NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 444

ONE-YEAR INITIATION/PROMOTION STUDY OF *o*-BENZYL-*p*-CHLOROPHENOL

(CAS NO. 120-32-1)

IN SWISS (CD-1®) MICE

(MOUSE SKIN STUDY)

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. 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 Technical 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. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

ONE-YEAR INITIATION/PROMOTION STUDY OF *o*-BENZYL-*p*-CHLOROPHENOL

(CAS NO. 120-32-1)

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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May 1995

NTP TR 444

NIH Publication No. 95-3157

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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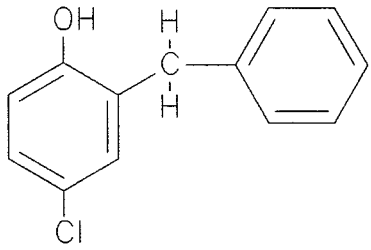
ABSTRACT



o-BENZYL-*p*-CHLOROPHENOL

CAS No. 120-32-1

Chemical Formula: $C_{13}H_{11}ClO$ Molecular Weight: 218.69

Synonyms: 4-Chloro-α-phenol-*o*-cresol; *p*-chloro-*o*-benzylphenol; 2-benzyl-4-chlorophenol; 2-hydroxy-5-chlorodiphenylmethane; 4-chloro-2-(phenylmethyl)phenol; 4-chloro-2-benzylphenol

Trade names: Bio-Clave; Chlorophene; Clorofene; Clorophene; Ketolin H; Nipacide BCPR; Preventol BPR; Santophen 1; Septiphene

o-Benzyl-*p*-chlorophenol (BCP), an aryl halide, is a broad spectrum germicide used in disinfectant solutions and soap formulations in United States hospitals and households. Human exposure to BCP occurs by absorption through the skin and mucous membranes and by ingestion. BCP was studied because of the widespread human exposure and because BCP is an irritant and certain phenolic compounds are weak promoters of skin neoplasia. Groups of Swiss (CD-1®) mice were used to study BCP in a 1-year mouse skin initiation/promotion protocol. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

1-YEAR INITIATION/PROMOTION STUDY

Groups of 50 male and 50 female Swiss (CD-1®) mice were topically exposed to BCP to study its effect as an initiator, promoter, and complete carcinogen. A number of control groups were included in these studies as a reference for the responses of the mouse skin to *o*-benzyl-*p*-chlorophenol (see following table).

Dose Regimen for Reference Controls in the 1-Year Initiation/Promotion Study of o-Benzyl-p-Chlorophenol[a]

Treatment		
Initiator[b]	Promoter[c]	Test Group
Acetone	Acetone	Vehicle Control
Acetone	20 μg DMBA	Reference Complete Carcinogen Control
50 μg DMBA	Acetone	Reference Initiator Control
50 μg DMBA	5 μg TPA	Reference Initiator/Promoter Control
5 μg TPA	5 μg TPA	Reference Promoter Control

[a] All dose volumes were 100 μL.
[b] Initiator doses were applied once during week 1 of the study, at which time mice were approximately 56 days old.
[c] With the exception of TPA, promoter doses were applied three times weekly from week 2 through week 52. TPA was applied three times weekly as a promoter for the first 6 months of the study, and once weekly for the last 6 months.

BCP in acetone was tested as an initiator with the promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The potential of BCP as an initiator was studied by applying a single 100 μL dose of BCP in acetone at a concentration of 10 mg/mL to the dorsal interscapular region of the backs of mice during week 1 of the study. Following the initial BCP application, mice were administered promoting doses of 5 μg TPA three times per week in 100 μL acetone for the first 6 months of the study and once weekly for the final 6 months of the study. BCP in acetone was tested as a promoter with the initiator 7,12-dimethylbenz(a)anthracene (DMBA). Mice were administered a single initiating dose of 50 μL DMBA in 100 μL acetone. Beginning on the second week of the study, mice received 100 μL applications of 0.1, 1.0, or 3.0 mg BCP in acetone three times weekly for up to 51 weeks. Comparative control groups used during the study of BCP as a promoter included: vehicle control (acetone/acetone); promoter control (TPA/TPA); and initiator control (DMBA/acetone). The potential for BCP to act as a complete carcinogen was studied by applying a single initiating dose of 10 mg BCP in 100 μL of acetone, followed by tri-weekly 100 μL applications of 0.1, 1.0, or 3.0 mg BCP to 50 male and 50 female Swiss (CD-1®) mice for 52 weeks. The responses of these groups were compared to vehicle control (acetone/acetone) and complete carcinogen control (acetone/DMBA) groups. The following table shows the various groups with BCP as a promoter, an initiator, and as a complete carcinogen.

Dose Regimen in the 1-Year Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol[a]

Treatment		
Initiator[b]	Promoter[c]	Test Group
10 mg BCP	0.1 mg BCP	Low-Dose as Complete Carcinogen
10 mg BCP	1.0 mg BCP	Mid-Dose as Complete Carcinogen
10 mg BCP	3.0 mg BCP	High-Dose as Complete Carcinogen
10 mg BCP	5 μg TPA	BCP as Initiator
50 μg DMBA	0.1 mg BCP	BCP Low-Dose as Promoter
50 μg DMBA	1.0 mg BCP	BCP Mid-Dose as Promoter
50 μg DMBA	3.0 mg BCP	BCP High-Dose as Promoter

[a] All dose volumes were 100 μL.
[b] Initiator doses were applied once during week 1 of the study, at which time mice were approximately 56 days old.
[c] With the exception of TPA, promoter doses were applied three times weekly from week 2 through week 52. TPA was applied three times weekly as a promoter for the first 6 months of the study, and once weekly for the last 6 months.

Results in the Study of BCP as a Complete Carcinogen

BCP acted as an irritant when tested as a complete carcinogen using a single initiating dose of 10 mg BCP followed by repetitive applications of 0.1, 1.0, or 3.0 mg BCP for up to 52 weeks, and many of the mice developed cutaneous lesions of scaling/crusts and ulceration. During the course of the study, a single papilloma was first observed after 12 weeks in one 0.1 mg BCP male mouse. One 3.0 mg BCP female was observed with a papilloma at week 10, and three 0.1 mg BCP females were observed with papillomas between weeks 22 and 27. No mice administered BCP/BCP had papillomas at the end of the study, and no malignant cutaneous epithelial tumors were observed at the application sites on any BCP/BCP mice. Thus, in the present study, BCP was not a complete carcinogen.

Results in the Study of BCP as an Initiator

One vehicle control (acetone/acetone) male mouse had developed crusts at the site of application at necropsy, but no male or female vehicle controls had developed papillomas. Mice administered BCP/TPA developed application site lesions including scaling/crusts, ulceration, and irritation; the incidences of these lesions were similar to those in the initiator/promoter control (DMBA/TPA) groups. After 22 weeks papillomas were observed in 12/50 male mice administered BCP/TPA. After 12 weeks papillomas were observed in 7/50 female mice administered BCP/TPA. However, the incidences of papillomas in mice administered BCP/TPA were lower than those in mice administered TPA/TPA (males, 16/50; females, 16/50) and were much lower than those in DMBA/TPA mice (males, 40/50; females, 48/50). Although the incidences of papillomas in mice administered BCP as an initiator were significantly greater than those in the vehicle controls, the incidences were not significantly different from those in TPA/TPA mice. Thus, in the present study, BCP did not demonstrate initiating potential.

Results in the Study of BCP as a Promoter

During the course of the study, incidences of scaling and/or crusts, ulceration, and irritation were observed at the site of application in DMBA/BCP male and female mice, and the incidences were dose related. Incidences of scaling and/or crusts, ulceration, and irritation in 3.0 mg BCP mice were similar to the incidences of these lesions in initiator/promoter control (DMBA/TPA) group, but much higher than the incidences of these lesions in the initiator control (DMBA/acetone) group. A dose-related increased incidence of papillomas was observed in males (DMBA/acetone, 8/50; DMBA/0.1 mg BCP, 3/50;

DMBA/1.0 mg BCP, 5/50; and DMBA/3.0 mg BCP, 14/50) and females (2/50, 6/50, 6/50, and 18/50). The incidence of papillomas in DMBA/3.0 mg BCP females was significantly greater ($P<0.001$) than that in DMBA/acetone females; the incidence of papillomas in DMBA/3.0 mg BCP males was marginally increased ($P=0.077$). No acetone/acetone mice developed papillomas. Although a higher percentage of DMBA/3.0 mg BCP mice developed papillomas over the course of the study than did DMBA/acetone controls, the time it took for half of the number of responding animals to develop papillomas was similar between DMBA/acetone groups and DMBA/3.0 mg BCP groups (DMBA/acetone males, week 38; DMBA/acetone females, week 34; DMBA/3.0 mg BCP males, week 36; DMBA/3.0 mg BCP females, week 37). However, the time to appearance of the first papilloma was shorter in DMBA/3.0 mg BCP mice (males, week 18; females, week 10) than in DMBA/acetone mice (males, week 26; females, week 27). BCP was considered to have promotion potential because the incidences of papillomas in mice treated with DMBA/3.0 mg BCP were greater than those in DMBA/acetone (initiator control) mice and because topical exposure to BCP alone caused no significant increased incidence of papillomas. However, the incidences of papillomas in DMBA/3.0 mg BCP mice (males, 14/50; females, 18/50) were much less than the incidences in DMBA/TPA (promoter control) mice (males, 40/50; females, 48/50); thus, BCP was classified as a weak promoter.

GENETIC TOXICOLOGY

o-Benzyl-*p*-chlorophenol did not induce gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. All tests were performed with and without S9 activation.

CONCLUSIONS

Under the conditions of this 1-year mouse skin initiation/promotion study in Swiss (CD-1®) mice, *o*-benzyl-*p*-chlorophenol was a cutaneous irritant and a weak skin tumor promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen*.

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* A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on *o*-benzyl-*p*-chlorophenol on November 16, 1993, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 16, 1993, the draft Technical Report on the initiation and promotion study of *o*-benzyl-*p*-chlorophenol (BCP) received public review by the National Toxicology Program Board of Scientific Counselors Technical Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. W.C. Eastin, NIEHS, introduced the initiation and promotion study of BCP in Swiss (CD-1®) mice by discussing the properties and uses of the chemical, describing the experimental design, and commenting on the compound-related skin lesions in male and female mice. The proposed conclusions for the study were that in Swiss (CD-1®) mice, BCP was a cutaneous irritant and a weak promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen.

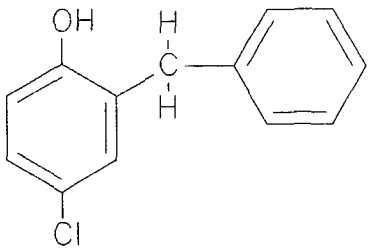
Dr. Ward, a principal reviewer, agreed in principle with the proposed conclusions except that he thought the words "skin tumor" should be added between "weak" and "promoter" in the first sentence of the conclusions. He suggested that the weak promoting activity of BCP may be due to skin wounding (irritation) rather than the effects of BCP on skin carcinogenesis/promotion, and provided copies of journal articles describing such effects. Dr. Eastin said he was familiar with the papers and would add information on wounding and skin irritation as well as discussion on other weak promoters.

Dr. Taylor, the second principal reviewer, said the proposed conclusion that BCP was a weak promoter was overstated considering that DMBA/acetone groups of mice developed many, if not more neoplasms than did the DMBA/BCP groups. Further, the inconclusiveness of the findings was compounded by the degree of irritation and skin wounding that occurred. Dr. Taylor thought that more discussion was needed.

Dr. Ryan, the third principal reviewer, commented that although the proposed conclusions were in line with the findings, she was concerned with the ambiguity of some of the results, such as the fact that higher incidences of some lesions were seen with DMBA/acetone than with DMBA/BCP. In response to Drs. Taylor and Ryan, Dr. Eastin said that the incidences of skin papillomas were clearly elevated in the high dose DMBA/BCP group relative to DMBA/acetone. The designation of "weak promoter" derived from a comparison of DMBA/BCP with the DMBA/TPA reference controls with TPA representing a strong or potent promoter. Dr. Ryan wondered how to interpret the results of this study in light of the gavage study and asked whether there had been studies of absorption of BCP following dermal administration. Dr. Eastin said that NIEHS studies have shown that BCP is well absorbed after dermal administration.

Dr. Ward moved that the Technical Report on *o*-benzyl-*p*-chlorophenol be accepted with the revisions discussed and the conclusions as written with the addition of "skin tumor" to read as follows: Under the conditions of this one-year mouse skin initiation/promotion study in Swiss (CD-1®) mice, *o*-benzyl-*p*-chlorophenol was a cutaneous irritant and a weak skin tumor promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen. Dr. Taylor seconded the motion, which was accepted unanimously with five votes.

INTRODUCTION



o-BENZYL-*p*-CHLOROPHENOL

CAS No. 120-32-1

Chemical Formula: $C_{13}H_{11}ClO$ Molecular Weight: 218.69

Synonyms: 4-Chloro-α-phenol-*o*-cresol; *p*-chloro-*o*-benzylphenol; 2-benzyl-4-chlorophenol; 2-hydroxy-5-chlorodiphenylmethane; 4-chloro-2-(phenylmethyl)phenol; 4-chloro-2-benzylphenol

Trade names: Bio-Clave; Chlorophene; Clorofene; Clorophene; Ketolin H; Nipacide BCPR; Preventol BPR; Santophen 1; Septiphene

CHEMICAL AND PHYSICAL PROPERTIES

o-Benzyl-*p*-chlorophenol (BCP) is a white to light tan or pink crystal or flake. The melting point is 46.5° to 48° C and the boiling point is 160° to 162° C (*Merck Index*, 1983). It is essentially insoluble in water, moderately soluble in acetone (100 mg/mL), and freely soluble in ethanol.

USE AND HUMAN EXPOSURE

o-Benzyl-*p*-chlorophenol, a phenolic aryl halide, is used extensively as a broad spectrum germicide in hospitals and households throughout the United States in disinfectant solutions and in soap formulations. Approximately 4 million pounds are used annually in the U.S., with the annual U.S. production estimated at 10 million pounds. There are a total of 347,636 workers (244,213 females) potentially exposed to BCP in 25 different industries. Ninety percent of these workers are in health services (286,603), business services (14,253), personal services (6,670), and general building contractors (6,136) (NIOSH, 1990). Human exposure can occur by absorption through the skin and mucous membranes or by ingestion. BCP applied at a concentration of 10% or greater is a primary irritant to skin and mucous membranes. Prolonged exposure to dilute solutions (0.03%) causes only mild cutaneous irritation (Monsanto).

ENVIRONMENTAL IMPACT

Disposal of BCP occurs primarily in municipal sewer systems. Ninety-five percent of the chemical is removed through biodegradation at wastewater treatment plants. Measured influent and effluent concentrations from 16 sites in the U.S. averaged 14.8 μg/L and 0.8 μg/L (Werner *et al.*, 1983). *o*-Benzyl-*p*-chlorophenol is rapidly metabolized by fish and has a low potential for bioconcentration in biota. Biodegradation and photolysis are the principal processes of BCP transformation in the environment; hydrolysis and volatilization rates are insignificant. Biodegradation of BCP occurs rapidly in systems such as river water, sewage, and activated sludge (Swisher and Gledhill, 1973). In unacclimated river water, 0.1 mg/L BCP degraded in 6 days; in sewage, 0.5 and 1.0 mg/L BCP degraded in 1 day; in acclimated sludge, 80% of 1.0 mg/L BCP degraded in 8 hours and 100% in 24 hours.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

o-Benzyl-*p*-chlorophenol metabolism and distribution studies have been conducted in male Sprague-Dawley rats administered a single oral dose of either 69 or 206 mg/kg body weight of [^{14}C]-labeled BCP in corn oil (Ridley *et al.*, 1986). Expired air, urine, feces, and blood were collected and the radioactivity was measured. Metabolites in urine and feces were separated by thin-layer chromatography and determined by gas chromatography and mass spectroscopy. BCP was rapidly eliminated and extensively metabolized by male rats, with about half the radioactivity eliminated in the urine and half in the feces 5 days after dosing. Most of the ^{14}C in the feces was unmetabolized and unabsorbed BCP. Only 0.28% to 0.30% of the radioactivity remained in the body 5 days after dosing; about half of this radioactivity was associated with the liver and kidney. The excretion of BCP was biphasic, with an initial rapid phase possessing a half-life of 8 to 9 hours and a slower phase with an estimated half-life of 52 to 140 hours. The majority of radioactivity in the urine (41% to 61%) was present as sulfate and/or glucuronide conjugates with sulfate esters as the predominant conjugate. Analysis of the urine and feces identified BCP and two metabolites with modified benzyl rings. The distribution and metabolism of BCP were studied in a series of experiments (Kao and Birnbaum, 1986). Male F344 rats received oral doses of 10, 100, or 1,000 mg/kg of [^{14}C]-BCP dissolved in corn oil. At the 10 mg/kg dose, almost all the radioactivity had been excreted in the urine and feces after 3 days. At a dose of 100 mg/kg, the rate of fecal excretion decreased and the rate of urinary excretion increased (54%). At the 1,000 mg/kg dose, fecal excretion was increased. Despite differences in dose concentrations, more than 92% of the radioactivity from BCP was excreted in 3 days by all dosed rats. An increased proportion of radioactivity from BCP in the feces after oral exposure indicated that [^{14}C]-BCP was incompletely absorbed.

The radioactivity excretion patterns of [^{14}C]-BCP administered intravenously (10 mg/kg body weight) and topically (10 mg/kg in acetone applied to the clipped interscapular area) to male F344 rats have been compared (Kao and Birnbaum, 1986). The skin site of application was protected with a perforated metal cap to prevent oral exposure. Approximately 88% of the intravenous dose was excreted in the urine and feces in 3 days, suggesting rapid elimination of BCP. BCP was well absorbed across the skin; 3 days after dosing, approximately 59% of the topical dose radioactivity was recovered in the urine and feces. Most of the remaining radioactivity (32%) was found at the skin site of application. The urine-to-feces ratio of recovered radioactivity was similar for intravenous and topical administrations, suggesting similar routes of excretion (Kao and Birnbaum, 1986).

For studies of biliary excretion, BCP was injected intravenously at doses of 5, 10, or 25 mg/kg body weight to male F344 rats (Kao and Birnbaum, 1986). Biliary excretion was affected by dose; excretion 6 hours after administration was 87% of a 5 mg/kg dose, 72% of a 10 mg/kg dose, and 56% of a 25 mg/kg dose. The principal *in vivo* metabolites were glucuronyl conjugates of BCP and hydroxyl-BCP. Results of *in vitro* metabolism studies indicated that microsomal oxidation and glutathione and glucuronyl conjugation were major pathways of BCP metabolism. The greatest concentrations of radioactivity from BCP were in the spleen, kidney, and liver.

Humans

No studies of the absorption, distribution, metabolism, or excretion of BCP in humans were found in the literature.

TOXICITY

Experimental Animals

The oral LD_{50} in rats has been reported as 2,800 mg/kg body weight (Monsanto). When administered in corn oil by gavage to F344 rats and B6C3F$_1$ mice at doses from 62.5 to 1,000 mg/kg body weight, BCP produced renal lesions in rats and mice in the higher dose groups (Deskin *et al.*, 1984). Rats were more sensitive to the nephrotoxic activity of BCP than mice. Renal lesions consistent with nephrosis were observed in rats receiving 480 mg/kg body weight of BCP for 13 weeks. The incidence and severity of renal lesions increased with increasing dose in rats (Deskin *et al.*, 1984). The effects of BCP treatment on the activity of drug-metabolizing enzymes in the liver and kidney of male F344 rats have been studied (Kao *et al.*, 1986). Treatment increased cytochrome P_{450} activity and decreased aryl hydrocarbon hydroxylase activity in liver and kidney microsomes. In the kidney, treatment of rats with

BCP increased cytochrome *c* reductase and uridine diphosphate glucuronyl transferase activity. In the kidney, the increases in total cytochrome P_{450} and glutathione were minimal. Liver glutathione concentration and glutathione transferase activity were unaltered by treatment with BCP. La Via and La Via (1979) have reported that phenolic derivatives have some immunodepressive activity in mice. CBA/J male mice were exposed to a disinfectant detergent solution containing three phenolic derivatives including BCP (4.5%) by being housed in cages washed with dilute solutions of a disinfectant detergent (also containing 5.0% *o*-phenylphenol and 1.0% *p-tert*-amylphenol). After a 4-week exposure, the mice developed depressed generation of plaque-forming cells when exposed to sheep erythrocytes *in vitro*. This depression was more severe after exposures of as long as 14 weeks.

BCP was evaluated as a sensitizing agent for contact hypersensitivity in $B6C3F_1$ female mice. Doses of BCP ranged from 3% to 10% in one part olive oil and four parts acetone for sensitization and was 20% for challenge. Mice received 20 μL by direct dermal application for 5 consecutive days to a prepared site. 1-fluoro-2,4-dinitrobenzene (DNFB) was used as a positive control at a concentration of 0.5%. Measurement of the contract hypersensitivity was accomplished by the radioisotopic assay and the mouse ear swelling test. A statistically significant dose-response contact hypersensitivity response to BCP was demonstrated in mice when the site of sensitization was prepared using shaving and dermabrasion with or without adjuvant (NTP, unpublished data).

Humans

o-Benzyl-*p*-chlorophenol used in excess of the recommended concentration as a disinfectant detergent for the cleaning of bassinets and mattresses in hospital nurseries has been linked to multiple cases of idiopathic hyperbilirubinemia in human infants (Wysowski *et al.*, 1978). Some infants required exchange transfusions and some had peak bilirubin concentrations of 23.9 to 42 mg/100 mL. The affected infants had no other illness. After disinfectant detergent use was discontinued, idiopathic hyperbilirubinemia cases ceased. Neither acute hemolysis nor hepatic dysfunction was determined to be the cause of the jaundice. However, the results of *in vitro* studies suggest that enzyme inhibition may have been a factor. A significant inhibition of hepatic bilirubin glucuronyl transferase activity was observed when phenol detergent (containing BCP) was added at dilutions of 1:128 or less to an *in vitro* assay system for the enzyme (Daum *et al.*, 1976). Signs and symptoms observed in human patients after overexposure to BCP have included sweating, thirst, nausea, diarrhea, abdominal pain, hyperactivity, convulsions or stupor, low blood pressure, and dyspnea.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No published studies of the reproductive or developmental toxicity of *o*-benzyl-*p*-chlorophenol in experimental animals were found. No information on the reproductive or developmental toxicity of BCP in humans was found.

CARCINOGENICITY

Experimental Animals

o-Benzyl-*p*-chlorophenol was negative as a promoter of cutaneous tumors in mouse skin when tested at a 20% concentration in benzene. The initiator, 0.3% 7,12-dimethylbenz(a)anthracene, was applied at a dose of 75 μg/animal. The promoter was applied twice weekly and observations were made for 21 weeks (Boutwell and Bosch, 1959).

In long-term studies (NTP, 1994) male and female Fischer 344/N rats were administered 0, 30, 60, or 120 mg BCP/kg body weight in corn oil by gavage for 5 days per week for up to 2 years. There were no chemical-related tumors observed in males. However, in females there was considered to be "equivocal evidence" of carcinogenic activity based on the occurrence of one renal transitional cell carcinoma in a high-dose female rat and one in a mid-dose female rat. Renal transitional cell carcinoma is a rare tumor in female F344/N rats; the NTP historical control incidence of the tumor in female F344/N rats is 0/1,068. $B6C3F_1$ mice were administered 0, 60, 120, or 240 mg BCP/kg body weight in corn oil by gavage for 5 days per week for up to 2 years. Renal tubule adenomas in all dosed groups of males (0/50, 3/50, 4/50, 5/50) and renal tubule carcinomas in 120 and 240 mg/kg males (0/50, 0/50, 3/50, 1/50) were considered "some evidence" of carcinogenic acitivity. The NTP historical control incidence for renal tubule adenomas in male $B6C3F_1$ mice is 4/949 and for

carcinomas is 0/949. There were no chemical-related tumors in female mice.

BCP was also nephrotoxic for male and female F344/N rats and B6C3F$_1$ mice (NTP, 1994). There was a dose-related increase in nephropathy severity in male and female rats, and the incidence and severity of nephropathy were increased in male and female mice.

Humans

No studies related to the carcinogenicity of BCP in humans were reported in the literature.

GENETIC TOXICITY

Little data exist on BCP mutagenicity. The compound did not induce gene mutations in *Salmonella typhimurium*, with or without exogenous metabolic activation (S9) (Mortelmans *et al.*, 1986), but did induce trifluorothymidine resistance in L5178Y mouse lymphoma and TK6 human lymphoblast cells without S9 (Caspary *et al.*, 1988).

STUDY RATIONALE

BCP was nominated for 2-year rodent toxicity and carcinogenicity studies by the National Cancer Institute because of widespread human exposure and the similarity of the chemical to the neurotoxin hexachlorophene, and as a representative biocide (Johnson *et al.*, 1984). Results of the 2-year gavage studies are reported in NTP Technical Report Series No. 424 (NTP, 1994). During the design of the 2-year carcinogenicity studies, it was recognized that as a substituted phenol, BCP may have tumor promotion potential (Boutwell and Bosch, 1959).

Historically, results of studies on mouse skin were the first to suggest distinct initiation and promotion steps in tumorigenesis (Berenblum, 1941; Rous and Kidd, 1941; Berenblum and Shubik, 1947). Now it is generally accepted that carcinogenesis is a multi-step process and evidence has been seen for multi-step processes in organ systems in addition to skin (Slaga, 1983). The general consensus is that initiators induce genotypic changes and that promotion results in the clonal expansion of initiated cells. Although BCP does not seem to be mutagenic in *Salmonella typhimurium* strains (Mortelmans *et al.*, 1986), repeated applications of phenol and some phenolic derivatives to mouse skin, including BCP, have been reported to promote tumor development (Boutwell and Bosch, 1959). Because BCP is a phenolic compound that has widespread use, it was selected for an initiation/promotion companion study to the 2-year toxicology/carcinogenesis gavage studies. The mouse skin model for initiation and promotion of skin tumors can be readily and predictably standardized for routine testing purposes (Eastin, 1989). In this model, skin tumors can be induced by the sequential application of a subthreshold dose of a carcinogen (initiation phase) followed by repetitive treatment with a promoter (promotion stage) (Boutwell, 1964). This system can be used to determine not only the tumor-initiating and -promoting activities of a compound, but also whether it is a complete carcinogen with both tumor-initiating and -promoting activities (Slaga, 1984).

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

o-Benzyl-*p*-chlorophenol

o-Benzyl-*p*-chlorophenol (BCP) was obtained from Monsanto Chemical Company (St. Louis, MO) in one lot (KM11195), which was used throughout the study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) and the results were confirmed by the study laboratory.

The chemical, white to pink flakes, was identified as *o*-benzyl-*p*-chlorophenol by infrared, nuclear magnetic resonance, and ultraviolet/visible spectroscopy and gas chromatography. The purity was determined by elemental analysis, Karl Fischer water analysis, non-aqueous titration of the phenol group, and thin-layer and gas chromatographies. Elemental analysis for chlorine was slightly high and those for carbon and hydrogen were in agreement with the theoretical values. Karl Fischer water analysis indicated the presence of less than 0.05% water. Titration of the phenol group indicated a purity of 101% ± 1%. The overall purity was determined to be approximately 97%.

Stability studies were performed with gas chromatography, and results indicated that BCP was stable as a bulk chemical when stored protected from light for at least 2 weeks at temperatures up to 25° C. During the 1-year study, the stability of the bulk chemical was monitored by the study laboratory using gas chromatography and potentiometric titration or ultraviolet spectroscopy. Analyses were performed at the study laboratory four times during the 1-year study; no degradation of the study material was observed.

7,12-Dimethylbenz(a)anthracene

7,12-Dimethylbenz(a)anthracene (DMBA) was obtained from Eastman Kodak Company (Rochester, NY) as lot K4. The chemical was purified by the analytical chemistry laboratory and assigned the lot number M111384, which was then used throughout the study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, and confirmed by the study laboratory.

The chemical, a yellow powder, was identified as 7,12-dimethylbenz(a)anthracene by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity was determined by elemental analyses, Karl Fischer water analysis, and thin-layer and gas chromatographies. Elemental analyses for carbon and hydrogen were in agreement with theoretical values for DMBA. Karl Fischer water analysis indicated the presence of less than 0.4% water. The overall purity was determined to be greater than 99%.

Stability studies were performed by the analytical chemistry laboratory. The results indicated that DMBA was stable as a bulk chemical for 2 weeks when stored in the dark at temperatures up to 60° C. The stability of the bulk chemical was monitored at the beginning of the 1-year study and every 4 months by the study laboratory using gas chromatography and ultraviolet spectroscopy. No degradation of the study material was observed.

12-*O*-Tetradecanoylphorbol-13-acetate

Lots OE511999 and 411999 of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were obtained from Pharmacia PL Biochemicals (Milwaukee, WI). A second shipment of lot 411999 was received from Pharmacia PL Biochemicals and was assigned a new lot number (UN2811) to assist in tracking. Consolidated Midland Corporation (Brewster, NY) supplied a fourth lot (031). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, and confirmed by the study laboratory.

Each lot of the chemical was identified as 12-*O*-tetradecanoylphorbol-13-acetate by nuclear magnetic resonance spectroscopy and mass spectrometry. The purity was determined by thin-layer chromatography, mass spectrometry, and high-performance liquid chromatography. The overall purity of the lots was determined to range from 97% to 99%.

A limited stability study was performed on lot 031, and results indicated TPA was stable as a bulk chemical when stored exposed to air and light for 6 days. During the 1-year study, the stability of the bulk chemical was monitored by the study laboratory using high-performance liquid chromatography. No degradation of the bulk chemical was observed.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

o-Benzyl-*p*-chlorophenol

Dose formulation solutions were prepared by dissolving BCP in acetone and storing in amber glass bottles for up to 3 weeks. Stability analysis of a 250 mg/mL dose formulation was performed using gas chromatography. Stability was confirmed when solutions were stored in the dark at room temperature for 3 weeks. Periodic analyses of the dose formulations of BCP were conducted at the study laboratory and at the analytical chemistry laboratory. Dose formulations were analyzed once every 2 months during the 1-year study. All dose formulations except one were within specifications. Four of the first five samples retrieved from the dosing containers after administration had concentrations more than 10% greater than the theoretical concentrations. This problem was traced to solvent evaporation during dosing and was corrected using each bottle for only 1 dosing day. Previously, each bottle was used for 3 dosing days. Results of 1-year study dose formulation analyses are presented in Table D2. Periodic referee analysis of BCP solutions were performed by the analytical chemistry laboratory and their results indicated good agreement with the results obtained by the study laboratory (Table D3).

7,12-Dimethylbenz(a)anthracene

The dose formulations were prepared by mixing DMBA and acetone to give the required concentrations (Table D1). Dose formulations were prepared every 2 weeks. Stability analyses of the dose formulations were conducted by the analytical chemistry laboratory, using high-performance liquid chromatography. Stability of the formulations was established for at least 3 weeks, when stored in the dark at room temperature. Periodic analyses of the dose formulations of DMBA were conducted at the study laboratory using ultraviolet spectroscopy and at the analytical chemistry laboratory using high-performance liquid chromatography. All dose formulations analyzed prior to administration during the 1-year study were within specifications. The initial samples retrieved from the dosing container after use had concentrations more than 10% greater than the theoretical concentration. This problem was resolved by storing all dosing solutions at 4° C. Dose formulation analysis results are presented in Table D2. Results of two referee analyses performed by the analytical chemistry laboratory indicated good agreement with results obtained by the study laboratory (Table D3).

