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COMPARATIVE INITIATION/PROMOTION SKIN PAINT STUDIES OF $B6C3F_1$ MICE, SWISS (CD-1®) MICE, AND SENCAR MICE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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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. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

COMPARATIVE INITIATION/PROMOTION SKIN PAINT STUDIES OF B6C3F$_1$ MICE, SWISS (CD-1®) MICE, AND SENCAR MICE

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ABSTRACT

In 1983, an *ad hoc* panel on chemical carcinogenesis testing and evaluation recommended additional methods that should be used by the National Toxicology Program (NTP) for the detection and evaluation of chemical carcinogens. One recommendation was that there should be an increased emphasis on short-term tests to detect agents that do not exert genetic effects such as some promoting agents.

Initiation/promotion models have been used routinely to identify chemicals with promoting potential and to study tumorigenesis. In one model, a topical subcarcinogenic dose of a chemical is first applied to the back of the skin (initiation) followed by repeated topical applications of one or more chemicals (promotion) and the skin is monitored for tumor development. Mouse skin has been shown to be more responsive (i.e., develops tumors using this protocol) than other commonly used laboratory rodent models. However, not all mouse strains are equally sensitive.

The skin tumor response of the $B6C3F_1$ mouse using the initiation/promotion protocol was not known. Since the $B6C3F_1$ mouse is commonly used in NTP carcinogenesis studies and much is known of its biology and response to chemical carcinogens, known initiators and promoters were used to compare the tumor response sensitivity of $B6C3F_1$ mouse skin to that of two often-used responsive strains, Swiss (CD-1®) and SENCAR mice. The combination of 7,12-dimethylbenz(a)anthracene (DMBA) initiation and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promotion was selected because this pair is routinely used to study tumorigenesis. However, DMBA requires metabolic activation to achieve initiation and it was possible that the $B6C3F_1$ mouse metabolism might not make this conversion (DiGiovanni and Juchau, 1980). Therefore, a second study was conducted using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), a direct acting carcinogen, as the initiator. MNNG is not used as frequently for mouse skin studies as is DMBA. In addition to the promoter TPA, benzoyl peroxide (BPO), a non-phorbol ester and known promoter after DMBA initiation, was also used (Slaga *et al.*, 1981). Each initiating chemical was used in combination with each promoting chemical as described on the following page.

Additional groups of male and female mice of each strain were treated with repeated applications of acetone (vehicle control), repeated applications of promoter (TPA or BPO) without prior initiation treatment (promoter reference controls), or a single application of the initiator (DMBA or MNNG) followed by repeated applications of acetone (initiator controls)

All three strains of mice demonstrated sensitivity by developing skin tumors after topical application of the chemicals under study (DMBA, MNNG, TPA, and BPO). The most sensitive of the three strains appeared to be SENCAR mice, in the sense that lower doses of the test chemical were generally required to produce effects equivalent to those in the other two strains. Skin tumors also tended to develop earlier and with greater multiplicity in SENCAR mice than in the other two strains. By these criteria, the overall sensitivity of Swiss (CD-1®) mice was intermediate, and $B6C3F_1$ mice showed the least overall sensitivity to dermal carcinogenicity.

In response to recommendations regarding specific short-term tests and also on the skin tumor response sensitivity of various initiators and promoters, SENCAR mice would be the most acceptable strain to use for such studies. Though the $B6C3F_1$ mice were less responsive in the skin initiation/promotion protocol, promotion data from this strain may, at times, be of more use in explaining mechanisms of tumor development (e.g. when there is a strain-specific response observed in 2-year carcinogenicity studies or effects on melanocytes are suspected).

Study Design for the 1-Year Comparative Initiation/Promotion Skin Paint Studies

Initiation/Promotion[a]	Mouse Strain		
	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Design A			
0.25 μg DMBA/TPA[b]		X	X
2.5 μg DMBA/TPA	X	X	X
25 μg DMBA/TPA	X	X	X
50 μg DMBA/TPA	X		
2.5 μg DMBA/BPO[c]	X	X	X
25 μg DMBA/BPO	X	X	X
Design B			
100 μg MNNG/TPA	X	X	X
1,000 μg MNNG/TPA	X	X	X
100 μg MNNG/BPO	X	X	X
500 μg MNNG/BPO	X	X	X
1,000 μg MNNG/BPO	X	X	X
Complete Carcinogen[d]			
2.5 μg DMBA/2.5 μg DMBA	X	X	X
100 μg MNNG/100 μg MNNG	X	X	X

[a] Mice received a single initiating application followed by repeated promotion applications for up to 52 weeks.
[b] B6C3F$_1$ and Swiss (CD-1®) mice received 5 μg TPA; SENCAR mice received 1 μg TPA.
[c] BPO applications contained 20 mg BPO.
[d] Mice received repeated applications of DMBA and MNNG.

The 1-year complete carcinogen studies used repeated applications of low concentrations of the carcinogens DMBA and MNNG. The skin tumor response in all three strains under these conditions was more similar than in the initiation and promotion studies. There was a high incidence of skin tumors in all three strains with both carcinogens. More B6C3F$_1$ and SENCAR mice developed skin tumors and averaged more tumors per mouse than did Swiss (CD-1®) mice. Skin tumors developed earlier in SENCAR mice than in B6C3F$_1$ and Swiss (CD-1®) mice. Although B6C3F$_1$ mice exhibited the lowest overall sensitivity to the initiation/promotion protocol when compared to Swiss (CD-1®) and SENCAR mice, the response of B6C3F$_1$ mice was similar to Swiss (CD-1®) and SENCAR mice for complete carcinogen studies.

A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 8.

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 the comparative initiation/promotion skin paint studies on June 21, 1994, 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 four 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, and
- to judge the significance of the experimental results by scientific criteria.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 21, 1994, the draft Technical Report on these comparative initiation/promotion skin paint studies of B6C3F$_1$ mice, Swiss (CD-1®) mice, and SENCAR mice received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports 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 report by relating that in 1983, an *ad hoc* panel on chemical carcinogenesis testing and evaluation commissioned by the NTP had recommended that the NTP increase emphasis on short-term tests to detect agents that do not exert genetic effects such as some promoting agents. For the B6C3F$_1$ mouse, the strain commonly used for NTP carcinogenesis studies and for which a large database exists, the skin tumor response using the initiation protocol was not known. Therefore, the objectives of this research project were to compare the tumor response sensitivity of B6C3F$_1$ mouse skin to that of two often-used responsive strains, Swiss (CD-1®) and SENCAR mice, using known chemical initiators and promoters, as well as complete carcinogens.

Dr. Eastin described the study design and techniques used for in-life data collection for these 1-year studies and provided a detailed report of the study results. For the initiation/promotion studies, all three strains of mice demonstrated sensitivity by developing skin tumors after topical applications of the chemicals under study (DMBA, MNNG, TPA, and BPO). At the concentrations tested, the most sensitive of the three strains appeared to be SENCAR mice, in the sense that lower doses of test chemical were generally required to produce effects equivalent to the other two strains. Skin tumors also tended to develop earlier and to exhibit increased multiplicity in SENCAR mice relative to the other two strains. By these criteria, the overall sensitivity of Swiss (CD-1®) mice was intermediate, and B6C3F$_1$ mice showed the least overall sensitivity to dermal carcinogenicity. In the complete carcinogen studies, the skin tumor response in all three strains was more similar than in the initiation/promotion studies. There was a high incidence of skin tumors in all three strains with both carcinogens. More B6C3F$_1$ and SENCAR mice developed skin tumors and averaged more tumors per mouse than did Swiss (CD-1®) mice. Skin tumors developed earlier in SENCAR mice than in B6C3F$_1$ and Swiss (CD-1®) mice.

Dr. Ryan, a principal reviewer, suggested that there should be some discussion regarding the increased sensitivity of the SENCAR strain in terms of survival in the TPA/TPA promoter reference group and whether this complicated the statistical analyses. Dr. Eastin explained that many of these animals were not really dying but were being removed from the study after lesions had developed. Dr. Ryan also questioned the implication of tumors appearing in the groups receiving TPA without DMBA or MNNG initiation. Dr. Eastin said there should have been tumors only in groups receiving initiation with promotion or those receiving repeated application of carcinogens (DMBA or MNNG). Dr. Ryan also asked why a standard survival analysis on time to tumor was not done. Dr. J.K. Haseman, NIEHS, responded that the analysis was based on the time of appearance of the first tumor, an in-life observation.

Dr. Bailey, the second principal reviewer, noted that, as stated in the report, these studies were designed to provide mechanistic tumorigenesis data and to determine if this model would be a useful adjunct to the NTP toxicity/carcinogenesis studies. Dr. Eastin responded that the NTP wanted the Subcommittee's advice on whether this model was a useful adjunct. Dr. Bailey said there should be a statement in the front of the report that the most sensitive strains of mice to tumor promotion were also those that were significantly more sensitive to the irritant effects of the chemicals as evidenced by a marked inflammatory reaction.

Dr. Miller, the third principal reviewer, said that there was a need for explanation of the possible effects of dose errors in the DMBA/TPA promoter reference group upon the study results. Dr. Eastin noted that the correct dose was given for 50 of the 52 weeks, so he doubted that the error would have affected the outcomes. Dr. Miller thought that the effect on the findings of the much lower dose of TPA promoter in SENCAR mice as compared with the other two strains should be discussed. Dr. Miller asked for a clearer explanation of why this study was

conducted and what conclusions can be drawn about performing such studies in $B6C3F_1$ mice.

Dr. Ryan moved that the Technical Report on comparative initiation/promotion skin paint studies of $B6C3F_1$ mice, Swiss (CD-1®) mice, and SENCAR mice be accepted. Dr. Bailey seconded the motion, which was accepted unanimously with eleven votes.

Regarding the usefulness of the initiation/promotion model for providing mechanistic data as an adjunct to the NTP toxicology and carcinogenesis studies, Dr. Reddy said that with limited resources, the NTP should not be doing initiation/promotion studies on most test chemicals. Dr. Bailey thought that there had been a forum or review of this subject several years ago by the Environmental Protection Agency. Dr. R. Griesemer, NIEHS, said that this was correct and there was also a review by the International Agency for Research on Cancer that dealt with initiation/promotion in all organs where data existed, not just skin. The newer approaches to understanding cell cycle stage specificity might diminish priority for standard initiation/promotion studies. Dr. Klaassen said use of this protocol would need to be more selective and based on some scientific rationale, for example, as with chemicals associated with thyroid tumors that act through a promotional mechanism. Dr. Klaassen cautioned that a major goal of toxicology was not to find the most sensitive test or species but rather the species or test most predictive for humans. Dr. Reddy commented that most promoters were organ specific. Dr. G. Lucier, NIEHS, stated that the NTP would like to be able to select from a variety of possibilities, including initiation/promotion, transgenic mice, mechanistic studies of chemical interactions with receptors or target genes, or alternative methods, with the ultimate goal being to develop information that will be more predictive of what might happen in humans.

INTRODUCTION

The National Toxicology Program (NTP) Board of Scientific Counselors established an *ad hoc* panel on chemical carcinogenesis testing and evaluation to recommend methods that NTP should use for the detection and evaluation of chemical carcinogens (NTP, 1984).

The panel concluded that, while considerable progress has been made in establishing the sensitivity and positive predictive value of the commonly used assays for genetic toxicity/potential carcinogenicity, there is a recognized deficiency of short-term tests to detect agents that do not exert genetic effects (such as some promoting agents) and research should be emphasized in these areas. One of the areas discussed in their review was the research being conducted to study the mechanism of tumorigenesis. Mouse skin initiation/promotion is one model routinely used to study this process.

Historically, results of skin "painting" studies in mice were the first to suggest distinctive steps in tumorigenesis (Berenblum, 1941; Berenblum and Shubik, 1941; Rous and Kidd, 1941). An initiation/promotion protocol, in which a single subthreshold application of a carcinogen followed by repeated applications of croton oil was used to produce skin tumors, was first used by Mottram (1944a,b). Since these early studies, investigators have continued to use this protocol to study the process of tumorigenesis (Boutwell, 1967; Hennings and Boutwell, 1969; VanDuuren, 1969; Slaga *et al.*, 1982). Now it is generally accepted that skin carcinogenesis is a multistep process and evidence for multistep processes in other organ systems have been shown to exist (Slaga, 1983). More recent studies support the concept that initiation is the result of a mutagenic event that causes a heritable change to some epidermal cells and that the promoter acts on these cells in some way to produce tumors after repeated exposure (Bizub *et al.*, 1986; Quintanilla *et al.*, 1986). In the mouse skin protocol, treatment with a subthreshold dose of an initiator without further treatment does not produce tumors. Subsequent treatment of initiated cells with a second, noncarcinogenic agent having promotion potential can produce clonal expansion, expression of the neoplastic change, and result in the formation of benign squamous papillomas. In contrast to the initiation step, the effects of most promoters are reversible and require continued exposure to cause papilloma formation. Papillomas are benign tumors, but some of these can progress to a malignant tumor, a change believed to be associated with an increased frequency of genetic changes. Many reviews describing these stages in tumorigenesis based on the findings of the mouse skin initiation/promotion studies can be found in the literature (Hennings *et al.*, 1993; Warren *et al.*, 1993).

This mouse skin model can be used not only to determine the tumor-initiating and -promoting activities of a compound but also whether it is a complete carcinogen, i.e., whether it has both tumor-initiating and -promoting activities (Slaga, 1984). Because these studies are designed to provide mechanistic tumorigenesis data, it was of interest to determine if this model would be a useful adjunct to the NTP toxicity/carcinogenesis studies.

While the mouse is a more responsive model for skin initiation/promotion studies than most other rodent species, not all strains of mice are equally sensitive (Slaga and Fischer, 1983) and the tumor response of untested strains using this protocol is not predictable. The reason for differences in skin tumor response to initiation and promotion is not clear, but seems to involve metabolic and genetic differences between mouse strains (DiGiovanni, 1992).

The NTP has directed more than 400 toxicity/carcinogenesis studies using $B6C3F_1$ mice, but this strain has not been used in initiation/promotion studies. Thus these studies were designed to compare the tumor response of the $B6C3F_1$ mouse with Swiss (CD-1®) and SENCAR mice in topical initiation/promotion studies and to compare the potential of the different mouse strains to identify complete carcinogens.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Benzoyl Peroxide (CAS No. 94-36-0)

Benzoyl peroxide was obtained from Akzo Chemie America (Maple Shade, NJ) in one lot (WM-40). Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix D) and confirmed by the study laboratory. Reports on analyses performed in support of the 1-year studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a white powdered solid, was identified as benzoyl peroxide by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Purity was determined by elemental analyses, nuclear magnetic resonance spectroscopy, functional group titration, thin-layer chromatography, and high-performance liquid chromatography. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for benzoyl peroxide. Nuclear magnetic resonance spectroscopy indicated 19.7% ± 0.5% water. Functional group titration indicated a purity of 97% ± 2% calculated on an anhydrous basis. Thin-layer chromatography by two systems indicated one major spot. High-performance liquid chromatography revealed a major peak and two impurities with areas totaling 0.4% of the major peak area. The overall purity was determined to be approximately 99% when corrected for water content.

Based on half-life data from the manufacturer the analytical chemistry laboratory recommended that the bulk chemical be stored protected from light at 5° C. Bulk benzoyl peroxide was stored under refrigeration. During the 1-year studies, the stability of the bulk chemical was monitored by the study laboratory using high-performance liquid chromatography and ultraviolet spectroscopy; no degradation of the bulk chemical was observed.

7,12-Dimethylbenz(a)anthracene (CAS No. 57-97-6)

7,12-Dimethylbenz(a)anthracene was obtained from Eastman Kodak Company (Rochester, NY) in one lot (K-4) and was purified by the analytical chemistry laboratory. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory (Appendix D). Reports on analyses performed in support of the 1-year studies are on file at the NIEHS.

The chemical, a light yellow powder, was identified as 7,12-dimethylbenz(a)anthracene by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Purity was determined by elemental analyses, Karl Fischer water analysis, thin-layer chromatography, and gas chromatography. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for 7,12-dimethylbenz(a)anthracene. Karl Fischer water analysis indicated less than 0.4% water. Thin-layer chromatography indicated a major spot and one trace impurity using two systems. Gas chromatography using two systems indicated one major peak and no impurities with areas greater than 0.1% relative to the major peak. The overall purity was determined to be greater than 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using gas chromatography. These studies indicated that 7,12-dimethylbenz(a)anthracene was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at −20° C in amber glass bottles. The stability of the bulk chemical was monitored periodically by the study laboratory using ultraviolet spectroscopy and gas chromatography. No degradation of the bulk chemical was observed.

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (CAS No. 70-25-7)

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (8228CK). Identity and purity analyses were conducted by the analytical chemistry laboratory (Appendix D). Reports on analyses performed in support of the 1-year studies are on file at the NIEHS.

The chemical, a light yellow crystalline solid, was identified as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Purity was determined by elemental analyses, nuclear magnetic resonance spectroscopy, thin-layer chromatography, and high-performance liquid chromatography. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Nuclear magnetic resonance spectroscopy and elemental analysis indicated that the water content was negligible. Thin-layer chromatography indicated a major spot and a minor spot by one system and indicated a major spot with a minor, a trace, and a slight impurity by a second system. High-performance liquid chromatography revealed a major peak and no impurities with areas greater than 0.1% of the major peak area. The overall purity was determined to be approximately 99%.

The manufacturer's information and literature sources specified that the bulk chemical be stored at −20° C protected from light. The bulk chemical was stored at −20° C in amber glass bottles. The stability of the bulk chemical was monitored periodically by the study laboratory using gas chromatography and high-performance liquid chromatography. No degradation of the bulk chemical was observed.

12-*O*-Tetradecanoylphorbol-13-acetate (CAS No. 16561-29-8)

12-*O*-Tetradecanoylphorbol-13-acetate (TPA) was obtained from Consolidated Midland Corporation (Brewster, NY) in one lot (031) and from Pharmacia PL Biochemical (Milwaukee, WI) in two lots (00411999 and 0E11999). A second shipment of lot 00411999 was received from Pharmacia PL Biochemical and was assigned a new number (UN2811) to assist in tracking. TPA was also obtained from L.C. Services Corporation (Woburn, MA) in one lot (F-121). All five lots were used during the 1-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory (Appendix D). Reports on analyses performed in support of the 1-year studies are on file at the NIEHS.

Each lot of the chemical was identified as TPA by nuclear magnetic resonance spectroscopy and mass spectrometry. The purity of the five lots was determined by thin-layer chromatography and high-performance liquid chromatography. Thin-layer chromatography indicated one major spot for all five lots. For lot 00411999, along with one major spot, one trace impurity was detected. For lot 031, along with one major spot, one system detected a trace impurity and a very slight trace impurity, and a second system identified a trace impurity, a slight trace impurity, and two very slight trace impurities. High-performance liquid chromatography indicated one major peak in all five lots. In addition, high-performance liquid chromatography of lots 031 and UN2811 indicated between seven and 11 trace impurities with peak areas that were approximately 3% of the major peak. High-performance liquid chromatography indicated between two and five trace impurities in lots 00411999, 0E11999, and F-121 with peak areas that were approximately 1% of the major peak. The overall purity was determined to be 97% for lots 031 and UN2811, and 99% for lots 00411999, 0E11999, and F-121.

The stability of the chemical was determined using high-performance liquid chromatography. There was no decomposition in samples exposed to air and light at ambient temperature for up to 6 days. The study laboratory stored the chemical in sealed vials at −20° C.

Preparation and Analysis of Dose Formulations

Benzoyl Peroxide

The dose formulations were prepared by dissolving benzoyl peroxide in acetone (Table D1). Stability studies of the dose formulations were performed by the analytical chemistry laboratory using high-performance liquid chromatography. The stability of the dose formulations was confirmed for at least 3 weeks at 5° C when stored in sealed vials protected from light and for 3 hours when the dose formulation was exposed to light and air.

Periodic analyses of the dose formulations of benzoyl peroxide were conducted at the study laboratory and analytical chemistry laboratory using high-performance liquid chromatography. The dose formulations were analyzed at least every 8 weeks (Table D2). Of the dose formulations analyzed, 94% (15/16) were within 10% of the target concentration. Results of the periodic referee analyses performed by the analytical chemistry laboratory were in agreement with the results obtained by the study laboratory for two of the four formulations (Table D3).

7,12-Dimethylbenz(a)anthracene

The dose formulations were prepared by dissolving 7,12-dimethylbenz(a)anthracene in acetone (Table D1). Stability studies were performed by the analytical chemistry laboratory using high-performance liquid chromatography. The stability of the dose formulations was confirmed for at least 3 weeks when stored in the dark at room temperature and less than 3 hours when exposed to light and air.

Periodic analyses of the dose formulations of 7,12-dimethylbenz(a)anthracene were conducted at the study laboratory and analytical chemistry laboratory using ultraviolet spectroscopy. During the 1-year studies, the dose formulations were analyzed at least every 8 weeks (Table D2). All 23 of the dose formulations analyzed were within 10% of the target concentration. Results of periodic referee analyses performed by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table D3).

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine

The dose formulations were prepared by dissolving *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in acetone (Table D1). Stability studies of dose formulations were performed by the analytical chemistry laboratory using high-performance liquid chromatography. The stability of the dose formulations was confirmed for at least 3 weeks stored at room temperature in sealed vials in the dark and for 3 hours when stored exposed to light and air.

Periodic analyses of the dose formulations of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were conducted at the study laboratory and analytical chemistry laboratory using high-performance liquid chromatography. During the 1-year studies, the dose formulations were analyzed at least every 8 weeks (Table D2). Of the dose formulations analyzed, 95% (19/20) were within 10% of the target concentration. Results of periodic referee analyses performed by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table D3).

12-*O*-Tetradecanoylphorbol-13-acetate

The dose formulations were prepared by dissolving 12-*O*-tetradecanoylphorbol-13-acetate in acetone (Table D1). Stability studies of the dose formulations were performed by the analytical chemistry laboratory using high-performance liquid chromatography. The stability of the dose formulations was confirmed for at least 3 weeks at room temperature when stored in amber glass bottles.

Periodic analyses of the dose formulations of 12-*O*-tetradecanoylphorbol-13-acetate were conducted at the study laboratory and analytical chemistry laboratory using high-performance liquid chromatography. During the 1-year studies, the dose formulations were analyzed at least every 8 weeks (Table D2). Of the dose formulations analyzed, 92% (24/26) were within 10% of the target concentration. Results of periodic referee analyses performed by the analytical chemistry laboratory agreed with the results obtained by the study laboratory for three of the six formulations (Table D3).

General Study Design

Two 1-year studies (design A and design B) were conducted to compare the skin response of B6C3F$_1$ mice, Swiss (CD-1®) mice, and SENCAR mice to known tumor initiators and promoters. In study design A, groups of 30 male and 30 female mice were administered 7,12-dimethylbenz(a)anthracene (DMBA) as an initiator treatment, followed by weekly applications of either 12-*O*-tetradecanoylphorbol-13-acetate (TPA), benzoyl peroxide (BPO), or acetone as a promoter treatment. Additional groups of 30 male and 30 female mice received weekly applications of acetone, TPA, BPO, or DMBA for the entire period of dosing. In study design B, groups of 30 male and 30 female mice were administered *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) as the initiator treatment followed by weekly applications of either TPA, BPO, or acetone as the promoter treatment. Additional groups of

30 male and 30 female mice received weekly applications of acetone, TPA, BPO, or MNNG for the entire period of dosing. In both study designs doses were applied to the intrascapular region of the back. The hair at the site of application was clipped 24 to 48 hours prior to the first chemical treatment and weekly thereafter. Initiators were applied once during the first week of dosing. The first promoter dose was applied one week after the initiator treatment and weekly thereafter for the remaining 51 weeks of dosing. Animals receiving only acetone, TPA, BPO, DMBA, or MNNG were treated beginning at week 1 of the study. All DMBA, MNNG, TPA, and acetone doses were administered in a volume of 0.1 mL. Due to relatively low solubility of BPO in the vehicle, BPO doses were administered by the application of two consecutive volumes of 0.1 mL, each containing 10 mg BPO, to achieve the total dose of 20 mg per mouse.

In both study designs A and B, the original protocol prescribed TPA promoter doses of 5 μg for all mouse strains. However, after approximately 3 months, the skin application site of SENCAR mice receiving repeated applications of 5 μg TPA had excessive irritation, scales, and ulcers. Animals from these groups were removed from the study without further evaluation. A dose range pilot study was performed in which groups of five male and five female SENCAR mice received repeated applications of 0.5, 1, 2, or 5 μg TPA per mouse once a week for 8 weeks. These studies indicated that a 1 μg TPA dose per mouse gave a minimal hyperplastic response without ulceration. Additional SENCAR mice groups receiving TPA were restarted at dose concentrations of 1 μg TPA.

Study Design A

The various combinations of initiation and promotion treatments in study design A can be ordered into five conceptual groups (Table 1):

Vehicle Control: Groups of 30 male and 30 female mice of each of the three strains were administered acetone topically once per week for 52 weeks.

Initiator Reference: Groups of 30 male and 30 female mice were administered single DMBA initiation doses of 0.25 μg [Swiss (CD-1®) and SENCAR], 2.5 μg (all strains), 25 μg (all strains), or 50 μg ($B6C3F_1$), followed by acetone as a promotion treatment applied once per week for the remaining 51 weeks of the study.

Promoter Reference: Groups of 30 male and 30 female mice received topical applications of 1 μg TPA (SENCAR), 5 μg TPA [$B6C3F_1$ and Swiss (CD-1®)], or 20 mg BPO (all strains) for 52 weeks. Due to a dose formulation error in study design A, all mice were initiated with 16 mg BPO, and all $B6C3F_1$ mice received one dose of 16 mg BPO as a promotion treatment in week 2.

Initiation/Promotion: Groups of 30 male and 30 female mice were administered single DMBA initiation doses of 0.25 μg [Swiss (CD-1®) and SENCAR], 2.5 μg (all strains), 25 μg (all strains), or 50 μg ($B6C3F_1$). Initiation was followed by the administration of 5 μg TPA [$B6C3F_1$ and Swiss (CD-1®)] or 1 μg TPA (SENCAR) as a promotion treatment once per week for the remaining 51 weeks of the study.

Additional groups of 30 male and 30 female mice (all strains) were administered 2.5 or 25 μg DMBA once in the first week of the study followed by 20 mg BPO once per week for the remaining 51 weeks.

Complete Carcinogen: Groups of 30 male and 30 female mice of all three strains were administered 2.5 μg DMBA topically as a complete carcinogen once per week for 52 weeks.

TABLE 1
Design A in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation[a]

	Mouse Strain		
	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Initiation/Promotion Treatment[b,c]			
Vehicle Control			
Acetone/Acetone	X	X	X
Initiator Reference			
0.25 μg DMBA/Acetone		X	X
2.5 μg DMBA/Acetone	X	X	X
25 μg DMBA/Acetone	X	X	X
50 μg DMBA/Acetone	X		
Promoter Reference			
1 μg TPA/1 μg TPA[d]			X
5 μg TPA/5 μg TPA	X	X	
20 mg BPO/20 mg BPO	X	X	X
Initiation/Promotion			
0.25 μg DMBA/1 μg TPA			X
2.5 μg DMBA/1 μg TPA			X
25 μg DMBA/1 μg TPA			X
0.25 μg DMBA/5 μg TPA		X	
2.5 μg DMBA/5 μg TPA	X	X	
25 μg DMBA/5 μg TPA	X	X	
50 μg DMBA/5 μg TPA	X		
2.5 μg DMBA/20 mg BPO	X	X	X
25 μg DMBA/20 mg BPO	X	X	X
Complete Carcinogen			
2.5 μg DMBA/2.5 μg DMBA	X	X	X

[a] 30 males and 30 females/group
[b] All dose volumes were 0.1 mL except for BPO doses which were delivered as two 0.1 mL volumes containing half the total dose.
[c] Initiators were applied once during the first week of dosing; promoters were applied one week after initiator application, and then once weekly for the remaining 51 weeks of dosing.
[d] After 3 months of dosing, SENCAR mice were determined to be intolerant of the 5 μg TPA dose. Thus, SENCAR groups administered 5 μg TPA were replaced with groups receiving 1 μg TPA.

Study Design B

As in study design A, the various combinations of initiation and promotion treatments in study design B can be ordered into five conceptual groups (Table 2):

Vehicle Control: Groups of 30 male and 30 female mice of each of the three strains were administered acetone topically once per week for 52 weeks.

Initiator Reference: Groups of 30 male and 30 female mice (all strains) were administered 100, 500, or 1,000 μg MNNG in a single dose during week 1, followed by acetone as a promotion treatment applied once per week for the remaining 51 weeks of the study.

Promoter Reference: Groups of 30 male and 30 female mice received topical application of 1 μg TPA (SENCAR), 5 μg TPA [B6C3F$_1$ and Swiss (CD-1®)], or 20 mg BPO (all strains) for 52 weeks.

Initiation/Promotion: Groups of 30 male and 30 female mice (all strains) were administered 100, 500, or 1,000 μg MNNG as an initiator in a single dose during week 1, followed by 20 mg BPO as a promoter treatment once per week for the remaining 51 weeks of the study.

Additional groups of 30 male and 30 female mice were administered 100 or 1,000 μg MNNG once in the first week of the study followed by 5 μg TPA [B6C3F$_1$ and Swiss (CD-1®)] or 1 μg TPA (SENCAR) once per week for the remaining 51 weeks.

Complete Carcinogen: Groups of 30 male and 30 female mice of all three strains were administered 100 μg MNNG topically as a complete carcinogen once per week for 52 weeks.

TABLE 2
Design B in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation[a]

	Mouse Strain		
	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Initiation/Promotion Treatment[b,c]			
Vehicle Control			
Acetone/Acetone	X	X	X
Initiator Reference			
100 μg MNNG/Acetone	X	X	X
500 μg MNNG/Acetone	X	X	X
1,000 μg MNNG/Acetone	X	X	X
Promoter Reference			
1 μg TPA/1 μg TPA[d]			X
5 μg TPA/5 μg TPA	X	X	
20 mg BPO/20 mg BPO	X	X	X
Initiation/Promotion			
100 μg MNNG/20 mg BPO	X	X	X
500 μg MNNG/20 mg BPO	X	X	X
1,000 μg MNNG/20 mg BPO	X	X	X
100 μg MNNG/5 μg TPA	X	X	
1,000 μg MNNG/5 μg TPA	X	X	
100 μg MNNG/1 μg TPA			X
1,000 μg MNNG/1 μg TPA			X
Complete Carcinogen			
100 μg MNNG/100 μg MNNG	X	X	X

[a] 30 males and 30 females/group
[b] All dose volumes were 0.1 mL except for BPO doses which were delivered as two 0.1 mL volumes containing half the total dose.
[c] Initiators were applied once during the first week of dosing; promoters were applied one week after initiator application, and then once weekly for the remaining 51 weeks of dosing.
[d] After 3 months of dosing, SENCAR mice were determined to be intolerant of the 5 μg TPA dose. Thus, SENCAR groups administered 5 μg TPA were replaced with groups receiving 1 μg TPA.

Source and Specification of Animals

Male and female B6C3F$_1$ and SENCAR mice were obtained from Frederick Cancer Research Facility (Frederick, MD) and male and female Swiss (CD-1®) mice were obtained from Charles River Breeding Laboratories (Portage, MI) for use in the studies. Mice were quarantined for 27 to 30 days before the beginning of the studies. Five male and five female mice per strain per study design were selected for parasite evaluation and gross observation of disease. Serology samples were collected for viral screening. Mice were approximately 8 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to 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 biweekly. 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

All animals were observed twice daily. Clinical findings were recorded weekly; animals were weighed weekly during the first three months of the study and monthly thereafter.

Clinical observations of the skin were recorded to follow the appearance and progression of any tumor development. To provide some consistency for these observations, guidelines were developed to define how the skin tumor response would be recorded. Thus, at first appearance, each skin tumor was recorded as a tissue mass. A tissue mass that was at least 2 mm in diameter and was present for 14 days was called a papilloma. A papilloma that became necrotic in appearance and attached to underlying tissue was called a carcinoma. Clinical observations were directly entered onto diskettes and transmitted to NIEHS using the Toxicology Data Management System (TDMS).

Using the mouse skin initiation/promotion protocol, the appearance and progression of tumor development can be observed. Since the skin was 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 studies, it was expected that some dosed 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.

A necropsy was performed on all mice. 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. Histopathologic examinations were performed on skin (site of application and untreated) (Table 3).

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into TDMS. 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 samples of skin from the site of application as well as samples from untreated sites.

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. 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).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Animals found dead of other than natural causes and those missing or missexed 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 neoplasms or nonneoplastic lesions as presented in Tables A1, A3, B1, B3, C1, and C3 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 neoplasms and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms, as is the case for skin tissue, before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence, 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 observed in-life was a 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 at the time of death of the animal.

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, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in these studies 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.

Analysis of Continuous Variables

Body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955). The number of papillomas was analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Quality Assurance Methods

The study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 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.

TABLE 3
Experimental Design and Materials and Methods in the Comparative Initiation/Promotion Skin Paint Studies of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice

B6C3F$_1$	Swiss (CD-1®)	SENCAR
Study Laboratory		
Battelle Columbus (Columbus, OH)	Battelle Columbus (Columbus, OH)	Battelle Columbus (Columbus, OH)
Animal Source		
Frederick Cancer Research Facility (Frederick, MD)	Charles River Breeding Laboratories (Portage, MI)	Frederick Cancer Research Facility (Frederick, MD)
Size of Study Groups		
30 males and 30 females	30 males and 30 females	30 males and 30 females
Time Held Before Studies		
A: 27 days B: 29 days	A: 29 days B: 29 days	A: 30 days B: 30 days Restart: 29 days
Average Age When Studies Began		
A: 56 days B: 57 days	A: 58 days B: 58 days	A: 58 days B: 58 days Restart: 57 days
Date of First Dose		
A: 22 April 1985 B: 22 May 1985	A: 3 May 1985 B: 31 May 1985	A: 9 May 1985 B: 6 June 1985 Restart: 16 October 1985
Duration of Dosing		
A: Initiator dose 1×, 22 April 1985; Promoter doses, 1× weekly for 51 weeks, 29 April 1985 - 14 April 1986 B: Initiator dose 1×, 22 May 1985; Promoter doses 1× weekly for 51 weeks, 29 May 1985 - 14 May 1986	A: Initiator dose 1×, 3 May 1985; Promoter doses, 1× weekly for 51 weeks, 10 May 1985 - 25 April 1986 B: Initiator dose 1×, 31 May 1985; Promoter doses 1× weekly for 51 weeks, 7 June 1985 - 23 May 1986	A: Initiator dose 1×, 9 May 1985; Promoter doses, 1× weekly for 51 weeks, 16 May 1985 - 1 May 1986 B: Initiator dose 1×, 6 June 1985; Promoter doses 1× weekly for 51 weeks, 13 June 1985 - 29 May 1986 Restart: Initiator dose 1×, 16 October 1985; Promoter doses 1× weekly for 51 weeks, 23 October 1985 - 8 October 1986
Date of Last Dose		
A: 14 April 1986 B: 14 May 1986	A: 25 April 1986 B: 23 May 1986	A: 1 May 1986 B: 29 May 1986 Restart: 8 October 1986

TABLE 3
Experimental Design and Materials and Methods in the Comparative Initiation/Promotion Skin Paint Studies of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice (continued)

B6C3F$_1$	Swiss (CD-1®)	SENCAR
Method of Sacrifice		
Carbon dioxide asphyxiation	Carbon dioxide asphyxiation	Carbon dioxide asphyxiation
Necropsy Dates		
A: 21-25 April 1986 B: 19-23 May 1986	A: 28 April - 2 May 1986 B: 27-30 May 1986	A: 5-8 May 1986 B: 2-4 June 1986 Restart: 13 October 1986
Average Age at Necropsy		
60-61 weeks	60-61 weeks	60-61 weeks
Method of Animal Distribution		
Animals were randomly assigned to groups of approximately equal initial mean body weight by a computer generated randomization procedure	Same as B6C3F$_1$	Same as B6C3F$_1$
Animals per Cage		
1	1	1
Method of Identification		
Toe mark	Toe mark	Toe mark
Diet		
NIH-07 open formula meal (Zeigler Brothers, Gardners, PA), available *ad libitum*; changed weekly	Same as B6C3F$_1$	Same as B6C3F$_1$
Maximum Storage Time for Feed		
120 days	120 days	120 days
Feeders		
Stainless-steel hopper-type (Lab Products, Inc., Garfield, NJ)	Same as B6C3F$_1$	Same as B6C3F$_1$
Water		
Tap water (Columbus municipal supply) via automatic watering system; available *ad libitum*	Same as B6C3F$_1$	Same as B6C3F$_1$
Cages		
Polycarbonate (Lab Products Inc., Garfield, NJ); changed weekly	Same as B6C3F$_1$	Same as B6C3F$_1$
Bedding		
BetaChips® (Northeastern Products Corp., Warrensburg, NY)	Same as B6C3F$_1$	Same as B6C3F$_1$

TABLE 3
Experimental Design and Materials and Methods in the Comparative Initiation/Promotion Skin Paint Studies of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice (continued)

B6C3F$_1$	Swiss (CD-1®)	SENCAR
Cage Filters		
Spun-bonded DuPont 2024 polyester (Snow Filtration Co., Cincinnati, OH); changed every 2 weeks	Same as B6C3F$_1$	Same as B6C3F$_1$
Racks		
Stainless steel drawer-type (Lab Products Inc., Garfield, NJ); changed every 2 weeks	Same as B6C3F$_1$	Same as B6C3F$_1$
Animal Room Environment		
Temperature: 21°-24° C Relative humidity: 35%-65% Fluorescent light: 12 hours/day Room air changes: 15 changes/hour	Temperature: 21°-24° C Relative humidity: 35%-65% Fluorescent light: 12 hours/day Room air changes: 15 changes/hour	Temperature: 21°-24° C Relative humidity: 35%-65% Fluorescent light: 12 hours/day Room air changes: 15 changes/hour
Doses		
See Tables 1 and 2	See Tables 1 and 2	See Tables 1 and 2
Type and Frequency of Observation		
Animals observed twice daily; animals were weighed weekly during the first three months, and monthly thereafter; clinical findings recorded weekly	Animals observed twice daily; animals were weighed weekly during the first three months, and monthly thereafter; clinical findings recorded weekly	Animals observed twice daily; animals were weighed weekly during the first three months, and monthly thereafter; clinical findings recorded weekly
Necropsy		
Necropsy performed on all animals	Necropsy performed on all animals	Necropsy performed on all animals
Histopathology		
Histopathology was performed on skin from the site of application and from untreated sites.	Histopathology was performed on skin from the site of application and from untreated sites.	Histopathology was performed on skin from the site of application and from untreated sites.

RESULTS

STUDY DESIGN A

PROTOCOL CONTROL GROUPS

The mouse skin initiation/promotion model identifies chemicals with skin tumor promotion potential. In this model, the combination of a single subthreshold topical application of a carcinogen followed by repeated doses of a second chemical with promotion potential will produce skin tumors. Based on the assumptions of the model, topical applications of vehicle alone, a single initiating subthreshold dose of a carcinogen without promotion, or repeated doses of a promoter without initiation should not produce tumors. In study design A, 7,12-dimethylbenz(a)-anthracene (DMBA) was used as the initiator, and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and benzoyl peroxide (BPO) were used as promoters. A number of initiator and promoter control groups were included in the study design as a measure of how well this mouse skin initiation/promotion model functioned. These control groups included groups of male and female mice of each strain that received the acetone vehicle alone, as well as groups that received a single application of one of three concentrations of DMBA [0.25, 2.5, or 25 μg/mouse in Swiss (CD-1®) and SENCAR and 2.5, 25, or 50 μg/mouse in $B6C3F_1$] followed by repeated applications of the non-promoter acetone, and groups that received repeated topical applications of either TPA [5 μg/mouse in $B6C3F_1$ and Swiss (CD-1®) and 1 μg/mouse in SENCAR] or BPO (20 mg/mouse) without DMBA initiation (Table 1).

Survival of Protocol Controls

For each strain, survival of male and female mice receiving any of the three initiator DMBA concentrations followed by acetone was similar to that of the vehicle controls of the respective strain (Table 4). Survival of males and females of each strain receiving repeated applications of BPO without prior initiation was also similar to that of the respective vehicle control groups. In male and female $B6C3F_1$ and Swiss (CD-1®) groups receiving repeated applications of TPA without prior initiation, survival was similar to that of the vehicle controls of the same strain. However, in male and female SENCAR groups receiving repeated applications of TPA without prior initiation, survival was significantly lower than that of the vehicle controls (Table 4).

Body Weights and Clinical Findings in Protocol Controls

Final mean body weights of the protocol control groups were similar to those of the respective vehicle controls (Table 4).

The primary clinical findings included irritation and ulcers. Irritation was defined as the skin being inflamed or appearing sore. Ulcer was defined as localized skin surface excavation and/or broken skin. The incidences of these clinical signs of toxicity in male and female DMBA initiator control mice of all three strains were similar to those in the vehicle controls. Some non-initiated male and female Swiss (CD-1®) and SENCAR mice receiving repeated applications of TPA were observed with skin irritation throughout the study and with ulcers that appeared after about 10 to 12 weeks, peaked between weeks 24 and 28, and decreased in incidence thereafter (Table 5). Clinical signs were not observed at the site of application in male or female $B6C3F_1$ or SENCAR mice or female Swiss (CD-1®) mice receiving repeated applications of BPO. Less than 7% of male Swiss (CD-1®) mice developed signs of irritation and no more than 12% developed signs of ulcer.

