noted that less of the *para* isomer is often formed *in vivo* than *in vitro*. Therefore, it is difficult to speculate which of the isomers is excreted as the sulfate conjugate in rat urine, and it is possible that both isomers are present.

## Plasma Concentrations of [$^{14}$C]-*p-tert*-Butylcatechol in Rats

The concentrations of *tert*-butylcatechol equivalents in plasma for rats administered 200 mg/kg by gavage or 60 mg/kg dermally are shown in Table G13. Peak concentrations were observed 30 minutes after gavage dosing and 1 hour after dermal application; no parent compound was detected in plasma extracts for either route.

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**TABLE G1**
**Cumulative Excretion of Radioactivity by Male F344/N Rats after a Single Intravenous Injection of 3 mg/kg [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Time (hours after dosing) | Urine | Feces | Total |
|---|---|---|---|
| 6 | 53.7 ± 1.8 | —[b] | 53.7 ± 1.8 |
| 24 | 81.0 ± 2.4 | 4.45 ± 3.31 | 85.5 ± 3.6 |
| 48 | 86.3 ± 1.9 | 5.69 ± 3.02 | 91.9 ± 1.9 |
| 72 | 89.8 ± 1.9 | 5.97 ± 2.98 | 95.8 ± 1.5 |

[a] Four animals were examined; data are presented as cumulative percentage of dose (mean ± standard deviation).
[b] Feces were not collected at this time point.

**TABLE G2**
**Tissue Distribution of Radioactivity in Male F344/N Rats 72 Hours after a Single Intravenous Injection of 3 mg/kg [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Tissue | *p-tert*-Butylcatechol Equivalents in Tissue (ng/g) | Tissue-to-Blood Ratio | Dose in Total Tissue (%) |
|---|---|---|---|
| Adipose | 2.90 ± 0.37 | 0.0748 ± 0.0245 | 0.00643 ± 0.00065 |
| Adrenal gland | 26.9 ± 2.5 | 0.684 ± 0.172 | 0.000161 ± 0.000025 |
| Blood | 40.9 ± 9.6 | —[b] | 0.0678 ± 0.0175 |
| Brain | 7.36 ± 0.91 | 0.190 ± 0.059 | 0.00160 ± 0.00021 |
| Cecum[c] | | | 0.0222 ± 0.0822 |
| Intestine, large[c] | | | 0.0152 ± 0.0060 |
| Intestine, small[c] | | | 0.0292 ± 0.0044 |
| Kidney | 61.7 ± 10.3 | 1.60 ± 0.58 | 0.0142 ± 0.0015 |
| Liver | 46.8 ± 5.1 | 1.20 ± 0.35 | 0.0580 ± 0.0034 |
| Lung | 63.0 ± 10.5 | 1.62 ± 0.51 | 0.0134 ± 0.0016 |
| Muscle | 2.88 ± 0.70 | 0.0759 ± 0.0312 | 0.0437 ± 0.0098 |
| Prostate gland | 5.60 ± 0.83 | 0.143 ± 0.043 | 0.000163 ± 0.000047 |
| Seminal vesicle | 4.45 ± 1.06 | 0.116 ± 0.051 | 0.000393 ± 0.000072 |
| Skin | 13.5 ± 6.1 | 0.356 ± 0.227 | 0.0724 ± 0.0307 |
| Spleen | 49.9 ± 18.1 | 1.34 ± 0.73 | 0.00380 ± 0.00141 |
| Stomach[c] | | | 0.00320 ± 0.00058 |
| Testis | 3.00 ± 0.35 | 0.0753 ± 0.0133 | 0.000992 ± 0.000145 |
| Total in tissues | | | 0.283 ± 0.023 |

[a] Four animals were examined; data are presented as mean ± standard deviation.
[b] Unity
[c] Includes contents

**TABLE G3**
**Cumulative Excretion of Radioactivity by Male F344/N Rats after a Single Gavage Dose of [$^{14}$C]-*p-tert*-Butylcatechol**[a]

| Dose | Time (hours after dosing) | Urine | Feces | Total |
|---|---|---|---|---|
| 2 mg/kg | | | | |
| | 6 | 38.7 ± 11.3 | —[b] | 38.7 ± 11.3 |
| | 24 | 74.5 ± 10.6 | 3.77 ± 0.76 | 78.3 ± 10.6 |
| | 48 | 83.5 ± 7.8 | 4.59 ± 0.70 | 88.1 ± 7.7 |
| | 72 | 89.0 ± 4.5 | 4.90 ± 0.75 | 93.9 ± 4.1 |
| 200 mg/kg | | | | |
| | 6 | 16.0 ± 11.4 | — | 16.0 ± 11.4 |
| | 24 | 72.2 ± 2.0 | 4.35 ± 1.75 | 76.5 ± 1.1 |
| | 48 | 87.0 ± 2.3 | 5.69 ± 1.59 | 92.7 ± 1.8 |
| | 72 | 94.4 ± 4.0 | 6.45 ± 1.34 | 101 ± 3 |
| 1,000 mg/kg | | | | |
| | 6 | 4.74 ± 1.45 | — | 4.74 ± 1.45 |
| | 24 | 18.9 ± 2.3 | 1.80 ± 0.47 | 20.8 ± 2.2 |
| | 48 | 40.0 ± 7.1 | 18.9 ± 9.8 | 58.9 ± 7.3 |
| | 72 | 56.6 ± 7.9 | 24.7 ± 9.1 | 81.3 ± 1.8 |