12-*O*-Tetradecanoylphorbol-13-acetate

The dose formulation solutions were prepared by mixing TPA and acetone to give the required concentrations (Table D1). Dose formulations were prepared every 2 weeks. Stability analyses of the acetone solutions were conducted by the analytical chemistry laboratory using high-performance liquid chromatography. Stability of the formulation was established for at least 3 weeks when stored at 4° C in amber glass bottles. Periodic analyses of the dose formulations of TPA were conducted at the study laboratory and at the analytical chemistry laboratory. During the 1-year study, all dose formulations were within 10% of the target concentrations (Table D2). Results of periodic referee analysis by the analytical chemistry laboratory were not always in agreement with the results obtained by the study laboratory. One sample differed by 24% and another differed by 18% (Table D3).

Dose Selection

A 3-week pilot topical application study was performed to determine appropriate concentrations of BCP for doses in the 1-year initiation/promotion study. Ten male and 10 female Swiss (CD-1®) mice were administered topical applications of BCP in 1 mL acetone at concentrations of 0.3, 1.0, 3.0, 10, or 30 mg 5 days per week for a total of 16 doses. Doses were applied to the clipped interscapular region of the backs of the mice. Mice administered 30 mg BCP developed brown discoloration at the site of application after 4 days; these areas became crusty during the second and third week of the study and appeared scaly at necropsy. During microscopic examination of 30 mg mice, hyperkeratosis, acanthosis, and inflammation were observed at the site of application. Seven males and seven females administered 10 mg developed hyperkeratosis, and the progression of these lesions during the in-life portion of the study was similar to that of animals receiving 30 mg; however, the latency of development of the lesions in

10 mg mice was longer than that in 30 mg mice. No lesions were observed during the study or at necropsy in 3.0 mg mice, but hyperkeratosis was observed in one male and one female during microscopic examination. Promoting BCP dose concentrations selected for the 1-year study were 0.3, 1.0, and 3.0 mg. Fifty micrograms of DMBA and 5 μg TPA were selected because these doses were typically used in CD-1 mice based on published reports on initiation/promotion studies (Slaga *et al.*, 1976; Verma and Boutwell, 1980; Argyris, 1981). Acetone was selected because all test chemicals were soluble in this vehicle.

Study Design

Groups of 50 male and 50 female Swiss (CD-1®) mice were topically administered a variety of doses of a well-known initiator (DMBA) and promoter (TPA) and of *o*-benzyl-*p*-chlorophenol in a 1-year study designed to test the capability of BCP to initiate and/or promote tumors (Tables 1 and 2). In addition, groups of 50 male and 50 female Swiss (CD-1®) mice were topically administered 0.1, 1.0, or 3.0 mg BCP in a protocol testing the ability of BCP to act as a complete carcinogen. Dose applications were administered in 100 μL of acetone to the dorsal interscapular region of the back. The site of application was clipped within 48 hours prior to administration of initiator doses and thereafter as often as necessary. Initiator doses were a single application during the first week of the study. Promoter doses were generally applied three times weekly beginning on the second week of the study; the promoter TPA was applied three times weekly for the first 6 months of the study and once weekly for the last 6 months of the study. In the assessment of BCP as a complete carcinogen, the compound was applied both as an initiator and as a promoter. Because of the large number of mice required, the study was carried out in two separate animal rooms and the start of the study was staggered. Half of the mice in each dose group were initiated on 25 March 1985 and promotion was begun on 1 April 1985; the remainder were initiated on 1 April 1985 and promotion was started on 8 April 1985.

TABLE 1
Dose Regimen for Reference Controls in the 1-Year Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol[a]

Treatment		
Initiator[b]	Promoter[c]	Test Group
Acetone	Acetone	Vehicle Control
Acetone	20 μg DMBA	Reference Complete Carcinogen Control
50 μg DMBA	Acetone	Reference Initiator Control
50 μg DMBA	5 μg TPA	Reference Initiator/Promoter Control
5 μg TPA	5 μg TPA	Reference Promoter Control

[a] All dose volumes were 100 μL.
[b] Initiator doses were applied once during week 1 of the study, at which time mice were approximately 56 days old.
[c] With the exception of TPA, promoter doses were applied three times weekly from week 2 through week 52. TPA was applied three times weekly as a promoter for the first 6 months of the study, and once weekly for the last 6 months.

TABLE 2
Dose Regimen in the 1-Year Initiation/Promotion Study of o-Benzyl-p-Chlorophenol[a]

Treatment		
Initiator[b]	Promoter[c]	Test Group
10 mg BCP	0.1 mg BCP	Low-Dose as Complete Carcinogen
10 mg BCP	1.0 mg BCP	Mid-Dose as Complete Carcinogen
10 mg BCP	3.0 mg BCP	High-Dose as Complete Carcinogen
10 mg BCP	5 μg TPA	BCP as Initiator
50 μg DMBA	0.1 mg BCP	BCP Low-Dose as Promoter
50 μg DMBA	1.0 mg BCP	BCP Mid-Dose as Promoter
50 μg DMBA	3.0 mg BCP	BCP High-Dose as Promoter

[a] All dose volumes were 100 μL.
[b] Initiator doses were applied once during week 1 of the study, at which time mice were approximately 56 days old.
[c] With the exception of TPA, promoter doses were applied three times weekly from week 2 through week 52. TPA was applied three times weekly as a promoter for the first 6 months of the study, and once weekly for the last 6 months.

BCP as a Complete Carcinogen: The potential for BCP to act as a complete carcinogen was studied using the following initiator/promoter combinations: acetone/acetone (vehicle control); acetone/20 μg DMBA (complete carcinogen control); 10 mg BCP/0.1 mg BCP (low-dose complete carcinogen); 10 mg BCP/1.0 mg BCP (mid-dose complete carcinogen); and 10 mg BCP/3.0 mg BCP (high-dose complete carcinogen).

BCP as an Initiator: The potential of BCP for tumor initiation was studied using these initiator/promoter combinations: acetone/acetone (vehicle control); 5 μg TPA/5 μg TPA (promoter control); 50 μg DMBA/5 μg TPA (initiator/promoter control); and 10 mg BCP/5 μg TPA (initiator).

BCP as a Promoter: The potential of BCP for tumor promotion was studied using the following initiator/promoter combinations: acetone/acetone (vehicle control); 50 μg DMBA/acetone (initiator control); 50 μg DMBA/5 μg TPA (initiator/promoter control); 50 μg DMBA/0.1 mg BCP (low-dose promoter); 50 μg DMBA/1.0 mg BCP (mid-dose promoter); and 50 μg DMBA/3.0 mg BCP (high-dose promoter).

Source and Specification of Animals

Male and female Swiss (CD-1®) mice were obtained in two shipments arriving 1 week apart from Charles River Breeding Laboratories (Kingston, NY). All animals were quarantined for 13 days prior to the beginning of the study. Five male and five female mice were examined for parasites and gross observation of disease. Mice were approximately 8 weeks old at the beginning of the study. Serum samples were obtained from 10 males and 30 females during quarantine, from eight males and eight females at 3 months, from nine males and nine females at 6 months, and from 10 male and 10 female vehicle controls (acetone/acetone) at the end of the study. In addition, sera were collected from moribund animals at various times during the study. Sera from sentinel animals were evaluated using the protocols of the NTP Sentinel Animal Program (Appendix F).

Animal Maintenance

Mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated once every 2 weeks.

Using the mouse skin initiation/promotion protocol, the appearance and progression of tumor development can be observed. Since the skin is the primary

target organ, the effect of chemical treatment would be expected to manifest itself at this site before affecting internal organs. In the present study, it was expected that some treated mice would develop lesions on the skin and an aggressive moribund sacrifice policy was maintained. Mice with large masses or other conditions that interfered with feed or water consumption and mice with ulcerations, debilitating conditions, or conditions indicating pain or suffering as judged by the laboratory animal veterinarian were killed.

Further details of animal maintenance are given in Table 3. Information on feed composition and contaminants is provided in Appendix E.

Clinical Examinations and Pathology

Animals were observed twice daily. Body weights were recorded weekly through the first 3 months, then monthly until the end of the study. Clinical findings were recorded once weekly. Clinical observations of the skin were recorded once weekly to follow the appearance and progression of any tumor development. To provide consistency for these observations, guidelines were developed to define how skin tumor response would be recorded. Thus, at first appearance, each skin tumor was recorded as a tissue mass. A tissue mass that was present for 14 days and was at least 2 mm in diameter was termed a papilloma. A papilloma that became necrotic in appearance and attached to an underlying tissue was termed a carcinoma.

A gross necropsy was performed on all mice and the kidneys, liver, and thymus were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. Histopathologic examinations were performed on skin (up to five tumors), nose, liver, kidney, thymus, and on all tissues with grossly visible lesions. For all paired organs (i.e., kidney, ovary, adrenal gland), samples from each organ are examined. Tissues examined are listed in Table 3.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscopic 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 evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist reviewed the kidney, liver, nose, skin, and thymus of mice for accuracy and consistency of diagnosis.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which a disagreement in diagnosis between the laboratory and quality assessment pathologists existed. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologist, or lesions of general interest were presented by the chair to the PWG for review. Tissues examined included: skin from sites of application, and (when appropriate) from other sites; kidney; liver; nose; and thymus. In addition, the pathologist examined a single lesion diagnosed by the study pathologist as a chemodectoma in a male mouse. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

All animals were necropsied and received a complete gross examination for masses or lesions. In addition, the kidney, liver, nose, and thymus were examined microscopically because these tissues were potential target sites based on clinical signs and histopathology results from 13-week gavage studies (NTP, 1994). The intent was to determine if these organs would be affected by topical treatment of BCP for up to

50 weeks. There were no chemical-related increased incidences of tumors or nonneoplastic lesions in the kidney, liver, nose, or thymus of mice when BCP was applied as a promoter or a complete carcinogen.

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of tumors or nonneoplastic lesions as presented in Appendixes A and B are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most tumors and all nonneoplastic lesions are given as the number of affected animals and the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect tumors in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) before microscopic evaluation, or when tumors had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidences

Because clinical observations were routinely recorded once a week and included the presence of skin tumors, it was possible to determine the approximate time of skin tumor onset for each animal. Consequently, the primary statistical analysis for skin tumors was the life table test (Cox, 1972; Tarone, 1975) based on the observed time of tumor onset. Most (but not all) of these tumors were also present when the animal died. Time of onset for the remaining tumors could not be determined, and the primary statistical method used was logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The tumor incidences of dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, see Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time.

Quality Assurance Methods

The 1-year study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 1-year study were submitted to the NTP Archives, the study was audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of *o*-benzyl-*p*-chlorophenol was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and chromosomal damage in cultured Chinese hamster ovary cells. The protocols for these studies and the results are given in Appendix C.

The genetic toxicity studies of *o*-benzyl-*p*-chlorophenol are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between the potential electrophilicity of a chemical (structural alert to DNA reactivity), mutagenicity in *S. typhimurium*, and carcinogenicity in rats and mice at single or multiple tissue sites (Ashby and Tennant, 1991). The other *in vitro* tests do not correlate well with carcinogenicity in rodents (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical under investigation. Data from NTP studies show that a positive response in *S. typhimurium* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the mutagens were rodent carcinogens), and that there is no complimentarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *S. typhimurium* test improved the predictivity of the *S. typhimurium* test alone. The predictivity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 3
Experimental Design and Materials and Methods in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol

Study Laboratory
Battelle Columbus Laboratories

Strain and Species
Swiss (CD-1®) mice

Animal Source
Charles River Breeding Laboratories (Kingston, NY)

Time Held Before Studies
13 days

Average Age When Studies Began
8 weeks

Date of First Dose
25 March 1985 or 1 April 1985

Duration of Dosing
52 weeks

Date of Last Dose
21 March 1986 or 28 March 1986

Necropsy Dates
25-28 March 1986 or 31 March-4 April 1986

Average Age at Necropsy
60 weeks

Size of Study Groups
50 males and 50 females

Method of Distribution
Mice weighed and placed into one of two weight classes then randomly separated by sex into individual cages; randomized using a Xybion computer randomization program.

Animals per Cage
1

Method of Animal Identification
Toe mark

Diet
Zeigler NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*

Maximum Storage Time for Feed
120 days post-milling

Water Distribution
Tap water supplied via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*

Cages
Polycarbonate (Lab Products, Inc., Garfield, NJ), changed weekly

TABLE 3
Experimental Design and Materials and Methods in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol (continued)

Bedding
BetaChip hardwood chips (Northeastern Products, Inc., Warrensburg, NY), changed weekly

Cage Filters
Spun-bonded polyester filter sheets (DuPont 2024) (Snow Filtration, Company, Cincinnati, OH), changed weekly

Racks
Stainless steel (Lab Products, Inc., Garfield, NJ)

Animal Room Environment
Average temperature: 21.8° ± 2.7° C
Relative humidity: 28% to 78%
Fluorescent light: 12 hours/day
Room air: 15 changes of room air/hour

Type and Frequency of Observation
Observed twice daily for morbidity and mortality with the exception of 3 days early in the study, when animals in one of the study rooms were checked only daily; observed once weekly for clinical signs. Clinical observations of the skin were made weekly. Animals weighed weekly through the first 3 months, then monthly until the end of the study.

Method of Sacrifice
CO_2 asphyxiation

Necropsy
Necropsy performed on all animals. The kidneys, liver, and thymus were weighed.

Histopathology
In addition to gross lesions, histopathology was performed on the skin, nose, liver, kidney, and thymus of all mice.

RESULTS

REFERENCE CONTROLS

The reference controls for this initiation/promotion study were: acetone/acetone (vehicle control): 7,12-dimethylbenz(a)anthracene (DMBA)/acetone (initiator control); 12-*O*-tetradecanoylphorbol-13-acetate (TPA)/TPA (promoter control); DMBA/TPA (initiator/promoter control); and acetone/DMBA (complete carcinogen control).

Survival in the Reference Controls

The survival rates of all reference control groups were lower than those of the vehicle control, except for that of males and females receiving DMBA/acetone (Table 4). Decreased survival was most notable in male and female mice administered the initiator/promoter control (DMBA/TPA) or the complete carcinogen control (acetone/DMBA). A primary feature of initiation/promotion studies using the mouse skin model to study tumorigenesis is the ability to observe the first appearance of a neoplasm and monitor the progressive stages of development without killing the animal. At the same time, the study laboratory is required to maintain an aggressive moribund sacrifice policy; once neoplasms appear, their progression is closely followed, and animals determined to be in distress are killed. Animals in the complete carcinogen (acetone/DMBA) and the initiator/promoter (DMBA/TPA) control groups would have been expected to develop neoplasms earlier and in greater frequency than animals in any of the other groups. Animals promoted with TPA had early signs of irritation and ulcers and the signs persisted throughout the study, suggesting that these animals may have appeared distressed. The lower survival rates in these groups were primarily due to moribund sacrifice.

TABLE 4
Survival of Mice in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol: Summary of the Reference Controls

	Acetone/ Acetone	DMBA/ Acetone	DMBA/TPA	TPA/TPA	Acetone/ DMBA
Male					
Animals initially in study	50	50	50	50	50
Accidental deaths[a]	0	0	1	0	0
Moribund	2	3	26	13	38
Natural deaths	1	5	16	8	12
Animals surviving to study termination	47	42	7	29	0
Percent probability of survival at end of study[b]	94	84	14	58	0
Mean survival (days)[c]	358	360	257	331	256
Survival analysis[d]		P=0.203	P<0.001	P<0.001	P<0.001
Female					
Animals initially in study	50	50	50	50	50
Accidental deaths[a]	1	0	1	0	0
Moribund	5	0	23	13	42
Natural deaths	0	3	13	3	8
Animals surviving to study termination	44	47	13	34	0
Percent probability of survival at end of study	90	94	27	68	0
Mean survival (days)	348	359	286	341	255
Survival analysis		P=0.687N	P<0.001	P=0.016	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the reference control columns. A lower mortality in a reference control group is indicated by N.

Clinical Findings in the Reference Controls

Vehicle control mice and mice administered DMBA/acetone had few lesions at the application site. Chemical-related lesions at the application site included ulceration, scaling, crusting, and discoloration. At the end of the study, scaling/crusts, ulceration, and irritation were most frequently observed in groups of male and female mice promoted with TPA and in acetone/DMBA mice. Within dose groups, lesion type and incidence between males and females were similar.

Tumor Response in the Reference Controls

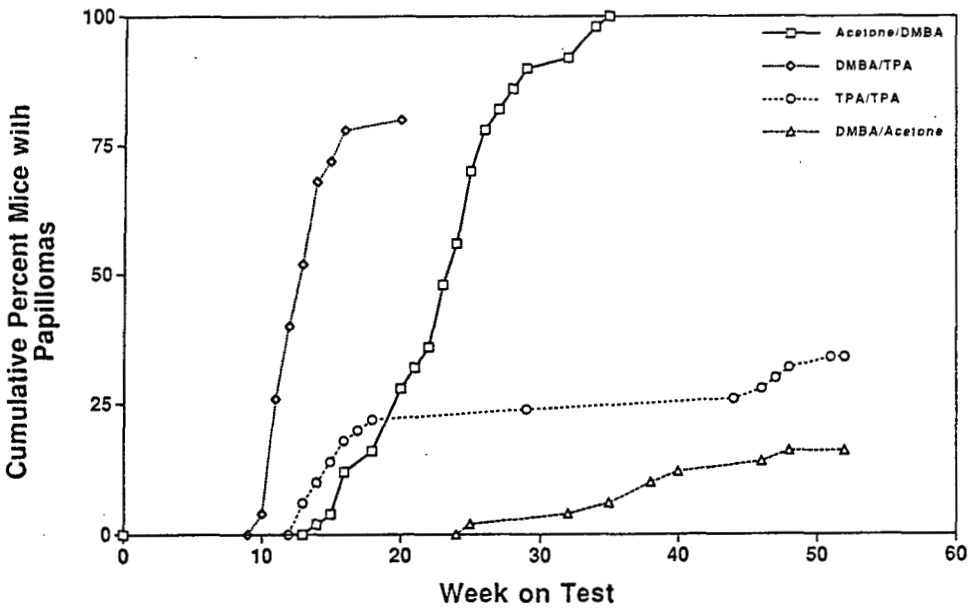
No papillomas or carcinomas were observed in vehicle control mice. The percentage of mice developing neoplasms in each treatment group, the length of time to first neoplasm appearance, the number of neoplasms per neoplasm-bearing animal, and the neoplasm types were similar between males and females (Table 5; Figure 1). Eight males and two females administered DMBA/acetone developed papillomas, and the first neoplasms were observed in males during week 26 and in females during week 27. Statistical analyses of the incidences of skin neoplasms observed in the reference controls are presented in Table 6.

Control groups were included in this study to serve as references. The initiator reference control was a subthreshold single dose of DMBA, a known carcinogen, followed by repetitive applications of the acetone vehicle. The concentration of DMBA to be administered as an initiator was selected from the literature, and under optimum conditions DMBA/acetone mice should not have developed any chemical-related skin neoplasms. In the present study, however, eight males and two females developed skin neoplasms, indicating that the initiating dose level of 50 μg may have been slightly above the threshold level for Swiss (CD-1®) mice. The initiation/promotion reference control (DMBA/TPA), routinely used to study tumorigenesis in mouse skin protocols, was used to compare the response to known promoters of tumorigenesis with the response to promotion with BCP. Initiation with DMBA followed by promotion with TPA produced neoplasms in 80% of the males in the study and in 96% of the females. Mice in another reference control group, the complete carcinogen control group, were administered repetitive applications of 20 μg DMBA and the effects on these mice were compared to the effects on mice administered BCP only. Mice in both the initiation/promotion reference control and the complete carcinogen reference control groups developed neoplasms. However, more complete carcinogen control mice developed carcinomas (males, 16/50; females, 19/50) than did initiation/promotion reference control mice (males, 1/50; females, 2/50).

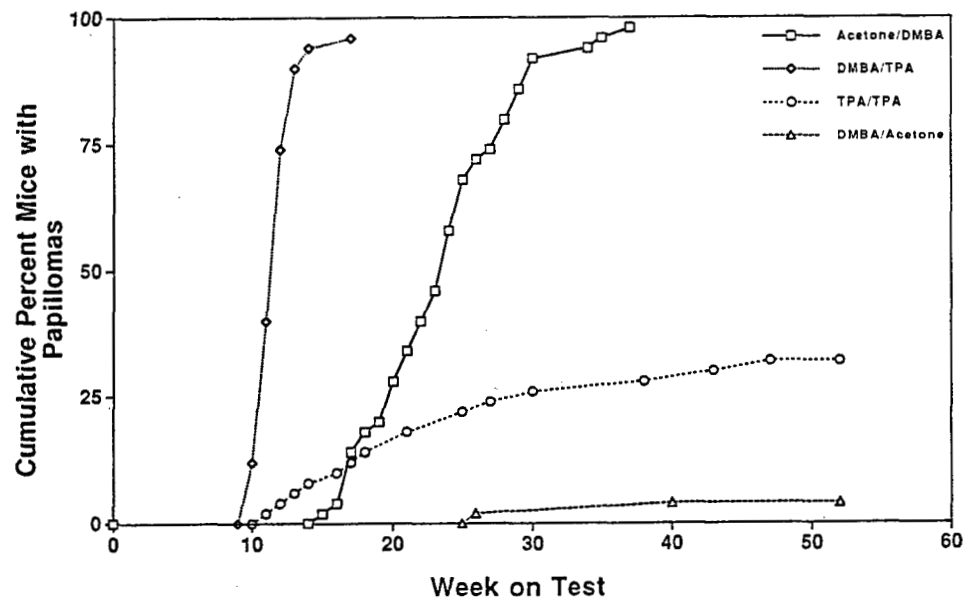
TABLE 5
Average Number of Papillomas Observed In-Life per Neoplasm-Bearing Mouse in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol: Tumor Response in the Reference Controls[a]

	Male					Female				
Week of Study	10	20	30	40	52	10	20	30	40	52
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
DMBA/Acetone	0	0	1.00	1.20	1.14	0	0	1.00	1.00	1.00
TPA/TPA	0	1.27	1.36	1.50	1.60	0	1.14	1.11	1.17	1.20
DMBA/TPA	1.00	7.20	5.50	6.27	5.33	0	8.63	7.12	7.11	5.86
Acetone/DMBA	0	1.64	2.58	1.90	0	0	1.27	2.81	1.53	0

[a] Average number of papillomas per mouse is expressed as total number of papillomas/number of mice with papillomas.



Male



Female

FIGURE 1
Tumor Response of Male and Female Swiss (CD-1®) Mice to DMBA and TPA Alone and in Combination

TABLE 6
Incidence of Skin Papillomas and Carcinomas Observed In-Life in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol: Tumor Response in the Reference Controls

	Acetone/Acetone	DMBA/Acetone	TPA/TPA	DMBA/TPA	Acetone/DMBA
Male					
Papilloma					
Overall rates[a]	0/50 (0%)	8/50 (16%)	16/50 (32%)	40/50 (80%)	50/50 (100%)
Adjusted rates[b]	0.0%	16.2%	36.9%	80.0%	100%
First incidence (days)	–[d]	170	91	65	98
Life table test[c]		P=0.005	P<0.001	P<0.001	P<0.001
Carcinoma					
Overall rates	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	16/50 (32%)
Adjusted rates	0.0%	0%	0.0%	2.9%	52.3%
First incidence (days)	–	–	–	233	149
Life table test		–	–	P=0.427	P<0.001
Female					
Papilloma					
Overall rates	0/50 (0%)	2/50 (4%)	16/50 (32%)	48/50 (96%)	49/50 (98%)
Adjusted rates	0.0%	4.1%	32.7%	96.0%	100%
First incidence (days)	–	179	74	74	109
Life table test		P=0.240	P<0.001	P<0.001	P<0.001
Carcinoma					
Overall rates	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)	19/50 (38%)
Adjusted rates	0.0%	0.0%	0.0%	8.5%	63.0%
First incidence (days)	–	–	–	214	151
Life table test		–	–	P=0.123	P<0.001

[a] Number of animals with neoplasm/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Beneath the dosed group incidences are the P values corresponding to pairwise comparisons between the vehicle control and that dosed group. Life table test results are based on the time of the first observation of papilloma or carcinoma.
[d] Not applicable; no neoplasms in animal group

BCP AS A COMPLETE CARCINOGEN

Survival in the Study of BCP as a Complete Carcinogen

Estimates of survival probabilities for male and female mice in the study of BCP as a complete carcinogen are presented in Table 7 and in the Kaplan-Meier survival curves (Figure 2). The survival rates of males and females initiated and promoted with 3.0 mg BCP were significantly lower than those of the vehicle controls, but significantly greater than those of mice administered the complete carcinogen control (acetone/DMBA) groups.

Body Weights and Clinical Findings in the Study of BCP as a Complete Carcinogen

The final mean body weights of males administered BCP as a complete carcinogen were similar and female body weights were decreased compared with those of the vehicle controls (Figure 3).

The incidences of scales and/or crusts and irritation were generally greater in male and female mice initiated with 10 mg BCP and promoted with 3.0 mg BCP than in those promoted with 0.1 or 1.0 mg BCP (Table 8). Males and females administered promoting doses of 3.0 mg BCP generally had incidences of scales, ulcers, and irritation similar to those in the complete carcinogen controls (acetone/DMBA).

TABLE 7
Survival of Mice in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as a Complete Carcinogen

	Acetone/ Acetone	Acetone/ DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Male					
Animals initially in study	50	50	50	50	50
Accidental deaths[a]	0	0	0	1	0
Moribund	2	38	8	6	9
Natural deaths	1	12	2	3	5
Animals surviving to study termination	47	0	40[d]	40	36
Percent probability of survival at end of study[b]	94	0	80	82	72
Mean survival (days)[c]	358	256	345	341	328
Survival analysis[e]	P=0.022		P=0.071	P=0.109	P=0.007
Survival analysis[f]		P<0.001			
Female					
Animals initially in study	50	50	51	50	50
Accidental deaths[a]	1	0	1	0	1
Moribund	5	42	9	7	10
Natural deaths	0	8	4	2	4
Animals surviving to study termination	44	0	37	41	35[d]
Percent probability of survival at end of study	90	0	74	82	72
Mean survival (days)	348	255	319	332	324
Survival analysis	P=0.170		P=0.060	P=0.372	P=0.033
Survival analysis		P<0.001			

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] Includes one animal that died during the last week of the study
[e] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle control are in the dosed columns.
[f] The result of the life table pairwise comparison with the vehicle control is in the Acetone/DMBA column.

