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NONPRESCRIPTION DRUG MANUFACTURERS ASSOCIATION

February 26, 1999

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Acting Director, Division of OTC
   Drug Products (HFD-560)
Deputy Director, Office of Drug
   Evaluation V
Center for Drug Evaluation and Research
9201 Corporate Boulevard
Rockville, Maryland 20850

Re: FDA Docket No. 81N-0114

Dear Dr. Bowen:

Enclosed for FDA review is an "Update on Safety Studies with Benzoyl Peroxide" submitted by the NDMA Benzoyl Peroxide Study Group. Included in the update are reports of the interim (1 year) sacrifices in the 2-year dermal carcinogenicity studies in F344 rats and B6C3F1 mice and the final report for the 1-year photo co-carcinogenicity study. In addition, we have included reports from investigative studies with benzoyl peroxide conducted to further understand relative species differences in skin penetration and potential interactions with ultraviolet radiation.

Topical benzoyl peroxide has been used for over 30 years in the treatment of acne with no reports of adverse effects that could be related to skin cancer. This positive clinical experience is supported by the results of epidemiological studies and chronic animal carcinogenicity studies. And now the results from the chronic safety studies in animals presented in this update further uphold the conclusions from earlier studies.

Three copies of this entire submission are being sent to the FDA Dockets Management Branch.

Please let me know if you or others at FDA have questions about this submission.

Sincerely yours,

Lorna C. Totman, Ph.D., DABT
Director of Scientific Affairs

Enclosures: Nine (9) volumes (in submission NB)

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Update on Safety Studies
with
<u>Benzoyl Peroxide</u>

Submission to FDA Docket No. 81N-0114
February 26, 1999

Nonprescription Drug Manufacturers Association
Benzoyl Peroxide Study Group
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List of Attachments

1. Overview

This submission from the Nonprescription Drug Manufacturers Association (NDMA) Benzoyl Peroxide Study Group provides an update on the chronic safety studies with benzoyl peroxide carbopol gels, which were conducted at the request of the Agency. We have attached reports of the interim (1 year) sacrifices in the 2-year dermal carcinogenicity studies in F344 rats (**Attachment 1**) and B6C3F1 mice (**Attachment 2**) and the final report for the 1-year photo co-carcinogenicity study (**Attachment 3**). The final report describing the preparation and stability of the benzoyl peroxide carbopol gels used in the photo co-carcinogenicity study is also provided (**Attachment 4**). In addition, we have included reports from investigative studies with benzoyl peroxide conducted to further understand relative species differences in skin penetration and potential interactions with ultraviolet radiation (**Attachment 5**).

In summary, there were no macroscopic or microscopic findings of carcinogenicity in the skin or in select internal organs related to the daily topical application of benzoyl peroxide carbopol gel to the backs of F344 rats or B6C3F1 mice for 1 year. In the 12-month photo co-carcinogenicity study, there was no evidence for a dose-dependent enhancement of photocarcinogenesis in SKH1 (hr/hr) albino hairless mice, after repeated topical treatment with benzoyl peroxide carbopol gels.

The absence of an enhancement of solar-simulated ultraviolet radiation (UVR)-induced skin tumor formation by benzoyl peroxide, even under the conditions of exaggerated exposure in the photo co-carcinogenicity study, is supported by investigative research studies that showed benzoyl peroxide has no demonstrable effects on: (a) UVB (290-320 nm)- or UVA (320-400 nm)-induced oxidative DNA damage in cell cultures; (b) UVB-induced skin damage, such as sunburn cell formation in SKH1(hr/hr) albino hairless mice, after 12 weeks concurrent exposure; (c) promotion of skin tumors in SKH1(hr/hr) albino hairless mice initiated with UVB; and (d) solar-simulated UVR-induced human skin damage after 5 weeks of repeated exposure.

Collectively, these data support the view that over-the-counter (OTC) acne treatments containing benzoyl peroxide are safe for human use. This conclusion is consistent with the position stated in the past by NDMA, which is supported by a large body of existing data and the judgment of professional organizations including the American Academy of Dermatology[1] and experts in the field of toxicology, photobiology, and cancer research[2].

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[1]Letter from Stephen B. Webster, M.D., President, American Academy of Dermatology, September 25, 1991, to William Gilbertson, Pharm.D., FDA.
[2] Letter from NDMA, July 2, 1990, to FDA Docket No. 81N-0114.