[a] Four animals were examined in the 2 and 200 mg/kg groups and three were examined in the 1,000 mg/kg group; data are presented as cumulative percentage of dose (mean ± standard deviation).
[b] Feces were not collected at this time point.

**TABLE G4**
**Tissue Distribution of Radioactivity in Male F344/N Rats 72 Hours after a Single Gavage Dose of [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Dose | Tissue | *p-tert*-Butylcatechol Equivalents in Tissue (ng/g) | Tissue-to-Blood Ratio | Dose in Total Tissue (%) |
|---|---|---|---|---|
| 2 mg/kg | | | | |
| | Adipose | 0.381 ± 0.279 | 0.0205 ± 0.0227 | 0.00135 ± 0.00098 |
| | Adrenal gland | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | Blood | 25.1 ± 13.5 | —[b] | 0.0670 ± 0.0366 |
| | Brain | 0.0564 ± 0.0639 | 0.00345 ± 0.00542 | 0.0000191 ± 0.0000224 |
| | Cecum[c] | | | 0.0221 ± 0.0075 |
| | Intestine, large[c] | | | 0.0124 ± 0.0009 |
| | Intestine, small[c] | | | 0.0384 ± 0.0029 |
| | Kidney | 79.4 ± 25.8 | 3.47 ± 0.92 | 0.0305 ± 0.0114 |
| | Liver | 27.3 ± 1.9 | 1.33 ± 0.63 | 0.0603 ± 0.0019 |
| | Lung | 22.3 ± 10.0 | 0.982 ± 0.502 | 0.00782 ± 0.00412 |
| | Muscle | 0.0377 ± 0.0260 | 0.00199 ± 0.00200 | 0.000919 ± 0.000629 |
| | Prostate gland | 1.15 ± 1.13 | 0.072 ± 0.102 | 0.0000585 ± 0.0000562 |
| | Seminal vesicle | 0.602 ± 0.595 | 0.0388 ± 0.0532 | 0.0000864 ± 0.0000581 |
| | Skin | 1.43 ± 0.84 | 0.0811 ± 0.0876 | 0.0123 ± 0.0070 |
| | Spleen | 26.3 ± 8.95 | 1.13 ± 0.24 | 0.00344 ± 0.00120 |
| | Stomach[c] | | | 0.125 ± 0.038 |
| | Testis | 0.184 ± 0.117 | 0.0107 ± 0.0120 | 0.000101 ± 0.000070 |
| | Total in tissues | | | 0.18 ± 0.05 |
| 200 mg/kg | | | | |
| | Adipose | 69.8 ± 18.9 | 0.0580 ± 0.0182 | 0.00272 ± 0.00075 |
| | Adrenal gland | 105 ± 24 | 0.0871 ± 0.0214 | 0.0000118 ± 0.0000028 |
| | Blood | 1,220 ± 127 | — | 0.0353 ± 0.0035 |
| | Brain | 19.2 ± 6.6 | 0.0159 ± 0.0060 | 0.0000676 ± 0.0000199 |
| | Cecum[c] | | | 0.0468 ± 0.0140 |
| | Intestine, large[c] | | | 0.0216 ± 0.0070 |
| | Intestine, small[c] | | | 0.0468 ± 0.0106 |
| | Kidney | 2,860 ± 1,060 | 2.39 ± 0.96 | 0.0105 ± 0.0037 |
| | Liver | 2,840 ± 137 | 2.34 ± 0.26 | 0.0638 ± 0.0119 |
| | Lung | 1,390 ± 219 | 1.14 ± 0.16 | 0.00489 ± 0.00123 |
| | Muscle | 22.0 ± 6.2 | 0.0178 ± 0.0042 | 0.00586 ± 0.00160 |
| | Prostate gland | 139 ± 78 | 0.119 ± 0.079 | 0.0000773 ± 0.0000411 |
| | Seminal vesicle | 58.7 ± 29.5 | 0.0487 ± 0.0260 | 0.0000823 ± 0.0000393 |
| | Skin | 368 ± 281 | 0.299 ± 0.223 | 0.0347 ± 0.0264 |
| | Spleen | 3,110 ± 2,880 | 2.42 ± 2.03 | 0.00412 ± 0.00367 |
| | Stomach[c] | | | 0.0102 ± 0.0020 |
| | Testis | 28.2 ± 10.3 | 0.0232 ± 0.0088 | 0.000167 ± 0.000054 |
| | Total in tissues | | | 0.16 ± 0.05 |