TABLE 4
Survival and Body Weights of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design A

Initiator/Promoter	Survival[a]	Final Mean Body Weight[b] (g)	Final Weight Relative to Controls (%)
B6C3F$_1$			
Male			
Acetone/Acetone	26/30	48.4 ± 0.6	
2.5 μg DMBA/Acetone	30/30	47.6 ± 0.5	98
25 μg DMBA/Acetone	29/30	48.3 ± 0.6	100
50 μg DMBA/Acetone	30/30	47.0 ± 0.6	97
5 μg TPA/5 μg TPA	29/30	46.8 ± 0.8	97
20 mg BPO/20 mg BPO	30/30	46.2 ± 0.8	95
Female			
Acetone/Acetone	30/30	44.4 ± 0.9	
2.5 μg DMBA/Acetone	29/30	45.5 ± 1.1	102
25 μg DMBA/Acetone	30/30	45.8 ± 1.0	103
50 μg DMBA/Acetone	30/30	46.5 ± 0.9	105
5 μg TPA/5 μg TPA	30/30	42.0 ± 0.9	94
20 mg BPO/20 mg BPO	27/30	43.1 ± 0.9	97
Swiss (CD-1®)			
Male			
Acetone/Acetone	27/30	50.0 ± 1.3	
0.25 μg DMBA/Acetone	25/30	51.7 ± 1.2	103
2.5 μg DMBA/Acetone	29/30	51.7 ± 1.1	103
25 μg DMBA/Acetone	28/30	51.4 ± 1.2	103
5 μg TPA/5 μg TPA	21/30	47.5 ± 1.4	95
20 mg BPO/20 mg BPO	25/30	51.6 ± 1.3	103
Female			
Acetone/Acetone	27/30	40.1 ± 1.5	
0.25 μg DMBA/Acetone	29/30	40.4 ± 1.2	101
2.5 μg DMBA/Acetone	29/30	39.3 ± 1.1	98
25 μg DMBA/Acetone	28/30	41.0 ± 1.3	102
5 μg TPA/5 μg TPA	23/30	38.2 ± 0.8	95
20 mg BPO/20 mg BPO	30/30	40.8 ± 1.1	102

(continued)

TABLE 4
Survival and Body Weights of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design A (continued)

Initiator/Promoter	Survival	Final Mean Body Weight (g)	Final Weight Relative to Controls (%)
SENCAR			
Male			
Acetone/Acetone	28/30	51.7 ± 1.3	
Acetone/Acetone[c]	29/30	51.7 ± 1.4	
0.25 µg DMBA/Acetone	27/30	54.0 ± 1.6	104
2.5 µg DMBA/Acetone	27/29	54.1 ± 1.2	105
25 µg DMBA/Acetone	30/31	52.1 ± 1.4	101
1 µg TPA/1 µg TPA	9/30**	48.8 ± 2.8	94
20 mg BPO/20 mg BPO	23/30	50.7 ± 1.4	98
Female			
Acetone/Acetone	27/30	44.5 ± 1.4	
Acetone/Acetone[c]	27/30	45.7 ± 1.7	
0.25 µg DMBA/Acetone	29/30	43.0 ± 1.4	97
2.5 µg DMBA/Acetone	30/31	43.8 ± 1.4	99
25 µg DMBA/Acetone	28/29	44.7 ± 1.4	100
1 µg TPA/1 µg TPA	10/30**	44.1 ± 2.6	97
20 mg BPO/20 mg BPO	28/30	41.1 ± 1.2	92

** Significantly different ($P \leq 0.01$) from the vehicle control group by life table pairwise comparison
[a] Number of animals surviving/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[b] Weights are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[c] Vehicle control group for TPA restart

TABLE 5
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design A

	Irritation					Ulcer				
Week of Study	1-10	11-20	21-30	31-40	41-50	1-10	11-20	21-30	31-40	41-50
Male										
B6C3F$_1$										
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
2.5 µg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
25 µg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
50 µg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
5 µg TPA/5 µg TPA	0	0	0	0	0	0	1	0	1	0
20 mg BPO/20 mg BPO	0	0	0	0	0	0	0	0	0	0
Female										
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
2.5 µg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
25 µg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
50 µg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
5 µg TPA/5 µg TPA	0	0	0	0	0	0	1	0	0	3
20 mg BPO/20 mg BPO	0	3	3	0	0	0	0	0	1	0
Swiss (CD-1®)										
Male										
Acetone/Acetone	2	3	3	5	4	1	2	3	0	0
0.25 µg DMBA/Acetone	0	0	0	0	0	1	0	1	4	3
2.5 µg DMBA/Acetone	2	2	0	0	0	2	2	0	0	0
25 µg DMBA/Acetone	0	0	1	3	3	2	3	3	1	0
5 µg TPA/5 µg TPA	1	6	9	13	19	28	33	40	40	22
20 mg BPO/20 mg BPO	0	0	1	0	6	2	1	4	13	9
Female										
Acetone/Acetone	0	0	0	0	1	1	0	1	2	0
0.25 µg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
2.5 µg DMBA/Acetone	1	2	0	0	0	3	1	0	1	1
25 µg DMBA/Acetone	0	0	0	0	0	0	0	0	0	1
5 µg TPA/5 µg TPA	0	2	5	19	23	28	19	24	25	23
20 mg BPO/20 mg BPO	0	0	0	0	0	2	0	0	0	1

(continued)

TABLE 5
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design A (continued)

	Irritation					Ulcer				
Week of Study	1-10	11-20	21-30	31-40	41-50	1-10	11-20	21-30	31-40	41-50
SENCAR										
Male										
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
0.25 μg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
2.5 μg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
25 μg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
1 μg TPA/1 μg TPA	0	23	54	51	31	7	12	5	8	7
20 mg BPO/20 mg BPO	0	6	0	0	0	3	3	6	1	1
Female										
Acetone/Acetone	0	0	0	0	0	0	0	0	0	0
Acetone/Acetone	1	0	0	0	0	0	0	0	0	0
0.25 μg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
2.5 μg DMBA/Acetone	0	0	0	0	0	0	0	0	0	0
25 μg DMBA/Acetone	0	0	0	0	0	0	0	0	2	0
1 μg TPA/1 μg TPA	0	25	32	21	13	9	14	9	10	1
20 mg BPO/20 mg BPO	0	0	0	0	0	0	0	0	0	1

Tumor Response in Protocol Controls

In B6C3F$_1$ and SENCAR mice, the incidences of skin tumors in protocol control groups were similar to those in the vehicle controls. In Swiss (CD-1®) mice, the incidences of skin tumors in male and female TPA protocol controls were significantly greater than those in the vehicle controls (Table 6).

TABLE 6
Incidences of Skin Tumors in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design A[a]

	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Male			
Acetone/Acetone	0/30	0/30	0/30
Acetone/Acetone[b]	–[c]	–	0/30
0.25 µg DMBA/Acetone	–	1/30	0/30
2.5 µg DMBA/Acetone	0/30	0/30	0/29
25 µg DMBA/Acetone	0/30	0/30	0/31
50 µg DMBA/Acetone	0/30	–	–
20 mg BPO/20 mg BPO	0/30	0/30	0/30
5 µg TPA/5 µg TPA	0/30	5/30*	–
1 µg TPA/1 µg TPA[d]	–	–	2/30
Female			
Acetone/Acetone	0/30	0/30	0/29
Acetone/Acetone	–	–	0/29
0.25 µg DMBA/Acetone	–	0/30	0/30
2.5 µg DMBA/Acetone	0/30	0/30	0/31
25 µg DMBA/Acetone	0/30	0/30	0/29
50 µg DMBA/Acetone	0/30	–	–
20 mg BPO/20 mg BPO	0/30	0/30	0/30
5 µg TPA/5 µg TPA	0/30	4/30*	–
1 µg TPA/1 µg TPA	–	–	0/30

* Significantly different ($P \leq 0.05$) from the vehicle control group by logistic regression
[a] Number of animals with tumors per number of animals with skin examined in-life. Incidences represent clinical observations of papilloma.
[b] Vehicle control for TPA restart (SENCAR mice)
[c] Dose level not administered to this strain
[d] SENCAR restart group

DMBA Initiation and TPA Promotion

Survival

Survival of all $B6C3F_1$ groups was similar to that of the vehicle controls. Survival of male and female Swiss (CD-1®) and SENCAR mice initiated with 0.25, 2.5, or 25 μg DMBA was lower than that of the respective vehicle controls (Table 7). However, many of the Swiss (CD-1®) and SENCAR mice receiving 25 μg DMBA/TPA were removed from the study in accordance with the moribund sacrifice policy.

Body Weights and Clinical Findings

Final mean body weights of $B6C3F_1$ and Swiss (CD-1®) mice in the DMBA/TPA groups were similar to those of the respective vehicle controls (Table 7).

The primary clinical findings recorded for mice receiving DMBA/TPA were irritation and ulcer (Table 8). Irritation was defined as inflamed skin or sore appearance. Ulcer was defined as localized skin surface excavation and/or broken skin. Irritation and ulcer were seldom observed in male or female $B6C3F_1$ mice. Swiss (CD-1®) mice had the highest incidences of skin irritation, and SENCAR mice had the most ulcers. Irritation was first observed in males and females in all three DMBA/TPA groups of Swiss (CD-1®) mice during week 3 and did not appear to be related to the concentration of DMBA. In SENCAR mice, irritation was observed in fewer animals and appeared to occur at a later time than in Swiss (CD-1®) mice. Generally, the highest percentages of Swiss (CD-1®) and SENCAR mice with incidences of irritation were observed during the first 10 weeks of the study; incidences of ulcer became more frequent during the later weeks of the study.

TABLE 7
Survival and Body Weights of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and TPA Promotion

Initiator/Promoter	Survival[a]	Final Mean Body Weight[b] (g)	Final Weight Relative to Controls (%)
B6C3F$_1$			
Male			
Acetone/Acetone	26/30	48.4 ± 0.6	
5 μg TPA/5 μg TPA	29/30	46.8 ± 0.8	97
2.5 μg DMBA/5 μg TPA	30/30	45.7 ± 0.8	94
25 μg DMBA/5 μg TPA	29/30	46.9 ± 0.8	97
50 μg DMBA/5 μg TPA	26/30	46.5 ± 0.8	96
Female			
Acetone/Acetone	30/30	44.4 ± 0.9	
5 μg TPA/5 μg TPA	30/30	42.0 ± 0.9	94
2.5 μg DMBA/5 μg TPA	30/30	42.1 ± 0.7	95
25 μg DMBA/5 μg TPA	28/30	41.7 ± 1.1	94
50 μg DMBA/5 μg TPA	27/30	42.1 ± 1.1	95
Swiss (CD-1®)			
Male			
Acetone/Acetone	27/30	50.0 ± 1.3	
5 μg TPA/5 μg TPA	21/30	47.5 ± 1.4	95
0.25 μg DMBA/5 μg TPA	20/30	48.6 ± 1.4	97
2.5 μg DMBA/5 μg TPA	15/30**	45.1 ± 1.2▲	90
25 μg DMBA/5 μg TPA	15/30**	45.1 ± 1.7▲	90
Female			
Acetone/Acetone	27/30	40.1 ± 1.5	
5 μg TPA/5 μg TPA	23/30	38.2 ± 0.8	95
0.25 μg DMBA/5 μg TPA	22/30	38.2 ± 0.9	95
2.5 μg DMBA/5 μg TPA	19/30*	38.6 ± 0.7	96
25 μg DMBA/5 μg TPA	16/30**	38.4 ± 1.3	96
SENCAR			
Male			
Acetone/Acetone	29/30	51.7 ± 1.4	
1 μg TPA/1 μg TPA	9/30**	48.8 ± 2.8	94
0.25 μg DMBA/1 μg TPA	6/30**	48.6 ± 3.7	94
2.5 μg DMBA/1 μg TPA	2/30**	46.5 ± 1.3	90
25 μg DMBA/1 μg TPA	1/30**	38.2	74
Female			
Acetone/Acetone	27/30	45.7 ± 1.7	
1 μg TPA/1 μg TPA	10/30**	44.1 ± 2.6	97
0.25 μg DMBA/1 μg TPA	8/30**	47.2 ± 2.9	103
2.5 μg DMBA/1 μg TPA	6/30**	39.1 ± 1.5	86
25 μg DMBA/1 μg TPA	1/30**	39.2	86

* Significantly different (P≤0.05) from the vehicle control group by life table pairwise comparison
** Significantly different (P≤0.01) from the vehicle control group by life table pairwise comparison
▲ Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
[a] Number of animals surviving/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[b] Weights are given as mean ± standard error. No standard errors were calculated for groups with high mortality.

TABLE 8
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and TPA Promotion

	Irritation					Ulcer				
Week of Study	1-10	11-20	21-30	31-40	41-50	1-10	11-20	21-30	31-40	41-50
Male										
B6C3F$_1$										
2.5 µg DMBA/5 µg TPA	0	2	0	1	1	0	0	0	0	0
25 µg DMBA/5 µg TPA	0	2	1	1	4	0	0	0	0	0
50 µg DMBA/5 µg TPA	0	3	1	1	2	0	0	0	0	0
Swiss (CD-1®)										
0.25 µg DMBA/5 µg TPA	30	27	27	31	25	9	12	5	4	8
2.5 µg DMBA/5 µg TPA	26	33	13	5	11	1	5	16	20	22
25 µg DMBA/5 µg TPA	20	18	10	7	11	1	3	6	22	30
SENCAR										
0.25 µg DMBA/1 µg TPA	7	14	6	4	0	0	22	32	48	24
2.5 µg DMBA/1 µg TPA	7	9	4	4	2	1	28	51	62	66
25 µg DMBA/1 µg TPA	6	5	4	6	0	0	15	47	63	67
Female										
B6C3F$_1$										
2.5 µg DMBA/5 µg TPA	0	1	0	0	0	0	0	0	0	0
25 µg DMBA/5 µg TPA	0	0	1	4	10	0	0	0	0	3
50 µg DMBA/5 µg TPA	0	0	0	2	6	0	0	0	0	2
Swiss (CD-1®)										
0.25 µg DMBA/5 µg TPA	26	13	5	3	9	0	8	11	7	0
2.5 µg DMBA/5 µg TPA	22	5	3	2	4	0	1	7	5	13
25 µg DMBA/5 µg TPA	30	27	19	29	32	0	0	2	10	11
SENCAR										
0.25 µg DMBA/1 µg TPA	4	5	2	3	0	0	16	36	42	22
2.5 µg DMBA/1 µg TPA	4	7	4	3	0	0	9	30	47	56
25 µg DMBA/1 µg TPA	4	9	10	8	1	0	8	44	39	44

Tumor Response

Three parameters were used to measure the skin sensitivity of each mouse strain to chemical-induced tumor development: the number of animals in each group that developed tumors, the group mean time to appearance of the first tumor, and the number of tumors per mouse. The responses of the three mouse strains initiated with different concentrations of DMBA followed by repeated applications of TPA are shown in Figures 1 and 2. The response is displayed as the cumulative percentage of mice that developed skin papillomas over the course of the 1-year study. In some groups, survival was reduced because of the aggressive moribund sacrifice policy maintained for this study. In addition, some tumors may have regressed or been removed by mechanical grooming. No mice were removed from the cumulative count. The number of mice per group that developed skin papillomas is given in Table 9.

Incidences of skin papillomas increased with increasing concentration of the initiator DMBA in males and females of each mouse strain. Pairwise comparisons within strains between the different DMBA initiating concentrations and the TPA/TPA promoter controls indicated that the skin papilloma response of all mice receiving DMBA was greater than that of the respective promoter controls (Table 9).

The mean time to the appearance of the first papilloma (based on the animals that developed papillomas) is shown in Table 9. The mean time to the first appearance of papilloma was greater for B6C3F$_1$ mice than for Swiss (CD-1®) and SENCAR mice in spite of the fact that B6C3F$_1$ mice received an initiating DMBA concentration that was twice as great as the concentration administered to the other mouse strains. The mean times to first appearance of papilloma in the three DMBA/TPA groups for Swiss (CD-1®) and SENCAR mice were remarkably similar even though the concentration of the TPA promoter was five times greater for Swiss (CD-1®) than for SENCAR mice.

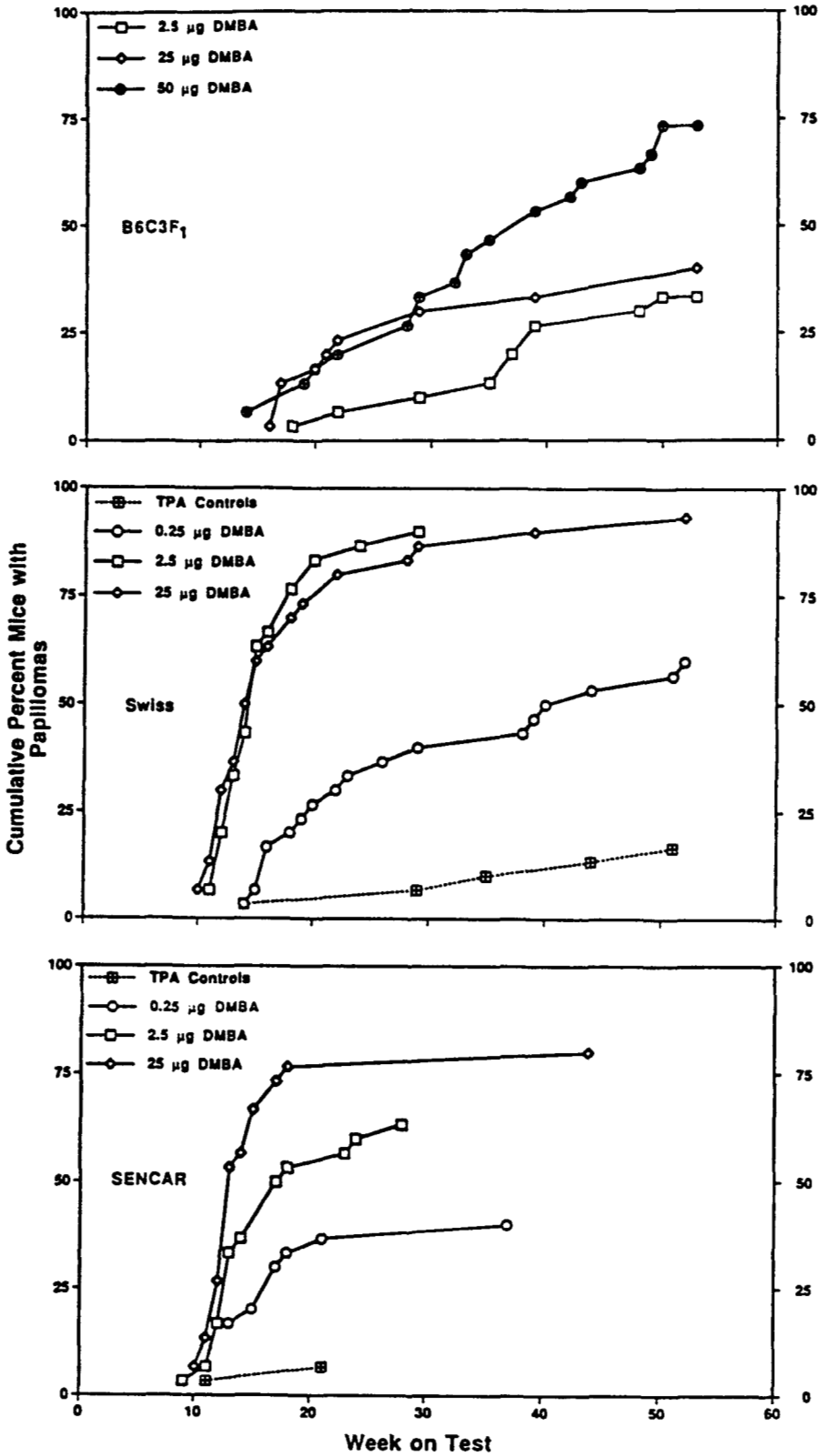

FIGURE 1
Tumor Response of Male Mice of the Three Strains Initiated with Different Concentrations of DMBA Followed by 5 µg [B6C3F$_1$ and Swiss (CD-1®)] or 1 µg (SENCAR) TPA Promotion

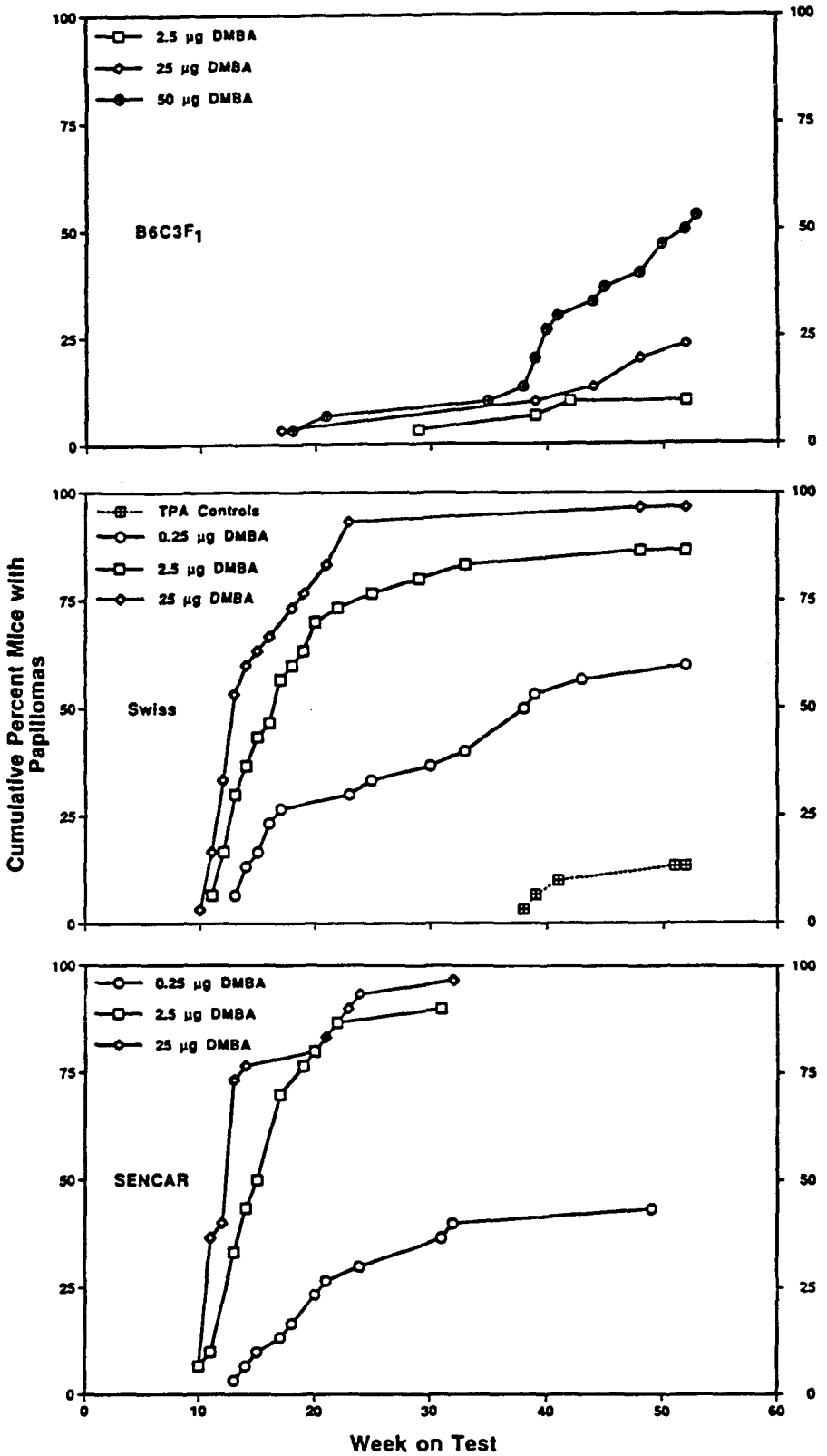

FIGURE 2
Tumor Response of Female Mice of the Three Strains Initiated with Different Concentrations of DMBA Followed by 5 µg [B6C3F$_1$ and Swiss (CD-1®)] or 1 µg (SENCAR) TPA Promotion

TABLE 9
Papilloma Response in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and TPA Promotion

B6C3F$_1$	DMBA/ Acetone	5 μg TPA/ 5 μg TPA	2.5 μg DMBA/ 5 μg TPA	25 μg DMBA/ 5 μg TPA	50 μg DMBA/ 5 μg TPA
Male					
Mean weeks to first papilloma[a]	–[e]	–	35.4 ± 3.21	27.6 ± 3.82	32.5 ± 2.46
Overall rate[b]	0/30	0/30	10/30	12/30	22/30
Life table test[c]			P<0.001	P<0.001	P<0.001
Life table test[d]		P<0.001	P<0.001	P<0.001	P<0.001
Female					
Mean weeks to first papilloma	–	–	36.7 ± 3.93	41.0 ± 4.40	40.8 ± 2.47
Overall rate	0/30	0/30	3/30	7/30	16/30
Life table test			P=0.124	P=0.007	P<0.001
Life table test		P<0.001	P=0.118	P=0.007	P<0.001

Swiss (CD-1®)	DMBA/ Acetone	5 μg TPA/ 5 μg TPA	0.25 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ 5 μg TPA	25 μg DMBA/ 5 μg TPA
Male					
Mean weeks to first papilloma	–[f]	34.6 ± 6.38	27.7 ± 3.04	15.4 ± 0.79	17.6 ± 1.78
Overall rate		5/30	18/30	27/30	28/30
Life table test			P<0.001	P<0.001	P<0.001
Life table test		P<0.001	P<0.001	P<0.001	P<0.001
Female					
Mean weeks to first papilloma	–	42.3 ± 2.98	26.5 ± 2.95	18.0 ± 1.61	16.0 ± 1.37
Overall rate	0/30	4/30	18/30	26/30	29/30
Life table test			P<0.001	P<0.001	P<0.001
Life table test		P<0.001	P<0.001	P<0.001	P<0.001

SENCAR	DMBA/ Acetone	1 μg TPA/ 1 μg TPA	0.25 μg DMBA/ 1 μg TPA	2.5 μg DMBA/ 1 μg TPA	25 μg DMBA/ 1 μg TPA
Male					
Mean weeks to first papilloma	–	16.0 ± 5.00	17.2 ± 1.93	15.5 ± 1.15	14.5 ± 1.35
Overall rate	0/30	2/30	12/30	19/30	24/30
Life table test			P<0.001	P<0.001	P<0.001
Life table test		P<0.001	P<0.001	P<0.001	P<0.001
Female					
Mean weeks to first papilloma	–	–	23.5 ± 2.79	15.9 ± 0.86	14.4 ± 0.99
Overall rate	0/30	0/30	13/30	27/30	29/30
Life table test			P<0.001	P<0.001	P<0.001
Life table test		P<0.001	P<0.001	P<0.001	P<0.001

(continued)

TABLE 9
Papilloma Response in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and TPA Promotion (continued)

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[a] Mean ± standard error
[b] Number of animals with tumor per number of animals with skin examined in-life
[c] Beneath the 2.5 μg DMBA/TPA, 25 μg DMBA/TPA, and 50 μg DMBA/TPA (B6C3F$_1$) or the 0.25 μg DMBA/TPA, 2.5 μg DMBA/TPA, and 25 μg DMBA/TPA [Swiss (CD-1®) and SENCAR] group incidences are the P values corresponding to pairwise comparisons between the corresponding DMBA/Acetone group and those groups.
[d] In the TPA/TPA columns are the P values associated with the trend test. Beneath the 2.5 μg DMBA/TPA, 25 μg DMBA/TPA, and 50 μg DMBA/TPA (B6C3F$_1$) or the 0.25 μg DMBA/TPA, 2.5 μg DMBA/TPA, and 25 μg DMBA/TPA [Swiss (CD-1®) and SENCAR] group incidences are the P values corresponding to pairwise comparisons between the TPA/TPA group and those groups.
[e] Not applicable; no papillomas observed in animal group unless otherwise noted
[f] The 0.25 μg DMBA/Acetone group had an overall rate of 1/30 and mean weeks to first papilloma of 50.0; the 2.5 and 25 μg DMBA/Acetone groups had overall rates of 0/30.

The average number of papillomas per mouse was calculated two ways. In the first method, the total number of tumors in each group was divided by the number of animals that developed tumors. In the second method, the total number of tumors in each group was divided by the total number of animals in each group. The values from both methods are presented in Table 10. The data used to calculate tumor multiplicity were collected at 10-week intervals over the course of the study. Swiss (CD-1®) and SENCAR mice had more tumors per mouse than did $B6C3F_1$ mice, and the tumor multiplicity increased with increasing initiating DMBA concentration, in a significant trend.

TABLE 10
Papillomas Observed During the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and TPA Promotion

Week of Study	10		20		30		40		50	
$B6C3F_1$										
Male										
2.5 µg DMBA/5 µg TPA	0[a]	0[b]	1.00	0.03	1.00	0.03	1.33	0.27	1.75	0.47
25 µg DMBA/5 µg TPA	0	0	2.00	0.33	1.88	0.50	1.90	0.63	2.50	0.69
50 µg DMBA/5 µg TPA	0	0	1.00	0.14	1.50	0.41	2.13	1.10	2.53	1.46
Female										
2.5 µg DMBA/5 µg TPA	0	0	0	0	1.00	0.03	2.00	0.13	1.67	0.17
25 µg DMBA/5 µg TPA	0	0	1.00	0.03	1.00	0.03	1.00	0.10	2.00	0.28
50 µg DMBA/5 µg TPA	0	0	1.00	0.03	1.50	0.10	1.29	0.32	1.75	0.78
Swiss (CD-1®)										
Male										
0.25 µg DMBA/5 µg TPA	0	0	2.14	0.05	2.13	0.63	1.90	0.79	1.70	0.75
2.5 µg DMBA/5 µg TPA	1.00	0	3.72	3.07▲	4.44	3.83▲	3.19	2.88▲	2.69	1.94▲
25 µg DMBA/5 µg TPA	2.25	0.10	5.50	3.87▲	4.88	4.43▲	4.28	3.19	2.60	1.88
Female										
0.25 µg DMBA/5 µg TPA	0	0	2.29	0.53	3.14	0.73	2.27	0.93	3.14	1.00
2.5 µg DMBA/5 µg TPA	0	0	3.90	2.33▲	4.10	2.83▲	3.47	2.54▲	3.15	2.05▲
25 µg DMBA/5 µg TPA	1.00	0.03	6.61	5.07▲	7.04	6.52▲	4.91	4.70▲	3.87	3.50▲
SENCAR										
Male										
0.25 µg DMBA/1 µg TPA	0	0	1.73	0.82	2.38	1.19	2.17	1.00	1.00	0.50
2.5 µg DMBA/1 µg TPA	1.00	0.03	3.82	1.68▲▼	3.50	2.15▲	2.75	1.38▲	1.00	0.50
25 µg DMBA/1 µg TPA	1.00	0.07	5.74	1.62▲▼	5.64	2.89▲▼	4.60	2.30	1.00	0.50
Female										
0.25 µg DMBA/1 µg TPA	0	0	1.67	0.40	1.67	0.48	1.88	0.94	1.67	1.25
2.5 µg DMBA/1 µg TPA	2.00	0.14	4.24	3.07▲	3.56	2.33▲	3.18	1.94▲	2.00	1.63▲
25 µg DMBA/1 µg TPA	2.00	0.13	7.17	6.38▲	6.53	4.45▲	5.86	4.70▲	0	0

▲ Significantly different ($P \leq 0.05$) from similarly treated $B6C3F_1$ groups by the Mann-Whitney U test
▼ Significantly different ($P \leq 0.05$) from similarly treated Swiss (CD-1®) groups by the Mann-Whitney U test
[a] Average number of papillomas expressed as the total number of papillomas/number of mice with papillomas
[b] Average number of papillomas expressed as the total number of papillomas/number of mice surviving

DMBA Initiation and BPO Promotion

Survival

Survival of male and female $B6C3F_1$ and Swiss (CD-1®) and female SENCAR mice was similar to that of the respective vehicle controls. Survival of male SENCAR mice receiving the 25 μg DMBA initiating dose was lower than that of the vehicle controls (Table 11).

Body Weights and Clinical Findings

Final mean body weights of DMBA/BPO study groups were similar to those of the vehicle controls (Table 11).

Clinical signs of chemical exposure, irritation and ulcer, were minimal. Over the course of the study these signs never averaged more than 15% for irritation or more than 10% for ulcers (Table 12). In general, these signs were more frequently observed in male than in female mice. Signs of irritation were first noted in week 3 for male and female Swiss (CD-1®) and female SENCAR mice and in week 5 for male SENCAR mice. Signs of irritation in the $B6C3F_1$ males appeared during weeks 11 through 20 and in females during weeks 21 through 30.

TABLE 11
Survival and Body Weights of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and BPO Promotion

Initiator/Promoter	Survival[a]	Final Mean Body Weight[b] (g)	Final Weight Relative to Controls (%)
B6C3F$_1$			
Male			
Acetone/Acetone	26/30	48.4 ± 0.6	
20 mg BPO/20 mg BPO	30/30	46.2 ± 0.8	95
2.5 µg DMBA/20 mg BPO	27/30	47.0 ± 0.7	97
25 µg DMBA/20 mg BPO	30/30	46.5 ± 0.9	96
Female			
Acetone/Acetone	30/30	44.4 ± 0.9	
20 mg BPO/20 mg BPO	27/30	43.1 ± 0.9	97
2.5 µg DMBA/20 mg BPO	28/30	41.9 ± 1.0	94
25 µg DMBA/20 mg BPO	29/30	43.1 ± 0.8	97
Swiss (CD-1®)			
Male			
Acetone/Acetone	27/30	50.0 ± 1.3	
20 mg BPO/20 mg BPO	25/30	51.6 ± 1.3	103
2.5 µg DMBA/20 mg BPO	27/30	50.0 ± 1.3	100
25 µg DMBA/20 mg BPO	24/30	50.7 ± 1.4	101
Female			
Acetone/Acetone	27/30	40.1 ± 1.5	
20 mg BPO/20 mg BPO	30/30	40.8 ± 1.1	102
2.5 µg DMBA/20 mg BPO	28/30	40.4 ± 1.0	101
25 µg DMBA/20 mg BPO	30/30	42.6 ± 1.3	106
SENCAR			
Male			
Acetone/Acetone	28/30	51.7 ± 1.3	
20 mg BPO/20 mg BPO	23/30	50.7 ± 1.4	98
2.5 µg DMBA/20 mg BPO	23/30	49.6 ± 1.5	96
25 µg DMBA/20 mg BPO	17/30**	46.1 ± 1.6▲	89
Female			
Acetone/Acetone	27/30	44.5 ± 1.4	
20 mg BPO/20 mg BPO	28/30	41.1 ± 1.2	92
2.5 µg DMBA/20 mg BPO	21/30	40.8 ± 1.2	92
25 µg DMBA/20 mg BPO	23/30	41.9 ± 1.0	94

** Significantly different (P≤0.01) from the vehicle control group by life table pairwise comparison
▲ Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
[a] Number of animals surviving/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[b] Weights are given as mean ± standard error.

TABLE 12
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and BPO Promotion

	Irritation					Ulcer				
Week of Study	1-10	11-20	21-30	31-40	41-50	1-10	11-20	21-30	31-40	41-50
Male										
B6C3F$_1$										
2.5 µg DMBA/20 mg BPO	0	3	6	4	7	0	0	2	7	4
25 µg DMBA/20 mg BPO	0	1	6	7	10	0	1	3	5	4
Swiss (CD-1®)										
2.5 µg DMBA/20 mg BPO	3	3	5	10	7	0	2	1	5	8
25 µg DMBA/20 mg BPO	4	1	4	13	13	1	2	1	0	1
SENCAR										
2.5 µg DMBA/20 mg BPO	2	7	10	1	3	0	0	0	3	5
25 µg DMBA/20 mg BPO	4	14	15	4	9	1	2	4	10	6
Female										
B6C3F$_1$										
2.5 µg DMBA/20 mg BPO	0	0	3	7	10	0	0	1	8	10
25 µg DMBA/20 mg BPO	0	0	0	0	0	0	0	0	0	0
Swiss (CD-1®)										
2.5 µg DMBA/20 mg BPO	1	0	1	1	1	0	0	0	0	0
25 µg DMBA/20 mg BPO	1	0	0	1	1	0	0	0	0	0
SENCAR										
2.5 µg DMBA/20 mg BPO	1	2	9	0	2	0	0	0	0	1
25 µg DMBA/20 mg BPO	3	2	7	0	0	0	2	0	0	2

Tumor Response

The response of mice receiving 2.5 or 25 μg DMBA initiation followed by repeated applications of BPO is shown in Figure 3. The response is displayed as the cumulative percentage of mice that developed skin papillomas over the course of the 1-year study. Some tumors may have regressed or been removed by mechanical grooming. No mice were removed from the cumulative count. The number of mice per group that developed skin papillomas is given in Table 13.

TABLE 13
Papilloma Response in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and BPO Promotion

	DMBA/ Acetone	20 mg BPO/ 20 mg BPO	2.5 μg DMBA/ 20 mg BPO	25 μg DMBA/ 20 mg BPO
B6C3F$_1$				
Male				
Mean weeks to first papilloma[a]	–[e]	–	48.0	39.0
Overall rate[b]	0/30	0/30	1/30	1/30
Life table test[c]			P=0.493	P=0.507
Life table test[d]		P=0.551	P=0.493	P=0.500
Female				
Mean weeks to first papilloma	–	–	51.0 ± 0.58	44.0 ± 7.00
Overall rate	0/30	0/30	4/30	2/30
Life table test			P=0.056	P=0.229
Life table test		P=0.601	P=0.065	P=0.248
Swiss (CD-1®)				
Male				
Mean weeks to first papilloma	–	–	52.0	47.0 ± 2.25
Overall rate	0/30	0/30	1/30	6/30
Life table test			P=0.486	P=0.009
Life table test		P=0.002	P=0.515	P=0.012
Female				
Mean weeks to first papilloma	–	–	51.0	39.2 ± 4.07
Overall rate	0/30	0/30	1/30	5/30
Life table test			P=0.493	P=0.034
Life table test		P=0.010	P=0.486	P=0.031

(continued)

TABLE 13
Papilloma Response in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and BPO Promotion (continued)

	DMBA/ Acetone	20 mg BPO/ 20 mg BPO	2.5 μg DMBA/ 20 mg BPO	25 μg DMBA/ 20 mg BPO
SENCAR				
Male				
Mean weeks to first papilloma	–	–	31.0 ± 2.25	31.0 ± 2.46
Overall rate	0/30	0/30	20/30	22/30
Life table test			P<0.001	P<0.001
Life table test		P<0.001	P<0.001	P<0.001
Female				
Mean weeks to first papilloma	–	–	31.2 ± 2.04	33.6 ± 1.88
Overall rate	0/30	0/30	22/30	20/30
Life table test			P<0.001	P<0.001
Life table test		P<0.001	P<0.001	P<0.001

[a] Mean ± standard deviation
[b] Number of animals with tumor per number of animals with skin examined in-life
[c] Beneath the 2.5 μg DMBA/20 mg BPO and 25 μg DMBA/20 mg BPO group incidences are the P values corresponding to pairwise comparisons between the corresponding DMBA/Acetone group and those groups.
[d] In the 20 mg BPO/20 mg BPO columns are the P values associated with the trend test. Beneath the 2.5 μg DMBA/20 mg BPO and 25 μg DMBA/20 mg BPO group incidences are the P values corresponding to pairwise comparisons between the 20 mg BPO/20 mg BPO group and those groups.
[e] Not applicable; no papillomas observed in animal group

The incidences of papilloma were significantly greater than those in controls in male and female Swiss (CD-1®) mice initiated with 25 μg DMBA and in male and female SENCAR mice initiated with 2.5 or 25 μg DMBA; the incidences in B6C3F$_1$ mice were greater than those in controls, but the increase was not statistically significant.

The mean time to the appearance of the first papilloma (based on the animals that developed papillomas) is shown in Table 13. Papillomas first appeared in SENCAR mice between weeks 9 and 10 and the mean time to first papilloma appearance was weeks 31 to 34. B6C3F$_1$ and Swiss (CD-1®) mice that received initiating concentrations of 25 μg DMBA appeared to develop tumors at an earlier time in the study than did those mice that received initiating concentrations of 2.5 μg DMBA, although there were incidences of only one tumor in 2.5 μg DMBA male B6C3F$_1$ and male and female Swiss (CD-1®) mice.

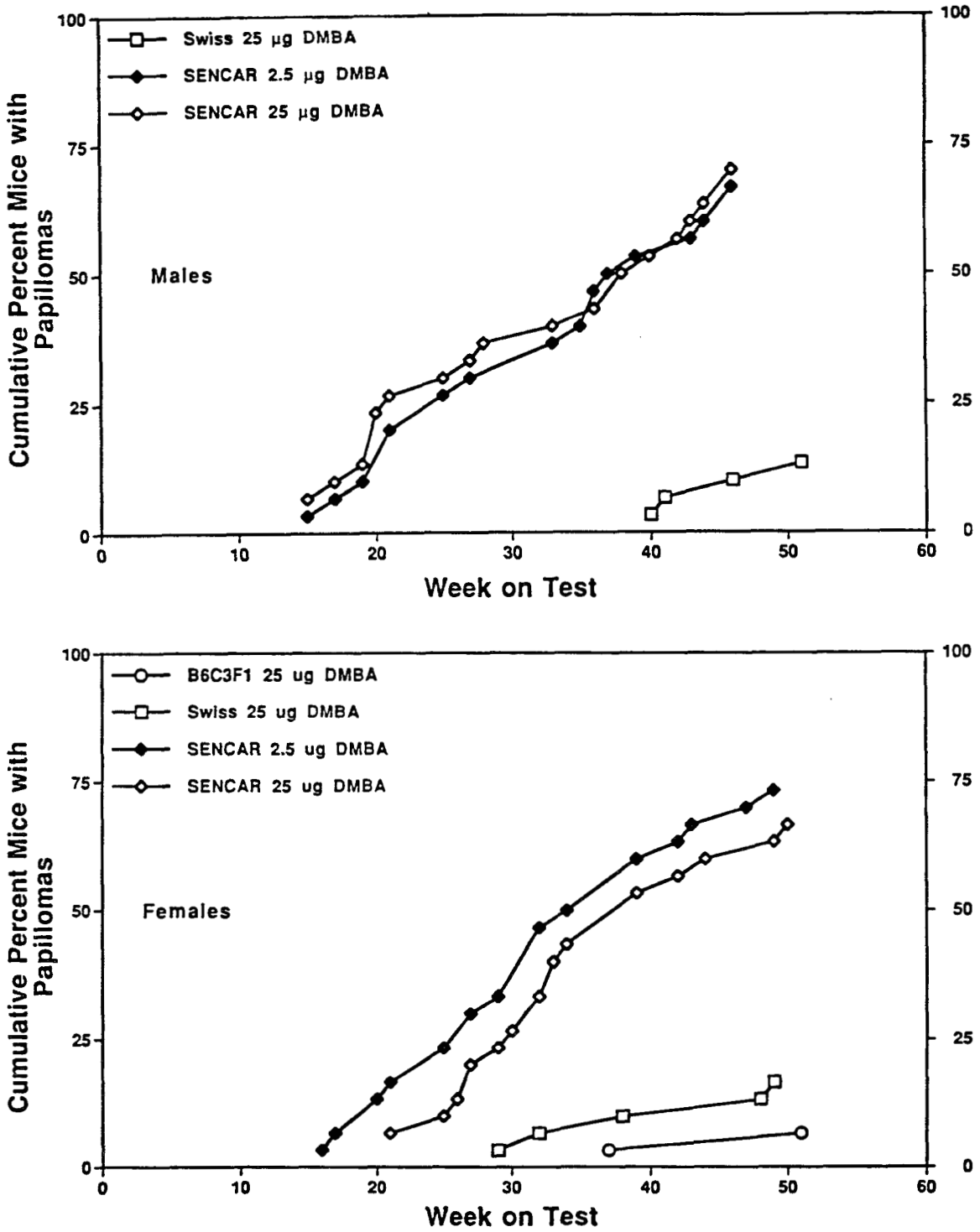


FIGURE 3
Tumor Response of the Three Mouse Strains Initiated with 2.5 or 25 µg DMBA Followed by 20 mg BPO Promotion

The average number of papillomas per mouse was calculated two ways as described previously, and these averages are presented in Table 14. The data used to calculate tumor multiplicity were collected at 10-week intervals over the course of the study. SENCAR mice receiving initiating concentrations of 2.5 μg and 25 μg DMBA averaged similar numbers of papillomas per mouse. SENCAR mice averaged more papillomas per mouse than did B6C3F$_1$ or Swiss (CD-1®) mice.

TABLE 14
Papillomas Observed During the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and BPO Promotion

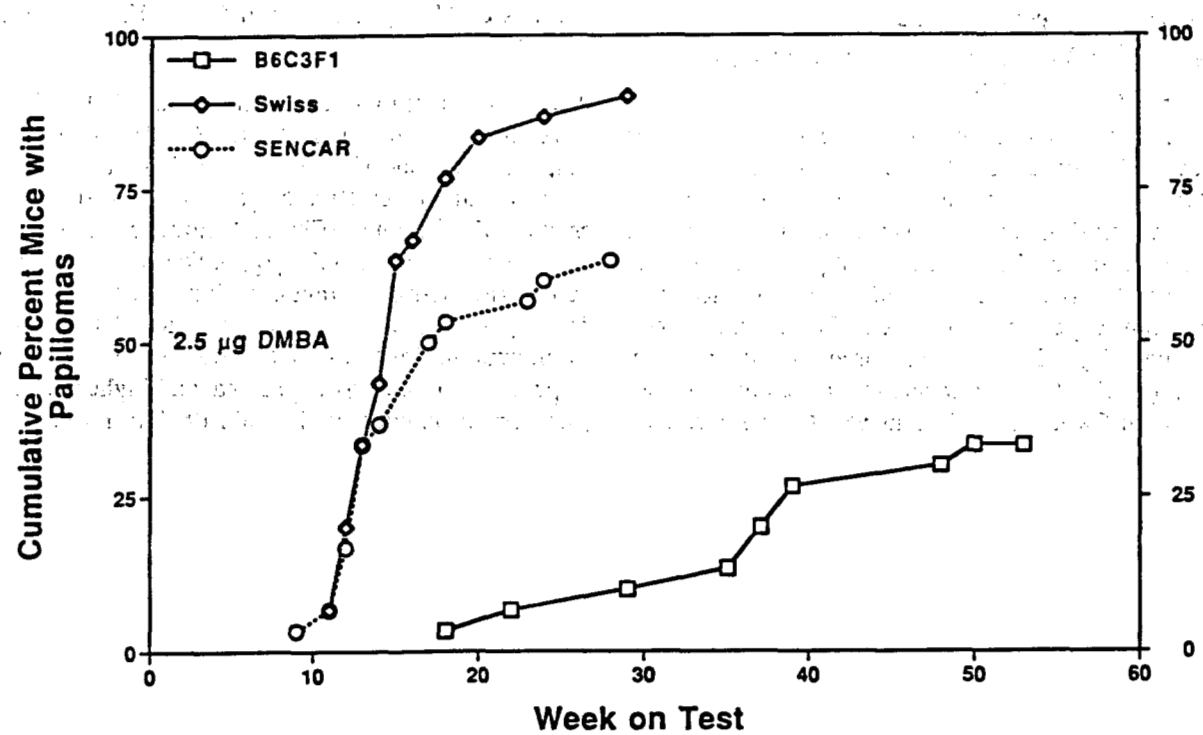
Week of Study	10		20		30		40		50	
B6C3F$_1$										
Male										
2.5 μg DMBA/20 mg BPO	0[a]	0[b]	0	0	0	0	0	0	1.00	0.04
25 μg DMBA/20 mg BPO	0	0	0	0	0	0	1.00	0.03	0	0
Female										
2.5 μg DMBA/20 mg BPO	0	0	0	0	0	0	0	0	1.00	0.07
25 μg DMBA/20 mg BPO	0	0	0	0	0	0	1.00	0.03	1.00	0.03
Swiss (CD-1®)										
Male										
2.5 μg DMBA/20 mg BPO	0	0	0	0	0	0	0	0	0	0
25 μg DMBA/20 mg BPO	0	0	0	0	0	0	1.00	0.04	1.00	0.08
Female										
2.5 μg DMBA/20 mg BPO	0	0	0	0	0	0	0	0	0	0
25 μg DMBA/20 mg BPO	0	0	0	0	1.00	0.03	1.50	0.10	1.50	0.20
SENCAR										
Male										
2.5 μg DMBA/20 mg BPO	0	0	3.00	0.31	4.00	1.24▲▼	2.21	1.07▲▼	2.73	1.64▲▼
25 μg DMBA/20 mg BPO	0	0	1.43	0.33▲▼	3.00	1.03▲▼	3.60	1.38▲▼	3.20	1.68▲▼
Female										
2.5 μg DMBA/20 mg BPO	0	0	1.50	0.20▲▼	3.20	1.10▲▼	3.06	1.86▲▼	1.69	1.23▲▼
25 μg DMBA/20 mg BPO	0	0	0	0	1.88	0.54▲▼	3.07	1.70▲▼	3.00	1.96▲▼

▲ Significantly different (P≤0.05) from similarly treated B6C3F$_1$ groups by the Mann-Whitney U test
▼ Significantly different (P≤0.05) from similarly treated Swiss (CD-1®) groups by the Mann-Whitney U test
[a] Average number of papillomas expressed as the total number of papillomas/number of mice with papillomas
[b] Average number of papillomas expressed as the total number of papillomas/number of mice surviving

Comparison of the Mouse Strain Response to DMBA as an Initiator and TPA or BPO as a Promoter

DMBA/TPA: The response of the three strains of mice to 2.5 or 25 μg DMBA initiation and 1 μg (SENCAR) or 5 μg [B6C3F$_1$ and Swiss (CD-1®)] TPA promotion over the 1-year study is shown in Figures 4 and 5. The incidences of papilloma in male and female Swiss (CD-1®) and SENCAR mice were significantly greater than those in B6C3F$_1$ mice when 2.5 and 25 μg DMBA were used as the initiation doses (Table 15). The incidences of papilloma were similar in Swiss (CD-1®) and SENCAR mice, even though Swiss (CD-1®) mice received higher concentrations of TPA (5 μg) than did the SENCAR mice (1 μg).