2. Integrated Human Safety Assessment

Benzoyl peroxide has a long history of safe and effective use in OTC acne treatment products. The NDMA Benzoyl Peroxide Study Group has submitted extensive scientific documentation supporting the conclusion that benzoyl peroxide is safe for human exposure.[3] The majority of this evidence has been available since the issuance of the 1991 amendment to the Acne Final Monograph[4] and includes:

- estimates of consumer use of OTC acne treatments containing benzoyl peroxide showing that the duration of exposure represents a small fraction of the human life span[5];

- epidemiological evidence that acne treatments containing benzoyl peroxide are not a risk factor for skin cancer[6];

- results from long- and short-term rodent studies which have shown no reproducible evidence of carcinogenic activity by benzoyl peroxide[7];

- absence of an effect of benzoyl peroxide on UVR-induced skin tumor formation in hairless mice.[8]

The details supporting this integrated human safety assessment, including estimates of human exposure and hazard identification, are briefly reviewed in the following sections.

2.1 Estimates of Human Exposure

Although initially introduced as a prescription drug in 1958, benzoyl peroxide has been available since the mid-60s as an OTC medication to treat the abnormal skin condition commonly known as acne.[9] The NDMA Benzoyl Peroxide Study Group has estimated consumer exposure to benzoyl peroxide from the application of OTC acne-treatment products for the average and top 5% users. This information was presented during the 34th meeting of

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[3] Letter from NDMAC-NDMA, July 14, 1987, to FDA Docket No. 81N-0114. Letter from NDMAC-NDMA, June 8, 1989, to FDA Docket No. 81N-0114. Letter from NDMA, May 15, 1990, to FDA Docket No. 81N-0114. Letter from NDMA, September 26, 1990, to FDA Docket No. 81N-0114.

[4] Food and Drug Administration (1991), *Fed Reg* 56:37622-37635

[5] Transcript of 34th Meeting of the Dermatological Drugs Advisory Committee, April 9, 1992. Letter from NDMA, May 23, 1996, to FDA Docket No. 81N-0114.

[6] Hogan *et al.* (1991), *Br J Dermatol* 125:343-348. Cartwright *et al.* (1988), *Br J Dermatol* 118:239-242.

[7] Binder *et al.* (1995) in Growth Factors and Tumor Promotion: Implications for Risk Assessment, Slaga *et al.*, eds., pp. 245-294. Kraus *et al.* (1995), *Reg Toxicol Pharmacol* 21:87-107

[8] Iversen (1986), *J Invest Dermatol* 86:442-448. Epstein (1988) *J Invest Dermatol* 91:114-116. Iversen (1988), *Carcinogenesis* 9:803-809.

[9] Chalker *et al.* (1983), *J Am Acad Dermatol* 6:933-936. Burke *et al.* (1983), *Br J Dermatol* 108:199-204. Berson and Shalita (1995), *J Am Acad Dermatol* 32:S31-41. Leyden (1997), *N Engl J Med* 336:1156-1162.

the Dermatological Drugs Advisory Committee and detailed in a later submission to the Agency.[5]

In brief, human exposure to benzoyl peroxide from the use of acne treatment products has been evaluated using the results from controlled clinical acne trials, consumer research studies, i.e., habits and practices, and *per capita* product consumption obtained from marketing and sales of benzoyl peroxide-containing products. The average and top 5% users of OTC acne treatments containing benzoyl peroxide apply product intermittently to affected sites and have extended periods of non-use. The duration of product use is, on average, between 2 and 5 years and, for the top 5% users, is less than 10 years out of a lifetime (70 years). Since the average user buys one tube, 28.35 g, of a product containing 10% benzoyl peroxide (i.e., maximum concentration in OTC acne treatment products) per year and assuming intermittent application over the entire face, 200 cm^2, then exposure to benzoyl peroxide would be 14.175 mg benzoyl peroxide/cm^2/year. For the top 5% users (e.g., 2.2 tubes/year or 62.37 g of a 10% benzoyl peroxide-containing product/year), exposure would be 31.39 mg benzoyl peroxide/cm^2/year. Thus, estimates of human exposure to benzoyl peroxide from the use of OTC acne treatment products need to incorporate intermittent use periods, spot-treatment, and duration of use over a lifetime, all of which need to be carefully factored into any assessment of human risk based on the outcome from daily lifetime exposure to benzoyl peroxide in rodent photo co-carcinogenicity and carcinogenicity models.

2.2   Hazard Identification

2.2.1. Epidemiology

Acne treatments containing benzoyl peroxide have not been found to be a risk factor for development of nonmelanoma or melanoma skin cancers in retrospective, case-control epidemiology studies.[6,10] The largest of such epidemiology studies was conducted by Hogan *et al.* using a detailed tumor registry that has been maintained for over 30 years in Saskatchewan, Canada. In this retrospective study, 964 cases with facial skin cancer and 3,856 controls completed a one-page questionnaire designed to determine possible risk factors for facial skin cancer, with emphasis on acne and past treatments for acne, particularly use of benzoyl peroxide preparations. Neither acne nor the use of any acne medication had a statistically significant association with the risk of facial skin cancer. Known risk factors, including fair skin,

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[10]Hogan *et al.* (1990), A report to the NDMA/NDMA of Canada on the Saskatchewan Study. To *et al.* (1991), *Am J Epidemiol* <u>134</u>:772.

light hair color, family history of skin cancer, ethnic origin, and susceptibility to sunburn, were confirmed in this study, and benzoyl peroxide did not exacerbate these risk factors. The results of Hogan *et al.* are consistent with the findings reported in other epidemiology studies[6,10] and support the view that neither acne nor use of acne treatments containing benzoyl peroxide is a risk factor for skin cancer.