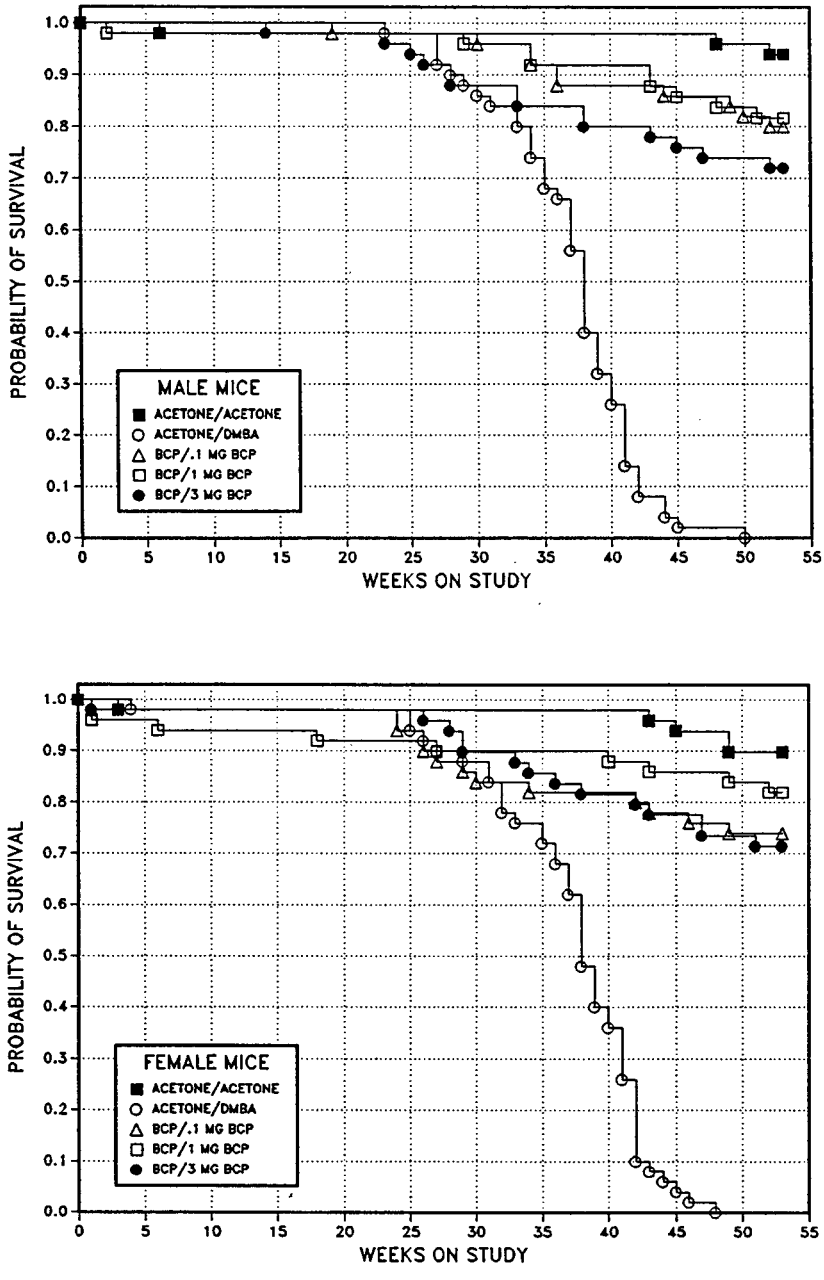


FIGURE 2
Kaplan-Meier Survival Curves for the 1-Year Study of BCP as a Complete Carcinogen in Male and Female Swiss (CD-1®) Mice

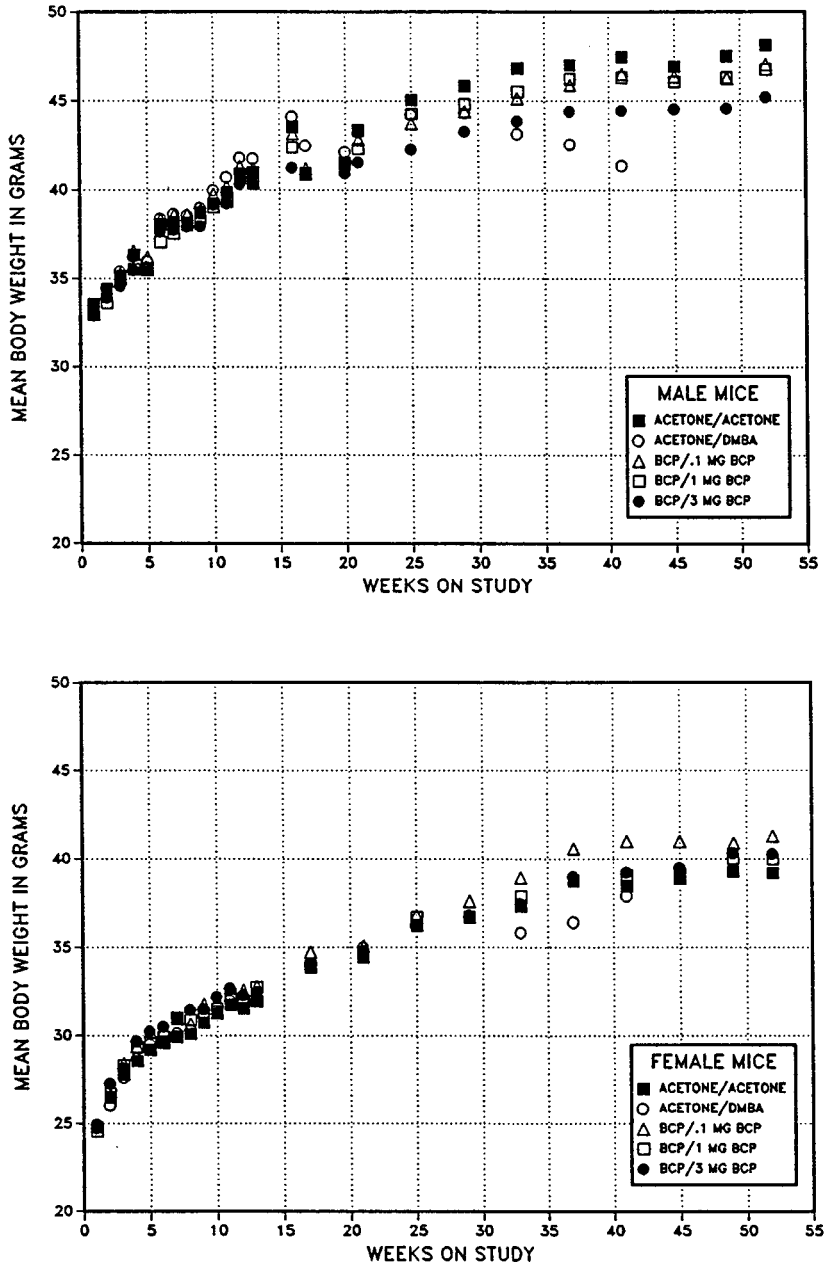


FIGURE 3
Growth Curves for the 1-Year Study of BCP as a Complete Carcinogen in Male and Female Swiss (CD-1®) Mice

TABLE 8
Incidences of Selected Cutaneous Application Site Lesions Observed In-Life in the 1-Year Mouse Skin Initiation/Promotion Study of o-Benzyl-p-Chlorophenol as a Complete Carcinogen

Initiator/ Promoter	Lesion			
	Scales	Crusts	Ulcer	Irritation
n	50	50	50	50
Male				
Acetone/Acetone	2[a]	1	1	1
Acetone/DMBA	47**	7*	28**	42**
10 mg BCP/0.1 mg BCP	23**	36**	23**	13**
10 mg BCP/1.0 mg BCP	30**	35**	21**	15**
10 mg BCP/3.0 mg BCP	48**	46**	33**	32**
Female				
Acetone/Acetone	3	0	0	1
Acetone/DMBA	40**	6**	32**	40**
10 mg BCP/0.1 mg BCP[b]	21**	15**	44**	15**
10 mg BCP/1.0 mg BCP	26**	13**	41**	14**
10 mg BCP/3.0 mg BCP	49**	16**	39**	34**

* P<0.05 vs. Acetone/Acetone (life table test)
** P<0.01
[a] Number of animals with observation recorded during the course of the study
[b] n=51

Tumor Response in the Study of BCP as a Complete Carcinogen

Papillomas were first observed in male and female complete carcinogen controls (acetone/DMBA) about 14 weeks after study initiation (Figure 1). By week 30, papillomas were observed in 82% to 84% of the animals and in all males and all but one female by week 35. The response of male and female Swiss (CD-1®) mouse skin to the effects of repetitive applications of DMBA appears to be similar judging by the pattern of time to neoplasm. Complete carcinogen control males and females averaged 1.90 and 1.53 papillomas, respectively (Table 9). No skin papillomas were observed grossly in male or female vehicle controls (Table 9).

BCP had essentially no activity when evaluated as a complete carcinogen (BCP/BCP). One low-dose male developed a papilloma after 11 weeks of application; three low-dose females developed papillomas after 22 weeks and one high-dose female developed two papillomas after 10 weeks (Table 9). None of the mice in the BCP-initiated/BCP-promoted groups had papillomas at the end of the study. No carcinomas were identified at the application site of any BCP-initiated mice administered a promoting dose of BCP. In contrast, most mice administered the complete carcinogen control (acetone/DMBA) developed papillomas (Table 10) and many also developed carcinomas (Table A1c and B1c).

TABLE 9
Average Number of Papillomas Observed In-Life per Neoplasm-Bearing Mouse in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as a Complete Carcinogen[a]

	Male					Female				
Week of Study	10	20	30	40	52	10	20	30	40	52
Initiator/Promoter										
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
Acetone/DMBA	0	1.64	2.58	1.90	0	0	1.27	2.81	1.53	0
10 mg BCP/0.1 mg BCP	0	0	0	0	0	0	0	2.00	0	0
10 mg BCP/1.0 mg BCP	0	0	0	0	0	0	0	0	0	0
10 mg BCP/3.0 mg BCP	0	0	0	0	0	1.00	1.00	0	0	0

[a] Average is expressed as total number of papillomas/number of mice with papillomas.

TABLE 10
Incidence of Skin Papillomas Observed In-Life in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as a Complete Carcinogen

	Acetone/ Acetone	Acetone/ DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Male					
Overall rates[a]	0/50 (0%)	50/50 (100%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rates[b]	0.0%	100%	2.0%	0.0%	0.0%
First incidence (days)	–[e]	98	72	–	–
Life table test[c]	P=0.498N		P=0.504	–	–
Life table test[d]		P<0.001			
Female					
Overall rates	0/50 (0%)	49/50 (98%)	3/51 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rates	0.0%	100%	6.4%	0.0%	2.0%
First incidence (days)	–	109	151	–	67
Life table test	P=0.582		P=0.116	–	P=0.504
Life table test		P<0.001			

[a] Number of animals with neoplasm/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Beneath the vehicle control incidences are the P values associated with the trend test for the BCP/BCP and Acetone/Acetone groups. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. Life table test results are based on the time of the first observation of papilloma. A negative trend is indicated by N.
[d] Beneath the Acetone/DMBA group incidence are the P values corresponding to pairwise comparisons between that group and the Acetone/Acetone controls. Life table test results are based on the time of the first observation of papilloma.
[e] Not applicable; no neoplasms in animal group

BCP AS AN INITIATOR

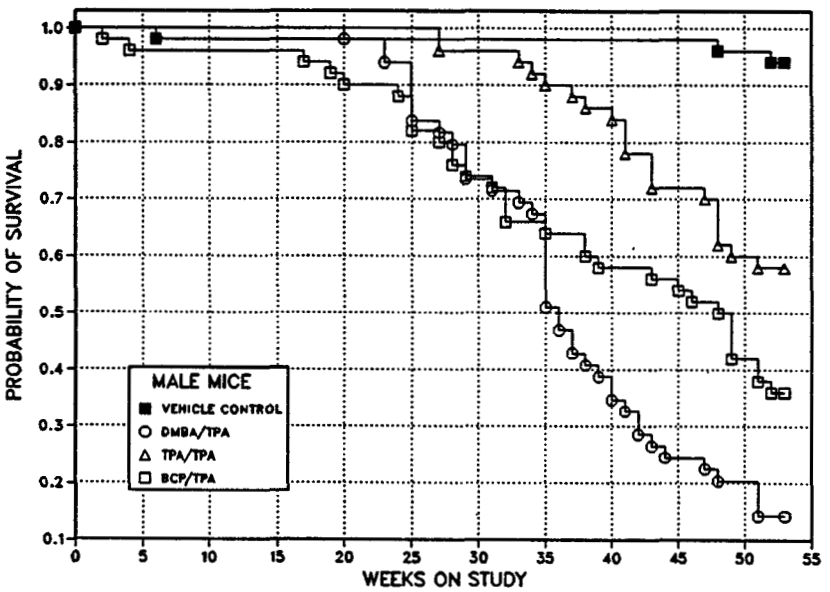
Survival in the Study of BCP as an Initiator

Estimates of survival probabilities for male and female mice in the study of BCP as an initiator are presented in Table 11 and in the Kaplan-Meier survival curves (Figure 4). The survival rates of males and females initiated with BCP and promoted with TPA were lower than those of the vehicle controls and promoter control (TPA/TPA) mice, but greater than those of mice receiving the initiator/promoter control (DMBA/TPA).

TABLE 11
Survival of Mice in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as an Initiator

	Acetone/ Acetone	DMBA/ Acetone	DMBA/TPA	TPA/TPA	BCP/TPA
Male					
Animals initially in study	50	50	50	50	50
Accidental deaths[a]	0	0	1	0	0
Moribund	2	3	26	13	22
Natural deaths	1	5	16	8	10
Animals surviving to study termination	47	42	7	29	18
Percent probability of survival at end of study[b]	94	84	14	58	36
Mean survival (days)[c]	358	360	257	331	280
Survival analysis[d]		P=0.203	P<0.001	P<0.001	P<0.001
Female					
Animals initially in study	50	50	50	50	50
Accidental deaths[a]	1	0	1	0	0
Moribund	5	0	23	13	11
Natural deaths	0	3	13	3	13
Animals surviving to study termination	44	47	13	34	26
Percent probability of survival at end of study	90	94	27	68	52
Mean survival (days)	348	359	286	341	283
Survival analysis		P=0.687N	P<0.001	P=0.016	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the reference control and dose columns. A lower mortality in a dose group is indicated by N.



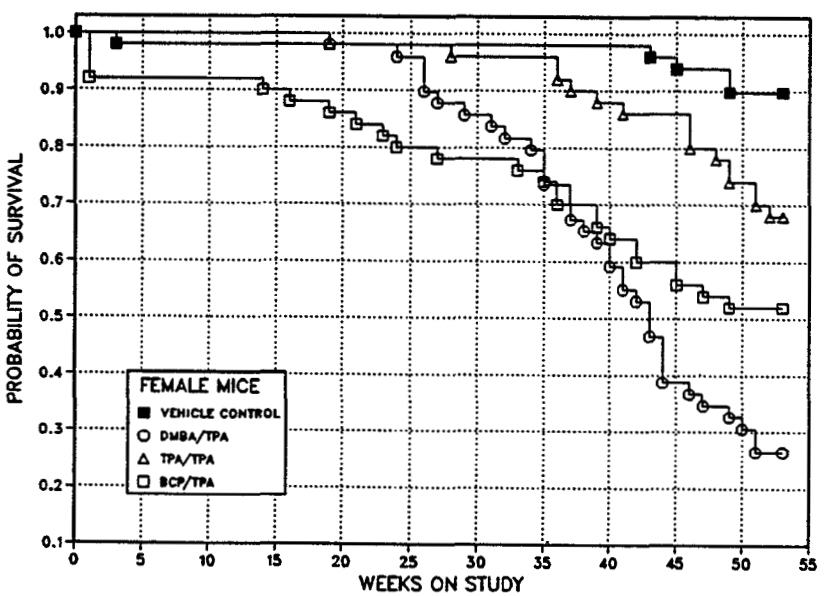


FIGURE 4
Kaplan-Meier Survival Curves for the 1-Year Study of BCP as an Initiator in Male and Female Swiss (CD-1®) Mice

Body Weights and Clinical Findings in the Study of BCP as an Initiator

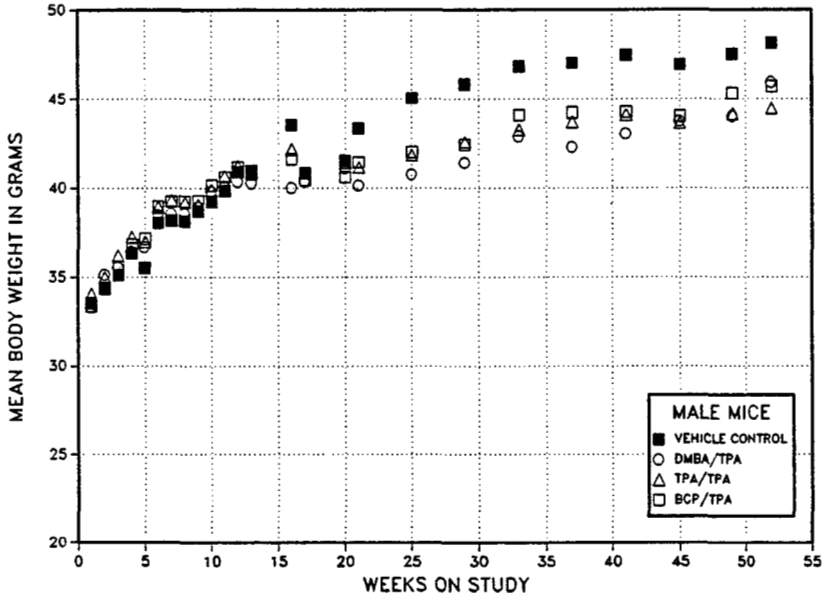
The final mean body weight of males initiated with BCP (BCP/TPA) was 95% of the vehicle control; the final mean body weight of BCP/TPA females was similar to that of the vehicle controls (Figure 5). The final mean body weights of BCP/TPA males and females were similar to those of the initiator/promoter and promoter control groups.

The use of TPA as a promoter with or without initiation was quite irritating to mouse skin (Table 12); ulcers and irritation were recorded for BCP/TPA mice at day 11. However, observations recorded at the time BCP/TPA mice were removed from the study showed fewer mice with these lesions (especially ulcers and irritation), suggesting a recovery from the initial insult. In addition, the frequency of TPA administration was reduced to once weekly during week 28 of the study; after this change, the frequency and severity of skin irritation was reduced.

TABLE 12
Incidences of Selected Cutaneous Application Site Lesions Observed In-Life in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as an Initiator

Initiator/ Promoter	Lesion			
	Scales	Crusts	Ulcer	Irritation
n	50	50	50	50
Male				
Acetone/Acetone	2[a]	1	1	1
DMBA/TPA	48**	18**	32**	44**
TPA/TPA	50**	14**	19**	47**
BCP/TPA	48**	40**	35**	44**
DMBA/Acetone	3	0	1	2
Female				
Acetone/Acetone	3	0	0	1
DMBA/TPA	46**	10**	26**	45**
TPA/TPA	50**	6*	14**	36**
BCP/TPA	46**	19**	42**	44**
DMBA/Acetone	8	0	0	3

* $P<0.05$ vs. Acetone/Acetone (life table test)
** $P<0.01$
[a] Number of animals with observation recorded during the course of the study



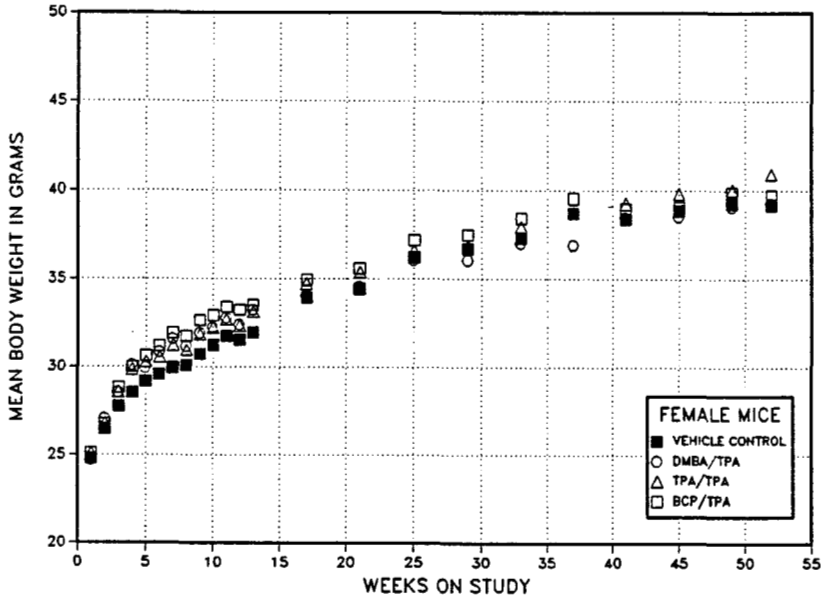


FIGURE 5
Growth Curves for the 1-Year Study of BCP as an Initiator in Male and Female Swiss (CD-1®) Mice

Tumor Response in the Study of BCP as an Initiator

Eight males and two females initiated with DMBA and receiving no promoter treatment (DMBA/acetone; initiator control) were first observed with papillomas after approximately 26 weeks of exposure (Tables 13 and 14). Mice administered DMBA/TPA (initiator/promoter control) developed multiple papillomas between weeks 10 and 20. At the end of the study, males administered DMBA/TPA had an average of 5.33 papillomas, and DMBA/TPA females had an average of 5.86 (Table 13). The average number of papillomas did not increase after 20 weeks. However, there appeared to be a slight difference in neoplasm response between males and females (Figure 6); papillomas were observed in 40 males and 48 females.

Males and females responded equally to administration of TPA/TPA (promoter control) (Figure 6). Papillomas began to appear about week 11 and approximately 32% of the animals were observed with a papilloma at some time during the study (Table 14). Some TPA/TPA mice observed to have papillomas during the course of the study did not have papillomas when they were removed from study.

Males and females responded similarly to initiation with BCP followed by promotion with TPA (Figure 6). Two females were observed with papillomas at week 13, but no more females were observed with neoplasms until week 25, about the same time papillomas were first observed in males. Papillomas were observed in about twice as many males as females at some time during the study (Table 14). The incidence of papillomas in mice administered TPA/TPA was similar to that in mice initiated with BCP and promoted with TPA (Table 14). Thus, it was not possible to observe any effect of initiation with BCP. However, BCP clearly does not have the same potential for initiation of tumorigenesis seen in mice administered the initiation/promotion control (DMBA/TPA).

No papillomas were observed grossly in male or female vehicle controls (Table 13).

TABLE 13
Average Number of Papillomas Observed In-Life per Neoplasm-Bearing Mouse in the 1-Year Mouse Skin Initiation/Promotion Study of o-Benzyl-p-Chlorophenol as an Initiator[a]

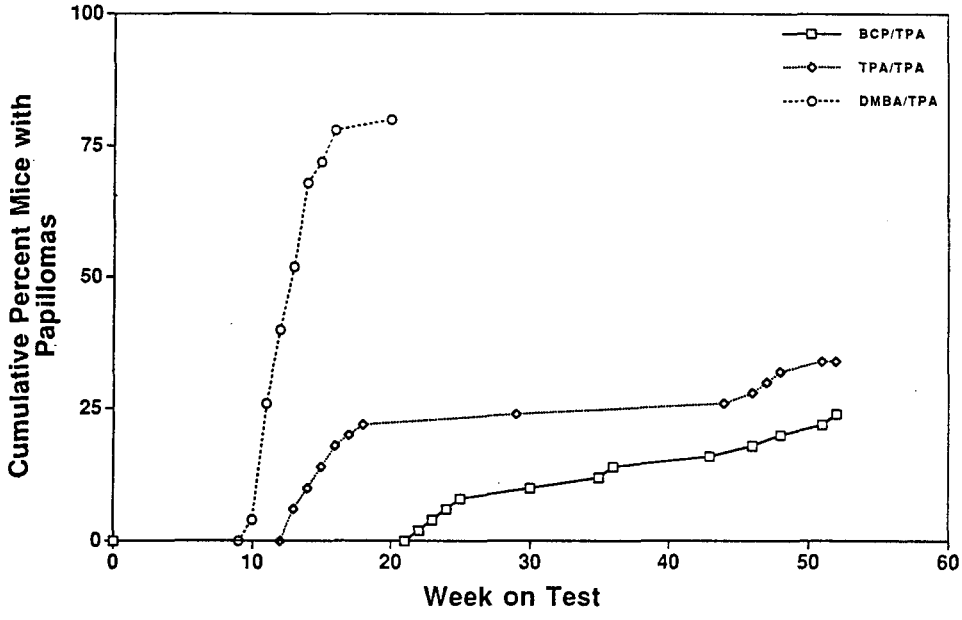
	Male					Female				
Week of Study	10	20	30	40	52	10	20	30	40	52
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
DMBA/Acetone	0	0	1.00	1.20	1.14	0	0	1.00	1.00	1.00
DMBA/TPA	1.00	7.20	5.50	6.27	5.33	0	8.63	7.12	7.11	5.86
TPA/TPA	0	1.27	1.36	1.50	1.60	0	1.14	1.11	1.17	1.20
BCP/TPA	0	0	1.00	1.20	1.13	0	1.00	1.00	1.00	1.00

[a] Average number of papillomas per mouse is expressed as total number of papillomas/number of mice with papillomas.

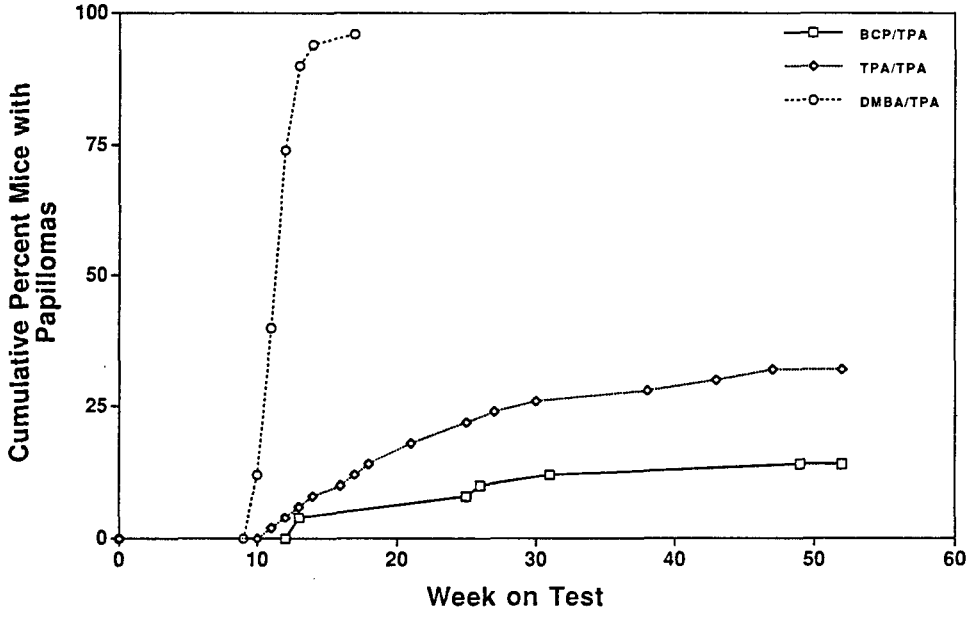
TABLE 14
Incidence of Skin Papillomas Observed In-Life in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as an Initiator

	Acetone/ Acetone	DMBA/ Acetone	TPA/TPA	BCP/TPA	DMBA/TPA
Male					
Overall rates[a]	0/50 (0%)	8/50 (16%)	16/50 (32%)	12/50 (24%)	40/50 (80%)
Adjusted rates[b]	0.0%	16.2%	36.9%	43.7%	80.0%
First incidence (days)	–[e]	170	91	156	65
Comparison with Acetone/Acetone[c]		P=0.005	P<0.001	P<0.001	P<0.001
Comparison with TPA/TPA[d]				P=0.363N	
Female					
Overall rates	0/50 (0%)	2/50 (4%)	16/50 (32%)	7/50 (14%)	48/50 (96%)
Adjusted rates	0.0%	4.1%	32.7%	18.3%	96.0%
First incidence (days)	–	179	74	88	74
Comparison with Acetone/Acetone		P=0.240	P<0.001	P=0.004	P<0.001
Comparison with TPA/TPA				P=0.070N	

[a] Number of animals with neoplasm/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Life table test; pairwise comparison with the Acetone/Acetone controls. Life table test results are based on the time of the first observation of papilloma.
[d] Life table test; pairwise comparison between the TPA/TPA group. Life table test results are based on the time of the first observation of papilloma. A lower incidence in the BCP/TPA group is indicated by N.
[e] Not applicable; no neoplasms in animal group



Male



Female

FIGURE 6
Cumulative Percent of Male and Female Swiss (CD-1®) Mice with Papillomas in the 1-Year Initiation/Promotion Study of BCP as an Initiator

BCP AS A PROMOTER

Survival in the Study of BCP as a Promoter

Estimates of survival probabilities for male and female mice in the study of BCP as a promoter are presented in Table 15 and in the Kaplan-Meier survival curves (Figure 7). The survival rates of males and females initiated with DMBA and promoted with BCP were similar to those of the initiator (DMBA/acetone) control groups and were significantly greater than those of the initiator/promoter (DMBA/TPA) control groups.

Body Weights and Clinical Findings in the Study of BCP as a Promoter

The mean body weights of males and females administered BCP as a promoter were similar to those of the initiator control throughout the study (Figure 8).

Few application-site lesions were observed in mice administered DMBA/0.1 mg BCP or DMBA/1.0 mg BCP (Table 16). The incidences of scales and irritation on the skin of mice promoted with 3.0 mg BCP were generally similar to the incidences in DMBA/TPA mice, but fewer ulcers were observed on the skin of the 3.0 mg BCP mice than on the skin of mice promoted with TPA.

TABLE 15
Survival of Mice in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as a Promoter

	Acetone/ Acetone	DMBA/ Acetone	DMBA/ 0.1 mg BCP	DMBA/ 1.0 mg BCP	DMBA/ 3.0 mg BCP	DMBA/ TPA
Male						
Animals initially in study	50	50	50	50	50	50
Accidental deaths[a]	0	0	0	0	0	1
Moribund	2	3	6	6	6	26
Natural deaths	1	5	1	3	5	16
Animals surviving to study termination	47	42	43	41	39	7
Percent probability of survival at end of study[b]	94	84	86	82	78	14
Mean survival (days)[c]	358	360	351	348	344	257
Comparison with DMBA/Acetone[d]		P=0.307	P=1.00N	P=0.916	P=0.488	
Comparison with Acetone/Acetone[e]		P=0.203	P=0.309	P=0.119	P=0.040	P<0.001
Female						
Animals initially in study	50	50	50	50	50	50
Accidental deaths[a]	1	0	0	0	0	1
Moribund	5	0	2	2	7	23
Natural deaths	0	3	5	4	3	13
Animals surviving to study termination	44	47	43	44	40	13
Percent probability of survival at end of study	90	94	86	88	80	27
Mean survival (days)	348	359	339	350	342	286
Comparison with DMBA/Acetone		P=0.117	P=0.302	P=0.478	P=0.072	
Comparison with Acetone/Acetone		P=0.687N	P=0.725	P=0.992	P=0.264	P<0.001

[a] Censored from survival analyses
[b] Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the DMBA/Acetone column, and the results of the life table pairwise comparisons (Cox, 1972) with the DMBA/Acetone group are in the dosed columns. A lower incidence in a dose group is indicated by N.
[e] The results of the life table pairwise comparisons (Cox, 1972) with the vehicle control are in the DMBA/Acetone, DMBA/TPA, and dosed group columns.