The in-life papilloma observations for DMBA/TPA mice are summarized in Table 16. The papilloma response of B6C3F$_1$ mice receiving the highest concentration of DMBA (50 μg) was most similar to Swiss (CD-1®) and SENCAR mice receiving the lowest concentration (0.25 μg) of DMBA. At equivalent initiating concentrations of DMBA, Swiss (CD-1®) and SENCAR mice had higher papilloma incidences, shorter group mean time to the appearance of the first papilloma, and higher average numbers of papillomas than did B6C3F$_1$ mice.



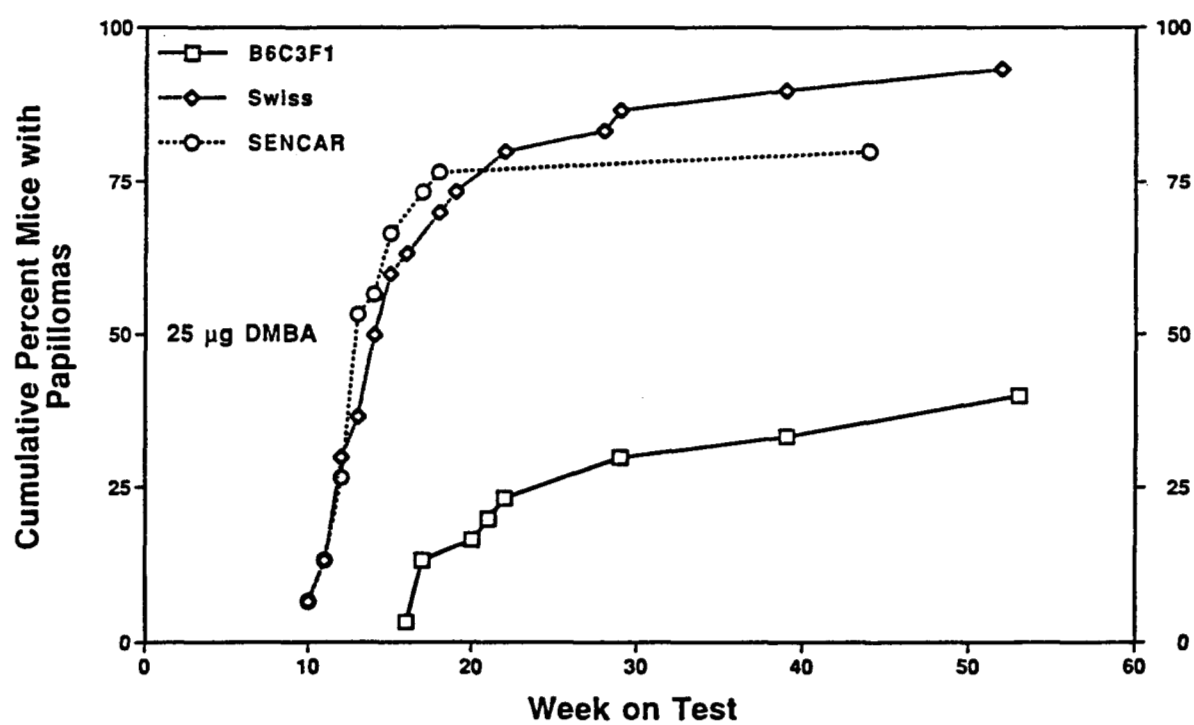
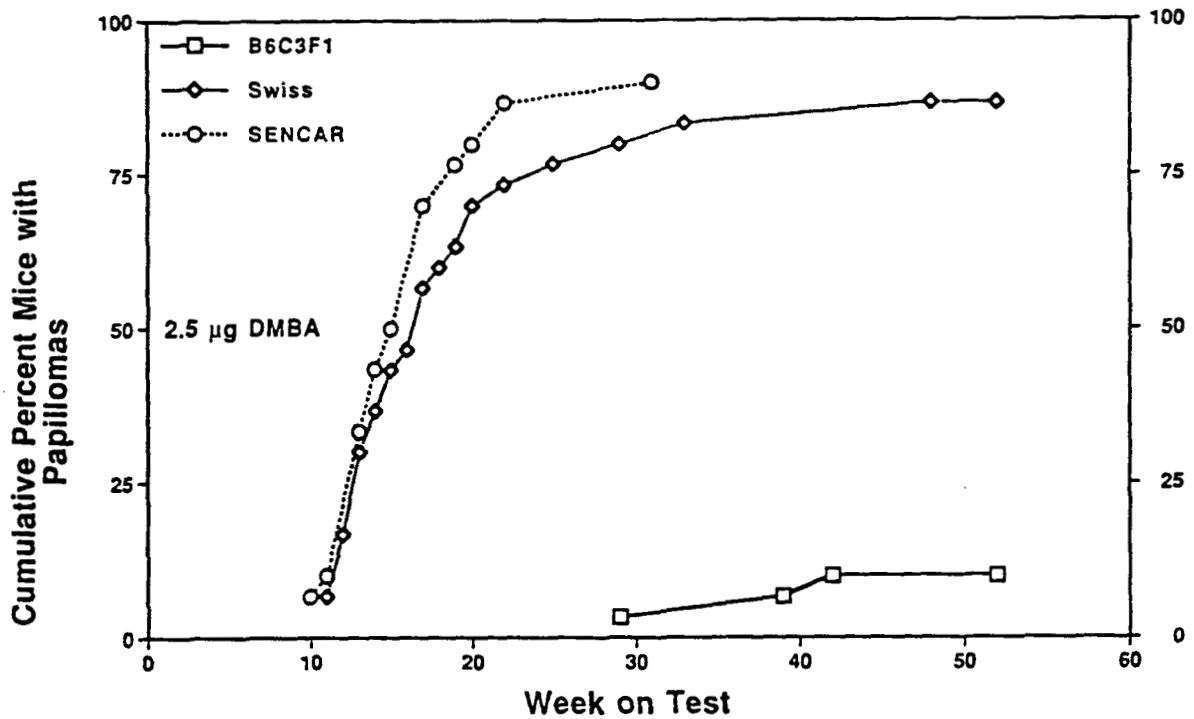


FIGURE 4
Tumor Response of Male Mice of the Three Strains Initiated with 2.5 or 25 µg DMBA Followed by 5 µg [B6C3F$_1$ and Swiss (CD-1®)] or 1 µg (SENCAR) TPA Promotion



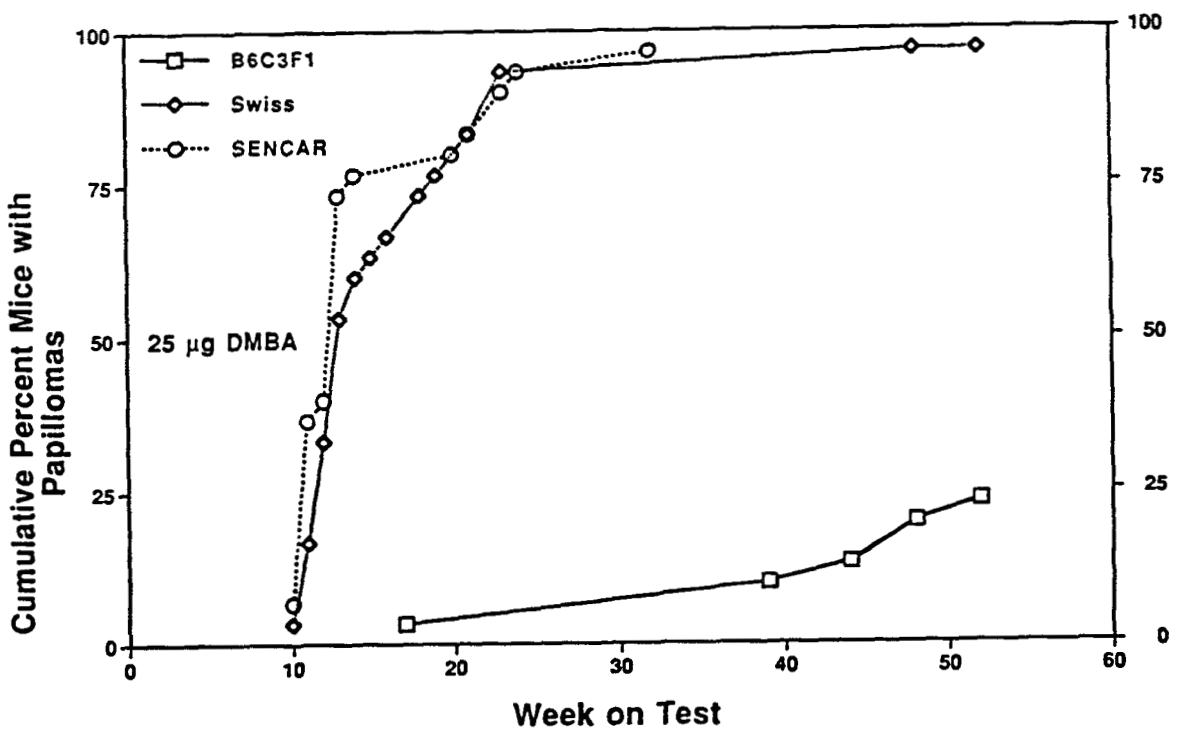


FIGURE 5
Tumor Response of Female Mice of the Three Strains Initiated with 2.5 or 25 µg DMBA Followed by 5 µg [B6C3F$_1$ and Swiss (CD-1®)] or 1 µg (SENCAR) TPA Promotion

TABLE 15
Comparisons of Papilloma Response Between B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: *DMBA Initiation and TPA Promotion*

	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Male			
2.5 μg DMBA/TPA[a]			
Overall rate[b]	10/30	27/30	19/30
Life table test[c]		P<0.001	P<0.001
Life table test[d]			P=0.106N
25 μg DMBA/TPA			
Overall rate	12/30	28/30	24/30
Life table test		P<0.001	P<0.001
Life table test			P=0.484N
Female			
2.5 μg DMBA/TPA			
Overall rate	3/30	26/30	27/30
Life table test		P<0.001	P<0.001
Life table test			P=0.119
25 μg DMBA/TPA			
Overall rate	7/30	29/30	29/30
Life table test		P<0.001	P<0.001
Life table test			P=0.208

[a] B6C3F$_1$ mice and Swiss (CD-1®) mice received 5 μg TPA; SENCAR mice received 1 μg TPA.
[b] Number of animals with tumor per number of animals with skin examined in-life
[c] P values correspond to pairwise comparisons with B6C3F$_1$ mice.
[d] P values correspond to pairwise comparisons Swiss (CD-1®) mice. A lower incidence in a dose group is indicated by N.

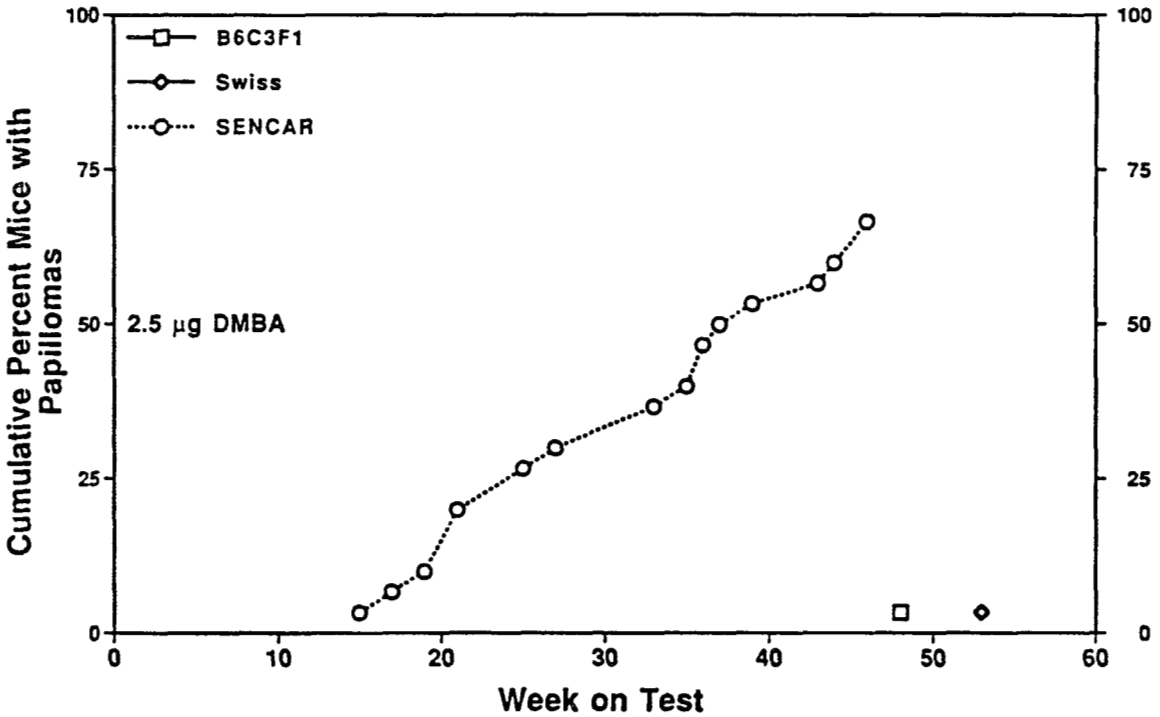
TABLE 16
Overview of DMBA/TPA Sensitivity in the Comparative Initiation/Promotion Skin Paint Studies

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
B6C3F$_1$				
Male				
2.5 µg DMBA/5 µg TPA	10/30	35.4	0.57	1.70
25 µg DMBA/5 µg TPA	12/30	27.6	1.13	2.83
50 µg DMBA/5 µg TPA	22/30	32.5	1.95	2.66
Female				
2.5 µg DMBA/5 µg TPA	3/30	36.7	0.27	2.67
25 µg DMBA/5 µg TPA	7/30	41.0	0.37	1.57
50 µg DMBA/5 µg TPA	16/30	40.8	1.10	2.06
Swiss (CD-1®)				
Male				
0.25 µg DMBA/5 µg TPA	18/30	27.7	1.07	1.78
2.5 µg DMBA/5 µg TPA	27/30	15.4	4.63	5.15
25 µg DMBA/5 µg TPA	28/30	17.6	5.60	6.00
Female				
0.25 µg DMBA/5 µg TPA	18/30	26.5	1.43	2.39
2.5 µg DMBA/5 µg TPA	26/30	18.0	3.77	4.35
25 µg DMBA/5 µg TPA	29/30	16.0	7.10	7.34
SENCAR				
Male				
0.25 µg DMBA/1 µg TPA	12/30	17.2	1.10	2.75
2.5 µg DMBA/1 µg TPA	19/30	15.5	3.03	4.79
25 µg DMBA/1 µg TPA	24/30	14.5	6.77	8.46
Female				
0.25 µg DMBA/1 µg TPA	13/30	23.5	0.87	2.00
2.5 µg DMBA/1 µg TPA	27/30	15.9	5.23	5.81
25 µg DMBA/1 µg TPA	29/30	14.4	9.23	9.55

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

DMBA/BPO: The responses of the three strains of mice to 2.5 or 25 μg DMBA initiation (doses in common) and BPO promotion over the 1-year study are shown in Figures 6 and 7. SENCAR mice were the most responsive to this combination of chemicals. The incidence of papilloma in SENCAR mice was significantly greater than that in $B6C3F_1$ and Swiss (CD-1®) mice for both initiation concentrations of DMBA. The papilloma response was similar in both male and female $B6C3F_1$ and Swiss (CD-1®) mice that received 2.5 μg DMBA, but was higher in male Swiss (CD-1®) mice than in $B6C3F_1$ males for groups receiving 25 μg DMBA (Table 17).

The in-life papilloma observations for DMBA/BPO mice are summarized in Table 18. In addition to a higher papilloma incidence, SENCAR mice had shorter mean group time to first papilloma, and a greater average number of tumors than did the $B6C3F_1$ or Swiss (CD-1®) mice.



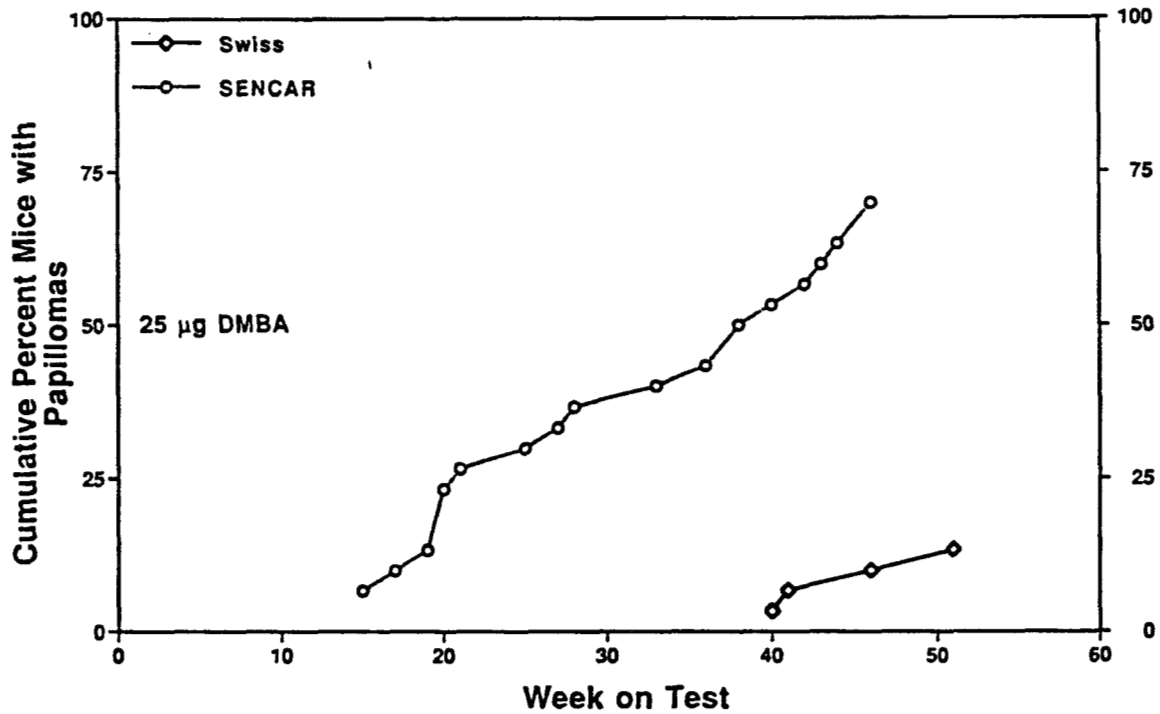


FIGURE 6
Tumor Response of Male Mice of the Three Strains Initiated with 2.5 or 25 µg DMBA Followed by 20 mg BPO Promotion

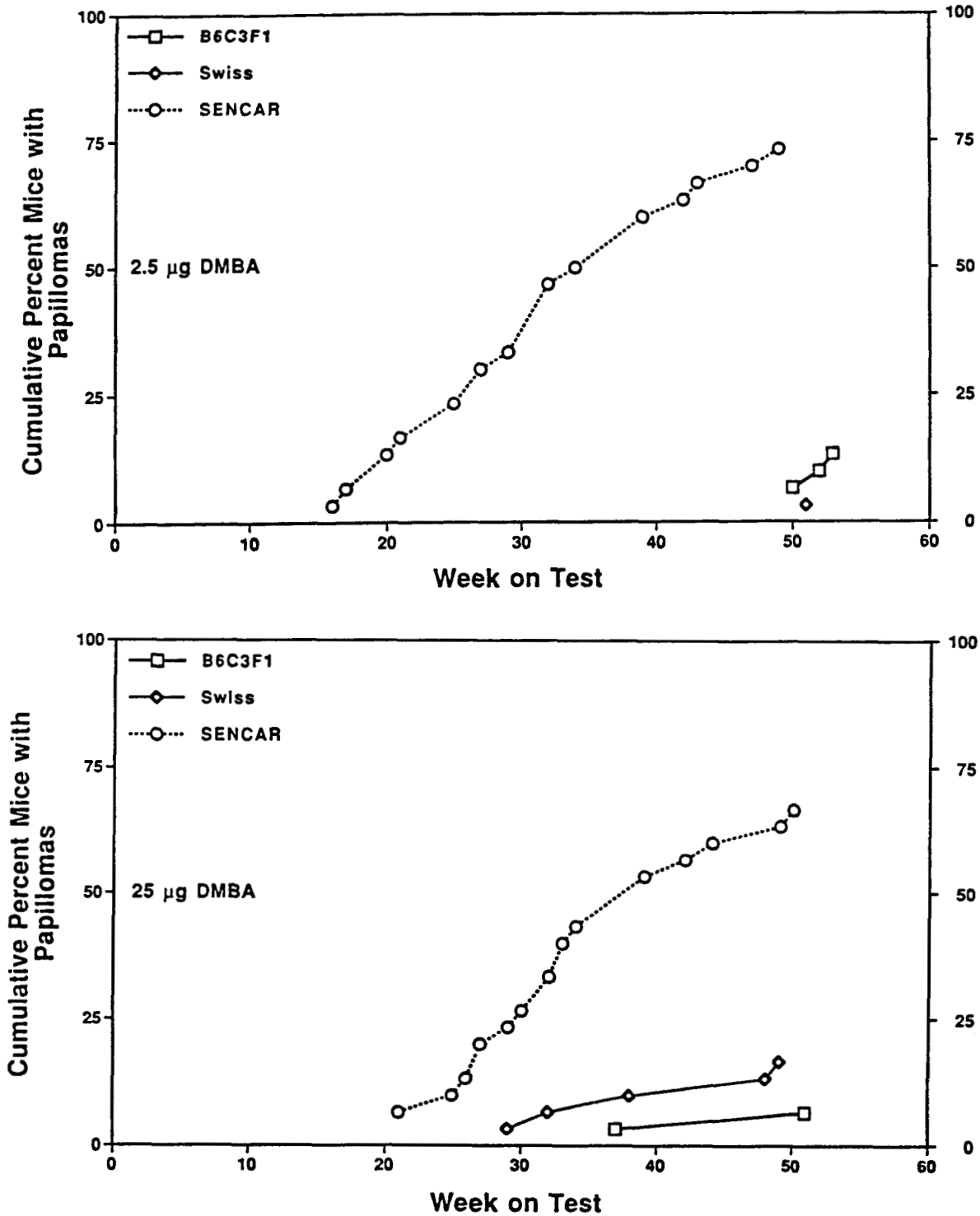


FIGURE 7
Tumor Response of Female Mice of the Three Strains Initiated with 2.5 or 25 µg DMBA Followed by 20 mg BPO Promotion

TABLE 17
Comparisons of Papilloma Response Between B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: DMBA Initiation and BPO Promotion

	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Male			
2.5 μg DMBA/20 mg BPO			
Overall rate[a]	1/30	1/30	20/30
Life table test[b]		P=0.757	P<0.001
Life table test[c]			P<0.001
25 μg DMBA/20 mg BPO			
Overall rate	1/30	6/30	22/30
Life table test		P=0.032	P<0.001
Life table test			P<0.001
Female			
2.5 μg DMBA/20 mg BPO			
Overall rate	4/30	1/30	22/30
Life table test		P=0.175N	P<0.001
Life table test			P<0.001
25 μg DMBA/20 mg BPO			
Overall rate	2/30	5/30	20/30
Life table test		P=0.212	P<0.001
Life table test			P<0.001

[a] Number of animals with tumor per number of animals with skin examined in-life
[b] P values correspond to pairwise comparisons with B6C3F$_1$ mice. A lower incidence in a dose group is indicated by N.
[c] P values correspond to pairwise comparisons with Swiss (CD-1®) mice.

TABLE 18
Overview of DMBA/BPO Sensitivity in the Comparative Initiation/Promotion Skin Paint Studies

	Number of Mice with Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
B6C3F$_1$				
Male				
2.5 μg DMBA/20 mg BPO	1/30	48.0	0.03	1.00
25 μg DMBA/20 mg BPO	1/30	39.0	0.03	1.00
Female				
2.5 μg DMBA/20 mg BPO	4/30	51.0	0.13	1.00
25 μg DMBA/20 mg BPO	2/30	44.0	0.07	1.00
Swiss (CD-1®)				
Male				
2.5 μg DMBA/20 mg BPO	1/30	52.0	0.03	1.00
25 μg DMBA/20 mg BPO	6/30	47.0	0.20	1.00
Female				
2.5 μg DMBA/20 mg BPO	1/30	51.0	0.03	1.00
25 μg DMBA/20 mg BPO	5/30	39.2	0.30	1.80
SENCAR				
Male				
2.5 μg DMBA/20 mg BPO	20/30	31.0	2.40	3.60
25 μg DMBA/20 mg BPO	22/30	31.0	2.77	3.77
Female				
2.5 μg DMBA/20 mg BPO	22/30	31.2	2.33	3.18
25 μg DMBA/20 mg BPO	20/30	33.6	2.50	3.75

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

Study Design B

Protocol Control Groups

In study design B, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was used as the initiator with TPA and BPO as promoters. As in study design A, a number of initiator and promoter control groups were included as a measure of how well the mouse skin initiation/promotion model functioned. These control groups included groups of male and female mice of each strain that received the acetone vehicle alone, as well as groups that received a single application of one of three concentrations of MNNG (100, 500, or 1,000 μg/mouse) followed by repeated applications of the non-promoter acetone, and groups that received repeated applications of either TPA [5 μg/mouse in B6C3F$_1$ and Swiss (CD-1®) and 1 μg/mouse in SENCAR] or BPO (20 mg/mouse) without MNNG initiation (Table 2).

Survival of Protocol Controls

Survival of mice receiving 1,000 μg MNNG followed by the non-promoter acetone was significantly lower than that of the vehicle controls for male and female mice of all strains except male B6C3F$_1$ mice (Table 19). Survival of male Swiss (CD-1®) and SENCAR mice receiving 500 μg MNNG followed by acetone was significantly lower than that of the vehicle controls. Survival of male and female SENCAR mice and male Swiss (CD-1®) mice receiving repeated applications of TPA was also significantly lower than that of the vehicle controls.

Body Weights and Clinical Findings in Protocol Controls

Final mean body weights of male and female Swiss (CD-1®), male B6C3F$_1$, and female SENCAR mouse protocol control groups were similar to those of the respective vehicle controls (Table 19). The final mean body weights of female B6C3F$_1$ mice receiving repeated applications of TPA or BPO were lower than those of the vehicle controls. The final mean body weight of SENCAR males receiving 1,000 μg MNNG followed by acetone was lower than that of the vehicle controls.

Table 19
Survival and Body Weights of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design B

Initiator/Promoter	Survival[a]	Final Mean Body Weight[b] (g)	Final Weight Relative to Controls (%)
B6C3F$_1$			
Male			
Acetone/Acetone	30/30	47.5 ± 0.7	
100 μg MNNG/Acetone	30/30	47.9 ± 0.5	101
500 μg MNNG/Acetone	29/30	47.6 ± 0.7	100
1,000 μg MNNG/Acetone	28/30	45.8 ± 0.9	96
5 μg TPA/5 μg TPA	30/30	48.1 ± 0.6	101
20 mg BPO/20 mg BPO	30/30	46.8 ± 0.8	99
Female			
Acetone/Acetone	30/30	47.9 ± 1.2	
100 μg MNNG/Acetone	30/30	47.3 ± 1.2	99
500 μg MNNG/Acetone	30/30	46.5 ± 1.1	97
1,000 μg MNNG/Acetone	23/30*	46.5 ± 1.1	97
5 μg TPA/5 μg TPA	30/30	45.0 ± 0.7▲	94
20 mg BPO/20 mg BPO	29/30	43.4 ± 0.9▲▲	91

(continued)

TABLE 19
Survival and Body Weights of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design B (continued)

Initiator/Promoter	Survival	Final Mean Body Weight (g)	Final Weight Relative to Controls (%)
Swiss (CD-1®)			
Male			
Acetone/Acetone	27/30	50.7 ± 1.3	
100 μg MNNG/Acetone	28/30	50.6 ± 1.4	100
500 μg MNNG/Acetone	17/30**	51.6 ± 1.6	102
1,000 μg MNNG/Acetone	9/30**	48.5 ± 2.9	96
5 μg TPA/5 μg TPA	17/30**	48.1 ± 1.4	95
20 mg BPO/20 mg BPO	20/30	48.4 ± 1.6	95
Female			
Acetone/Acetone	27/30	39.0 ± 1.1	
100 μg MNNG/Acetone	29/30	39.6 ± 1.0	102
500 μg MNNG/Acetone	26/30	39.8 ± 1.0	102
1,000 μg MNNG/Acetone	15/30**	37.9 ± 1.7	97
5 μg TPA/5 μg TPA	23/30	37.4 ± 1.3	96
20 mg BPO/20 mg BPO	29/30	40.7 ± 1.1	104
SENCAR			
Male			
Acetone/Acetone	29/30	52.0 ± 1.2	
Acetone/Acetone[c]	29/30	51.7 ± 1.4	
100 μg MNNG/Acetone	28/30	51.6 ± 1.2	99
500 μg MNNG/Acetone	16/30**	48.6 ± 1.5	93
1,000 μg MNNG/Acetone	7/30**	45.5 ± 2.2▲	87
1 μg TPA/1 μg TPA	9/30**	48.8 ± 2.8	94
20 mg BPO/20 mg BPO	26/30	52.5 ± 1.0	101
Female			
Acetone/Acetone	26/30	44.9 ± 1.4	
Acetone/Acetone[c]	27/30	45.7 ± 1.7	
100 μg MNNG/Acetone	30/30	44.3 ± 1.3	99
500 μg MNNG/Acetone	22/30	42.6 ± 1.4	95
1,000 μg MNNG/Acetone	11/30**	43.8 ± 1.6	98
1 μg TPA/1 μg TPA	10/30**	44.1 ± 2.6	97
20 mg BPO/20 mg BPO	27/30	46.2 ± 1.8	103

* Significantly different (P≤0.05) from the vehicle control group by life table pairwise comparison
** Significantly different (P≤0.01) from the vehicle control group by life table pairwise comparison
▲ Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
▲▲ Significantly different (P≤0.01) from the vehicle control group by Dunnett's test
[a] Number of animals surviving/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[b] Weights are given as mean ± standard error.
[c] Vehicle control group for TPA restart

The primary clinical findings were irritation and ulcers. Irritation was defined as the skin being inflamed or appearing sore. Ulcer was defined as localized skin surface excavation and/or broken skin. Incidences of irritation and ulcer are presented as weekly averages for the first 10 weeks of the study and as weekly averages at 10-week intervals for the remaining 40 weeks (Table 20). In male and female $B6C3F_1$ and SENCAR mice receiving 100 μg MNNG initiation, the incidences of irritation and ulcer were zero or near zero for the duration of the study. Some male and female Swiss (CD-1®) mice receiving 100 μg MNNG initiation followed by acetone developed irritation that peaked around weeks 2 to 3 and then decreased to zero by weeks 7 to 8. Between weeks 2 and 4, 7% of the 100 μg MNNG Swiss (CD-1®) males were observed with ulcers, but no ulcers were present after week 5. Female Swiss (CD-1®) mice receiving 100 μg MNNG initiation never developed ulcers. The percent incidence of irritation in all strains and ulcer in Swiss (CD-1®) and SENCAR mice exhibited a dose-related increase at initiating concentrations of 500 and 1,000 μg MNNG.

Tumor Response in Protocol Controls

Microscopic evaluation of skin at the site of application revealed no tumors in the vehicle controls (Table 21). The incidences of skin tumors in any group receiving 100 μg MNNG or in male and female $B6C3F_1$ mice receiving 500 μg MNNG followed by acetone were similar to those of the vehicle controls. The incidences of skin tumors in Swiss (CD-1®) and SENCAR mice receiving 500 μg MNNG and in mice of all three strains receiving 1,000 μg MNNG were significantly greater than those of the vehicle controls.

In all three strains, the incidences of skin tumors in mice receiving repeated applications of BPO were similar to those of the respective vehicle controls. The incidences of skin tumors in male and female $B6C3F_1$ and SENCAR mice receiving repeated applications of TPA were similar to those of vehicle controls. However, the incidences of skin tumors in male and female Swiss (CD-1®) mice receiving repeated applications of TPA were greater than those of the vehicle controls (Table 21).

TABLE 20
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design B

Week of Study	1	2	3	4	5	6	7	8	9	10	11-20	21-30	31-40	41-50
Male														
Irritation														
B6C3F$_1$														
100 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	1
500 µg MNNG/Acetone	0	0	3	0	0	0	0	0	0	0	0	0	0	2
1,000 µg MNNG/Acetone	0	0	33	20	0	0	0	0	0	0	0	0	9	20
5 µg TPA/5 µg TPA	0	0	0	0	0	0	0	0	0	0	0	0	2	5
20 mg BPO/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Swiss (CD-1®)														
100 µg MNNG/Acetone	3	47	37	30	20	3	3	0	0	0	0	1	0	4
500 µg MNNG/Acetone	3	87	100	100	100	87	63	43	43	31	25	20	16	5
1,000 µg MNNG/Acetone	17	97	100	97	97	87	67	57	50	53	36	23	17	3
5 µg TPA/5 µg TPA	3	31	41	69	93	100	66	38	38	43	52	34	20	15
20 mg BPO/20 mg BPO	0	7	10	20	43	27	7	7	7	7	13	13	8	3
SENCAR														
100 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	2	1
500 µg MNNG/Acetone	0	27	23	30	10	3	3	0	7	3	11	16	13	21
1,000 µg MNNG/Acetone	0	13	20	23	20	20	28	28	34	45	43	34	45	42
1 µg TPA/1 µg TPA	0	0	0	3	0	7	7	23	23	10	12	5	8	7
20 mg BPO/20 mg BPO	0	0	0	0	0	0	3	3	3	3	4	3	0	2
Ulcer														
B6C3F$_1$														
100 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1,000 µg MNNG/Acetone	0	0	0	3	0	0	0	0	0	0	0	0	0	0
5 µg TPA/5 µg TPA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 mg BPO/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swiss (CD-1®)														
100 µg MNNG/Acetone	0	3	7	7	0	0	0	0	0	0	0	0	0	1
500 µg MNNG/Acetone	0	30	73	67	53	43	37	33	33	31	30	25	5	1
1,000 µg MNNG/Acetone	0	43	87	83	67	47	47	43	40	37	43	27	7	1
5 µg TPA/5 µg TPA	0	7	3	7	3	3	3	3	3	4	14	21	24	20
20 mg BPO/20 mg BPO	0	0	0	3	3	3	3	0	3	3	5	6	3	1
SENCAR														
100 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500 µg MNNG/Acetone	0	20	7	10	7	3	3	7	7	3	7	14	6	13
1,000 µg MNNG/Acetone	0	60	17	43	47	40	31	34	41	45	39	26	11	10
1 µg TPA/1 µg TPA	0	0	0	0	0	0	0	0	0	0	23	54	51	31
20 mg BPO/20 mg BPO	0	0	0	0	0	0	0	0	0	0	2	4	0	0

(continued)

TABLE 20
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design B (continued)

Week of Study	1	2	3	4	5	6	7	8	9	10	11-20	21-30	31-40	41-50
Female														
Irritation														
B6C3F$_1$														
100 µg MNNG/Acetone	10	0	0	0	0	0	0	0	0	0	0	0	0	0
500 µg MNNG/Acetone	70	7	3	0	0	0	0	0	0	0	0	0	0	0
1,000 µg MNNG/Acetone	93	80	47	13	7	3	3	0	0	0	2	2	3	6
5 µg TPA/5 µg TPA	90	43	10	0	0	0	0	0	0	0	0	0	0	1
20 mg BPO/20 mg BPO	7	0	0	0	0	0	0	0	0	0	0	0	3	4
Swiss (CD-1®)														
100 µg MNNG/Acetone	0	27	43	17	7	3	0	0	0	0	0	0	0	0
500 µg MNNG/Acetone	20	93	97	93	97	87	48	24	7	10	5	3	3	0
1,000 µg MNNG/Acetone	40	83	100	100	100	97	90	41	34	34	25	22	11	5
5 µg TPA/5 µg TPA	7	10	73	87	80	77	67	47	17	20	22	21	19	22
20 mg BPO/20 mg BPO	0	7	40	20	20	23	0	0	0	0	0	1	2	3
SENCAR														
100 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	1
500 µg MNNG/Acetone	0	13	3	0	0	0	0	0	0	0	0	2	5	6
1,000 µg MNNG/Acetone	10	37	7	10	17	10	3	7	17	27	21	16	15	20
1 µg TPA/1 µg TPA	0	0	7	0	3	10	10	20	23	20	14	9	10	1
20 mg BPO/20 mg BPO	0	0	0	0	0	0	0	0	9	3	3	0	1	2
Ulcer														
B6C3F$_1$														
100 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1,000 µg MNNG/Acetone	0	0	3	7	3	3	0	3	3	3	3	3	1	1
5 µg TPA/5 µg TPA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 mg BPO/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	2	4
Swiss (CD-1®)														
100 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500 µg MNNG/Acetone	3	57	43	30	17	13	10	10	10	10	4	2	0	0
1,000 µg MNNG/Acetone	13	80	63	67	63	50	45	41	31	31	34	16	1	2
5 µg TPA/5 µg TPA	0	0	0	0	0	0	0	0	0	0	5	15	13	14
20 mg BPO/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	3	4
SENCAR														
100 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500 µg MNNG/Acetone	0	0	0	0	0	0	0	0	0	0	0	0	1	0
1,000 µg MNNG/Acetone	0	7	3	3	3	3	13	13	17	20	20	10	9	7
1 µg TPA/1 µg TPA	0	0	0	0	0	0	0	0	3	0	25	32	21	13
20 mg BPO/20 mg BPO	0	0	0	0	0	0	3	0	0	0	1	0	0	0

TABLE 21
Incidences of Skin Tumors in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Protocol Controls for Study Design B[a]

	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Male			
Acetone/Acetone	0/30	0/30	0/30
Acetone/Acetone[b]	–[c]	–	0/30
100 μg MNNG/Acetone	0/30	1/30	2/30
500 μg MNNG/Acetone	1/30	7/30**	19/30**
1,000 μg MNNG/Acetone	13/30**	15/30**	22/30**
20 mg BPO/20 mg BPO	0/30	0/30	0/30
5 μg TPA/5 μg TPA	0/30	4/30*	–
1 μg TPA/1 μg TPA[d]	–	–	2/30
Female			
Acetone/Acetone	0/30	0/30	0/30
Acetone/Acetone[b]	–	–	0/29
100 μg MNNG/Acetone	0/30	0/30	0/30
500 μg MNNG/Acetone	0/30	6/29*	8/30**
1,000 μg MNNG/Acetone	13/30**	9/30**	25/30**
20 mg BPO/20 mg BPO	0/30	0/30	1/30
5 μg TPA/5 μg TPA	0/30	8/28**	–
1 μg TPA/1 μg TPA[d]	–	–	0/30

* Significantly different (P≤0.05) from the vehicle control group by life table pairwise comparison
** P≤0.01
[a] Number of animals with tumors per number of animals with skin examined in-life. Incidences represent clinical observations of papilloma.
[b] Vehicle control for TPA restart (SENCAR mice)
[c] Dose level not administered to this strain
[d] SENCAR restart group

Because of the high incidences of irritation, ulcer, and skin tumors in the 1,000 μg MNNG initiator protocol control mice and the high incidence of skin tumors in 500 μg MNNG SENCAR protocol control mice, these groups did not meet the criteria for the initiator subthreshold carcinogenic dose. Therefore, groups receiving 1,000 μg MNNG and SENCAR mice receiving 500 μg MNNG were excluded from the evaluation of mouse strain skin tumor sensitivity in the initiation/promotion studies.

MNNG Initiation and TPA Promotion

Survival

Survival of male Swiss (CD-1®) and both male and female SENCAR mice receiving 100 μg MNNG was lower than that of the respective vehicle controls; all other 100 μg MNNG/TPA groups were similar to their respective vehicle controls (Table 22). Survival of all mice receiving 1,000 μg MNNG/TPA was significantly lower than that of the respective vehicle controls. Many of the mice in these groups were removed from the study in accordance with the moribund sacrifice policy.

Body Weights and Clinical Findings

The final mean body weight of female $B6C3F_1$ mice receiving 100 μg MNNG/TPA was significantly lower than that of the vehicle controls; final mean body weights of all other 100 μg MNNG/TPA groups were similar to those of the respective vehicle controls (Table 22).

The primary clinical findings included irritation and ulcer. Irritation was defined as inflamed skin or sore appearance. Ulcer was defined as localized skin surface excavation and/or broken skin. In all strains, the percentage of mice with signs of irritation peaked between weeks 2 and 3 and fell to zero or near zero for male and female $B6C3F_1$ and SENCAR mice (Table 23). Signs of irritation were observed in a higher percentage of male and female Swiss (CD-1®) than in either $B6C3F_1$ or SENCAR mice. This irritation decreased during the duration of the study but still involved more than 38% of the male and 20% of the female Swiss (CD-1®) mice. The percentage of mice with irritation was similar to that of mice receiving TPA alone without initiation (Tables 20 and 23).