2.2.2. Animal Studies

2.2.2.1. Chronic Dermal Studies

Lifetime animal studies offer important insight into the mechanism(s) of carcinogenesis and are often used to assess the potential carcinogenic risk of a drug in the absence of human epidemiology data. Benzoyl peroxide has been tested for complete carcinogenic potential in multiple species, including rats, mice, and Syrian hamsters, after repeated oral, subcutaneous, and dermal administration (for review see Binder *et al.* and Kraus *et al.*[7]). Several of these studies were of lifetime duration, and most were part of investigations of the skin tumor promoting potential of benzoyl peroxide in experimental models of multistage carcinogenesis.[11]

Although many of the 23 chronic studies with benzoyl peroxide do not meet the present-day design criteria for assessing carcinogenicity, collectively they provide a large body of reproducible data supporting the singular conclusion that benzoyl peroxide is not a complete carcinogen. In complete contrast to this wealth of data, a study by Kurokawa *et al.*[12] reported that benzoyl peroxide alone increased the formation of benign and malignant skin tumors in SENCAR mice. The authors interpreted their results as indicating that benzoyl peroxide possibly acted as a complete carcinogen. The outcome of this individual study was not reproduced in a number of other laboratories using the same mouse stock and similar dosing regimens. Moreover, the interpretation of these data has been questioned, even by the author[13] of the published report. Thus, based on the weight of the evidence, we have concluded that benzoyl peroxide is not a complete carcinogen.

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[11] Slaga *et al.* (1981), *Science* 213:1023-5. DiGovanni (1992), *Pharmacol Ther* 54:63-128.
[12] Kurokawa *et al.* (1984), *Cancer Lett* 24:299-304.
[13] Letter from Y. Kurokawa to A. Sivak (1986) included in NDMA submission, August 7, 1991, to FDA Docket No. 81N-114A.

2.2.2.2. Studies with Concurrent UVR Exposure

Because exposure to UVR is a known human carcinogen[14] that represents a significant public health concern, laboratory studies have been conducted to determine whether benzoyl peroxide treatment influences UVR-induced skin tumor formation in rodents.[8] The effect of a commercial formulation with and without benzoyl peroxide on UVR-induced skin carcinogenesis in hairless Oslo mice has been evaluated by Iversen.[8] In these studies, benzoyl peroxide gel did not exacerbate UVR-induced skin carcinogenesis. Separately, Epstein[8] examined the effect of a commercial preparation containing benzoyl peroxide on the appearance of skin tumors in UCSD hairless mice after treatment with initiating doses of UVR. In this tumor promotion study, benzoyl peroxide did not affect the rate or number of UV-induced skin tumors. Thus, there was no evidence of skin tumor promotion by benzoyl peroxide after UVR initiation. These data demonstrate the lack of an effect of benzoyl peroxide on UVR-induced mouse skin carcinogenesis.

2.2.2.3. Conclusions from Chronic Animal Studies

The weight of evidence from 23 long-term studies using 3 different exposure routes, 3 species, several strains or stocks of mice, including SENCAR, selectively bred for skin cancer sensitivity, and 4 vehicles in the topical studies supports the conclusion that benzoyl peroxide is not an animal carcinogen. Further, negative results in a UVR initiation/benzoyl peroxide promotion study and two photo co-carcinogenicity studies with benzoyl peroxide gel show there is no reproducible evidence of an interaction between UVR and benzoyl peroxide. Thus, consistent with findings in epidemiology studies, these animal studies support the absence of complete carcinogenic activity or an enhancing effect on skin tumor development in UVR-exposed skin.

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[14] IARC Monograph (1992), Vol 55, Solar and Ultraviolet Radiation.

3. FDA Human Safety Concerns

FDA reclassified benzoyl peroxide from its originally proposed monograph status[15] of Category I, generally recognized as safe and effective, to Category III, more data needed, in a 1991 amendment[4] to the Tentative Final Monograph for Topical Acne Drug Products for OTC Human Use. This action was based on studies conducted in rats, mice, and hamsters demonstrating the tumor promoting effect of benzoyl peroxide[11] and, as discussed above, a single study suggesting that benzoyl peroxide may act as a complete carcinogen.[12] Although a substantial body of data shows benzoyl peroxide is not a complete carcinogen, the Agency wanted more evidence that it was not a weak, slow-acting one. In the opinion of the Agency, there was sufficient uncertainty regarding potential human health effects from exposure to OTC acne treatments containing benzoyl peroxide to request additional safety studies be conducted.