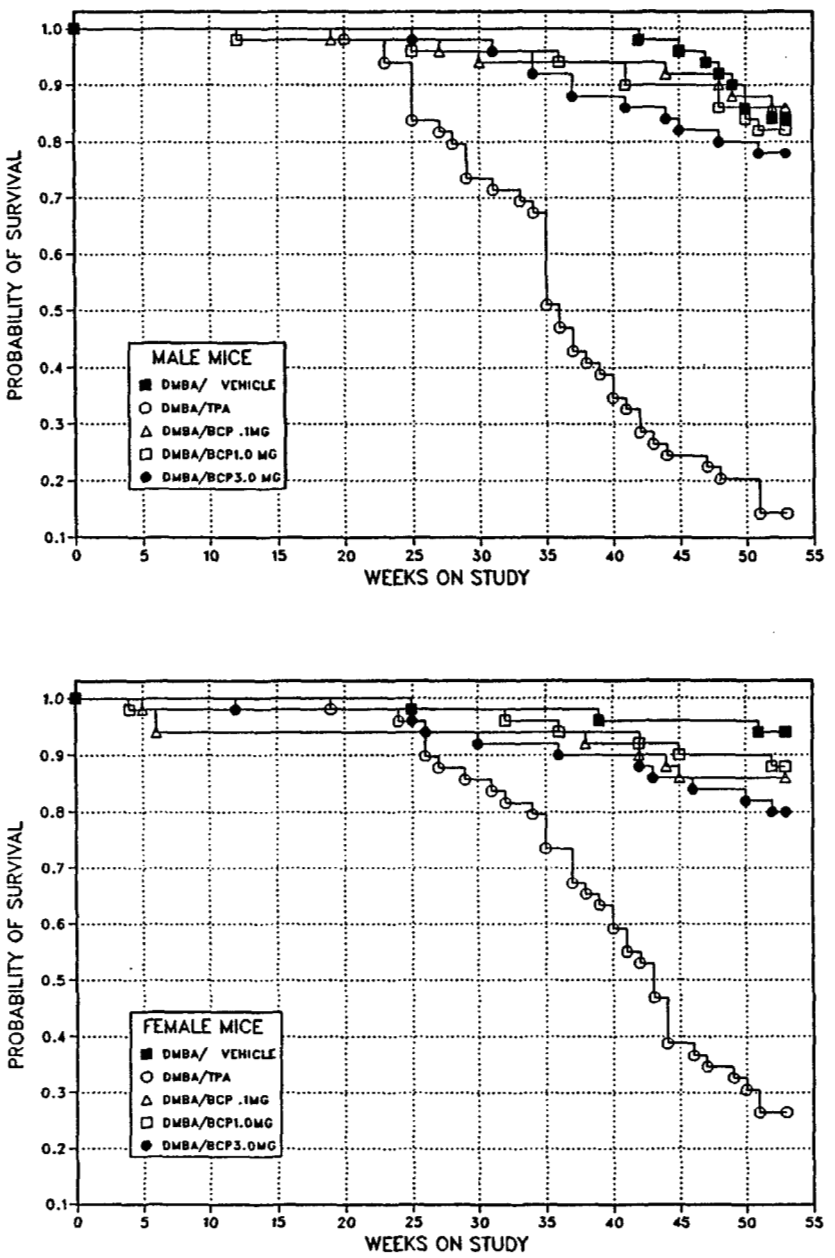


FIGURE 7
Kaplan-Meier Survival Curves for the 1-Year Study of BCP as a Promoter in Male and Female Swiss (CD-1®) Mice

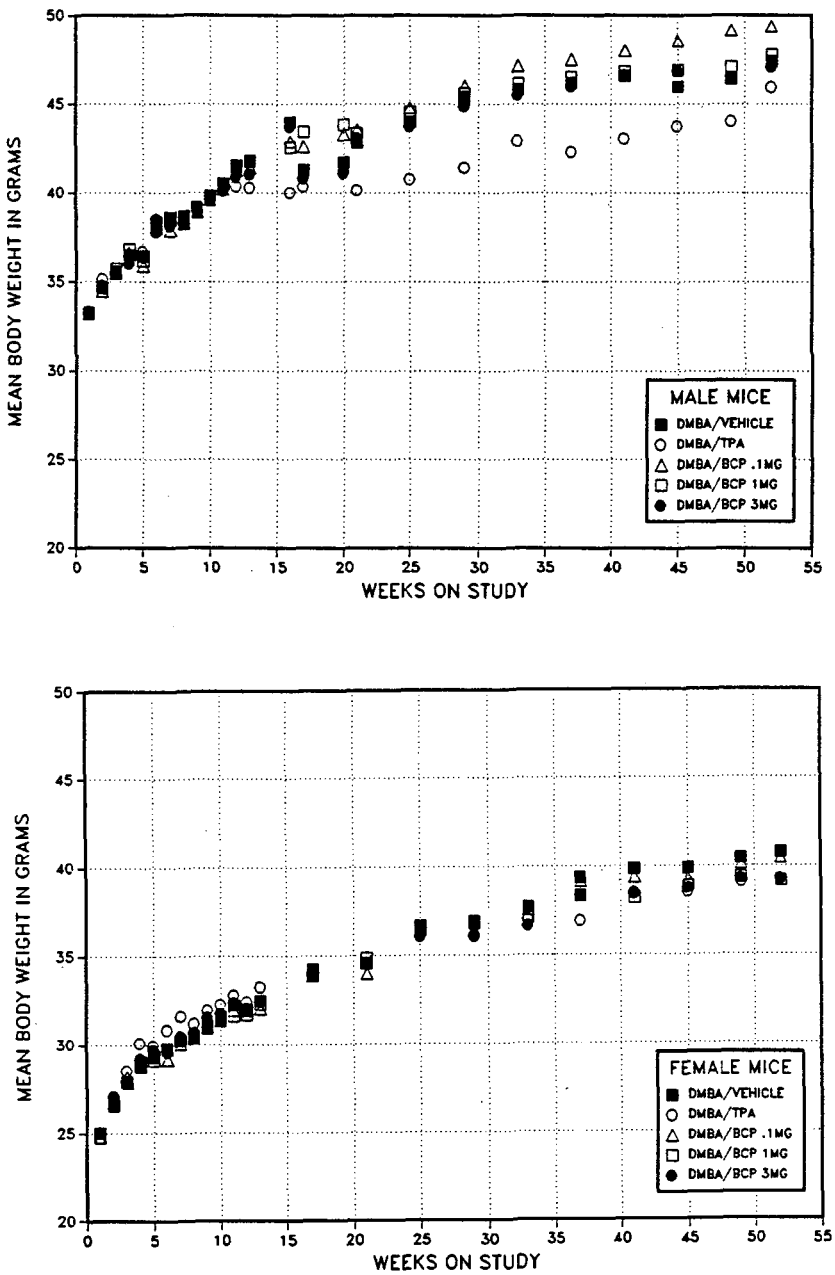


FIGURE 8
Growth Curves for the 1-Year Study of BCP as a Promoter in Male and Female Swiss (CD-1®) Mice

TABLE 16
Incidences of Selected Cutaneous Application Site Lesions Observed In-Life in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as a Promoter

Initiator/ Promoter	Lesion			
	Scales	Crusts	Ulcer	Irritation
n	50	50	50	50
Male				
Acetone/Acetone	2[a]	1	1	1
DMBA/Acetone	3	0	1	2
DMBA/TPA	48**	18**	32**	44**
DMBA/0.1 mg BCP	5	1	1	3
DMBA/1.0 mg BCP	13**	2	1	1
DMBA/3.0 mg BCP	49**	16**	17**	28**
TPA/TPA	50**	14**	19**	47**
Female				
Acetone/Acetone	3	0	0	1
DMBA/Acetone	8	0	0	3
DMBA/TPA	46**	10**	26**	45**
DMBA/0.1 mg BCP	1	0	0	1
DMBA/1.0 mg BCP	8	1	2	3
DMBA/3.0 mg BCP	48**	5*	8**	29**
TPA/TPA	50**	6*	14**	36**

* $P<0.05$ vs. Acetone/Acetone (and also vs. DMBA/Acetone) (life table test)
** $P<0.01$
[a] Number of animals with observation recorded during the course of the study

Tumor Response and Nonneoplastic Lesion Development in the Study of BCP as a Promoter

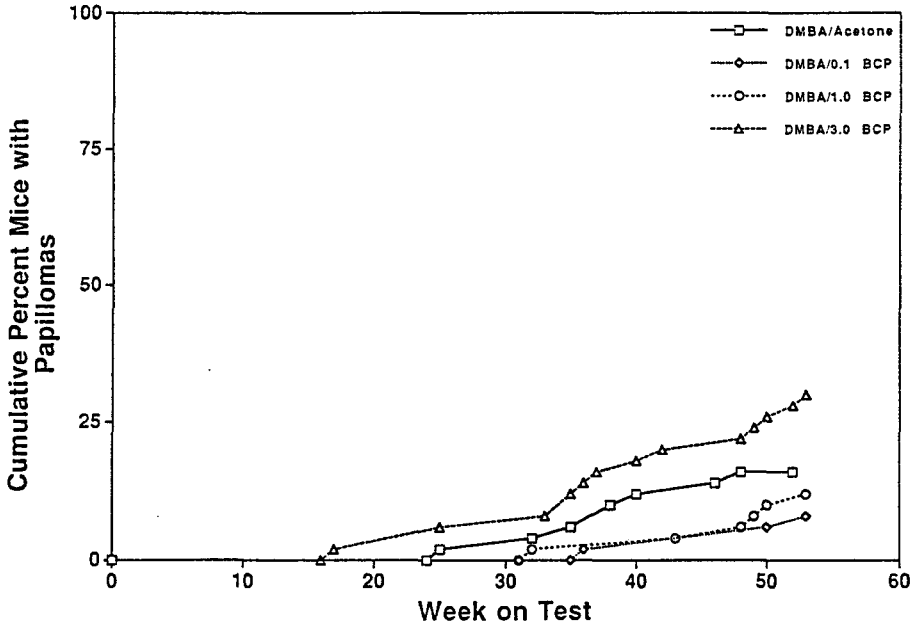
Female Swiss (CD-1®) mice administered the vehicle control (acetone/acetone) developed few nonneoplastic lesions. Topical applications of the vehicle control to males and of the initiator control (DMBA/acetone) to males and females produced slight nonneoplastic effects, primarily hyperkeratosis, acanthosis, and chronic inflammation. Male and female mice promoted with BCP had dose-related increased incidences of all categories of nonneoplastic lesions at the site of application.

The average number of papillomas per neoplasm-bearing mouse is shown in Table 17, and the pattern of mouse skin papilloma response over time is shown in Figure 9. Over the course of the study, a greater number of male and female mice developed papillomas after initiation with DMBA followed by promotion with 3.0 mg BCP than did males or females administered DMBA/acetone (initiator control). Papillomas were first observed at 18 weeks in males administered DMBA/3.0 mg BCP and at 26 weeks in DMBA/acetone control males. Papillomas were first observed at 10 weeks in females administered DMBA/3.0 mg BCP and at 27 weeks in females administered the initiator control (Figure 9). During the course of the study, 14 males and 18 females receiving DMBA/3.0 mg BCP developed papillomas, while only eight males and two females administered DMBA/acetone developed papillomas. The number of males and females receiving DMBA/0.1 mg BCP and developing papillomas was similar to the incidences in the DMBA/1.0 mg BCP groups (Figure 9). Males and females initiated with DMBA and promoted with TPA (initiator/promoter control) developed more than seven neoplasms per tumor-bearing mouse. Fewer numbers of mice in other test groups developed papillomas, and those mice with papillomas developed an average of less than two per mouse. There were no statistically significant differences in tumor multiplicity between DMBA/BCP and DMBA/acetone groups. Statistical analyses of skin papillomas in the study of BCP as a promoter are presented in Table 18.

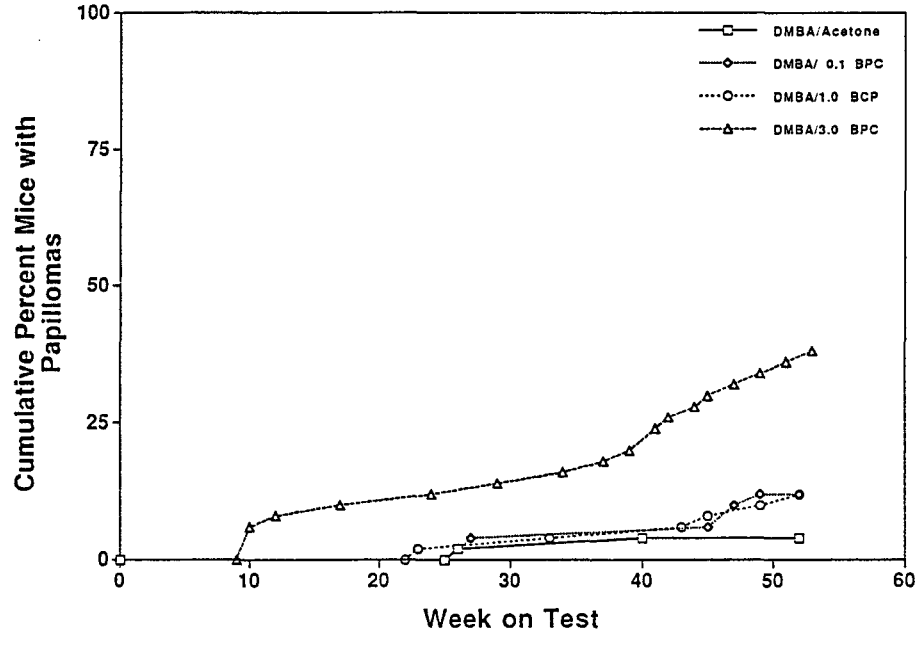
TABLE 17
Average Number of Papillomas Observed In-Life per Neoplasm-Bearing Mouse in the 1-Year Mouse Skin Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol as a Promoter[a]

	Male					Female				
Week of Study	**10**	**20**	**30**	**40**	**52**	**10**	**20**	**30**	**40**	**52**
Initiator/Promoter										
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
DMBA/Acetone	0	0	1.00	1.20	1.14	0	0	1.00	1.00	1.00
DMBA/TPA	1.00	7.20	5.50	6.27	5.33	0	8.63	7.12	7.11	5.86
DMBA/0.1 mg BCP	0	0	0	1.00	1.00	0	0	1.00	1.00	1.00
DMBA/1.0 mg BCP	0	0	0	1.00	1.00	0	0	1.00	1.50	1.60
DMBA/3.0 mg BCP	0	1.00	1.00	1.00	1.57	2.00	1.00	1.33	1.57	1.42

[a] Average number of papillomas per neoplasm-bearing mouse is expressed as total number of papillomas/number of mice with papillomas.



Male



Female

FIGURE 9
Occurrence of Papilloma versus Time of Occurrence in Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter

TABLE 18
Incidence of Skin Papillomas and Carcinomas Observed In-Life in the 1-Year Mouse Skin Initiation/Promotion Study of o-Benzyl-p-Chlorophenol as a Promoter

	Acetone/ Acetone	DMBA/ Acetone	DMBA/ 0.1 mg BCP	DMBA/ 1.0 mg BCP	DMBA/ 3.0 mg BCP
Male					
Papilloma					
Overall rates[a]	0/50 (0%)	8/50 (16%)	3/50 (6%)	5/50 (10%)	14/50 (28%)
Adjusted rates[b]	0.0%	16.2%	6.7%	11.2%	31.3%
First incidence (days)	–[e]	170	247	219	114
Comparison with DMBA/Acetone[c]		P=0.030	P=0.102N	P=0.278N	P=0.077
Comparison with Acetone/Acetone[d]	P<0.001		P=0.108	P=0.027	P<0.001
Female					
Papilloma					
Overall rates	0/50 (0%)	2/50 (4%)	6/50 (12%)	6/50 (12%)	18/50 (36%)
Adjusted rates	0.0%	4.1%	13.6%	13.1%	37.5%
First incidence (days)	–	179	186	158	67
Comparison with DMBA/Acetone	P<0.001	P=0.114	P=0.125	P<0.001	
Comparison with Acetone/Acetone	P<0.001		P=0.015	P=0.017	P<0.001
Carcinoma					
Overall rates	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)

[a] Number of animals with neoplasm/number of animals examined microscopically
[b] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[c] Beneath the DMBA/Acetone incidences are the P values associated with the trend test. Beneath the other dosed group incidences are the P values corresponding to pairwise comparisons between the reference control and that dosed group. Life table test results are based on the time of the first observation of papilloma. A lower incidence in a dose group is indicated by N.
[d] Beneath the vehicle control incidences are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. Life table test results are based on the time of the first observation of papilloma.
[e] Not applicable; no neoplasms in animal group

Effects of BCP on Other Target Sites

The kidney, liver, nose, and thymus were examined microscopically because these organs were potential target sites based on clinical signs and histopathology results from 13-week gavage studies (NTP, 1994). The intent was to determine if these organs would be affected by topical treatment with BCP for up to 50 weeks. There were no chemical-related increased incidences of neoplasms or nonneoplastic lesions in the kidney, liver, nose, or thymus of mice when BCP was applied as an initiator, a promoter, or as a complete carcinogen (Appendixes A and B).

Sentinel Animals

Titers for the virus of epizootic diarrhea of infant mice were detected in 20/20 mice in the study rooms at the beginning of the quarantine period (Table F1) and in 10/20 mice in the study rooms at the end of quarantine period. This virus causes disease in infant mice and no signs or lesions of infection were detected; thus, it seems unlikely that infection had an adverse effect on the results of the study.

DISCUSSION AND CONCLUSIONS

Boutwell and Bosch (1959) studied the tumor-promoting effects of a wide variety of substituted phenols. In their studies, 23 mice were treated topically with a single application of 0.3% DMBA in benzene followed by twice weekly 25 μL applications of a 20% solution of *o*-benzyl-*p*-chlorophenol (BCP) in benzene. BCP-treated mice had no observed papillomas by week 21, but one mouse had a carcinoma at week 34. No skin tumors were observed in the benzene control group mice. The authors considered BCP to have little tumor-promoting activity. When the National Toxicology Program toxicology and carcinogenicity 2-year studies were designed, a mouse skin initiation/promotion study was included to further examine the tumor-promoting potential of BCP.

In the present study, groups of male and female Swiss (CD-1®) mice were initiated with a single dose of DMBA followed by one of three concentrations of BCP three times weekly for up to 51 weeks. The vehicle in this study was acetone. Papillomas were first observed in male mice receiving promoting doses of 3.0 mg BCP at about week 18 and in 3.0 mg BCP females at about week 10. Mice initiated with DMBA and promoted with BCP showed a dose-related decrease in time to first tumor observation, and an increase in the number of mice responding and number of tumors per neoplasm-bearing mouse. In addition, the groups initiated and promoted with BCP had no skin tumors at the end of the study, suggesting that BCP alone was not responsible for the tumor response. However, since male mice initiated with DMBA and promoted with acetone developed as many, if not more, tumors as did mice administered DMBA/0.1 mg BCP or DMBA/1.0 mg BCP, the promotion effect of BCP in males is difficult to assess. The results of the present study coincide with results of the earlier studies by Boutwell and Bosch (1959), suggesting that BCP has weak promotion potential in responsive female and possibly in male Swiss (CD-1®) mice.

Treatment of mice with 10 mg BCP as an initiator and TPA as a promoter did not adequately assess the initiation potential of BCP. Mice treated only with TPA developed more tumors than did mice administered BCP/TPA. In the present study, the concentration to be applied to the hair-clipped backs of Swiss (CD-1®) mice was selected from the literature; the three times weekly applications appeared to be excessive, based on the skin lesions that developed. However, BCP appeared to be a weak promoter based on the results from mice initiated with DMBA and promoted with BCP. When mice were initiated with 10 mg BCP and promoted with 0.1, 1.0, or 3.0 mg BCP, no tumors were observed at the end of the study, suggesting that BCP is not an initiator.

The applied dose concentrations of BCP and TPA were irritating to the mouse skin, and BCP caused dose-related epidermal hyperplasia and ulceration. Chemical promoters and mechanical wounding can cause epidermal hyperplasia. Repeated abrasion or wounding of female Swiss (CD-1®) mice previously initiated with a single application of 200 nmol (~ 50 μg) DMBA produced epidermal tumors. However, no tumors were produced by abrasion or wounding when there had been no chemical initiation (Argyris, 1980). Other mouse strains known to be responsive to chemical initiation and promotion have also been shown to produce tumors after repetitive wounding of DMBA-initiated skin (Argyris and Slaga, 1981). In addition, mouse strains resistant to chemical initiation and promotion did not produce tumors after wounding (DiGiovanni *et al.*, 1993). A possible relationship between wounding and chemical induced skin tumor promotion may exist (reviewed in Parkinson, 1985 and Parkinson and Balmain, 1990). In the current studies the irritation and ulcers caused by the repetitive applications of BCP on the mouse skin would not be unlike abrasion and wounding. The results of the current studies with concentrations of BCP that caused hyperplasia, irritation, and ulcers are similar to those when Swiss (CD-1®) mice were abraded or wounded, i.e., initiated mice developed papillomas; uninitiated ones did not. Therefore, it is not clear whether the promoting effect of BCP is due to a direct effect on the initiated cells or an indirect effect resulting from the skin's hyperplastic response to the irritation and ulceration.

The results of the NTP 16-day and 13-week BCP gavage studies indicated chemical-related effects, and several potential target organs were identified. NTP designed the present topical BCP initiation/promotion study to run concurrently with the 2-year gavage studies to relate the results of the two studies as well as to further examine the tumor promotion potential of BCP. The four target organs identified in the 13-week gavage toxicology studies were the kidney, liver, nose, and thymus. There were no chemical-related increased incidences of tumors or nonneoplastic lesions in these organs in the present initiation/promotion study. In the 2-year gavage studies (NTP, 1994), mice were administered 120, 240, or 480 mg/kg BCP. There was "some evidence" of carcinogenic activity of BCP in male B6C3F$_1$ mice based on increased incidences of renal tubule cell adenoma or carcinoma (combined) (0/50, 2/50, 6/50, 6/50). There was "no evidence" of carcinogenic activity of BCP in female B6C3F$_1$ mice. Male and female F344/N rats were administered doses of 30, 60, or 120 mg/kg BCP and there was "no evidence" of carcinogenic activity in males and "equivocal evidence" in females, based on the occurrence of two rare renal transitional cell carcinomas. For comparison with the gavage exposure, the doses for the topical exposure study of BCP/BCP were estimated to be 2.4, 15.4, and 39.3 mg/kg body weight per day (males) or 2.9, 18.3, and 46.5 mg/kg body weight per day (females). These estimated doses take into account the total amount of chemical applied over the course of the study including the 10 mg per mouse initiating dose divided by the number of weekdays on test (gavage study was weekdays only). The greatest topical dose was only about one-third of the lowest dose used in the 2-year gavage mouse study. However, the preliminary topical dose range-finding study indicated that concentrations higher than 3 mg/100 μL would not have been tolerated by the mouse skin. At these concentrations and dosing frequencies, male and female Swiss (CD-1®) mice exposed topically to BCP for approximately 1 year did not experience the same systemic effects as did male and female B6C3F$_1$ mice exposed to BCP by gavage for 2 years.

The carcinogenic effect of BCP in the 2-year gavage studies was only observed in one organ of one species, and this would be considered a "weak" carcinogen. However, other phenolic disinfectants were negative for induction of renal tumors in rats and mice (NCI, 1978a; NCI, 1980; NTP, 1986). Chemicals active as renal tubule carcinogens include both genotoxic and non-genotoxic substances (NCI, 1977; NCI, 1978b; NTP, 1988; NTP, 1992). Thus, both primary and secondary mechanisms are probably operative in the induction of the renal tumors. In the 2-year NTP BCP gavage studies the kidney tumors were accompanied by renal tubule hyperplasia and this supported a conclusion of a proliferative response of the renal tubule epithelium to BCP administration. It was suggested that the cellular proliferative lesions in response to the increased nephropathy caused by BCP possible provided a background for genetic mutations resulting in the renal tumors in male mice.

CONCLUSIONS

Under the conditions of this 1-year mouse skin initiation/promotion study in Swiss (CD-1®) mice, *o*-benzyl-*p*-chlorophenol was a cutaneous irritant and a weak skin tumor promoter relative to strong promoters such as TPA. BCP had no activity as an initiator or as a complete carcinogen*.

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* A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE MICE IN THE 1-YEAR INITIATION/PROMOTION STUDY OF *o*-BENZYL-*p*-CHLOROPHENOL

TABLE A1a
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator[a]

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	2	26	13	22
Natural deaths	1	16	8	10
Survivors				
Terminal sacrifice	47	7	29	18
Animals examined microscopically	50	50	50	50
Alimentary System				
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma, single	3 (6%)	2 (4%)	6 (12%)	2 (4%)
Cardiovascular System				
None				
Endocrine System				
None				
General Body System				
None				
Genital System				
None				
Hematopoietic System				
Lymph node	(1)	(11)	(7)	(13)
Axillary, squamous cell carcinoma, metastatic, skin		1 (9%)		
Mediastinal, schwannoma malignant		1 (9%)		
Lymph node, mandibular	(4)	(15)	(7)	(18)
Lymph node, mesenteric	(3)	(12)	(4)	(15)
Spleen	(6)	(42)	(27)	(33)
Thymus	(46)	(37)	(43)	(40)
Thymoma benign	1 (2%)			

TABLE A1a
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Control, squamous cell papilloma, single		1 (2%)		
Site of application-mass, keratoacanthoma, multiple		2 (4%)		
Site of application-mass, keratoacanthoma, single		2 (4%)	1 (2%)	
Site of application-mass, squamous cell carcinoma		2 (4%)	1 (2%)	
Site of application-mass, squamous cell carcinoma, multiple		1 (2%)		
Site of application-mass, squamous cell carcinoma, single		4 (8%)	3 (6%)	
Site of application-mass, squamous cell papilloma, multiple		21 (42%)	3 (6%)	3 (6%)
Site of application-mass, squamous cell papilloma, single		8 (16%)	8 (16%)	6 (12%)
Subcutaneous tissue, hemangioma		1 (2%)		
Subcutaneous tissue, site of application-mass, fibrosarcoma, single		2 (4%)		
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(4)	(4)	(4)	(2)
Alveolar/bronchiolar adenoma, single		1 (25%)	1 (25%)	1 (50%)
Alveolar/bronchiolar carcinoma, single		1 (25%)	1 (25%)	
Mediastinum, schwannoma malignant		1 (25%)		
Special Senses System				
Harderian gland		(1)		(1)
Adenoma				1 (100%)
Urinary System				
None				
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Lymphoma malignant undifferentiated cell	1 (2%)			

TABLE A1a
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Neoplasm Summary				
Total animals with primary neoplasms[c]	5	30	19	12
Total primary neoplasms	5	50	24	13
Total animals with benign neoplasms	4	30	16	12
Total benign neoplasms	4	38	19	13
Total animals with malignant neoplasms	1	9	5	
Total malignant neoplasms	1	12	5	
Total animals with metastatic neoplasms		1		
Total metastatic neoplasms		1		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A1b
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter[a]

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death		1			
Moribund	3	26	6	6	6
Natural deaths	5	16	1	3	5
Survivors					
Terminal sacrifice	42	7	43	41	39
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Liver	(50)	(50)	(50)	(50)	(50)
Hepatocellular adenoma, multiple					2 (4%)
Hepatocellular adenoma, single	2 (4%)	2 (4%)	6 (12%)	3 (6%)	2 (4%)
Cardiovascular System					
None					
Endocrine System					
None					
General Body System					
None					
Genital System					
None					
Hematopoietic System					
Lymph node	(2)	(11)	(2)	(2)	(4)
Axillary, squamous cell carcinoma, metastatic, skin		1 (9%)			
Mediastinal, schwannoma malignant		1 (9%)			
Lymph node, mesenteric	(5)	(12)	(2)	(1)	(5)
Spleen	(7)	(42)	(8)	(8)	(12)
Thymus	(44)	(37)	(47)	(45)	(45)

TABLE A1b
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 µg DMBA/ Acetone	50 µg DMBA/ 5 µg TPA	50 µg DMBA/ 0.1 mg BCP	50 µg DMBA/ 1.0 mg BCP	50 µg DMBA/ 3.0 mg BCP
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(50)
Squamous cell papilloma, single				1 (2%)	
Control, squamous cell papilloma, single		1 (2%)			
Sebaceous gland, other, adenoma			1 (2%)		
Sebaceous gland, site of application-mass, adenoma, multiple	1 (2%)				
Sebaceous gland, site of application-mass, adenoma, single	1 (2%)		1 (2%)	1 (2%)	
Site of application-mass, keratoacanthoma	1 (2%)				
Site of application-mass, keratoacanthoma, multiple		2 (4%)			1 (2%)
Site of application-mass, keratoacanthoma, single		2 (4%)	1 (2%)	1 (2%)	2 (4%)
Site of application-mass, squamous cell carcinoma		2 (4%)			
Site of application-mass, squamous cell carcinoma, multiple		1 (2%)			
Site of application-mass, squamous cell carcinoma, single		4 (8%)			
Site of application-mass, squamous cell papilloma					1 (2%)
Site of application-mass, squamous cell papilloma, multiple		21 (42%)	1 (2%)		
Site of application-mass, squamous cell papilloma, single	4 (8%)	8 (16%)		1 (2%)	5 (10%)
Subcutaneous tissue, hemangioma		1 (2%)			
Subcutaneous tissue, site of application-mass, fibrosarcoma					1 (2%)
Subcutaneous tissue, site of application-mass, fibrosarcoma, single	1 (2%)	2 (4%)			
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lung	(5)	(4)	(5)		
Alveolar/bronchiolar adenoma, single	5 (100%)	1 (25%)	2 (40%)		
Alveolar/bronchiolar carcinoma, single		1 (25%)			
Mediastinum, schwannoma malignant		1 (25%)			

TABLE A1b
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Special Senses System					
None					
Urinary System					
None					
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Lymphoma malignant mixed					1 (2%)
Lymphoma malignant undifferentiated cell					1 (2%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	13	30	11	7	14
Total primary neoplasms	15	50	12	7	16
Total animals with benign neoplasms	12	30	11	7	12
Total benign neoplasms	14	38	12	7	13
Total animals with malignant neoplasms	1	9			3
Total malignant neoplasms	1	12			3
Total animals with metastatic neoplasms		1			
Total metastatic neoplasms		1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A1c
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen[a]

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death				1	
Moribund	2	38	8	6	9
Natural deaths	1	12	2	3	5
Survivors					
Died last week of study			1		
Terminal sacrifice	47		39	40	36
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Liver	(50)	(50)	(50)	(50)	(50)
Hepatocellular adenoma, multiple			1 (2%)	1 (2%)	
Hepatocellular adenoma, single	3 (6%)		1 (2%)	1 (2%)	3 (6%)
Mast cell tumor malignant					1 (2%)
Cardiovascular System					
None					
Endocrine System					
None					
General Body System					
None					
Genital System					
None					
Hematopoietic System					
Lymph node	(1)	(6)	(4)	(3)	(4)
Axillary, mast cell tumor malignant					1 (25%)
Axillary, squamous cell carcinoma, metastatic, skin		3 (50%)			
Axillary, lumbar, squamous cell carcinoma, metastatic, skin		2 (33%)			
Lymph node, mandibular	(4)	(4)	(8)	(4)	(7)
Mast cell tumor malignant					1 (14%)
Lymph node, mesenteric	(3)	(13)	(1)	(3)	(3)
Mast cell tumor malignant					1 (33%)
Spleen	(6)	(47)	(11)	(11)	(16)
Mast cell tumor malignant					1 (6%)
Thymus	(46)	(31)	(42)	(48)	(39)
Thymoma benign	1 (2%)				

TABLE A1c
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(50)
Squamous cell carcinoma, single		1 (2%)			
Control, squamous cell carcinoma, single		2 (4%)			
Site of application-mass, basosquamous tumor malignant, single		1 (2%)			
Site of application-mass, keratoacanthoma, multiple		1 (2%)			
Site of application-mass, keratoacanthoma, single		4 (8%)			
Site of application-mass, squamous cell carcinoma		1 (2%)			
Site of application-mass, squamous cell carcinoma, multiple		20 (40%)			
Site of application-mass, squamous cell carcinoma, single		23 (46%)			
Site of application-mass, squamous cell papilloma, multiple		4 (8%)			
Site of application-mass, squamous cell papilloma, single		4 (8%)			
Subcutaneous tissue, mast cell tumor malignant					1 (2%)
Subcutaneous tissue, control, mast cell tumor malignant					1 (2%)
Subcutaneous tissue, site of application-mass, mast cell tumor malignant					1 (2%)
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lung	(4)	(10)	(2)	(1)	(1)
Alveolar/bronchiolar adenoma, single			1 (50%)	1 (100%)	
Mast cell tumor malignant					1 (100%)
Squamous cell carcinoma, metastatic, skin		6 (60%)			
Squamous cell carcinoma, metastatic, uncertain primary site		1 (10%)			
Nose	(50)	(50)	(50)	(50)	(50)
Mast cell tumor malignant					1 (2%)

TABLE A1c
Summary of the Incidence of Neoplasms in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Special Senses System					
Harderian gland					(1)
Mast cell tumor malignant					1 (100%)
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Mast cell tumor malignant					1 (2%)
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Lymphoma malignant undifferentiated cell	1 (2%)			1 (2%)	
Neoplasm Summary					
Total animals with primary neoplasms[c]	5	48	3	4	4
Total primary neoplasms	5	61	3	4	15
Total animals with benign neoplasms	4	10	3	3	3
Total benign neoplasms	4	13	3	3	3
Total animals with malignant neoplasms	1	44		1	1
Total malignant neoplasms	1	48		1	12
Total animals with metastatic neoplasms		10			
Total metastatic neoplasms		12			
Total animals with metastatic neoplasms-uncertain primary site		1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2a
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator[a]

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	2	26	13	22
Natural deaths	1	16	8	10
Survivors				
Terminal sacrifice	47	7	29	18
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon		(1)		
Inflammation, chronic		1 (100%)		
Intestine small, duodenum		(1)	(1)	
Inflammation, chronic		1 (100%)	1 (100%)	
Intestine small, jejunum		(1)	(1)	
Inflammation, chronic		1 (100%)	1 (100%)	
Liver	(50)	(50)	(50)	(50)
Amyloid deposition		1 (2%)	1 (2%)	5 (10%)
Eosinophilic focus		1 (2%)		
Hematopoietic cell proliferation	1 (2%)	10 (20%)	8 (16%)	6 (12%)
Hepatodiaphragmatic nodule	1 (2%)	2 (4%)		
Inflammation, granulomatous	1 (2%)			1 (2%)
Inflammation, suppurative		10 (20%)	7 (14%)	5 (10%)
Necrosis, coagulative	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Vacuolization cytoplasmic	39 (78%)	8 (16%)	14 (28%)	13 (26%)
Pancreas		(1)	(2)	
Serosa, inflammation, acute		1 (100%)	2 (100%)	
Salivary glands		(3)	(2)	(1)
Abscess		2 (67%)	1 (50%)	
Inflammation, acute		1 (33%)	1 (50%)	
Stomach, forestomach				(1)
Acanthosis				1 (100%)
Hyperkeratosis				1 (100%)
Stomach, glandular			(2)	
Serosa, inflammation, acute			2 (100%)	
Tongue			(1)	
Abscess			1 (100%)	
Cardiovascular System				
Heart		(1)	(1)	(2)
Bacterium			1 (100%)	
Myocardium, inflammation, suppurative		1 (100%)	1 (100%)	2 (100%)

