DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 333

[Docket No. 81N-114A]

RIN 0905-AA06

Topical Acne Drug Products for Over-the-Counter Human Use; Amendment of Tentative Final Monograph

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice of proposed rulemaking.

SUMMARY: The Food and Drug Administration (FDA) is issuing a notice of proposed rulemaking amending the tentative final monograph (proposed rule) for over-the-counter (OTC) topical acne drug products. This amendment reclassifies the topical acne active ingredient benzoyl peroxide from its previously proposed monograph status (Category I) to "more-data-needed" (Category III) status. FDA is issuing this notice of proposed rulemaking after considering data and information on the safety of benzoyl peroxide. This proposal is part of the ongoing review of OTC drug products conducted by FDA.

DATES: Written comments, objections, or requests for oral hearing on the proposed regulation before the Commissioner of Food and Drugs by October 7, 1991. New Data by August 7, 1992. Comments on the new data by October 7, 1992. Written comments on the agency's economic impact determination by October 7, 1991.

ADDRESSES: Written comments, objections, new data, or requests for oral hearing to the Dockets Management Branch (HFA-305), Food and Drug Administration, Room 1-23, 12420 Parklawn Drive, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: William E. Gilbertson, Center for Drug Evaluation and Research (HFD-210), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-295-8000.

SUPPLEMENTARY INFORMATION: In the **Federal Register** of March 23, 1982 (47 FR 12430) FDA published, under § 330.10(a)(6) (21 CFR 330.10(a)(6)), an advance notice of proposed rulemaking to establish a monograph for OTC topical acne drug products, together with the recommendations of the Advisory Review Panel on OTC Antimicrobial (II) Drug Products (Antimicrobial II Panel), which was the advisory review panel responsible for evaluating data on the active ingredients in this drug class. Interested persons were invited to submit comments by June 21, 1982. Reply comments in response to comments filed in the initial comment period could be submitted by July 21, 1982.

In accordance with § 330.10(a)(10), the data and information considered by the Panel were placed on display in the Dockets Management Branch (address above), after deletion of a small amount of trade secret information.

The agency's proposed regulation, in the form of a tentative final monograph, for OTC topical acne drug products was published in the **Federal Register** of January 15, 1985 (50 FR 2172). Interested persons were invited to file by May 15, 1985 written comments, objections, or requests for oral hearing before the Commissioner of Food and drugs regarding the proposal. New data could have been submitted until January 15, 1986, and comments on the new data until March 17, 1986.

The OTC drug procedural regulations (21 CFR 330.10) now provide that any testing necessary to resolve the safety or effectiveness issues that formerly resulted in a Category III classification, and submission to FDA of the results of that testing or any other data, must be done during the OTC drug rulemaking process before the establishment of a final monograph. Accordingly, FDA will no longer use the terms "Category I" (generally recognized as safe and effective and not misbranded), "Category II" (not generally recognized as safe and effective or misbranded), and "Category III" (available data are insufficient to classify as safe and effective, and further testing is required) at the final monograph stage, but will use instead the terms "monograph conditions" (old Category I) and "nonmonograph conditions" (old Categories II and III). This document retains the concepts of Categories I, II, and III at this amended tentative final monograph stage.

In response to the proposed rule on OTC topical acne drug products, two drug manufacturers associations submitted comments on the safety of benzoyl peroxide. Copies of the comments received are on public display in the Dockets Management Branch (address above). Additional information on benzoyl peroxide that has come to the agency's attention since publication of the proposed rule is also on public display in the Dockets Management Branch.

The Antimicrobial II Panel in its advance notice of proposed rulemaking (47 FR 12430 at 12475) and the agency in its tentative final monograph (50 FR 2172 at 2181) proposed monograph status for the ingredient benzoyl peroxide for OTC topical use in the treatment of acne. However, subsequently the agency became aware of a 1981 study by Slage, et al. (Ref. 1) that raised a safety concern regarding benzoyl peroxide as a tumor promoter in mice and a 1984 study by Kurokawa, et al. (Ref. 2) that reported benzoyl peroxide to have tumor initiation potential. Neither of these studies was discussed by the Panel or by the agency in the **Federal Register** publications identified above.

Subsequently also, a drug manufacturers association submitted data and information in support of the safety of benzoyl peroxide (Refs. 3 through 6). FDA has evaluated these data and information and determined that the studies show that benzoyl peroxide is a skin tumor promoter in more than one strain of mice as well as in other laboratory animals tested. To date, topical studies (which have shown only tumor promotion) have been of short duration (about 52 weeks), which the agency considers insufficient to rule out the potential for carcinogenicity. Although extensive animal data and human epidemiology data are available, the agency is unable to state that benzoyl peroxide is generally recognized as safe at this time. The agency has determined that further study is necessary to adequately assess the tumorigenic potential of benzoyl peroxide. The agency believes that studies of 18 to 24 months in two species of animals (mouse and rat) are needed to rule out the possibility of carcinogenicity. While the agency finds that additional studies are needed to address concerns about benzoyl peroxide's possible tumor initiating and promotion potential, the agency is unable to state that this ingredient is unsafe for OTC use while these studies are being conducted. The agency acknowledges that it may take several years for these studies to be conducted and analyzed, and for a final determination to be made on benzoyl peroxide's safety. Because animal studies have shown that benzoyl peroxide is a skin tumor promoter and the relevance to humans is unknown, the agency is concerned about continued OTC marketing availability pending resolution of these unresolved safety issues. The agency specifically invites comments on this issue. The agency plans to discuss its concerns and comments received on the agency's conclusions on the data and on continued marketing of benzoyl peroxide with one of its advisory committees at a public meeting to be held in the near future. Notice of this

meeting will appear in a future issue of the Federal Register.

Based on the above, the agency is amending the tentative final monograph for OTC topical acne drug products to reclassify benzoyl peroxide from Category I to Category III. As a result, in subpart D, it is being proposed that the ingredient benzoyl peroxide be removed from § 333.310 (21 CFR 333.310) and that the warning proposed for products containing benzoyl peroxide in § 333.350(c)(2) (21 CFR 333.350(c)(2)) be removed. The agency will publish its final decision on benzoyl peroxide in a future issue of the Federal Register.

This amendment of the tentative final monograph concerns only the ingredient benzoyl peroxide and labeling related to this specific ingredient. It does not concern any other OTC topical acne active ingredients or the labeling of products containing such ingredients. The agency advises that a final decision on benzoyl peroxide, if it is included in the final monograph for OTC topical acne drug products at a later date, will be effective 12 months after the date of publication of the final decision in the Federal Register. If a safety problem is identified for benzoyl peroxide, resulting in it being a nonmonograph condition, a shorter deadline may be set for removal of that ingredient from OTC drug products. On or after the effective date of any final rule pertaining to benzoyl peroxide, no OTC drug product containing benzoyl peroxide may be initially introduced or initially delivered for introduction into interstate commerce unless benzoyl peroxide is included in the final monograph for OTC topical acne drug products or, alternatively, the product is the subject of an approved application, if one is required for marketing.

I. The Agency's Conclusions on the Data

Since publication of the tentative final monograph for OTC topical acne drug products on January 15, 1985, the agency has evaluated substantial additional data on benzoyl peroxide. The data included in vitro and in vivo studies, epidemiological studies, and studies published in the literature. The agency's evaluation of these data follows.

A. Initiation/Promotion Studies

In a study by Sharrat, et al. (Ref. 7) albino mice or rats, 25 per dose per sex (strain and age not specified), were fed 0, 28, 280, or 2,800 milligrams/kilograms (mg/kg) benzoyl peroxide in a commercial diet with a commercial flour bleach consisting of 18 percent benzoyl peroxide, 78 percent calcium sulfate, and 4 percent magnesium carbonate. Controls received untreated flour in the diet. Test diets were fed to mice and rats for 80 and 120 weeks, respectively.

At 104 weeks, the number of surviving male/female rats was 12/14, 12/7, 13/9, and 11/11, respectively. No significant intergroup difference in tumor incidence between groups was observed; however, the incidence of testicular atrophy was higher in the male rats receiving 2,800 mg/kg benzoyl peroxide. At study termination, the number of surviving male/female mice per dose was 3/9, 10/11, 0/9, and 2/11, respectively. No significant difference in tumor incidence between groups was observed.

Using another protocol, groups of albino mice, 25 per sex (strain and age not specified), were fed a diet containing 2,800 mg/kg benzoyl peroxide and received simultaneous subcutaneous injection of 50 mg benzoyl peroxide in 20 percent starch solution. Mice were also painted 6 days per week with approximately 50 mg benzoyl peroxide from a 50 percent suspension of benzoyl peroxide in flour paste. At study termination (80 weeks), 3 males and 11 females survived. No intergroup difference in tumor incidence was observed.

Additional groups of albino mice, 25 per sex (strain and age not specified), were fed a diet containing 2,800 mg/kg benzoyl peroxide and received simultaneous subcutaneous injections of 120 mg benzoyl peroxide in 20 percent starch solution for 120 weeks. At 104 weeks, 14 males and 10 females were surviving. The overall tumor incidence was similar in the control and benzoyl peroxide groups.