3.1.   Recommendation of the Dermatologic Drugs Advisory Committee

Based on the concerns outlined above, the Agency considered whether continued marketing of benzoyl peroxide-containing products should be suspended until additional safety data were obtained. To aid in this decision, the Agency convened a meeting of the Dermatologic Drugs Advisory Committee (DDAC) in April 1992 to discuss the continued OTC availability of benzoyl peroxide-containing acne treatments. In accordance with FDA procedures, a panel of experts appointed by the Agency reviewed data and information related to the human safety of benzoyl peroxide and unanimously concluded that benzoyl peroxide should remain available as an OTC active drug ingredient at concentrations up to 10%. In addition, DDAC was informed that the Agency had recommended the interaction between benzoyl peroxide and UVR be included in the lifetime animal carcinogenicity studies. DDAC agreed that the previous studies evaluating the interaction between UVR and benzoyl peroxide were insufficient to resolve fully this issue because they were not lifetime studies and insufficient numbers of animals had been used. Further, based on its assessment of the experimental design of earlier studies, DDAC questioned whether the studies provided assurance that benzoyl peroxide's tumor promoting potential was definitively evaluated. DDAC therefore recommended that a new photo co-carcinogenicity study be conducted[5] to confirm results obtained in earlier studies.[8]

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[15] Food and Drug Administration (1982), *Fed Reg* 47:12430-12477. Food and Drug Administration (1985), *Fed Reg* 50: 2172-2182.

3.2. Agreement to Conduct Additional Chronic Safety Studies

NDMA member companies who manufacture acne treatment products containing benzoyl peroxide agreed that additional studies would be conducted to assess potential human safety concerns related to the chronic topical use of these products. Specifically, the companies agreed to sponsor 2-year dermal carcinogenicity studies in two species (i.e., mice and rats)[16] and a photocarcinogenicity study, in hairless mice, with benzoyl peroxide.[17]

These studies, which are summarized in the following sections, were performed even though: (a) human exposure to OTC acne treatments containing benzoyl peroxide resulting from intermittent product application is limited and represents a small fraction of human life span; (b) neither acne nor use of acne treatments containing benzoyl peroxide is a risk factor for skin cancer in retrospective, case-control epidemiology studies; (c) studies of chronic topical application in multiple species support the lack of complete carcinogenicity; (d) no inter-action occurs between benzoyl peroxide and UVR; and (e) the views of leading experts in the field of dermatology, toxicology, photobiology and cancer research support the human safety of benzoyl peroxide.

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[16] Letter from NDMA, Jan. 10, 1990, to FDA (Dr. Gilbertson), Docket No. 81N-0114.
[17] Letter from NDMA, June 9, 1992, to FDA Docket No. 81N-0114.

4. Industry-Sponsored Studies

4.1. Dermal Carcinogenicity Studies

4.1.1. Selection of Vehicle and Doses for 2-Year Studies

The NDMA Benzoyl Peroxide Study Group conducted several studies to select the appropriate vehicle for the 2-year dermal carcinogenicity studies and 1-year photo co-carcinogenicity study. From the results of multiple 14-day studies, carbopol gel was concluded to be the most suitable vehicle for evaluation of benzoyl peroxide in the chronic studies. The key reasons underlying this decision were: (a) higher absolute doses of benzoyl peroxide could be applied to skin in a carbopol gel; (b) carbopol gel is a prototypic aqueous vehicle used in currently marketed OTC acne treatments containing benzoyl peroxide; and (c) the maximal target-organ response obtained in the 14-day studies was observed at the top doses of benzoyl peroxide suspended in carbopol gel, and even twice-daily application of solubilized benzoyl peroxide in acetone did not produce stronger target-organ (i.e., skin) responses. Complete study reports along with supporting material used as the basis for selecting this vehicle are detailed in a previous submission.[18]

Dose selection for the 2-year carcinogenicity studies using B6C3F1 mice and F344 rats was based on results of 13-week sub-chronic studies conducted at the International Research and Development Corporation, Mattawan, Michigan (now known as MPI Research). The doses selected for these 2-year studies were: 25, 5, and 1 mg/day for B6C3F1 mice and 45, 15, and 3 mg/day for F344 rats. (The low dose for the rats was changed to 5 mg/day, as was recommended by FDA.) The criteria used for selecting these doses were based on the recommendations arising from two workshops conducted by the U.S. Environmental Protection Agency (EPA) regarding proposed guidelines for establishing a maximum tolerated dose (MTD) for dermal carcinogenicity studies.[19] The dose range selected for each species was equally spaced and included doses that elicited the spectrum of dermal responses observed in the 13-week studies. In addition, the top two doses selected produced skin effects consistent with reaching but not exceeding the MTD and had no

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[18] Letter from NDMA, Oct. 20, 1993, to FDA Docket No. 81N-0114. Letter from FDA (Dr. Gilbertson), Dec. 23, 1993, to NDMA. Binder *et al.* (1997), *The Toxicologist* 37:188.