[a] Four animals were examined in each group; data are presented as mean ± standard deviation.
[b] Unity
[c] Includes contents

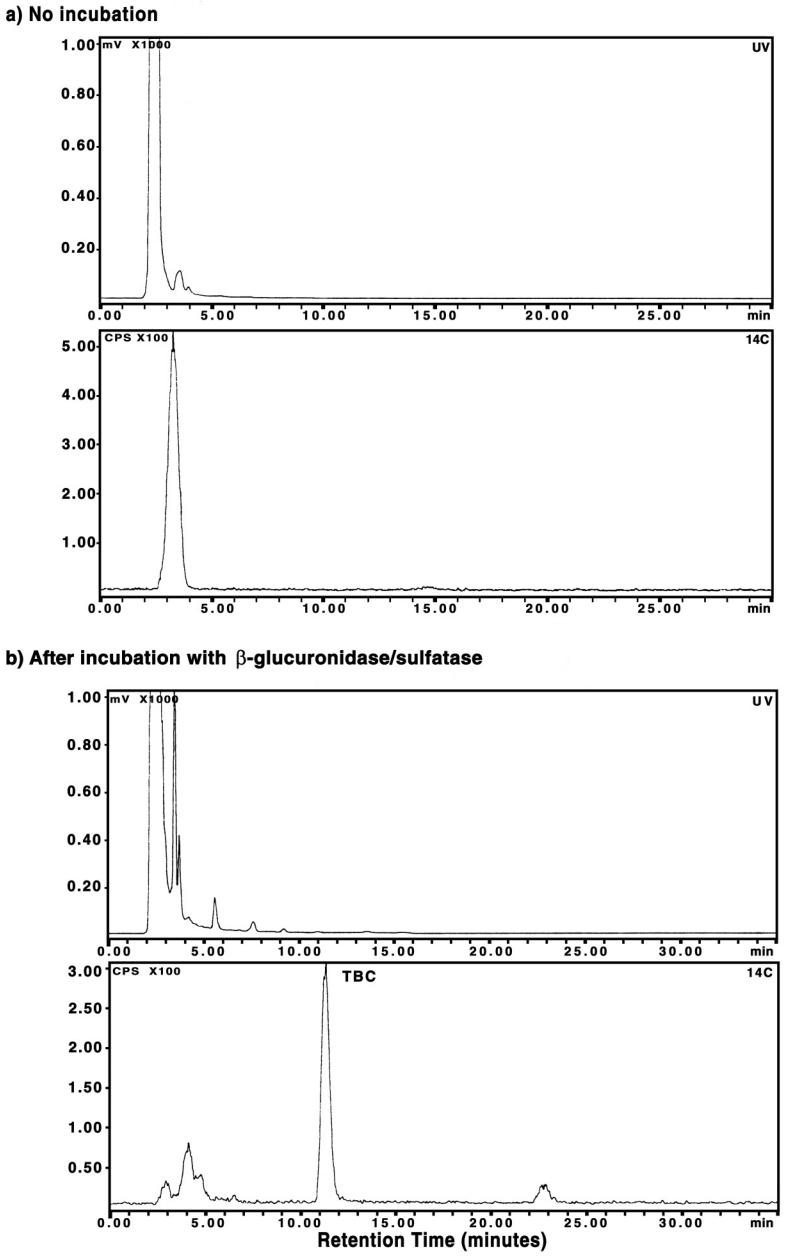


**FIGURE G1**
**High-Performance Liquid Chromatographic Radiochromatogram of Urine Collected from 0 to 6 Hours after a Single Gavage Dose of 2 mg/kg [$^{14}$C]-*p-tert*-Butylcatechol to Male F344/N Rats**