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

TABLE A2a
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Endocrine System				
Islets, pancreatic	(1)			
Hyperplasia	1 (100%)			
Pituitary gland	(1)	(1)		(1)
Cyst	1 (100%)			
General Body System				
None				
Genital System				
Penis	(1)	(6)		(2)
Inflammation, chronic	1 (100%)	3 (50%)		1 (50%)
Inflammation, necrotizing		3 (50%)		1 (50%)
Preputial gland	(1)	(2)	(3)	(6)
Abscess		1 (50%)	2 (67%)	5 (83%)
Inflammation, chronic			1 (33%)	
Duct, dilatation	1 (100%)	1 (50%)	1 (33%)	3 (50%)
Seminal vesicle	(3)	(5)	(1)	(2)
Hemorrhage	1 (33%)			
Inflammation, chronic		3 (60%)		
Hematopoietic System				
Lymph node	(1)	(11)	(7)	(13)
Axillary, abscess		1 (9%)	1 (14%)	1 (8%)
Axillary, hyperplasia, plasma cell		7 (64%)	6 (86%)	9 (69%)
Deep cervical, hyperplasia, plasma cell		2 (18%)	1 (14%)	1 (8%)
Inguinal, hyperplasia, plasma cell		2 (18%)		
Lumbar, hyperplasia, plasma cell		1 (9%)		
Mediastinal, hyperplasia, plasma cell		2 (18%)	1 (14%)	2 (15%)
Pancreatic, hyperplasia, plasma cell		1 (9%)		
Renal, hyperplasia, plasma cell		1 (9%)		2 (15%)
Lymph node, mandibular	(4)	(15)	(7)	(18)
Abscess		4 (27%)	2 (29%)	3 (17%)
Hyperplasia, plasma cell	3 (75%)	14 (93%)	7 (100%)	15 (83%)
Inflammation, acute		1 (7%)		1 (6%)
Sinus, ectasia				3 (17%)
Lymph node, mesenteric	(3)	(12)	(4)	(15)
Hematopoietic cell proliferation	2 (67%)	2 (17%)	3 (75%)	5 (33%)
Hyperplasia, lymphoid		1 (8%)		
Hyperplasia, plasma cell		6 (50%)		6 (40%)
Sinus, ectasia	2 (67%)	5 (42%)	2 (50%)	10 (67%)
Spleen	(6)	(42)	(27)	(33)
Amyloid deposition	1 (17%)	6 (14%)	4 (15%)	10 (30%)
Hematopoietic cell proliferation	3 (50%)	39 (93%)	23 (85%)	31 (94%)
Hyperplasia, lymphoid			2 (7%)	1 (3%)
Inflammation, acute		1 (2%)		
Inflammation, chronic			1 (4%)	
Thymus	(46)	(37)	(43)	(40)
Cortex, atrophy	1 (2%)	10 (27%)	11 (26%)	12 (30%)

TABLE A2a
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Abscess		6 (12%)	3 (6%)	8 (16%)
Acanthosis	3 (6%)	23 (46%)	15 (30%)	25 (50%)
Amyloid deposition			1 (2%)	2 (4%)
Cyst epithelial inclusion	1 (2%)		1 (2%)	5 (10%)
Edema				1 (2%)
Hyperkeratosis	3 (6%)	20 (40%)	15 (30%)	24 (48%)
Inflammation, chronic	3 (6%)	22 (44%)	15 (30%)	26 (52%)
Ulcer	3 (6%)	22 (44%)	12 (24%)	24 (48%)
Control, acanthosis		23 (46%)	21 (42%)	28 (56%)
Control, amyloid deposition			4 (8%)	4 (8%)
Control, cyst epithelial inclusion			1 (2%)	1 (2%)
Control, hyperkeratosis	2 (4%)	28 (56%)	29 (58%)	36 (72%)
Control, inflammation, chronic	1 (2%)	17 (34%)	9 (18%)	13 (26%)
Control, ulcer		6 (12%)	1 (2%)	
Sebaceous gland, hypertrophy	2 (4%)	6 (12%)	3 (6%)	10 (20%)
Sebaceous gland, control, hypertrophy		2 (4%)	4 (8%)	5 (10%)
Sebaceous gland, site of application-no mass, hypertrophy	2 (4%)	14 (28%)	22 (44%)	16 (32%)
Sebaceous gland, site of application-mass, hypertrophy			1 (2%)	
Site of application-no mass, abscess		2 (4%)	2 (4%)	4 (8%)
Site of application-no mass, acanthosis	5 (10%)	46 (92%)	49 (98%)	50 (100%)
Site of application-no mass, amyloid deposition		2 (4%)	18 (36%)	17 (34%)
Site of application-no mass, cyst epithelial inclusion		6 (12%)		7 (14%)
Site of application-no mass, hyperkeratosis	10 (20%)	45 (90%)	47 (94%)	48 (96%)
Site of application-no mass, inflammation, chronic	3 (6%)	42 (84%)	42 (84%)	44 (88%)
Site of application-no mass, ulcer	1 (2%)	22 (44%)	13 (26%)	19 (38%)
Musculoskeletal System				
Bone		(2)	(2)	(2)
Humerus, abscess			1 (50%)	
Synovial tissue, inflammation, suppurative		2 (100%)	1 (50%)	2 (100%)
Skeletal muscle	(1)	(4)	(2)	
Inflammation, suppurative	1 (100%)	4 (100%)	2 (100%)	
Nervous System				
None				

TABLE A2a
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Respiratory System				
Lung	(4)	(4)	(4)	(2)
Emphysema			1 (25%)	
Hemorrhage			1 (25%)	
Alveolus, inflammation, subacute	1 (25%)			
Artery, thrombosis		1 (25%)		
Pleura, inflammation, chronic	1 (25%)	1 (25%)		
Special Senses System				
Ear	(2)	(6)	(3)	(4)
Pinna, inflammation, chronic	1 (50%)	1 (17%)		
Pinna, ulcer	1 (50%)	4 (67%)	3 (100%)	4 (100%)
Eye			(1)	(2)
Cornea, inflammation, necrotizing				1 (50%)
Sclera, inflammation, chronic			1 (100%)	
Harderian gland		(1)		(1)
Abscess		1 (100%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition	22 (44%)	2 (4%)	12 (24%)	6 (12%)
Bacterium		1 (2%)	1 (2%)	
Hydronephrosis	2 (4%)	2 (4%)		
Inflammation, acute		2 (4%)	5 (10%)	
Inflammation, chronic	7 (14%)	15 (30%)	15 (30%)	17 (34%)
Urinary bladder		(8)		(3)
Hemorrhage		1 (13%)		
Inflammation, chronic		3 (38%)		

TABLE A2b
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter[a]

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death		1			
Moribund	3	26	6	6	6
Natural deaths	5	16	1	3	5
Survivors					
Terminal sacrifice	42	7	43	41	39
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Intestine large, colon		(1)			
Inflammation, chronic		1 (100%)			
Intestine small, duodenum		(1)			
Inflammation, chronic		1 (100%)			
Intestine small, jejunum		(1)			(2)
Amyloid deposition					1 (50%)
Inflammation, chronic		1 (100%)			1 (50%)
Liver	(50)	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)	1 (2%)		2 (4%)	1 (2%)
Basophilic focus	2 (4%)				
Cyst	1 (2%)				
Eosinophilic focus		1 (2%)			
Hematopoietic cell proliferation	1 (2%)	10 (20%)	2 (4%)	1 (2%)	3 (6%)
Hepatodiaphragmatic nodule	1 (2%)	2 (4%)		1 (2%)	
Inflammation, granulomatous			1 (2%)		1 (2%)
Inflammation, suppurative	2 (4%)	10 (20%)	1 (2%)	3 (6%)	2 (4%)
Necrosis, coagulative		1 (2%)	1 (2%)		4 (8%)
Vacuolization cytoplasmic	28 (56%)	8 (16%)	33 (66%)	27 (54%)	22 (44%)
Pancreas	(1)	(1)		(1)	(1)
Duct, ectasia					1 (100%)
Interlobular, edema	1 (100%)			1 (100%)	
Serosa, inflammation, acute		1 (100%)			
Salivary glands		(3)			
Abscess		2 (67%)			
Inflammation, acute		1 (33%)			
Cardiovascular System					
Heart	(2)	(1)	(1)		
Atrium, thrombosis	1 (50%)		1 (100%)		
Myocardium, amyloid deposition	1 (50%)		1 (100%)		
Myocardium, inflammation, suppurative		1 (100%)			
Endocrine System					
None					

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

TABLE A2b
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
General Body System					
None					
Genital System					
Penis	(1)	(6)			(1)
Inflammation, chronic	1 (100%)	3 (50%)			
Inflammation, necrotizing		3 (50%)			1 (100%)
Preputial gland	(2)	(2)		(1)	(2)
Abscess	1 (50%)	1 (50%)			
Cyst multilocular	1 (50%)				
Inflammation, chronic				1 (100%)	1 (50%)
Duct, dilatation		1 (50%)			2 (100%)
Seminal vesicle	(1)	(5)	(4)		(2)
Inflammation, chronic		3 (60%)	2 (50%)		1 (50%)
Testes		(1)	(1)	(1)	
Abscess			1 (100%)		
Tunic, fat, necrosis				1 (100%)	
Hematopoietic System					
Lymph node	(2)	(11)	(2)	(2)	(4)
Axillary, abscess		1 (9%)			
Axillary, hyperplasia, lymphoid			1 (50%)		
Axillary, hyperplasia, plasma cell	1 (50%)	7 (64%)	1 (50%)		2 (50%)
Axillary, infiltration cellular, plasma cell	1 (50%)				
Axillary, infiltration cellular, histiocyte			1 (50%)		
Deep cervical, hyperplasia, plasma cell		2 (18%)			
Inguinal, hyperplasia, plasma cell		2 (18%)			
Inguinal, infiltration cellular, histiocyte	1 (50%)				
Lumbar, hyperplasia, plasma cell		1 (9%)			1 (25%)
Mediastinal, abscess				1 (50%)	
Mediastinal, hyperplasia, plasma cell		2 (18%)		2 (100%)	1 (25%)
Pancreatic, hyperplasia, plasma cell		1 (9%)			
Renal, hyperplasia, plasma cell		1 (9%)			
Lymph node, mandibular	(4)	(15)	(4)	(5)	(7)
Abscess	1 (25%)	4 (27%)	1 (25%)	1 (20%)	2 (29%)
Hyperplasia, plasma cell	3 (75%)	14 (93%)	4 (100%)	5 (100%)	7 (100%)
Infiltration cellular, histiocyte	1 (25%)				
Inflammation, acute		1 (7%)			
Sinus, ectasia				1 (20%)	
Lymph node, mesenteric	(5)	(12)	(2)	(1)	(5)
Hematopoietic cell proliferation	1 (20%)	2 (17%)			2 (40%)
Hyperplasia, lymphoid		1 (8%)			
Hyperplasia, plasma cell	1 (20%)	6 (50%)			2 (40%)
Infiltration cellular, histiocyte	1 (20%)				
Sinus, ectasia	3 (60%)	5 (42%)	2 (100%)	1 (100%)	2 (40%)

TABLE A2b
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Hematopoietic System (continued)					
Spleen	(7)	(42)	(8)	(8)	(12)
Amyloid deposition		6 (14%)	1 (13%)	2 (25%)	1 (8%)
Hematopoietic cell proliferation	4 (57%)	39 (93%)	5 (63%)	7 (88%)	11 (92%)
Inflammation, acute		1 (2%)			
Thymus	(44)	(37)	(47)	(45)	(45)
Cortex, atrophy	1 (2%)	10 (27%)	2 (4%)	2 (4%)	4 (9%)
Medulla, hyperplasia				1 (2%)	1 (2%)
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(50)
Abscess	2 (4%)	6 (12%)		2 (4%)	2 (4%)
Acanthosis	4 (8%)	23 (46%)	3 (6%)	4 (8%)	9 (18%)
Amyloid deposition					1 (2%)
Cyst epithelial inclusion				3 (6%)	3 (6%)
Edema	2 (4%)		1 (2%)		
Hyperkeratosis	4 (8%)	20 (40%)	3 (6%)	5 (10%)	8 (16%)
Inflammation, chronic	4 (8%)	22 (44%)	2 (4%)	5 (10%)	9 (18%)
Mineralization			1 (2%)		
Ulcer	4 (8%)	22 (44%)	4 (8%)	4 (8%)	7 (14%)
Control, acanthosis		23 (46%)	2 (4%)	6 (12%)	21 (42%)
Control, amyloid deposition			1 (2%)	3 (6%)	3 (6%)
Control, edema			1 (2%)		
Control, hyperkeratosis	2 (4%)	28 (56%)	3 (6%)	9 (18%)	25 (50%)
Control, inflammation, chronic		17 (34%)	1 (2%)	1 (2%)	7 (14%)
Control, mineralization			1 (2%)		
Control, ulcer		6 (12%)	1 (2%)		
Sebaceous gland, hypertrophy		6 (12%)	1 (2%)	4 (8%)	1 (2%)
Sebaceous gland, control, hypertrophy		2 (4%)	1 (2%)		8 (16%)
Sebaceous gland, site of application-no mass, hypertrophy	2 (4%)	14 (28%)	1 (2%)	6 (12%)	34 (68%)
Sebaceous gland, site of application-mass, hypertrophy					2 (4%)
Site of application-no mass, abscess		2 (4%)			3 (6%)
Site of application-no mass, acanthosis	6 (12%)	46 (92%)	5 (10%)	21 (42%)	45 (90%)
Site of application-no mass, amyloid deposition	1 (2%)	2 (4%)		12 (24%)	31 (62%)
Site of application-no mass, cyst epithelial inclusion		6 (12%)			1 (2%)
Site of application-no mass, hemorrhage	1 (2%)				
Site of application-no mass, hyperkeratosis	8 (16%)	45 (90%)	15 (30%)	28 (56%)	48 (96%)
Site of application-no mass, inflammation, chronic	3 (6%)	42 (84%)	5 (10%)	5 (10%)	41 (82%)
Site of application-no mass, ulcer	1 (2%)	22 (44%)			10 (20%)
Site of application-mass, acanthosis					3 (6%)
Vein, site of application-mass, angiectasis	1 (2%)				

TABLE A2b
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Musculoskeletal System					
Bone	(1)	(2)	(2)		
Cranium, abscess			1 (50%)		
Synovial tissue, inflammation, suppurative	1 (100%)	2 (100%)	1 (50%)		
Skeletal muscle	(1)	(4)			
Inflammation, suppurative		4 (100%)			
Head, atrophy	1 (100%)				
Nervous System					
None					
Respiratory System					
Lung	(5)	(4)	(5)		
Abscess			1 (20%)		
Hemorrhage			1 (20%)		
Artery, thrombosis		1 (25%)			
Interstitium, inflammation, chronic			2 (40%)		
Pleura, inflammation, chronic		1 (25%)			
Special Senses System					
Ear	(1)	(6)	(1)	(3)	(1)
Pinna, inflammation, chronic		1 (17%)			1 (100%)
Pinna, ulcer	1 (100%)	4 (67%)	1 (100%)	3 (100%)	1 (100%)
Eye	(3)		(1)	(1)	
Cornea, inflammation, chronic	2 (67%)				
Cornea, inflammation, necrotizing	1 (33%)				
Lids, inflammation, necrotizing				1 (100%)	
Harderian gland	(1)	(1)	(1)		
Abscess	1 (100%)	1 (100%)			
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Amyloid deposition	28 (56%)	2 (4%)	16 (32%)	24 (48%)	15 (30%)
Bacterium		1 (2%)			1 (2%)
Cyst	1 (2%)				
Hydronephrosis	5 (10%)	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Inflammation, acute		2 (4%)	2 (4%)		2 (4%)
Inflammation, chronic	9 (18%)	15 (30%)	10 (20%)	12 (24%)	13 (26%)
Urinary bladder		(8)	(1)		(2)
Hemorrhage		1 (13%)			
Inflammation, chronic		3 (38%)			1 (50%)
Transitional epithelium, hyperplasia			1 (100%)		

TABLE A2c
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen[a]

	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death			1	
Moribund	2	8	6	9
Natural deaths	1	2	3	5
Survivors				
Died last week of study		1		
Terminal sacrifice	47	39	40	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum			(1)	
Bacterium			1 (100%)	
Inflammation, acute			1 (100%)	
Intestine small, duodenum				(1)
Inflammation, acute				1 (100%)
Intestine small, jejunum				(1)
Amyloid deposition				1 (100%)
Inflammation, acute				1 (100%)
Liver	(50)	(50)	(50)	(50)
Amyloid deposition			1 (2%)	
Basophilic focus				1 (2%)
Hematopoietic cell proliferation	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Hepatodiaphragmatic nodule	1 (2%)			
Inflammation, granulomatous	1 (2%)			
Inflammation, suppurative		2 (4%)	2 (4%)	2 (4%)
Necrosis, coagulative	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Vacuolization cytoplasmic	39 (78%)	20 (40%)	18 (36%)	19 (38%)
Mesentery			(1)	
Inflammation, chronic			1 (100%)	
Salivary glands		(1)		
Abscess		1 (100%)		
Stomach, glandular			(1)	
Mucosa, hyperplasia			1 (100%)	
Cardiovascular System				
Heart				(1)
Myocardium, inflammation, suppurative				1 (100%)
Endocrine System				
Islets, pancreatic	(1)			
Hyperplasia	1 (100%)			
Pituitary gland	(1)			
Cyst	1 (100%)			

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

TABLE A2c
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
General Body System				
None				
Genital System				
Penis	(1)			(2)
Inflammation, chronic	1 (100%)			2 (100%)
Preputial gland	(1)	(2)		(2)
Abscess		2 (100%)		2 (100%)
Duct, dilatation	1 (100%)	1 (50%)		1 (50%)
Seminal vesicle	(3)	(1)	(4)	(3)
Hemorrhage	1 (33%)			
Inflammation, chronic			2 (50%)	
Testes		(1)		(3)
Inflammation, chronic				1 (33%)
Seminiferous tubule, atrophy		1 (100%)		2 (67%)
Seminiferous tubule, mineralization				1 (33%)
Hematopoietic System				
Lymph node	(1)	(4)	(3)	(4)
Axillary, abscess		1 (25%)		
Axillary, hyperplasia, plasma cell		3 (75%)	1 (33%)	2 (50%)
Deep cervical, hyperplasia, plasma cell		1 (25%)	2 (67%)	
Mediastinal, hyperplasia, plasma cell		1 (25%)		
Renal, hyperplasia, plasma cell				1 (25%)
Lymph node, mandibular	(4)	(8)	(4)	(7)
Abscess		3 (38%)	1 (25%)	2 (29%)
Hyperplasia, plasma cell	3 (75%)	4 (50%)	4 (100%)	6 (86%)
Infiltration cellular, histiocyte		1 (13%)		
Sinus, ectasia		1 (13%)		
Lymph node, mesenteric	(3)	(1)	(3)	(3)
Hematopoietic cell proliferation	2 (67%)		1 (33%)	
Hyperplasia, plasma cell			1 (33%)	
Sinus, ectasia	2 (67%)	1 (100%)	1 (33%)	2 (67%)
Spleen	(6)	(11)	(11)	(16)
Amyloid deposition	1 (17%)	1 (9%)	1 (9%)	
Hematopoietic cell proliferation	3 (50%)	9 (82%)	6 (55%)	11 (69%)
Hyperplasia, plasma cell				1 (6%)
Inflammation, acute			1 (9%)	
Thymus	(46)	(42)	(48)	(39)
Cortex, atrophy	1 (2%)	1 (2%)	2 (4%)	4 (10%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Abscess		4 (8%)	3 (6%)	4 (8%)
Acanthosis	3 (6%)	10 (20%)	7 (14%)	11 (22%)
Amyloid deposition		1 (2%)	1 (2%)	2 (4%)
Cyst epithelial inclusion	1 (2%)	2 (4%)		
Edema		1 (2%)		

TABLE A2c
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Integumentary System (continued)				
Skin (continued)				
Hyperkeratosis	3 (6%)	10 (20%)	6 (12%)	10 (20%)
Inflammation, chronic	3 (6%)	10 (20%)	7 (14%)	11 (22%)
Ulcer	3 (6%)	8 (16%)	7 (14%)	10 (20%)
Control, acanthosis		2 (4%)	8 (16%)	23 (46%)
Control, amyloid deposition			2 (4%)	6 (12%)
Control, hyperkeratosis	2 (4%)	9 (18%)	15 (30%)	31 (62%)
Control, inflammation, chronic	1 (2%)	1 (2%)	2 (4%)	12 (24%)
Control, ulcer		1 (2%)		
Sebaceous gland, hypertrophy	2 (4%)	3 (6%)	3 (6%)	5 (10%)
Sebaceous gland, control, hypertrophy			1 (2%)	13 (26%)
Sebaceous gland, site of application-no mass, hypertrophy	2 (4%)	9 (18%)	24 (48%)	38 (76%)
Site of application-no mass, abscess				1 (2%)
Site of application-no mass, acanthosis	5 (10%)	23 (46%)	40 (80%)	49 (98%)
Site of application-no mass, amyloid deposition		3 (6%)	17 (34%)	29 (58%)
Site of application-no mass, cyst epithelial inclusion		2 (4%)		1 (2%)
Site of application-no mass, hyperkeratosis	10 (20%)	29 (58%)	41 (82%)	50 (100%)
Site of application-no mass, inflammation, chronic	3 (6%)	12 (24%)	17 (34%)	46 (92%)
Site of application-no mass, ulcer	1 (2%)	5 (10%)	5 (10%)	8 (16%)
Musculoskeletal System				
Bone		(1)	(1)	(2)
Cranium, inflammation, granulomatous		1 (100%)		
Phalanges, fracture				1 (50%)
Synovial tissue, inflammation, suppurative				1 (50%)
Skeletal muscle	(1)		(1)	
Inflammation, suppurative	1 (100%)		1 (100%)	
Nervous System				
None				
Respiratory System				
Lung	(4)	(2)	(1)	(1)
Inflammation, granulomatous		1 (50%)		
Alveolus, inflammation, subacute	1 (25%)			
Pleura, inflammation, chronic	1 (25%)			
Special Senses System				
Ear	(2)	(3)	(4)	(4)
Middle ear, inflammation, chronic			1 (25%)	
Pinna, inflammation, chronic	1 (50%)	1 (33%)	1 (25%)	
Pinna, ulcer	1 (50%)	2 (67%)	2 (50%)	4 (100%)

TABLE A2c
Summary of the Incidence of Nonneoplastic Lesions in Male Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition	22 (44%)	18 (36%)	16 (32%)	16 (32%)
Bacterium			1 (2%)	1 (2%)
Hydronephrosis	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Inflammation, acute			1 (2%)	2 (4%)
Inflammation, chronic	7 (14%)	12 (24%)	5 (10%)	8 (16%)
Urinary bladder		(2)		(3)
Inflammation, chronic				1 (33%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE MICE IN THE 1-YEAR INITIATION/PROMOTION STUDY OF *o*-BENZYL-*p*-CHLOROPHENOL

TABLE B1a
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator[a]

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1	1		
Moribund	5	23	13	11
Natural deaths		13	3	13
Survivors				
Terminal sacrifice	44	13	34	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Liver	(50)	(50)	(50)	(50)
Hemangioma, single	1 (2%)			
Squamous cell carcinoma, metastatic, skin		1 (2%)		
Cardiovascular System				
None				
Endocrine System				
None				
General Body System				
None				
Genital System				
Ovary	(15)	(13)	(20)	(14)
Granulosa cell tumor benign			1 (5%)	
Uterus	(26)	(5)	(23)	(9)
Leiomyoma	1 (4%)			
Polyp stromal	1 (4%)		1 (4%)	
Hematopoietic System				
Lymph node		(12)	(12)	(4)
Axillary, squamous cell carcinoma, metastatic, skin		1 (8%)		
Lumbar, squamous cell carcinoma, metastatic, skin		1 (8%)		
Lymph node, mandibular	(2)	(10)	(11)	(11)
Lymph node, mesenteric	(4)	(14)	(9)	(6)
Spleen	(4)	(40)	(23)	(22)
Thymus	(49)	(40)	(45)	(48)

TABLE B1a
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		
Squamous cell papilloma, single				1 (2%)
Control, squamous cell papilloma, single			1 (2%)	
Sebaceous gland, site of application-mass, adenoma, single		1 (2%)		1 (2%)
Site of application-mass, basal cell carcinoma, single		1 (2%)		
Site of application-mass, fibrosarcoma		2 (4%)		
Site of application-mass, keratoacanthoma		1 (2%)		
Site of application-mass, keratoacanthoma, multiple		3 (6%)		
Site of application-mass, keratoacanthoma, single		3 (6%)	1 (2%)	1 (2%)
Site of application-mass, squamous cell carcinoma		2 (4%)		
Site of application-mass, squamous cell carcinoma, multiple		3 (6%)		
Site of application-mass, squamous cell carcinoma, single		13 (26%)	1 (2%)	
Site of application-mass, squamous cell papilloma, multiple		23 (46%)	1 (2%)	1 (2%)
Site of application-mass, squamous cell papilloma, single		8 (16%)	7 (14%)	1 (2%)
Subcutaneous tissue, site of application-mass, lymphoma malignant lymphocytic		1 (2%)		
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(1)	(1)		(1)
Alveolar/bronchiolar adenoma, single	1 (100%)			
Special Senses System				
None				
Urinary System				
Kidney	(50)	(50)	(50)	(50)

TABLE B1a
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Lymphoma malignant lymphocytic		1 (2%)		1 (2%)
Lymphoma malignant undifferentiated cell		1 (2%)	1 (2%)	
Neoplasm Summary				
Total animals with primary neoplasms[c]	4	42	14	6
Total primary neoplasms	4	63	14	6
Total animals with benign neoplasms	4	34	12	5
Total benign neoplasms	4	39	12	5
Total animals with malignant neoplasms		23	2	1
Total malignant neoplasms		24	2	1
Total animals with metastatic neoplasms		2		
Total metastatic neoplasms		3		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B1b
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter[a]

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death		1			
Moribund		23	2	2	7
Natural deaths	3	13	5	4	3
Survivors					
Terminal sacrifice	47	13	43	44	40
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Liver	(50)	(50)	(50)	(50)	(50)
Hepatocellular adenoma, single			1 (2%)		
Squamous cell carcinoma, metastatic, skin		1 (2%)			
Cardiovascular System					
None					
Endocrine System					
None					
General Body System					
None					
Genital System					
Ovary	(16)	(13)	(25)	(15)	(18)
Cystadenoma, papillary					1 (6%)
Uterus	(21)	(5)	(30)	(31)	(24)
Leiomyoma			1 (3%)		
Polyp stromal				1 (3%)	
Sarcoma stromal					1 (4%)
Hematopoietic System					
Lymph node	(1)	(12)	(3)	(3)	(2)
Axillary, squamous cell carcinoma, metastatic, skin		1 (8%)			
Lumbar, squamous cell carcinoma, metastatic, skin		1 (8%)			

TABLE B1b
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Hematopoietic System (continued)					
Lymph node, mandibular	(1)	(10)	(2)	(2)	(4)
Lymph node, mesenteric	(8)	(14)	(3)	(4)	(4)
Spleen	(6)	(40)	(6)	(8)	(14)
Thymus	(50)	(40)	(48)	(50)	(47)
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)			
Other, squamous cell papilloma					1 (2%)
Sebaceous gland, adenoma, single					1 (2%)
Sebaceous gland, site of application-mass, adenoma, multiple				2 (4%)	
Sebaceous gland, site of application-mass, adenoma, single	1 (2%)	1 (2%)	2 (4%)		
Site of application-mass, basal cell carcinoma, single		1 (2%)			
Site of application-mass, fibrosarcoma		2 (4%)			
Site of application-mass, keratoacanthoma		1 (2%)			
Site of application-mass, keratoacanthoma, multiple		3 (6%)			
Site of application-mass, keratoacanthoma, single		3 (6%)		1 (2%)	1 (2%)
Site of application-mass, squamous cell carcinoma		2 (4%)			1 (2%)
Site of application-mass, squamous cell carcinoma, multiple		3 (6%)			
Site of application-mass, squamous cell carcinoma, single	1 (2%)	13 (26%)			1 (2%)
Site of application-mass, squamous cell papilloma					5 (10%)
Site of application-mass, squamous cell papilloma, multiple		23 (46%)			
Site of application-mass, squamous cell papilloma, single		8 (16%)	3 (6%)	3 (6%)	6 (12%)
Subcutaneous tissue, site of application-mass, hemangioma					1 (2%)
Subcutaneous tissue, site of application-mass, lymphoma malignant lymphocytic		1 (2%)			
Musculoskeletal System					
None					
Nervous System					
None					

TABLE B1b
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Respiratory System					
Lung	(3)	(1)	(6)	(2)	(2)
Alveolar/bronchiolar adenoma, single	3 (100%)		4 (67%)	1 (50%)	1 (50%)
Squamous cell carcinoma, metastatic, skin					1 (50%)
Nose	(50)	(50)	(50)	(50)	(50)
Special Senses System					
Harderian gland		(2)	(2)		
Adenoma			1 (50%)		
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(50)	(50)	(50)
Lymphoma malignant histiocytic	1 (2%)				
Lymphoma malignant lymphocytic		1 (2%)	1 (2%)		
Lymphoma malignant undifferentiated cell		1 (2%)	1 (2%)		1 (2%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	6	42	11	6	18
Total primary neoplasms	6	63	14	8	21
Total animals with benign neoplasms	4	34	9	6	16
Total benign neoplasms	4	39	12	8	17
Total animals with malignant neoplasms	2	23	2		4
Total malignant neoplasms	2	24	2		4
Total animals with metastatic neoplasms		2			1
Total metastatic neoplasms		3			1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B1c
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen[a]

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Disposition Summary					
Animals initially in study	50	50	51	50	50
Early deaths					
Accidental deaths	1		1		1
Moribund	5	42	9	7	10
Natural deaths		8	4	2	4
Survivors					
Died last week of study					1
Terminal sacrifice	44		37	41	34
Animals examined microscopically	50	50	51	50	50
Alimentary System					
Liver	(50)	(50)	(51)	(50)	(50)
Hemangioma, single	1 (2%)				
Hepatocellular adenoma, single			1 (2%)	1 (2%)	
Cardiovascular System					
None					
Endocrine System					
Pituitary gland				(1)	
Pars distalis, adenoma				1 (100%)	
General Body System					
None					
Genital System					
Uterus	(26)	(1)	(20)	(18)	(19)
Leiomyoma	1 (4%)				
Polyp stromal	1 (4%)				
Hematopoietic System					
Lymph node		(14)	(2)	(4)	(5)
Axillary, squamous cell carcinoma, metastatic, skin		8 (57%)			
Axillary, lumbar, squamous cell carcinoma, metastatic, skin		1 (7%)			
Lymph node, mandibular	(2)	(4)	(6)	(4)	(14)
Axillary, squamous cell carcinoma, metastatic, skin		1 (25%)			
Lymph node, mesenteric	(4)	(9)	(5)	(2)	(4)
Spleen	(4)	(46)	(11)	(10)	(19)
Thymus	(49)	(41)	(49)	(47)	(46)