[19] EPA (1989), Summary of the Second EPA Workshop on Carcinogenesis Bioassay via the Dermal Route, May 18-19, 1988, Research Triangle Park, NC. U.S. Environmental Protection Agency: EPA 560-6-89-003. EPA (1987): Report of the EPA Workshop on the Development of Risk Assessment Methodologies for Tumor Promoters. U.S. Environmental Protection Agency: EPA/600/9-87/013.

significant systemic effects. Moreover, the top two doses represent the maximum (top dose) and near-maximum (mid-dose) skin effects observed in the 13-week studies. A full explanation of these data along with the study reports are on file at the Agency.[20] The FDA response to our dose recommendation stated "We concur with the doses selected for the mouse (1, 5, and 25 mg/day) and rat (5, 15, 45 mg/day) 2-year dermal carcinogenicity studies."[21]

4.1.2. Interim Sacrifice: Findings after Daily Dosing for 1 Year

4.1.2.1. F344 Rats (Covance Study No. 6711-101)

A 2-year cancer bioassay of benzoyl peroxide suspended in carbopol gel and administered by the dermal route to F344 rats was conducted at Covance Laboratories, Vienna, Virginia. Dosing in this 2-year study was initiated on March 12, 1996, and the terminal necropsy was performed on March 10-15, 1998. The results from the interim sacrifice at 52 weeks of treatment are summarized below. Full details of the results are provided in the "Pathology Report" that is **Attachment 1**.

The overall study design for the 2-year dermal carcinogenicity study in F344 rats is summarized in Table 1 (on the next page) and in the attached pathology report for the 1-year interim sacrifice. Carbopol gel containing various levels of benzoyl peroxide was administered 7 days/week to Groups 2-4. Group 1 received the gel vehicle with no benzoyl peroxide throughout the study. The rats in Group 5 (discontinued group) received the high dose of benzoyl peroxide for 52 weeks and the gel vehicle for the remainder of the study.

The first 10 males and 10 females in each of dose groups 1-4 and 6 were designated for the interim sacrifice at 52 weeks. All of those rats that survived at 52 weeks (99 of the 100 designated) were humanely killed and subjected to complete necropsies. Skin (treated and untreated) and kidneys were micro-scopically evaluated by a board-certified veterinary pathologist. A complete list of organs and tissues was preserved in 10% neutral-buffered formalin, in accordance with the study protocol.

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[20] Letter from NDMA, June 30, 1995, to FDA Docket No. 81N-0114. Nash *et al.* (1997), *The Toxicologist* 37:188.
[21] Letter from FDA (Dr. Gilbertson), Oct 13, 1995, to NDMA.

Table 1. Summary of study design for 2-year dermal carcinogenicity study in F344 rats

Group	Number of rats/sex		Benzoyl Peroxide
	Total	Interim sacrifice	Dose (mg/rat/day)
1. Vehicle control	60	10	0
2. Low dose	60	10	5
3. Mid dose	60	10	15
4. High dose	60	10	45
5. High discontinued	50	0	45
6. Untreated control	60	10	0

One of the rats designated for the interim sacrifice, a male in Group 2, died at week 52. Among the rats sacrificed at 52 weeks, mean terminal body weights and organ weights were unaffected by treatment with benzoyl peroxide. There were no macroscopic pathology findings suggestive of any treatment-related effect.

In the 13-week dose range-finding study, statistically significant increases in kidney-to-body weight ratios were observed in male and female rats given doses of 75 and 150 mg/day, although no microscopic alterations were noted. Daily application of 45 mg benzoyl peroxide in the first year of the 2-year study was not associated with changes in absolute or relative kidney weights, and microscopic examination revealed no evidence of any kidney effects.

Benzoyl peroxide induced similar cutaneous responses in male and female rats. There were dose-dependent increases in the incidence and severity of hyperkeratosis, which were graded as minimal to mild. Similarly, there was a dose-dependent increase in acanthosis (i.e., epithelial thickening), which was generally graded as minimal in severity. One high-dose female exhibited mild acanthosis in treated skin. The acanthosis was due to both an increase in epithelial cell layers (i.e., hyperplasia) and an increase in cell size (i.e., hypertrophy). These cutaneous effects are consistent with reaching but not exceeding the dermal MTD, based on criteria developed in U.S. EPA sponsored workshops.[18]

4.1.2.2. B6C3F1 Mice (Covance Study No. 6711-100)

A 2-year cancer bioassay of benzoyl peroxide suspended in carbopol gel by the dermal route of administration in B6C3F1 mice was conducted at Covance Laboratories, Vienna, Virginia. Dosing in the 2-year study was initiated on March 26, 1996, and the terminal necropsy was performed on March 26 to April 4, 1998. The results from an interim sacrifice at 52 weeks are summarized here. Full details are provided in the "Pathology Report" that is **Attachment 2**.