**TABLE G5**
**Cumulative Excretion of Radioactivity by Male F344/N Rats after a Single Dermal Application of [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Dose | Time (hours after dosing) | Urine | Feces | Total |
|---|---|---|---|---|
| 0.6 mg/kg | | | | |
| | 6 | 1.45 ± 1.82 | —[b] | 1.45 ± 1.82 |
| | 24 | 16.3 ± 2.4 | 0.363 ± 0.135 | 16.7 ± 2.6 |
| | 48 | 24.6 ± 5.3 | 1.11 ± 0.77 | 25.7 ± 5.6 |
| | 72 | 31.7 ± 7.8 | 1.34 ± 0.88 | 33.1 ± 8.2 |
| 6 mg/kg | | | | |
| | 6 | 7.46 ± 2.16 | — | 7.46 ± 2.16 |
| | 24 | 27.4 ± 5.7 | 0.641 ± 0.221 | 28.1 ± 5.9 |
| | 48 | 38.9 ± 6.4 | 1.04 ± 0.30 | 39.9 ± 6.7 |
| | 72 | 46.8 ± 6.8 | 1.69 ± 0.83 | 48.5 ± 6.8 |
| 60 mg/kg | | | | |
| | 6 | 12.2 ± 16.5 | — | 12.2 ± 16.5 |
| | 24 | 64.3 ± 4.3 | 2.43 ± 0.67 | 66.7 ± 4.2 |
| | 48 | 73.2 ± 5.0 | 3.37 ± 0.87 | 76.6 ± 4.6 |
| | 72 | 79.5 ± 5.2 | 4.09 ± 1.41 | 83.6 ± 4.2 |

[a] Four animals were examined per group; data are presented as cumulative percentage of dose (mean ± standard deviation).
[b] Feces were not collected at this time point.

**TABLE G6**
**Distribution of Radioactivity in Male F344/N Rats 72 Hours after a Single Dermal Application of [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Site | 0.6 mg/kg | 6 mg/kg | 60 mg/kg |
|---|---|---|---|
| **Absorbed Dose** | | | |
| Tissues | 0.35 ± 0.11 | 0.52 ± 0.19 | 0.995 ± 0.444 |
| Site of application | 10.2 ± 4.3 | 8.24 ± 2.48 | 2.84 ± 0.17 |
| Feces | 1.34 ± 0.88 | 1.69 ± 0.83 | 4.09 ± 1.41 |
| Urine | 31.7 ± 7.8 | 46.8 ± 6.8 | 79.5 ± 5.2 |
| Total | 43.7 ± 9.8 | 57.2 ± 4.7 | 87.4 ± 4.3 |
| **Unabsorbed Dose** | | | |
| Appliance | 26.6 ± 12.9 | 16.7 ± 3.1 | 1.67 ± 1.02 |
| Skin gauze | 5.35 ± 1.93 | 11.5 ± 3.1 | 0.606 ± 0.532 |
| Skin wash | 8.41 ± 4.04 | 0.73 ± 0.48 | 0.463 ± 0.131 |
| Total | 40.4 ± 9.8 | 28.9 ± 6.1 | 2.74 ± 0.76 |

[a] Four animals were examined per group; data are presented as percentage of dose (mean ± standard deviation).

**TABLE G7**
**Cumulative Excretion of Radioactivity by Male B6C3F$_1$ Mice after a Single Intravenous Injection of 3 mg/kg [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Time (hours after dosing) | Urine | Feces | Total |
|---|---|---|---|
| **Initial Study** | | | |
| 6 | 13.3 ± 9.7 | —[b] | 13.3 ± 9.7 |
| 24 | 37.3 ± 17.6 | 16.4 ± 13.4 | 53.7 ± 21.7 |
| 48 | 43.2 ± 19.9 | 21.0 ± 15.5 | 64.3 ± 20.3 |
| 72 | 59.9 ± 22.3 | 28.1 ± 17.2 | 88.0 ± 8.3 |
| **Repeat Study** | | | |
| 6 | 8.26 ± 7.74 | — | 8.26 ± 7.74 |
| 24 | 28.7 ± 14.5 | 13.1 ± 13.1 | 41.8 ± 27.7 |
| 48 | 37.6 ± 22.8 | 24.5 ± 0.8 | 62.1 ± 22.0 |
| 72 | 40.8 ± 21.2 | 25.7 ± 1.1 | 66.6 ± 20.0 |
| Cage rinse | 63.2 ± 7.8 | 25.7 ± 1.1 | 88.9 ± 6.7 |

[a] Four animals were examined in the initial study and two were examined in the repeat study; data are presented as cumulative percentage of dose (mean ± standard deviation).
[b] Feces were not collected at this time point.