In another protocol, albino mice, 25 per sex (age and strain not specified), received a single subcutaneous injection of 50 mg of a 20 percent suspension of benzoyl peroxide in starch solution or starch solution alone. The mice were sacrificed at 80 weeks. Male/female survivors were 9/7 in the test group and 0/6 in the control group. There were no tumors found in any group.

In a similar protocol with albino rats, after a single subcutaneous injection of 120 mg benzoyl peroxide, animals were followed for 120 weeks and then sacrificed. Mortality was checked at 26, 52, 78, and 104 weeks. Survivors at 104 weeks included 10 males and 9 females in the test group and 16 males and 17 females in the control group. No intergroup differences were observed in tumor incidence.

Hueper (Ref. 8) performed a 24-month controlled study on Bethesda (National Institutes of Health) black rats (20 males and 15 females). Animals received a subcutaneous implantation of 50 mg benzoyl peroxide in a gelatin capsule at the nape of the neck. Controls (21 males and 14 females) received a silicone rubber implant without benzoyl peroxide. The rats were followed for 24 months. Tumors were found at the implantation site in 10 control animals but in none of the benzoyl-peroxide-treated animals. Tumors found at other sites in test and control rats were reported to be spontaneous and not dose-related.

A vehicle controlled study by Poirier, et al. (Ref. 9) included 20 male rats (age not specified) in each group. Intramuscular injections of 2.9 mg benzoyl peroxide in 0.2 milliliters (mL) trioctanoin were given into the right hind leg twice a week for 12 weeks. No mortality or tumors were found during the 14-month study.

Saffiotti and Shubik (Ref. 10) used 0.5 percent benzoyl peroxide in acetone applied dermally to 21 female Swiss mice (age not specified) twice a week for 80 weeks. A second group of mice received a similar dose of benzoyl peroxide for 3 weeks, and after 1 week the mice were treated with 5 percent croton oil (in mineral oil) twice a week for 67 weeks. No skin tumors were observed.

In a study by Van Duuren, et al. (Ref. 11) the backs of 30 male ICR/Ha mice (8 weeks old) were painted 3 times per week with approximately 1,000 mg benzoyl peroxide in 5 percent benzene. Controls consisted of 150 mice divided into 4 groups and treated with benzene alone. The median survival time for benzoyl-peroxide-treated animals was 292 days, and 262 to 412 days for the control groups. One test mouse developed a skin papilloma, while 11 skin neoplasma (including 1 carcinoma) were observed in control mice. No benzoyl-peroxide-related increase in skin tumors was observed.

Sharrat, et al. (Ref. 7) conducted a study in which albino mice, 25 per sex, received dermal application of a 50 percent benzoyl peroxide suspension in flour paste (approximately 50 mg benzoyl peroxide per application) on the back of the neck six times per week for 80 weeks. Controls were painted with flour paste only. No skin neoplasma were found and overall tumor incidence did not differ significantly among groups.

Slaga, et al. (Ref. 1) conducted a 52-week study using female SENCAR mice (7 to 9 weeks old). Thirty mice were used per dose of benzoyl peroxide. One group of animals received a single dermal painting of 10 nanomole 7, 12-methylbenz(a)anthracene in 0.2 mL acetone followed by topical application of 0, 1, 10, 20, or 40 mg benzoyl peroxide in acetone twice a week. A second

group of animals received only the various doses of benzoyl peroxide in acetone 2 times per week, while a third group received a single application of the 0 to 40 mg doses of benzoyl peroxide in acetone followed 1 week later by twice-weekly applications of 2 micrograms (μg) 12-O-tetradecanoylphorbol 13-acetate in acetone for 52 weeks.

In the first group, the incidence of papillomas at week 30 was as follows: Control (1/28), 1 mg benzoyl peroxide (9/29), 10 mg benzoyl peroxide (20/28), 20 mg benzoyl peroxide (21/27), 40 mg benzoyl peroxide (20/24). At the end of the study, the number of carcinomas was as follows: Control (0/28), 1 mg benzoyl peroxide (1/29), 10 mg benzoyl peroxide (6/28), 20 mg benzoyl peroxide (12/27), 40 mg benzoyl peroxide (10/24). In the second group studied, while no intergroup differences in papillomas or carcinomas were observed, the single application of benzoyl peroxide produced marked epidermal hyperplasia and a large number of dark basal keratinocytes. The group treated with only benzoyl peroxide showed no carcinomas or intergroup differences in the incidence of papillomas. It was inferred that benzoyl peroxide was not a complete carcinogen in SENCAR mice.

Klein-Szanto and Slaga (Ref. 12) gave female SENCAR mice (7 to 9 weeks old) a single topical application of 10, 20, or 40 mg of benzoyl peroxide in 0.2 mL acetone (16 to 20 mice per dose). Controls (12 mice) received only acetone. On days 1, 2, 4, 6, 8, and 10, groups of 2 to 4 mice were sacrificed. Skin sections were examined to count the number of darkly stained basal cells versus total number of basal cells. Beginning 48 hours after treatment, the mid- and high-level dosed animals demonstrated a marked incidence of epidermal hyperplasia characterized by acanthosis with hyperorthokeratinization. Within 2 to 4 days after application of benzoyl peroxide, the epidermis exhibited a 5-fold increase in dark cells. The agency considers these results as indicating a potent tumor-promoting ability of benzoyl peroxide.

Kurokawa, et al. (Ref. 2) conducted a 52-week study involving 15 to 20 female SENCAR mice (6 weeks old) that received a single application of 20 nanomole 7, 12-methylbenz(a)-anthracene in 0.2 mL acetone or acetone alone. One week later, the mice received an application of 10 percent benzoyl peroxide (20 mg), 12-O-tetradecanoylphorbol 13-acetate (2 μg), or acetone. These applications were continued for 51 weeks. Another group of animals received twice weekly applications of 10 percent benzoyl peroxide or acetone for 51 weeks. The frequency and distribution of neoplasms was as follows:

SKIN TUMOR PROMOTION TESTS IN FEMALE SENCAR MICE INITIATED WITH DMBA [1]

Group	Chemical	No. of effective mice	No. of mice with skin tumors at week				Maximum No. of skin tumors per mouse	No. of mice with squamous cell carcinoma (percent)	No. of mice with epidermal hyperplasia (percent)
			13	26	38	52			
2	Benzoyl peroxide	20	7	16	19	[2] 20	[2] 15.6	[2] [3] 18 (90)	[2] 20 (100)
7	TPA [4]	20	20	20	20	20	[2] 40.1	[2] [3] 20 (100)	[2] 20 (100)
8	Acetone	15	0	0	0	0	0	0	0

[1] 7, 12-methylbenz(a)anthracene.
[2] Significantly different from Group 8 ($p<0.01$).
[3] One lymph node metastasis.
[4] 12-O-tetradecanoylphorbol 13-acetate.

COMPETE SKIN CARCINOGENICITY TESTS IN FEMALE SENCAR MICE

Group	Chemical	No. of effective mice	No. of mice with skin tumors t week				Maximum No. of skin tumors per mouse	No. of mice with squamous cell carcinoma (percent)	No. of mice with epidermal hyperplasia (percent)
			13	26	38	51			
2	Benzoyl peroxide	20	0	2	6	[1] 8	2.0	5 (25)	6(30)
7	Acetone	15	0	0	0	0	0	0	0

[1] Significantly different from Group 7 ($p < 0.05$).

Irrespective of the treatment protocol, a relatively high incidence of adenocarcinomas of the mammary gland and adenomas of the lung and uterus were observed in all groups. No intergroup differences in mean survival time were noted.

Reiners, et al. (Ref. 13) treated the shaved backs of female C57BL/6 and SENCAR mice (7 to 8 weeks old, 30 to 40 per group) with acetone, benzo (a) pyrene, or 7, 12-methylbenz (a) anthracene dissolved in acetone. One week after these treatments, the animals received twice-weekly applications of 2 μg (SENCAR) or 4 μg (C57BL/6) 12-O-tetradecanoylphorbol 13-acetate or 20 mg benzoyl peroxide. A large number of the benzoyl-peroxide-treated C57BL/6 mice developed skin carcinomas. The number of carcinomas following benzoyl peroxide promotion was greater compared to 12-O-tetradecanoylphorbol 13-acetate-promotion. Benzoyl peroxide significantly reduced the latency period for appearance of first skin tumor compared to 12-O-tetradecanoylphorbol 13-acetate.

The C57BL/6 mice promoted with benzoyl peroxide almost exclusively developed caracinomas, while SENCAR mice predominantly developed papillomas. However, 50 percent of the SENCAR mice did develop carcinomas by week 48 of the study.

In a study by Odukoya and Shklar (Ref. 14), 66 young adult Syrian golden hamsters (Lakeview strain) of both sexes were treated as follows:

Group 1: (8 pr sex) The left buccal pouches were painted 3 times per week for 10 weeks with a 0.1 percent solution of 7, 12-methylbenz (a) anthracene in heavy mineral oil. Two animals per sex

were sacrificed at weeks 22, 23, 24, and 25.

Group 2: (8 per sex) After 10 weeks of 7, 12-mehtylbenz (a) anthracene painting, a 6-week treatment-free period followed. During weeks 17 to 22, the left buccal pouches were painted 3 times per week with 40 percent (20 mg) benzoyl peroxide in acetone. Animals were sacrificed as in Group 1.