The overall study design was nearly identical to that for the F344 rats and is summarized in Table 2 and further described in the attached pathology report. Carbopol gel containing various levels of benzoyl peroxide was administered 7 days/week to Groups 2-4. Group 1 received the gel vehicle with no benzoyl peroxide throughout the study. The mice in Group 5 (discontinued group) received the high dose of benzoyl peroxide, 25 mg/day, for 52 weeks and the gel vehicle for the remainder of the study.

Table 2. Summary of study design for 2-year dermal carcinogenicity study in B6C3F1 mice

Group	Number of mice/sex		Benzoyl Peroxide
	Total	Interim sacrifice	Dose (mg/mouse/day)
1. Vehicle control	60	10	0
2. Low dose	60	10	1
3. Mid dose	60	10	5
4. High dose	60	10	25*
5. High discontinued	50	0	25
6. Untreated control	60	10	0

*Group 4 received 25 mg/day (weeks 1-56), 0 mg/day (weeks 57-58), 15 mg/day (weeks 59-84), 0 mg/day (weeks 85-86), 15 mg/day (weeks 87-92), and 0 mg/day (weeks 93-104). The reasons for this are described in the text.

The first 10 males and females in each of dose groups 1-4 and 6 were designated for the interim sacrifice at 52 weeks. All of those mice that survived at 52 weeks (97 of the 100 designated) were humanely killed and subjected to complete necropsies. Skin (treated and untreated) and livers were

microscopically evaluated by a board-certified veterinary pathologist. A complete list of organs and tissues was preserved in 10% neutral-buffered formalin.

Three of the mice designated for the interim sacrifice were found dead (two Group 6 males and one Group 2 female). The cause of death of the Group 6 males was not evident from the gross and microscopic examinations of the tissues. The death of the Group 2 female was attributed to amyloidosis primarily involving the kidney. Among the mice sacrificed at 52 weeks, mean terminal body weights were unaffected by treatment with benzoyl peroxide.

In the 13-week dose range-finding study, a statistically significant increase in the liver-to-body weight ratio was observed in male mice receiving benzoyl peroxide at the 50-mg/day level, although no microscopic alterations were noted. In the present study, there was no evidence of a treatment-related effect on liver weight in mice receiving benzoyl peroxide at up to 25 mg/day for 52 weeks. In addition, no treatment-related histomorphologic alterations in the liver were found. The presence of liver tumors in some mice in Groups 2, 3, 4, and 6 was considered unrelated to the test material because there was no evidence of a dose-response and such tumors are relatively common in B6C3F1 mice.[22]

The microscopic evaluation of the mice in the interim sacrifice group found that benzoyl peroxide induced similar cutaneous responses in mice of both sexes. These responses consisted of minimal-to-mild acanthosis and hyperkeratosis, minimal sub-acute inflammation in Groups 3 and 4, and minimal-to-moderate hyperplasia of the sebaceous glands in males in Groups 3 and 4 and females in Groups 2, 3, and 4. The incidence and/or severity of these effects were dose-dependent.

Some Group 4 mice receiving the 25 mg/day dose of benzoyl peroxide developed persistent skin ulceration. Two of those mice, a male and a female in Group 4, were among those designated for the interim sacrifice. The ulcer detected during clinical observation of the Group 4 male correlated with the

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[22] Drinkwater et al. (1989), Toxicol. Lett 49:255-265.

microscopic findings of degeneration/necrosis, crust, chronic active inflammation, and fibrosis, and the grossly observed ulcer in the female correlated with the microscopic findings of ulcer, crust, and chronic active inflammation. These macroscopic and microscopic cutaneous effects are evidence that the MTD had been exceeded based on the EPA dermal MTD criteria.[18]

During the second year of the study, the incidence of persistent visible skin ulcers increased in mice that received benzoyl peroxide at the 25-mg/day dose. This was interpreted as evidence of exceeding the MTD. Because chronic skin ulceration is a risk factor for skin cancer[23], mice that had developed persistent ulcers were removed from the study to avoid confounding interpretation of any effects truly related to the test substance. Further, the high dose was lowered from 25 mg of benzoyl peroxide/day to 15 mg/day. Mice were rested by administration of vehicle for 2 weeks starting at week 57, to increase the chances that they would tolerate the 15-mg/day dosage. FDA concurred with this approach.[24]