**TABLE G8**
**Tissue Distribution of Radioactivity in Male B6C3F$_1$ Mice 72 Hours after a Single Intravenous Injection of 3 mg/kg [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Tissue | *p-tert*-Butylcatechol Equivalents in Tissue (ng/g) | Tissue-to-Blood Ratio | Dose in Total Tissue (%) |
|---|---|---|---|
| **Initial Study**[b] | | | |
| Adipose | 2.51 ± 1.15 | 0.0482 ± 0.0278 | 0.00780 ± 0.00367 |
| Adrenal gland | 9.66 ± 2.64 | 0.185 ± 0.070 | 0.000111 ± 0.000026 |
| Blood | 54.6 ± 12.8 | —[c] | 0.131 ± 0.029 |
| Brain | 7.59 ± 1.17 | 0.141 ± 0.016 | 0.00337 ± 0.00038 |
| Cecum[d] | | | 0.00535 ± 0.00716 |
| Intestine, large[d] | | | 0.0215 ± 0.0405 |
| Intestine, small[d] | | | 0.0104 ± 0.0042 |
| Kidney | 25.2 ± 6.5 | 0.479 ± 0.172 | 0.0119 ± 0.0028 |
| Liver | 12.2 ± 1.9 | 0.235 ± 0.071 | 0.0216 ± 0.0126 |
| Lung | 136 ± 37 | 2.68 ± 1.23 | 0.0270 ± 0.0113 |
| Muscle | 3.61 ± 2.35 | 0.0708 ± 0.0558 | 0.0519 ± 0.0348 |
| Prostate gland | 9.62 ± 9.83 | 0.176 ± 0.197 | 0.000134 ± 0.000146 |
| Seminal vesicle | 2.57 ± 1.02 | 0.0492 ± 0.0239 | 0.000417 ± 0.000168 |
| Skin | 10.8 ± 3.9 | 0.207 ± 0.100 | 0.0497 ± 0.0189 |
| Spleen | 23.0 ± 4.4 | 0.430 ± 0.092 | 0.00177 ± 0.00031 |
| Stomach[d] | | | 0.0155 ± 0.0098 |
| Testis | 2.75 ± 1.38 | 0.0535 ± 0.0338 | 0.000662 ± 0.000368 |
| Total in tissues | | | 0.36 ± 0.09 |
| **Repeat Study**[e] | | | |
| Adipose | 16.7 ± 7.6 | 0.521 ± 0.137 | 0.0663 ± 0.0271 |
| Adrenal gland | 27.5 ± 7.2 | 0.876 ± 0.052 | 0.000273 ± 0.000030 |
| Blood | 31.2 ± 6.4 | — | 0.0971 ± 0.0148 |
| Brain | 7.1 ± 2.0 | 0.225 ± 0.017 | 0.00500 ± 0.00168 |
| Cecum[d] | | | 0.0574 ± 0.0262 |
| Intestine, large[d] | | | 0.0299 ± 0.0177 |
| Intestine, small[d] | | | 0.0787 ± 0.0470 |
| Kidney | 31.7 ± 5.7 | 1.02 ± 0.03 | 0.0239 ± 0.0016 |
| Liver | 41.9 ± 7.4 | 1.35 ± 0.04 | 0.0765 ± 0.0067 |
| Lung | 73.0 ± 18.9 | 2.33 ± 0.13 | 0.0185 ± 0.0037 |
| Muscle | 9.6 ± 2.4 | 0.308 ± 0.015 | 0.177 ± 0.035 |
| Prostate gland | 22.5 ± 7.2 | 0.714 ± 0.086 | 0.00149 ± 0.00019 |
| Seminal vesicle | 7.8 ± 4.5 | 0.241 ± 0.094 | 0.00153 ± 0.00030 |
| Skin | 26.0 ± 5.7 | 0.834 ± 0.013 | 0.154 ± 0.026 |
| Spleen | 18.2 ± 3.9 | 0.583 ± 0.005 | 0.00178 ± 0.00001 |
| Stomach[d] | | | 0.0123 ± 0.0049 |
| Testis | 8.6 ± 0.8 | 0.277 ± 0.030 | 0.00266 ± 0.00027 |
| Total in tissues | | | 0.805 ± 0.214 |

[a] Four animals were examined in the initial study and two were examined in the repeat study; data are presented as mean ± standard deviation.
[b] Formulated in isotonic saline
[c] Unity
[d] Includes contents
[e] Formulated in 10% Emulphor in B6C3F$_1$ mouse plasma

**TABLE G9**
**Cumulative Excretion of Radioactivity by Male B6C3F$_1$ Mice after a Single Gavage Dose of 300 mg/kg [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Time (hours after dosing) | Urine | Feces | Total |
|---|---|---|---|
| 6 | 21.3 ± 15.5 | —[b] | 21.3 ± 15.5 |
| 24 | 40.2 ± 18.1 | 14.2 ± 6.2 | 54.4 ± 15.4 |
| 48 | 47.6 ± 18.6 | 20.5 ± 6.2 | 68.1 ± 12.8 |
| 72 | 50.1 ± 18.1 | 25.6 ± 9.5 | 75.7 ± 10.3 |
| Cage rinse | 64.6 ± 11.1 | 25.6 ± 9.5 | 90.2 ± 1.6 |

[a] Four animals were examined; data are presented as cumulative percentage of dose (mean ± standard deviation).
[b] Feces were not collected at this time point.