TABLE 22
Survival and Body Weights of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and TPA Promotion

Initiator/Promoter	Survival[a]	Final Mean Body Weight[b] (g)	% Relative to Controls
B6C3F$_1$			
Male			
Acetone/Acetone	30/30	47.5 ± 0.7	
5 µg TPA/5 µg TPA	30/30	48.1 ± 0.6	101
100 µg MNNG/5 µg TPA	29/30	45.9 ± 0.7	97
1,000 µg MNNG/5 µg TPA	16/30**	44.1 ± 1.1▲▲	93
Female			
Acetone/Acetone	30/30	47.9 ± 1.2	
5 µg TPA/5 µg TPA	30/30	45.0 ± 0.7▲	94
100 µg MNNG/5 µg TPA	29/30	44.5 ± 0.9▲	93
1,000 µg MNNG/5 µg TPA	22/30**	42.9 ± 1.1▲▲	90
Swiss (CD-1®)			
Male			
Acetone/Acetone	27/30	50.7 ± 1.3	
5 µg TPA/5 µg TPA	17/30**	48.1 ± 1.4	95
100 µg MNNG/5 µg TPA	10/30**	46.1 ± 2.3	91
1,000 µg MNNG/5 µg TPA	4/30**	40.0 ± 4.8	79
Female			
Acetone/Acetone	27/30	39.0 ± 1.1	
5 µg TPA/5 µg TPA	23/30	37.4 ± 1.3	96
100 µg MNNG/5 µg TPA	22/30	40.1 ± 1.1	103
1,000 µg MNNG/5 µg TPA	14/30**	37.2 ± 1.0	95
SENCAR			
Male			
Acetone/Acetone	29/30	51.7 ± 1.4	
1 µg TPA/1 µg TPA	9/30**	48.8 ± 2.8	94
100 µg MNNG/1 µg TPA	8/30**	51.1 ± 2.5	99
1,000 µg MNNG/1 µg TPA	1/30**	38.3[c]	74
Female			
Acetone/Acetone	27/30	45.7 ± 1.7	
1 µg TPA/1 µg TPA	10/30**	44.1 ± 2.6	97
100 µg MNNG/1 µg TPA	13/30**	43.2 ± 1.7	94
1,000 µg MNNG/1 µg TPA	2/30**	42.9 ± 2.9	94

** Significantly different (P≤0.01) from the vehicle control group by life table pairwise comparison
▲ Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
▲▲ Significantly different (P≤0.01) from the vehicle control group by Dunnett's test
[a] Number of animals surviving/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[b] Weights are given as mean ± standard error.
[c] No standard error calculated due to high mortality

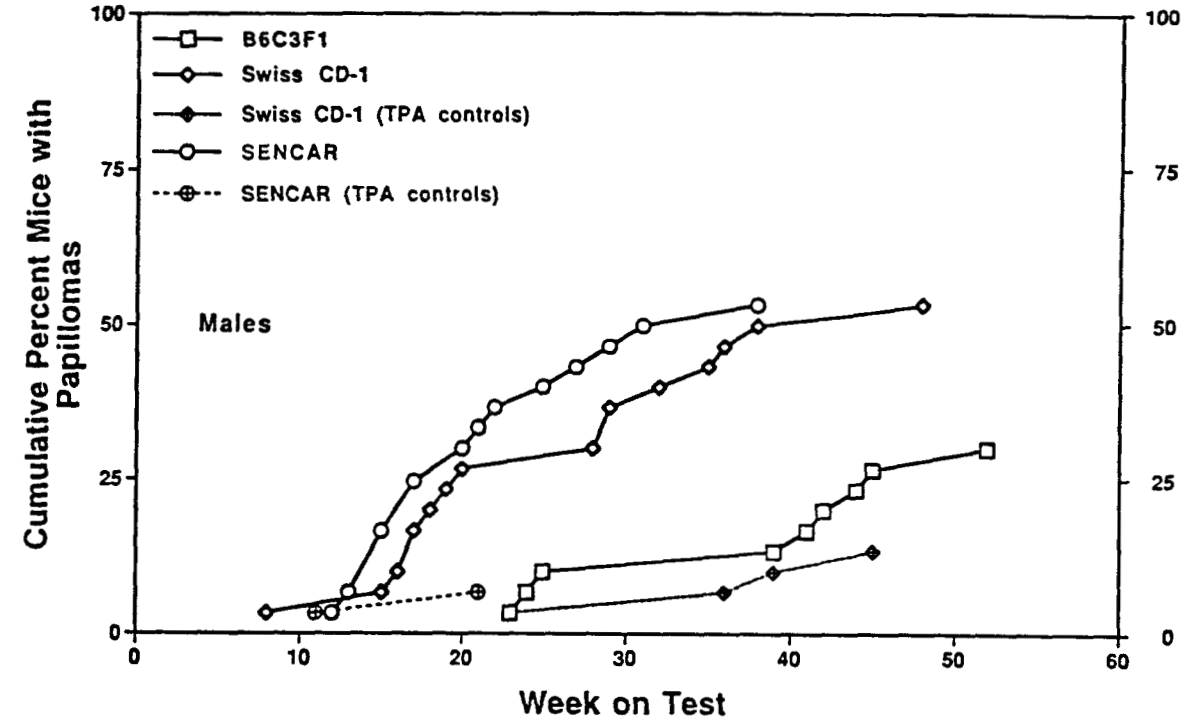
TABLE 23
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and TPA Promotion

Week of Study	1	2	3	4	5	6	7	8	9	10	11-20	21-30	31-40	41-50
Male														
Irritation														
B6C3F$_1$														
100 μg MNNG/5 μg TPA	0	0	40	3	0	3	0	0	0	0	0	0	3	7
Swiss (CD-1®)														
100 μg MNNG/5 μg TPA	0	53	90	97	97	93	83	33	53	47	58	42	41	38
SENCAR														
100 μg MNNG/1 μg TPA	0	0	17	0	0	0	0	0	0	0	6	7	8	1
Ulcer														
B6C3F$_1$														
100 μg MNNG/5 μg TPA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swiss (CD-1®)														
100 μg MNNG/5 μg TPA	0	10	43	27	13	10	10	7	7	13	27	42	29	24
SENCAR														
100 μg MNNG/1 μg TPA	0	0	0	0	0	0	0	0	0	0	15	37	53	43
Female														
Irritation														
B6C3F$_1$														
100 μg MNNG/5 μg TPA	0	30	10	0	0	0	0	0	0	0	0	0	0	1
Swiss (CD-1®)														
100 μg MNNG/5 μg TPA	0	67	90	83	93	73	63	50	30	23	20	23	22	28
SENCAR														
100 μg MNNG/1 μg TPA	0	0	10	0	0	0	0	0	3	0	7	5	3	0
Ulcer														
B6C3F$_1$														
100 μg MNNG/5 μg TPA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swiss (CD-1®)														
100 μg MNNG/5 μg TPA	0	10	0	0	0	0	0	0	0	0	6	6	21	28
SENCAR														
100 μg MNNG/1 μg TPA	0	0	0	0	0	0	0	0	0	0	12	25	27	34

Tumor Response

As in study design A, three parameters were used to measure the skin sensitivity of each mouse strain: the number of animals in each group that developed tumors, the group mean time to appearance of the first tumor, and the number of tumors per mouse. The response of male and female mice of the three strains initiated with 100 μg MNNG followed by repeated applications of TPA is shown in Figure 8. The papilloma response of male and female Swiss (CD-1®) and male SENCAR mice receiving TPA alone (non-initiated) is included for comparison because the tumor response for these groups was significantly higher than that of the vehicle controls, although male and female $B6C3F_1$ and female SENCAR mice receiving TPA alone did not develop papillomas. The response is displayed as the cumulative percentage of mice that developed skin papillomas over the course of the 1-year study. In some groups survival was reduced because of the aggressive moribund sacrifice policy maintained for this study. In addition, some tumors may have regressed or been removed by mechanical grooming. No mice were removed from the cumulative count. The papilloma response of male and female Swiss (CD-1®) and male SENCAR mice was markedly greater than that of the non-initiated TPA controls. The number of mice per group that developed skin papillomas is given in Table 24.

The average number of papillomas per mouse was calculated as in design A and the values are presented in Table 25. The data used to calculate tumor multiplicity were collected at 10-week intervals over the course of the study. $B6C3F_1$ mice developed fewer tumors per mouse than either of the other strains. Male and female Swiss (CD-1®) and SENCAR mice averaged about the same number of papillomas per mouse.



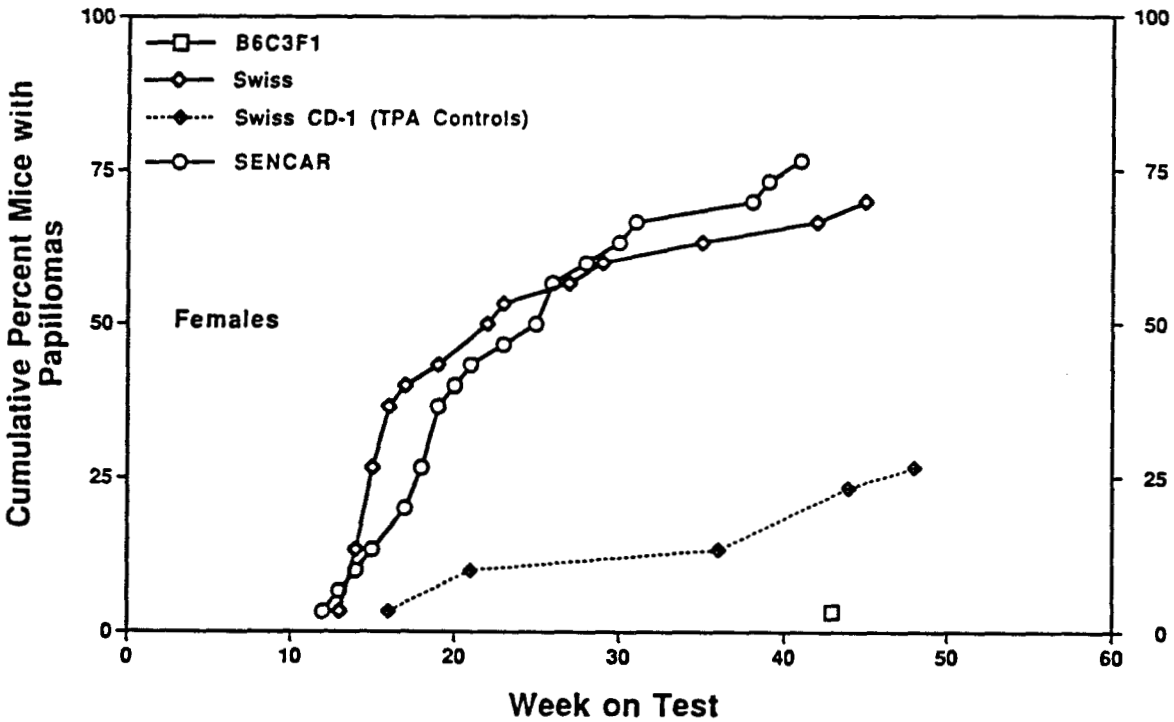


FIGURE 8
Tumor Response of the Three Mouse Strains Initiated with 100 μg MNNG Followed by 5 μg [B6C3F$_1$ and Swiss (CD-1®)] or 1 μg (SENCAR) TPA Promotion

TABLE 24
Comparisons of Papilloma Response Between B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and TPA Promotion

	100 μg MNNG/ Acetone	5 μg TPA/ 5 μg TPA	100 μg MNNG/ 5 μg TPA
B6C3F$_1$			
Male			
Mean weeks to first papilloma[a]	–[e]	–	37.2 ± 3.52
Overall rate[b]	0/30	0/30	9/30
Life table test[c]			P=0.002
Life table test[d]			P=0.002
Female			
Mean weeks to first papilloma	–	–	43.0
Overall rate	0/30	0/30	1/30
Life table test			P=0.493
Life table test			P=0.493
Swiss (CD-1®)			
Male			
Mean weeks to first papilloma	15.0 ± 0	35.8 ± 4.64	25.3 ± 2.64
Overall rate	1/30	4/30	16/30
Life table test			P<0.001
Life table test			P<0.001
Female			
Mean weeks to first papilloma	–	34.1 ± 4.63	21.1 ± 2.05
Overall rate	0/30	8/30	21/30
Life table test			P<0.001
Life table test			P<0.001

	100 μg MNNG/ Acetone	1 μg TPA/ 1 μg TPA	100 μg MNNG/ 1 μg TPA
SENCAR			
Male			
Mean weeks to first papilloma	47.5 ± 4.50	16.0 ± 5.00	20.8 ± 1.86
Overall rate	2/30	2/30	16/30
Life table test			P<0.001
Life table test			P<0.001
Female			
Mean weeks to first papilloma	–	–	23.0 ± 1.71
Overall rate	0/30	0/30	23/30
Life table test			P<0.001
Life table test			P<0.001

[a] Mean ± standard deviation
[b] Number of animals with tumor per number of animals with skin examined in-life
[c] Beneath the 100 μg MNNG/TPA group incidence is the P value corresponding to pairwise comparison with the 100 μg MNNG/Acetone group.
[d] Beneath the 100 μg MNNG/TPA group incidence is the P value corresponding to pairwise comparison with the TPA/TPA group.
[e] Not applicable; no papillomas observed in animal group

TABLE 25
Papillomas Observed During the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and TPA Promotion

Week of Study	11		21		31		41		51	
$B6C3F_1$										
Male										
100 μg MNNG/5 μg TPA	0[a]	0[b]	0	0	1.00	0.10	1.00	0.13	1.00	0.21
Female										
100 μg MNNG/5 μg TPA	0	0	0	0	0	0	0	0	1.00	0.03
Swiss (CD-1®)										
Male										
100 μg MNNG/5 μg TPA	1.00	0.03	1.50	0.28	0	0	2.44	1.10	2.00	1.09
Female										
100 μg MNNG/5 μg TPA	0	0	1.85	0.83▲	0	0	2.57	1.29▲	3.22	1.26▲
SENCAR										
Male										
100 μg MNNG/1 μg TPA	0	0	1.43	0.34	1.75	0.67▲	3.50	1.58	3.75	1.67▲▼
Female										
100 μg MNNG/1 μg TPA	0	0	2.00	0.64▲	2.14	1.24▲▼	1.81	1.53▲	2.13	1.31▲

▲ Significantly different ($P \leq 0.05$) from similarly treated $B6C3F_1$ groups by the Mann-Whitney U test
▼ Significantly different ($P \leq 0.05$) from similarly treated Swiss (CD-1®) groups by the Mann-Whitney U test
[a] Average number of papillomas expressed as the total number of papillomas/number of mice with papillomas
[b] Average number of papillomas expressed as the total number of papillomas/number of mice surviving

MNNG Initiation and BPO Promotion

Survival

Survival of male and female mice of all three strains initiated with 100 μg MNNG was similar to that of respective vehicle controls (Table 26). Survival of $B6C3F_1$ mice initiated with 500 μg MNNG was also similar to that of the vehicle controls. Survival of male and female Swiss (CD-1®) mice receiving 500 μg MNNG initiation was lower than that of the vehicle controls.

Body Weights and Clinical Findings

Final mean body weights of female $B6C3F_1$ mice initiated with 100, 500, or 1,000 μg MNNG were significantly lower than that of the vehicle controls; final mean body weights of all other MNNG/BPO groups were similar to that of their respective vehicle controls (Table 26).

Skin irritation and ulcer were observed in less than 10% of male and female $B6C3F_1$ and SENCAR mice receiving 100 μg MNNG initiation (Table 27). $B6C3F_1$ mice receiving 500 μg MNNG exhibited an increase in the percentage of mice with irritation in the first 3 weeks, which then decreased to zero or near zero before increasing again to an average of 16% in males and 10% in females during the last 10 weeks of the study. There were no ulcers observed in $B6C3F_1$ mice receiving 500 μg MNNG.

Male and female Swiss (CD-1®) mice exhibited a dose-related response in the signs of irritation and ulcer. The percentage of 100 μg MNNG mice observed with irritation peaked at week 3 then decreased to less than 15% in males and 5% in females for the remainder of the study. Ulcer was never observed in more than 6% of either male or female Swiss (CD-1®) mice receiving 100 μg MNNG. All Swiss (CD-1®) mice receiving 500 μg MNNG showed signs of irritation with the peak incidence occurring between weeks 3 and 5. By week 10, the percentage of animals with signs of irritation in this group had decreased and did not average greater than 24% in males or 11% in females.

The percentage of 500 μg MNNG Swiss (CD-1®) mice observed with ulcer also increased during weeks 2 and 3, then decreased, and after week 10 did not exceed 17%.

TABLE 26
Survival and Body Weights of B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and BPO Promotion

Initiator/Promoter	Survival[a]	Final Mean Body Weight[b] (g)	Final Weight Relative to Controls (%)
B6C3F$_1$			
Male			
Acetone/Acetone	30/30	47.5 ± 0.7	
20 mg BPO/20 mg BPO	30/30	46.8 ± 0.8	99
100 µg MNNG/20 mg BPO	30/30	46.2 ± 0.8	97
500 µg MNNG/20 mg BPO	29/30	46.7 ± 0.7	98
1,000 µg MNNG/20 mg BPO	28/30	46.6 ± 0.8	98
Female			
Acetone/Acetone	30/30	47.9 ± 1.2	
20 mg BPO/20 mg BPO	29/30	43.4 ± 0.9▲▲	91
100 µg MNNG/20 mg BPO	30/30	43.8 ± 1.1▲▲	91
500 µg MNNG/20 mg BPO	27/30	43.2 ± 1.0▲▲	90
1,000 µg MNNG/20 mg BPO	25/30	43.1 ± 1.1▲▲	90
Swiss (CD-1®)			
Male			
Acetone/Acetone	27/30	50.7 ± 1.3	
20 mg BPO/20 mg BPO	20/30	48.4 ± 1.6	95
100 µg MNNG/20 mg BPO	26/30	49.4 ± 1.2	97
500 µg MNNG/20 mg BPO	18/30*	49.9 ± 1.2	98
1,000 µg MNNG/20 mg BPO	8/30**	47.9 ± 2.1	94
Female			
Acetone/Acetone	27/30	39.0 ± 1.1	
20 mg BPO/20 mg BPO	29/30	40.7 ± 1.1	104
100 µg MNNG/20 mg BPO	28/30	40.1 ± 1.0	103
500 µg MNNG/20 mg BPO	20/30*	36.9 ± 1.3	94
1,000 µg MNNG/20 mg BPO	17/30**	37.6 ± 1.2	96
SENCAR			
Male			
Acetone/Acetone	29/30	52.0 ± 1.2	
20 mg BPO/20 mg BPO	26/30	52.5 ± 1.0	101
100 µg MNNG/20 mg BPO	28/30	51.2 ± 1.2	99
500 µg MNNG/20 mg BPO	16/30**	49.5 ± 2.0	95
1,000 µg MNNG/20 mg BPO	6/30**	46.1 ± 2.4▲	89
Female			
Acetone/Acetone	26/30	44.9 ± 1.4	
20 mg BPO/20 mg BPO	27/30	46.2 ± 1.8	103
100 µg MNNG/20 mg BPO	29/30	46.5 ± 1.3	103
500 µg MNNG/20 mg BPO	23/30	46.2 ± 1.7	103
1,000 µg MNNG/20 mg BPO	14/30**	42.7 ± 2.0	95

* Significantly different (P≤0.05) from the vehicle control group by life table pairwise comparison
** Significantly different (P≤0.01) from the vehicle control group by life table pairwise comparison
▲ Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
▲▲ Significantly different (P≤0.01) from the vehicle control group by Dunnett's test
[a] Number of animals surviving/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[b] Weights are given as mean ± standard error.

TABLE 27
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and BPO Promotion

Week of Study	1	2	3	4	5	6	7	8	9	10	11-20	21-30	31-40	41-50
Male														
Irritation														
B6C3F$_1$														
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	3	3
500 µg MNNG/20 mg BPO	0	0	17	0	0	0	0	0	0	0	0	1	2	16
Swiss (CD-1®)														
100 µg MNNG/20 mg BPO	0	47	53	37	30	10	0	3	0	3	8	10	14	3
500 µg MNNG/20 mg BPO	10	83	100	97	100	80	43	24	17	21	16	19	24	18
SENCAR														
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	3	0	3	5	7
Ulcer														
B6C3F$_1$														
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	2	3
500 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swiss (CD-1®)														
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	4	6	4
500 µg MNNG/20 mg BPO	0	7	33	37	17	20	20	17	14	14	17	16	5	10
SENCAR														
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	1	3	2

(continued)

TABLE 27
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and BPO Promotion (continued)

Week of Study	1	2	3	4	5	6	7	8	9	10	11-20	21-30	31-40	41-50
Female														
Irritation														
B6C3F$_1$														
100 µg MNNG/20 mg BPO	7	0	0	0	0	0	0	0	0	0	0	0	0	2
500 µg MNNG/20 mg BPO	70	3	0	0	0	0	0	0	0	0	0	0	5	10
Swiss (CD-1®)														
100 µg MNNG/20 mg BPO	0	43	77	40	0	0	0	3	3	3	4	2	1	0
500 µg MNNG/20 mg BPO	37	93	100	97	97	73	30	17	7	10	11	7	4	3
SENCAR														
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	3	0	2	0	0	4
Ulcer														
B6C3F$_1$														
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swiss (CD-1®)														
100 µg MNNG/20 mg BPO	0	3	3	0	0	0	0	0	0	0	2	0	0	0
500 µg MNNG/20 mg BPO	0	47	30	20	13	7	7	7	7	7	13	5	0	0
SENCAR														
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0	0	0	0	0

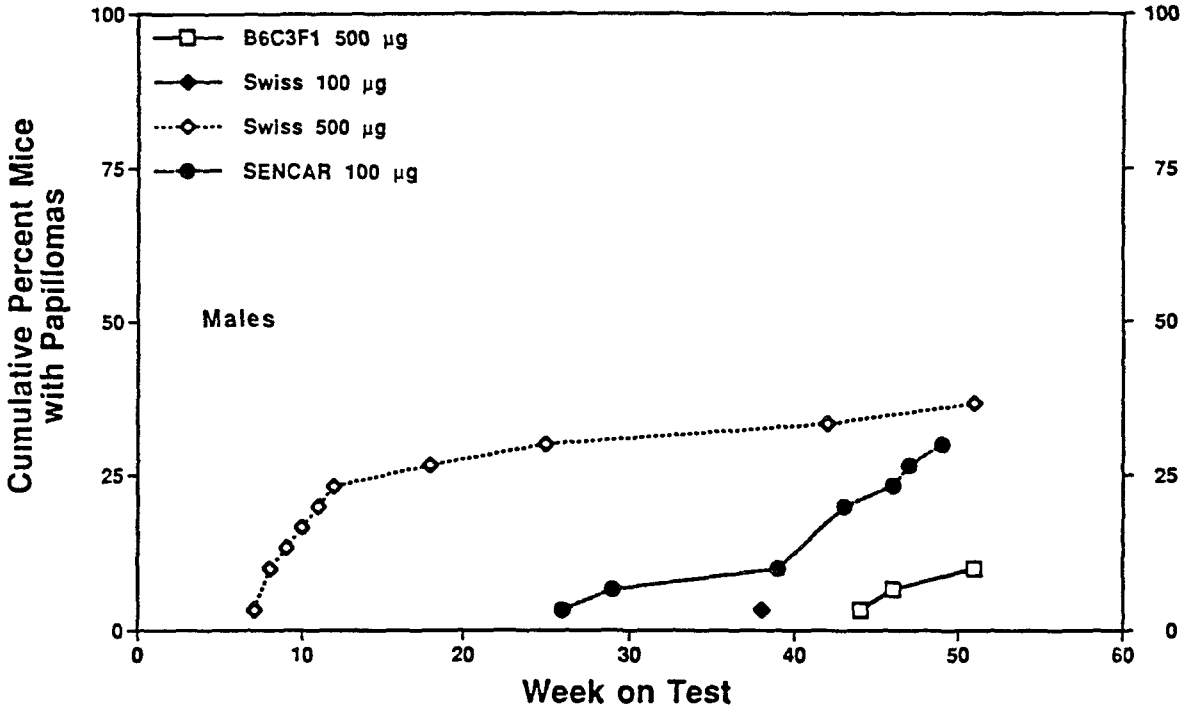
Tumor Response

The response of mice receiving 100 μg MNNG initiation followed by repeated applications of BPO is shown in Figure 9. The response is displayed as the cumulative percentage of mice that developed skin papillomas over the course of the 1-year study. In some groups survival was reduced because of the aggressive moribund sacrifice policy maintained for this study. In addition, some tumors may have regressed or been removed by mechanical grooming. No mice were removed from the cumulative count.

One female $B6C3F_1$, one male Swiss (CD-1®), and three female Swiss (CD-1®) mice receiving 100 μg MNNG initiation developed papillomas. Thirty percent of male and female SENCAR mice receiving 100 μg MNNG initiation developed papillomas (Table 28).

Higher incidences of papillomas were observed in $B6C3F_1$ and Swiss (CD-1®) mice receiving 500 μg MNNG than in the vehicle control or 100 μg MNNG groups, but this difference was only significant in Swiss (CD-1®) mice. The incidences of papilloma in $B6C3F_1$ mice receiving 100 or 500 μg MNNG and Swiss (CD-1®) mice receiving 100 μg MNNG were similar to the respective vehicle controls.

The mean time to the appearance of the first papilloma is given in Table 28. The mean time to the appearance of the first papilloma in $B6C3F_1$ mice was 43 to 47 weeks. In Swiss (CD-1®) mice, the 100 μg MNNG groups had a mean time to the appearance of the first papilloma of 38 weeks in males and 31 weeks in females. This time was greatly shortened in 500 μg MNNG Swiss (CD-1®) mice, in which the mean times to the appearance of the first papilloma were 18.3 weeks in males and 8.4 weeks in females. In SENCAR mice receiving 100 μg MNNG, the mean time to first tumor appearance was 40.6 weeks for males and 38.1 weeks for females.



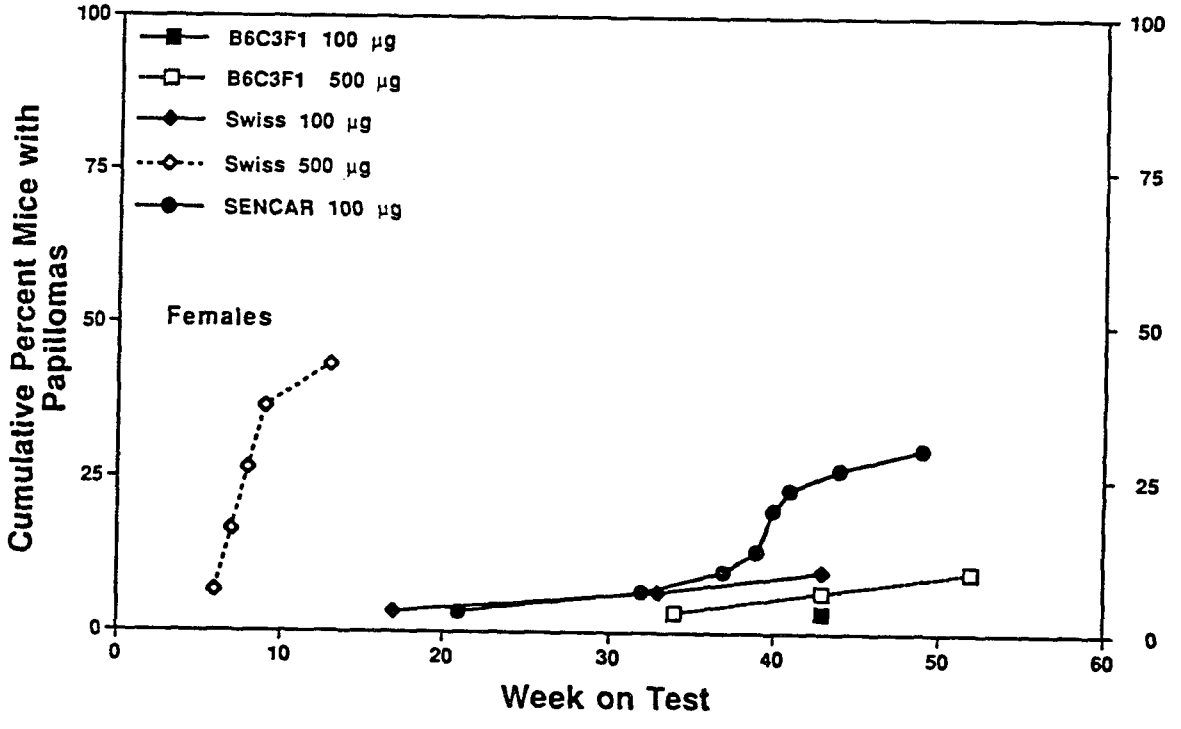


FIGURE 9
Tumor Response of the Three Mouse Strains Initiated with 100 µg MNNG Followed by 20 mg BPO Promotion

TABLE 28
Comparisons of Papilloma Response Between B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and BPO Promotion

	100 µg MNNG/ Acetone	500 µg MNNG/ Acetone	20 mg BPO/ 20 mg BPO	100 µg MNNG/ 20 mg BPO	500 µg MNNG/ 20 mg BPO
B6C3F$_1$					
Male					
Mean weeks to first papilloma[a]	–[e]	34.0	–	–	47.0 ± 2.08
Overall rate[b]	0/30	1/30	0/30	0/30	3/30
Life table test[c]				–[f]	P=0.308
Life table test[d]			P=0.028	–	P=0.118
Female					
Mean weeks to first papilloma	–	–	–	44.0	43.0 ± 5.20
Overall rate	0/30	0/30	0/30	1/30	3/30
Life table test				P=0.500	P=0.114
Life table test			P=0.074	P=0.500	P=0.116
Swiss (CD-1®)					
Male					
Mean weeks to first papilloma	15.0 ± 0	14.9 ± 1.60	–	38.0	18.3 ± 4.54
Overall rate	1/30	7/30	0/30	1/30	11/30
Life table test				P=0.752	P=0.164
Life table test			P<0.001	P=0.522	P<0.001
Female					
Mean weeks to first papilloma	–	18.0 ± 6.93	–	31.0 ± 7.57	8.4 ± 0.65
Overall rate	0/30	6/30	0/30	3/30	13/30
Life table test				P=0.120	P=0.032
Life table test			P<0.001	P=0.116	P<0.001
SENCAR					
Male					
Mean weeks to first papilloma	47.5 ± 4.50	13.9 ± 2.39	–	40.6 ± 2.66	12.9 ± 1.91
Overall rate	2/30	19/30	0/30	9/30	25/30
Life table test				P=0.021	P=0.079
Life table test			P<0.001	P=0.003	P<0.001
Female					
Mean weeks to first papilloma	–	25.5 ± 6.40	47.0 ± 0	38.1 ± 2.64	25.8 ± 4.43
Overall rate	0/30	8/30	1/30	9/30	16/30
Life table test				P=0.002	P=0.046
Life table test			P<0.001	P=0.008	P<0.001

[a] Mean ± standard deviation
[b] Number of animals with tumor per number of animals with skin examined in-life
[c] Beneath the 100 µg MNNG/20 mg BPO and 500 µg MNNG/20 mg BPO group incidences are the P values corresponding to pairwise comparisons between the appropriate MNNG/Acetone group and those groups.
[d] In the 20 mg BPO/20 mg BPO columns are the P values associated with the trend test. Beneath the 100 µg MNNG/20 mg BPO and 500 µg MNNG/20 mg BPO group incidences are the P values corresponding to pairwise comparisons between the 20 mg BPO/20 mg BPO group and those groups.
[e] Not applicable; no papillomas observed in animal group
[f] Value of statistic cannot be computed

The average numbers of papillomas per mouse, calculated as for the MNNG/TPA groups, are presented in Table 29. Of the mice that developed tumors, there was only one papilloma per mouse except for female SENCAR mice which averaged 1.17 papillomas at week 41 and 2.00 papillomas at week 52.

TABLE 29
Papillomas Observed During the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and BPO Promotion

Week of Study	11		21		31		41		51	
B6C3F$_1$										
Male										
100 µg MNNG/20 mg BPO	0[a]	0[b]	0	0	0	0	0	0	0	0
500 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	1.00	0.07
Female										
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	1.00	0.03
500 µg MNNG/20 mg BPO	0	0	0	0	0	0	0	0	0	0
Swiss (CD-1®)										
Male										
100 µg MNNG/20 mg BPO	0	0	0	0	0	0	1.00	0.04	1.00	0.04
500 µg MNNG/20 mg BPO	1.40	0.24▲	1.83	0.39▲	0	0.08	0	0	1.00	0.11
Female										
100 µg MNNG/20 mg BPO	0	0	1.00	0.03	0	0	1.00	0.04	1.00	0.04
500 µg MNNG/20 mg BPO	1.09	0.47▲	1.15	0.47▲	0	0.24▲	1.00	0.17▲	1.00	0.15▲
SENCAR										
Male										
100 µg MNNG/20 mg BPO	0	0	0	0	1.00	0.07	1.00	0.07	1.17	0.39▲
Female										
100 µg MNNG/20 mg BPO	0	0	1.00	0.03	0	0	1.17	0.24▲▼	2.00	0.55▲▼

▲ Significantly different ($P \leq 0.05$) from similarly treated B6C3F$_1$ groups by the Mann-Whitney U test
▼ Significantly different ($P \leq 0.05$) from similarly treated Swiss (CD-1®) groups by the Mann-Whitney U test
[a] Average number of papillomas expressed as the total number of papillomas/number of mice with papillomas
[b] Average number of papillomas expressed as the total number of papillomas/number of mice surviving

Comparison of Mouse Strain Response to MNNG as an Initiator with TPA or BPO as a Promoter

MNNG/TPA: The response of the three strains of mice to 100 μg MNNG initiation and 1 μg (SENCAR) or 5 μg [B6C3F$_1$ and Swiss (CD-1®)] TPA promotion over the 1-year study is shown in Figure 8. The incidences of papilloma in male and female Swiss (CD-1®) and SENCAR mice were significantly greater than those in B6C3F$_1$ mice (Table 30). The incidences of papilloma in Swiss (CD-1®) and SENCAR mice were similar, even though Swiss (CD-1®) mice received higher concentrations of TPA (5 μg) than did the SENCAR mice (1 μg).

The in-life papilloma observations for MNNG/TPA mice are summarized in Table 31. The papilloma response of B6C3F$_1$ mice receiving 100 μg MNNG was less than that of Swiss (CD-1®) and SENCAR mice in all parameters: fewer mice developed papillomas, the mean time to the appearance of the first tumor was longer, and the average number of tumors was lower than either of the other strains. Swiss (CD-1®) and SENCAR mice had similar papilloma incidences, group mean time to the appearance of the first papilloma, and average numbers of papillomas.

MNNG/BPO: The response of the three strains of mice to 100 μg MNNG initiation and BPO promotion over the 1-year study is shown in Figure 9. SENCAR mice were the most responsive to this combination of chemicals. The incidences of papilloma in SENCAR mice were significantly greater than those in male and female B6C3F$_1$ and male Swiss (CD-1®) mice (Table 32). The papilloma response for male and female B6C3F$_1$ and Swiss (CD-1®) mice that received 100 μg MNNG was similar to that of the vehicle controls (Table 28).

Table 30
Comparisons of Papilloma Response Between B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and TPA Promotion

	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Male			
100 μg MNNG/TPA[a]			
Mean time to first papilloma (weeks)	37.2	25.3	20.8
Overall rate[b]	9/30	16/30	16/30
Life table test[c]		P=0.002	P<0.001
Life table test[d]			P=0.234
Female			
100 μg MNNG/TPA			
Mean time to first papilloma (weeks)	43.0	21.1	23.0
Overall rate	1/30	21/30	23/30
Life table test		P<0.001	P<0.001
Life table test			P=0.296

[a] B6C3F$_1$ mice and Swiss (CD-1®) mice received 5 μg TPA; SENCAR mice received 1 μg TPA.
[b] Number of animals with tumor per number of animals with skin examined in-life
[c] P values correspond to pairwise comparison with B6C3F$_1$ mice.
[d] P values correspond to pairwise comparison with Swiss (CD-1®) mice.

TABLE 31
Overview of MNNG/TPA Sensitivity in the Comparative Initiation/Promotion Skin Paint Studies

	Number of Mice with Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
$B6C3F_1$				
Male				
100 μg MNNG/5 μg TPA	9/30	37.2	0.30	1.00
Female				
100 μg MNNG/5 μg TPA	1/30	43.0	0.03	1.00
Swiss (CD-1®)				
Male				
100 μg MNNG/5 μg TPA	16/30	25.3	1.40	2.63
Female				
100 μg MNNG/5 μg TPA	21/30	21.1	2.27	3.24
SENCAR				
Male				
100 μg MNNG/1 μg TPA	16/30	20.8	1.47	2.75
Female				
100 μg MNNG/1 μg TPA	23/30	23.0	2.13	2.78

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE 32
Comparisons of Papilloma Response Between B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: MNNG Initiation and BPO Promotion

	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Male			
100 μg MNNG/20 mg BPO			
Mean weeks to first papilloma	–[d]	38.0	40.6
Overall rate[a]	0/30	1/30	9/30
Life table test[b]		P=0.493	P=0.002
Life table test[c]			P=0.011
Female			
100 μg MNNG/20 mg BPO			
Mean weeks to first papilloma	44.0	31.0	38.1
Overall rate	1/30	3/30	9/30
Life table test		P=0.284	P=0.007
Life table test			P=0.062

[a] Number of animals with tumor per number of animals with skin examined in-life
[b] P values correspond to pairwise comparison with B6C3F$_1$ mice.
[c] P values correspond to pairwise comparison with Swiss (CD-1®) mice.
[d] Not applicable; no papillomas in animal group

The in-life papilloma observations for MNNG/BPO mice are summarized in Table 33. In addition to a higher papilloma incidence, SENCAR mice had a greater average number of tumors per mouse than did the other two strains. Swiss (CD-1®) female mice had a shorter mean time to the appearance of the first tumor, but only three mice developed papillomas and the mean time to appearance of the first tumor in Swiss (CD-1®) and SENCAR males was similar.

TABLE 33
Overview of MNNG/BPO Sensitivity in the Comparative Initiation/Promotion Skin Paint Studies

	Number of Mice with Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
B6C3F$_1$				
Male				
100 µg MNNG/20 mg BPO	0/30	–[c]	–	–
Female				
100 µg MNNG/20 mg BPO	1/30	44.0	0.03	1.00
Swiss (CD-1®)				
Male				
100 µg MNNG/20 mg BPO	1/30	38.0	0.03	1.00
Female				
100 µg MNNG/20 mg BPO	3/30	31.0	0.13	1.33
SENCAR				
Male				
100 µg MNNG/20 mg BPO	9/30	40.6	0.50	1.67
Female				
100 µg MNNG/20 mg BPO	9/30	38.1	0.60	2.00

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group
[c] Not applicable; no papillomas in animal group

DMBA AND MNNG AS COMPLETE CARCINOGENS

To compare the mouse strain skin tumor response to DMBA and MNNG as complete carcinogens, groups of male and female mice of the three strains were dosed once a week with either 2.5 μg DMBA or 100 μg MNNG for the duration of the 1-year study.

Clinical Observations of Skin at the Site of Application

In mice receiving DMBA, the percentage of mice with signs of irritation was minimal (Table 34). Less than 10% of the animals were observed with this condition except for female Swiss (CD-1®) mice, which averaged 18% during weeks 31 through 40, and male and female Swiss (CD-1®) mice and male SENCAR mice, which averaged less than 20% during weeks 41 through 50. The percentage of mice with ulcers was never greater than 8% in any strain during the study.

A greater percentage of mice with irritation and ulcer were observed in MNNG groups (Table 35). In the first 30 weeks of the study, a very low percentage of $B6C3F_1$ mice (3% during one week) were observed with skin irritation. However, skin irritation averaged 20% in males and 23% in females during weeks 31 through 40 and increased to an average of 35% in males and females during weeks 41 through 50. Ulcers in $B6C3F_1$ mice were not noted before the last 10 weeks of the study during which time the incidence averaged 1% in males and 4% in females. SENCAR mice were slightly more sensitive to MNNG than $B6C3F_1$ mice. Irritation appeared during the second week of the study, increasing to an average of 20% in males at week 3; the percentage of females with irritation rose to 7% at week 4 and remained relatively constant until weeks 21 through 30. The percentage of male and female SENCAR mice with irritation gradually increased to an average of 56% males and 64% females by weeks 41 through 50. The percentage of SENCAR mice with ulcer was near zero for most of the first 30 weeks of the study, and never exceeded 7% for females during the entire study. The average incidence of ulcers increased to 10% in SENCAR males during weeks 31 through 40 and to 20% during weeks 41 through 50. The highest percentage of mice with signs of irritation was observed in Swiss (CD-1®) mice, in which approximately 90% of males and females were observed with irritation at weeks 3 to 4. The average percentage generally decreased through week 30 before increasing to 40% in males and 24% in females during weeks 41 through 50. Ulcers were observed in the Swiss (CD-1®) mice during weeks 2 through 5, and remained low (less than 17%) until weeks 41 through 50 during which time the average was 19% in males and 15% in females.

TABLE 34
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Repeated Applications of 2.5 μg DMBA

Week on Study	1-10	11-20	21-30	31-40	41-50
Male					
Irritation					
B6C3F$_1$	0	0	0	0	0
Swiss (CD-1®)	0	3	3	7	19
SENCAR	1	4	9	5	17
Ulcer					
B6C3F$_1$	0	0	0	0	0
Swiss (CD-1®)	1	4	4	6	1
SENCAR	0	1	4	1	5
Female					
Irritation					
B6C3F$_1$	0	0	0	0	2
Swiss (CD-1®)	0	0	0	18	15
SENCAR	0	1	5	1	7
Ulcer					
B6C3F$_1$	0	0	0	0	0
Swiss (CD-1®)	0	0	0	1	8
SENCAR	0	0	0	0	6

TABLE 35
Incidences of Irritation and Ulcer (Expressed as Percentage) in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Repeated Applications of 100 μg MNNG

Week on Study	1	2	3	4	5	6	7	8	9	10	11-20	21-30	31-40	41-50
Male														
Irritation														
B6C3F$_1$	0	0	0	0	3	0	0	0	0	0	0	5	20	35
Swiss (CD-1®)	0	43	83	93	73	62	38	17	21	34	17	15	29	40
SENCAR	0	17	20	17	13	3	3	10	20	17	14	25	50	56
Ulcer														
B6C3F$_1$	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Swiss (CD-1®)	0	3	17	13	7	0	0	0	0	0	3	3	7	19
SENCAR	0	0	0	0	0	0	0	0	0	0	2	1	10	20
Female														
Irritation														
B6C3F$_1$	3	0	0	0	0	0	0	0	0	0	0	1	23	35
Swiss (CD-1®)	0	70	90	83	83	87	50	27	10	13	4	4	17	24
SENCAR	0	3	3	7	7	7	7	7	7	7	6	18	33	64
Ulcer														
B6C3F$_1$	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Swiss (CD-1®)	0	3	3	0	0	0	0	0	0	0	0	0	3	15
SENCAR	0	0	0	0	0	0	0	0	0	3	6	7	6	6

The tumor response in mice receiving topical applications of DMBA is shown in Figure 10. The mean time to the appearance of the first papilloma was shortest in SENCAR mice and longest in $B6C3F_1$ mice (Table 36). Pairwise comparison of strains at individual time points over the course of the study indicated that the response of mice developing tumors was significantly greater in SENCAR (shorter time to tumor) than in $B6C3F_1$ or Swiss (CD-1®) mice. The total number of mice observed with skin tumors was greatest in SENCAR mice and lowest in Swiss (CD-1®) mice.

The tumor response in mice receiving topical applications of MNNG is shown in Figure 11. The mean time to first appearance of papilloma was similar in $B6C3F_1$ and Swiss (CD-1®) mice, but was markedly shorter in SENCAR mice. Pairwise comparison of strains at individual time points over the course of the study indicated that the response of mice developing tumors was significantly greater in SENCAR mice than in male $B6C3F_1$ and male and female Swiss (CD-1®) mice and that the $B6C3F_1$ response was significantly greater than that of Swiss (CD-1®) mice (Table 37).