Group 3: (8 per sex) This protocol was similar to Group 2, except instead of benzoyl peroxide, the animals were painted with acetone.

Group 4: (3 per sex) After a 16-week treatment-free period, animals were painted 3 times per week with benzoylperoxide for 6 weeks and sacrificed in equal numbers at weeks 22 and 23.

Group 5: (3 per sex) This protocol was the same as in Group 4, except the animals were painted with acetone.

Group 6: (3 per sex) Untreated controls, sacrificed in equal numbers at weeks 22 and 23.

At termination of the study, no tumors in buccal pouches were found in Groups 1, 3, 4, 5, and 6. In Group 2, where carcinogenesis was initiated with 7, 12-methylbenz(a)anthracene and promoted with benzoyl peroxide, animals rapidly developed cincinomas. The subthreshold of 7, 12-methylbenz(a)anthracene in itself was sufficient to result in carcinoma.

O'Connell, et al. (Ref. 15) induced skin tumors (papillomas) in female SENCAR (5 to 7 weeks old) mice using a single topical application of 10 nanomole 7, 12-methylbenz(a)anthracene as the initiator on the shaved backs of the animals. Two weeks after initiation, promotion was accomplished by twice weekly application of 1 μg 12-O-tetradecanoylphorbol 13-acetate. At study week 21, 21 papilloma-bearing mice were continued on the biweekly 12-O-tetradecanoylphorbol 13-acetate treatments, while 20 other mice received an application of 20 mg benzoyl peroxide twice a week. These treatments were continued until week 40. Prior to the benzoyl peroxide applications, the papilloma incidence was similar in both groups of mice designated for 12-O-tetradecanoylphorbol 13-acetate and benzoyl peroxide applications. However, at week 40, benzoyl-peroxide-treated mice compared to 12-O-tetradecanoylphorbol 13-acetate-control mice showed 54 and 325 percent higher incidences of carcinoma and cumulative carcinoma, respectively. Histopathologic examinations revealed that 44 percent of skin tunors in benzoyl-peroxide-treated mice were keratoacanthomas and that 59 percent of these showed y-glutamyltransferase foci. The agency considers the presence of these foci in the keratocanthomas as suggesting a possible role for these lesions as precursors of squamous cell carcinomas. Results indicate the benzoyl peroxide enhanced the progression of pre-existing papillomas.

Iverson (Ref. 16) conducted a 60-week study using 11 groups of hairless hr/hr Oslo mice (16 per sex per group; age and weight not specified). Six of the groups received a single application of 51.2 μg, 12-methylbenz(a)anthracene (in 100 microliters (μl) acetone) prior to one of the following treatments: No other treatment, the gel vehicle (without benzoyl peroxide) twice a week, 5 percent benzoyl peroxide in a gel twice a week, ultraviolet radiation twice a week, or 5 percent benzoyl peroxide in a gel before ultraviolet radiation twice a week. The other five groups received one of the following treatments: Gel vehicle followed by ultraviolet radiation twice a week, 5 percent benzoyl peroxide in a gel followed by ultraviolet radiation twice a week, ultraviolet radiation twice a week, 5 percent benzoyl peroxide in a gel twice a week, or gradually increased ultraviolet radiation followed by 5 percent benzoyl peroxide in a gel twice a week. Reportedly, no spontaneous skin tumors have been observed in this strain of mice. Two mice (sex not specified) that received 5 percent benzoyl peroxide in a gel alone twice a week developed squamous cell carcinoma near the tail root, far from the site of drug application. This incidence was reported to be a "random event." None of the mice in this group developed papillomas.

In a study by Rotstein, et al. (Ref. 17), female SENCAR mice (7 to 9 weeks old) received a single application of 10 nanomole 7, 12-methylbenz(a)anthracene. Two weeks later, the mice received applications of 2 μg 12-O-tetradecanoylphorbol 13-acetate twice a week for 16 weeks, followed by a 4-week treatment-free period. Groups of at least 30 papilloma-bearing mice received 20 μl acetone or 20 mg benzoyl peroxide (in acetone) twice a week for 12 weeks beginning week 21 of the study. One group received benzoyl peroxide for only 4 weeks, followed by acetone treatment for 8 weeks.

Twelve weeks after the benzoyl peroxide treatment, all benzoyl-peroxide-treated mice showed a significantly greater incidence of carcinoma than controls (37 versus 16 percent). All carcinomas arose from pre-existing papillomas. Animals treated for 4 weeks with benzoyl peroxide showed a similar incidence of carcinoma as the 12-week treated animals. The agency believes that this result infers that free-radical generating promoters can enhance tumor progression within a short period.

A study conducted by the National Toxicology Program (Ref. 18) involved comparing the sensitivity of SENCAR, Swiss CD–1, and B6C3F1 strains of mice in a dermal initiation-promotion protocol using different combinations of initiators and promoters (i.e., 7, 12-methylbenz(a)anthracene, benzoyl peroxide, and N-methyl-N-nitro-N-nitrosoguanidine). After a single dose of 7, 12-methylbenz(a)anthracene (0.25, 2.5, or 25.0 μg) or N-methyl-N-nitro-N-nitrosoguanidine (100, 500, or 1,000 μg), groups of 30 male/female of each strain of mice received topical applications of 20 mg benzoyl peroxide in acetone, once a week for 52 weeks. Animals for complete carcinogen testing received 20 mg benzoyl peroxide throughout the study. Controls received two dose levels of initiators once and only acetone thereafter, and the vehicle control received only applications of acetone.

The gross incidence of papilloma was more prevalent in 7, 12-methylbenz(a)anthracene-initiated/12-O-tetradecanoylphorbol 13-acetate-promoted SENCAR and Swiss mice; however, all strains were equally sensitive to carcinoma induction. The 7, 12-methylbenz(a)anthracene-initiated/benzoyl-peroxide-promoted SENCAR mice were comparatively much more sensitive to papilloma induction. Gross incidence of carcinoma was observed only in SENCAR mice. The mean time to papilloma induction in 7, 12-methylbenz(a)anthracene/12-O-tetradecanoylphorbol 13-acetate groups was shorter in SENCAR and Swiss strains. In the 7, 12-methylbenz(a)anthracene-benzoyl peroxide groups, the induction time was much shorter in SENCAR mice. In 7, 12-methylbenz(a)anthracene/12-O-tetradecanoylphorbol 13-acetate groups, papillomas appeared in both sexes of SENCAR and Swiss mice by 10 weeks, and by 20 in B6C3F1 male mice. In 7, 12-methylbenz(a)anthracene/benzoyl peroxide groups, papillomas appeared in SENCAR mice at week 20, and at week 30 in both sexes of the 2 other strains.

A majority of mice in the 7, 12-methylbenz(a)anthracene/7, 12-methylbenz(a)anthracene groups developed papillomas. Neoplasm multiplicity was comparable in the 3 strains. The induction of papilloma in 12-O-tetradecanoylphorbol 13-acetate/12-O-tetradecanoylphorbol 13-acetate groups was observed in both sexes of

Swiss mice only. Regarding tumor induction, no one strain responded to benzoyl peroxide/benzoyl peroxide, 7, 12-methylbenz(a)anthracene/acetone combinations, or repeated application of acetone.

In N-methyl-N-nitro-N-nitrosoguanidine-initiated/12-O-tetradecanoylphorbol 13-acetate-promoted groups, SENCAR and Swiss strains were more sensitive to papilloma incidence and multiplicity of tumors. However, on gross examination, all strains were found to be equally sensitive to carcinoma incidence. The sensitivity in the N-methyl-N-nitro-N-nitrosoguanidine/benzoyl peroxide groups, when compared for gross incidence of papilloma, decreased in this order: SENCAR, Swiss, B6C3F1. Carcinoma incidence was similar in females, while in males sensitivity decreased in this order: SENCAR, Swiss, B6C3F1. The papillomas response time in the N-methyl-N-nitro-N-nitroso-guanidine/12-O-tetradecanoylphorbol 13-acetate groups decreased in the same order. In N-methyl-N-nitro-N-nitroso-guanidine/benzoyl peroxide groups, SENCAR and Swiss mice showed similar papilloma-response time. All strains were positive for papilloma and carcinoma induction in 100 μg N-methyl-N-nitro-N-nitrosoguanidine-initiated-100 mg N-methyl-N-nitro-N-nitrosoguanidine-promoted groups. SENCAR mice were found to be much more sensitive with benzoyl peroxide promotion and with 7, 12-methylbenz(a)anthracene or N-methyl-N-nitro-N-nitrosoguanidine initiation. On the whole, the SENCAR strain proved to be the most sensitive in two-stage tumorigenesis.

Iverson (Ref. 19) look at equal numbers of male and female SENCAR and hr/hr Oslo mice in a 52-week study. Where applicable, a single 51.2 μg dose of 7, 12-methylbenz(a)anthracene was used as an initiator. Skin tumors were subjected to histopathologic examination. The following groups were studies:

Group A: (32 hr/hr) 5 percent benzoyl peroxide in a gel vehicle twice per week in the evening of 1 day, followed by ultraviolet exposure the next morning.

Group B: (32 hr/hr) twice per week ultraviolet radiation.

Group C: (32 hr/hr) Gel vehicle twice per week in the afternoon of one day, ultraviolet exposure next morning.