**TABLE G10**
**Tissue Distribution of Radioactivity in Male B6C3F$_1$ Mice 72 Hours after a Single Gavage Dose of 300 mg/kg [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Tissue | *p-tert*-Butylcatechol Equivalents in Tissue (ng/g) | Tissue-to-Blood Ratio | Dose in Total Tissue (%) |
|---|---|---|---|
| Adipose | 373 ± 153 | 0.733 ± 0.090 | 0.0117 ± 0.0047 |
| Adrenal gland | 577 ± 161 | 1.22 ± 0.39 | 0.000137 ± 0.000165 |
| Blood | 495 ± 146 | —[b] | 0.0117 ± 0.0034 |
| Brain | 169 ± 55 | 0.35 ± 0.12 | 0.00103 ± 0.00044 |
| Cecum[c] | | | 0.0145 ± 0.0038 |
| Intestine, large[c] | | | 0.00771 ± 0.00522 |
| Intestine, small[c] | | | 0.0536 ± 0.0296 |
| Kidney | 1,160 ± 184 | 2.43 ± 0.40 | 0.00657 ± 0.00086 |
| Liver | 2,620 ± 332 | 5.53 ± 1.16 | 0.0412 ± 0.0048 |
| Lung | 920 ± 204 | 1.91 ± 0.35 | 0.00188 ± 0.00030 |
| Muscle | 206 ± 100 | 0.421 ± 0.228 | 0.0296 ± 0.0145 |
| Prostate gland | 398 ± 156 | 0.784 ± 0.177 | 0.000197 ± 0.000076 |
| Seminal vesicle | 191 ± 70 | 0.380 ± 0.054 | 0.000317 ± 0.000096 |
| Skin | 691 ± 305 | 1.39 ± 0.56 | 0.0320 ± 0.0140 |
| Spleen | 196 ± 80 | 0.384 ± 0.070 | 0.000169 ± 0.000056 |
| Stomach[c] | | | 0.0197 ± 0.0112 |
| Testis | 126 ± 40 | 0.253 ± 0.031 | 0.000298 ± 0.000081 |
| Total in tissues | | | 0.234 ± 0.065 |

[a] Four animals were examined; data are presented as mean ± standard deviation.
[b] Unity
[c] Includes contents

**TABLE G11**
**Cumulative Excretion of Radioactivity by Male B6C3F$_1$ Mice after a Single Dermal Application of [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Dose | Time (hours after dosing) | Urine | Feces | Total |
|---|---|---|---|---|
| 0.04 mg/animal | | | | |
| | 6 | 4.3 ± 5.2 | —[b] | 4.3 ± 5.2 |
| | 24 | 32.1 ± 9.6 | 7.45 ± 5.39 | 39.6 ± 13.6 |
| | 48 | 47.1 ± 10.7 | 8.15 ± 5.31 | 55.2 ± 15.3 |
| | 72 | 51.7 ± 8.9 | 12.4 ± 7.7 | 64.1 ± 15.7 |
| | Cage rinse | 57.0 ± 7.5 | 12.4 ± 7.7 | 69.4 ± 13.4 |
| 4 mg/animal | | | | |
| | 6 | 11.9 ± 14.0 | — | 11.9 ± 14.0 |
| | 24 | 54.3 ± 10.5 | 2.58 ± 1.30 | 56.9 ± 10.3 |
| | 48 | 68.3 ± 3.9 | 3.48 ± 1.51 | 71.8 ± 3.0 |
| | 72 | 71.4 ± 3.5 | 5.42 ± 2.55 | 76.8 ± 1.4 |
| | Cage rinse | 73.7 ± 3.2 | 5.42 ± 2.55 | 79.1 ± 2.1 |

[a] Four animals were examined per group; data are presented as cumulative percentage of dose (mean ± standard deviation).
[b] Feces were not collected at this time point.

**TABLE G12**
**Distribution of Radioactivity in Male B6C3F$_1$ Mice 72 Hours after a Single Dermal Application of [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Site | 0.04 mg/animal | 4 mg/animal |
|---|---|---|
| **Absorbed Dose** | | |
| Tissues | 11.6 ± 8.3 | 0.76 ± 0.30 |
| Site of application | 1.51 ± 0.43 | 6.18 ± 0.52 |
| Feces | 12.4 ± 7.7 | 5.4 ± 2.6 |
| Urine | 57.0 ± 7.5 | 73.7 ± 3.2 |
| Total | 72.2 ± 14.2 | 86.0 ± 2.7 |
| **Unabsorbed Dose** | | |
| Skin wash | 11.6 ± 8.3 | 2.25 ± 0.81 |
| **Total Recovery** | 83.7 ± 11.3 | 88.3 ± 2.7 |

[a] Four animals were examined per group; data are presented as percentage of dose (mean ± standard deviation).