TABLE B1c
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Integumentary System					
Skin	(50)	(50)	(51)	(50)	(50)
Squamous cell carcinoma, multiple		1 (2%)			
Squamous cell carcinoma, single		5 (10%)			
Sebaceous gland, site of application-mass, adenoma, single		3 (6%)			
Site of application-mass, basosquamous tumor malignant, single		1 (2%)			
Site of application-mass, squamous cell carcinoma, multiple		17 (34%)			
Site of application-mass, squamous cell carcinoma, single		30 (60%)			
Site of application-mass, squamous cell papilloma, multiple		1 (2%)			
Site of application-mass, squamous cell papilloma, single		8 (16%)			
Subcutaneous tissue, hemangiosarcoma, single			1 (2%)		
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lung	(1)	(17)		(1)	
Alveolar/bronchiolar adenoma, single	1 (100%)	1 (6%)			
Squamous cell carcinoma, metastatic, skin		12 (71%)			
Special Senses System					
Harderian gland					(1)
Adenoma					1 (100%)
Urinary System					
None					

TABLE B1c
Summary of the Incidence of Neoplasms in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Systemic Lesions					
Multiple organs[b]	(50)	(50)	(51)	(50)	(50)
Lymphoma malignant undifferentiated cell			1 (2%)		1 (2%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	4	48	3	2	2
Total primary neoplasms	4	67	3	2	2
Total animals with benign neoplasms	4	12	1	2	1
Total benign neoplasms	4	13	1	2	1
Total animals with malignant neoplasms		47	2		1
Total malignant neoplasms		54	2		1
Total animals with metastatic neoplasms		19			
Total metastatic neoplasms		22			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2a
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator[a]

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	1	1		
Moribund	5	23	13	11
Natural deaths		13	3	13
Survivors				
Terminal sacrifice	44	13	34	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine small, jejunum		(1)		(1)
Amyloid deposition				1 (100%)
Inflammation, acute		1 (100%)		
Intestine small, ileum				(1)
Amyloid deposition				1 (100%)
Liver	(50)	(50)	(50)	(50)
Amyloid deposition		3 (6%)	3 (6%)	2 (4%)
Basophilic focus		1 (2%)		
Hematopoietic cell proliferation	1 (2%)	9 (18%)	8 (16%)	5 (10%)
Inflammation, granulomatous	6 (12%)		2 (4%)	1 (2%)
Inflammation, suppurative		12 (24%)	8 (16%)	3 (6%)
Necrosis, coagulative	2 (4%)	2 (4%)		1 (2%)
Vacuolization cytoplasmic	2 (4%)	3 (6%)	6 (12%)	5 (10%)
Serosa, inflammation, chronic		1 (2%)		
Pancreas			(1)	
Abscess			1 (100%)	
Salivary glands		(1)	(1)	(2)
Abscess		1 (100%)		1 (50%)
Inflammation, acute			1 (100%)	
Stomach, forestomach	(1)	(3)	(1)	(1)
Serosa, inflammation, acute		1 (33%)		
Serosa, inflammation, chronic		1 (33%)		
Stomach, glandular		(2)	(2)	(2)
Ulcer			1 (50%)	
Mucosa, hyperplasia				1 (50%)
Mucosa, mineralization				1 (50%)
Serosa, inflammation, acute		2 (100%)	1 (50%)	
Tongue			(1)	
Abscess			1 (100%)	
Cardiovascular System				
Heart		(3)		(1)
Bacterium		1 (33%)		1 (100%)
Myocardium, abscess		1 (33%)		
Myocardium, inflammation, suppurative		2 (67%)		1 (100%)

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

TABLE B2a
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Endocrine System				
None				
General Body System				
None				
Genital System				
Ovary	(15)	(13)	(20)	(14)
Abscess				1 (7%)
Amyloid deposition	3 (20%)	1 (8%)	7 (35%)	6 (43%)
Angiectasis	1 (7%)			1 (7%)
Fat, necrosis	1 (7%)			
Follicle, cyst	8 (53%)	9 (69%)	11 (55%)	8 (57%)
Periovarian tissue, cyst	6 (40%)	4 (31%)	7 (35%)	2 (14%)
Periovarian tissue, inflammation, chronic			1 (5%)	
Uterus	(26)	(5)	(23)	(9)
Abscess			1 (4%)	
Amyloid deposition	4 (15%)			
Dilatation		1 (20%)		
Inflammation, chronic			1 (4%)	
Thrombosis		1 (20%)		
Endometrium, hyperplasia, cystic	26 (100%)	3 (60%)	19 (83%)	8 (89%)
Hematopoietic System				
Lymph node		(12)	(12)	(4)
Axillary, abscess		2 (17%)	3 (25%)	1 (25%)
Axillary, hyperplasia, plasma cell		7 (58%)	7 (58%)	2 (50%)
Deep cervical, abscess			1 (8%)	
Deep cervical, hyperplasia, plasma cell		2 (17%)	1 (8%)	
Deep cervical, inflammation, acute				1 (25%)
Lumbar, hyperplasia, plasma cell			2 (17%)	
Mediastinal, abscess			1 (8%)	
Mediastinal, hyperplasia, plasma cell		2 (17%)	3 (25%)	2 (50%)
Pancreatic, hyperplasia, plasma cell			2 (17%)	
Renal, hyperplasia, plasma cell		2 (17%)	1 (8%)	
Lymph node, mandibular	(2)	(10)	(11)	(11)
Abscess	1 (50%)	3 (30%)		2 (18%)
Hyperplasia, plasma cell		8 (80%)	9 (82%)	10 (91%)
Inflammation, acute			1 (9%)	
Sinus, ectasia	1 (50%)			2 (18%)
Lymph node, mesenteric	(4)	(14)	(9)	(6)
Hematopoietic cell proliferation	3 (75%)	5 (36%)	1 (11%)	3 (50%)
Hyperplasia, plasma cell		5 (36%)	4 (44%)	4 (67%)
Infiltration cellular, histiocyte	1 (25%)			
Sinus, ectasia	3 (75%)	9 (64%)	5 (56%)	2 (33%)

TABLE B2a
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Hematopoietic System (continued)				
Spleen	(4)	(40)	(23)	(22)
Amyloid deposition	1 (25%)	5 (13%)	6 (26%)	4 (18%)
Hematopoietic cell proliferation	3 (75%)	35 (88%)	20 (87%)	16 (73%)
Inflammation, chronic		2 (5%)		
Thymus	(49)	(40)	(45)	(48)
Cortex, atrophy	2 (4%)	14 (35%)	4 (9%)	11 (23%)
Medulla, hyperplasia	1 (2%)		2 (4%)	2 (4%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Abscess		6 (12%)	4 (8%)	5 (10%)
Acanthosis	2 (4%)	12 (24%)	8 (16%)	16 (32%)
Amyloid deposition				1 (2%)
Cyst epithelial inclusion		3 (6%)	2 (4%)	7 (14%)
Hyperkeratosis	2 (4%)	14 (28%)	9 (18%)	16 (32%)
Inflammation, chronic	2 (4%)	9 (18%)	9 (18%)	16 (32%)
Ulcer	2 (4%)	10 (20%)	6 (12%)	15 (30%)
Control, acanthosis		27 (54%)	25 (50%)	30 (60%)
Control, amyloid deposition		3 (6%)	12 (24%)	7 (14%)
Control, hyperkeratosis	1 (2%)	33 (66%)	34 (68%)	33 (66%)
Control, inflammation, chronic	1 (2%)	19 (38%)	19 (38%)	29 (58%)
Sebaceous gland, hypertrophy	2 (4%)	3 (6%)	2 (4%)	4 (8%)
Sebaceous gland, control, hypertrophy		7 (14%)	11 (22%)	14 (28%)
Sebaceous gland, site of application-no mass, hypertrophy		27 (54%)	28 (56%)	26 (52%)
Site of application-no mass, abscess		3 (6%)		
Site of application-no mass, acanthosis		45 (90%)	45 (90%)	45 (90%)
Site of application-no mass, amyloid deposition	1 (2%)	4 (8%)	21 (42%)	14 (28%)
Site of application-no mass, cyst epithelial inclusion		4 (8%)	5 (10%)	3 (6%)
Site of application-no mass, hyperkeratosis	2 (4%)	43 (86%)	47 (94%)	44 (88%)
Site of application-no mass, infiltration cellular, mast cell			1 (2%)	1 (2%)
Site of application-no mass, inflammation, chronic	1 (2%)	44 (88%)	45 (90%)	46 (92%)
Site of application-no mass, ulcer		18 (36%)	13 (26%)	17 (34%)
Musculoskeletal System				
Bone				(1)
Synovial tissue, inflammation, suppurative				1 (100%)
Skeletal muscle		(1)	(1)	
Inflammation, suppurative		1 (100%)	1 (100%)	
Nervous System				
None				

TABLE B2a
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as an Initiator (continued)

	Acetone/ Acetone	50 μg DMBA/ 5 μg TPA	5 μg TPA/ 5 μg TPA	10 mg BCP/ 5 μg TPA
Respiratory System				
None				
Special Senses System				
Ear	(1)	(3)	(2)	(2)
External ear, inflammation, chronic		1 (33%)		
Pinna, inflammation, chronic		1 (33%)	2 (100%)	
Pinna, ulcer	1 (100%)	2 (67%)		2 (100%)
Eye		(2)	(2)	(1)
Phthisis bulbi		1 (50%)		1 (100%)
Cornea, inflammation, chronic			1 (50%)	
Cornea, inflammation, necrotizing				1 (100%)
Harderian gland		(2)		
Inflammation, chronic		1 (50%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition	26 (52%)	4 (8%)	15 (30%)	11 (22%)
Bacterium		3 (6%)		
Cyst	1 (2%)			
Hydronephrosis	1 (2%)			
Inflammation, acute		4 (8%)	1 (2%)	1 (2%)
Inflammation, chronic	3 (6%)	24 (48%)	11 (22%)	11 (22%)

TABLE B2b
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter[a]

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Disposition Summary					
Animals initially in study	50	50	50	50	50
Early deaths					
Accidental death		1			
Moribund		23	2	2	7
Natural deaths	3	13	5	4	3
Survivors					
Terminal sacrifice	47	13	43	44	40
Animals examined microscopically	50	50	50	50	50
Alimentary System					
Intestine large, colon				(1)	
Inflammation, chronic				1 (100%)	
Intestine large, rectum				(1)	
Thrombosis				1 (100%)	
Intestine large, cecum		(1)		(1)	
Inflammation, chronic				1 (100%)	
Intestine small, duodenum					(2)
Inflammation, chronic					1 (50%)
Artery, amyloid deposition					1 (50%)
Intestine small, jejunum	(1)	(1)	(2)		(1)
Amyloid deposition	1 (100%)				
Inflammation, acute		1 (100%)	2 (100%)		
Inflammation, chronic	1 (100%)				1 (100%)
Intestine small, ileum				(1)	
Inflammation, chronic				1 (100%)	
Liver	(50)	(50)	(50)	(50)	(50)
Amyloid deposition		3 (6%)		1 (2%)	1 (2%)
Basophilic focus		1 (2%)			
Eosinophilic focus			1 (2%)		
Hematopoietic cell proliferation	1 (2%)	9 (18%)		1 (2%)	4 (8%)
Inflammation, granulomatous	3 (6%)		6 (12%)	4 (8%)	1 (2%)
Inflammation, suppurative		12 (24%)			4 (8%)
Necrosis, coagulative	1 (2%)	2 (4%)	1 (2%)		4 (8%)
Vacuolization cytoplasmic	1 (2%)	3 (6%)	3 (6%)	3 (6%)	9 (18%)
Serosa, inflammation, chronic		1 (2%)		1 (2%)	
Mesentery				(1)	
Fat, necrosis				1 (100%)	
Pancreas				(1)	
Acinus, atrophy				1 (100%)	
Salivary glands		(1)		(1)	
Abscess		1 (100%)		1 (100%)	
Stomach, forestomach	(2)	(3)	(1)		(2)
Acanthosis			1 (100%)		
Hyperkeratosis			1 (100%)		
Serosa, inflammation, acute		1 (33%)			1 (50%)
Serosa, inflammation, chronic		1 (33%)			
Stomach, glandular	(1)	(2)			(1)
Serosa, inflammation, acute		2 (100%)			1 (100%)

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

TABLE B2b
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Cardiovascular System					
Heart		(3)		(1)	(1)
Bacterium		1 (33%)			
Atrium, thrombosis				1 (100%)	
Myocardium, abscess		1 (33%)			
Myocardium, inflammation, suppurative		2 (67%)			1 (100%)
Endocrine System					
Islets, pancreatic				(1)	
Hyperplasia				1 (100%)	
General Body System					
None					
Genital System					
Ovary	(16)	(13)	(25)	(15)	(18)
Abscess				1 (7%)	
Amyloid deposition	8 (50%)	1 (8%)	14 (56%)	7 (47%)	3 (17%)
Bilateral, amyloid deposition					1 (6%)
Follicle, cyst	7 (44%)	9 (69%)	14 (56%)	5 (33%)	14 (78%)
Periovarian tissue, cyst	7 (44%)	4 (31%)	8 (32%)	9 (60%)	1 (6%)
Uterus	(21)	(5)	(30)	(31)	(24)
Abscess				1 (3%)	
Amyloid deposition	1 (5%)		2 (7%)	6 (19%)	
Dilatation		1 (20%)			
Inflammation, necrotizing			1 (3%)		
Thrombosis		1 (20%)			
Endometrium, hyperplasia, cystic	20 (95%)	3 (60%)	28 (93%)	29 (94%)	23 (96%)
Vagina				(1)	
Inflammation, chronic				1 (100%)	
Hematopoietic System					
Lymph node	(1)	(12)	(3)	(3)	(2)
Axillary, abscess		2 (17%)		1 (33%)	
Axillary, hyperplasia, plasma cell		7 (58%)			
Deep cervical, hyperplasia, lymphoid	1 (100%)				
Deep cervical, hyperplasia, plasma cell	1 (100%)	2 (17%)			
Mediastinal, ectasia			1 (33%)		
Mediastinal, hyperplasia, plasma cell		2 (17%)		1 (33%)	2 (100%)
Pancreatic, infiltration cellular, histiocyte				1 (33%)	
Renal, hyperplasia, plasma cell		2 (17%)			
Lymph node, mandibular	(1)	(10)	(2)	(2)	(4)
Abscess		3 (30%)			
Hyperplasia, plasma cell	1 (100%)	8 (80%)		1 (50%)	4 (100%)
Infiltration cellular, histiocyte			1 (50%)	1 (50%)	
Sinus, ectasia					1 (25%)

TABLE B2b
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Hematopoietic System (continued)					
Lymph node, mesenteric	(8)	(14)	(3)	(4)	(4)
Abscess				1 (25%)	1 (25%)
Hematopoietic cell proliferation	3 (38%)	5 (36%)	1 (33%)		1 (25%)
Hyperplasia, lymphoid				1 (25%)	
Hyperplasia, plasma cell	1 (13%)	5 (36%)			
Infiltration cellular, histiocyte	1 (13%)		1 (33%)		
Sinus, ectasia	5 (63%)	9 (64%)		2 (50%)	3 (75%)
Spleen	(6)	(40)	(6)	(8)	(14)
Amyloid deposition		5 (13%)		1 (13%)	1 (7%)
Hematopoietic cell proliferation	1 (17%)	35 (88%)	1 (17%)	3 (38%)	10 (71%)
Hyperplasia, lymphoid				1 (13%)	
Inflammation, chronic		2 (5%)			1 (7%)
Thymus	(50)	(40)	(48)	(50)	(47)
Cortex, atrophy	2 (4%)	14 (35%)	3 (6%)	6 (12%)	3 (6%)
Medulla, hyperplasia					1 (2%)
Integumentary System					
Skin	(50)	(50)	(50)	(50)	(50)
Abscess		6 (12%)			2 (4%)
Acanthosis		12 (24%)			6 (12%)
Cyst epithelial inclusion		3 (6%)			
Edema			1 (2%)		
Hyperkeratosis		14 (28%)			6 (12%)
Inflammation, chronic		9 (18%)			7 (14%)
Ulcer	1 (2%)	10 (20%)			6 (12%)
Control, abscess					1 (2%)
Control, acanthosis	1 (2%)	27 (54%)	3 (6%)	16 (32%)	23 (46%)
Control, amyloid deposition		3 (6%)		5 (10%)	5 (10%)
Control, hyperkeratosis	2 (4%)	33 (66%)	3 (6%)	15 (30%)	26 (52%)
Control, inflammation, chronic		19 (38%)	1 (2%)	1 (2%)	21 (42%)
Control, ulcer					1 (2%)
Sebaceous gland, hypertrophy		3 (6%)			4 (8%)
Sebaceous gland, control, hypertrophy		7 (14%)		2 (4%)	16 (32%)
Sebaceous gland, site of application-no mass, hypertrophy	4 (8%)	27 (54%)		4 (8%)	40 (80%)
Sebaceous gland, site of application-mass, hypertrophy					2 (4%)
Site of application-no mass, abscess		3 (6%)			
Site of application-no mass, acanthosis	6 (12%)	45 (90%)	5 (10%)	26 (52%)	47 (94%)
Site of application-no mass, amyloid deposition	1 (2%)	4 (8%)		10 (20%)	26 (52%)
Site of application-no mass, cyst epithelial inclusion	1 (2%)	4 (8%)			1 (2%)
Site of application-no mass, hyperkeratosis	9 (18%)	43 (86%)	8 (16%)	36 (72%)	47 (94%)
Site of application-no mass, inflammation, chronic	6 (12%)	44 (88%)	4 (8%)	9 (18%)	45 (90%)
Site of application-no mass, ulcer		18 (36%)			6 (12%)
Site of application-mass, acanthosis					3 (6%)
Subcutaneous tissue, site of application-mass, angiectasis					1 (2%)

TABLE B2b
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Promoter (continued)

	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	50 μg DMBA/ 0.1 mg BCP	50 μg DMBA/ 1.0 mg BCP	50 μg DMBA/ 3.0 mg BCP
Musculoskeletal System					
Bone					(1)
Synovial tissue, inflammation, suppurative					1 (100%)
Skeletal muscle		(1)			
Inflammation, suppurative		1 (100%)			
Nervous System					
Brain					(1)
Infarct					1 (100%)
Respiratory System					
None					
Special Senses System					
Ear	(1)	(3)	(1)		(3)
External ear, inflammation, chronic		1 (33%)			
Pinna, inflammation, chronic		1 (33%)	1 (100%)		
Pinna, ulcer	1 (100%)	2 (67%)			3 (100%)
Eye		(2)			(1)
Phthisis bulbi		1 (50%)			
Harderian gland		(2)	(2)		
Inflammation, chronic		1 (50%)			
Urinary System					
Kidney	(50)	(50)	(50)	(50)	(50)
Amyloid deposition	24 (48%)	4 (8%)	18 (36%)	25 (50%)	15 (30%)
Bacterium		3 (6%)	1 (2%)	1 (2%)	
Cyst				1 (2%)	
Hemorrhage			1 (2%)		
Hydronephrosis	2 (4%)				
Inflammation, acute		4 (8%)	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic	5 (10%)	24 (48%)	3 (6%)	6 (12%)	6 (12%)
Glomerulus, amyloid deposition				1 (2%)	
Interstitial tissue, amyloid deposition				1 (2%)	
Urinary bladder		(1)		(1)	
Inflammation, chronic				1 (100%)	

TABLE B2c
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen[a]

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Disposition Summary					
Animals initially in study	50	50	51	50	50
Early deaths					
Accidental deaths	1		1		1
Moribund	5	42	9	7	10
Natural deaths		8	4	2	4
Survivors					
Died last week of study					1
Terminal sacrifice	44		37	41	34
Animals examined microscopically	50	50	51	50	50
Alimentary System					
Intestine large, colon		(1)			
Inflammation, chronic		1 (100%)			
Intestine large, cecum		(1)			
Inflammation, chronic		1 (100%)			
Liver	(50)	(50)	(51)	(50)	(50)
Amyloid deposition		2 (4%)	1 (2%)	2 (4%)	
Hematopoietic cell proliferation	1 (2%)	27 (54%)	6 (12%)	2 (4%)	5 (10%)
Inflammation, granulomatous	6 (12%)		2 (4%)	1 (2%)	
Inflammation, suppurative		16 (32%)	2 (4%)	3 (6%)	2 (4%)
Necrosis, coagulative	2 (4%)	2 (4%)	1 (2%)	1 (2%)	
Vacuolization cytoplasmic	2 (4%)		6 (12%)	5 (10%)	3 (6%)
Mesentery		(1)		(1)	(1)
Inflammation, chronic		1 (100%)		1 (100%)	1 (100%)
Pancreas		(3)	(1)		(1)
Abscess		1 (33%)			1 (100%)
Interlobular, edema			1 (100%)		
Serosa, inflammation, acute		2 (67%)			
Stomach, forestomach	(1)	(1)		(2)	(2)
Hyperkeratosis					1 (50%)
Serosa, inflammation, acute		1 (100%)			
Stomach, glandular		(2)		(2)	
Serosa, inflammation, acute		2 (100%)			
Tooth		(2)			
Abscess		1 (50%)			
Gingiva, inflammation, chronic active		1 (50%)			
Cardiovascular System					
Heart				(1)	
Myocardium, inflammation, suppurative				1 (100%)	
Endocrine System					
Adrenal cortex				(1)	
Abscess				1 (100%)	
Adrenal medulla				(1)	
Abscess				1 (100%)	

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

TABLE B2c
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
General Body System					
None					
Genital System					
Clitoral gland			(2)		
Abscess			1 (50%)		
Duct, dilatation			1 (50%)		
Ovary	(15)	(6)	(19)	(16)	(19)
Abscess		1 (17%)			2 (11%)
Amyloid deposition	3 (20%)		8 (42%)	9 (56%)	7 (37%)
Angiectasis	1 (7%)				
Fat, necrosis	1 (7%)				
Follicle, cyst	8 (53%)	4 (67%)	9 (47%)	6 (38%)	9 (47%)
Periovarian tissue, cyst	6 (40%)	1 (17%)	11 (58%)	7 (44%)	7 (37%)
Uterus	(26)	(1)	(20)	(18)	(19)
Amyloid deposition	4 (15%)		1 (5%)	2 (11%)	
Thrombosis					1 (5%)
Endometrium, hyperplasia, cystic	26 (100%)	1 (100%)	20 (100%)	18 (100%)	17 (89%)
Vagina		(2)			(1)
Inflammation, chronic		2 (100%)			
Inflammation, necrotizing					1 (100%)
Hematopoietic System					
Lymph node		(14)	(2)	(4)	(5)
Axillary, abscess		4 (29%)	1 (50%)	1 (25%)	1 (20%)
Axillary, hyperplasia, plasma cell		5 (36%)	2 (100%)	2 (50%)	2 (40%)
Axillary, sinus, ectasia			1 (50%)		1 (20%)
Deep cervical, hyperplasia, plasma cell					1 (20%)
Mediastinal, hyperplasia, plasma cell		1 (7%)		1 (25%)	
Pancreatic, inflammation, acute				1 (25%)	
Renal, hyperplasia, plasma cell		1 (7%)			
Lymph node, mandibular	(2)	(4)	(6)	(4)	(14)
Abscess	1 (50%)		1 (17%)		3 (21%)
Hyperplasia, plasma cell		3 (75%)	4 (67%)	3 (75%)	13 (93%)
Inflammation, acute			2 (33%)		
Sinus, ectasia	1 (50%)			1 (25%)	1 (7%)
Lymph node, mesenteric	(4)	(9)	(5)	(2)	(4)
Abscess			1 (20%)		
Hematopoietic cell proliferation	3 (75%)	1 (11%)	1 (20%)	1 (50%)	1 (25%)
Hyperplasia, lymphoid		1 (11%)			
Hyperplasia, plasma cell		5 (56%)			1 (25%)
Infiltration cellular, histiocyte	1 (25%)	1 (11%)			
Sinus, ectasia	3 (75%)	3 (33%)	3 (60%)	1 (50%)	1 (25%)
Spleen	(4)	(46)	(11)	(10)	(19)
Amyloid deposition	1 (25%)	5 (11%)	1 (9%)	1 (10%)	1 (5%)
Hematopoietic cell proliferation	3 (75%)	45 (98%)	9 (82%)	6 (60%)	15 (79%)
Hyperplasia, plasma cell					1 (5%)
Inflammation, chronic		3 (7%)			

TABLE B2c
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Hematopoietic System (continued)					
Thymus	(49)	(41)	(49)	(47)	(46)
Ectopic parathyroid gland		1 (2%)			
Cortex, atrophy	2 (4%)	12 (29%)	3 (6%)	3 (6%)	4 (9%)
Medulla, hyperplasia	1 (2%)		1 (2%)	1 (2%)	
Integumentary System					
Skin	(50)	(50)	(51)	(50)	(50)
Abscess		1 (2%)	3 (6%)		2 (4%)
Acanthosis	2 (4%)	5 (10%)	5 (10%)	5 (10%)	14 (28%)
Amyloid deposition					1 (2%)
Cyst epithelial inclusion			1 (2%)	3 (6%)	3 (6%)
Hyperkeratosis	2 (4%)	6 (12%)	5 (10%)	4 (8%)	14 (28%)
Inflammation, chronic	2 (4%)	6 (12%)	6 (12%)	5 (10%)	13 (26%)
Ulcer	2 (4%)	5 (10%)	5 (10%)	6 (12%)	13 (26%)
Control, acanthosis		22 (44%)	8 (16%)	17 (34%)	21 (42%)
Control, amyloid deposition		1 (2%)		3 (6%)	2 (4%)
Control, hyperkeratosis	1 (2%)	38 (76%)	16 (31%)	20 (40%)	29 (58%)
Control, inflammation, chronic	1 (2%)	18 (36%)	8 (16%)	12 (24%)	18 (36%)
Control, ulcer		1 (2%)		1 (2%)	
Sebaceous gland, hypertrophy	2 (4%)		3 (6%)	2 (4%)	6 (12%)
Sebaceous gland, control, hypertrophy		1 (2%)	4 (8%)	14 (28%)	14 (28%)
Sebaceous gland, site of application-no mass, hypertrophy		2 (4%)	16 (31%)	30 (60%)	44 (88%)
Site of application-no mass, abscess		1 (2%)	2 (4%)	1 (2%)	
Site of application-no mass, acanthosis		41 (82%)	26 (51%)	36 (72%)	47 (94%)
Site of application-no mass, amyloid deposition	1 (2%)		3 (6%)	9 (18%)	24 (48%)
Site of application-no mass, cyst epithelial inclusion			1 (2%)		1 (2%)
Site of application-no mass, hyperkeratosis	2 (4%)	43 (86%)	31 (61%)	37 (74%)	48 (96%)
Site of application-no mass, inflammation, chronic	1 (2%)	35 (70%)	21 (41%)	29 (58%)	48 (96%)
Site of application-no mass, ulcer		15 (30%)	8 (16%)	6 (12%)	5 (10%)
Musculoskeletal System					
Bone					(3)
Mandible, abscess					1 (33%)
Synovial tissue, inflammation, suppurative					2 (67%)
Skeletal muscle		(2)			(2)
Inflammation, suppurative		2 (100%)			2 (100%)
Nervous System					
None					

TABLE B2c
Summary of the Incidence of Nonneoplastic Lesions in Female Swiss (CD-1®) Mice in the 1-Year Initiation/Promotion Study of BCP as a Complete Carcinogen (continued)

	Acetone/ Acetone	Acetone/ 20 μg DMBA	10 mg BCP/ 0.1 mg BCP	10 mg BCP/ 1.0 mg BCP	10 mg BCP/ 3.0 mg BCP
Respiratory System					
Lung	(1)	(17)		(1)	
Abscess		1 (6%)		1 (100%)	
Artery, thrombosis		1 (6%)			
Interstitium, inflammation, acute		1 (6%)			
Pleura, inflammation, chronic		3 (18%)			
Special Senses System					
Ear	(1)		(2)	(2)	(2)
Pinna, ulcer	1 (100%)		2 (100%)		2 (100%)
Eye		(1)		(1)	(1)
Cornea, inflammation, chronic				1 (100%)	1 (100%)
Cornea, inflammation, necrotizing		1 (100%)			
Urinary System					
Kidney	(50)	(50)	(51)	(50)	(50)
Amyloid deposition	26 (52%)	1 (2%)	19 (37%)	18 (36%)	15 (30%)
Bacterium					1 (2%)
Cyst	1 (2%)		1 (2%)	1 (2%)	
Hydronephrosis	1 (2%)				
Inflammation, acute		2 (4%)			2 (4%)
Inflammation, chronic	3 (6%)	31 (62%)	9 (18%)	9 (18%)	8 (16%)
Glomerulus, amyloid deposition					1 (2%)
Urinary bladder				(1)	
Amyloid deposition				1 (100%)	

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1986). *o*-Benzyl-*p*-chlorophenol was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of *o*-benzyl-*p*-chlorophenol. The high dose was limited by toxicity. All trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, not reproducible, or is of 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 was no minimum percentage or fold increase required for a chemical to the judged positive or weakly positive.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1985, 1987). *o*-Benzyl-*p*-chlorophenol was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of *o*-benzyl-*p*-chlorophenol. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with *o*-benzyl-*p*-chlorophenol in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing *o*-benzyl-*p*-chlorophenol was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with *o*-benzyl-*p*-chlorophenol, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no *o*-benzyl-*p*-chlorophenol and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence

of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with *o*-benzyl-*p*-chlorophenol for 10 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with *o*-benzyl-*p*-chlorophenol and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test. Because cell cycle delay was considered a possible confounding factor in this test, a subset of cells from the cultures harvested at the standard time of 12 to 14 hours was incubated for an additional several hours, followed by harvesting and analysis for chromosomal aberrations.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as percentage of cells with aberrations. Statistical analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.015$) are considered weak evidence for a positive response; significant differences for two or more doses indicate the trial is positive. A positive trend test in the absence of a statistically significant increase at any one dose results in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

RESULTS

o-Benzyl-*p*-chlorophenol (0.1 to 100 μg/plate) was not mutagenic to *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table C1; Mortelmans *et al.*, 1986). In cytogenetic tests with Chinese hamster ovary cells, *o*-benzyl-*p*-chlorophenol did not induce sister chromatid exchanges (Table C2) or chromosomal aberrations (Table C3), with or without Aroclor 1254-induced male Sprague-Dawley rat liver S9. The highest non-lethal dose tested in either of these mammalian cell assays was 16 μg/mL. In the Abs test, the second reported trial under each activation condition was a continuation of the cultures harvested in the first trial; the results of this second harvest indicated that cell cycle delay was not a factor in the observed lack of induced Abs following treatment with *o*-benzyl-*p*-chlorophenol.