Group D: (32 hr/hr) twice per week ultraviolet exposure followed 5 minutes later by 5 percent benzoyl peroxide in a gel vehicle.

Group E: (32 hr/hr) twice per week ultraviolet exposure followed 5 minutes later by gel vehicle.

Group F: (32 hr/hr) Single dose of 7, 12-methylbenz (a)-anthracene; starting 1 week later, twice per week application of gel vehicle.

Group G: (32 Sencar) Single application of 100 μl acetone.

Group H: (32 Sencar) Gel vehicle twice per week.

Group I: (32 Sencar) 5 percent benzoyl peroxide in a gel vehicle twice per week.

Group J: (32 Sencar) 7, 12-methylbenz (a) anthracene, followed by continuous treatment with 5 percent benzoyl peroxide in a gel vehicle twice per week.

Group K: (32 Sencar) 7, 12-methylbenz (a) anthracene, followed by continuous treatment with gel vehicle twice per week.

Group L: (48 Sencar) One application of 7, 12-methylbenz (a) anthracene.

Group M: (176 Sencar) One application of 7, 12-methylbenz (a) anthracene (historical control group).

Group N: (32 hr/hr) 7, 12-methylbenz (a) anthracene and gel vehicle.

There were no significant intergroup differences in survival rate (73 to 91 percent) observed in the SENCAR mice; however, in the hr/hr Oslo mice survival rate was very low (19 to 41 percent) due to radiation effects. Group B (ultraviolet radiation twice a week in hr/hr Oslo mice) had the highest number of tumor-bearing mice and total number of carcinomas, indicating that neither the gel vehicle nor 5 percent benzoyl peroxide promoted tumorigenesis. The 7, 12-methylbenz (a) anthracene treatment produced more tumors in SENCAR mice with 3 low-grade fibrosarcomas in Group G and squamous cell carcinomas as follows: four in Group L (the highest number observed), two in Group J, and one each in Group H and I.

Schweizer, et al. (Ref. 20) conducted a 16-month study using 12-week old, pathogen-free Syrian golden hamsters (weighing about 100 grams (g)). The hamsters were randomly assigned to 1 of 5 test groups, each containing 20 animals. All animals received the following application and were examined for skin lesions.

Group I: (Control) Application of 1 mL acetone 3 times per week on the shaved dorsal area.

Group II: Initiation with a single dose of 10 mg/kg 7, 12-methylbenz (a) anthracene.

Group III: Topical application, 3 times per week, with 160 mg benzoyl peroxide in 1 mL acetone.

Group IV: Initiation with 7, 12-methylbenz (a) anthracene (Group II) and promotion with 80 mg benzoyl peroxide 3 times per week.

Group V: Repetitive applications of benzoyl peroxide after 7, 12-methylbenz (a) anthracene initiation.

Benzoyl peroxide alone increased generalized hyperpigmentation and scaling, but no tumors were observed. The 7, 12-methylbenz (a) anthracene alone induced a moderate number of melanotic foci and a small number of palpable melanotic tumors, both in the dermis. Papillomas were found in the epithelia of the tongue, esophagus, and forestomach. The 7, 12-methylbenz (a) anthracene and benzoyl peroxide at both dose levels drastically increased the number of melanotic foci and the incidence of tumors at later stages, implying that benzoyl peroxide promoted the incidence of papilloma, carcinoma, and melanotic tumors.

Hergenhahn (Ref. 21) conducted a study in which NMRI mice (age and sex not specified) received a single dose of 7, 12-methylbenz (a) anthracene followed by dermal applications of 40 mg benzoyl peroxide (in acetone) twice a week for 24 weeks. This treatment was followed by application of a second promoter, retinoyl phorbol acetate, for another 24 weeks. In the second experiment, mice received 20 mg benzoyl peroxide (in acetone) twice a week for 16 weeks. All animals in both groups were observed for 48 weeks. No results were provided except for a comment that benzoyl peroxide did not induce skin tumors in any group.

B. Promotional Studies

Slaga, et al. (Ref. 1) assessed the intercellular communication between Chinese hamster V79 (6-thioguanine-sensitive) cells measured by evaluating the metabolic cooperation between hypoxanthine-guanine phosphoribosyl transferase positive and hypoxanthine-guanine phosphoribosyl transferase negative cells. Inhibition of metabolic cooperation in these cells is a property of many structurally diverse tumor promoters. Benzoyl peroxide inhibited the intercellular communication between the cells.

Yuspa, et al. (Ref. 22) used benzoyl peroxide in 10 to 20 mg concentration incubated with epidermal cells prepared from newborn BALB/c mice. The induction of epidermal transglutaminase has been used as an indication for terminal differentiation in cultured epidermal cells. The phorbol esters are potent inducers of transglutaminase in vivo and in vitro. The high concentration of benzoyl peroxide used did not induce transglutaminase but was significantly cytotoxic to the cells.

In a study by Lawrence, et al. (Ref. 23) human epidermal keratinocytes (strain R), derived from a young donor's skin, were used to assess the effect of benzoyl peroxide on the cellular

metabolic cooperation compared to acetone-treated control cells. Benzoyl peroxide at 0.5 μg/mL exhibited a small but significant effect, while doses between 1.0 and 3.6 μg/mL strongly inhibited metabolic cooperation. At the 3.6 μg/mL dose of benzoyl peroxide, the extent of nucleotide transfer compared to controls was only 31 percent. However, benzoyl peroxide showed no effect on cell survival, attachment, or keratinocyte morphology.

Armato, et al. (Ref. 24) tested on the activity of many tumor promoters by using primary liver cultures (with 40 to 50 percent hepatocytes) prepared from male and female Wistar rats (4 days old). Benzoyl peroxide was tested at 10^{-10} moles/liter (mol/L) dose. At this dose, benzoyl peroxide significantly stimulated the hepatocellular 24-hour deoxyribonucleic acid synthesis. The simulatory activity was inhibited by simultaneous addition of exogenous superoxide dismutase.

Fey and Sheldon (Ref. 25) incubated promoters with the Madin-Darby Canine Kidney cell line, which when injected into nude mice form highly differentiated epithelial colonies that are nontumorigenic. The epitheloid nature of these cuboidal cell colonies is altered on incubation with subnanomolar levels of promoters (i.e., individual cells become mobile, flattened, and elongated). Five structurally dissimilar complete or second-stage tumor promoters, including benzoyl peroxide, were shown to induce identical morphological changes (signatures) after 2 hours incubation with the Madin-Darby Canine Kidney cells.

Mass, et al. (Ref. 26) used tracheal cell cultures prepared from young pathogen-free male Fisher 344 rats to examine their proliferative response to a spectrum of known promoters. It has been observed that tumor promoters can induce terminal differentiation in a variety of cell types. This preneoplastic phenotype is characterized by the capacity of cells to grow in semi-solid medium, i.e., colony forming efficiency. It has also been reported that initiated mouse skin contains keratinocytes resistant to induction of terminal differentiation. Benzoyl peroxide neither stimulated nor diminished colony forming efficiency over the wide concentration range tested. It was inferred that benzoyl peroxide does not bind to the phorbol receptor and thus probably acts as a skin tumor promoter by a different mechanism than 12-O-tetradecanoyl-phorbol 13-acetate.

Gindhart, et al. (Ref. 27) used JB6 mouse epidermal cells (which are initiated and sensitive to further transformation by tumor promoters) to test the tumor promoting activity of benzoyl peroxide. In a dose-dependent fashion (10^{-9} to 10^{-5} Molar), benzoyl peroxide promoted further transformation of JB6 cells and caused a decrease in the net synthesis of the major ganglioside of epidermal cells, trisialoganglioside GT.

C. Reproductive and Developmental Toxicology Studies

Korhonen, et al. (Ref. 28) used various doses (0.05 to 1.7 micromole) of benzoyl peroxide in acetone injected into the inner shell membrane in the air chamber of 3-day old white Leghorn chicken eggs. Except for the lowest dose level tested, there was a dose-related increase in early embryonic deaths, with an estimated LD_{50} of 0.99 micromole per egg. At all dose levels, benzoyl peroxide increased malformation at a moderate frequency. The calculated median effective dose for mortality and malformations was 0.27 micromole per egg.

D. Genotoxicity Studies

Epstein, et al. (Ref. 29) used benzoyl peroxide given intraperitoneally at 52 and 64 mg/kg to 7 and 9 male ICR/Ha Swiss mice, respectively, in a dominant lethal assay evaluation. Each male was caged with three untreated virgin female mice for 1 week. Dams were replaced every week for 8 weeks, sacrificed, and examined for total implants, and early and late fetal deaths. Late fetal deaths were rare, while early fetal deaths and pre-implantation losses were within the control limits.

Litton Bionetics (Ref. 30) conducted a modified Ames assay using *Salmonella* strains TA 1535, 1537, and 1538, and *Saccharomyces cerevisiae* strain D-4 for a gene conversion assay. Benzoyl peroxide was evaluated at two concentration levels, one of which was half of the medium lethal concentration (1.8 mg/mL) value. All bacterial assays were performed with S-9 metabolic activation systems prepared from the lung, liver, and testes of mice, rats, and monkeys. Benzoyl peroxide was found to be nonmutagenic. The yeast assay performed in the presence/absence of S-9 fractions also gave negative results.