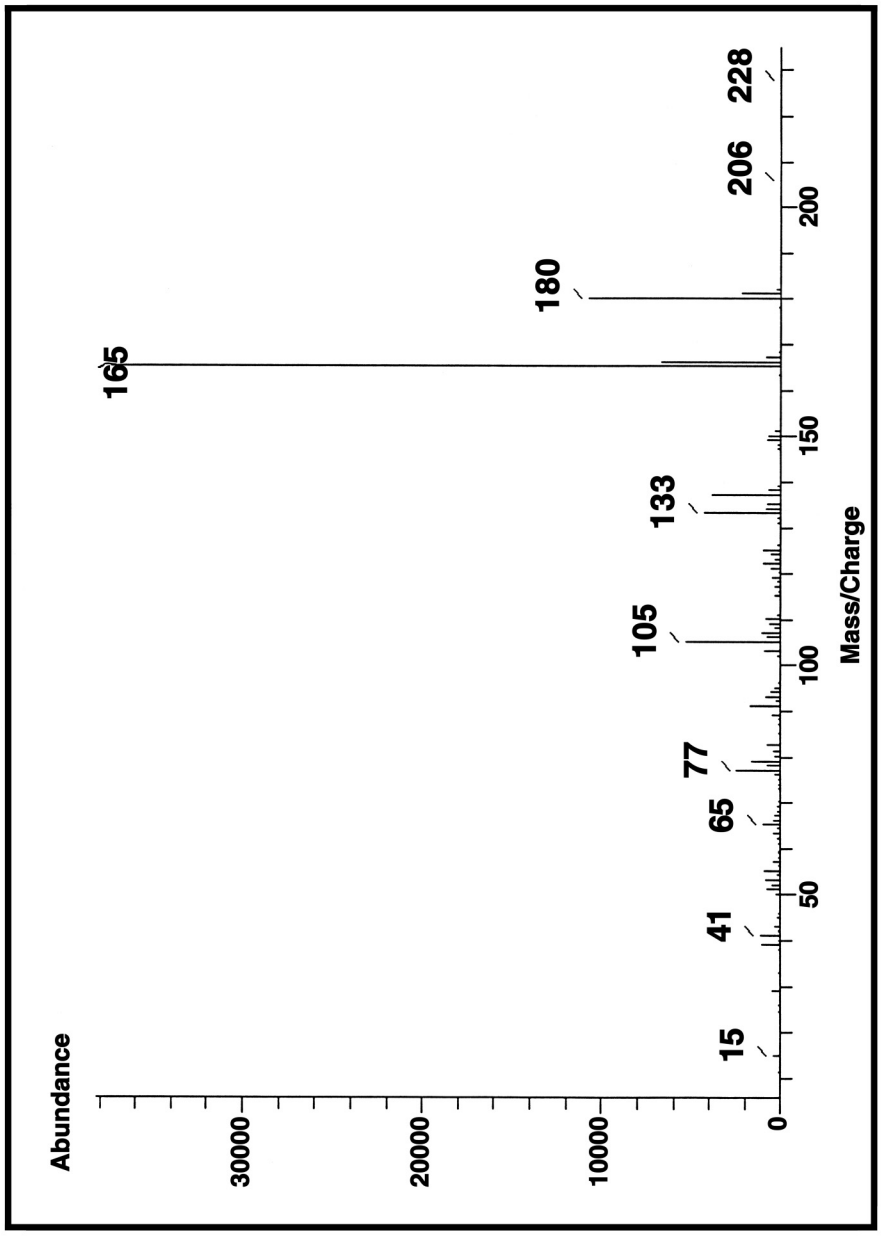


**FIGURE G2**
**Mass Spectrum of the Late-Eluting Deconjugated *p-tert*-Butylcatechol Metabolite**

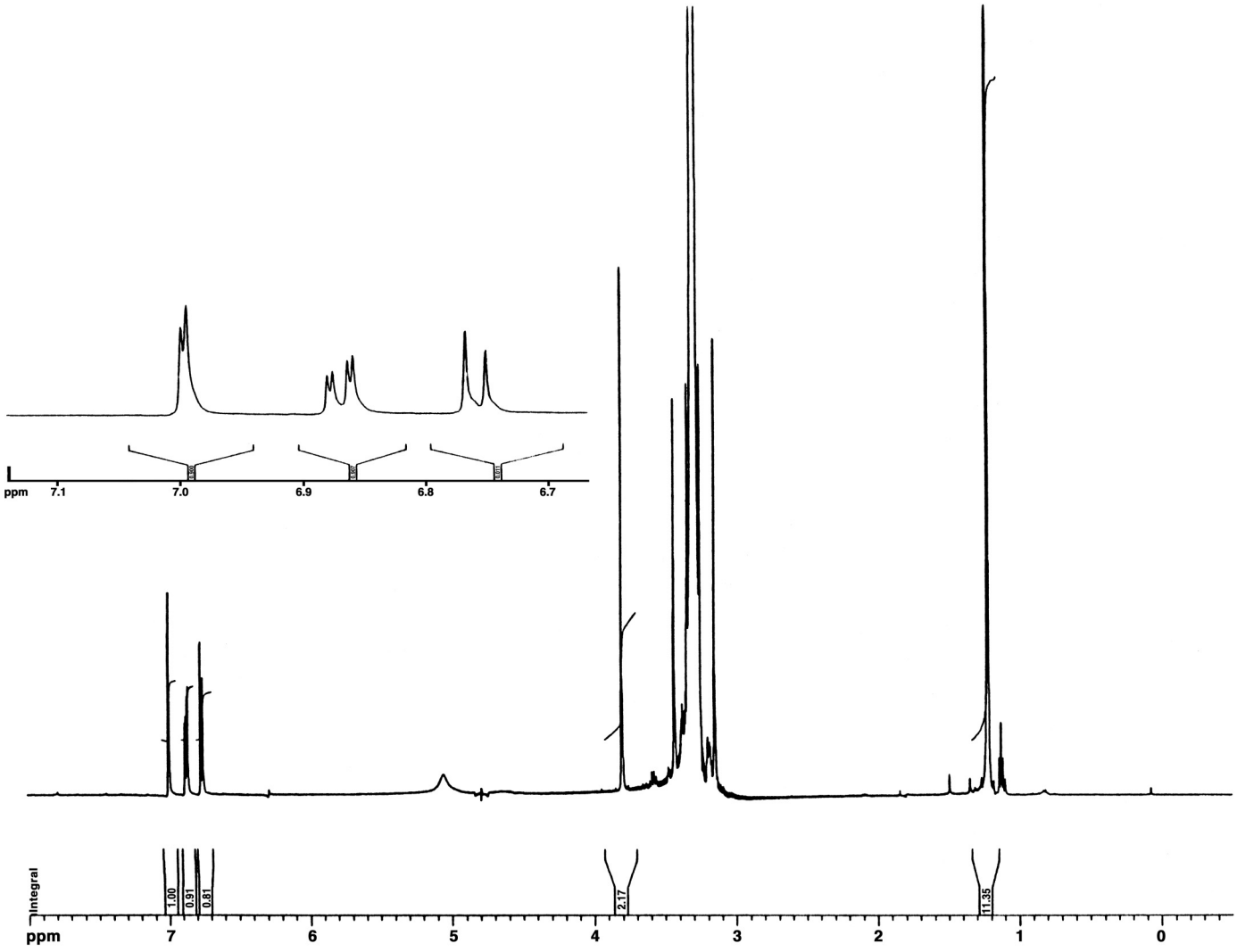


**FIGURE G3**
**Proton Nuclear Magnetic Resonance Spectrum**
**of the Late-Eluting Deconjugated *p-tert*-Butylcatechol Metabolite**

**TABLE G13**
**Concentration of *p-tert*-Butylcatechol Equivalents in the Plasma of Male F344/N Rats after a Single Gavage Dose or Dermal Application of [$^{14}$C]-*p-tert*-Butylcatechol[a]**

| Time (hours after dosing) | Rat 1 | Rat 2 | Rat 3 | Rat 4 | Mean ± Standard Deviation |
|---|---|---|---|---|---|
| **Gavage Dosing (200 mg/kg)** | | | | | |
| 0.25 | 36,700 | 32,100 | 42,200 | 34,900 | 36,500 ± 4,290 |
| 0.5 | 64,200 | 38,600 | 44,000 | 45,700 | 48,100 ± 11,100 |