Dosing with benzoyl peroxide at 15 mg/day was begun at study week 59. By week 83, more than 10% of the mice in this treatment group had developed ulcers at the treatment site. Therefore, treatment with this dose, 15 mg/day, of benzoyl peroxide in carbopol gel was suspended at week 85. Again, all mice that had developed persistent ulcers were removed from the study and subjected to complete necropsies as agreed to by the Agency.[25] After a 2-week rest during which mice received the vehicle, no additional skin ulcers were observed. Thus, dosing with the 15 mg/day benzoyl peroxide carbopol gel was resumed on week 87; however, treatment site ulcers were once again observed by week 92 on more than 10% of the surviving mice. It was therefore decided to stop administration of this dose of benzoyl peroxide gel at week 93 and to continue treating the mice in the high-dose group with the gel vehicle for the remainder of the study. As before, mice that had developed

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[23] Argyris (1980), *J Invest Dermatol.* 75:360-362. Argyris (1985), *Crit Rev Toxicol* 14:211-258.
[24] Letter from NDMA, April 23, 1997, to FDA Docket No. 81N-0114. Letter from FDA (Dr. Bowen), July 23, 1997, to NDMA.
[25] Letter from NDMA, November 12, 1997, to FDA Docket No. 81N-0114. Letter from FDA (Dr. Bowen), January 2, 1998, to NDMA.

persistent ulceration were removed from the study and subjected to complete necropsies. Again, FDA concurred with this approach.[26]

The reproducible induction of skin ulceration at the 15-mg/day dose provides clear evidence that this dose, in addition to the 25-mg/day dose, exceeded the dermal MTD. As noted above, two Group 4 mice with grossly detected skin ulceration were among those designated for interim sacrifice. In one case the ulcer was confirmed microscopically, and in the other case the tissue sections of the lesion evaluated exhibited necrosis/degeneration and crusting. The EPA dermal MTD criteria state that all of those skin alterations, which compromise the integrity and barrier function of the skin, are evidence for exceeding the MTD.

The mid-dose of benzoyl peroxide, 5 mg/day, produced effects after 52 weeks of dosing in the interim sacrifice mice, consistent with reaching but not exceeding the MTD. These mice had been continuously treated (7 days/week) with this dose of benzoyl peroxide since the beginning of the study without any signs of persistent ulceration. Thus, benzoyl peroxide was tested at an MTD in B6C3F1 mice in this 2-year dermal carcinogenicity study.

4.1.2.3. Summary of Findings in Rats and Mice at 1 Year

After 52 weeks of daily topical administration of benzoyl peroxide carbopol gels to F344 rats and B6C3F1 mice, there was no macroscopic or microscopic evidence for cutaneous carcinogenicity. There was evidence that the MTD for benzoyl peroxide was tested in these studies.

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[26] Letter from NDMA, January 22, 1998, to FDA Docket No. 81N-0114. Fax from FDA (Dr. Bowen from Dr. Jacobs via Dr. Wilkin) sent Feb. 12, 1998 to NDMA. Conference call 2/18/98 with FDA (Division of OTC Drug Products) and NDMA.

4.2.   Photo Co-Carcinogenicity Study with Benzoyl Peroxide Carbopol Gel (Argus Study No. C-314-001)

In addition to the 2-year dermal carcinogenicity studies, data concerning the potential effect of benzoyl peroxide on solar-simulated UVR-induced skin tumor formation were requested by the Agency. To this end, a 12-month photo co-carcinogenicity study was performed by Dr. P. Donald Forbes and coworkers at the Center for Photobiology, Argus Research Laboratories. The objective of this study was to determine whether repeated, topical application of benzoyl peroxide carbopol gels enhanced solar-simulated UVR-induced skin tumor formation in albino SKH1(hr/hr) hairless mice. The final report from the 12-month study entitled "12-Month Topical Study to Determine the Influence of Benzoyl Peroxide on Photocarcinogenesis in Albino Hairless Mice Crl:SKH1(hr/hr)BR" is provided in **Attachment 3**.

The protocols for the preliminary and definitive 12-month photo co-carcinogenicity studies were shared with and agreed to by the Agency.[27] The experimental design has been proposed as a photocarcinogenicity safety test to determine whether a product influences the time required for the known carcinogen, UVR, to produce skin tumors in a hairless, albino mouse model.[28] As part of this approach, preliminary studies were conducted to establish that benzoyl peroxide carbopol gels were neither phototoxic nor photoprotective after single or repeated (8 weeks) topical application. In addition, these data were used as the basis to select 50, 15, and 1 mg/ml as the doses for the 12-month study.[29]

In this 12-month photo co-carcinogenicity study, male (n = 39) and female (n = 39) SKH1(hr/hr) albino hairless mice were randomly assigned to treatment groups and treated topically with benzoyl peroxide carbopol gel [0 (vehicle), 1, 15, or 50 mg/ml] and solar-simulated UVR. Two groups of mice received either 600 or 1200 Robertson-Berger Units (RBU) of solar-simulated UVR alone, which served as internal response calibrators for this study. The benzoyl peroxide carbopol gels were applied to the backs and sides of hairless mice before solar-simulated UVR (600 RBU) on Monday, Wednesday, and Friday and after on Tuesday and Thursday for 40 weeks, followed by a 12-week observation period with no treatment. All mice in the study received a tumorigenic dose of solar-simulated UVR. The

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[27] Letter from NDMA, March 11, 1993, to FDA Docket No. 81N-0114. Letter from FDA, May 11, 1993, to NDMA. Letter from NDMA, August 13, 1993, to FDA Docket No. 81N-0114. Letter from FDA, Oct 26, 1993, to NDMA.