In another study, Yamaguchi and Yamashita (Ref. 31) conducted a slightly modified Ames assay using *Salmonella* strains TA 98 and 100. Benzoyl peroxide was tested in Tween 20T at a high dose of 300 μg per plate in the presence of a rat S-9 metabolic activation system. It was found to be nonmutagenic.

DeFlora, et al. (Ref. 32) evaluated benzoyl peroxide in the Ames test using *Salmonella typhimurium* (*S. typhimurium*) strains TA 1535, 1537, 1538, 98, and 100, and several isogenic strains of *Escherichia coli* (*E. coli*) (WP-2 wild type (repair-proficient), WP67-UVrA-, Pol A-, CM871-UVrA-, recA-, and lexA-). The names of the solvent used and the highest concentration tested were not given. Benzoyl peroxide was nonmutagenic but caused deoxyribonucleic acid damage in *E. coli* and was more toxic to deoxyribonucleic acid repair-deficient than to repair-proficient strains. It was lethal to WP-2 and WP 67 at 1,000 μg/mL and to CM 871 at 250 μg/mL, in both the absence and presence of S-9 fraction. In the presence of S-9 fraction, benzoyl peroxide was lethal to WP-2 (750 μg/mL) and to both repair-deficient strains (500 μg/mL).

Ishidate, et al. (Ref. 33) evaluated benzoyl peroxide in dimethyl sulfoxide (5 mg per plate) in *Salmonella* strains TA 1535, 1537, 92, 94, 98, and 100 in the presence and absence of S-9 fraction. In a second set of experiments, benzoyl peroxide in dimethyl sulfoxide (0.2 mg/mL) was tested for chromosomal aberrations in Chinese hamster fibroblasts. All assays gave negative results.

Jarventaus, et al. (Ref. 34) used Chinese hamster ovary cells to evaluate the effect of benzoyl peroxide on the incidence of sister chromatid exchange. Benzoyl peroxide induced a dose-dependent increase in the incidence of sister chromatid exchange only in the presence of S-9 fraction. At 1.0 millimole benzoyl peroxide concentration, sister chromatid exchange doubled.

Tainer (Ref. 35) reported benzoyl peroxide (in acetone or dimethyl sulfoxide) to be nonmutagenic, with or without hamster and rat liver S-9 fraction in *S. typhimurium* strain TA 1535, 97, 98, and 100.

Matula, et al. (Ref. 36) reported benzoyl peroxide (in acetone) was nonmutagenic with or without S-9 activating system in *S. typhimurium* strain TA 1535, 98, 100, and 102; however, it produced a dose-dependent increase in mutation with strain TA 97 in the absence of metabolic activation. Reportedly, these results were erratic due to high cell toxicity (dependent on the volume of acetone per plate). However, results were reconfirmed in a liposome vehicle with a commerical preparation in strain TA 97. Benzoyl peroxide (in acetone and a commerical lotion) also damaged deoxyribonucleic acid in the *E. coli* SOS test; however, a dose-dependent relationship was not observed. Weak mutagenic activity of

benzoyl peroxide was inferred from these results.

Swierenga (Ref. 37) investigated the cytotoxicity and genotoxicity of benzoyl peroxide for epithelial cells by using proliferating T-51-B cells or rat hepatocytes. Benzoyl peroxide was extremely toxic to these cells. Depending on cell density, exposure duration, and media composition, the median lethal concentration of benzoyl peroxide varied from 5 to 50 μg/mL. Deoxyribonucleic acid strand breaks, but no mutations, were observed at these concentrations. Hepatocytes tolerated up to 300 micromole benzoyl peroxide over a 24-hour period. Latent random cell death was observed in all cultures. When the assay conditions were adjusted to enhance cell survival, both strand breaks and mutation were observed. In addition, benzoyl peroxide showed some ability to induce deoxyribonucleic acid repair in hepatocytes and sister chromatid exchange in V79 cells. It was inferred that benzoyl peroxide showed weak genotoxicity at concentrations 10^4 fold lower than present in the commercial preparations.

Birnboim (Ref. 38) studied a spectrum of phorbol and nonphorbol promoters incubated with white blood cells isolated from human blood. Cells were examined for deoxyribonucleic acid strand breaks. Benzoyl peroxide induced a dose-dependent break in deoxyribonucleic acid strands.

Gensler and Bowden (Ref. 39) evaluated initiated epidermal JB6 cells for clastogenic events after a single treatment with a noncytotoxic dose (50 micromole) of benzoyl peroxide. Deoxyribonucleic acid single-strand scissions did not occur, suggesting a dissociation between the induction of deoxyribonucleic acid strand breaks and late-stage promotion.

Saladino, et al. (Ref. 40) assessed the effect of benzoyl peroxide and other drugs on clonal growth rate, squamous differentiation, deoxyribonucleic acid damage, ornithine decarboxylase activity, nucleic acid synthesis, aryl hydrocarbon hydroxide activity, and arachidonic acid and choline release measured in normal human bronchial epithelial cells. Benzoyl peroxide increased the promotion of cross-linked envelopes and depressed ribonucleic acid synthesis more than deoxyribonucleic acid synthesis. In addition, it produced detectable amounts of both single-strand breaks and deoxyribonucleic acid-protein cross links, and inhibited growth.

Hartley, et al. (Ref. 41) investigated the degree of deoxyribonucleic acid strand break in cultured keratinocytes (BALB/c mice) and the cell lines D, F, and 308 (derived from primary mouse epidermal cultures by carcinogen treatment) after exposure to phorbol esters and benzoyl peroxide. Benzoyl peroxide at 10^{-4} Molar concentration induced single strand breaks in basal keratinocytes within 1 hour, and attached cells exhibited extensive single strand breaks by 12 hours. It was inferred that benzoyl peroxide-produced breaks were due to a direct mechanism of deoxyribonucleic acid damage.

Birnboim (Ref. 42) reported that deoxyribonucleic acid strand breaks produced in human leukocytes by benzoyl peroxide (50 micromole), anthralin and 12-O-tetradecanoylphorbol 13-acetate were not repaired during a 30-minute period following treatment. However, under the same assay conditions, substantial repair of ionizing radiation-induced breaks was observed.

An abstract by Swierenga (Ref. 37) inferred that in vitro benzoyl peroxide induced deoxyribonucleic acid strand breaks in rat hepatocytes.

E. Biochemistry Studies

Molloy, et al. (Ref. 43) conducted a study in which the dorsal skins of female CD-1 (7 to 10 weeks old) mice were painted with benzoyl peroxide (in acetone) or acetone alone. Skins were excised, cultured, and pulse-labeled with ^{35}S-methionine 24 hours after treatment. Qualitative changes in synthesized epidermal proteins were examined using one- and two-dimensional gel electrophoresis. Benzoyl peroxide-treated epidermal proteins resembled those of controls compared to 12-O-tetradecanoyl-phorbol 13-acetate- and anthralin-treated skins. It was inferred that benzoyl peroxide may act by a mechanism distinct from the other two promoters.

Binder and Volpenhein (Ref. 44) investigated the induction of ornithine decarboxylase activity by 12-O-tetradecanoyl-phorbol 13-acetate and benzoyl peroxide in female SENCAR mice. The 12-O-tetradecanoylphorbol 13-acetate (2 μg) caused induction of ornithine decarboxylase activity 30 times greater than benzoyl peroxide (20 mg, in acetone) applied once to dorsal skin. The activity level was 10 percent greater after 3 or more doses of benzoyl peroxide (20 mg) applied 2 to 7 days apart. However, the additive effect of doses was reported as not responsible for the enhanced induction because ornithine decarboxylase activity was at the basal level at the time of the last dose. Benzoyl peroxide applied once a day for 5 consecutive days resulted in only one-tenth the enzyme activity by the same number of doses given 2 or more days apart. Pretreatment with benzoyl peroxide (20 mg) once a day for 4 days greatly enhanced the ornithine decarboxylase activity by a one-time application of 12-O-tetradecanoylphorbol 13-acetate (2 μg) 24 hours after the last benzoyl peroxide dose. It was inferred that while the 2 promoters operate through different mechanisms, their promotional effects are synergistic.

Kensler, et al. (Ref. 45) used skin-trapping and electron skin resonance techniques to characterize free-radical metabolites of benzoyl peroxide in target keratinocytes isolated from neonatal SENCAR mice. Cell incubation with benzoyl peroxide gave an electron skin resonance spectrum characteristic of alkyl radical adducts. No detectable electron skin resonance spectrum were observed in heat denatured cells or in the absence of benzoyl peroxide. It was inferred that the peroxide bond undergoes cleavage to yield benzoyloxyl radicals, which then break to form a phenyl radical (skin trapped species).

In another experiment, liposome-containing ^{14}C ring-labeled benzoyl peroxide was incubated with keratinocytes for 1 hour, and covalent binding to macromolecules was determined. Substantial covalent binding of radioactivity with proteins, but not deoxyribonucleic acid, was detected. The assumed limit of detection was in the range of 1.5 picomole/mg deoxyribonucleic acid. The results were consistent with the reported nil/low mutagenic, initiating and complete carcinogenic activity of benzoyl peroxide.