TABLE C1
Mutagenicity of *o*-Benzyl-*p*-Chlorophenol in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SRI, International							
TA100	0.0	99 ± 5.7	103 ± 4.0	123 ± 6.8	108 ± 5.4	125 ± 13.6	121 ± 7.0
	0.1	142 ± 9.7	133 ± 3.5				
	0.3	126 ± 13.1	124 ± 7.3				
	1.0	130 ± 3.8	114 ± 5.4	125 ± 10.3	107 ± 5.0	156 ± 3.9	129 ± 12.8
	3.0	140 ± 9.4	138 ± 5.4	111 ± 16.1	105 ± 4.3	158 ± 3.2	141 ± 9.1
	10.0	151 ± 8.1	133 ± 2.3	115 ± 5.2	109 ± 4.2	114 ± 10.5	135 ± 3.5
	33.0			125 ± 0.9	109 ± 9.4	115 ± 8.7	129 ± 3.8
	66.0						116 ± 10.1
	100.0			117 ± 13.0	84 ± 9.9[c]	41 ± 20.7[c]	
Trial summary		Equivocal	Negative	Negative	Negative	Negative	Negative
Positive control[d]		433 ± 17.5	281 ± 6.9	1,617 ± 71.3	1,112 ± 18.2	533 ± 44.5	572 ± 32.9
TA 1535	0.0	23 ± 2.8	22 ± 2.0	10 ± 3.3	10 ± 0.9	7 ± 0.3	11 ± 2.4
	0.1	34 ± 7.7	32 ± 5.8				
	0.3	44 ± 3.2	35 ± 3.7				
	1.0	42 ± 3.2	37 ± 2.4	14 ± 1.2	5 ± 0.3	11 ± 2.7	11 ± 3.3
	3.0	48 ± 1.3	31 ± 1.8	11 ± 1.0	6 ± 1.0	9 ± 2.5	11 ± 2.2
	10.0	45 ± 0.9	38 ± 5.2	7 ± 1.5	9 ± 1.9	10 ± 1.2	10 ± 3.2
	33.0			14 ± 1.2	9 ± 2.8	8 ± 2.2	8 ± 3.9
	66.0						13 ± 0.3
	100.0			7 ± 2.1	4 ± 0.3[c]	2 ± 1.2[c]	
Trial summary		Equivocal	Negative	Negative	Negative	Negative	Negative
Positive control		488 ± 6.0	295 ± 1.7	458 ± 12.6	312 ± 11.7	208 ± 5.8	129 ± 17.6
TA1537	0.0	6 ± 1.2	4 ± 1.0	5 ± 1.0	8 ± 1.2	4 ± 0.9	6 ± 0.9
	0.1	6 ± 0.0	6 ± 1.9				
	0.3	4 ± 0.7	4 ± 1.5				
	1.0	4 ± 1.2	6 ± 0.9	7 ± 0.9	6 ± 0.9	6 ± 1.7	8 ± 1.0
	3.0	5 ± 0.3	6 ± 1.5	9 ± 2.1	7 ± 0.3	4 ± 1.2	10 ± 1.2
	10.0	4 ± 0.3	5 ± 1.2	8 ± 0.6	4 ± 1.0	7 ± 0.6	7 ± 1.8
	33.0			9 ± 1.9	6 ± 0.3	7 ± 1.2	6 ± 0.9
	66.0						7 ± 0.7
	100.0			5 ± 1.7	2 ± 0.3[c]	2 ± 2.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		161 ± 6.7	329 ± 59.5	328 ± 6.2	335 ± 37.6	168 ± 4.7	146 ± 6.4
TA98	0.0	16 ± 1.8	16 ± 0.3	31 ± 3.6	26 ± 4.0	32 ± 0.6	24 ± 0.3
	0.1	16 ± 0.9	16 ± 1.0				
	0.3	17 ± 3.5	18 ± 1.0				
	1.0	15 ± 0.7	18 ± 3.8	32 ± 2.2	30 ± 3.5	30 ± 6.2	24 ± 2.4
	3.0	11 ± 4.0	14 ± 1.5	27 ± 1.8	24 ± 2.3	31 ± 2.6	31 ± 1.5
	10.0	14 ± 2.4	15 ± 2.1	22 ± 4.7	25 ± 2.6	28 ± 1.7	29 ± 1.7
	33.0			26 ± 1.9	19 ± 4.6	27 ± 2.2	32 ± 0.7
	66.0						27 ± 1.8
	100.0			19 ± 1.2	0 ± 0.0[c]	0 ± 0.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		908 ± 46.9	472 ± 27.7	1,438 ± 97.8	774 ± 37.1	535 ± 16.8	446 ± 24.0

TABLE C1
Mutagenicity of *o*-Benzyl-*p*-Chlorophenol in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Corporation									
TA100	0.0	181 ± 13.2	143 ± 4.8	151 ± 10.7	242 ± 5.9	128 ± 8.0	149 ± 10.7	133 ± 6.7	148 ± 11.2
	0.3	197 ± 4.5	143 ± 6.1	141 ± 4.8					
	1.0	190 ± 7.0	140 ± 5.9	146 ± 5.8	245 ± 4.1	118 ± 7.1	138 ± 11.2	124 ± 4.1	129 ± 8.6
	3.3	204 ± 2.3	143 ± 13.1	162 ± 3.8	241 ± 5.5	116 ± 6.7	138 ± 7.0	142 ± 11.0	135 ± 8.5
	10.0	199 ± 3.3	166 ± 7.7	174 ± 3.4	244 ± 4.6	135 ± 9.5	140 ± 4.6	149 ± 4.4	139 ± 4.2
	33.0	Toxic	138 ± 1.5[c]	Toxic	207 ± 7.0	124 ± 11.3	145 ± 3.5	141 ± 5.2	136 ± 3.0
	100.0				118 ± 19.6[c]	122 ± 3.6[c]	121 ± 15.0[c]	148 ± 2.1[c]	117 ± 5.1[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		890 ± 26.9	1,330± 22.3	1,503 ± 24.3	1,116 ± 91.9	1,017 ± 31.0	1,247 ± 26.8	700 ± 36.5	1,029 ± 47.4
TA1535	0.0	38 ± 1.2	30 ± 1.0	24 ± 2.0	48 ± 1.7	9 ± 1.0	10 ± 2.2	9 ± 0.3	11 ± 2.0
	0.3	35 ± 4.6	38 ± 1.7	28 ± 3.8					
	1.0	43 ± 2.6	36 ± 8.5	25 ± 3.9	49 ± 0.9	10 ± 2.1	11 ± 2.4	9 ± 1.5	9 ± 0.9
	3.3	38 ± 1.5	33 ± 2.9	30 ± 2.5	46 ± 0.6	10 ± 1.9	14 ± 1.5	8 ± 1.3	11 ± 0.9
	10.0	36 ± 5.5	30 ± 2.9	33 ± 2.0	46 ± 2.0	13 ± 0.6	12 ± 2.9	8 ± 0.9	11 ± 2.2
	33.0	14 ± 4.3[c]	34 ± 0.7[c]	Toxic	47 ± 3.1	10 ± 1.2	11 ± 2.0	6 ± 0.9	11 ± 2.1
	100.0				8 ± 2.7[c]	9 ± 2.1[c]	7 ± 1.9[c]	9 ± 0.7[c]	7 ± 1.8[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		694 ± 10.3	1,030 ± 31.7	1,207 ± 54.6	128 ± 17.6	86 ± 2.3	95 ± 6.1	73 ± 5.2	85 ± 1.8

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Corporation							
TA1537	0.0	7 ± 0.6	6 ± 1.2	6 ± 2.3	5 ± 0.6	6 ± 2.9	11 ± 2.9
	0.3	7 ± 1.5	5 ± 2.2				
	1.0	4 ± 1.2	4 ± 0.6	8 ± 1.7	8 ± 1.7	6 ± 0.7	6 ± 1.8
	3.3	4 ± 0.9	8 ± 0.3	8 ± 1.8	9 ± 0.6	8 ± 0.7	7 ± 0.6
	10.0	4 ± 0.3	4 ± 1.0	5 ± 0.7	2 ± 0.3	8 ± 1.0	8 ± 1.0
	33.0	Toxic	Toxic	8 ± 2.0	6 ± 1.5	6 ± 2.2	6 ± 0.9
	100.0			3 ± 0.6[c]	7 ± 0.6[c]	3 ± 1.0[c]	9 ± 0.3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		132 ± 29.2	340 ± 21.8	96 ± 0.7	111 ± 0.9	65 ± 5.2	68 ± 4.5

TABLE C1
Mutagenicity of *o*-Benzyl-*p*-Chlorophenol in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3
Study performed at EG&G Mason Research Corporation							
TA98	0.0	19 ± 3.3	17 ± 1.0	16 ± 0.3	32 ± 2.0	29 ± 1.3	31 ± 5.1
	0.3	19 ± 2.1	12 ± 1.5	15 ± 1.2			
	1.0	13 ± 0.6	15 ± 2.0	17 ± 1.8	24 ± 4.6	32 ± 3.8	30 ± 4.1
	3.3	15 ± 3.0	20 ± 0.7	18 ± 1.8	22 ± 1.5	26 ± 3.6	24 ± 1.8
	10.0	13 ± 2.0	19 ± 1.5	16 ± 3.8	20 ± 2.5	29 ± 2.6	30 ± 2.7
	33.0	3 ± 0.0[c]	12 ± 1.2[c]	Toxic	24 ± 2.3	25 ± 1.5	33 ± 3.5
	100.0				12 ± 1.8[c]	24 ± 2.8[c]	19 ± 1.8[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,439 ± 47.5	1,122 ± 47.7	1,363 ± 38.7	1,045 ± 46.0	842 ± 44.7	915 ± 27.3

Strain	Dose (μg/plate)	Revertants/plate +10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
TA98	0.0	23 ± 1.7	25 ± 5.2	25 ± 4.3
	0.3	19 ± 2.0	30 ± 2.7	25 ± 3.5
	1.0	24 ± 0.6	29 ± 4.2	24 ± 0.6
	3.3	31 ± 3.5	26 ± 4.0	28 ± 1.9
	10.0	30 ± 2.2	25 ± 2.9	20 ± 5.0
	33.0	14 ± 1.2[c]	24 ± 4.5[c]	18 ± 0.3[c]
Trial summary		Negative	Negative	Negative
Positive control		723 ± 31.8	550 ± 25.4	724 ± 31.1

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

TABLE C2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *o*-Benzyl-*p*-Chlorophenol[a]

Compound	Dose μg/mL	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Summary: Negative								
Dimethylsulfoxide		50	1,035	493	0.47	9.9	26.5	
Mitomycin-C	0.01	50	1,043	1,898	1.81	38.0	26.5	282.04
o-Benzyl-*p*-chlorophenol	0.5	50	1,038	525	0.50	10.5	26.5	6.18
	1.6	50	1,033	506	0.48	10.1	26.5	2.84
	5.0	50	1,041	550	0.52	11.0	26.5	10.92
	16.0	50	1,047	511	0.48	10.2	26.5	2.46
					P=0.255[c]			
+S9								
Summary: Negative								
Dimethylsulfoxide		50	1,041	581	0.55	11.6	26.0	
Cyclophosphamide	2.0	50	1,047	3,165	3.02	63.3	26.0	441.64
o-Benzyl-*p*-chlorophenol	0.05	50	1,049	552	0.52	11.0	26.0	-5.72
	0.16	50	1,035	501	0.48	10.0	26.0	-13.27
	0.50	50	1,039	509	0.48	10.2	26.0	-12.22
	1.60	50	1,046	501	0.47	10.0	26.0	-14.18
	5.00	50	1,042	504	0.48	10.1	26.0	-13.34
	16.00	50	1,044	554	0.53	11.1	26.0	-4.92
					P=0.931			

[a] Study performed at Environmental Health Research & Testing. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1985, 1987).
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose

TABLE C3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *o*-Benzyl-*p*-Chlorophenol[a]

Dose (μg/mL)	Total Cells	−S9 No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (μg/mL)	Total Cells	+S9 No. of Abs	Abs/ Cell	Cells with Abs (%)
Trial 1 – Harvest time: 12.0 hours Summary: Negative					**Trial 1** – Harvest time: 14.5 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	1	0.01	1.0		100	2	0.02	2.0
Mitomycin-C					Cyclophosphamide				
0.5	100	45	0.45	34.0	50.0	100	120	1.2	60.0
o-Benzyl-*p*-chlorophenol					*o*-Benzyl-*p*-chlorophenol				
0.16	100	0	0.00	0.0	0.5	100	0	0.00	0.0
0.50	100	5	0.05	2.0	1.6	100	2	0.02	2.0
1.60	100	3	0.03	3.0	5.0	100	2	0.02	2.0
5.00	100	0	0.00	0.0	16.0	100	4	0.04	3.0
16.00	100	2	0.02	2.0					
				P=0.265[b]					P=0.171
Trial 2 – Harvest time: 21.0 hours[c] Summary: Negative					**Trial 2** – Harvest time: 18.0 hours[c] Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	0	0.00	0.0		100	0	0.00	0.0
Mitomycin-C					Cyclophosphamide				
0.25	100	8	0.08	8.0	50.00	100	108	1.08	57.0
0.50	100	8	0.08	7.0					
o-Benzyl-*p*-chlorophenol					*o*-Benzyl-*p*-chlorophenol				
0.5	100	0	0.00	0.0	0.5	100	0	0.00	0.0
1.6	100	0	0.00	0.0	1.6	100	0	0.00	0.0
5.0	100	0	0.00	0.0	5.0	100	0	0.00	0.0
16.0	100	0	0.00	0.0	16.0	100	0	0.00	0.0
				P=0.500					P=0.500

[a] Study performed at Environmental Health Research & Testing. Abs=aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1985, 1987).
[b] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose
[c] To offset chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened.

APPENDIX D
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

o-Benzyl-p-chlorophenol

o-Benzyl-*p*-chlorophenol was obtained from Monsanto Chemical Company (St. Louis, MO) in one lot (KM11195), which was used throughout the study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI), Kansas City, MO, and confirmed by the study laboratory. Reports on analyses performed in support of the *o*-benzyl-*p*-chlorophenol studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, white to pink flakes with a melting point of 46.5° to 48° C, was identified as *o*-benzyl-p--chlorophenol by infrared, nuclear magnetic resonance, and ultraviolet/visible spectroscopy and gas chromatography. All spectra were consistent with those expected for the structure and with the literature spectra (*Sadtler Standard Spectra*) of *o*-benzyl-*p*-chlorophenol, as shown in Figures D1 and D2.

The purity was determined by elemental analysis, Karl Fischer water analysis, nonaqueous titration of the phenol group, and thin-layer (TLC) and gas chromatography. Titration of the phenol group was accomplished by dissolving samples of *o*-benzyl-*p*-chlorophenol in *N,N*-dimethylformamide and titrating with 0.1 N tetrabutylammonium hydroxide. The titration was monitored potentiometrically with a glass indicator electrode and a calomel reference electrode filled with methanolic 1 M tetrabutylammonium chloride. TLC was performed on silica gel 60 F-254 plates using two solvent systems: A) toluene: methanol:0.1 N glacial acetic acid (90:5:5); and B) hexanes:acetone:0.1 N glacial acetic acid (60:35:5). Visualization was accomplished with ultraviolet light (254 nm) and a spray of 0.4% 2,6-dichloro-quinone-4-chloroimide and 10% aqueous sodium carbonate. ß-Naphthol was used as the reference standard. Gas chromatography was performed using a flame ionization detector and a nitrogen carrier gas at 70 mL/minute with methylene chloride as the solvent. Two systems were used: 1) 1% SP-1000 on 100/120 Supelcoport glass column (1.8 m × 4 mm ID), oven temperature program of 50° C for 5 minutes, then 50° to 225° C at 10° C/minute, and 2) 3% SP-2100 on 100/120 Supelcoport glass column (1.8 m × 4 mm ID), an isothermal temperature of 190° C.

Elemental analysis for chlorine was slightly high and those for carbon and hydrogen were in agreement with the theoretical values. Karl Fischer water analysis indicated the presence of less than 0.05% water. Titration of the phenol group indicated a purity of 101 ± 1%. TLC analysis indicated one major spot, two minor spots, and one trace spot using system A and one major spot, one minor spot, and one trace spot using system B. Gas chromatography using system 1 resolved a major peak and six impurity peaks, the largest of which eluted after the major peak and had an area of 1.6% relative to the major peak area. The 1.6% impurity observed in the gas chromatographic profile of *o*-benzyl-*p*-chlorophenol was identified as an isomer of benzylchlorophenol. On the basis of mass spectral and synthesis considerations, MRI identified the impurity as *o*-chloro-*p*-benzylphenol. The remaining five impurities, which eluted before the major peak, had a combined area of 1.5% relative to the major peak area. Gas chromatography using system 2 indicated a major peak and four impurities, the largest of which eluted after the major peak and had an area of 1.1% relative to the major peak area. The remaining three impurities, which eluted before the major peak, had a combined area of 0.60% relative to the major peak area. The overall purity was determined to be approximately 97%.

Stability studies were performed with gas chromatography using system 2 described for the purity analyses. *n*-Hexadecane was used as an internal standard. The results indicated that *o*-benzyl-*p*-chlorophenol was stable as a bulk chemical when stored protected from light for 2 weeks at temperatures up to 25° C. Samples stored at 60° C underwent decomposition. During the 1-year study, the stability of the bulk

chemical was monitored by the study laboratory using gas chromatography and ultraviolet spectroscopy. Analyses were performed at the study laboratory four times during the 1-year study; no degradation of the bulk chemical was observed.

7,12-Dimethylbenz(a)anthracene

7,12-Dimethylbenz(a)anthracene was obtained from Eastman Kodak Company (Rochester, NY) as lot K4. The chemical was purified by the analytical chemistry laboratory through an alumina column and was recrystallized from isopropanol. The purified chemical was assigned the lot number M111384 and used throughout the study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and confirmed by the study laboratory. Reports on analyses performed in support of the 7,12-dimethylbenz(a)anthracene studies are on file at the NIEHS.

The chemical, a yellow powder, was identified as 7,12-dimethylbenz(a)anthracene by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra (*Sadtler Standard Spectra*) of 7,12-dimethylbenz(a)anthracene (Figures D3 and D4).

The purity was determined by elemental analyses, Karl Fischer water analysis, TLC, and gas chromatography. TLC was performed on silica gel 60 F-254 plates with two solvent systems: A) toluene:hexane (60:40) and B) hexane:chloroform (78:22). Plates were examined under shortwave (254 nm) and longwave (366 nm) ultraviolet light and a spray of 5% potassium dichromate in 40% sulfuric acid. Pyrene was used as the reference standard. Gas chromatography was performed using a flame ionization detector and a nitrogen carrier gas at 70 mL/minute with methylene chloride as the solvent. Two systems were used: 1) 3% Dexsil 400 on 80/100 Chromosorb W(AW), with an oven temperature program of 50° C for 5 minutes, then 50° to 300° C at 10° C/minute, and 2) 3% SP-2100 on 100/120 Supelcoport, with an oven temperature program of 75° C for one minute, then 75° to 275° C at 10° C/minute.

Elemental analyses for carbon and hydrogen were in agreement with theoretical values for 7,12-dimethylbenz(a)anthracene. Karl Fischer water analysis indicated the presence of less than 0.4% water. TLC using system A indicated one major spot and a trace spot; TLC using system B indicated one major spot. Gas chromatography indicated no impurities greater than or equal to 0.1% relative to the major peak. The overall purity was determined to be greater than 99%.

Stability studies were performed by the analytical chemistry laboratory. Gas chromatography was performed using system 1 employed in the purity studies, but with an oven temperature of 300° C, isothermal. Octacosane was used as the internal standard. The results indicated that 7,12-dimethylbenz(a)anthracene was stable as a bulk chemical for 2 weeks in the dark at temperatures up to 60° C. The stability of the bulk chemical was monitored at the beginning of the 1-year study by the study laboratory, and at months 5, 9, and 13. The study laboratory monitored the bulk chemical using gas chromatography system 1 described above. Ultraviolet spectroscopy was also performed at each analysis period. No degradation of the bulk chemical was observed throughout the study.

12-*O*-Tetradecanoylphorbol-13-acetate

Lot OE511999 and lot 411999 of 12-*O*-tetradecanoylphorbol-13-acetate were obtained from Pharmacia PL Biochemicals (Milwaukee, WI). A second shipment of lot 411999 was received from Pharmacia PL Biochemicals and was assigned a new lot number (UN2811) to assist in tracking. Consolidated Midland Corporation (Brewster, NY) supplied a third lot (031). Each lot was supplied in several 5- or 10-mg glass ampules. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, and confirmed by the study laboratory. Reports on analyses performed in support of the 12-*O*-tetradecanoylphorbol-13-acetate studies are on file at the NIEHS.

The chemical, a liquid, was identified as 12-*O*-tetradecanoyl-phorbol-13-acetate by nuclear magnetic resonance spectroscopy (Figure D5) and mass spectrometry. All spectra were consistent with those expected for the structure.

The purity was determined by TLC, mass spectrometry, and high-performance liquid chromatography (HPLC). TLC was performed on silica gel 60 F-254 plates using two solvent systems: A) 100% anhydrous diethyl ether and B) ethyl acetate:chloroform (60:40). Visualization was accomplished with ultraviolet light (254 and 366 nm) and a spray of 1% vanillin in concentrated sulfuric acid. The reference standard used was 2,2′,4,4′-tetrahydroxybenzophenone (1 μL of a 10 μg/μL solution in methanol). HPLC was performed using a DuPont Zorbax ODS column with ultraviolet detection at 229 nm, a flow rate of 1.0 mL/minute, and a solvent system of water:acetonitrile (10:90).

TLC using system A indicated one trace impurity and one very slight trace impurity in lot 031 and no impurities in lot 411999 or lot OE511999. TLC using system B indicated one trace impurity, one slight trace impurity, and two very slight trace impurities in lot 031. System B also indicated one very slight trace impurity in lot 411999 but no impurities in lot OE511999. Mass spectrometry indicated a very low mass spectral abundance of predicted ions characteristic of expected oxidation products of 12-*O*-tetradecanoylphorbol-13-acetate. This confirmed that no extensive oxidation occurred in any of the four samples tested. The mass spectra of the four samples were consistent with each other. HPLC indicated 11 impurity peaks in lot 031, with a combined area of 3.1% relative to the major peak. For lot 411999, HPLC indicated three impurity peaks with a combined area of 0.6% relative to the major peak. HPLC indicated a total of five impurity peaks in lot number OE511999; the combined impurity area relative to the major peak totaled 1.0% The overall purity of the lots was determined to range between 97% and 99%.

A limited stability study was performed on lot 031 using the HPLC system described in the purity studies. Two sample vials of 12-*O*-tetradecanoylphorbol-13-acetate were opened and exposed to air and light at ambient temperature. One vial was exposed for 1 day and the second for 6 days. After exposure, samples were analyzed concomitantly with sealed samples which had been stored at −20° C. The HPLC profiles of the exposed samples were then compared to those of the reference samples. Results indicated 12-*O*-tetradecanoylphorbol-13-acetate was stable as a bulk chemical when exposed to air and light for 6 days. During the 1-year study, the stability of the bulk chemical was monitored by the study laboratory using the HPLC system described above. No degradation of the bulk chemical was observed throughout the study.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

o-Benzyl-*p*-chlorophenol

Dose formulations were prepared every 2 weeks. For each dose concentration, *o*-benzyl-*p*-chlorophenol was weighed and then transferred to a 100 mL graduated mixing cylinder. Acetone was added and the cylinder stoppered and inverted until a solution was achieved. Dose formulations were placed into amber glass bottles and stored at 4° C for up to 3 weeks.

Stability analysis of a 250 mg/mL dose formulation was performed by gas chromatography using a flame ionization detector and a nitrogen carrier gas at 30 mL/minute. The system included a 3% SP-2100 on 100/120 Supelcoport glass column (1.8 m × 2 mm ID) and an isothermal oven temperature of 165° C. Stability was confirmed for 3 weeks when solutions were stored in the dark at room temperature and for 3 hours when stored open to air and light. Periodic analyses of the dose formulations of *o*-benzyl-*p*-chlorophenol were conducted at the study laboratory and at the analytical chemistry laboratory with the same gas chromatography method as described above, but with a carrier flow rate of 30 mL/minute. Dose formulations were analyzed once every 2 months during the 1-year study.

All dose formulations except one were within specifications. Four of the first five samples retrieved from the dosing containers after administration had concentrations more than 10% greater than the theoretical concentrations. This problem was traced to solvent evaporation during dosing and was corrected by using each bottle for only 1 dosing day.

Results of 1-year study dose formulation analyses are presented in Table D2. Periodic referee analysis of *o*-benzyl-*p*-chlorophenol solutions were performed by the analytical chemistry laboratory, and their results indicated good agreement with the results obtained by the study laboratory (Table D3).

7,12-Dimethylbenz(a)anthracene

The dose formulation solutions were prepared by mixing 7,12-dimethylbenz(a)anthracene and acetone to give the required concentrations (Table D1). Dose formulations were prepared every 2 weeks.

Stability analysis of the 2.5 μg/mL formulation was conducted by the analytical chemistry laboratory using HPLC. The formulation was evaporated under nitrogen and the residue was redissolved in 2 mL of an internal standard solution consisting of anthracene in mobile phase (water:acetonitrile 15:85). HPLC was performed using a Brownlee RP-18 column, a Waters 440 detector (365 nm), anthracene as an internal standard, and a flow rate of 1.0 mL/minute. Stability of the dose formulations was established for at least 3 weeks when stored in the dark at room temperature.

Periodic analyses of the dose formulations of 7,12-dimethylbenz(a)anthracene were conducted at the study laboratory using a spectroscopic method (363 nm) and at the analytical chemistry laboratory using HPLC. Formulations were analyzed using the same procedure described for the stability studies.

All dose formulations analyzed prior to administration during the 1-year study were within specifications. The initial samples retrieved from the dosing container after use had concentrations more than 10% greater than the theoretical concentration. Storing the solution at 4° C solved this problem. Dose formulation analyses for the 1-year study are presented in Table D2. Results of two referee analyses performed by the analytical chemistry laboratory indicated good agreement with results obtained by the study laboratory (Table D3).

12-*O*-Tetradecanoylphorbol-13-acetate

The dose formulations were prepared by mixing 12-*O*-tetradecanoylphorbol-13-acetate and acetone to give the required concentrations (Table D1). Dose formulations were prepared every 2 weeks.

Stability analyses of the acetone solutions were conducted by the analytical chemistry laboratory using the HPLC system used in the bulk chemical analyses except with a solvent ratio of 7:93. Stability of the formulation was established for at least 3 weeks when stored at 4° C in amber glass bottles.

Periodic analyses of the dose formulations of 12-*O*-tetradecanoylphorbol-13-acetate were conducted at the study laboratory and at the analytical chemistry laboratory by HPLC using a Waters μBondapak C_{18} column. During the 1-year study, all dose formulations were within 10% of the target concentrations (Table D2). Results of periodic referee analyses performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table D3).