| 1 | 52,100 | 36,200 | 30,500 | 36,400 | 38,800 ± 9,250 |
| 2 | 15,400 | 26,100 | 25,800 | 28,000 | 23,800 ± 5,680 |
| 4 | 29,400 | 27,200 | 26,400 | 30,400 | 28,300 ± 1,890 |
| 8 | 21,400 | 16,100 | 28,700 | 26,100 | 23,100 ± 5,550 |
| 24 | 2,540 | 2,370 | 2,700 | 1,710 | 2,330 ± 436 |
| **Dermal Application (60 mg/kg)** | | | | | |
| 0.25 | 15,500 | 15,200 | 10,000 | 10,300 | 12,700 ± 3,010 |
| 0.5 | 25,400 | 25,000 | 21,400 | 18,500 | 22,600 ± 3,270 |
| 1 | 28,700 | 29,900 | 22,200 | 28,600 | 27,300 ± 3,490 |
| 2 | 20,400 | 21,000 | 23,400 | 27,600 | 23,100 ± 3,260 |
| 4 | 10,000 | 10,700 | 12,500 | 8,660 | 10,500 ± 1,620 |
| 8 | 5,370 | 5,180 | 19,500 | 8,900 | 9,740 ± 6,730 |
| 24 | 717 | 745 | 1,010 | 628 | 776 ± 165 |

[a] Data are presented as ng equivalents per gram of plasma.