F. Absorption, Distribution, and Excretion Studies

Nacht, et al. (Ref. 46) assessed absorption and biodisposition of ^{14}C-benzoyl peroxide both in vitro (excised human skin) and in vivo (rhesus monkeys). In vitro, benzoyl peroxide penetrated through the stratum corneum, follicular openings, or both, and was recovered on the dermal side of the skin as benzoic acid. In vivo, following topical and intramuscular administration of ^{14}C-benzoyl peroxide, 45 and 98 percent, respectively, of the radioactivity was found in the benzoic acid in urine. Benzoyl peroxide penetrated into skin layers, was metabolized to benzoic acid, and then absorbed into the systemic circulation. No hippuric acid was found in monkey urine, implying that renal clearance of benzoyl peroxide metabolites was sufficiently rapid which precluded its hepatic conjugation with glycine.

Morsches and Holzmann (Ref. 47) used in vitro and in vivo methods to assess percutaneous penetration and metabolism of benzoyl peroxide in human skin and five patients with leg ulcers. Benzoyl peroxide absorbed in vitro was metabolized (preferably in the dermis) to benzoic acid. The portion which penetrated the intact skin was benzoic acid only.

In a study by Wepierre, et al. (Ref. 48), hairless Spraque Dawley male rats received topical application of ^{14}C-benzoyl peroxide 10 percent gel. Distribution and dissociation were studied at 3, 8, and 24 hours. Most of the applied dose was retained in the horny layer, where metabolic conversion to benzoic acid was low. In the dermis, conversion to benzoic acid increased sharply, and the metabolite was taken up by the systemic circulation.

G. Epidemiological Studies

Sakabe and Fukuda (Ref. 49) reported two cases of lung cancer in industrial workers in a small plant in Japan where benzoyl peroxide and benzoyl chloride were produced. The number of workers in the factory varied from 13 in 1952 to 40 in 1963. The first worker was a 40-year-old male smoker with 17 years of service in the manufacture of benzoyl peroxide and intermittent exposure to benzoyl chloride; the second was a 35-year-old male nonsmoker with squamous-cell carcinoma, who had had about 4 years of exposure to benzoyl peroxide production starting about 15 years prior to detection, and had worked for 1 year in benzoyl chloride production. Both workers would also have been exposed to a number of precursors in the production process, including benzotrichloride.

Wright, et al. (Ref. 50) reviewed 43 cases of cancer, grouped by occupation for unusual risk for melanoma. Eleven subjects had melanomas and 32 had other cancers. Cases included all white male chemists with cutaneous malignant melanoma diagnosed between 1972 and 1979. The control group included all white male chemists with other cancers diagnosed during the same period. Patients with melanoma were supposed to have been exposed to more individual chemicals than controls and reported more work with solvents, pesticides, plastics, ionizing radiation, and benzoyl peroxide. There was little difference between cases and controls for other chemical exposures.

Hogan (Ref. 51) reviewed 870 subjects and 1,250 age, sex- and location-matched controls for skin cancer. Analysis was performed for cases of basal cell carcinoma, squamous cell carcinoma of the lip, and cutaneous malignant melanoma. A family history of skin cancer and exposure to agrochemicals were the most significant risk factors analyzed for skin cancer. Other factors included prominent freckles during childhood, history of severe sunburn, light skin color, and skin types 1 and 2. The strongest risk factor for basal cell carcinoma was a family history of skin cancer. The past history of acne was the second strongest correlate with subsequent development of basal cell carcinoma in over 600 patients. However, the study failed to trace the treatment of patients to determine if they had applied benzoyl peroxide.

Elwood, et al. (Ref. 52) analyzed case histories of 651 patients with cutaneous malignant melanoma and matched controls. The distribution of pathologic lesions was as follows: 415 individuals exhibited superficial spreading melanoma, 128 nodular melanoma, 52 unclassified or borderline melanoma, and 56 lentigo maligna melanoma. Subjects more frequently used soaps and sulfur or resorcinol compounds; benzoyl peroxide preparations were used by very few subjects, and there were no differences in the types of drugs used by cases and controls. It was inferred that reported frequencies of acne and psoriasis were not related to any substantial increase or decrease in melanoma formation. Because benzoyl peroxide preparations were not used very extensively in these patients, no definite correlation was visible between benzoyl peroxide and melanoma risk.

Cartwright, et al. (Ref. 53) investigated cases of malignant skin melanoma in subjects under the age of 45, reported between 1984 and 1986 inclusive, from hospital and general practitioners' patient records. Data were compared with subjects of the same age and sex without malignant disease. The analyzed risk factors included acne, any skin medication, and prolonged exposure to sunlight. Of 213 identified melanomas, 159 (75 percent) were investigated further. The incidences of clinical and physiological acne were 15 and 85 percent, respectively. Reportedly, the study had several limitations. The number of subjects was limited, and no direct contact was made to trace whether they had purchased benzoyl peroxide without prescription. Irrespective of these limitations, the study authors concluded that benzoyl peroxide posed no major risk of an association with malignant melanoma.

Ewing, et al. (Ref. 54) designed a study to determine whether Stage I promotion could occur prior to initiation and to examine its role in carcinoma development. SENCAR mice received two applications of various complete, first, and second stage promoters prior to being initiated with 7, 12-methylbenz(a)-anthracene (2μg). Two weeks later animals received twice-weekly applications of mezerein (2μg). Benzoyl peroxide (20 mg) given either 2, 5, or 10 weeks prior to initiation had no effect on the subsequent promoting activity of mezerein.

In a 62-week study, Epstein (Ref. 55) examined the effect of benzoyl peroxide on ultraviolet-radiation-initiated tumor formation. Uscd strain albino hairless mice (4 months old) received 270 millijoule/square centimeter of ultraviolet (280 to 320 nanometer) radiation 3 times a week for 8 weeks to the posterior halves of their backs. Four weeks later, mice were treated with one of the following: Croton oil (in acetone) 5 times per week for 50 weeks; acetone alone; benzoyl peroxide in an aqueous diluent 5 times per week for 50 weeks, or benzoyl peroxide diluent alone. Results demonstrated that croton oil promoted ultraviolet-initiated tumor formation, but benzoyl peroxide did not.

In another 62-week study, Epstein (Ref. 56) compared the effects of chronic applications of croton oil and benzoyl peroxide on epidermal deoxyribonucleic acid synthesis in ultraviolet-initiated skin. Uscd strain albino hairless mice (3 to 4 months old) were irradiated with 125 millijoule/square centimeter of ultraviolet (280 to 320 nanometer) radiation energy 3 times a week for 8 weeks. Four weeks later, animals were treated with one of the following: 0.1 mL croton oil solution 5 times per week; acetone alone; 5 percent benzoyl peroxide; or aqueous base solution. Treatment continued for 50 weeks. Results indicated that croton oil applications stimulated a deoxyribonucleic acid synthesis level that was significantly greater than all other groups, including the mice receiving benzoyl peroxide. The mechanism of the promoting effects or croton oil and benzoyl peroxide to be different.

Naito, et al. (Ref. 57) examined histogolical effects of multiple applications of 12-O-tetradecanoylphorbol 13-acetate (6.8 nanomole), teleocidin (6.8 nanomole), chrysarobin (220 nanomole), mezerein (6.8 nanomole), 4-O-Methyl-12-O-tetrade-canoylphorbol 13-acetate (150 μg), and benzoyl peroxide (20 mg) on the skin of DBA/2 and C57BL/6 mice. Benzoyl peroxide and 4-O-Methyl-12-O-tetradecanoylphorbol 13-acetate (given twice a week for 2 weeks) induced only a week sustained epidermal hyperplasia, dark basal keratinocyte

response, and labeling index of similar magnitude in both strains of mice. No other morphological changes were attributed to benzoyl peroxide treatment.

Hartley, et al. (Ref. 58) used alkaline elution to examine deoxyribonucleic acid single-strand breaks in cultured normal and carcinogen-altered mouse keratinocytes exposed to 12-O-tetradecanoylphorbol 13-acetate and benzoyl peroxide. Benzoyl peroxide induced extensive strand breaks in normal keratinocytes at both 6 and 24 hours, and was associated with marked cytoxicity. Nine of 10 cell lines showed complete or partial resistance to strand breaks following benzoyl peroxide exposure. The differential resistance to deoxyribonucleic acid strand breaks and cytotoxicity among normal and carcinogen altered cells suggest a biological basis for the promoting action of benzoyl peroxide.

In a study by Pelling, et al. (Ref. 59), papillomas were induced in 7, 12-methylbenz (a) anthracene-initiated SENCAR mouse epidermis by complete promotion with benzoyl peroxide or 12-O-tetradecanoylphorbal 13-acetate and two-state promotion with 12-O-tetradecanoylphorbol 13-acetate for 2 weeks followed by mezerein for 9 weeks. Results of Northern blot hybridization analyses showed that early papillomas in 7, 12-methylbenz(a)-anthracene-initiated epidermis contained elevated levels of Ha-ras specific polyadenylated transfer ribonucleic acid irrespective of the tumor promoter regimen used.

The agency's detailed comments and evaluations on the data are on file in the Dockets Management Branch (Refs. 60 and 61).