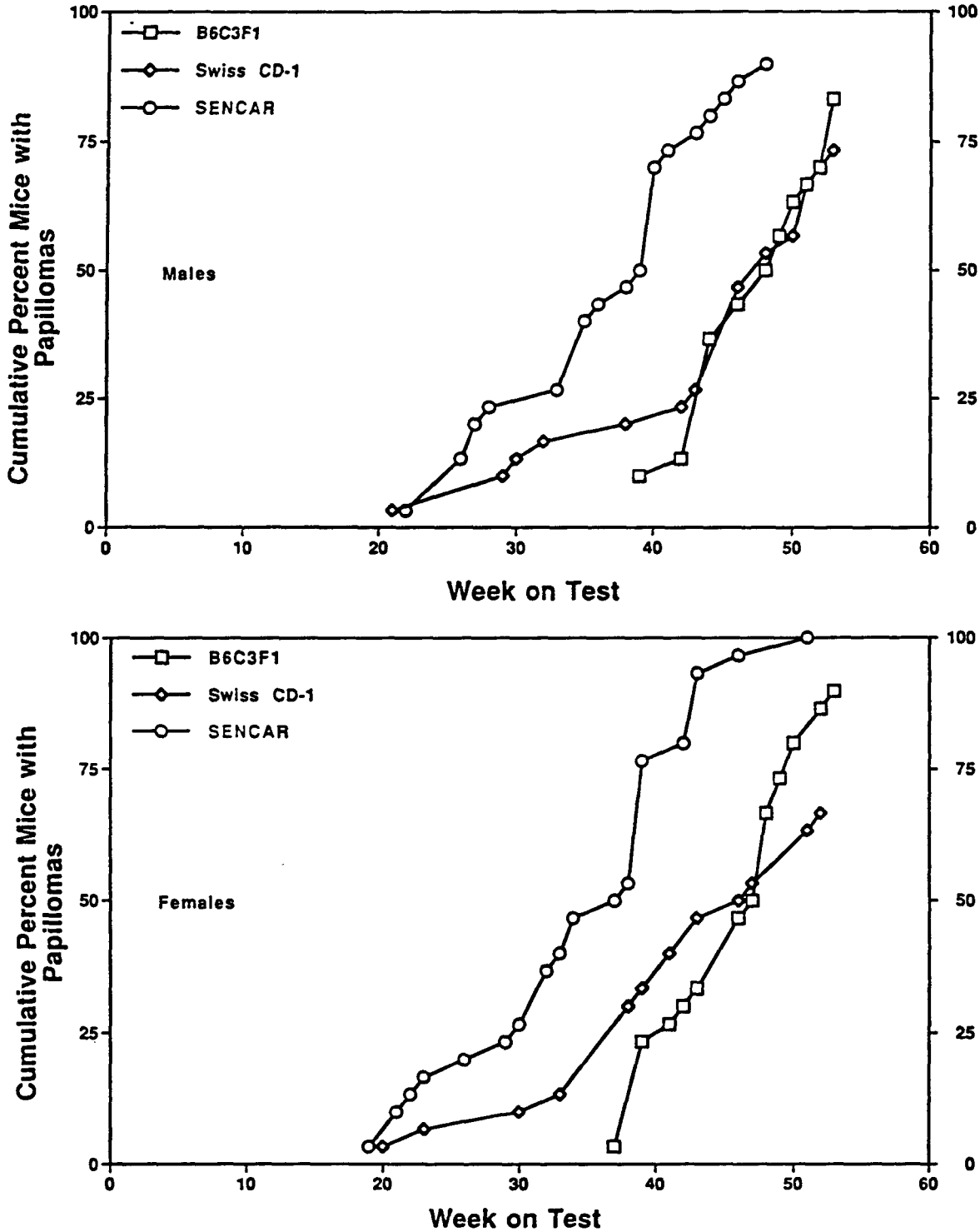


FIGURE 10
Tumor Response of the Three Mouse Strains Receiving Repeated Applications of 2.5 μg DMBA

TABLE 36
Comparisons of Papilloma Response Between B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Repeated Applications of 2.5 μg DMBA

	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Male			
Mean weeks to first papilloma	46.6 (0.86)[d]	42.9 (1.92)	36.1 (1.36)
Overall rate[a]	25/30	22/30	27/30
Life table test[b]		P=0.521N	P<0.001
Life table test[c]			P<0.001
Female			
Mean weeks to first papilloma	45.3 (0.92)	40.1 (1.97)	34.9 (1.49)
Overall rate	27/30	20/30	30/30
Life table test		P=0.242N	P<0.001
Life table test			P<0.001

[a] Number of animals with tumor per number of animals with skin examined in-life
[b] P values correspond to pairwise comparison with B6C3F$_1$ mice. A lower incidence in a dose group is indicated by N.
[c] P values correspond to pairwise comparison with Swiss (CD-1®) mice.
[d] Mean (standard deviation)

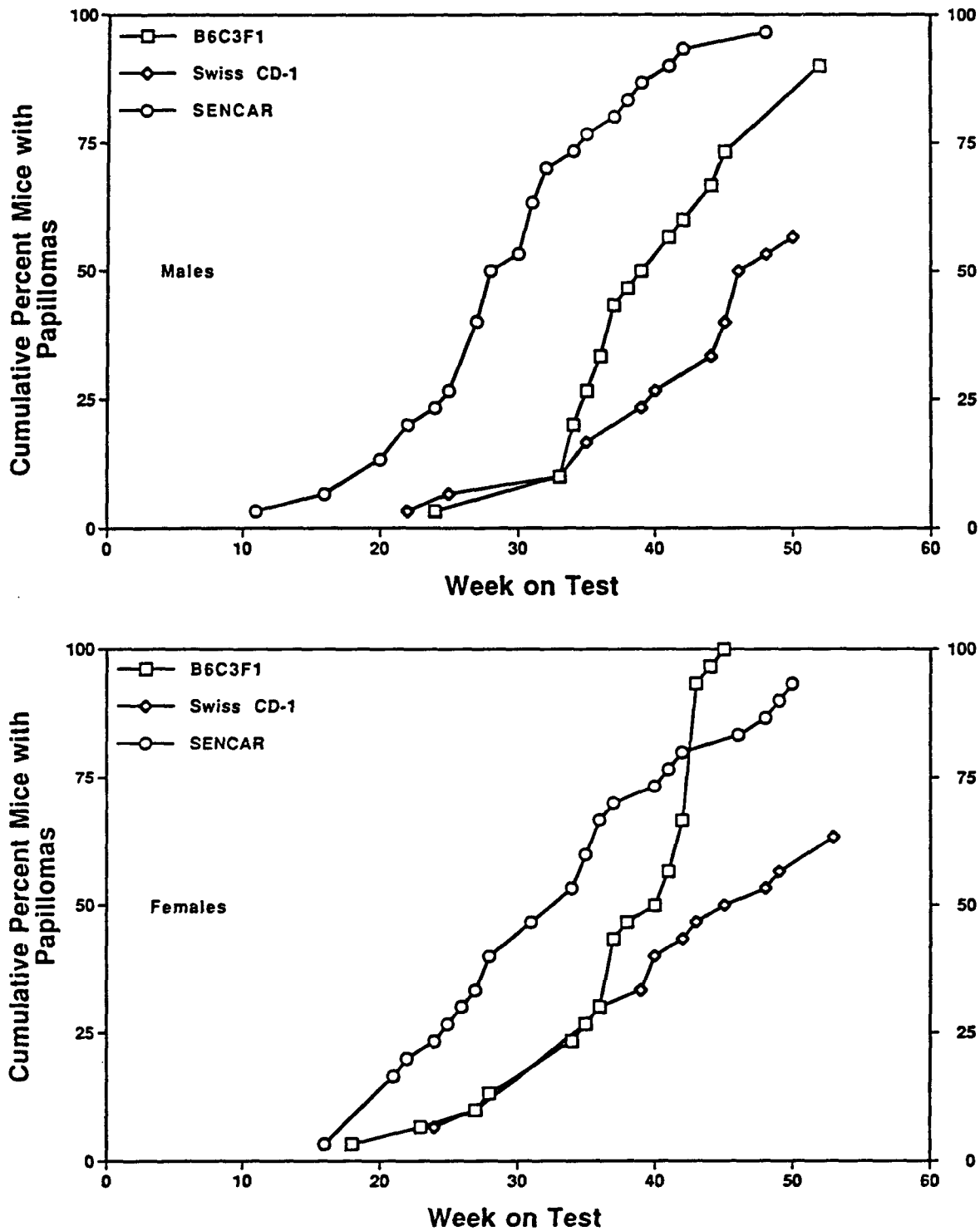


FIGURE 11
Tumor Response of the Three Mouse Strains Receiving Repeated Applications of 100 μg MNNG

TABLE 37
Comparisons of Papilloma Response Between B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Repeated Applications of 100 μg MNNG

	B6C3F$_1$	Swiss (CD-1®)	SENCAR
Male			
Mean weeks to first papilloma	40.1 (1.40)[d]	40.2 (1.93)	29.4 (1.50)
Overall rate[a]	27/30	17/30	29/30
Life table test[b]		P=0.017N	P<0.001
Life table test[c]			P<0.001
Female			
Mean weeks to first papilloma	37.9 (1.21)	38.7 (1.92)	32.3 (1.80)
Overall rate	30/30	19/30	28/30
Life table test		P<0.001N	P=0.150N
Life table test			P<0.001

[a] Number of animals with tumor per number of animals with skin examined in-life
[b] P values correspond to pairwise comparison with B6C3F$_1$ mice. A lower incidence in a dose group is indicated by N.
[c] P values correspond to pairwise comparison with Swiss (CD-1®) mice.
[d] Mean (standard deviation)

The overall sensitivity of mice to the complete carcinogen treatment is shown together with their response to the same concentration of the carcinogen as an initiator followed by TPA promotion (Tables 38 and 39). B6C3F$_1$ mice had a much higher incidence of tumors and developed more tumors per animal with the complete carcinogen treatment than they did with the initiation and promotion treatment. The mean time to the appearance of the first tumor was somewhat shorter in B6C3F$_1$ mice treated with DMBA/TPA than it was in mice receiving the DMBA complete carcinogen treatment. Differences in the papilloma incidence in Swiss (CD-1®) and SENCAR mice were less clear; however, the mean time to the appearance of the first tumor was shorter and the average number of tumors per animal was generally greater in mice receiving initiation/promotion treatment than in those receiving either DMBA or MNNG complete carcinogen treatment.

TABLE 38
Overview of DMBA Sensitivity in the Comparative Initiation/Promotion Skin Paint Studies

	Number of Mice with Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
B6C3F$_1$				
Male				
2.5 μg DMBA/5 μg TPA	10/30	35.4	0.57	1.70
2.5 μg DMBA/2.5 μg DMBA	25/30	46.6	2.13	2.56
Female				
2.5 μg DMBA/5 μg TPA	3/30	36.7	0.27	2.67
2.5 μg DMBA/2.5 μg DMBA	27/30	45.3	2.17	2.41
Swiss (CD-1®)				
Male				
2.5 μg DMBA/5 μg TPA	27/30	15.4	4.63	5.15
2.5 μg DMBA/2.5 μg DMBA	22/30	42.9	1.30	1.77
Female				
2.5 μg DMBA/5 μg TPA	26/30	18.0	3.77	4.35
2.5 μg DMBA/2.5 μg DMBA	20/30	40.1	1.27	1.90
SENCAR				
Male				
2.5 μg DMBA/1 μg TPA	19/30	15.5	3.03	4.79
2.5 μg DMBA/2.5 μg DMBA	27/30	36.1	2.53	2.81
Female				
2.5 μg DMBA/1 μg TPA	27/30	15.9	5.23	5.81
2.5 μg DMBA/2.5 μg DMBA	30/30	34.9	2.80	2.80

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE 39
Overview of MNNG Sensitivity in the Comparative Initiation/Promotion Skin Paint Studies

	Number of Mice with Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
B6C3F$_1$				
Male				
100 µg MNNG/5 µg TPA	9/30	37.2	0.30	1.00
100 µg MNNG/100 µg MNNG	27/30	40.1	1.43	1.59
Female				
100 µg MNNG/5 µg TPA	1/30	43.0	0.03	1.00
100 µg MNNG/100 µg MNNG	30/30	37.9	1.70	1.70
Swiss (CD-1®)				
Male				
100 µg MNNG/5 µg TPA	16/30	25.3	1.40	2.63
100 µg MNNG/100 µg MNNG	17/30	40.2	0.97	1.71
Female				
100 µg MNNG/5 µg TPA	21/30	21.1	2.27	3.24
100 µg MNNG/100 µg MNNG	19/30	38.7	0.87	1.37
SENCAR				
Male				
100 µg MNNG/1 µg TPA	16/30	20.8	1.47	2.75
100 µg MNNG/100 µg MNNG	29/30	29.4	2.00	2.07
Female				
100 µg MNNG/1 µg TPA	23/30	23.0	2.13	2.78
100 µg MNNG/100 µg MNNG	28/30	32.3	1.77	1.89

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

Microscopic evaluation of skin at the site of application in mice receiving the complete carcinogen treatment is summarized in Tables 40 and 41. Squamous cell carcinoma was the tumor with the highest incidence in all three strains of mice receiving DMBA or MNNG as complete carcinogens, followed by squamous cell papilloma. In mice receiving initiation and promotion treatment there were higher incidences of squamous cell papillomas than squamous cell carcinomas in all three strains of mice receiving DMBA and MNNG initiation.

TABLE 40
Incidences of Skin Tumors in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies

	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ 20 mg BPO
B6C3F$_1$			
Male	30	30	30
Sebaceous gland, adenoma	2	1	1
Melanoma NOS	1	1	0
Basal cell carcinoma	1	0	0
Keratoacanthoma	5	0	0
Squamous cell carcinoma	24	1	0
Squamous cell papilloma	12	8	0
Female	30	30	30
Sebaceous gland, adenoma	9	2	0
Melanoma NOS	0	2	1
Basal cell adenoma	1	0	0
Basal cell carcinoma	1	0	0
Squamous cell carcinoma	24	0	2
Squamous cell papilloma	11	3	3
Swiss (CD-1®)			
Male	30	30	30
Basal cell adenoma	1	0	0
Keratoacanthoma	1	1	0
Sarcoma	0	1	0
Squamous cell carcinoma	23	10	0
Squamous cell papilloma	8	17	3
Female	30	30	30
Sebaceous gland, adenoma	0	1	0
Keratoacanthoma	1	2	0
Squamous cell carcinoma	23	12	0
Squamous cell papilloma	8	15	5
SENCAR			
Male	30	30	30
Keratoacanthoma	3	0	2
Squamous cell carcinoma	27	9	11
Squamous cell papilloma	7	9	12
Female	30	30	30
Basal cell carcinoma	1	1	0
Keratoacanthoma	2	2	5
Squamous cell carcinoma	29	14	14
Squamous cell papilloma	9	11	11

TABLE 41
Incidences of Skin Tumors in B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies

	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 5 μg TPA	100 μg MNNG/ 20 mg BPO
B6C3F$_1$			
Male	30	30	30
Squamous cell carcinoma	29	0	0
Squamous cell papilloma	2	7	0
Subcutaneous tissue, sarcoma	0	1	0
Subcutaneous tissue, squamous cell carcinoma	1	0	0
Female	30	29	30
Squamous cell carcinoma	30	0	0
Squamous cell papilloma	1	1	1
Subcutaneous tissue, mast cell tumor benign	1	0	0
Subcutaneous tissue, sarcoma	1	0	0
Swiss (CD-1®)			
Male	30	30	30
Squamous cell carcinoma, metastatic, skin	1	0	0
Squamous cell carcinoma	14	1	0
Squamous cell papilloma	6	9	1
Female	30	30	30
Keratoacanthoma	0	1	1
Squamous cell carcinoma	15	6	0
Squamous cell papilloma	9	13	2

	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 1 μg TPA	100 μg MNNG/ 20 mg BPO
SENCAR			
Male	30	30	30
Basal cell adenoma	1	0	0
Keratoacanthoma	0	0	2
Squamous cell carcinoma	27	5	2
Squamous cell papilloma	7	4	8
Female	30	30	30
Basal cell adenoma	1	0	0
Keratoacanthoma	1	0	2
Squamous cell carcinoma	28	6	2
Squamous cell papilloma	7	10	6

Increased incidences of proliferative melanotic lesions occurred at the site of application in male and female $B6C3F_1$ mice in the DMBA study (study design A); however, the incidences were greatest in female mice (Table 42). Melanotic lesions occurred as increased numbers of deeply pigmented cells (melanocytes) within the dermis diagnosed as "pigmentation, melanin" or as "melanoma, NOS." The incidences of "pigmentation, melanin" were greatest in groups receiving DMBA initiation and TPA promotion and in groups receiving TPA with no initiation. The incidences of melanoma were greatest in groups receiving DMBA initiation and TPA promotion. Only two lesions were diagnosed grossly; most diagnoses were made upon microscopic examination of the skin. Melanotic lesions were not observed in Swiss (CD-1®) or SENCAR mice initiated with DMBA, nor were these lesions observed in mice initiated with MNNG or receiving MNNG as a complete carcinogen.

Lesions diagnosed as "pigmentation, melanin" consisted of focal to multifocal, loosely or densely aggregated melanocytes restricted to and randomly distributed within the dermis (Plates 1 and 2). These lesions were considered to represent hyperplastic melanocytes. Melanomas were discrete, focal to multifocal nodular masses that occurred primarily in the dermis (Plate 3) but frequently extended into the subcutaneous adipose tissue (Plate 4). These masses were expansive and displaced but did not invade adjacent dermal adnexal structures and compressed surrounding subcutaneous adipose tissue. They were composed of closely packed, heavily pigmented polygonal cells (Plate 5). Cellular detail was largely obscured because of the dense pigmentation; however, dendritic cellular processes were occasionally observed extending between cells.

TABLE 42
Incidences of Melanotic Skin Lesions in B6C3F$_1$ Mice at the Site of Application

	Pigmentation, Melanin	Melanoma	Melanoma, Multiple
Male			
Acetone/Acetone	0/30	0/30	0/30
2.5 μg DMBA/Acetone	0/30	0/30	0/30
25 μg DMBA/Acetone	0/30	0/30	0/30
50 μg DMBA/Acetone	0/30	0/30	0/30
2.5 μg DMBA/2.5 μg DMBA	0/30	1/30	0/30
5 μg TPA/5 μg TPA	23/30	0/30	0/30
20 mg BPO/20 mg BPO	0/30	0/30	0/30
2.5 μg DMBA/TPA	10/30	0/30	0/30
25 μg DMBA/TPA	17/30	2/30	2/30
50 μg DMBA/TPA	14/30	6/30	4/30
2.5 μg DMBA/20 mg BPO	3/30	0/30	0/30
25 μg DMBA/20 mg BPO	0/30	1/30	0/30
Female			
Acetone/Acetone	0/30	0/30	0/30
2.5 μg DMBA/Acetone	0/30	0/30	0/30
25 μg DMBA/Acetone	0/30	0/30	0/30
50 μg DMBA/Acetone	0/30	0/30	0/30
2.5 μg DMBA/2.5 μg DMBA	7/30	0/30	0/30
5 μg TPA/5 μg TPA	29/30	0/30	0/30
20 mg BPO/20 mg BPO	6/30	0/30	0/30
2.5 μg DMBA/TPA	28/30	2/30	0/30
25 μg DMBA/TPA	21/30	5/30	4/30
50 μg DMBA/TPA	11/30	10/30	9/30
2.5 μg DMBA/20 mg BPO	8/30	1/30	0/30
25 μg DMBA/20 mg BPO	2/30	4/30	0/30

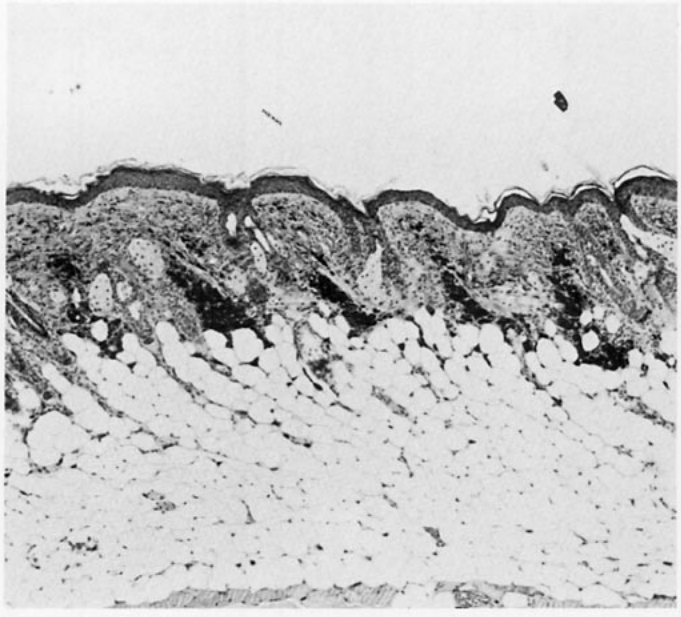


PLATE 1
Pigmentation, melanin in the skin (site of application) of a $B6C3F_1$ mouse receiving 5 μg TPA/5 μg TPA. Increased numbers of deeply pigmented melanocytes occur as multifocal aggregates in the reticular dermis and as loose aggregates within the papillary dermis. H&E, 16×

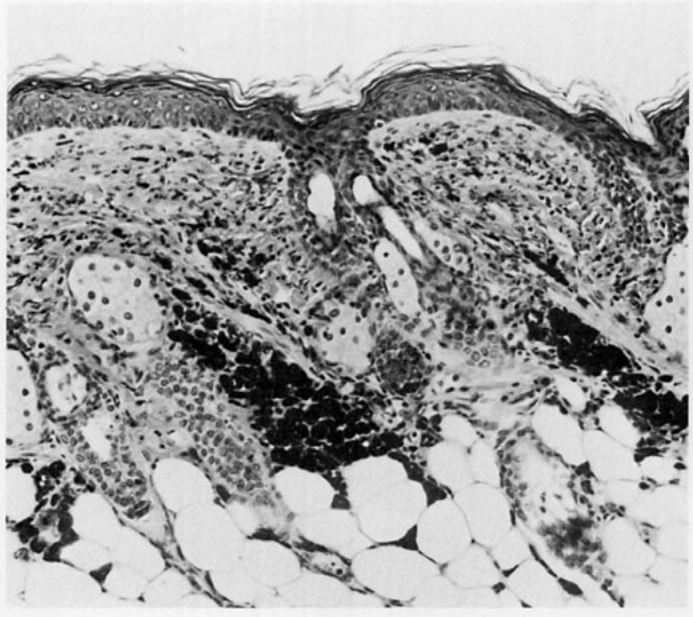


PLATE 2
Pigmentation, melanin in the skin (site of application) of a $B6C3F_1$ mouse receiving 5 μg TPA/5 μg TPA. Increased magnification of Plate 1 showing densely and loosely aggregated melanocytes within the dermis. H&E, 40×

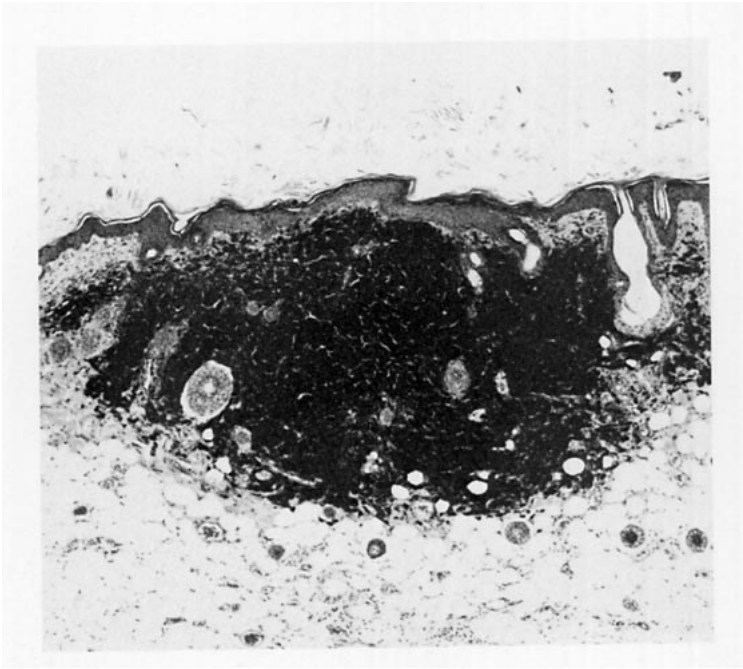


PLATE 3
Melanoma in the skin (site of application) of a $B6C3F_1$ mouse receiving 2.5 μg DMBA/2.5 μg DMBA. Discrete, heavily pigmented, nodular mass that is confined largely to the dermis with slight extension into the subcutaneous adipose tissue. H&E, 8×

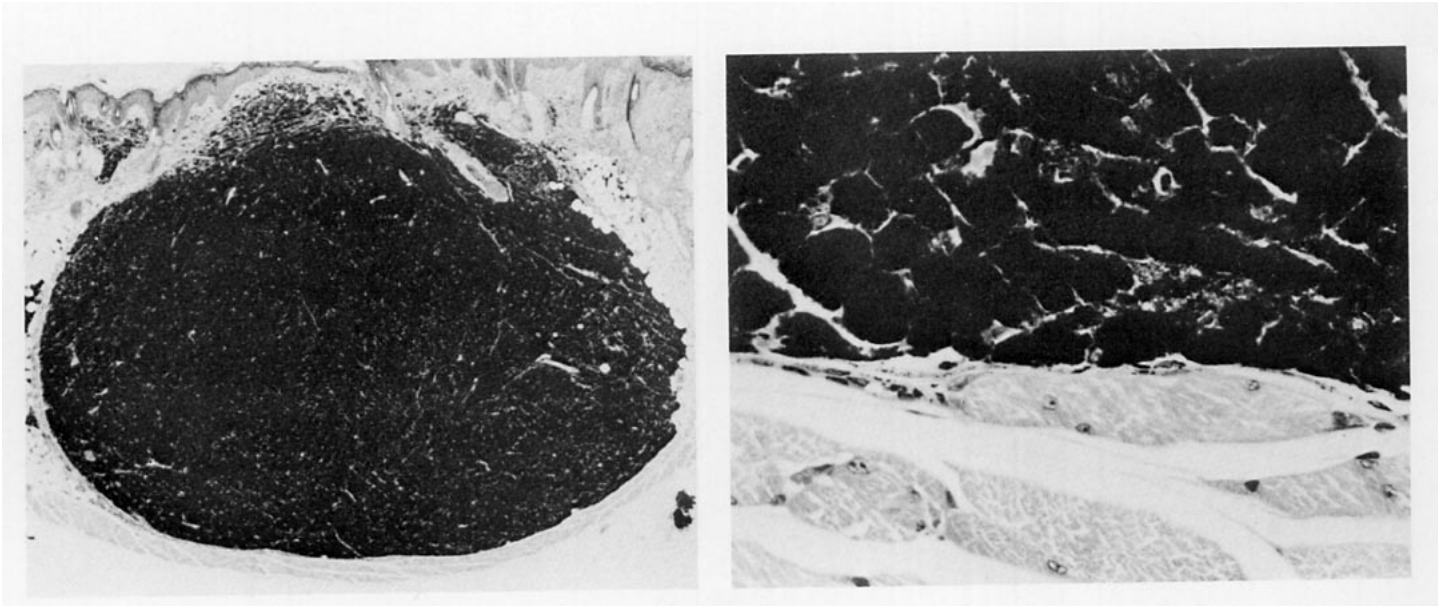


PLATE 4
Melanoma in the skin (site of application) of a $B6C3F_1$ mouse receiving 50 μg DMBA/5 μg TPA. Well-circumscribed, heavily pigmented, expansile, nodular mass extends deeply into the subcutis and compresses surrounding adipose tissue. H&E, 8×

PLATE 5
Melanoma in the skin (site of application) of a $B6C3F_1$ mouse 50 μg DMBA/5 μg TPA. Higher magnification of Plate 4. Melanoma is composed of heavily pigmented polygonal cells adjacent to the panniculus carnosus muscle fibers in the subcutis. Cellular detail is obscured by extensive accumulation of pigmented granules. H&E, 100×

DISCUSSION

These studies were developed to address the recommendation made that the NTP expand the use of short-term tests to detect agents that do not exert genetic effects (e.g., promoting agents). The non-invasive mouse skin initiation/promotion model allows the progress of tumor development to be monitored following treatment of the skin by various test agents. Studies of this type have provided valuable information about tumorigenesis (i.e., the progression from a genetic change through promotion of that change to the development of a tumor).

All of the NTP 2-year toxicology/carcinogenesis studies follow a well-defined standard protocol for study conduct and data collection. The F344/N rat and $B6C3F_1$ mouse are the rodent species used to evaluate these chemical effects. Data from NTP 2-year studies are captured electronically, frequently reviewed and summarized, and the biology of the F344/N rat and $B6C3F_1$ mouse are now well defined. Obtaining initiation/promotion data in the $B6C3F_1$ mouse with its large historical database would provide valuable information to increase our understanding about the tumorigenic potential of tested chemicals.

The current studies used combinations of well-studied chemicals in an initiation/promotion model to collect skin tumor response data in $B6C3F_1$, Swiss (CD-1®), and SENCAR mice. The data were used to compare the potential of the mouse strains to identify promoters and complete carcinogens using this protocol.

All three strains of mice demonstrated sensitivity by developing skin tumors in response to the initiation/promotion study using DMBA, MNNG, TPA, and BPO. The most sensitive of the three strains appeared to be SENCAR mice, in the sense that lower doses of the test chemical were generally required to produce effects equivalent to those in the other two strains. Skin tumors also tended to develop earlier and to increase in multiplicity in SENCAR mice relative to the other two strains. By these criteria, the overall sensitivity of Swiss (CD-1®) mice was intermediate, and $B6C3F_1$ mice showed the least overall sensitivity to dermal carcinogenicity.

There were clear differences in mouse skin nonneoplastic response to the chemicals used in these studies. Chemicals with promotion potential have been reported to cause inflammation and epidermal hyperplasia (Slaga, 1984). In the current studies, no irritation or ulcers were observed in acetone vehicle controls and were rarely observed in DMBA initiator controls for any of the strains. TPA did not cause irritation or ulcer in non-initiated $B6C3F_1$ mice and BPO did not produce significant incidences of these lesions in any of the strains. However, TPA without any chemical initiation caused skin irritation and ulcer in Swiss (CD-1®) and SENCAR mice. Also, 5 μg doses of TPA caused an increased incidence of papillomas in the non-initiated Swiss (CD-1®) mice compared with vehicle controls. When DMBA was used as the initiator followed by TPA promotion, the incidence of irritation and ulcer did not increase significantly in $B6C3F_1$ mice, but papilloma incidence was significantly increased. In Swiss (CD-1®) and SENCAR mice receiving DMBA/TPA treatment, the incidence of ulcer increased somewhat and these two strains also had significant incidences of papillomas compared with the respective vehicle controls. Fewer observations of irritation and ulcer were collected for mice exposed to DMBA initiation followed by BPO promotion than for mice of these strains that received TPA promotion. $B6C3F_1$ and Swiss (CD-1®) mice receiving DMBA initiation and BPO promotion also developed fewer tumors than did mice of these strains that received TPA promotion. However, more than 66% of SENCAR mice were observed with papillomas. The low incidence of irritation and ulcer as a result of TPA application in the $B6C3F_1$ mouse skin compared with Swiss (CD-1®) and SENCAR mice suggests a difference in skin sensitivity to the irritating properties of TPA. The higher incidence of ulcer in Swiss (CD-1®) and SENCAR mice receiving TPA may also have contributed to the higher papilloma incidence in these two strains (Argyris, 1981). Therefore, the skin papilloma response to BPO may be more reflective of chemical promotion potential.

DMBA and MNNG treatment as complete carcinogens produced a higher ratio of squamous cell carcinoma to squamous cell papilloma in all three

strains than it did in the mice receiving initiation and promotion treatment. This result would support the concept of an initiator producing some fixed number of genetic alterations followed by a non-mutagenic promoting effect. Also, more carcinomas would be expected given the greater number of times for a genetic alteration to occur after repeated treatment with a carcinogen (DiGiovanni and Juchau, 1980; Reddy and Fialkow, 1983).

There were increased incidences of melanomas in $B6C3F_1$ mice receiving DMBA. These lesions were not seen in groups receiving MNNG treatment and would not be observed in the unpigmented strains. Spontaneous cutaneous melanomas are rare in $B6C3F_1$ mice (Ward *et al.*, 1979; Haseman *et al.*, 1985) and occur principally in the dermis. The melanotic lesions observed in these studies are similar to those that occur spontaneously (Kanno, 1989; Bogovski, 1994). The melanomas diagnosed in $B6C3F_1$ mice in the DMBA studies were relatively small and considered benign based on morphology. However, spontaneous dermal melanomas are known to metastasize to the lungs and other organs upon attaining a large size. Melanomas have been reported in a variety of mouse strains following topical application of chemical carcinogens (Takizawa *et al.*, 1985; Kanno, 1989) and with few exceptions are confined to the dermis.

In response to the NTP *ad hoc* committee recommendations to use short-term tests, SENCAR mice would be the most acceptable strain to use for such studies, based on the skin tumor response sensitivity to various initiators and promoters. Though the $B6C3F_1$ mice were less responsive in the skin initiation/promotion protocol, promotion data from this strain may, at times, be of more use in explaining mechanisms of tumor development (e.g., when there is a strain-specific response observed in 2-year carcinogenicity studies or effects on melanocytes are suspected).

In recent years, transgenic strains of mice have been developed which may replace the existing mouse models. For example, NIEHS/NTP scientists have begun to study chemical effects on tumor development using the TG.AC transgenic line (Spalding *et al.*, 1993). TG.AC mice behave like genetically initiated mice, rapidly developing high numbers of epidermal papillomas in response to topical treatment with tumor promoters and carcinogens. In some studies, the time to first tumor appearance has been as short as 3 weeks, and all chemicals tested that produced papillomas did so in less than 20 weeks. The absence of the need for an initiator, the short term to tumor, and high tumor yield all suggest that the use of transgenic mice, such as the TG.AC line, has the potential to be extremely useful in the study of the mechanism of tumorigenesis.

A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 8.

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APPENDIX A
SUMMARY OF SKIN LESIONS IN MALE AND FEMALE B6C3F$_1$ MICE IN THE COMPARATIVE INITIATION/PROMOTION SKIN PAINT STUDIES

TABLE A1a Summary of the Incidence of Skin Neoplasms in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A 102
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TABLE A1a
Summary of the Incidence of Skin Neoplasms in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ 20 mg BPO	5 μg TPA/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						
Moribund	3		3		2	1
Natural deaths	1		1		1	
Survivors						
Terminal sacrifice	26	30	26	30	27	29
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Melanoma NOS				1 (3%)		
Sebaceous gland, site of application-mass, adenoma			1 (3%)	1 (3%)	1 (3%)	
Sebaceous gland, site of application-mass, adenoma, multiple			1 (3%)			
Site of application-no mass, melanoma NOS			1 (3%)			
Site of application-no mass, melanoma NOS, multiple						
Site of application-mass, basal cell carcinoma			1 (3%)			
Site of application-mass, keratoacanthoma			4 (13%)			
Site of application-mass, keratoacanthoma, multiple			1 (3%)			
Site of application-mass, squamous cell carcinoma			10 (33%)	1 (3%)		
Site of application-mass, squamous cell carcinoma, multiple			14 (47%)			
Site of application-mass, squamous cell papilloma			7 (23%)	4 (13%)		
Site of application-mass, squamous cell papilloma, multiple			5 (17%)	4 (13%)		
Subcutaneous tissue, sarcoma						1 (3%)
Subcutaneous tissue, site of application-mass, fibrosarcoma						
Subcutaneous tissue, site of application-mass, sarcoma			1 (3%)			
Neoplasm Summary						
Total animals with primary neoplasms[b]			29	11	1	1
Total primary neoplasms			46	11	1	1
Total animals with benign neoplasms			17	9	1	
Total benign neoplasms			19	9	1	
Total animals with malignant neoplasms			26	1		1
Total malignant neoplasms			26	1		1
Total animals with uncertain neoplasms-benign or malignant			1	1		
Total uncertain neoplasms			1	1		

TABLE A1a
Summary of the Incidence of Skin Neoplasms in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	25 µg DMBA/ Acetone	25 µg DMBA/ 5 µg TPA	25 µg DMBA/ 20 mg BPO	50 µg DMBA/ Acetone	50 µg DMBA/ 5 µg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental deaths	1				1	
Moribund		1			3	
Natural deaths						
Survivors						
Terminal sacrifice	29	29	30	30	26	30
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Melanoma NOS						
Sebaceous gland, site of application-mass, adenoma	1 (3%)	1 (3%)	1 (3%)		2 (7%)	
Sebaceous gland, site of application-mass, adenoma, multiple						
Site of application-no mass, melanoma NOS		2 (7%)	1 (3%)		6 (20%)	
Site of application-no mass, melanoma NOS, multiple		2 (7%)			4 (13%)	
Site of application-mass, basal cell carcinoma						
Site of application-mass, keratoacanthoma		2 (7%)	1 (3%)		2 (7%)	
Site of application-mass, keratoacanthoma, multiple		1 (3%)				
Site of application-mass, squamous cell carcinoma		1 (3%)	1 (3%)		2 (7%)	
Site of application-mass, squamous cell carcinoma, multiple					1 (3%)	
Site of application-mass, squamous cell papilloma		7 (23%)			7 (23%)	
Site of application-mass, squamous cell papilloma, multiple		3 (10%)			9 (30%)	
Subcutaneous tissue, sarcoma						
Subcutaneous tissue, site of application-mass, fibrosarcoma					1 (3%)	
Subcutaneous tissue, site of application-mass, sarcoma						
Neoplasm Summary (continued)						
Total animals with primary neoplasms	1	14	3		21	
Total primary neoplasms	1	19	4		34	
Total animals with benign neoplasms	1	11	2		16	
Total benign neoplasms	1	14	2		20	
Total animals with malignant neoplasms		1	1		4	
Total malignant neoplasms		1	1		4	
Total animals with uncertain neoplasms-benign or malignant		4	1		10	
Total uncertain neoplasms		4	1		10	

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

TABLE A1b
Summary of the Incidence of Skin Neoplasms in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	5 μg TPA/ 5 μg TPA	100 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 20 mg BPO	100 μg MNNG/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund				12		1
Natural deaths				2		
Survivors						
Terminal sacrifice	30	30	30	16	30	29
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Site of application-mass, keratoacanthoma						
Site of application-mass, squamous cell carcinoma				16 (53%)		
Site of application-mass, squamous cell carcinoma, multiple				13 (43%)		
Site of application-mass, squamous cell papilloma				2 (7%)		5 (17%)
Site of application-mass, squamous cell papilloma, multiple						2 (7%)
Site of application-mass, trichoepithelioma						
Subcutaneous tissue, site of application-no mass, lymphangioma						
Subcutaneous tissue, site of application-no mass, lymphangiosarcoma						
Subcutaneous tissue, site of application-no mass, sarcoma						
Subcutaneous tissue, site of application-mass fibrosarcoma						
Subcutaneous tissue, site of application-mass, hemangiosarcoma						
Subcutaneous tissue, site of application-mass, sarcoma						1 (3%)
Subcutaneous tissue, site of application-mass, sarcoma, multiple						
Subcutaneous tissue, site of application-mass, squamous cell carcinoma				1 (3%)		
Neoplasm Summary						
Total animals with primary neoplasms[b]				29		8
Total primary neoplasms				32		8
Total animals with benign neoplasms				2		7
Total benign neoplasms				2		7
Total animals with malignant neoplasms				29		1
Total malignant neoplasms				30		1

TABLE A1b
Summary of the Incidence of Skin Neoplasms in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	500 μg MNNG/ Acetone	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ Acetone	1,000 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	1	1	2	2	14	
Natural deaths						
Survivors						
Terminal sacrifice	29	29	28	28	16	30
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Site of application-mass, keratoacanthoma			1 (3%)			
Site of application-mass, squamous cell carcinoma		2 (7%)	3 (10%)	1 (3%)	4 (13%)	
Site of application-mass, squamous cell carcinoma, multiple				1 (3%)	1 (3%)	
Site of application-mass, squamous cell papilloma			1 (3%)	3 (10%)	3 (10%)	
Site of application-mass, squamous cell papilloma, multiple				1 (3%)	3 (10%)	
Site of application-mass, trichoepithelioma					2 (7%)	
Subcutaneous tissue, site of application-no mass, lymphangioma					1 (3%)	
Subcutaneous tissue, site of application-no mass, lymphangiosarcoma					1 (3%)	
Subcutaneous tissue, site of application-no mass, sarcoma			1 (3%)			
Subcutaneous tissue, site of application-mass, fibrosarcoma			1 (3%)			
Subcutaneous tissue, site of application-mass, hemangiosarcoma		1 (3%)				
Subcutaneous tissue, site of application-mass, sarcoma	1 (3%)	1 (3%)	3 (10%)	3 (10%)	10 (33%)	
Subcutaneous tissue, site of application-mass, sarcoma, multiple				1 (3%)	2 (7%)	
Subcutaneous tissue, site of application-mass, squamous cell carcinoma						
Neoplasm Summary (continued)						
Total animals with primary neoplasms	1	4	10	9	22	
Total primary neoplasms	1	4	10	10	27	
Total animals with benign neoplasms			2	4	8	
Total benign neoplasms			2	4	9	
Total animals with malignant neoplasms	1	4	8	6	16	
Total malignant neoplasms	1	4	8	6	18	

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

TABLE A1c
Summary of the Incidence of Skin Neoplasms in Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ 20 mg BPO	5 μg TPA/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						
Moribund			2		1	
Natural deaths		1	2		1	
Survivors						
Terminal sacrifice	30	29	26	30	28	30
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Squamous cell carcinoma						
Sebaceous gland, site of application-mass, adenoma			8 (27%)	2 (7%)		
Sebaceous gland, site of application-mass, adenoma, multiple			1 (3%)			
Site of application-no mass, melanoma NOS				2 (7%)	1 (3%)	
Site of application-no mass, melanoma NOS, multiple						
Site of application-mass, keratoacanthoma						
Site of application-mass, melanoma NOS						
Site of application-mass, basal cell adenoma			1 (3%)			
Site of application-mass, basal cell carcinoma			1 (3%)			
Site of application-mass, squamous cell carcinoma			16 (53%)		2 (7%)	1 (3%)
Site of application-mass, squamous cell carcinoma, multiple			8 (27%)			
Site of application-mass, squamous cell papilloma			3 (10%)	1 (3%)	3 (10%)	
Site of application-mass, squamous cell papilloma, multiple			8 (27%)	2 (7%)		
Subcutaneous tissue, site of application-mass, hemangiosarcoma						
Neoplasm Summary						
Total animals with primary neoplasms[b]			29	6	6	1
Total primary neoplasms			46	7	6	1
Total animals with benign neoplasms			14	4	3	
Total benign neoplasms			21	5	3	
Total animals with malignant neoplasms			25		2	1
Total malignant neoplasms			25		2	1
Total animals with uncertain neoplasms-benign or malignant				2	1	
Total uncertain neoplasms				2	1	

TABLE A1c
Summary of the Incidence of Skin Neoplasms in Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	25 μg DMBA/ Acetone	25 μg DMBA/ 5 μg TPA	25 μg DMBA/ 20 mg BPO	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						1
Moribund		1	1		2	2
Natural deaths		1			1	
Survivors						
Terminal sacrifice	30	28	29	30	27	27
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Squamous cell carcinoma		1 (3%)				
Sebaceous gland, site of application-mass, adenoma		3 (10%)			11 (37%)	
Sebaceous gland, site of application-mass, adenoma, multiple					2 (7%)	
Site of application-no mass, melanoma NOS		5 (17%)	4 (13%)		10 (33%)	
Site of application-no mass, melanoma NOS, multiple		4 (13%)			9 (30%)	
Site of application-mass, keratoacanthoma					1 (3%)	
Site of application-mass, melanoma NOS					1 (3%)	
Site of application-mass, basal cell adenoma						
Site of application-mass, basal cell carcinoma						
Site of application-mass, squamous cell carcinoma		3 (10%)			1 (3%)	
Site of application-mass, squamous cell carcinoma, multiple						
Site of application-mass, squamous cell papilloma		3 (10%)	1 (3%)		5 (17%)	
Site of application-mass, squamous cell papilloma, multiple		1 (3%)			5 (17%)	
Subcutaneous tissue, site of application-mass, hemangiosarcoma					1 (3%)	
Neoplasm Summary (continued)						
Total animals with primary neoplasms		13	5		24	
Total primary neoplasms		20	5		46	
Total animals with benign neoplasms		7	1		17	
Total benign neoplasms		7	1		24	
Total animals with malignant neoplasms		3			2	
Total malignant neoplasms		4			2	
Total animals with uncertain neoplasms-benign or malignant		9	4		19	
Total uncertain neoplasms		9	4		20	

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

TABLE A1d
Summary of the Incidence of Skin Neoplasms in Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	5 μg TPA/ 5 μg TPA	100 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 5 μg TPA	500 μg MNNG/ Acetone
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						
Moribund				13		
Natural deaths				4		
Survivors						
Terminal sacrifice	30	30	30	13	29	30
Missing					1	
Animals examined microscopically	30	30	30	30	29	30
Skin	(30)	(30)	(30)	(30)	(29)	(30)
Sarcoma						
Squamous cell carcinoma						
Sebaceous gland, site of application-mass, adenoma						
Site of application-mass, squamous cell carcinoma				24 (80%)		
Site of application-mass, squamous cell carcinoma, multiple				6 (20%)		
Site of application-mass, squamous cell papilloma				1 (3%)	1 (3%)	
Site of application-mass, squamous cell papilloma, multiple						
Subcutaneous tissue, control, sarcoma						
Subcutaneous tissue, site of application-no mass, lymphangioma						
Subcutaneous tissue, site of application-no mass, sarcoma						
Subcutaneous tissue, site of application-no mass, mast cell tumor benign				1 (3%)		
Subcutaneous tissue, site of application-mass, sarcoma				1 (3%)		
Subcutaneous tissue, site of application-mass, sarcoma, multiple						
Neoplasm Summary						
Total animals with primary neoplasms[b]				30	1	
Total primary neoplasms				33	1	
Total animals with benign neoplasms				2	1	
Total benign neoplasms				2	1	
Total animals with malignant neoplasms				30		
Total malignant neoplasms				31		