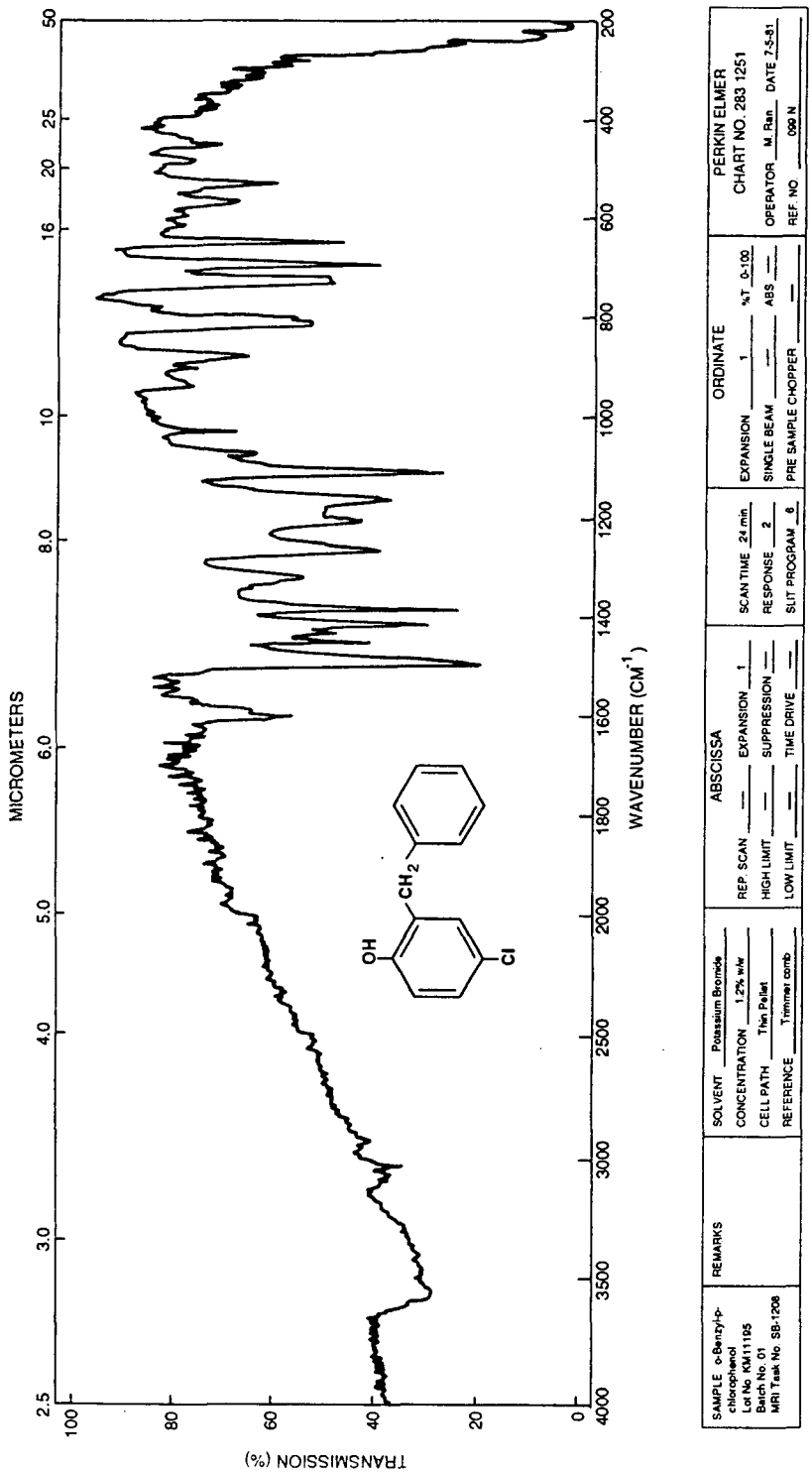

FIGURE D1
Infrared Absorption Spectrum of *o*-Benzyl-*p*-Chlorophenol

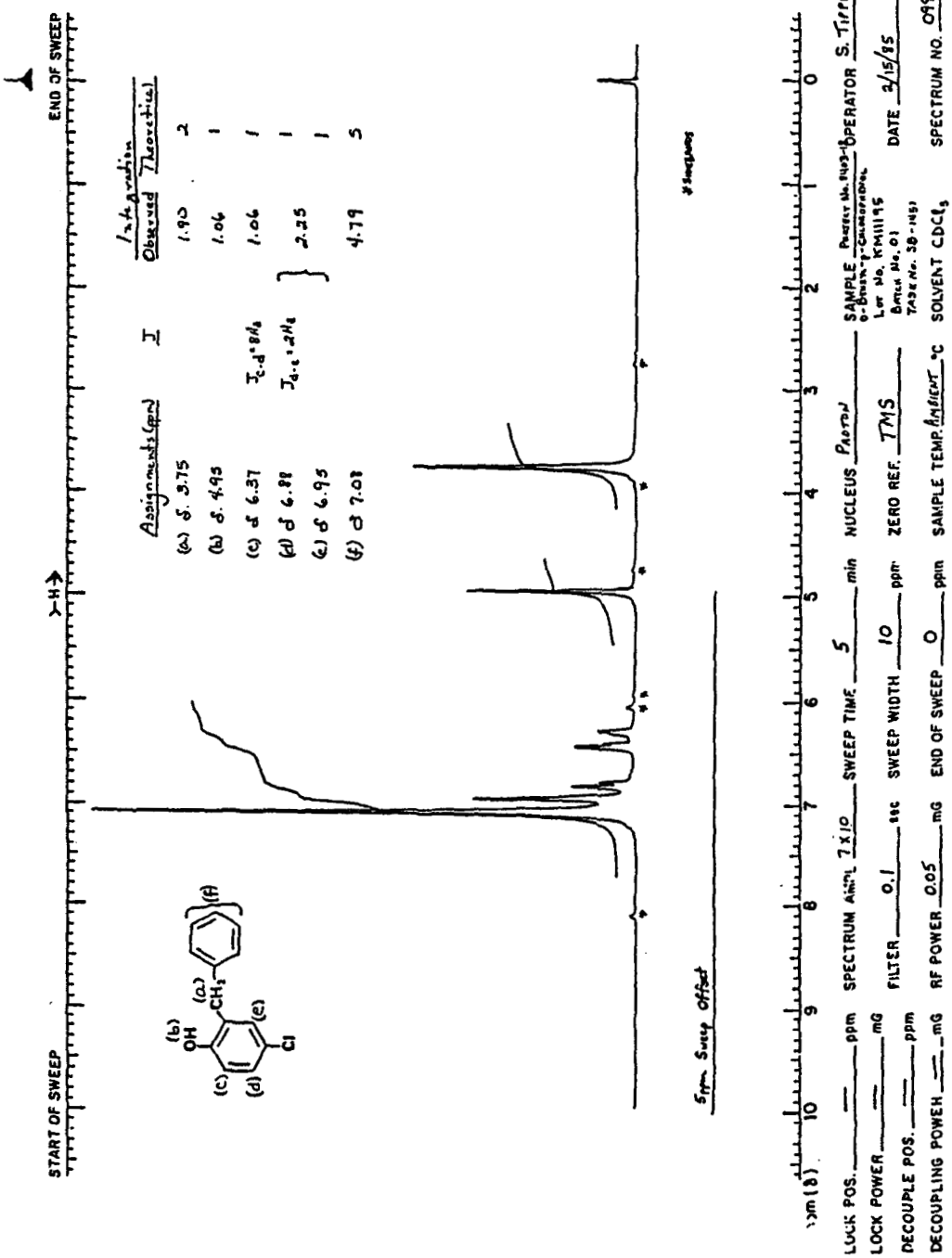

FIGURE D2
Nuclear Magnetic Resonance Spectrum of *o*-Benzyl-*p*-Chlorophenol

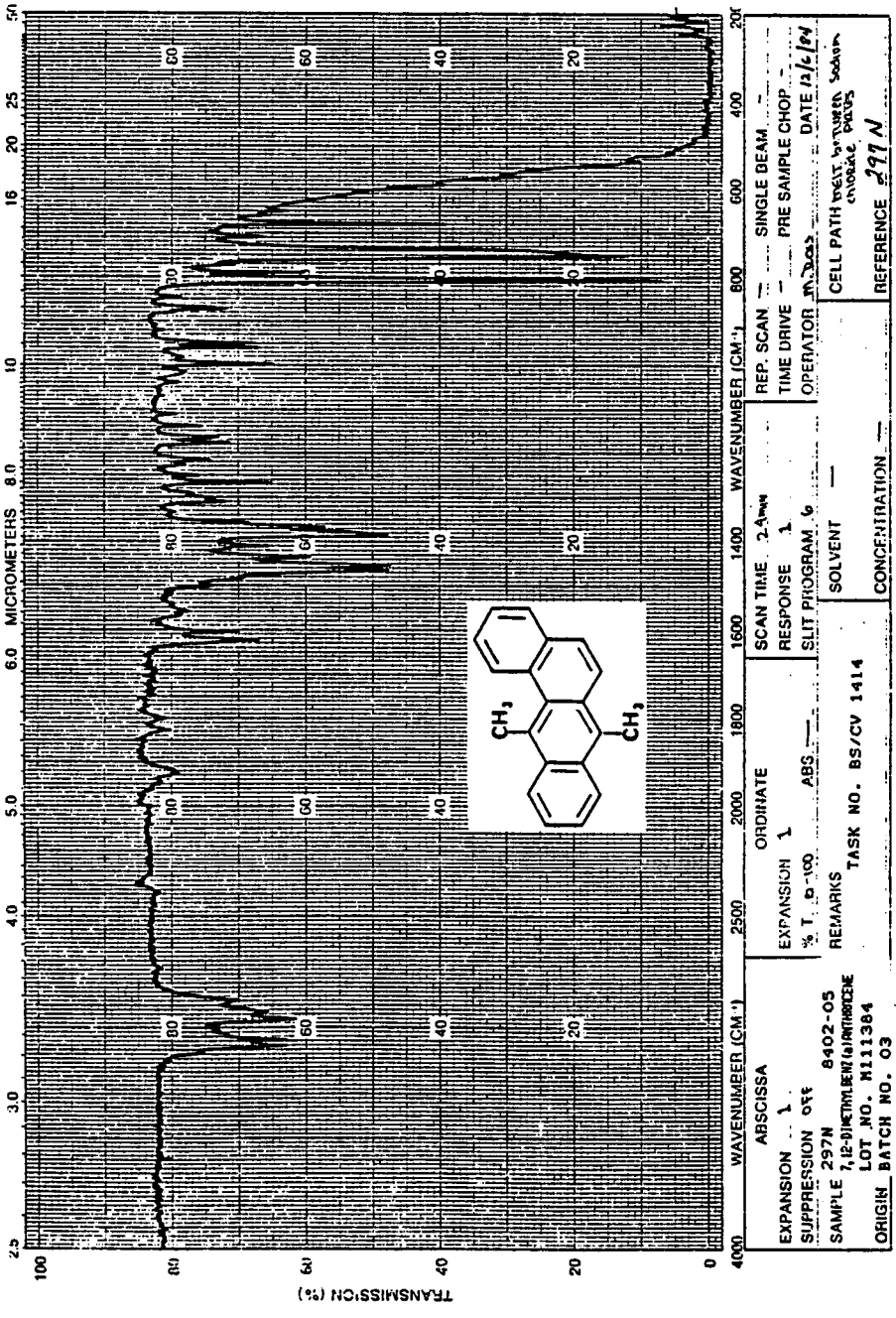

FIGURE D3
Infrared Absorption Spectrum of 7,12-Dimethylbenz(a)anthracene

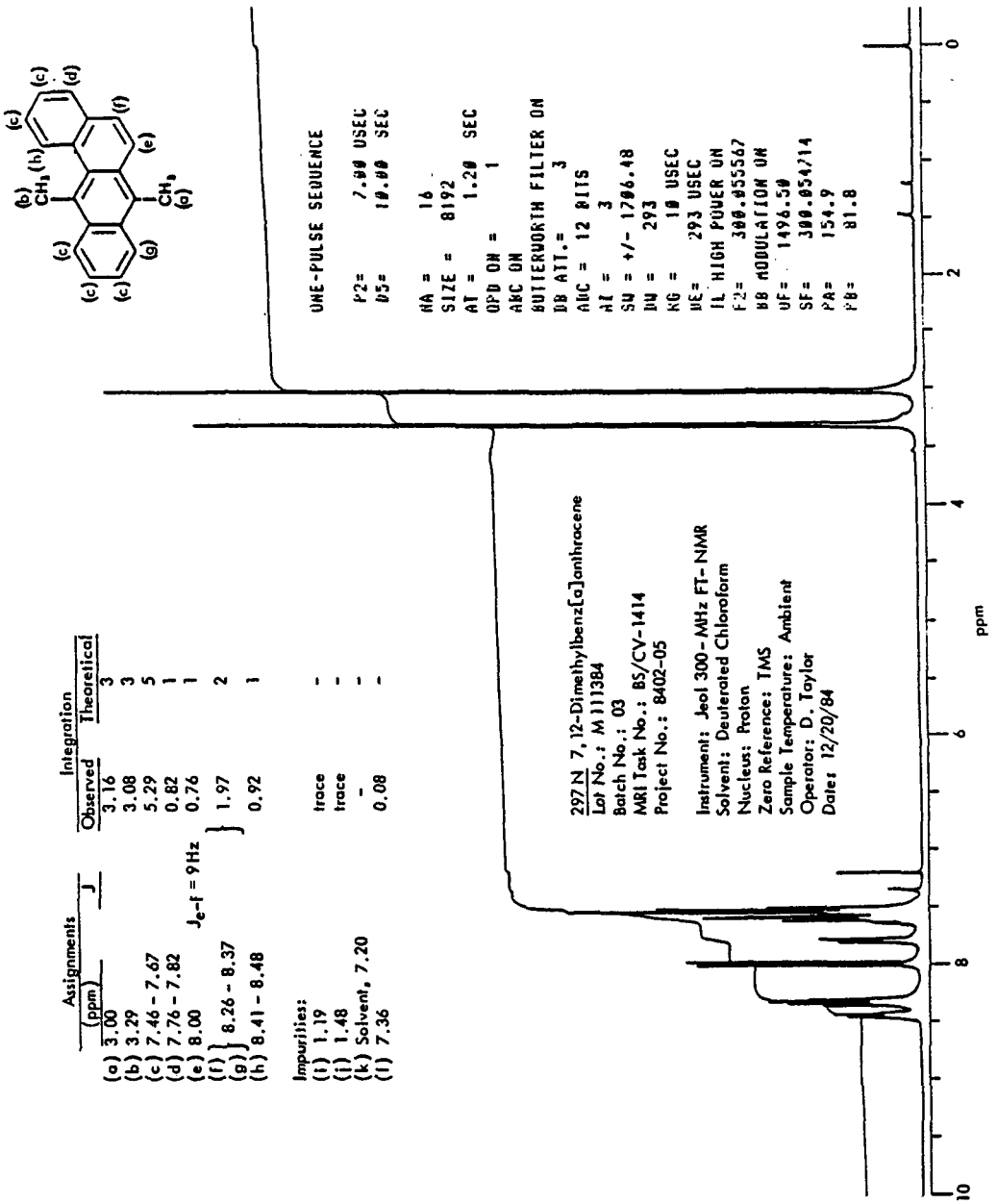

FIGURE D4
Nuclear Magnetic Resonance Spectrum of 7,12-Dimethylbenz(a)anthracene

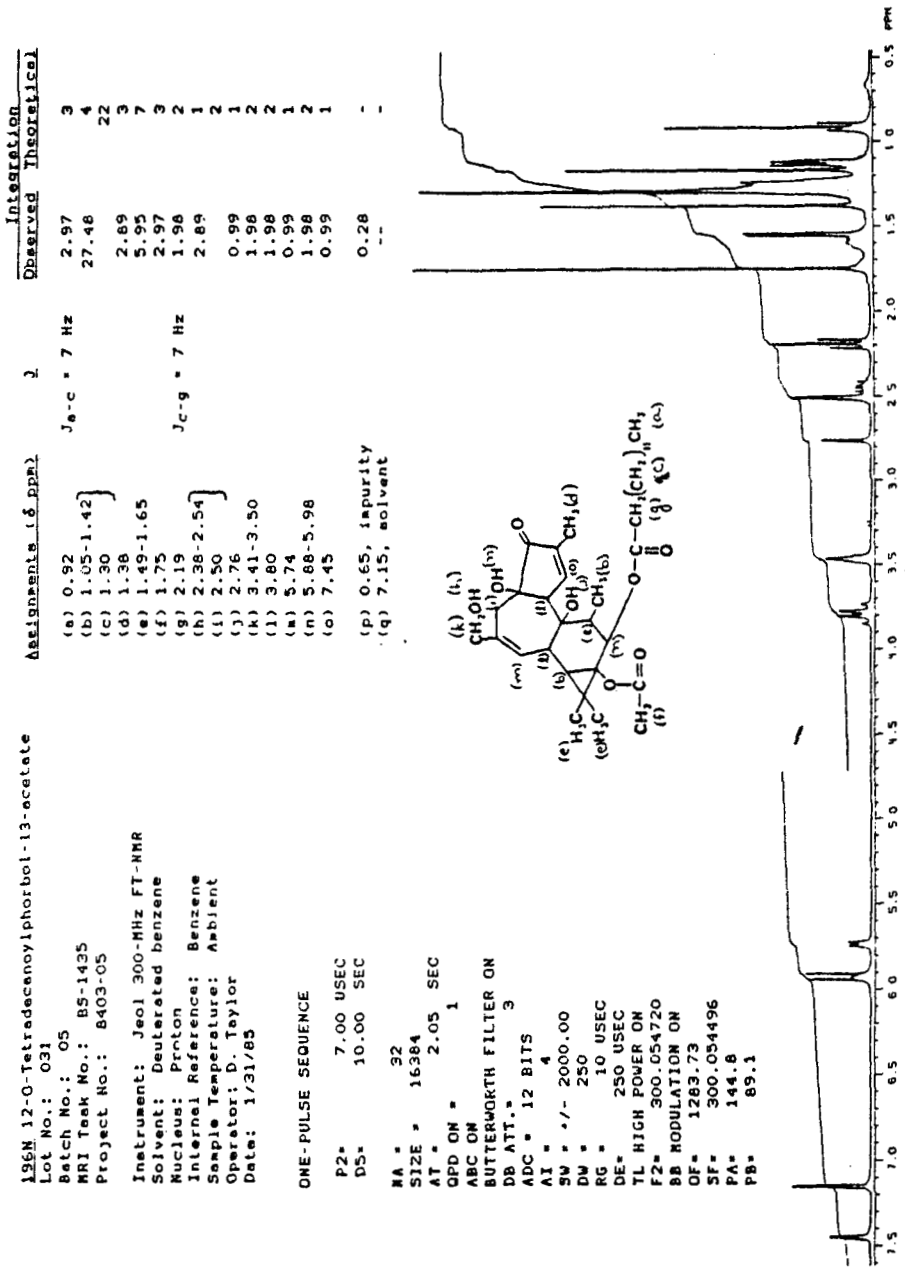

FIGURE D5
Nuclear Magnetic Resonance Spectrum of 12-*O*-Tetradecanoylphorbol-13-Acetate

TABLE D1
Preparation and Storage of Dose Formulations in the 1-Year Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol

o-Benzyl-*p*-chlorophenol	7,12-Dimethylbenz(a)anthracene	12-*O*-Tetradecanoylphorbol-13-acetate
Preparation		
o-Benzyl-*p*-chlorophenol was weighed and then transferred to a graduated cylinder. Acetone was added to the cylinder to obtain a solution at the required *o*-benzyl-*p*-chlorophenol concentration.	7,12-Dimethylbenz(a)anthracene was weighed and then transferred to a graduated cylinder. Acetone was added to the column to obtain a solution at the required 7,12-dimethylbenz(a)anthracene concentration.	Vials containing 12-*O*-tetradecanoyl-phorbol-13-acetate were filled with acetone and agitated. The solution was transferred to a graduated cylinder and each vial was rinsed with acetone. The rinses were transferred to the cylinder. Acetone was then added to obtain a solution with the required concentration of 12-*O*-tetradecanoylphorbol-13-acetate.
Chemical Lot Number		
KM11195	M111384	031, 411999, UN2811, and OE511999
Maximum Storage Time		
3 weeks	3 weeks	3 weeks
Storage Conditions		
Stored at 4° C in an amber glass bottle	Stored at 4° C in an amber glass bottle	Stored at 4° C in an amber glass bottle
Study Laboratory		
Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)	Battelle Columbus Laboratories (Columbus, OH)
Referee Laboratory		
Midwest Research Institute (Kansas City, MO)	Midwest Research Institute (Kansas City, MO)	Midwest Research Institute (Kansas City, MO)

TABLE D2
Results of Analysis of Dose Formulations Administered to Mice in the 1-Year Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	% Difference from Target
***o*-Benzyl-*p*-chlorophenol**				
20 March 1985	22 March 1985	1	1.07	+7
		10	10.8	+8
		30	32.4	+8
		100	106.8	+7
	3 April 1985[c]	1	1.05	+5
		10	12.8	+28
		30	37.4	+25
		100	130.3	+30
	9 April 1985[d]	100	133.8	+34
15 May 1985	21 May 1985	1	1.2	+20
		10	10.6	+6
		30	33.0	+10
9 July 1985	10 July 1985	1	0.96	–4
		10	9.9	–1
		30	28.4	–5
3 September 1985	6 September 1985	1	1.02	+2
		10	10.4	+4
		30	28.7	–4
	23 September 1985[c]	1	1.12	+12
		10	11.9	+19
		30	37.2	+24
29 October 1985	29 October 1985	1	1.01	+1
		10	9.9	–1
		30	31.2	+4
10 December 1985	13 December 1985	1	1.08[e]	+8
		10	10.6	+6
		30	31.7	+6
4 February 1986	6 February 1986	1	1.01	+1
		10	10.6	+6
		30	30.7	+2
	26 February 1986[c]	1	1.07	+7
		10	10.5	+5
		30	31.1	+4
7,12-Dimethylbenz(a)anthracene				
20 March 1985	21 March 1985	0.50	0.500	0
		0.02	0.021[f]	+5
	10 April 1985[c]	0.50	0.575	+15
		0.02	0.023[f]	+15

TABLE D2
Results of Analysis of Dose Formulations Administered to Mice in the 1-Year Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
7,12-Dimethylbenz(a)anthracene (continued)				
15 May 1985	17 May 1985	0.02	0.022[f]	+10
9 July 1985	10 July 1985	0.02	0.021[f]	+5
3 September 1985	4 September 1985	0.02	0.018[f]	–10
4 October 1985	4 October 1985	0.02	0.022	+10
	24 October 1985[c]	0.02	0.022	+10
29 October 1985	1 November 1985	0.02	0.022	+10
10 December 1985	11 December 1985	0.02	0.019	–5
4 February 1986	4 February 1986	0.02	0.020[f]	0
	25 February 1986[c]	0.02	0.020[f]	0
12-*O*-Tetradecanoylphorbol-13-Acetate				
22 March 1985	22 March 1985	0.05	0.054[e]	+8
	10 April 1985[c]	0.05	0.045	–10
		0.05	0.047	–6
15 May 1985	16 May 1985	0.05	0.049[e]	–2
9 July 1985	11 July 1985	0.05	0.052[e]	+4
3 September 1985	6 September 1985	0.05	0.051[e]	+2
1 October 1985	3 October 1985	0.05	0.051[e]	+2
	22 October 1985[c]	0.05	0.050[e]	0
29 October 1985	30 October 1985	0.05	0.050[e]	0
10 December 1985	11 December 1985	0.05	0.050[e]	0
4 February 1986	6 February 1986	0.05	0.048[e]	–4
	25 February 1986[c]	0.05	0.046[e]	–8

[a] Dosing volume = 10 μL
[b] Results of duplicate analyses
[c] Results of post-dosing analysis
[d] Analysis performed at direction of study lab principal investigator to follow up analysis of 3 April 1985.
[e] Results of quadruple analyses
[f] Results of single analysis

TABLE D3
Results of Referee Analysis of Dose Formulations Administered to Mice in the 1-Year Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol

Date Mixed	Target Concentration[a] (mg/mL)	Determined Concentration (mg/mL)	
		Study Laboratory[b]	Referee Laboratory
***o*-Benzyl-*p*-chlorophenol**			
15 May 1985	10.0	10.6	9.99 ± 0.01
29 October 1985	1.0	1.01	0.982 ± 0.003
4 February 1986	30.0	30.7	30.0 ± 0.3
7,12-Dimethylbenz(a)anthracene			
15 May 1985	0.02	0.022[c]	0.0219 ± 0.0001
10 December 1985	0.02	0.019	0.0177 ± 0.0
12-*O*-Tetradecanoylphorbol-13-acetate			
22 March 1985	0.05	0.054[d]	0.0412[c]
15 May 1985	0.05	0.049[d]	0.0402 ± 0.0002
3 September 1985	0.05	0.051[d]	0.0504 ± 0.0002
4 February 1986	0.05	0.048[d]	0.0473 ± 0.0001

[a] Dosing volume = 10 μL
[b] Results of duplicate analyses
[c] Results of single analysis
[d] Results of quadruple analyses

APPENDIX E
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE E1
Ingredients of NIH-07 Rat and Mouse Ration[a]

Ingredients[b]	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE E2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE E3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	21.96 ± 0.48	21.10 – 22.50	12
Crude Fat (% by weight)	5.49 ± 0.51	4.70 – 6.40	12
Crude Fiber (% by weight)	3.52 ± 0.61	3.00 – 5.40	12
Ash (% by weight)	6.59 ± 0.25	6.19 – 6.97	12
Amino Acids (% of total diet)			
Arginine	1.287 ± 0.084	1.100 – 1.390	10
Cystine	0.306 ± 0.075	0.181 – 0.400	10
Glycine	1.160 ± 0.050	1.060 – 1.220	10
Histidine	0.580 ± 0.024	0.531 – 0.608	10
Isoleucine	0.917 ± 0.034	0.867 – 0.965	10
Leucine	1.972 ± 0.052	1.850 – 2.040	10
Lysine	1.273 ± 0.051	1.200 – 1.370	10
Methionine	0.437 ± 0.115	0.306 – 0.699	10
Phenylalanine	0.994 ± 0.125	0.665 – 1.110	10
Threonine	0.896 ± 0.055	0.824 – 0.985	10
Tryptophan	0.223 ± 0.160	0.107 – 0.671	10
Tyrosine	0.677 ± 0.105	0.564 – 0.794	10
Valine	1.089 ± 0.057	0.962 – 1.170	10
Essential Fatty Acids (% of total diet)			
Linoleic	2.389 ± 0.233	1.830 – 2.570	9
Linolenic	0.277 ± 0.036	0.210 – 0.320	9
Vitamins			
Vitamin A (IU/kg)	10,233 ± 2477	7,600 – 15,000	12
Vitamin D (IU/kg)	4,450 ± 1382	3,000 – 6,300	4
α-Tocopherol (ppm)	37.95 ± 9.32	22.5 – 48.9	9
Thiamine (ppm)	19.75 ± 1.66	17.0 – 23.0	12
Riboflavin (ppm)	7.92 ± 0.93	6.10 – 9.00	10
Niacin (ppm)	103.38 ± 25.92	65.0 – 150.0	9
Pantothenic Acid (ppm)	30.30 ± 3.60	23.0 – 34.6	10
Pyridoxine (ppm)	9.25 ± 2.62	5.60 – 14.0	10
Folic Acid (ppm)	2.51 ± 0.64	1.80 – 3.70	10
Biotin (ppm)	0.254 ± 0.049	0.19 – 0.35	10
Vitamin B12 (ppb)	40.14 ± 20.04	10.6 – 65.0	10
Choline (ppm)	3,068 ± 314	2,400 – 3430	9
Minerals			
Calcium (%)	1.11 ± 0.11	0.98 – 1.41	12
Phosphorus (%)	0.93 ± 0.04	0.87 – 0.99	12
Potassium (%)	0.887 ± 0.067	0.772 – 0.971	8
Chloride (%)	0.526 ± 0.092	0.380 – 0.635	8
Sodium (%)	0.315 ± 0.344	0.258 – 0.370	10
Magnesium (%)	0.168 ± 0.008	0.151 – 0.180	10
Sulfur (%)	0.274 ± 0.063	0.208 – 0.420	10
Iron (ppm)	356.2 ± 90.0	255.0 – 523.0	10
Manganese (ppm)	92.24 ± 5.35	81.70 – 99.40	10
Zinc (ppm)	58.14 ± 9.91	46.10 – 81.60	10
Copper (ppm)	11.50 ± 2.40	8.090 – 15.39	10
Iodine (ppm)	3.70 ± 1.14	1.52 – 5.83	10
Chromium (ppm)	1.71 ± 0.45	0.85 – 2.09	9
Cobalt (ppm)	0.797 ± 0.23	0.490 – 1.150	6

TABLE E4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.74 ± 0.15	0.47 – 0.94	12
Cadmium (ppm)	<0.10		12
Lead (ppm)	0.58 ± 0.31	0.14 – 1.32	12
Mercury (ppm)	0.05		12
Selenium (ppm)	0.34 ± 0.09	0.17 – 0.48	12
Aflatoxins (ppb)	<5.0		12
Nitrate nitrogen (ppm)[b]	14.23 ± 4.68	2.80 – 22.0	12
Nitrite nitrogen (ppm)[b]	0.13 ± 0.11	<0.10 – 0.50	12
BHA (ppm)[c]	2.17 ± 0.58	<2.00 – 4.00	12
BHT (ppm)[c]	2.00 ± 1.13	<1.00 – 4.00	12
Aerobic plate count (CFU/g)[d]	43,255 ± 43,931	770 – 1,300,000	12
Coliform (MPN/g)[e]	3.67 ± 1.72	<3.00 – 9.00	12
E. coli (MPN/g)	<3.00		12
Total nitrosoamines (ppb)[f]	7.49 ± 3.96	4.00 – 16.00	12
N-Nitrosodimethylamine (ppb)[f]	6.29 ± 3.63	3.00 – 15.00	12
N-Nitrosopyrrolidine (ppb)[f]	1.20 ± 0.69	1.00 – 3.40	12
Pesticides (ppm)			
α-BHC[g]	<0.01		12
ß-BHC	<0.02		12
γ-BHC	<0.01		12
δ-BHC	<0.01		12
Heptachlor	<0.01		12
Aldrin	<0.01		12
Heptachlor epoxide	<0.01		12
DDE	<0.01		12
DDD	<0.01		12
DDT	<0.01		12
HCB	<0.01		12
Mirex	<0.01		12
Methoxychlor	<0.05		12
Dieldrin	<0.01		12
Endrin	<0.01		12
Telodrin	<0.01		12
Chlordane	<0.05		12
Toxaphene	<0.1		12
Estimated PCBs	<0.2		12
Ronnel	<0.01		12
Ethion	<0.02		12
Trithion	<0.05		12
Diazinon	<0.1		12
Methyl parathion	<0.02		12
Ethyl parathion	<0.02		12
Malathion[h]	0.34 ± 0.90	0.05 – 3.20	12
Endosulfan I	<0.01		12
Endosulfan II	<0.01		12
Endosulfan sulfate	<0.03		12

TABLE E4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Sources of contamination: alfalfa, grains, and fish meal
[c] Sources of contamination: soy oil and fish meal
[d] CFU=colony forming units
[e] MPN=most probable number
[f] All values were corrected for percent recovery
[g] BHC is hexachlorocyclohexane or benzene hexachloride
[h] The lot milled 7 May 1985 included one unusually large value of 3.20.

APPENDIX F
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Prior to the beginning of the 1-year initiation/promotion study, serum samples were collected from 10 male and 30 female sentinel mice during the quarantine period. Samples were also collected from nine males and nine females at 6 months and from 10 male and 10 female acetone/acetone (vehicle control) mice at the end of the study. In addition, serum samples were collected from moribund animals at various times throughout the study. Blood from each collection was appropriately processed, shipped to Microbiological Associates, Inc. (Bethesda, MD), and screened for the following:

Method of Analysis	Time of Analysis
Complement Fixation	
LCM (lymphocytic choriomeningitis virus)	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
ELISA	
Ectromelia virus	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
GDVII (mouse encephalomyelitis virus)	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
Mouse adenoma virus	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
MHV (mouse hepatitis virus)	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
Mycoplasma arthritidis	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
Mycoplasma pulmonis	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
PVM (pneumonia virus of mice)	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
Reovirus 3	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
Sendai	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
Immunofluorescence Assay	
EDIM (epizootic diarrhea of infant mice)	Quarantine, 2 weeks, 1, 7, 8 months, and study end
Hemagglutination Inhibition	
K (papovavirus)	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
MVM (minute virus of mice)	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end
Polyoma virus	Quarantine, 2 weeks, 1, 6, 7, 8 months, and study end

Results of serology testing for sentinel animals are presented in Table F1.

TABLE F1
Murine Virus Antibody Determinations for Mice in the 1-Year Initiation/Promotion Study of *o*-Benzyl-*p*-Chlorophenol

Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
Quarantine screen	30/40	EDIM
2 weeks	0/2	None positive
1 month	0/1	None positive
6 months	1/18	*M. arthritidis*[a]
7 months	0/5	None positive
8 months	1/19	Reovirus 3[b]
Study termination	0/20	None positive

[a] Further evaluation of samples positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titers may be due to cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. Only sporadic samples were positive and there were no clinical signs or histopathologic changes of *M. arthritidis* infection in mice with positive titers. Accordingly, sporadic *M. arthritidis* positive titers were considered to be false positive.
[b] Positive with ELISA and with immunofluorescence assay

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TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichlorethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4′-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl)phosphonium Sulfate & Tetrakis(hydroxymethyl)phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3

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TR No.	CHEMICAL
336	Penicillin VK
337	Nitrofurazone
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	α-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	α-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
384	1,2,3-Trichloropropane
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3′-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
394	Acetaminophen
395	Probenecid
396	Monochloroacetic Acid
397	C.I. Direct Blue 15
398	Polybrominated Biphenyls
399	Titanocene Dichloride
400	2,3-Dibromo-1-propanol
401	2,4-Diaminophenol Dihydrochloride
402	Furan
403	Resorcinol
404	5,5-Diphenylhydantoin
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
408	Mercuric Chloride
409	Quercetin
410	Naphthalene
411	C.I. Pigment Red 23
412	4,4-Diamino-2,2-stilbenedisulfonic Acid
413	Ethylene Glycol
414	Pentachloroanisole
415	Polysorbate 80
416	*o*-Nitroanisole
417	*p*-Nitrophenol
418	*p*-Nitroaniline
419	HC Yellow 4
420	Triamterene
421	Talc
422	Coumarin
423	Dihydrocoumarin
424	*o*-Benzyl-*p*-chlorophenol
425	Promethazine Hydrochloride
426	Corn Oil, Safflower Oil, and Tricaprylin
427	Turmeric Oleoresin
428	Manganese (II) Sulfate Monohydrate
430	C.I. Direct Blue 218
431	Benzyl Acetate
432	Barium Chloride Dihydrate
433	Tricresyl Phosphate
434	1,3-Butadiene
435	4,4′-Thiobis(6-*t*-butyl-*m*-cresol)
437	Hexachlorocyclopentadiene
440	Ozone and Ozone/NNK
442	*p*-Nitrobenzoic Acid
443	Oxazepam

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NIH Publication No. 95-3157
May 1995