H. Tumor Promoters

The agency notes that a prominent feature of skin tumor promoters is that they all cause release of free oxygen radicals. These species stimulate cells to produce active forms of oxygen (Ref. 62). The agency believes that evidence that promotion involves free radicals is supported by the following observations: Free-radical generating compounds are promoters; 12-O-tetradecanoylphorbol 13-acetate-type promoters have been shown to stimulate formation of oxyradicals; promoters can modulate the anti-oxidant defense mechanisms; and antioxidants are antipromoters. Presumably, promotion of mouse skin transformation occurs in two stages, both of which involve active oxygen (Ref. 63). Free radicals, especially peroxyl radicals, may be involved in both the initiation and promotion stages of multistage carcinogenesis (Ref. 64). A second characteristic of skin tumor promoters is that they all induce epidermal hyperplasia, i.e., the appearance of dark basal cells in the epidermis (Ref. 1). The agency points out that these dark basal cells are normally present in large numbers in embryonic skin, papillomas, and carcinomas and are considered a reliable marker of stage I promotion (Refs. 63, 65, 66, and 67). Stage II promotion is accompanied by various biochemical changes, many of which are related to the stimulation of cell proliferation.

These include increased levels of polyamines, prostaglandins, and induction of some embryonic conditions; decreased activity of two detoxifying enzymes (i.e., superoxide dismutase and catalase); and increased activity of ornithine decarboxylase in the skin (Refs. 68 and 69). The agency notes that the induction of ornithine decarboxylase activity and increased levels of polyamines are considered necessary indicators for tumor promotion by phorbol esters. It is also noted that another enzyme activated by the tumor promoter 12-O-tetradecanoylphorbol 13-acetate is protein kinase C, which is generally regarded as being synonymous with the phorbol ester receptor (Refs. 70, 71, and 72).

I. Conclusions

A significant amount of research has been conducted on benzoyl peroxide since the Panel's deliberations were completed in 1980. Some of this research was conducted after the 1985 tentative final monograph for OTC topical acne drug products (50 FR 2172) was published. The agency has determined from its evaluation of the data that some of the studies contained procedural deficiencies including the following: Inadequate numbers of animals, low doses, inadequate data on animal survival, and lack of adequate controls. In addition body weight, age, strain, and sex of the animals were not provided for some studies and, in certain other studies, data for both sexes of the animals were pooled. The agency finds that, despite all the research conducted to date, a definitive study to assess the complete carcinogenicity of benzoyl peroxide has not, as yet, been conducted.

Benzoyl peroxide was initially shown to be a promoter in a two-stage, initiation-promotion skin carcinogenesis study in mice (Ref. 1). Because mouse skin is responsive to the two-stage system of tumor promotion, it has been widely used for initiation-promotion studies. In fact, the agency notes that all national and international regulatory agencies have accepted mice as a standard model for testing potential carcinogens. The agency's position is that because many of the known human tumor initiators, promoters, and carcinogens have initially been identified in rodents, positive results in this species would suggest the need for further investigation.

The agency considers the status of benzoyl peroxide as a free-radical-generating compound to be well established (Ref. 45). The agency believes that there is strong evidence to suggest that the free-radical generating ability of benzoyl peroxide is responsible for its promotional effects. These include an increase in dark basal keratinocytes and epidermal hyperplasia, increased terminal differentiation and ornithine decarboxylase levels, and inhibition of intracellular communication in mouse, hamster, and human cells (Refs. 1, 12, 23, 40, and 44). In addition, benzoyl peroxide activates protein kinase C (Ref. 73), and promotes chemically-initated transformation of mouse epidermal cells (Refs. 1, 11, 27, 74, 75, and 76).

The agency notes that most of the topical studies with benzoyl peroxide have been conducted in mice. While promotion was observed in almost all studies, carcinogenesis was observed in a select few that primarily used SENCAR (i.e., sensitive to carcinogens) mice, bred to have a unique sensitivity to cancer. Benzoyl peroxide has also promoted tumor development in C57BL/6 mice (Ref. 75) and demonstrated tumor-promoting activity in another species, the Syrian golden hamster (Ref. 14).

The agency contends that benzoyl peroxide not only shares most of the tumor-promoting features of 12-O-tetradecanoyl-phorbol 13-acetate, but also exhibits several properties of complete carcinogenesis not shared by 12-O-tetradecanoylphorbol 13-acetate. Included among these are resistance to inhibition by retinoic acid and induction of a high ratio of papillomas to carcinomas (Refs. 12, 75, and 77). In addition, benzoyl peroxide characteristically induced single-strand breaks in deoxyribonucleic acid, and it increased the rate of malignant progression of benign epidermal papillomas to squamous cell carcinomas (Refs. 15 and 39).

The agency considers benzoyl peroxide to have exhibited weak mutagenic activity in the Ames test (with adequate dissolution) (Ref. 36). It has been shown to produce single-strand deoxyribonucleic acid breaks in human bronchial epithelial and mouse

epidermal cells, deoxyribonucleic acid-protein cross-linking in human cells, neoplastic transformation in mouse epidermal cells, and sister chromatid exchange (Refs. 27, 34, 39, 40, and 41). Also, the agency notes that a single teratology study in white Leghorn chicken eggs indicated that benzoyl peroxide increased malformations at a moderate frequency and, except for the lowest dose level, there was a dose-related increase in embryonic deaths (Ref. 28).

The agency concludes that the evidence (as described above) is substantial to establish benzoyl peroxide as a potent skin tumor promoter in more than one strain of mice and other laboratory animals tested. In addition, it appears that benzoyl peroxide shares a spectrum of characteristic features with the true (complete carcinogen) initiators. The most critical of these features is that benzoyl peroxide increased the rate of malignant progression of benign epidermal papillomas to squamous cell carcinomas in mice. While the promotional activity of benzoyl peroxide appears to predominate over initiator activity, the agency believes that it is possible that benzoyl peroxide could have a longer latency period as an initiator. The agency finds that initiation and complete carcinogenicity have not been evaluated in adequate studies of sufficient duration.

To date, benzoyl peroxide has not been subjected to the normally expected long-term (18 to 24 months) carcinogenicity studies in rodents. The agency considers the short duration (about 52 weeks) of topical studies which have shown only "promotion" to be insufficient to rule out the possibility of "initiation." In a complete carcinogenicity test by Kurokawa, et al. (Ref. 2) using female SENCAR mice, treatment with benzoyl peroxide alone resulted in two mice with skin tumors at 6 months. There were six mice with skin tumors at 9 months and eight mice with skin tumors at 12 months, a three- and four-fold increase, respectively.

Because of the general observation that most chemically-induced tumors have not become apparent until 18 months, the agency has extended the duration of bioassay for potential carcinogens to 24 months. In addition, current agency criteria are that a carcinogenicity study must cover a major part of an animal's lifespan (i.e., 18 months in the mouse, and 24 months in the rat).

In view of these findings, the agency concludes that it is unable to state, at this time, that benzoyl peroxide is generally recognized as safe. The agency's position is that long-term topical studies (18 to 24 months) in two species (mouse and rat) need to be conducted to adequately address the issue of benzoyl peroxide's safety as an OTC topical acne ingredient. Accordingly, the agency is amending the tentative final monograph for OTC topical acne drug products to reclassify benzoyl peroxide from Category I to Category III.

On May 18, 1990, the agency received a submission (Ref. 78) from a drug manufacturers association in response to the agency's letter of February 1, 1990 (Ref. 60). The drug manufacturers association stated that it had carefully considered the agency's evaluations of the data and information regarding the safety of benzoyl peroxide, but it did not agree with all of the agency's interpretations of the data. The drug manufacturers association remains convinced that benzoyl peroxide fulfills monograph conditions (i.e., generally recognized as safe and effective). However, the association agreed (as previously stated in a letter to the agency dated January 10, 1990 (Ref. 79)) that an additional animal study would be appropriate to more fully confirm benzoyl peroxide's safety. In addition, the association included in its response new data (an abstract of a recently completed epidemiologic study (Ref. 78) on benzoyl peroxide). The association mentioned that the results from this study indicate that there is no statistically significant association between benzoyl peroxide use and the subsequent development of skin cancer in humans.

The agency met with industry representatives on June 28, 1990 (Ref. 61) to discuss its evaluation of the benzoyl peroxide data and the additional long-term studies that need to be conducted. This meeting did not change the agency's position that additional long-term studies in animals are needed before benzoyl peroxide can be declared generally recognized as safe as an OTC topical acne active ingredient, as stated above in this amended tentative final monograph.

J. Labeling

One comment addressed a warning that the agency had proposed in the tentative final monograph of January 15, 1985 for products containing benzoyl peroxide. That warning in proposed § 333.350(c)(2) (50 FR 2172 at 2181) read as follows:

Do not use this medication if you have very sensitive skin or if you are sensitive to benzoyl peroxide. This product may cause irritation, characterized by redness, burning, itching, peeling, or possibly swelling. More frequent use or higher concentrations may aggravate such irritation. Mild irritation may be reduced by using the product less frequently or in a lower concentration. If irritation becomes severe, discontinue use; if irritation still continues, consult a doctor. Keep away from eyes, lips, and mouth. This product may bleach hair or dyed fabrics.