TABLE A1d
Summary of the Incidence of Skin Neoplasms in Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	100 μg MNNG/ 20 mg BPO	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ Acetone	1,000 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death		1				
Moribund		2	6	5	5	1
Natural deaths			1		3	
Survivors						
Terminal sacrifice	30	27	23	25	22	29
Missing						
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Sarcoma			1 (3%)			
Squamous cell carcinoma			1 (3%)			
Sebaceous gland, site of application-mass, adenoma				1 (3%)		
Site of application-mass, squamous cell carcinoma			5 (17%)	4 (13%)	1 (3%)	
Site of application-mass, squamous cell carcinoma, multiple						
Site of application-mass, squamous cell papilloma	1 (3%)			2 (7%)	1 (3%)	
Site of application-mass, squamous cell papilloma, multiple					1 (3%)	
Subcutaneous tissue, control, sarcoma			1 (3%)			
Subcutaneous tissue, site of application-no mass, lymphangioma					1 (3%)	
Subcutaneous tissue, site of application-no mass, sarcoma				1 (3%)		
Subcutaneous tissue, site of application-no mass, mast cell tumor benign						
Subcutaneous tissue, site of application-mass, sarcoma		1 (3%)	4 (13%)	3 (10%)	7 (23%)	
Subcutaneous tissue, site of application-mass, sarcoma, multiple			1 (3%)		1 (3%)	
Neoplasm Summary (continued)						
Total animals with primary neoplasms	1	1	9	11	10	
Total primary neoplasms	1	1	13	11	12	
Total animals with benign neoplasms	1			3	3	
Total benign neoplasms	1			3	3	
Total animals with malignant neoplasms		1	9	8	8	
Total malignant neoplasms		1	13	8	9	

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

TABLE A2a
Overview of the Sensitivity of Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
5 μg TPA/5 μg TPA	0/30	–	0.00	0.00
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
2.5 μg DMBA/Acetone	0/30	–	0.00	0.00
25 μg DMBA/Acetone	0/30	–	0.00	0.00
50 μg DMBA/Acetone	0/30	–	0.00	0.00
2.5 μg DMBA/5 μg TPA	10/30	35.4	0.57	1.70
25 μg DMBA/5 μg TPA	12/30	27.6	1.13	2.83
50 μg DMBA/5 μg TPA	22/30	32.5	1.95	2.66
2.5 μg DMBA/20 mg BPO	1/30	48.0	0.03	1.00
25 μg DMBA/20 mg BPO	1/30	39.0	0.03	1.00
2.5 μg DMBA/2.5 μg DMBA	25/30	46.6	2.13	2.56

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE A2b
Overview of the Sensitivity of Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
5 μg TPA/5 μg TPA	0/30	–	0.00	0.00
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
100 μg MNNG/Acetone	0/30	–	0.00	0.00
500 μg MNNG/Acetone	1/30	34.0	0.03	1.00
1,000 μg MNNG/Acetone	13/30	23.8	0.43	1.00
100 μg MNNG/5 μg TPA	9/30	37.2	0.30	1.00
1,000 μg MNNG/5 μg TPA	26/30	16.6	1.30	1.50
100 μg MNNG/20 mg BPO	0/30	–	0.00	0.00
500 μg MNNG/20 mg BPO	3/30	47.0	0.10	1.00
1,000 μg MNNG/20 mg BPO	9/30	34.4	0.33	1.11
100 μg MNNG/100 μg MNNG	27/30	40.1	1.43	1.59

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE A2c
Overview of the Sensitivity of Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
5 μg TPA/5 μg TPA	0/30	–	0.00	0.00
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
2.5 μg DMBA/Acetone	0/30	–	0.00	0.00
25 μg DMBA/Acetone	0/30	–	0.00	0.00
50 μg DMBA/Acetone	0/30	–	0.00	0.00
2.5 μg DMBA/5 μg TPA	3/30	36.7	0.27	2.67
25 μg DMBA/5 μg TPA	7/30	41.0	0.37	1.57
50 μg DMBA/5 μg TPA	16/30	40.8	1.10	2.06
2.5 μg DMBA/20 mg BPO	4/30	51.0	0.13	1.00
25 μg DMBA/20 mg BPO	2/30	44.0	0.07	1.00
2.5 μg DMBA/2.5 μg DMBA	27/30	45.3	2.17	2.41

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE A2d
Overview of the Sensitivity of Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
5 μg TPA/5 μg TPA	0/30	–	0.00	0.00
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
100 μg MNNG/Acetone	0/30	–	0.00	0.00
500 μg MNNG/Acetone	0/30	–	0.00	0.00
1,000 μg MNNG/Acetone	13/30	16.0	0.57	1.31
100 μg MNNG/5 μg TPA	1/30	43.0	0.03	1.00
1,000 μg MNNG/5 μg TPA	16/30	25.8	0.70	1.31
100 μg MNNG/20 mg BPO	1/30	44.0	0.03	1.00
500 μg MNNG/20 mg BPO	3/30	43.0	0.10	1.00
1,000 μg MNNG/20 mg BPO	13/30	24.5	0.50	1.15
100 μg MNNG/100 μg MNNG	30/30	37.9	1.70	1.70

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE A3a
Summary of the Incidence of Nonneoplastic Skin Lesions in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ 20 mg BPO	5 μg TPA/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						
Moribund	3		3		2	1
Natural deaths	1		1		1	
Survivors						
Terminal sacrifice	26	30	26	30	27	29
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Site of application-no mass, acanthosis			23 (77%)	28 (93%)	27 (90%)	30 (100%)
Site of application-no mass, cyst epithelial inclusion			1 (3%)			
Site of application-no mass, granuloma						
Site of application-no mass, inflammation, chronic active			5 (17%)	28 (93%)	27 (90%)	29 (97%)
Site of application-no mass, pigmentation, melanin				10 (33%)	3 (10%)	23 (77%)
Site of application-no mass, ulcer			1 (3%)	1 (3%)	2 (7%)	1 (3%)
Site of application-no mass, ulcer, multiple			2 (7%)		1 (3%)	
Site of application-mass, acanthosis			2 (7%)			

TABLE A3a
Summary of the Incidence of Nonneoplastic Skin Lesions in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	25 μg DMBA/ Acetone	25 μg DMBA/ 5 μg TPA	25 μg DMBA/ 20 mg BPO	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death	1				1	
Moribund		1			3	
Natural deaths						
Survivors						
Terminal sacrifice	29	29	30	30	26	30
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Site of application-no mass, acanthosis	8 (27%)	29 (97%)	30 (100%)	7 (23%)	29 (97%)	27 (90%)
Site of application-no mass, cyst epithelial inclusion						
Site of application-no mass, granuloma						1 (3%)
Site of application-no mass, inflammation, chronic active	1 (3%)	28 (93%)	27 (90%)		28 (93%)	26 (87%)
Site of application-no mass, pigmentation, melanin		17 (57%)			14 (47%)	
Site of application-no mass, ulcer			1 (3%)		1 (3%)	
Site of application-no mass, ulcer, multiple			2 (7%)			
Site of application-mass, acanthosis		2 (7%)			1 (3%)	

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

TABLE A3b
Summary of the Incidence of Nonneoplastic Skin Lesions in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	5 μg TPA/ 5 μg TPA	100 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 20 mg BPO	100 μg MNNG/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund				12		1
Natural deaths				2		
Survivors						
Terminal sacrifice	30	30	30	16	30	29
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Inflammation, chronic active						
Hair follicle, site of application-no mass, cyst				2 (7%)	2 (7%)	
Sebaceous gland, site of application-mass, hyperplasia						
Site of application-no mass, acanthosis		30 (100%)	2 (7%)	21 (70%)	22 (73%)	29 (97%)
Site of application-no mass, inflammation, chronic active		12 (40%)		15 (50%)	9 (30%)	20 (67%)
Site of application-no mass, ulcer		2 (7%)		9 (30%)	3 (10%)	1 (3%)
Site of application-mass, acanthosis						1 (3%)
Site of application-mass, hyperplasia, basal cell						
Subcutaneous tissue, site of application-no mass, granuloma				1 (3%)		

TABLE A3b
Summary of the Incidence of Nonneoplastic Skin Lesions in Male B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	500 μg MNNG/ Acetone	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ Acetone	1,000 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	1	1	2	2	14	
Natural deaths						
Survivors						
Terminal sacrifice	29	29	28	28	16	30
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Inflammation, chronic active			1 (3%)	1 (3%)		
Hair follicle, site of application-no mass, cyst		1 (3%)	5 (17%)	1 (3%)	1 (3%)	
Sebaceous gland, site of application-mass, hyperplasia				1 (3%)		
Site of application-no mass, acanthosis	18 (60%)	27 (90%)	19 (63%)	25 (83%)	28 (93%)	24 (80%)
Site of application-no mass, inflammation, chronic active	1 (3%)	9 (30%)	5 (17%)	19 (63%)	20 (67%)	12 (40%)
Site of application-no mass, ulcer		1 (3%)	4 (13%)	3 (10%)	4 (13%)	
Site of application-mass, acanthosis		1 (3%)	1 (3%)	1 (3%)	2 (7%)	
Site of application-mass, hyperplasia, basal cell				1 (3%)		
Subcutaneous tissue, site of application-no mass, granuloma						

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

TABLE A3c
Summary of the Incidence of Nonneoplastic Skin Lesions in Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ 20 mg BPO	5 μg TPA/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						
Moribund			2		1	
Natural deaths		1	2		1	
Survivors						
Terminal sacrifice	30	29	26	30	28	30
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Sebaceous gland, site of application-mass, hyperplasia			1 (3%)			
Site of application-no mass, acanthosis			24 (80%)	30 (100%)	29 (97%)	30 (100%)
Site of application-no mass, granuloma			1 (3%)			
Site of application-no mass, inflammation, chronic active			9 (30%)	30 (100%)	29 (97%)	30 (100%)
Site of application-no mass, mineralization						
Site of application-no mass, pigmentation, melanin			7 (23%)	28 (93%)	8 (27%)	29 (97%)
Site of application-no mass, ulcer			3 (10%)		2 (7%)	1 (3%)
Site of application-no mass, ulcer, multiple			2 (7%)	1 (3%)	2 (7%)	1 (3%)
Site of application-mass, acanthosis			1 (3%)	1 (3%)		

TABLE A3c
Summary of the Incidence of Nonneoplastic Skin Lesions in Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	25 μg DMBA/ Acetone	25 μg DMBA/ 5 μg TPA	25 μg DMBA/ 20 mg BPO	50 μg DMBA/ Acetone	50 μg DMBA/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						1
Moribund		1	1		2	2
Natural deaths		1			1	
Survivors						
Terminal sacrifice	30	28	29	30	27	27
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Sebaceous gland, site of application-mass, hyperplasia						
Site of application-no mass, acanthosis	5 (17%)	30 (100%)	28 (93%)	18 (60%)	30 (100%)	29 (97%)
Site of application-no mass, granuloma						
Site of application-no mass, inflammation, chronic active	1 (3%)	30 (100%)	30 (100%)	2 (7%)	30 (100%)	27 (90%)
Site of application-no mass, mineralization		1 (3%)				
Site of application-no mass, pigmentation, melanin		21 (70%)	2 (7%)		11 (37%)	6 (20%)
Site of application-no mass, ulcer		1 (3%)			1 (3%)	
Site of application-no mass, ulcer, multiple		3 (10%)			3 (10%)	1 (3%)
Site of application-mass, acanthosis						

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

TABLE A3d
Summary of the Incidence of Nonneoplastic Skin Lesions in Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	5 μg TPA/ 5 μ TPA	100 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 5 μg TPA	500 μg MNNG/ Acetone
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						
Moribund				13		
Natural deaths				4		
Survivors						
Terminal sacrifice	30	30	30	13	29	30
Missing					1	
Animals examined microscopically	30	30	30	30	29	30
Skin	(30)	(30)	(30)	(30)	(29)	(30)
Hair follicle, site of application-no mass, cyst				1 (3%)		
Sebaceous gland, site of application-mass, hyperplasia						
Site of application-no mass, acanthosis		30 (100%)	2 (7%)	25 (83%)	29 (100%)	20 (67%)
Site of application-no mass, inflammation, chronic active		27 (90%)		17 (57%)	29 (100%)	6 (20%)
Site of application-no mass, ulcer		1 (3%)		12 (40%)		
Site of application-mass, acanthosis		1 (3%)				
Subcutaneous tissue, site of application-no mass, fibrosis				1 (3%)		
Subcutaneous tissue, site of application-no mass, infiltration cellular, focal, mast cell						
Subcutaneous tissue, site of application-no mass, mineralization						

TABLE A3d
Summary of the Incidence of Nonneoplastic Skin Lesions in Female B6C3F$_1$ Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	100 μg MNNG/ 20 mg BPO	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ Acetone	1,000 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death		1				
Moribund		2	6	5	5	1
Natural deaths			1		3	
Survivors						
Terminal sacrifice	30	27	23	25	22	29
Missing						
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst		1 (3%)	1 (3%)	1 (3%)		
Sebaceous gland, site of application-mass, hyperplasia					1 (3%)	
Site of application-no mass, acanthosis	22 (73%)	27 (90%)	18 (60%)	27 (90%)	28 (93%)	17 (57%)
Site of application-no mass, inflammation, chronic active	17 (57%)	20 (67%)	13 (43%)	23 (77%)	24 (80%)	15 (50%)
Site of application-no mass, ulcer		3 (10%)	2 (7%)	2 (7%)	5 (17%)	3 (10%)
Site of application-mass, acanthosis		1 (3%)				
Subcutaneous tissue, site of application-no mass, fibrosis						
Subcutaneous tissue, site of application-no mass, infiltration cellular, focal, mast cell		1 (3%)				
Subcutaneous tissue, site of application-no mass, mineralization					1 (3%)	

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

APPENDIX B
SUMMARY OF SKIN LESIONS IN MALE AND FEMALE SWISS (CD-1®) MICE IN THE COMPARATIVE INITIATION/PROMOTION SKIN PAINT STUDIES

TABLE B1a Summary of the Incidence of Skin Neoplasms in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A 122
TABLE B1b Summary of the Incidence of Skin Neoplasms in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B 124
TABLE B1c Summary of the Incidence of Skin Neoplasms in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A 126
TABLE B1d Summary of the Incidence of Skin Neoplasms in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B 128
TABLE B2a Overview of the Sensitivity of Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A 130
TABLE B2b Overview of the Sensitivity of Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B 130
TABLE B2c Overview of the Sensitivity of Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A 131
TABLE B2d Overview of the Sensitivity of Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B 131
TABLE B3a Summary of the Incidence of Nonneoplastic Skin Lesions in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A 132
TABLE B3b Summary of the Incidence of Nonneoplastic Skin Lesions in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B 134
TABLE B3c Summary of the Incidence of Nonneoplastic Skin Lesions in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A 136
TABLE B3d Summary of the Incidence of Nonneoplastic Skin Lesions in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B 138

TABLE B1a
Summary of the Incidence of Skin Neoplasms in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	0.25 μg DMBA/ Acetone	0.25 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	1	2	6	1	7	8
Natural deaths	2	3	4		4	7
Survivors						
Terminal sacrifice	27	25	20	29	19	15
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Squamous cell carcinoma						1 (3%)
Squamous cell papilloma						
Site of application-mass, basal cell adenoma					1 (3%)	
Site of application-mass, keratoacanthoma			1 (3%)		1 (3%)	
Site of application-mass, keratoacanthoma, multiple						1 (3%)
Site of application-mass, sarcoma						1 (3%)
Site of application-mass, squamous cell carcinoma					12 (40%)	6 (20%)
Site of application-mass, squamous cell carcinoma, multiple					11 (37%)	4 (13%)
Site of application-mass, squamous cell papilloma		1 (3%)	7 (23%)		6 (20%)	6 (20%)
Site of application-mass, squamous cell papilloma, multiple			9 (30%)		2 (7%)	11 (37%)
Subcutaneous tissue, control, lymphoma malignant lymphocytic						
Subcutaneous tissue, hemangiosarcoma	1 (3%)					
Subcutaneous tissue, sarcoma			1 (3%)			
Subcutaneous tissue, site of application-mass, lymphoma malignant lymphocytic						
Neoplasm Summary						
Total animals with primary neoplasms[b]	1	1	16		24	21
Total primary neoplasms	1	1	18		33	30
Total animals with benign neoplasms		1	16		9	17
Total benign neoplasms		1	17		10	18
Total animals with malignant neoplasms	1		1		23	11
Total malignant neoplasms	1		1		23	12

TABLE B1a
Summary of the Incidence of Skin Neoplasms in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	2.5 μg DMBA/ 20 mg BPO	5 μg TPA/ 5 μg TPA	25 μg DMBA/ Acetone	25 μg DMBA/ 5 μg TPA	25 μg DMBA/ 20 mg BPO	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	2	9	1	13	6	2
Natural deaths	1		1	2		3
Survivors						
Terminal sacrifice	27	21	28	15	24	25
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Squamous cell carcinoma						
Squamous cell papilloma					1 (3%)	
Site of application-mass, basal cell adenoma						
Site of application-mass, keratoacanthoma		1 (3%)				
Site of application-mass, keratoacanthoma, multiple				1 (3%)		
Site of application-mass, sarcoma				1 (3%)	1 (3%)	
Site of application-mass, squamous cell carcinoma				10 (33%)		
Site of application-mass, squamous cell carcinoma, multiple				4 (13%)		
Site of application-mass, squamous cell papilloma	2 (7%)	2 (7%)		4 (13%)	6 (20%)	1 (3%)
Site of application-mass, squamous cell papilloma, multiple	1 (3%)		1 (3%)	13 (43%)	1 (3%)	
Subcutaneous tissue, control, lymphoma malignant lymphocytic					1 (3%)	
Subcutaneous tissue, hemangiosarcoma						
Subcutaneous tissue, sarcoma						
Subcutaneous tissue, site of application-mass, lymphoma malignant lymphocytic					1 (3%)	
Neoplasm Summary (continued)						
Total animals with primary neoplasms	3	3	1	21	10	1
Total primary neoplasms	3	3	1	33	10	1
Total animals with benign neoplasms	3	3	1	17	8	1
Total benign neoplasms	3	3	1	18	8	1
Total animals with malignant neoplasms				14	2	
Total malignant neoplasms				15	2	

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

TABLE B1b
Summary of the Incidence of Skin Neoplasms in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	5 μg TPA/ 5 μg TPA	100 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 20 mg BPO	100 μg MNNG/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	3	11	1	15	3	17
Natural deaths		2	1	1	1	3
Survivors						
Terminal sacrifice	27	17	28	14	26	10
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Squamous cell carcinoma		1 (3%)				
Squamous cell carcinoma, metastatic, skin				1 (3%)		
Control, lymphoma malignant lymphocytic				1 (3%)		
Site of application-no mass, lymphoma malignant lymphocytic				1 (3%)		
Site of application-mass, keratoacanthoma						
Site of application-mass, keratoacanthoma, multiple						
Site of application-mass, squamous cell carcinoma				9 (30%)		
Site of application-mass, squamous cell carcinoma, multiple				5 (17%)		1 (3%)
Site of application-mass, squamous cell papilloma		3 (10%)		6 (20%)	1 (3%)	4 (13%)
Site of application-mass, squamous cell papilloma, multiple		1 (3%)				5 (17%)
Subcutaneous tissue, sarcoma						
Subcutaneous tissue, site of application-no mass, fibroma				1 (3%)		
Subcutaneous tissue, site of application-mass, hemangioma						
Subcutaneous tissue, site of application-mass, hemangiosarcoma						
Subcutaneous tissue, site of application-mass, sarcoma				6 (20%)		1 (3%)
Neoplasm Summary						
Total animals with primary neoplasms[b]		5		23	1	10
Total primary neoplasms		5		28	1	11
Total animals with benign neoplasms		4		7	1	9
Total benign neoplasms		4		7	1	9
Total animals with malignant neoplasms		1		21		2
Total malignant neoplasms		1		21		2
Total animals with metastatic neoplasms				1		
Total metastatic neoplasms				1		

TABLE B1b
Summary of the Incidence of Skin Neoplasms in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	500 μg MNNG/ Acetone	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ Acetone	1,000 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	12	9	19	17	22	9
Natural deaths	1	3	2	5	4	1
Survivors						
Terminal sacrifice	17	18	9	8	4	20
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Squamous cell carcinoma			1 (3%)		1 (3%)	
Squamous cell carcinoma, metastatic, skin					1 (3%)	
Control, lymphoma malignant lymphocytic						
Site of application-no mass, lymphoma malignant lymphocytic						
Site of application-mass, keratoacanthoma					2 (7%)	
Site of application-mass, keratoacanthoma, multiple					1 (3%)	
Site of application-mass, squamous cell carcinoma	1 (3%)		3 (10%)	4 (13%)	4 (13%)	
Site of application-mass, squamous cell carcinoma, multiple			1 (3%)			
Site of application-mass, squamous cell papilloma		1 (3%)	2 (7%)	3 (10%)	6 (20%)	
Site of application-mass, squamous cell papilloma, multiple					3 (10%)	
Subcutaneous tissue, sarcoma			1 (3%)			
Subcutaneous tissue, site of application-no mass, fibroma						
Subcutaneous tissue, site of application-mass, hemangioma				1 (3%)		
Subcutaneous tissue, site of application-mass, hemangiosarcoma					1 (3%)	
Subcutaneous tissue, site of application-mass, sarcoma	2 (7%)	4 (13%)	7 (23%)	9 (30%)	11 (37%)	
Neoplasm Summary (continued)						
Total animals with primary neoplasms	3	5	13	16	20	
Total primary neoplasms	3	5	15	17	29	
Total animals with benign neoplasms		1	2	4	10	
Total benign neoplasms		1	2	4	12	
Total animals with malignant neoplasms	3	4	12	13	17	
Total malignant neoplasms	3	4	13	13	17	
Total animals with metastatic neoplasms					1	
Total metastatic neoplasms					1	

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

TABLE B1c
Summary of the Incidence of Skin Neoplasms in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	0.25 μg DMBA/ Acetone	0.25 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						
Moribund	1		6		7	7
Natural deaths	1	1	2	1	3	3
Survivors						
Died last week of study	1					1
Terminal sacrifice	27	29	22	29	20	19
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Squamous cell papilloma						
Sebaceous gland, site of application-mass, adenoma						1 (3%)
Site of application-no mass, squamous cell carcinoma						
Site of application-mass, keratoacanthoma			3 (10%)			1 (3%)
Site of application-mass, keratoacanthoma, multiple					1 (3%)	1 (3%)
Site of application-mass, squamous cell carcinoma			5 (17%)		16 (53%)	8 (27%)
Site of application-mass, squamous cell carcinoma, multiple			2 (7%)		7 (23%)	4 (13%)
Site of application-mass, squamous cell papilloma			5 (17%)		3 (10%)	3 (10%)
Site of application-mass, squamous cell papilloma, multiple			10 (33%)		5 (17%)	12 (40%)
Subcutaneous tissue, site of application-mass, fibrosarcoma					1 (3%)	
Subcutaneous tissue, site of application-mass, sarcoma						1 (3%)
Neoplasm Summary						
Total animals with primary neoplasms[b]			18		25	20
Total primary neoplasms			25		33	31
Total animals with benign neoplasms			16		8	15
Total benign neoplasms			18		9	18
Total animals with malignant neoplasms			7		23	13
Total malignant neoplasms			7		24	13

TABLE B1c
Summary of the Incidence of Skin Neoplasms in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	2.5 μg DMBA/ 20 mg BPO	5 μg TPA/ 5 μg TPA	25 μg DMBA/ Acetone	25 μg DMBA/ 5 μg TPA	25 μg DMBA/ 20 mg BPO	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death		1				
Moribund	1	4		10		
Natural deaths	1	1	1	4		
Survivors						
Died last week of study		1	1			
Terminal sacrifice	28	23	28	16	30	30
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Squamous cell papilloma			1 (3%)			
Sebaceous gland, site of application-mass, adenoma						
Site of application-no mass, squamous cell carcinoma				1 (3%)		
Site of application-mass, keratoacanthoma				1 (3%)	1 (3%)	
Site of application-mass, keratoacanthoma, multiple						
Site of application-mass, squamous cell carcinoma				11 (37%)	2 (7%)	
Site of application-mass, squamous cell carcinoma, multiple				6 (20%)		
Site of application-mass, squamous cell papilloma	4 (13%)	5 (17%)		4 (13%)	2 (7%)	
Site of application-mass, squamous cell papilloma, multiple	1 (3%)	1 (3%)		18 (60%)	3 (10%)	
Subcutaneous tissue, site of application-mass, fibrosarcoma						
Subcutaneous tissue, site of application-mass, sarcoma				1 (3%)		
Neoplasm Summary (continued)						
Total animals with primary neoplasms	5	6	1	28	6	
Total primary neoplasms	5	6	1	42	8	
Total animals with benign neoplasms	5	6	1	22	5	
Total benign neoplasms	5	6	1	23	6	
Total animals with malignant neoplasms				18	2	
Total malignant neoplasms				19	2	

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

TABLE B1d
Summary of the Incidence of Skin Neoplasms in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	5 μg TPA/ 5 μg TPA	100 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 5 μg TPA	500 μg MNNG/ Acetone
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	2	4	1	9	6	2
Natural deaths	1	1		2	2	1
Survivors						
Terminal sacrifice	27	23	29	19	22	26
Other		2				1
Animals examined microscopically	30	28	30	30	30	29
Skin	(30)	(28)	(30)	(30)	(30)	(29)
Squamous cell papilloma				1 (3%)		
Site of application-no mass, squamous cell carcinoma					1 (3%)	
Site of application-no mass, squamous cell carcinoma, multiple						
Site of application-mass, keratoacanthoma					1 (3%)	
Site of application-mass, squamous cell carcinoma				11 (37%)	5 (17%)	2 (7%)
Site of application-mass, squamous cell carcinoma, multiple				4 (13%)		
Site of application-mass, squamous cell papilloma		4 (14%)		4 (13%)	5 (17%)	1 (3%)
Site of application-mass, squamous cell papilloma, multiple				5 (17%)	8 (27%)	1 (3%)
Subcutaneous tissue, lymphoma malignant lymphocytic		1 (4%)				
Subcutaneous tissue, sarcoma						
Subcutaneous tissue, site of application-no mass, sarcoma						
Subcutaneous tissue, site of application-mass, fibroma						1 (3%)
Subcutaneous tissue, site of application-mass, hemangioma					1 (3%)	
Subcutaneous tissue, site of application-mass, sarcoma				4 (13%)		2 (7%)
Neoplasm Summary						
Total animals with primary neoplasms[b]		5		19	15	6
Total primary neoplasms		5		29	21	7
Total animals with benign neoplasms		4		9	13	3
Total benign neoplasms		4		10	15	3
Total animals with malignant neoplasms		1		17	6	4
Total malignant neoplasms		1		19	6	4

TABLE B1d
Summary of the Incidence of Skin Neoplasms in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	100 μg MNNG/ 20 mg BPO	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ Acetone	1,000 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	2	9	13	10	13	1
Natural deaths		1	2	3	2	
Survivors						
Terminal sacrifice	28	20	15	17	14	29
Other					1	
Animals examined microscopically	30	30	30	30	29	30
Skin (continued)	(30)	(30)	(30)	(30)	(29)	(30)
Squamous cell papilloma						
Site of application-no mass, squamous cell carcinoma					1 (3%)	
Site of application-no mass, squamous cell carcinoma, multiple				1 (3%)		
Site of application-mass, keratoacanthoma	1 (3%)	1 (3%)				
Site of application-mass, squamous cell carcinoma		3 (10%)	3 (10%)	3 (10%)	3 (10%)	
Site of application-mass, squamous cell carcinoma, multiple			1 (3%)	1 (3%)	1 (3%)	
Site of application-mass, squamous cell papilloma	2 (7%)	6 (20%)	2 (7%)	2 (7%)	7 (24%)	
Site of application-mass, squamous cell papilloma, multiple					2 (7%)	
Subcutaneous tissue, lymphoma malignant lymphocytic						
Subcutaneous tissue, sarcoma		1 (3%)		2 (7%)	1 (3%)	
Subcutaneous tissue, site of application-no mass, sarcoma			1 (3%)			
Subcutaneous tissue, site of application-mass, fibroma						
Subcutaneous tissue, site of application-mass, hemangioma						
Subcutaneous tissue, site of application-mass, sarcoma		3 (10%)	5 (17%)	6 (20%)	3 (10%)	
Neoplasm Summary (continued)						
Total animals with primary neoplasms	3	13	10	13	15	
Total primary neoplasms	3	14	12	15	18	
Total animals with benign neoplasms	3	7	2	2	9	
Total benign neoplasms	3	7	2	2	9	
Total animals with malignant neoplasms		6	9	12	8	
Total malignant neoplasms		7	10	13	9	

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

TABLE B2a
Overview of the Sensitivity of Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
5 μg TPA/5 μg TPA	5/30	34.6	0.17	1.00
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
0.25 μg DMBA/Acetone	1/30	50.0	0.03	1.00
2.5 μg DMBA/Acetone	0/30	–	0.00	0.00
25 μg DMBA/Acetone	0/30	–	0.00	0.00
0.25 μg DMBA/5 μg TPA	18/30	27.7	1.07	1.78
2.5 μg DMBA/5 μg TPA	27/30	15.4	4.63	5.15
25 μg DMBA/5 μg TPA	28/30	17.6	5.60	6.00
2.5 μg DMBA/20 mg BPO	1/30	52.0.	0.03	1.00
25 μg DMBA/20 mg BPO	6/30	47.0	0.20	1.00
2.5 μg DMBA/2.5 μg DMBA	22/30	42.9	1.30	1.77

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE B2b
Overview of the Sensitivity of Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
5 μg TPA/5 μg TPA	4/30	35.8	0.20	1.50
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
100 μg MNNG/Acetone	1/30	15.0	0.03	1.00
500 μg MNNG/Acetone	7/30	14.9	0.30	1.29
1,000 μg MNNG/Acetone	15/30	15.9	0.73	1.47
100 μg MNNG/5 μg TPA	16/30	25.3	1.40	2.63
1,000 μg MNNG/5 μg TPA	23/30	12.9	1.87	2.43
100 μg MNNG/20 mg BPO	1/30	38.0	0.03	1.00
500 μg MNNG/20 mg BPO	11/30	18.3	0.63	1.73
1,000 μg MNNG/20 mg BPO	13/30	10.9	0.83	1.92
100 μg MNNG/100 μg MNNG	17/30	40.2	0.97	1.71

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE B2c
Overview of the Sensitivity of Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
5 μg TPA/5 μg TPA	4/30	42.3	0.13	1.00
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
0.25 μg DMBA/Acetone	0/30	–	0.00	0.00
2.5 μg DMBA/Acetone	0/30	–	0.00	0.00
25 μg DMBA/Acetone	0/30	–	0.00	0.00
0.25 μg DMBA/5 μg TPA	18/30	26.5	1.43	2.39
2.5 μg DMBA/5 μg TPA	26/30	18.0	3.77	4.35
25 μg DMBA/5 μg TPA	29/30	16.0	7.10	7.34
2.5 μg DMBA/20 mg BPO	1/30	51.0	0.03	1.00
25 μg DMBA/20 mg BPO	5/30	39.2	0.30	1.80
2.5 μg DMBA/2.5 μg DMBA	20/30	40.1	1.27	1.90

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE B2d
Overview of the Sensitivity of Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
5 μg TPA/5 μg TPA	8/30	34.1	0.33	1.25
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
100 μg MNNG/Acetone	0/30	–	0.00	0.00
500 μg MNNG/Acetone	6/30	18.0	0.27	1.33
1,000 μg MNNG/Acetone	9/30	11.7	0.40	1.33
100 μg MNNG/5 μg TPA	21/30	21.1	2.27	3.24
1,000 μg MNNG/5 μg TPA	21/30	18.8	1.23	1.76
100 μg MNNG/20 mg BPO	3/30	31.0	0.13	1.33
500 μg MNNG/20 mg BPO	13/30	8.4	0.62	1.42
1,000 μg MNNG/20 mg BPO	19/30	17.8	0.90	1.42
100 μg MNNG/100 μg MNNG	19/30	38.7	0.87	1.37

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.

TABLE B3a
Summary of the Incidence of Nonneoplastic Skin Lesions in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	0.25 μg DMBA/ Acetone	0.25 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	1	2	6	1	7	8
Natural deaths	2	3	4		4	7
Survivors						
Terminal sacrifice	27	25	20	29	19	15
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst						
Site of application-no mass, acanthosis	4 (13%)	8 (27%)	30 (100%)	4 (13%)	26 (87%)	30 (100%)
Site of application-no mass, alopecia						
Site of application-no mass, inflammation, chronic active	4 (13%)	7 (23%)	30 (100%)	5 (17%)	25 (83%)	29 (97%)
Site of application-no mass, ulcer	1 (3%)		5 (17%)		9 (30%)	11 (37%)
Site of application-no mass, ulcer, multiple	1 (3%)	2 (7%)	10 (33%)		1 (3%)	8 (27%)
Site of application-mass, acanthosis					1 (3%)	

TABLE B3a
Summary of the Incidence of Nonneoplastic Skin Lesions in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	2.5 μg DMBA/ 20 mg BPO	5 μg TPA/ 5 μg TPA	25 μg DMBA/ Acetone	25 μg DMBA/ 5 μg TPA	25 μg DMBA/ 20 mg BPO	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	2	9	1	13	6	2
Natural deaths	1		1	2		3
Survivors						
Terminal sacrifice	27	21	28	15	24	25
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst	1 (3%)			1 (3%)		
Site of application-no mass, acanthosis	25 (83%)	30 (100%)	5 (17%)	30 (100%)	29 (97%)	27 (90%)
Site of application-no mass, alopecia				1 (3%)		
Site of application-no mass, inflammation, chronic active	24 (80%)	30 (100%)	5 (17%)	30 (100%)	28 (93%)	28 (93%)
Site of application-no mass, ulcer	5 (17%)	7 (23%)		7 (23%)	2 (7%)	6 (20%)
Site of application-no mass, ulcer, multiple	2 (7%)	7 (23%)		8 (27%)	5 (17%)	4 (13%)
Site of application-mass, acanthosis				1 (3%)		

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

TABLE B3b
Summary of the Incidence of Nonneoplastic Skin Lesions in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	5 μg TPA/ 5 μg TPA	100 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 20 mg BPO	100 μg MNNG/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	3	11	1	15	3	17
Natural deaths		2	1	1	1	3
Survivors						
Terminal sacrifice	27	17	28	14	26	10
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst						
Sebaceous gland, site of application-mass, hyperplasia						
Site of application-no mass, acanthosis	4 (13%)	30 (100%)	7 (23%)	29 (97%)	22 (73%)	29 (97%)
Site of application-no mass, hemorrhage						
Site of application-no mass, inflammation, chronic active	2 (7%)	24 (80%)	2 (7%)	19 (63%)	11 (37%)	28 (93%)
Site of application-no mass, ulcer	2 (7%)	8 (27%)	3 (10%)	9 (30%)	7 (23%)	11 (37%)
Site of application-mass, acanthosis		1 (3%)	1 (3%)	2 (7%)		
Site of application-mass, hyperplasia, mast cell						
Site of application-mass, ulcer						

TABLE B3b
Summary of the Incidence of Nonneoplastic Skin Lesions in Male Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	500 μg MNNG/ Acetone	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ Acetone	1,000 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	12	9	19	17	22	9
Natural deaths	1	3	2	5	4	1
Survivors						
Terminal sacrifice	17	18	9	8	4	20
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst				1 (3%)	2 (7%)	
Sebaceous gland, site of application-mass, hyperplasia		1 (3%)				
Site of application-no mass, acanthosis	13 (43%)	25 (83%)	16 (53%)	25 (83%)	28 (93%)	25 (83%)
Site of application-no mass, hemorrhage				1 (3%)		
Site of application-no mass, inflammation, chronic active	13 (43%)	20 (67%)	21 (70%)	20 (67%)	25 (83%)	15 (50%)
Site of application-no mass, ulcer	9 (30%)	8 (27%)	9 (30%)	10 (33%)	8 (27%)	3 (10%)
Site of application-mass, acanthosis			2 (7%)			1 (3%)
Site of application-mass, hyperplasia, mast cell	1 (3%)					
Site of application-mass, ulcer					1 (3%)	

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

TABLE B3c
Summary of the Incidence of Nonneoplastic Skin Lesions in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	0.25 μg DMBA/ Acetone	0.25 μg DMBA/ 5 μg TPA	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA	2.5 μg DMBA/ 5 μg TPA
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death						
Moribund	1		6		7	7
Natural deaths	1	1	2	1	3	3
Survivors						
Died last week of study	1					1
Terminal sacrifice	27	29	22	29	20	19
Animals examined microscopically	30	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst						
Site of application-no mass, acanthosis	5 (17%)	1 (3%)	29 (97%)	3 (10%)	25 (83%)	30 (100%)
Site of application-no mass, inflammation, chronic active	9 (30%)	5 (17%)	29 (97%)	10 (33%)	27 (90%)	30 (100%)
Site of application-no mass, ulcer	1 (3%)				5 (17%)	3 (10%)
Site of application-no mass, ulcer, multiple	1 (3%)		5 (17%)		2 (7%)	5 (17%)
Site of application-mass, acanthosis					1 (3%)	

TABLE B3c
Summary of the Incidence of Nonneoplastic Skin Lesions in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	2.5 μg DMBA/ 20 mg BPO	5 μg TPA/ 5 μg TPA	25 μg DMBA/ Acetone	25 μg DMBA/ 5 μg TPA	25 μg DMBA/ 20 mg BPO	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Accidental death		1				
Moribund	1	4		10		
Natural deaths	1	1	1	4		
Survivors						
Died last week of study		1	1			
Terminal sacrifice	28	23	28	16	30	30
Animals examined microscopically	30	30	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst				1 (3%)		
Site of application-no mass, acanthosis	30 (100%)	30 (100%)		30 (100%)	28 (93%)	30 (100%)
Site of application-no mass, inflammation, chronic active	30 (100%)	29 (97%)	5 (17%)	30 (100%)	28 (93%)	30 (100%)
Site of application-no mass, ulcer	1 (3%)	4 (13%)		4 (13%)		
Site of application-no mass, ulcer, multiple	2 (7%)	5 (17%)		11 (37%)		1 (3%)
Site of application-mass, acanthosis		1 (3%)		1 (3%)		

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

TABLE B3d
Summary of the Incidence of Nonneoplastic Skin Lesions in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	5 μg TPA/ 5 μg TPA	100 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG	100 μg MNNG/ 5 μg TPA	500 μg MNNG/ Acetone
Disposition Summary						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	2	4	1	9	6	2
Natural deaths	1	1		2	2	1
Survivors						
Terminal sacrifice	27	23	29	19	22	26
Other		2				1
Animals examined microscopically	30	28	30	30	30	29
Skin	(30)	(28)	(30)	(30)	(30)	(29)
Hair follicle, site of application-no mass, cyst						2 (7%)
Site of application-no mass, acanthosis	2 (7%)	27 (96%)	7 (23%)	27 (90%)	25 (83%)	14 (48%)
Site of application-no mass, hemorrhage				1 (3%)		
Site of application-no mass, inflammation, chronic active	1 (3%)	25 (89%)	6 (20%)	27 (90%)	28 (93%)	13 (45%)
Site of application-no mass, ulcer	1 (3%)	7 (25%)	1 (3%)	7 (23%)	8 (27%)	2 (7%)
Site of application-mass, acanthosis		1 (4%)		1 (3%)	1 (3%)	1 (3%)
Site of application-mass, ulcer						
Subcutaneous tissue, infiltration cellular, mixed cell						

TABLE B3d
Summary of the Incidence of Nonneoplastic Skin Lesions in Female Swiss (CD-1®) Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	100 μg MNNG/ 20 mg BPO	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ Acetone	1,000 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 5 μg TPA	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)						
Animals initially in study	30	30	30	30	30	30
Early deaths						
Moribund	2	9	13	10	13	1
Natural deaths		1	2	3	2	
Survivors						
Terminal sacrifice	28	20	15	17	14	29
Other					1	
Animals examined microscopically	30	30	30	30	29	30
Skin (continued)	(30)	(30)	(30)	(30)	(29)	(30)
Hair follicle, site of application-no mass, cyst			2 (7%)		1 (3%)	
Site of application-no mass, acanthosis	28 (93%)	28 (93%)	22 (73%)	24 (80%)	26 (90%)	21 (70%)
Site of application-no mass, hemorrhage						
Site of application-no mass, inflammation, chronic active	26 (87%)	26 (87%)	19 (63%)	20 (67%)	27 (93%)	16 (53%)
Site of application-no mass, ulcer		2 (7%)	6 (20%)	5 (17%)	9 (31%)	2 (7%)
Site of application-mass, acanthosis						
Site of application-mass, ulcer				1 (3%)		
Subcutaneous tissue, infiltration cellular, mixed cell			1 (3%)			

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

APPENDIX C
SUMMARY OF SKIN LESIONS IN MALE AND FEMALE SENCAR MICE IN THE COMPARATIVE INITIATION/PROMOTION SKIN PAINT STUDIES

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TABLE C1a
Summary of the Incidence of Skin Neoplasms in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	0.25 μg DMBA/ Acetone	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA
Disposition Summary				
Animals initially in study	30	30	29	30
Early deaths				
Moribund	1	1	1	15
Natural deaths	1	2	1	4
Survivors				
Terminal sacrifice	28	27	27	11
Animals examined microscopically	30	30	29	30
Skin	(30)	(30)	(29)	(30)
Squamous cell papilloma			1 (3%)	2 (7%)
Site of application-mass, keratoacanthoma				3 (10%)
Site of application-mass, keratoacanthoma, multiple				
Site of application-mass, squamous cell carcinoma				14 (47%)
Site of application-mass, squamous cell carcinoma, multiple				13 (43%)
Site of application-mass, squamous cell papilloma				5 (17%)
Site of application-mass, squamous cell papilloma, multiple				1 (3%)
Subcutaneous tissue, site of application-mass, fibrosarcoma				
Neoplasm Summary				
Total animals with primary neoplasms[b]			1	28
Total primary neoplasms			1	38
Total animals with benign neoplasms			1	9
Total benign neoplasms			1	11
Total animals with malignant neoplasms				27
Total malignant neoplasms				27

TABLE C1a
Summary of the Incidence of Skin Neoplasms in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	2.5 μg DMBA/ 20 mg BPO	25 μg DMBA/ Acetone	25 μg DMBA/ 20 mg BPO	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)				
Animals initially in study	30	31	30	30
Early deaths				
Moribund	6		12	5
Natural deaths	1	1	1	2
Survivors				
Terminal sacrifice	23	30	17	23
Animals examined microscopically	30	31	30	30
Skin (continued)	(30)	(31)	(30)	(30)
Squamous cell papilloma			1 (3%)	
Site of application-mass, keratoacanthoma	1 (3%)		3 (10%)	
Site of application-mass, keratoacanthoma, multiple	1 (3%)			
Site of application-mass, squamous cell carcinoma	8 (27%)		8 (27%)	
Site of application-mass, squamous cell carcinoma, multiple	3 (10%)		5 (17%)	
Site of application-mass, squamous cell papilloma	2 (7%)		6 (20%)	
Site of application-mass, squamous cell papilloma, multiple	10 (33%)		5 (17%)	
Subcutaneous tissue, site of application-mass, fibrosarcoma			1 (3%)	
Neoplasm Summary (continued)				
Total animals with primary neoplasms	19		18	
Total primary neoplasms	25		29	
Total animals with benign neoplasms	13		12	
Total benign neoplasms	14		15	
Total animals with malignant neoplasms	11		14	
Total malignant neoplasms	11		14	

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

TABLE C1b
Summary of the Incidence of Skin Neoplasms in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	100 μg MNNG/ Acetone	500 μg MNNG/ Acetone	1,000 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG
Disposition Summary					
Animals initially in study	30	30	30	30	30
Early deaths					
Moribund		2	10	20	23
Natural deaths	1		4	3	3
Survivors					
Died last week of study					
Terminal sacrifice	29	28	16	7	4
Animals examined microscopically	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)
Squamous cell carcinoma					
Squamous cell carcinoma, multiple				1 (3%)	
Sebaceous gland, site of application-mass, adenoma					
Site of application-no mass, squamous cell carcinoma			1 (3%)		
Site of application-mass, basal cell adenoma			2 (7%)		1 (3%)
Site of application-mass, keratoacanthoma					
Site of application-mass, squamous cell carcinoma			5 (17%)	6 (20%)	19 (63%)
Site of application-mass, squamous cell carcinoma, multiple			1 (3%)	2 (7%)	8 (27%)
Site of application-mass, squamous cell papilloma		1 (3%)	1 (3%)	6 (20%)	5 (17%)
Site of application-mass, squamous cell papilloma, multiple			1 (3%)	2 (7%)	2 (7%)
Site of application-mass, trichoepithelioma					
Subcutaneous tissue, sarcoma			1 (3%)	1 (3%)	
Subcutaneous tissue, site of application-no mass, sarcoma					2 (7%)
Subcutaneous tissue, site of application-mass, fibroma					
Subcutaneous tissue, site of application-mass, sarcoma			4 (13%)	3 (10%)	1 (3%)
Subcutaneous tissue, site of application-mass, sarcoma, multiple			1 (3%)		
Subcutaneous tissue, site of application-mass, squamous cell carcinoma				1 (3%)	
Neoplasm Summary					
Total animals with primary neoplasms[b]		1	14	18	28
Total primary neoplasms		1	17	22	38
Total animals with benign neoplasms		1	4	8	8
Total benign neoplasms		1	4	8	8
Total animals with malignant neoplasms			13	14	27
Total malignant neoplasms			13	14	30

TABLE C1b
Summary of the Incidence of Skin Neoplasms in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	20 mg BPO/ 20 mg BPO	100 μg MNNG/ 20 mg BPO	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 20 mg BPO
Disposition Summary (continued)				
Animals initially in study	30	30	30	30
Early deaths				
Moribund	3	2	11	21
Natural deaths			3	3
Survivors				
Died last week of study	1			
Terminal sacrifice	26	28	16	6
Animals examined microscopically	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)
Squamous cell carcinoma				1 (3%)
Squamous cell carcinoma, multiple				
Sebaceous gland, site of application-mass, adenoma			1 (3%)	
Site of application-no mass, squamous cell carcinoma				
Site of application-mass, basal cell adenoma				1 (3%)
Site of application-mass, keratoacanthoma		2 (7%)	3 (10%)	
Site of application-mass, squamous cell carcinoma		1 (3%)	9 (30%)	15 (50%)
Site of application-mass, squamous cell carcinoma, multiple		1 (3%)	1 (3%)	3 (10%)
Site of application-mass, squamous cell papilloma	1 (3%)	6 (20%)	3 (10%)	6 (20%)
Site of application-mass, squamous cell papilloma, multiple		2 (7%)	4 (13%)	
Site of application-mass, trichoepithelioma				1 (3%)
Subcutaneous tissue, sarcoma				
Subcutaneous tissue, site of application-no mass, sarcoma				
Subcutaneous tissue, site of application-mass, fibroma			1 (3%)	
Subcutaneous tissue, site of application-mass, sarcoma			5 (17%)	5 (17%)
Subcutaneous tissue, site of application-mass, sarcoma, multiple				
Subcutaneous tissue, site of application-mass, squamous cell carcinoma				
Neoplasm Summary (continued)				
Total animals with primary neoplasms	1	11	18	27
Total primary neoplasms	1	12	27	32
Total animals with benign neoplasms	1	9	11	8
Total benign neoplasms	1	10	12	8
Total animals with malignant neoplasms		2	14	23
Total malignant neoplasms		2	15	24

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

TABLE C1c
Summary of the Incidence of Skin Neoplasms in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Restart[a]

	Acetone/ Acetone	0.25 μg DMBA/ 1 μg TPA	2.5 μg DMBA/ 1 μg TPA	25 μg DMBA/ 1 μg TPA
Disposition Summary				
Animals initially in study	30	30	30	30
Early deaths				
Moribund	1	22	22	25
Natural deaths		2	6	4
Survivors				
Terminal sacrifice	29	6	2	1
Animals examined microscopically	30	30	30	30
Skin	(30)	(30)	(30)	(30)
Site of application-mass, keratoacanthoma		1 (3%)		
Site of application-mass, keratoacanthoma, multiple				2 (7%)
Site of application-mass, basal cell carcinoma				
Site of application-mass, squamous cell carcinoma		4 (13%)	7 (23%)	16 (53%)
Site of application-mass, squamous cell carcinoma, multiple		2 (7%)	2 (7%)	3 (10%)
Site of application-mass, squamous cell papilloma		3 (10%)	4 (13%)	4 (13%)
Site of application-mass, squamous cell papilloma, multiple		1 (3%)	5 (17%)	5 (17%)
Subcutaneous tissue, site of application-no mass, sarcoma				
Subcutaneous tissue, site of application-mass, sarcoma				
Neoplasm Summary				
Total animals with primary neoplasms[b]		8	14	21
Total primary neoplasms		11	18	30
Total animals with benign neoplasms		5	9	9
Total benign neoplasms		5	9	11
Total animals with malignant neoplasms		6	9	19
Total malignant neoplasms		6	9	19

TABLE C1c
Summary of the Incidence of Skin Neoplasms in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Restart (continued)

	1 μg TPA/ 1 μg TPA	100 μg MNNG/ 1 μg TPA	1,000 μg MNNG/ 1 μg TPA
Disposition Summary (continued)			
Animals initially in study	30	30	30
Early deaths			
Moribund	17	20	26
Natural deaths	4	2	3
Survivors			
Terminal sacrifice	9	8	1
Animals examined microscopically	30	30	30
Skin (continued)	(30)	(30)	(30)
Site of application-mass, keratoacanthoma			
Site of application-mass, keratoacanthoma, multiple			
Site of application-mass, basal cell carcinoma			1 (3%)
Site of application-mass, squamous cell carcinoma	1 (3%)	3 (10%)	14 (47%)
Site of application-mass, squamous cell carcinoma, multiple		2 (7%)	4 (13%)
Site of application-mass, squamous cell papilloma		1 (3%)	2 (7%)
Site of application-mass, squamous cell papilloma, multiple		3 (10%)	2 (7%)
Subcutaneous tissue, site of application-no mass, sarcoma			1 (3%)
Subcutaneous tissue, site of application-mass, sarcoma			1 (3%)
Neoplasm Summary (continued)			
Total animals with primary neoplasms	1	8	20
Total primary neoplasms	1	9	25
Total animals with benign neoplasms		4	4
Total benign neoplasms		4	4
Total animals with malignant neoplasms	1	5	20
Total malignant neoplasms	1	5	21

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

TABLE C1d
Summary of the Incidence of Skin Neoplasms in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	0.25 μg DMBA/ Acetone	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA
Disposition Summary				
Animals initially in study	30	30	31	30
Early deaths				
Moribund	2	1	1	19
Natural deaths	1			1
Survivors				
Terminal sacrifice	27	29	30	10
Animals examined microscopically	30	30	31	30
Skin	(29)	(30)	(31)	(30)
Squamous cell carcinoma				
Squamous cell papilloma				
Sebaceous gland, site of application-mass, adenoma				
Site of application-mass, basal cell carcinoma				1 (3%)
Site of application-mass, keratoacanthoma				1 (3%)
Site of application-mass, keratoacanthoma, multiple				1 (3%)
Site of application-mass, squamous cell carcinoma				17 (57%)
Site of application-mass, squamous cell carcinoma, multiple				12 (40%)
Site of application-mass, squamous cell papilloma				3 (10%)
Site of application-mass, squamous cell papilloma, multiple				6 (20%)
Subcutaneous tissue, site of application-mass, fibrosarcoma				1 (3%)
Neoplasm Summary				
Total animals with primary neoplasms[b]				29
Total primary neoplasms				42
Total animals with benign neoplasms				9
Total benign neoplasms				11
Total animals with malignant neoplasms				29
Total malignant neoplasms				31

TABLE C1d
Summary of the Incidence of Skin Neoplasms in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	2.5 μg DMBA/ 20 mg BPO	25 μg DMBA/ Acetone	25 μg DMBA/ 20 mg BPO	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)				
Animals initially in study	30	29	30	30
Early deaths				
Moribund	8	1	6	2
Natural deaths	1		1	
Survivors				
Terminal sacrifice	21	28	23	28
Animals examined microscopically	30	29	30	30
Skin (continued)	(30)	(29)	(30)	(30)
Squamous cell carcinoma			1 (3%)	
Squamous cell papilloma		1 (3%)		
Sebaceous gland, site of application-mass, adenoma			1 (3%)	
Site of application-mass, basal cell carcinoma				
Site of application-mass, keratoacanthoma	4 (13%)		4 (13%)	
Site of application-mass, keratoacanthoma, multiple	1 (3%)		1 (3%)	
Site of application-mass, squamous cell carcinoma	11 (37%)		9 (30%)	
Site of application-mass, squamous cell carcinoma, multiple	3 (10%)		2 (7%)	
Site of application-mass, squamous cell papilloma	6 (20%)		8 (27%)	
Site of application-mass, squamous cell papilloma, multiple	5 (17%)		8 (27%)	
Subcutaneous tissue, site of application-mass, fibrosarcoma				
Neoplasm Summary (continued)				
Total animals with primary neoplasms	22	1	20	
Total primary neoplasms	30	1	34	
Total animals with benign neoplasms	14	1	18	
Total benign neoplasms	16	1	22	
Total animals with malignant neoplasms	14		11	
Total malignant neoplasms	14		12	

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

TABLE C1e
Summary of the Incidence of Skin Neoplasms in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	100 μg MNNG/ Acetone	500 μg MNNG/ Acetone	1,000 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG
Disposition Summary					
Animals initially in study	30	30	30	30	30
Early deaths					
Accidental death	1				
Moribund	1		5	13	25
Natural deaths	2		3	6	2
Survivors					
Terminal sacrifice	26	30	22	11	3
Animals examined microscopically	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)
Squamous cell carcinoma					
Site of application-no mass, squamous cell carcinoma				1 (3%)	
Site of application-mass, basal cell adenoma					1 (3%)
Site of application-mass, keratoacanthoma			1 (3%)		1 (3%)
Site of application-mass, sarcoma					
Site of application-mass, squamous cell carcinoma			5 (17%)	14 (47%)	17 (57%)
Site of application-mass, squamous cell carcinoma, multiple				1 (3%)	11 (37%)
Site of application-mass, squamous cell papilloma			1 (3%)	3 (10%)	5 (17%)
Site of application-mass, squamous cell papilloma, multiple			1 (3%)	1 (3%)	2 (7%)
Subcutaneous tissue, lymphoma malignant undifferentiated cell type					1 (3%)
Subcutaneous tissue, control, lymphoma malignant undifferentiated cell type					1 (3%)
Subcutaneous tissue, site of application-no mass, lymphoma malignant undifferentiated cell type					1 (3%)
Subcutaneous tissue, site of application-mass, sarcoma			1 (3%)	4 (13%)	3 (10%)
Neoplasm Summary					
Total animals with primary neoplasms[b]			7	20	28
Total primary neoplasms			9	24	41
Total animals with benign neoplasms			2	4	8
Total benign neoplasms			2	4	9
Total animals with malignant neoplasms			7	19	28
Total malignant neoplasms			7	20	32

TABLE C1e
Summary of the Incidence of Skin Neoplasms in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	20 mg BPO/ 20 mg BPO	100 μg MNNG/ 20 mg BPO	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 20 mg BPO
Disposition Summary (continued)				
Animals initially in study	30	30	30	30
Early deaths				
Accidental death				
Moribund	1	1	7	12
Natural deaths	2			4
Survivors				
Terminal sacrifice	27	29	23	14
Animals examined microscopically	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)
Squamous cell carcinoma				1 (3%)
Site of application-no mass, squamous cell carcinoma				
Site of application-mass, basal cell adenoma				
Site of application-mass, keratoacanthoma		2 (7%)		
Site of application-mass, sarcoma			1 (3%)	
Site of application-mass, squamous cell carcinoma		1 (3%)	5 (17%)	8 (27%)
Site of application-mass, squamous cell carcinoma, multiple		1 (3%)		
Site of application-mass, squamous cell papilloma	1 (3%)	3 (10%)	4 (13%)	6 (20%)
Site of application-mass, squamous cell papilloma, multiple		3 (10%)	1 (3%)	1 (3%)
Subcutaneous tissue, lymphoma malignant undifferentiated cell type				
Subcutaneous tissue, control, lymphoma malignant undifferentiated cell type				
Subcutaneous tissue, site of application-no mass, lymphoma malignant undifferentiated cell type				
Subcutaneous tissue, site of application-mass, sarcoma			3 (10%)	4 (13%)
Neoplasm Summary (continued)				
Total animals with primary neoplasms	1	10	13	18
Total primary neoplasms	1	10	14	20
Total animals with benign neoplasms	1	8	5	7
Total benign neoplasms	1	8	5	7
Total animals with malignant neoplasms		2	9	13
Total malignant neoplasms		2	9	13

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

TABLE C1f
Summary of the Incidence of Skin Neoplasms in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Restart[a]

	Acetone/ Acetone	0.25 μg DMBA/ 1 μg TPA	2.5 μg DMBA/ 1 μg TPA	25 μg DMBA/ 1 μg TPA
Disposition Summary				
Animals initially in study	30	30	30	30
Early deaths				
Moribund	1	18	19	23
Natural deaths	2	4	5	6
Survivors				
Terminal sacrifice	27	8	6	1
Animals examined microscopically	30	30	30	30
Skin	(29)	(30)	(30)	(30)
Basal cell carcinoma			1 (3%)	
Squamous cell papilloma		1 (3%)		
Site of application-mass, keratoacanthoma			1 (3%)	1 (3%)
Site of application-mass, keratoacanthoma, multiple			1 (3%)	
Site of application-mass, squamous cell carcinoma		2 (7%)	10 (33%)	12 (40%)
Site of application-mass, squamous cell carcinoma, multiple			4 (13%)	7 (23%)
Site of application-mass, squamous cell papilloma		6 (20%)	7 (23%)	5 (17%)
Site of application-mass, squamous cell papilloma, multiple		3 (10%)	4 (13%)	9 (30%)
Subcutaneous tissue, site of application-mass, sarcoma				
Subcutaneous tissue, site of application-mass, sarcoma, multiple				
Neoplasm Summary				
Total animals with primary neoplasms[b]		10	20	24
Total primary neoplasms		12	28	34
Total animals with benign neoplasms		10	12	14
Total benign neoplasms		10	13	15
Total animals with malignant neoplasms		2	14	19
Total malignant neoplasms		2	15	19
Total animals with uncertain neoplasms-benign or malignant				
Total uncertain neoplasms				

TABLE C1f
Summary of the Incidence of Skin Neoplasms in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Restart (continued)

	1 μg TPA/ 1 μg TPA	100 μg MNNG/ 1 μg TPA	1,000 μg MNNG/ 1 μg TPA
Disposition Summary (continued)			
Animals initially in study	30	30	30
Early deaths			
Moribund	19	15	24
Natural deaths	1	2	4
Survivors			
Terminal sacrifice	10	13	2
Animals examined microscopically	30	30	30
Skin (continued)	(30)	(30)	(30)
Basal cell carcinoma			
Squamous cell papilloma			
Site of application-mass, keratoacanthoma			
Site of application-mass, keratoacanthoma, multiple			
Site of application-mass, squamous cell carcinoma		6 (20%)	6 (20%)
Site of application-mass, squamous cell carcinoma, multiple			2 (7%)
Site of application-mass, squamous cell papilloma	1 (3%)	3 (10%)	2 (7%)
Site of application-mass, squamous cell papilloma, multiple		7 (23%)	3 (10%)
Subcutaneous tissue, site of application-mass, sarcoma			3 (10%)
Subcutaneous tissue, site of application-mass, sarcoma, multiple			1 (3%)
Neoplasm Summary (continued)			
Total animals with primary neoplasms	1	15	13
Total primary neoplasms	1	16	17
Total animals with benign neoplasms	1	10	5
Total benign neoplasms	1	10	5
Total animals with malignant neoplasms		6	11
Total malignant neoplasms		6	12
Total animals with uncertain neoplasms-benign or malignant			
Total uncertain neoplasms			

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

TABLE C2a
Overview of the Sensitivity of Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
Acetone/Acetone[c]	0/30	–	0.00	0.00
1 μg TPA/1 μg TPA	2/30	16.0	0.10	1.50
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
0.25 μg DMBA/Acetone	0/30	–	0.00	0.00
2.5 μg DMBA/Acetone	0/29	–	0.00	0.00
25 μg DMBA/Acetone	0/31	–	0.00	0.00
0.25 μg DMBA/1 μg TPA	12/30	17.2	1.10	2.75
2.5 μg DMBA/1 μg TPA	19/30	15.5	3.03	4.79
25 μg DMBA/1 μg TPA	24/30	14.5	6.77	8.46
2.5 μg DMBA/20 mg BPO	20/30	31.0	2.40	3.60
25 μg DMBA/20 mg BPO	22/30	31.0	2.77	3.77
2.5 μg DMBA/2.5 μg DMBA	27/30	36.1	2.53	2.81

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.
[c] Vehicle control for restart groups

TABLE C2b
Overview of the Sensitivity of Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
Acetone/Acetone[c]	0/30	–	0.00	0.00
1 μg TPA/1 μg TPA	2/30	16.0	0.10	1.50
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
100 μg MNNG/Acetone	2/30	47.5	0.07	1.00
500 μg MNNG/Acetone	19/30	13.9	1.37	2.16
1000 μg MNNG/Acetone	22/30	12.0	1.57	2.14
100 μg MNNG/1 μg TPA	16/30	20.8	1.47	2.75
1000 μg MNNG/1 μg TPA	24/30	11.8	2.67	3.33
100 μg MNNG/20 mg BPO	9/30	40.6	0.50	1.67
500 μg MNNG/20 mg BPO	25/30	12.9	1.77	2.12
1000 μg MNNG/20 mg BPO	25/30	11.9	1.70	2.04
100 μg MNNG/100 μg MNNG	29/30	29.4	2.00	2.07

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.
[c] Vehicle control for restart groups

TABLE C2c
Overview of the Sensitivity of Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
Acetone/Acetone[c]	0/30	–	0.00	0.00
1 μg TPA/1 μg TPA	0/30	–	0.00	0.00
20 mg BPO/20 mg BPO	0/30	–	0.00	0.00
0.25 μg DMBA/Acetone	0/30	–	0.00	0.00
2.5 μg DMBA/Acetone	0/31	–	0.00	0.00
25 μg DMBA/Acetone	0/29	–	0.00	0.00
0.25 μg DMBA/1 μg TPA	13/30	23.5	0.87	2.00
2.5 μg DMBA/1 μg TPA	27/30	15.9	5.23	5.81
25 μg DMBA/1 μg TPA	29/30	14.4	9.23	9.55
2.5 μg DMBA/20 mg BPO	22/30	31.2	2.33	3.18
25 μg DMBA/20 mg BPO	20/30	33.6	2.50	3.75
2.5 μg DMBA/2.5 μg DMBA	30/30	34.9	2.80	2.80

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.
[c] Vehicle control for restart groups

TABLE C2d
Overview of the Sensitivity of Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B

	Number of Mice With Tumors	Mean Time to Tumor (Weeks)	Average Number of Tumors/ Total Mice[a]	Average Number of Tumors/ Mice with Tumors[b]
Acetone/Acetone	0/30	–	0.00	0.00
Acetone/Acetone[c]	0/30	–	0.00	0.00
1 μg TPA/1 μg TPA	0/30	–	0.00	0.00
20 mg BPO/20 mg BPO	1/30	47.0	0.03	1.00
100 μg MNNG/Acetone	0/30	–	0.00	0.00
500 μg MNNG/Acetone	8/30	25.5	0.06	2.25
1000 μg MNNG/Acetone	25/30	14.0	1.87	2.24
100 μg MNNG/1 μg TPA	23/30	23.0	2.13	2.78
1000 μg MNNG/1 μg TPA	20/30	9.6	1.93	2.90
100 μg MNNG/20 mg BPO	9/30	38.1	0.60	2.00
500 μg MNNG/20 mg BPO	16/30	25.8	0.73	1.38
1000 μg MNNG/20 mg BPO	21/30	12.5	1.50	2.14
100 μg MNNG/100 μg MNNG	28/30	32.3	1.77	1.89

[a] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of animals per group.
[b] Maximum number of tumors recorded in-life per mouse were summed and divided by the total number of mice that developed tumors in each group.
[c] Vehicle control for restart groups

TABLE C3a
Summary of the Incidence of Nonneoplastic Skin Lesions in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	0.25 μg DMBA/ Acetone	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA
Disposition Summary				
Animals initially in study	30	30	29	30
Early deaths				
Moribund	1	1	1	15
Natural deaths	1	2	1	4
Survivors				
Terminal sacrifice	28	27	27	11
Animals examined microscopically	30	30	29	30
Skin	(30)	(30)	(29)	(30)
Hair follicle, site of application-no mass, cyst				
Site of application-no mass, acanthosis	1 (3%)	4 (13%)	1 (3%)	23 (77%)
Site of application-no mass, inflammation, chronic active	1 (3%)	2 (7%)		18 (60%)
Site of application-no mass, ulcer	1 (3%)	1 (3%)		4 (13%)
Site of application-no mass, ulcer, multiple				2 (7%)
Site of application-mass, acanthosis				1 (3%)
Site of application-mass, cyst epithelial inclusion				

TABLE C3a
Summary of the Incidence of Nonneoplastic Skin Lesions in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	2.5 μg DMBA/ 20 mg BPO	25 μg DMBA/ Acetone	25 μg DMBA/ 20 mg BPO	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)				
Animals initially in study	30	31	30	30
Early deaths				
Moribund	6		12	5
Natural deaths	1	1	1	2
Survivors				
Terminal sacrifice	23	30	17	23
Animals examined microscopically	30	31	30	30
Skin (continued)	(30)	(31)	(30)	(30)
Hair follicle, site of application-no mass, cyst			1 (3%)	
Site of application-no mass, acanthosis	29 (97%)	1 (3%)	28 (93%)	30 (100%)
Site of application-no mass, inflammation, chronic active	29 (97%)		28 (93%)	29 (97%)
Site of application-no mass, ulcer	1 (3%)		3 (10%)	
Site of application-no mass, ulcer, multiple	2 (7%)		4 (13%)	
Site of application-mass, acanthosis				
Site of application-mass, cyst epithelial inclusion			1 (3%)	

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

TABLE C3b
Summary of the Incidence of Nonneoplastic Skin Lesions in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	100 μg MNNG/ Acetone	500 μg MNNG/ Acetone	1,000 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG
Disposition Summary					
Animals initially in study	30	30	30	30	30
Early deaths					
Moribund		2	10	20	23
Natural deaths	1		4	3	3
Survivors					
Died last week of study					
Terminal sacrifice	29	28	16	7	4
Animals examined microscopically	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst			2 (7%)	2 (7%)	
Site of application-no mass, acanthosis	1 (3%)	6 (20%)	13 (43%)	9 (30%)	25 (83%)
Site of application-no mass, alopecia					
Site of application-no mass, inflammation, chronic active			15 (50%)	17 (57%)	26 (87%)
Site of application-no mass, ulcer			8 (27%)	11 (37%)	15 (50%)
Site of application-mass, acanthosis		2 (7%)		2 (7%)	
Subcutaneous tissue, site of application-no mass, edema					
Subcutaneous tissue, site of application-no mass, infiltration cellular, mixed cell					1 (3%)

TABLE C3b
Summary of the Incidence of Nonneoplastic Skin Lesions in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	20 mg BPO/ 20 mg BPO	100 μg MNNG/ 20 mg BPO	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 20 mg BPO
Disposition Summary (continued)				
Animals initially in study	30	30	30	30
Early deaths				
Moribund	3	2	11	21
Natural deaths			3	3
Survivors				
Died last week of study	1			
Terminal sacrifice	26	28	16	6
Animals examined microscopically	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst		1 (3%)	2 (7%)	
Site of application-no mass, acanthosis	25 (83%)	28 (93%)	27 (90%)	28 (93%)
Site of application-no mass, alopecia	1 (3%)			
Site of application-no mass, inflammation, chronic active	26 (87%)	29 (97%)	28 (93%)	27 (90%)
Site of application-no mass, ulcer	2 (7%)	1 (3%)	5 (17%)	5 (17%)
Site of application-mass, acanthosis		1 (3%)	3 (10%)	
Subcutaneous tissue, site of application-no mass, edema			1 (3%)	
Subcutaneous tissue, site of application-no mass, infiltration cellular, mixed cell				

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

TABLE C3c
Summary of the Incidence of Nonneoplastic Skin Lesions in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Restart[a]

	Acetone/ Acetone	0.25 μg DMBA/ 1 μg TPA	2.5 μg DMBA/ 1 μg TPA	25 μg DMBA/ 1 μg TPA
Disposition Summary				
Animals initially in study	30	30	30	30
Early deaths				
Moribund	1	22	22	25
Natural deaths		2	6	4
Survivors				
Terminal sacrifice	29	6	2	1
Animals examined microscopically	30	30	30	30
Skin	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst		5 (17%)	6 (20%)	9 (30%)
Site of application-no mass, acanthosis	2 (7%)	30 (100%)	30 (100%)	29 (97%)
Site of application-no mass, alopecia				
Site of application-no mass, inflammation, chronic active	2 (7%)	30 (100%)	30 (100%)	28 (93%)
Site of application-no mass, ulcer		9 (30%)	14 (47%)	6 (20%)
Site of application-no mass, ulcer, multiple		12 (40%)	9 (30%)	15 (50%)
Site of application-mass, acanthosis				
Site of application-mass, ulcer				

TABLE C3c
Summary of the Incidence of Nonneoplastic Skin Lesions in Male SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Restart (continued)

	1 μg TPA/ 1 μg TPA	100 μg MNNG/ 1 μg TPA	1,000 μg MNNG/ 1 μg TPA
Disposition Summary (continued)			
Animals initially in study	30	30	30
Early deaths			
Moribund	17	20	26
Natural deaths	4	2	3
Survivors			
Terminal sacrifice	9	8	1
Animals examined microscopically	30	30	30
Skin (continued)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst	15 (50%)	10 (33%)	3 (10%)
Site of application-no mass, acanthosis	30 (100%)	23 (77%)	21 (70%)
Site of application-no mass, alopecia			1 (3%)
Site of application-no mass, inflammation, chronic active	30 (100%)	29 (97%)	29 (97%)
Site of application-no mass, ulcer	5 (17%)	21 (70%)	21 (70%)
Site of application-no mass, ulcer, multiple	13 (43%)		
Site of application-mass, acanthosis		1 (3%)	
Site of application-mass, ulcer		1 (3%)	

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

TABLE C3d
Summary of the Incidence of Nonneoplastic Skin Lesions in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A[a]

	Acetone/ Acetone	0.25 μg DMBA/ Acetone	2.5 μg DMBA/ Acetone	2.5 μg DMBA/ 2.5 μg DMBA
Disposition Summary				
Animals initially in study	30	30	31	30
Early deaths				
Moribund	2	1	1	19
Natural deaths	1			1
Survivors				
Terminal sacrifice	27	29	30	10
Animals examined microscopically	30	30	31	30
Skin	(29)	(30)	(31)	(30)
Site of application-no mass, acanthosis		2 (7%)	3 (10%)	23 (77%)
Site of application-no mass, inflammation, chronic active	2 (7%)	2 (7%)	5 (16%)	22 (73%)
Site of application-no mass, ulcer			1 (3%)	3 (10%)
Site of application-no mass, ulcer, multiple				1 (3%)

TABLE C3d
Summary of the Incidence of Nonneoplastic Skin Lesions in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design A (continued)

	2.5 μg DMBA/ 20 mg BPO	25 μg DMBA/ Acetone	25 μg DMBA/ 20 mg BPO	20 mg BPO/ 20 mg BPO
Disposition Summary (continued)				
Animals initially in study	30	29	30	30
Early deaths				
Moribund	8	1	6	2
Natural deaths	1		1	
Survivors				
Terminal sacrifice	21	28	23	28
Animals examined microscopically	30	29	30	30
Skin (continued)	(30)	(29)	(30)	(30)
Site of application-no mass, acanthosis	25 (83%)	3 (10%)	27 (90%)	16 (53%)
Site of application-no mass, inflammation, chronic active	30 (100%)	3 (10%)	27 (90%)	28 (93%)
Site of application-no mass, ulcer	3 (10%)		1 (3%)	
Site of application-no mass, ulcer, multiple	1 (3%)		1 (3%)	

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

TABLE C3e
Summary of the Incidence of Nonneoplastic Skin Lesions in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B[a]

	Acetone/ Acetone	100 μg MNNG/ Acetone	500 μg MNNG/ Acetone	1,000 μg MNNG/ Acetone	100 μg MNNG/ 100 μg MNNG
Disposition Summary					
Animals initially in study	30	30	30	30	30
Early deaths					
Accidental death	1				
Moribund	1		5	13	25
Natural deaths	2		3	6	2
Survivors					
Terminal sacrifice	26	30	22	11	3
Animals examined microscopically	30	30	30	30	30
Skin	(30)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst				2 (7%)	
Site of application-no mass, abscess		1 (3%)			
Site of application-no mass, acanthosis		1 (3%)	17 (57%)	11 (37%)	24 (80%)
Site of application-no mass, inflammation, chronic active			16 (53%)	16 (53%)	27 (90%)
Site of application-no mass, ulcer			1 (3%)	8 (27%)	6 (20%)
Site of application-mass, acanthosis				2 (7%)	

TABLE C3e
Summary of the Incidence of Nonneoplastic Skin Lesions in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Design B (continued)

	20 mg BPO/ 20 mg BPO	100 μg MNNG/ 20 mg BPO	500 μg MNNG/ 20 mg BPO	1,000 μg MNNG/ 20 mg BPO
Disposition Summary (continued)				
Animals initially in study	30	30	30	30
Early deaths				
Accidental death				
Moribund	1	1	7	12
Natural deaths	2			4
Survivors				
Terminal sacrifice	27	29	23	14
Animals examined microscopically	30	30	30	30
Skin (continued)	(30)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst				1 (3%)
Site of application-no mass, abscess				
Site of application-no mass, acanthosis	27 (90%)	28 (93%)	28 (93%)	27 (90%)
Site of application-no mass, inflammation, chronic active	27 (90%)	28 (93%)	28 (93%)	29 (97%)
Site of application-no mass, ulcer	1 (3%)		2 (7%)	6 (20%)
Site of application-mass, acanthosis			1 (3%)	

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

TABLE C3f
Summary of the Incidence of Nonneoplastic Skin Lesions in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Restart[a]

	Acetone/ Acetone	0.25 μg DMBA/ 1 μg TPA	2.5 μg DMBA/ 1 μg TPA	25 μg DMBA/ 1 μg TPA
Disposition Summary				
Animals initially in study	30	30	30	30
Early deaths				
Moribund	1	18	19	23
Natural deaths	2	4	5	6
Survivors				
Terminal sacrifice	27	8	6	1
Animals examined microscopically	30	30	30	30
Skin	(29)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst		12 (40%)	8 (27%)	9 (30%)
Site of application-no mass, acanthosis		30 (100%)	30 (100%)	28 (93%)
Site of application-no mass, inflammation, chronic active	1 (3%)	30 (100%)	30 (100%)	27 (90%)
Site of application-no mass, ulcer	1 (3%)	8 (27%)	15 (50%)	13 (43%)
Site of application-no mass, ulcer, multiple		12 (40%)	6 (20%)	9 (30%)
Site of application-mass, acanthosis			1 (3%)	1 (3%)
Site of application-mass, cyst epithelial inclusion				

TABLE C3f
Summary of the Incidence of Nonneoplastic Skin Lesions in Female SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies: Study Restart (continued)

	1 μg TPA/ 1 μg TPA	100 μg MNNG/ 1 μg TPA	1,000 μg MNNG/ 1 μg TPA
Disposition Summary (continued)			
Animals initially in study	30	30	30
Early deaths			
Moribund	19	15	24
Natural deaths	1	2	4
Survivors			
Terminal sacrifice	10	13	2
Animals examined microscopically	30	30	30
Skin (continued)	(30)	(30)	(30)
Hair follicle, site of application-no mass, cyst	16 (53%)	5 (17%)	3 (10%)
Site of application-no mass, acanthosis	30 (100%)	24 (80%)	24 (80%)
Site of application-no mass, inflammation, chronic active	30 (100%)	28 (93%)	23 (77%)
Site of application-no mass, ulcer	5 (17%)	19 (63%)	17 (57%)
Site of application-no mass, ulcer, multiple	11 (37%)		
Site of application-mass, acanthosis	1 (3%)	1 (3%)	2 (7%)
Site of application-mass, cyst epithelial inclusion			1 (3%)

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

APPENDIX D
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Benzoyl Peroxide

Benzoyl peroxide was obtained from Akzo Chemie America (Maple Shade, NJ) in one lot (WM-40) which was used throughout the 1-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) and confirmed by the study laboratory. Reports on analyses performed in support of the 1-year studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a white powdered solid, was identified as benzoyl peroxide by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the literature spectra (*Sadtler Standard Spectra*) of benzoyl peroxide (Figures D1 and D2).

Purity was determined by elemental analyses, nuclear magnetic resonance spectroscopy, functional group titration, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Functional group titration was performed by dissolving samples in acetone, adding potassium:iodide solution (1:2), and immediately titrating the samples with 0.1 N sodium thiosulfate to a colorimetric endpoint. TLC was performed on Silica Gel 60 F-254 plates with two solvent systems: 1) toluene:carbon tetrachloride:glacial acetic acid (66:33:1), and 2) chloroform:ether (75:25). Benzoic anhydride was used as a reference standard. Plates were examined under 254 nm ultraviolet light and a spray of ferrous thiocyanate. HPLC was performed with a Waters μBondapak C_{18} column, with a flow rate of 1.0 mL/minute, detection at 254 nm, and a solvent system of water:acetonitrile (40:60).

Elemental analyses for carbon and hydrogen agreed with theoretical values. Nuclear magnetic resonance spectroscopy indicated 19.7% ± 0.5(s)% water. Functional group titration indicated a purity of 97% ± 2(s)% calculated on an anhydrous basis. TLC indicated a major spot by each system. HPLC indicated two impurities with peak areas totaling 0.4% relative to the major peak. The overall purity was determined to be approximately 99% when corrected for water content.

Based on half-life data from the manufacturer the analytical chemistry laboratory recommended that the bulk chemical be stored protected from light at 5° C. Bulk benzoyl peroxide was stored under refrigeration. During the 1-year studies, the stability of the bulk chemical was monitored by the study laboratory using HPLC and ultraviolet spectroscopy. No degradation of the bulk chemical was observed.

7,12-Dimethylbenz(a)anthracene

7,12-Dimethylbenz(a)anthracene was obtained from the Eastman Kodak Company (Rochester, NY) in one lot (K-4). The lot was purified by the analytical chemistry laboratory. The chemical was dissolved in benzene, passed through a neutral alumina column, and crystallized from isopropanol. The purified material was assigned lot number M111384 and was used throughout the 1-year studies. Reports on the identity, purity, and stability analyses performed by the analytical chemistry laboratory in support of the 1-year studies are on file at the NIEHS.

The chemical, a light yellow powder, was identified as 7,12-dimethylbenz(a)anthracene by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent 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 using two solvent systems:

1) toluene:hexane (60:40) and 2) hexane:chloroform (78:22). Pyrene dissolved in toluene (10μg/mL) was used as a reference standard. Plates were examined under 254 nm and 366 nm ultraviolet light and a spray of 5% potassium dichromate in 40% sulfuric acid. Gas chromatography was performed using a flame ionization detector with a nitrogen carrier gas at a flow rate of 70 mL/minute. Two systems were used:

A) 3% Dexsil 400 on 80/100 Chromosorb W(AW) column, with an oven temperature program of 50° C for 5 minutes, then 50° to 300° C at 10° C per minute, and

B) 3% SP-2100 on 100/120 Supelcoport column, with an oven temperature program of 75° C for 1 minute, then 75° to 275° C at 10° C per minute.

Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for 7,12-dimethylbenz(a)anthracene. Karl Fischer water analysis indicated less than 0.4% water. TLC by system 1 indicated one major spot and one trace spot, and system 2 indicated only a major spot. Gas chromatography using both systems indicated one major peak and no impurities with peaks greater than 0.1% relative to the major peak area. The overall purity was determined to be greater than 99%.

Stability studies were performed with gas chromatography system A described above except with an oven temperature of 300° C and 2.3 mg/mL octacosane added as an internal standard. These studies indicated that 7,12-dimethylbenz(a)anthracene was stable as a bulk chemical for at least 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored protected from light at approximately −20° C. The stability of the bulk chemical was monitored periodically by the study laboratory using ultraviolet spectroscopy and gas chromatography. No degradation of the bulk chemical was observed.

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (8228CK) which was used throughout the 1-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory and confirmed by the study laboratory. Reports on analyses performed in support of the 1-year studies are on file at the NIEHS.

The chemical, a light yellow crystalline solid, was identified as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the literature spectra (*Sadtler Standard Spectra*) of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Figures D5 and D6).

Purity was determined by elemental analyses, nuclear magnetic resonance spectroscopy, TLC, and HPLC. TLC was performed on Silica Gel 60 F-254 plates using two solvent systems: 1) toluene:acetone (9:1), and 2) toluene:ethyl acetate (1:1), with *p*-nitroaniline as a reference standard. Plates were examined under 254 nm ultraviolet light, with a titaneous chloride spray. HPLC was performed with an Alltech Spherisorb CN column, with a flow rate of 1.0 mL/minute, detection at 254 nm, and a solvent system of hexane:methylene chloride (60:40).

Elemental analyses for carbon, hydrogen, and nitrogen agreed with theoretical values. The nuclear magnetic resonance spectrum and elemental analysis results indicated that the water content of the chemical was negligible. TLC indicated a major spot and a minor impurity by system 1 and a major spot with a minor, a trace, and a slight trace impurity by system 2. HPLC indicated no impurities with peak areas greater than 0.1% relative to the major peak. The overall purity was determined to be approximately 99%.

The manufacturer's information and literature sources specified that the bulk chemical be stored at −20° C protected from light. The bulk chemical was stored at −20° C in amber glass bottles. The stability of the

bulk chemical was monitored periodically by the study laboratory using HPLC and ultraviolet spectroscopy. No degradation of the bulk chemical was observed.

12-*O*-Tetradecanoylphorbol-13-acetate

12-*O*-Tetradecanoylphorbol-13-acetate was obtained from Consolidated Midland Corporation (Brewster, NY) in one lot (031), from Pharmacia PL Biochemical (Milwaukee, WI) in two lots (00411999 and 0E11999), and from L.C. Services Corporation (Woburn, MA) in one lot (F-121). A second shipment of lot 00411999 was received from Pharmacia PL Biochemical and was assigned a new number (UN2811) to assist in tracking. All five lots were used during the 1-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory. Reports on analyses performed in support of the study are on file at the NIEHS.

Each lot of the chemical was identified as 12-*O*-tetradecanoylphorbol-13-acetate by nuclear magnetic resonance spectroscopy and mass spectrometry. Both spectra were consistent with that expected for 12-*O*-tetradecanoylphorbol-13-acetate (Figure D7).

The purity of the five lots was determined by TLC and HPLC. TLC was performed on Silica Gel 60 F-254 plates using two solvent systems: 1) anhydrous diethyl ether (100%), and 2) ethyl acetate:chloroform (60:40). Visualization was at 254 nm (and 366 nm for lot 00411999) with a spray of 1% vanillin in concentrated sulfuric acid, followed by heating at 120° C for 10 to 20 minutes. HPLC was performed with a DuPont Zorbax ODS column, with a flow rate of 1 mL/minute, detection at 229 nm, and a solvent system of water:acetonitrile (10:90).

TLC for lots UN2811, 0E11999, and F-121 revealed one major spot with each system. TLC for lot 00411999 revealed only one major spot using system 1 and one major spot and one very slight trace impurity using system 2. TLC of lot 031 using system 1 revealed one major spot, one trace impurity, and one very slight trace impurity, while system 2 revealed one major spot, one trace impurity, one slight trace impurity, and two very slight trace impurities. HPLC of lot 031 revealed one major peak and 11 impurities with areas greater than or equal to 0.1% of the major peak area and a combined area of 3.1% relative to the major peak area. HPLC of lot UN2811 indicated one major peak and seven impurities with areas greater than or equal to 0.1% of the major peak area and a combined area of 2.9% relative to the major peak area. For lot 00411999, HPLC indicated one major peak and three impurities with areas greater than or equal to 0.1% of the major peak area and a combined area of 0.6% relative to the major peak. HPLC of lot 0E11999 indicated one major peak and five impurities with areas greater than or equal to 0.1% of the major peak area and a combined area of 1.0% relative to the major peak area. For lot F-121, HPLC indicated one major peak and two impurities with areas greater than or equal to 0.1% of the major peak area and a combined area of 0.8% relative to the major peak area. The overall purity was determined to be 99% for lots F-121, 0E11999, and 00411999 and 97% for lots 031 and UN2811.

The stability of the chemical was determined using the HPLC system described in the purity analysis. The study indicated that no decomposition had occurred in samples exposed to air and light at ambient temperature for up to 6 days.

Preparation and Analysis of Dose Formulations

Benzoyl Peroxide

Dose formulations were prepared by dissolving benzoyl peroxide in acetone to give the required concentrations (Table D1). The dose formulations were stored at 4° C or at room temperature protected from light, and were discarded 3 weeks after preparation.

Dose formulation stability studies were performed by the analytical chemistry laboratory. The stability study samples were prepared by dissolving benzoyl peroxide in acetone and sonicating.

Samples to be analyzed from the 21-day stability study were first diluted to volume with acetone. Samples from the 3-hour study were brought to their original weight by adding acetone, and aliquots (4 mL) were diluted to volume with acetone. Aliquots of diluted samples were combined with a phenanthrene solution (0.5 mg/mL in acetonitrile:water [75:25]) then filtered (0.45 μ pore size) and analyzed by HPLC using a Waters μBondapak C_{18} column, with a flow rate of 1 mL/minute, a mobile phase of acetonitrile:water (75:25), with benzoyl peroxide 64 μg/mL, and detection at 280 nm. The stability of benzoyl peroxide solutions in acetone (100 mg/mL) was confirmed for at least 3 weeks when the solutions were stored in sealed vials in the dark at 5° C, and for 3 hours when the chemical was exposed to light and air.

Periodic analysis of dose formulations of benzoyl peroxide were performed by the study laboratory and the analytical chemistry laboratory using HPLC. The dose formulations were analyzed at least every 8 weeks (Table D2). During the 1-year studies, 94% (15/16) of the dose formulations were within 10% of the target concentration. Results of the periodic referee analyses performed by the analytical chemistry laboratory were in agreement with the results obtained by the study laboratory for two of the four formulations (Table D3).

7,12-Dimethylbenz(a)anthracene

The dose formulations were prepared by dissolving 7,12-dimethylbenz(a)anthracene and acetone to give the required concentration (Table D1). The dose formulations were stored at 4° C protected from light, and discarded 3 weeks after preparation.

Stability analyses of the 0.1 mg/mL and 0.0025 mg/mL dose formulations were performed by the analytical chemistry laboratory. Aliquots were diluted with acetone, then mixed with 5 mL of the internal standard solution, anthracene (0.2 mg/mL in acetonitrile:water [85:15], and further diluted with acetonitrile:water [85:15]). HPLC was performed using a Brownlee RP-18 column, with a flow rate of 1 mL/minute, a mobile phase of acetonitrile:water (85:15), with anthracene added as an internal standard, and detection at 365 nm. The stability of the dose formulations was confirmed for at least 3 weeks at room temperature when stored in the dark, and for less than 3 hours when exposed to light and air.

Periodic analyses of the dose formulations of 7,12-dimethylbenz(a)anthracene were conducted by the study laboratory and analytical chemistry laboratory using ultraviolet spectroscopy at 363 nm. During the 1-year studies, 100% (23/23) of the dose formulations were within 10% of the target concentrations (Table D2). Results of periodic referee analyses performed by the analytical chemistry laboratory were in agreement with the results obtained by the study laboratory (Table D3).

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine

Dose formulations were prepared by dissolving *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in acetone to give the required concentrations (Table D1). The dose formulations were stored at 4° C protected from light, and were discarded 3 weeks after preparation.

Dose formulation stability studies were performed by the analytical chemistry laboratory. Samples were prepared by dissolving 50 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in acetone and diluting to 100 mL. Aliquots (2 mL) of these solutions were mixed with 3 mL of dimethylnitrosamine (45 mg/mL in methylene chloride) and diluted to 100 mL with n-hexane:methylene chloride (50:50). HPLC was performed using an Alltech Associates Spherisorb S-5-CN column, with a flow rate of 1 mL/minute, a mobile phase of n-hexane:methylene chloride (50:50), with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (10.1 μg/mL) added as an internal standard, and detection at 280 nm. The stability of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was confirmed for at least 3 weeks in sealed vials, in the dark, at room temperature, and for 3 hours when exposed to light and air.

Periodic analyses of dose formulations of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were performed by the study laboratory and the analytical chemistry laboratory using HPLC. The dose formulations were analyzed at least every 8 weeks (Table D2). During the 1-year studies, 95% (19/20) of the dose formulations were within 10% of the target concentration. Results of the periodic referee analyses performed by the analytical laboratory were in agreement with the results obtained by the study laboratory for two of the three formulations.

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. The dose formulations were stored at 4° C protected from light, and were discarded 3 weeks after the date of preparation.