One comment contended this proposed warning was overly lengthy and, thus, might discourage consumers from reading it. The comment added that the language used could be ambiguous and confusing to consumers. Therefore, the comment proposed an alternative warning, which it felt was more direct and more easily understood, as follows:

This product may cause irritation if you have very sensitive skin or are sensitive to benzoyl peroxide. Should your skin become red and you experience itching, burning, peeling or swelling, discontinue use. If these symptoms persist, consult a physician. Mild irritation may be reduced by using the product less frequently or in a lower concentration. Keep away from eyes, lips and mouth. This product may bleach hair or dyed fabrics.

The agency's and the comment's proposed warnings differ in several ways. The agency's warning alerts individuals who have very sensitive skin or who are sensitive to benzoyl peroxide not to use acne preparations containing this ingredient. The Panel noted that certain types of complexion are more sensitive to environmental factors as well as topical drugs and that people with an atopic background (an inherited tendency to develop allergy) may also be more easily irritated by certain topical preparations (47 FR 12430 at 12444). Benzoyl peroxide is known to produce a primary irritant dermatitis in certain people with sensitive skin. There is evidence that the higher the concentration of benzoyl peroxide, the greater the irritation. Therefore, the Panel believed people should be warned that if they have excessive irritation or allergic reaction to benzoyl peroxide, they should not use this ingredient (Ref. 80). The alternative warning recommended by the comment only indicates that individuals with one or the other of these sensitivities may experience irritation from use of products containing benzoyl peroxide. It does not state that these individuals should not use the product but only tells them to discontinue use if symptoms of irritation occur. The agency does not find this approach to be adequate because some individuals should not use the ingredient under any conditions. Therefore, the agency cannot agree with the comment's suggestion.

The agency considers its proposed warning as more clearly describing the characteristics of a potential irritant type skin reaction than the comment's proposed alternative. The agency's proposed warning emphasizes irritation as the main side effect that may occur and then describes the nature of that irritation, whereas the comment's proposed warning does not as clearly link the irritation that may occur with the descriptive symptoms. However, the agency agrees with the comment's argument regarding the ambiguity of some of the language (i.e., "more frequent use or higher concentrations may aggravate such irritation") included in its proposed warning. The agency believes that the sentence "mild irritation may be reduced by using the product less frequently or in a lower concentration," contained in both warnings, clearly conveys the agency's intended message, and that the sentence "more frequent use or higher concentrations may aggravate such irritation," in the agency's proposal, is duplicative and not needed. Accordingly, the proposed warning in § 333.350 for products containing benzoyl peroxide would be revised to state:

Do not use this medication if you have very sensitive skin or if you are sensitive to benzoyl peroxide. This product may cause irritation, characterized by redness, burning, itching, peeling, or possibly swelling. Mild irritation may be reduced by using the product less frequently or in a lower concentration. If irritation becomes severe, discontinue use; if irritation still continues, consult a doctor. Keep away from eyes, lips, and mouth. This product may bleach hair or dyed fabrics.

This revised warning will be added to the final monograph for OTC topical acne drug products if benzoyl peroxide is determined to be generally recognized as safe in the final rule pertaining to this ingredient, which will be published in a future issue of the **Federal Register**. Other general labeling issues for OTC topical acne drug products will be discussed in the final rule for these products, which will be published in a future issue of the **Federal Register**. That final rule will represent final agency action on all conditions in this rulemaking except for the ingredient benzoyl peroxide.

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II. Summary of the Agency's Changes to the Proposed Rule

A. Ingredient Changes

1. The agency is reclassifying the ingredient benzoyl peroxide from Category I to Category III. Based on new data and information, the agency has determined that additional information is needed to adequately assess the tumor initiation potential of benzoyl peroxide. To date, topical studies have been of short duration (about 52 weeks).

The agency has determined that studies of 18 to 24 months duration in two species of animals (mouse and rat) are needed to definitively address the safety status of benzoyl peroxide for the topical treatment of acne. (See section I. paragraph I. above.)

2. The agency is removing benzoyl peroxide from the proposed list of Category I active ingredients in § 333.310(a) and redesignating paragraphs (b) through (f) as paragraphs (a) through (e) in § 333.310 of this amended tentative final monograph.

3. The agency is revising the § 333.310 cross-references that appear in § 333.320 to reflect the redesignations that have occurred in § 333.310.

B. Labeling Changes

1. The agency is deleting the warning proposed in § 333.350(c)(2) of the previous tentative final monograph. This warning was proposed specifically for products containing benzoyl peroxide. Should benzoyl peroxide be included in the final monograph, the agency will slightly modify the previously proposed warning. (See section I. paragraph J. above.)

2. The warnings in § 333.350 (c)(3) and (c)(4) are redesignated (c)(2) and (c)(3), respectively.

The agency has examined the economic consequences of this proposed rulemaking in conjunction with other rules resulting from the OTC drug review. In a notice published in the Federal Register of February 8, 1983 (48 FR 5806), the agency announced the availability of an assessment of these economic impacts. The assessment determined that the combined impacts of all the rules resulting from the OTC drug review do not constitute a major rule according to the criteria established by Executive Order 12291. The agency therefore concludes that not one of these rules, including this amendment of the tentative final monograph for OTC topical acne drug products, is a major rule.

In the economic assessment, the agency also concluded that the overall OTC drug review was not likely to have a significant economic impact on a substantial number of small entities as defined in the Regulatory Flexibility Act (Pub. L. 96-354). That assessment included a discretionary regulatory flexibility analysis in the event that an individual rule might impose an unusual or disproportionate impact on small entities. However, this particular rulemaking for OTC topical acne drug products is not expected to pose such an impact on small businesses. Therefore, the agency certifies that this proposed rule, if implemented, will not have a significant economic impact on a substantial number of small entities.

The agency invites public comment regarding any substantial or significant economic impact that this rulemaking would have on OTC topical acne drug products. Types of impact may include, but are not limited to, costs associated with product testing, relabeling, repackaging, or reformulating. Comments regarding the impact of this rulemaking on OTC topical acne drug products should be accompanied by appropriate documentation. A period of 60 days from the date of publication of this proposed rulemaking in the Federal Register will be provided for comments on this subject to be developed and submitted. The agency will evaluate any comments and supporting data that are received and will reassess the economic impact of this rulemaking in the preamble to the final rule on benzoyl peroxide in OTC topical acne drug products.

The agency has determined under 21 CFR 25.24(c)(6) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

Interested persons may, on or before October 7, 1991, submit to the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857, written comments, objections, or requests for oral hearing before the Commissioner on the proposed regulation. A request for an oral hearing must specify points to be covered and time requested. Written comments on the agency's economic impact determination may be submitted on or before October 7, 1991. Three copies of all comments, objections, and requests are to be submitted, except that individuals may submit one copy. Comments, objections, and requests are to be identified with the docket number found in brackets in the heading of this document and may be accompanied by a supporting memorandum or brief. Comments, objections, and requests may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday. Any scheduled oral hearing will be announced in the Federal Register.

Interested persons, on or before August 7, 1992, may also submit in writing new data demonstrating the safety of those conditions not classified in Category I. Written comments on the new data may be submitted on or before October 7, 1992. These dates are consistent with the time periods specified in the agency's final rule revising the procedural regulations for reviewing and classifying OTC drugs, published in the Federal Register of September 29, 1981 (46 FR 47730). Three copies of all data and comments on the data are to be submitted, except that individuals may submit one copy, and all data and comments are to be identified with the docket number found in brackets in the heading of this document. Data and comments should be addressed to the Dockets Management Branch (HFA-305) (address above). Received data and comments may also be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

In establishing a final monograph, the agency will ordinarily consider only data submitted prior to the closing of the administrative record on October 7, 1992. Data submitted after the closing of the administrative record will be reviewed by the agency only after a final monograph is published in the Federal Register, unless the Commissioner finds good cause has been shown that warrants earlier consideration.

List of Subjects in 21 CFR Part 333

Labeling, Over-the-counter drugs, Topical acne drug products.

Therefore, under the Federal Food, Drug, and Cosmetic Act, it is proposed that part 333 of subchapter D of chapter I of title 21 of the Code of Federal Regulations (as proposed in the Federal Register of January 15, 1985; 50 FR 2172) be amended as follows:

PART 333—TOPICAL ANTIMICROBIAL DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

1. The authority citation for 21 CFR part 333 continues to read as follows:

Authority: Secs. 201, 501, 502, 503, 505, 510, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 351, 352, 353, 355, 360, 371).

§ 333.310 [Amended]

2. Section 333.310 *Acne active ingredients* is amended by removing paragraph (a) and redesignating paragraphs (b) through (f) as paragraphs (a) through (e).

3. Section 333.320 is revised to read as follows:

§ 333.320 Permitted combinations of active ingredients.

(a) Resorcinol identified in § 333.310(a) when combined with sulfur identified in § 333.310(e) provided the product is labeled according to § 333.350.

(b) Resorcinol monoacetate identified in § 333.310(b) when combined with sulfur identified in § 333.310(e) provided

the product is labeled according to § 333.350.

§ 333.350 [Amended]

4. Section 333.350 *Labeling of acne drug products* is amended by removing paragraph (c)(2) and redesignating paragraphs (c)(3) and (c)(4) as paragraphs (c)(2) and (c)(3).

Dated: June 4, 1991.

David A. Kessler,

Commissioner of Food and Drugs.

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