Stability analyses of the dose formulations were conducted by the analytical chemistry laboratory, using the HPLC system used in the bulk chemical analyses of 12-*O*-tetradecanoylphorbol-13-acetate except with a solvent ratio of 7:93. Stability of the formulation was established for at least 3 weeks when stored at room temperature in amber glass bottles.

Periodic analyses of the dose formulations of 12-*O*-tetradecanoylphorbol-13-acetate were conducted by the study laboratory and by the analytical chemistry laboratory with the same HPLC method as that used in the stability study. During the 1-year studies, 92% (24/26) of the formulations analyzed were within 10% of the target concentrations (Table D2). Results of periodic referee analyses performed by the analytical chemistry laboratory were in agreement with the results obtained by the study laboratory in three of the six formulations (Table D3).

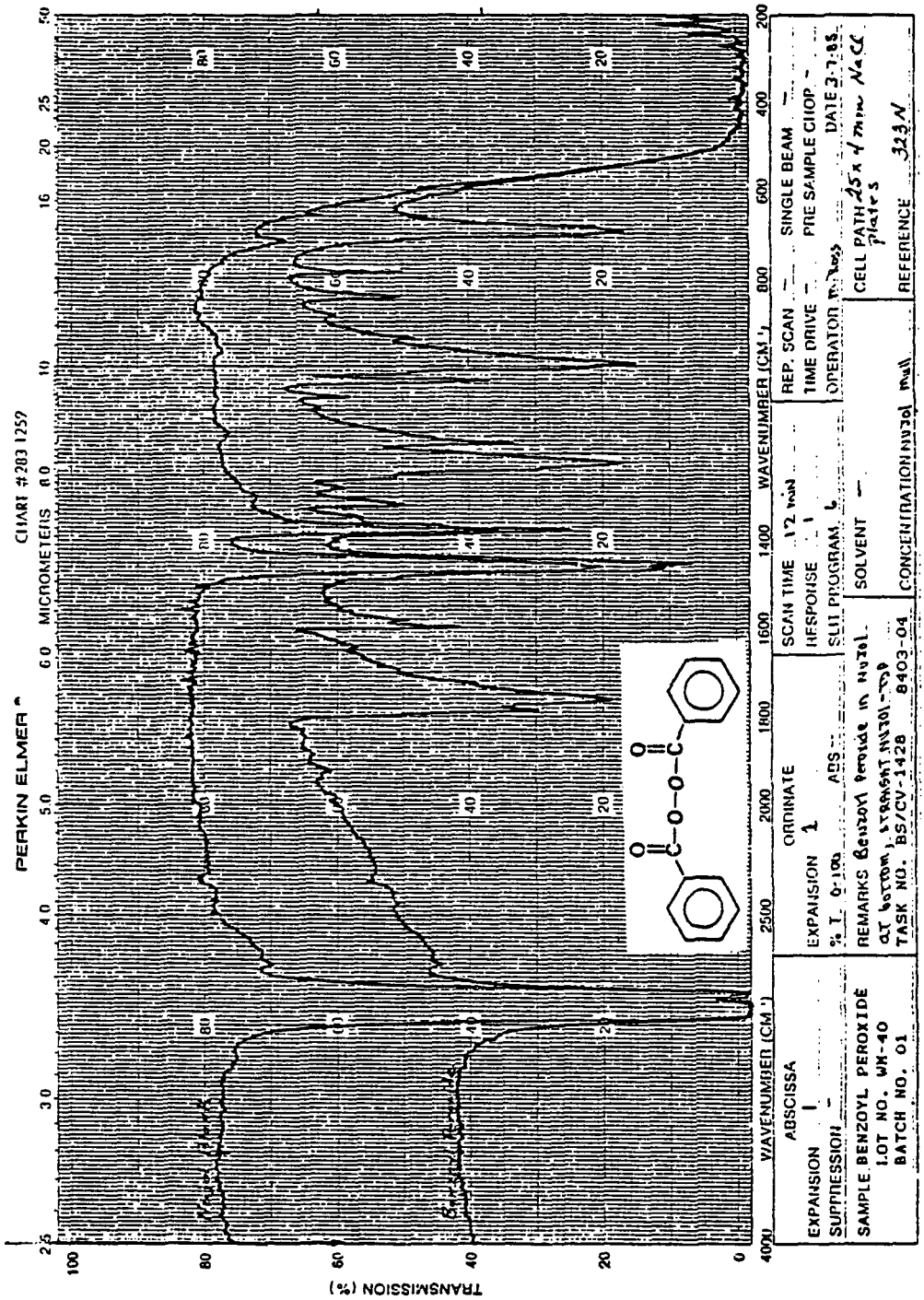


FIGURE D1
Infrared Absorption Spectrum of Benzoyl Peroxide

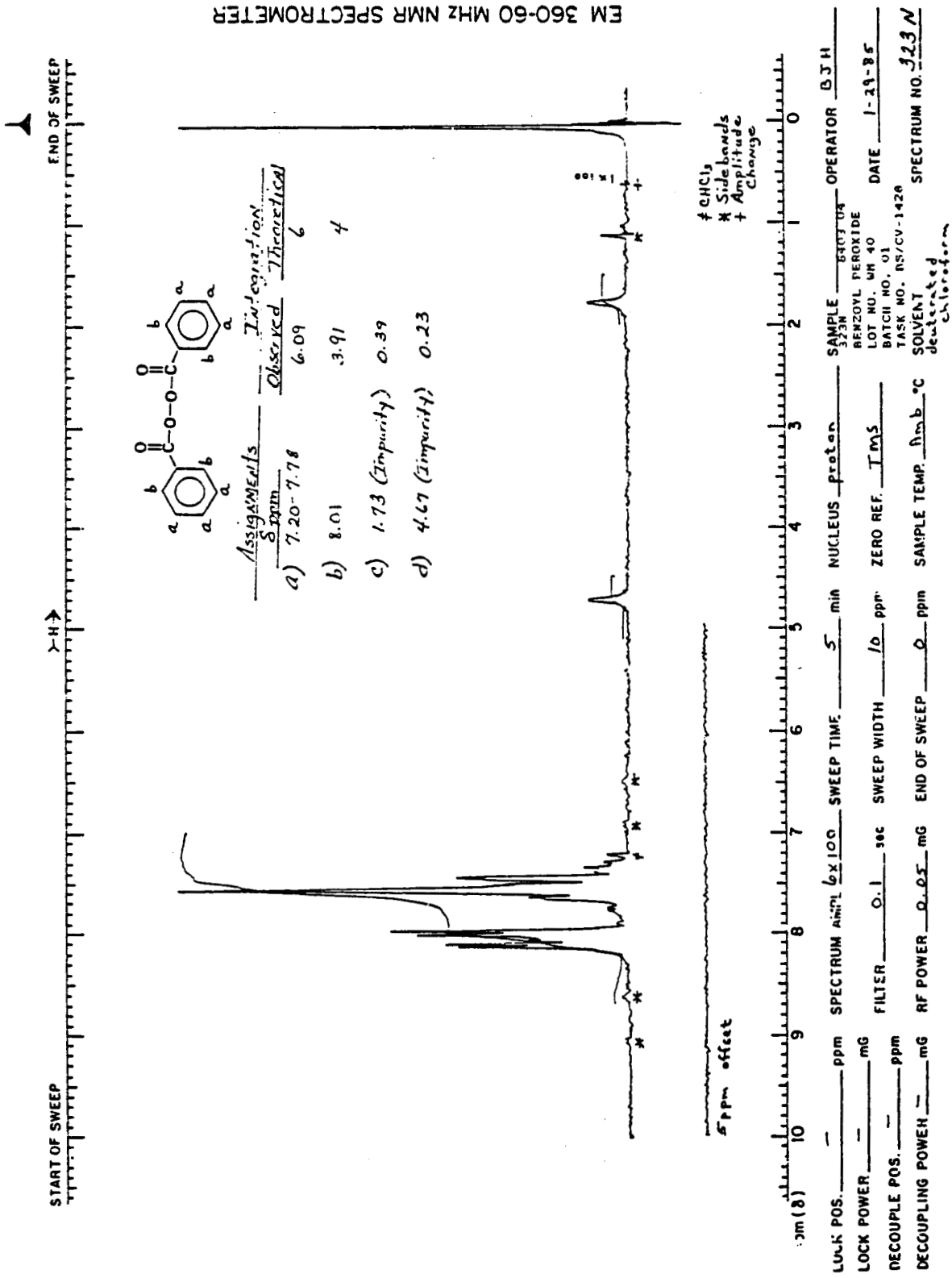


FIGURE D2
Nuclear Magnetic Resonance Spectrum of Benzoyl Peroxide

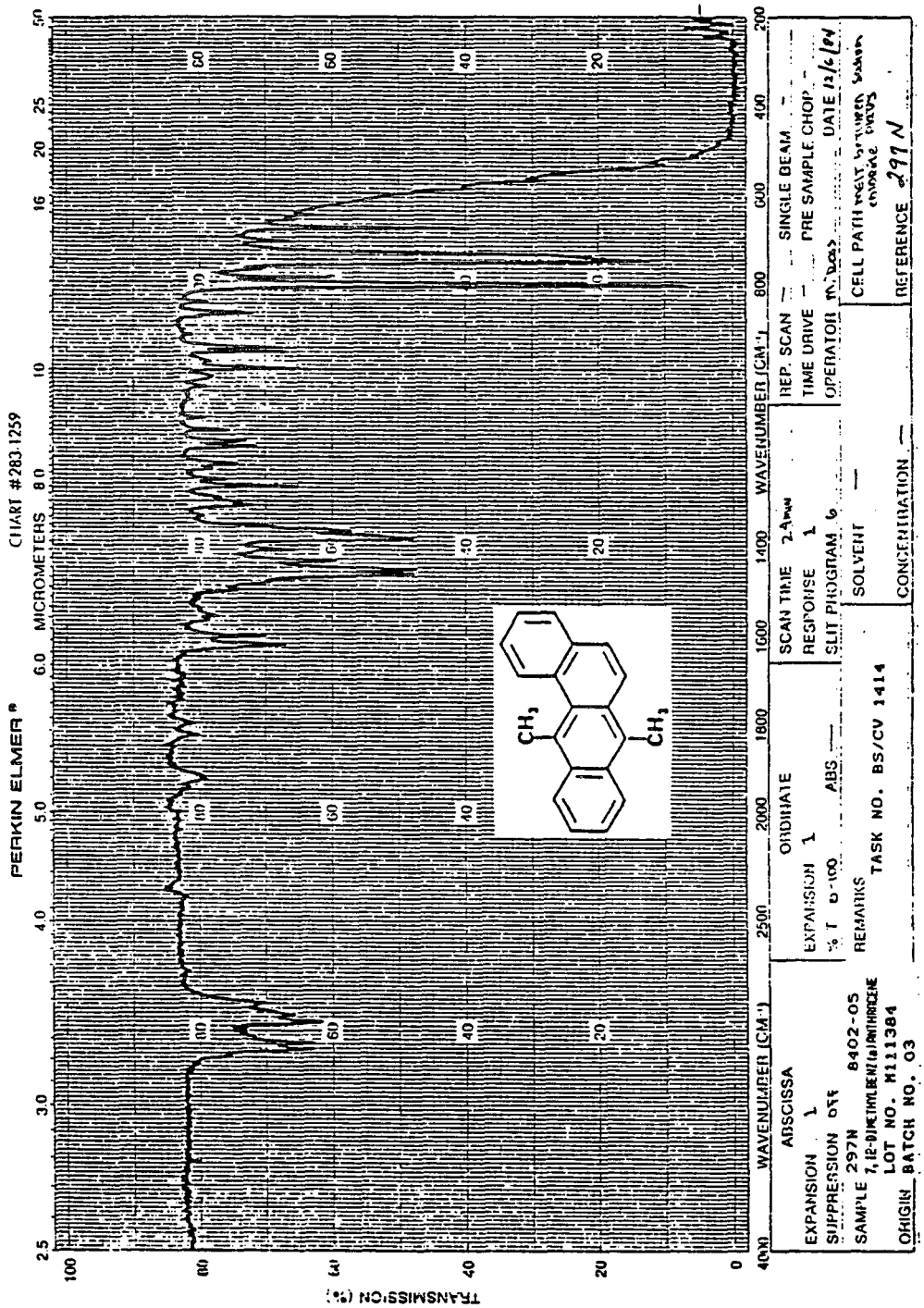
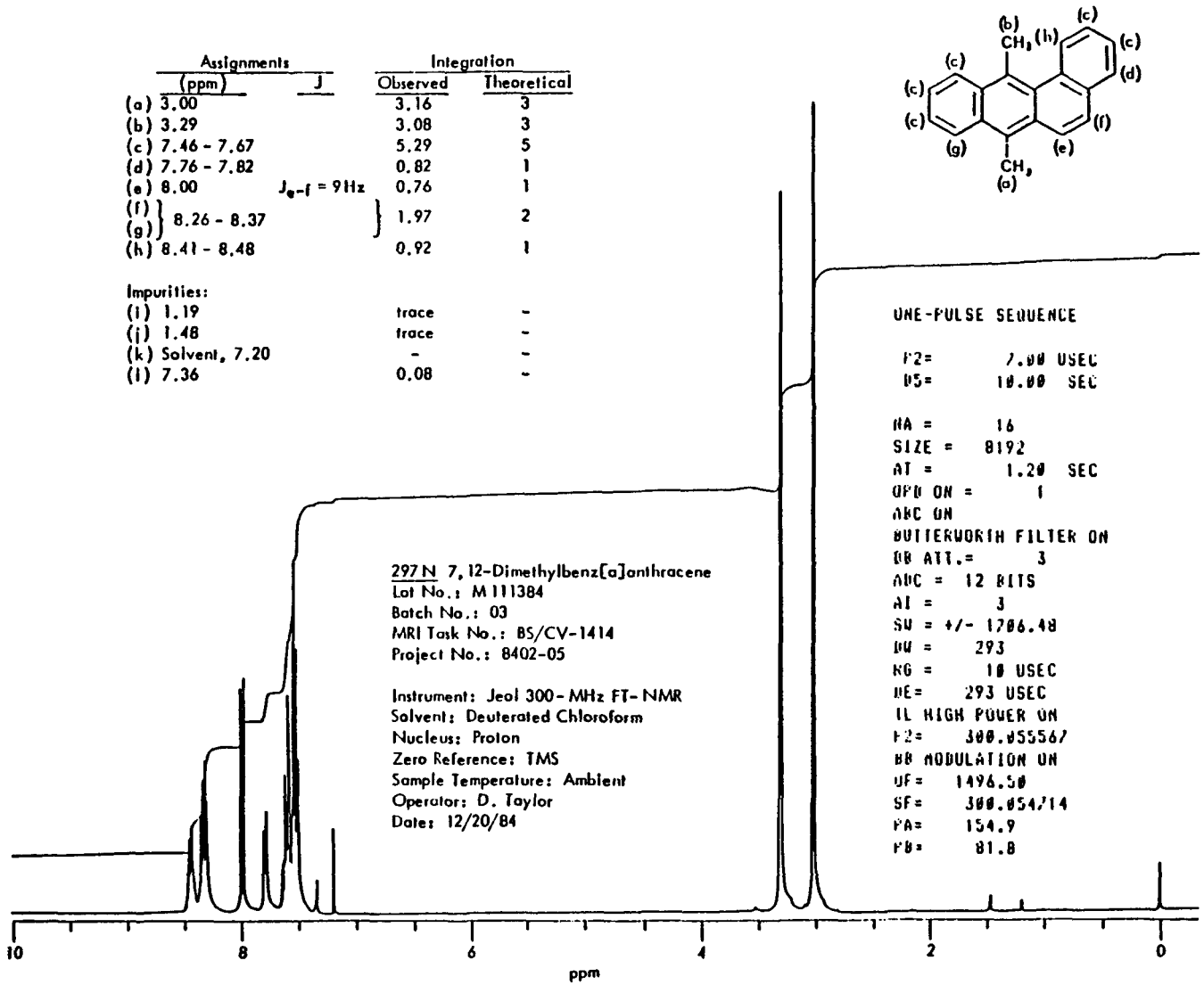


FIGURE D3
Infrared Absorption Spectrum of 7,12-Dimethylbenz(a)anthracene



Assignments (ppm)	J	Integration Observed	Integration Theoretical
(a) 3.00		3.16	3
(b) 3.29		3.08	3
(c) 7.46 - 7.67		5.29	5
(d) 7.76 - 7.82		0.82	1
(e) 8.00	J_{e-f} = 9Hz	0.76	1
(f), (g) 8.26 - 8.37		1.97	2
(h) 8.41 - 8.48		0.92	1
Impurities:			
(i) 1.19		trace	-
(j) 1.48		trace	-
(k) Solvent, 7.20		-	-
(l) 7.36		0.08	-

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

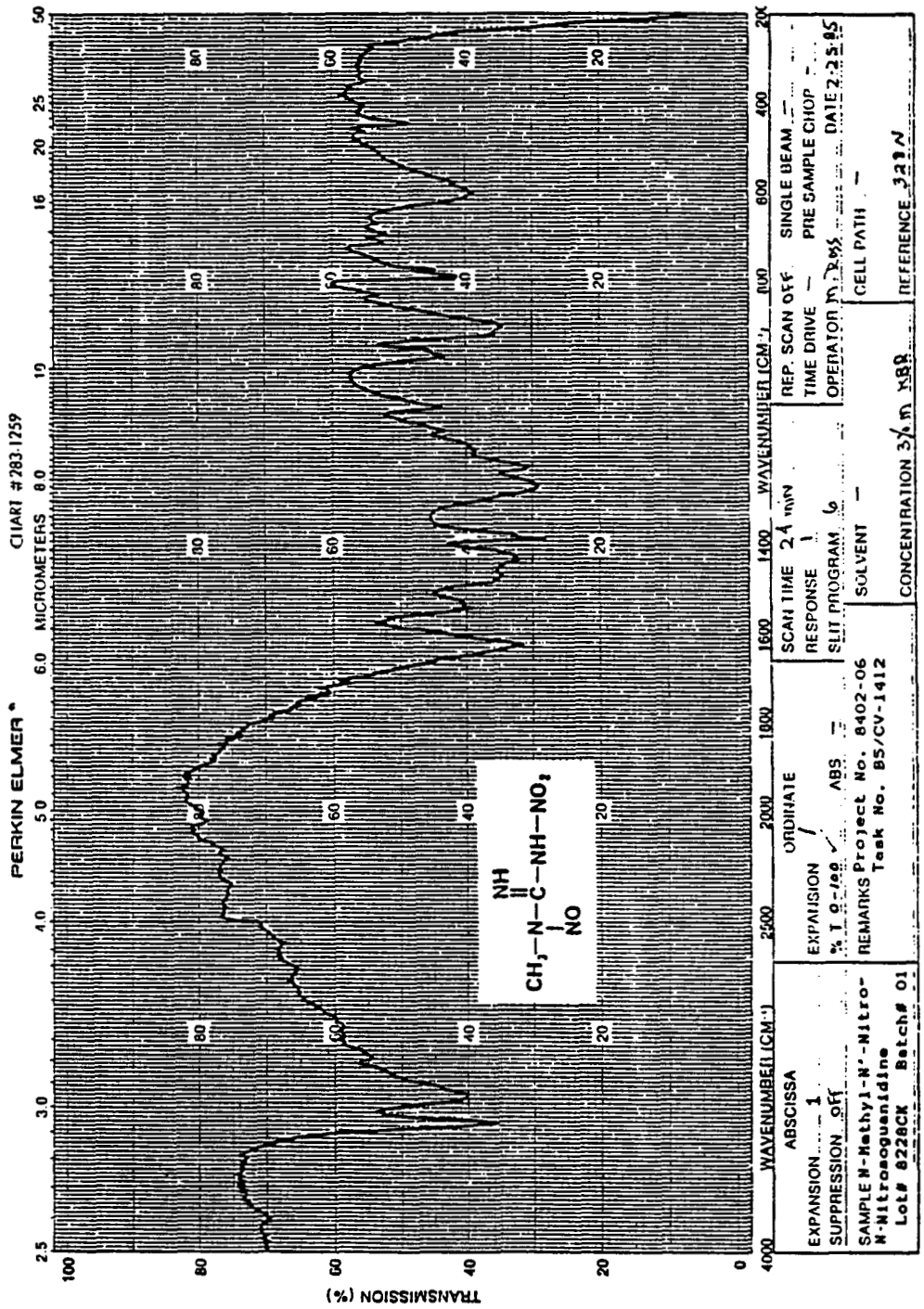


FIGURE D5
Infrared Absorption Spectrum of *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine

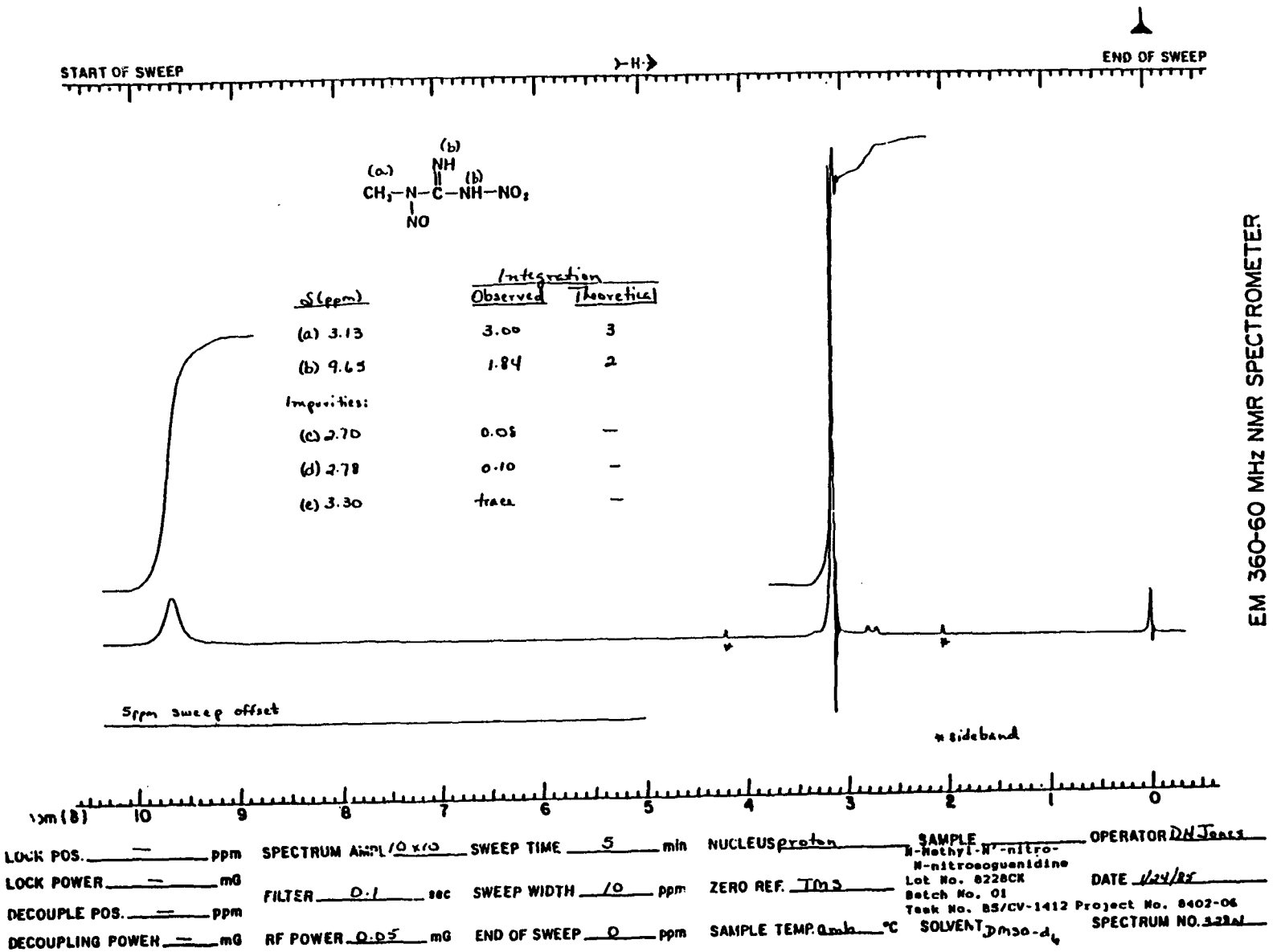


FIGURE D6
Nuclear Magnetic Resonance Spectrum of *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine

196H 12-O-Tetradecanoylphorbol-13-acetate
Lot No.: 031
Batch No.: 05
NRI Task No.: BS-1435
Project No.: 8403-05

Instrument: Jeol 300-MHz FT-NMR
Solvent: Deuterated benzene
Nucleus: Proton
Internal Reference: Benzene
Sample Temperature: Ambient
Operator: D. Taylor
Date: 1/31/85

ONE-PULSE SEQUENCE

P2= 7.00 USEC
D5= 10.00 SEC

NA = 32
SIZE = 16384
AT = 2.05 SEC
OPD ON = 1
ABC ON
BUTTERWORTH FILTER ON
DB ATT.= 3
ADC = 12 BITS
AI = 4
SW = +/- 2000.00
DW = 250
RG = 10 USEC
DE= 250 USEC
TL HIGH POWER ON
F2= 300.054720
BB MODULATION ON
OF= 1283.73
SF= 300.054496
PA= 144.8
PB= 89.1

Assignments (δ ppm)	J	Integration Observed	Integration Theoretical
(a) 0.92	J_{a-c} = 7 Hz	2.97	3
(b) 1.05-1.42		27.48	4
(c) 1.30			22
(d) 1.38		2.89	3
(e) 1.49-1.65		5.95	7
(f) 1.75		2.97	3
(g) 2.19	J_{c-g} = 7 Hz	1.98	2
(h) 2.38-2.54		2.89	1
(i) 2.50			2
(j) 2.76		0.99	1
(k) 3.41-3.50		1.98	2
(l) 3.80		1.98	2
(m) 5.74		0.99	1
(n) 5.88-5.98		1.98	2
(o) 7.45		0.99	1
(p) 0.65, impurity		0.28	-
(q) 7.15, solvent		--	-

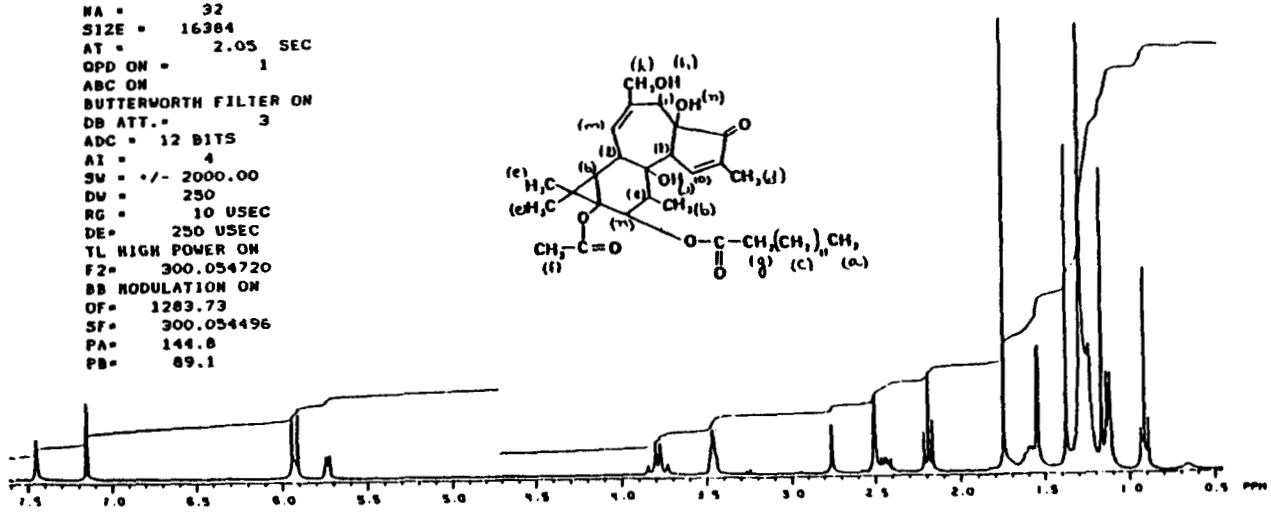


FIGURE D7
Nuclear Magnetic Resonance Spectrum of 12-*O*-Tetradecanoylphorbol-13-acetate

TABLE D1
Preparation and Storage of Dose Formulations in the Comparative Initiation/Promotion Skin Paint Studies

Benzoyl Peroxide	7,12-Dimethylbenz(a)anthracene	*N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine	12-*O*-Tetradecanoylphorbol-13-acetate
Preparation			
Benzoyl peroxide was weighed and then transferred to a graduated cylinder. Acetone was added to obtain a solution with the required benzoyl peroxide concentration.	7,12-Dimethylbenz(a)anthracene was weighed and then transferred to a graduated cylinder. Acetone was added to obtain a solution with the required 7,12-dimethylbenz(a)anthracene concentration.	*N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine was weighed and then transferred to a graduated cylinder. Acetone was added to obtain a solution with the required *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine concentration.	12-*O*-Tetradecanoylphorbol-13-acetate was weighed and then transferred to a graduated cylinder. Acetone was added to obtain a solution with the required 12-*O*-tetradecanoylphorbol-13-acetate concentration.
Chemical Lot Number			
WM-40	M111384	8228CK	031, UN2811, 00411999, 0E11999, and F-121
Maximum Storage Time			
3 weeks	3 weeks	3 weeks	3 weeks
Storage Conditions			
Stored at 4 °C protected from light, except doses prepared June 10-25, 1985 (stored at room temperature)	Stored at 4 °C protected from light	Stored at 4 °C protected from light	Stored at 4 °C protected from light
Study Laboratory			
Battelle Columbus Division (Columbus, OH)	Battelle Columbus Division (Columbus, OH)	Battelle Columbus Division (Columbus, OH)	Battelle Columbus Division (Columbus, OH)
Referee Laboratory			
Midwest Research Institute (Kansas City, MO)	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 B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies

Date Prepared	Date Analyzed	Target Concentration (mg/mL)[a]	Determined Concentration (mg/mL)[b]	% Difference from Target
Benzoyl Peroxide				
18 April 1985	19 April 1985	100.0	96.04	−4
	1 May 1985[c]	100.0	106.24	+6
10 June 1985	14 June 1985	100.0	96.36	−4
5 August 1985	7 August 1985	100.0	98.67	−1
	5 September 1985[c]	100.0	107.59	+8
30 September 1985	2 October 1985	100.0	97.41	−3
		100.0	101.97	+2
26 November 1985	26 November 1985	100.0	94.50	−6
		100.0	93.14	−7
21 January 1986	23 January 1986	100.0	101.17	+1
		100.0	99.03	−1
	18 February 1986[c]	100.0	115.56	+16
		100.0	109.06	+9
18 March 1986	20 March 1986	100.0	97.20	−3
		100.0	98.28	−2
12 May 1986	14 May 1986	100.0	98.66	−1
7,12-Dimethylbenz(a)anthracene				
18 April 1985	19 April 1985	0.0025	0.0023[d]	−8
		0.025	0.0240[d]	−4
		0.250	0.2480	−1
		0.500	0.4960	−1
	2 May 1985[c]	0.0025	0.00236[d]	−6
		0.025	0.02631[d]	+5
		0.250	0.26624	+6
		0.500	0.49708	−1
1 May 1985	2 May 1985	0.025	0.02451[d]	−2
11 June 1985	13 June 1985	0.025	0.02593[d]	+4
5 August 1985	6 August 1985	0.025	0.02529[d]	+1
1 October 1985	3 October 1985	0.025	0.02609[d]	+4
	24 October 1985[c]	0.025	0.02648	+6
8 October 1985	10 October 1985	0.025	0.02588[d]	+4
	24 October 1985[c]	0.025	0.02685	+7

TABLE D2
Results of Analysis of Dose Formulations Administered to B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
7,12-Dimethylbenz(a)anthracene (continued)				
11 October 1985	11 October 1985	0.0025	0.00258[d]	+3
		0.250	0.2522	+1
	24 October 1985[c]	0.0025	0.00262	+5
		0.250	0.2551	+2
25 November 1985	25 November 1985	0.025	0.02606	+4
21 January 1986	23 January 1986	0.025	0.02607[d]	+4
18 March 1986	18 March 1986	0.025	0.02589[d]	+4
	10 April 1986[c]	0.025	0.02635[d]	+5
***N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine**				
17 May 1985	20 May 1985	1.0	1.00	0
		5.0	5.46	+9
		10.0	10.66	+7
	3-4 June 1985[c]	1.0	1.10	+1
		5.0	5.42[e]	+8
		10.0	12.71[f]	+27
	5 June 1985[c]	5.0	5.43	+9
		10.0	10.69	+7
8 July 1985	9 July 1985	1.0	0.96	−4
5 September 1985	6 September 1985	1.0	1.01	+1
8 October 1985	15 October 1985	10.0	10.34	+3
	21 October 1985[c]	10.0	10.95	+10
15 October 1985	15 October 1985	1.0	0.94[e]	−7
	18 October 1985[c]	1.0	0.93	−7
28 October 1985	31 October 1985	1.0	0.96	−4
	19 November 1985[c]	1.0	1.07	+7
23 December 1985	27 December 1985	1.0	0.95	−5
18 February 1986	19 February 1986	1.0	0.94	−6
14 April 1986	15 April 1986	1.0	1.03	+3
	7 May 1986[c]	1.0	0.96	−4

TABLE D2
Results of Analysis of Dose Formulations Administered to B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
12-*O*-Tetradecanoylphorbol-13-acetate				
18 April 1985	19 April 1985	0.05	0.0537	+7
	3 May 1985[c]	0.05	0.0518	+4
1 May 1985	3 May 1985	0.05	0.0519	+4
10 June 1985	12 June 1985	0.05	0.0479	−4
5 August 1985	6 August 1985	0.05	0.05664	+13
7 August 1985	7 August 1985	0.05	0.05326	+7
30 September 1985	3 October 1985	0.05	0.05182	+4
	22 October 1985[c]	0.05	0.04964	−1
8 October 1985	14 October 1985	0.01	0.00961	−4
	22 October 1985[c]	0.01	0.00991	−1
25 November 1985	26 November 1985	0.05	0.04971	−1
	2 December 1985	0.01	0.00998	0
21 January 1986	25 January 1986	0.05	0.04680	−6
		0.01	0.01016	+2
18 March 1986	19 March 1986	0.05	0.04664	−6
	21 March 1986	0.01	0.01036	+4
	8 April 1986[c]	0.05	0.05451	+9
		0.05	0.05489	+10
		0.01	0.01385	+39
12 May 1986	13 May 1986	0.05	0.04561	−9
		0.01	0.00904	−10
	4 June 1986[c]	0.01	0.00988	−1
		0.05	0.04855	−3
7 July 1986	8 July 1986	0.01	0.01041[e]	+4
2 September 1986	3 September 1986	0.01	0.01013	+1
	22 September 1986[c]	0.01	0.01006	+1

[a] Dosing volume = 0.1 mL for 7,12-dimethylbenz(a)anthracene, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and 12-*O*-tetradecanoylphorbol-13-acetate. Dosing volume for benzoyl peroxide was 0.2 mL (two consecutive administrations of 0.1 mL)
[b] Results of duplicate analyses
[c] Animal room sample
[d] Results of single analysis
[e] Results of triplicate analyses
[f] Results of quadruplicate analyses

TABLE D3
Results of Referee Analysis of Dose Formulations Administered to B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies

Date Mixed	Target Concentration (mg/mL)[a]	Determined Concentration (mg/mL) Study Laboratory[b]	Determined Concentration (mg/mL) Referee Laboratory[c]
Benzoyl Peroxide			
18 April 1985	100	96.04	82.7 ± 0.8
5 August 1985	100	98.67	80.9 ± 1.9
26 November 1985	100	94.50	99.9 ± 0.1
18 March 1986	100	97.20	106.3 ± 0.6
7,12-Dimethylbenz(a)anthracene			
18 April 1985	0.025	0.024	0.0208 ± 0.0
1 May 1985	0.025	0.02451	0.0239 ± 0.0
1 October 1985	0.025	0.02609	0.0249 ± 0.0
18 March 1986	0.025	0.02589	0.0242 ± 0.1
***N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine**			
17 May 1985	1.0	1.00	1.05 ± 0.01
28 October 1985	1.0	0.96	0.98 ± 0.02
14 April 1986	1.0	1.03	1.22 ± 0.03
12-*O*-Tetradecanoylphorbol-13-acetate			
18 April 1985	0.05	0.0537	0.0375[d]
1 May 1985	0.05	0.0519	0.0387 ± 0.0003
7 August 1985	0.05	0.05326	0.0473 ± 0.0004
25 November 1985	0.01	0.00998	0.0097 ± 0.0[b]
21 January 1986	0.05	0.0468	0.0467 ± 0.0002
12 May 1986	0.01	0.00904	0.0098 ± 0.0

[a] Dosing volume = 0.1 mL for 7,12-dimethylbenz(a)anthracene, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and 12-*O*-tetradecanoylphorbol-13-acetate. Dosing volume for benzoyl peroxide was 0.2 mL (two consecutive administrations of 0.1 mL)
[b] Results of duplicate analyses
[c] Results of triplicate analyses
[d] Single analysis

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 188
TABLE E2 Vitamins and Minerals in NIH-07 Rat and Mouse Ration 188
TABLE E3 Nutrient Composition of NIH-07 Rat and Mouse Ration 189
TABLE E4 Contaminant Levels in NIH-07 Rat and Mouse Ration 190

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)	21.97 ± 0.43	21.10 – 22.50	15
Crude fat (% by weight)	5.57 ± 0.50	4.70 – 6.40	15
Crude fiber (% by weight)	3.48 ± 0.58	2.70 – 5.40	15
Ash (% by weight)	6.53 ± 0.26	6.13 – 6.97	15
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)	9,707 ± 2,523	6,200 – 15,000	15
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 – 6,300	4
α-Tocopherol (ppm)	36.92 ± 9.32	22.5 – 48.9	9
Thiamine (ppm)	19.80 ± 1.52	17.0 – 23.0	15
Riboflavin (ppm)	7.92 ± 0.93	6.10 – 9.00	10
Niacin (ppm)	100.95 ± 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)	0.267 ± 0.049	0.17 – 0.35	10
Vitamin B_{12} (ppb)	40.14 ± 20.04	10.6 – 65.0	10
Choline (ppm)	3,068 ± 314	2,400 – 3,430	9
Minerals			
Calcium (%)	1.16 ± 0.10	0.98 – 1.49	15
Phosphorus (%)	0.93 ± 0.04	0.87 – 0.99	15
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)	0.76 ± 0.14	0.47 – 0.94	15
Cadmium (ppm)	<0.10		15
Lead (ppm)	0.58 ± 0.28	0.14 – 1.32	15
Mercury (ppm)	<0.05		15
Selenium (ppm)	0.35 ± 0.09	0.17 – 0.48	15
Aflatoxins (ppb)	<5.0		15
Nitrate nitrogen (ppm)[b]	14.92 ± 4.41	2.80 – 22.0	15
Nitrite nitrogen (ppm)[b]	0.13 ± 0.10	<0.10 – 0.50	15
BHA (ppm)[c]	2.47 ± 0.99	<2.00 – 5.00	15
BHT (ppm)[c]	1.80 ± 1.08	<1.00 – 4.00	15
Aerobic plate count (CFU/g)[d]	44,411 ± 43,967	770 – 130,000	15
Coliform (MPN/g)[e]	4.40 ± 2.41	3.00 – 9.00	15
E. coli (MPN/g)	<3.00		15
Salmonella (MPN/g)	Negative		15
Total nitrosoamines (ppb)[f]	7.20 ± 3.84	3.80 – 16.00	15
N-Nitrosodimethylamine (ppb)	6.04 ± 3.56	2.80 – 15.00	15
N-Nitrosopyrrolidine (ppb)	1.16 ± 0.62	1.00 – 3.40	15
Pesticides (ppm)			
α-BHC[g]	<0.01		15
β-BHC	<0.02		15
γ-BHC	<0.01		15
δ-BHC	<0.01		15
Heptachlor	<0.01		15
Aldrin	<0.01		15
Heptachlor epoxide	<0.01		15
DDE	<0.01		15
DDD	<0.01		15
DDT	<0.01		15
HCB	<0.01		15
Mirex	<0.01		15
Methoxychlor	<0.05		15
Dieldrin	<0.01		15
Endrin	<0.01		15
Telodrin	<0.01		15
Chlordane	<0.05		15
Toxaphene	<0.1		15
Estimated PCBs	<0.2		15
Ronnel	<0.01		15
Ethion	<0.02		15
Trithion	<0.05		15
Diazinon	<0.1		15
Methyl parathion	<0.02		15
Ethyl parathion	<0.02		15
Malathion[h]	0.33 ± 0.81	<0.05 – 3.20	15
Endosulfan I	<0.01		15
Endosulfan II	<0.01		15
Endosulfan sulfate	<0.03		15

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 as the mean.
[b] Sources of contamination: alfalfa, grains, and fish meal
[c] Sources of contamination: soy oil and fish meal
[d] CFU = colony forming unit
[e] MPN = most probable number
[f] All values were corrected for percent recovery.
[g] BHC is hexachlorocyclohexane or benzene hexachloride
[h] One lot milled on 7 May 1985 contained more than 0.7 ppm.

APPENDIX F
SENTINEL ANIMAL PROGRAM

METHODS 194
TABLE F1 Murine Virus Antibody Determinations for B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies 196

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.

Serum samples were collected from randomly selected sentinel mice during the quarantine period and at 6 months, and from Acetone/Acetone control animals at study termination. Serum samples were also collected from several moribund animals throughout the studies. A special screening for mouse hepatitis virus was performed on sentinel mice in study design A of all three strains. Blood from each animal was collected, allowed to clot and the serum separated. The samples were processed appropriately and sent to Microbiological Associates, Inc. (Bethesda, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

B6C3F$_1$ MICE

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

Swiss (CD-1®) Mice

Method and Test	Time of Analysis
Complement Fixation	
LCM	Quarantine, 6 months, study termination
ELISA	
Ectromelia virus	Quarantine, 6 months, study termination
GDVII	Quarantine, 6 months, study termination
Mouse adenoma virus	Quarantine, 6 months, study termination
MHV	Quarantine, special screening, 6 months, study termination
M. arthritidis	Quarantine, 6 months, study termination
M. pulmonis	Quarantine, 6 months, study termination
PVM	Quarantine, 6 months, study termination
Reovirus 3	Quarantine, 6 months, study termination
Sendai	Quarantine, 6 months, study termination
Hemagglutination Inhibition	
K	Quarantine, 6 months, study termination
MVM	Quarantine, 6 months, study termination
Polyoma virus	Quarantine, 6 months, study termination
Immunofluorescence Assay	
EDIM	Quarantine, 6 months, study termination
Reovirus 3	Study termination
Sendai	Study termination

SENCAR Mice

Method and Test	Time of Analysis
Complement Fixation	
LCM	Quarantine, 6 months, study termination
ELISA	
Ectromelia virus	Quarantine, 6 months, study termination
GDVII	Quarantine, 6 months, study termination
Mouse adenoma virus	Quarantine, 6 months, study termination
MHV	Quarantine, special screening, 6 months, study termination
M. arthritidis	Quarantine, 6 months, study termination
M. pulmonis	Quarantine, 6 months, study termination
PVM	Quarantine, 6 months, study termination
Reovirus 3	Quarantine, 6 months, study termination
Sendai	Quarantine, 6 months, study termination
Hemagglutination Inhibition	
K	Quarantine, 6 months, study termination
MVM	Quarantine, 6 months, study termination
Polyoma virus	Quarantine, 6 months, study termination
Immunofluorescence Assay	
EDIM	Quarantine, 6 months, study termination
MVM	6 Months
MHV	Special screening

Results of serology tests are presented in Table F1.

TABLE F1
Murine Virus Antibody Determinations for B6C3F$_1$ Mice, Swiss (CD-1®) Mice, and SENCAR Mice in the Comparative Initiation/Promotion Skin Paint Studies

Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
B6C3F$_1$ Mice		
Quarantine	0/40	None positive
Special screening	0/10	None positive
6 Months	0/19	None positive
Study termination	0/20	None positive
Swiss (CD-1®) Mice		
Quarantine	0/40	None positive
Special screening	0/10	None positive
6 Months	1/19	GDVII
	1/19	Sendai
7 Months[a]	2/3	Mouse adenoma virus
Study termination	1/20	*M. arthritidis*[b]
	3/19	Reovirus 3
	1/20	Sendai
SENCAR Mice		
Quarantine	0/50	None positive
Special screening	1/9	MHV
6 Months	1/26	EDIM
Study termination	1/30	*M. arthritidis*[b]
	2/30	Reovirus 3

[a] Evaluation of 3 moribund animals
[b] 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, *M. arthritidis* positive titers were considered to be false positives.

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