[28] Sambuco *et al.* (1991), *Toxicol Methods* 1:75-83. Forbes *et al.* (1993), *J Am Coll Toxicol* 12:417-424.

[29] Letter from NDMA, June 15, 1995, to FDA Docket No. 81N-0114. Nash *et al.* (1997), *J Invest Dermatol* 108: 772.

endpoints used to assess the effect of a test material, specifically benzoyl peroxide carbopol gel, on UVR-induced skin tumor formation in this photo co-carcinogenicity model were the time for tumors to appear (i.e., tumor latency) and the number of tumors per mouse (i.e., tumor yield).

There was no statistically significant dose-dependent enhancement of UVR-induced skin tumor formation (tumor latency) or the number of tumors per animal (tumor yield) by benzoyl peroxide carbopol gels in this study. For all tumor sizes, the highest dose of benzoyl peroxide carbopol gel, 50 mg/ml, had no statistically significant effect on tumor latency or yield compared to vehicle + low UVR or low UVR alone. Importantly, the absence of a dose-related effect is probably not attributable to any photoprotection produced by benzoyl peroxide carbopol gels, since the highest concentration used in this study, 50 mg/ml, was shown to have no effect on UVR-induced skin responses in the preliminary studies. Furthermore, the isolated values (i.e., sex-specific, dose differences in tumor prevalence or yield) that were statistically significant in uncorrected multiple pairwise comparisons were small and not dose-dependent, were within the magnitude of variation seen in historical control data, and are most readily understood on the basis of sampling error.

In conclusion, there is no evidence for enhancement of photocarcinogenesis by benzoyl peroxide carbopol gels in the SKH1(hr/hr) albino hairless mouse. This conclusion is based on the lack of enhancement by the highest dose of benzoyl peroxide carbopol gel (50 mg/ml) and the negative association between test article dose and UVR-induced skin tumor production. The isolated effects observed at the lower doses of benzoyl peroxide carbopol gels approximated the smallest change detectable in this test system. Thus, under the conditions of this rodent photo co-carcinogenicity study, benzoyl peroxide in a carbopol gel vehicle is not a photocarcinogenicity risk factor.

5. Benzoyl Peroxide Carbopol Gels: Preparation, Analysis, and Stability

The final report, entitled "Preparation of Gels Containing 0, 0.1, 1.5, and 5% (w/w) Benzoyl Peroxide for Use in a Photocarcinogenicity Study" (MPI 657-012), detailing the preparation of benzoyl peroxide carbopol gels used in the rodent photo co-carcinogenicity study is provided in **Attachment 4**. A brief description is given here: 8 kg of each concentration of benzoyl peroxide carbopol gel was prepared and immediately packaged in 2-ounce glass jars with Teflon-coated lids. The glass jars were kept frozen at -20° C until the day of use. Before the gels were used in the photo co-carcinogenicity study, random samples from the beginning, middle, and end of each batch were analyzed for the concentration of benzoyl peroxide, the absence of microbial contamination, pH, specific gravity, color, characteristic appearance and smell, and particle size. Analysis of each concentration of the benzoyl peroxide carbopol gel was conducted approximately half way through and at the end of the 40-week dosing period. At each time, the concentration of benzoyl peroxide was within the specified range. Collectively, these data support the stability and homogeneity of the benzoyl peroxide carbopol gels.

Benzoyl peroxide carbopol gels used in the 2-year dermal carcinogenicity studies in B6C3F1 mice and F344 rats were prepared in a separate project, with a similar approach for determining the stability and homogeneity of the gels. The protocol and final report for that work (MPI 657-013) includes 3-year stability testing and quantitative analysis of each concentration of benzoyl peroxide at 6-month intervals throughout the 2-year dosing period. The final report containing those data will be submitted with the complete final reports from the 2-year dermal carcino-genicity studies in F344 rats and B6C3F1 mice.

6. Investigative Studies

The NDMA Benzoyl Peroxide Study Group conducted several investigative research studies with benzoyl peroxide as part of our effort to better understand skin penetration and potential interaction with UVR. Specifically, we compared the *in vitro* skin penetration of benzoyl peroxide among human, F344 rat, B6C3F1 mouse and SKH1 hairless mouse skin explants. In addition, the potential interaction between benzoyl peroxide and UVR was studied in collaboration with leading experts in the areas of genotoxicity, carcinogenicity, photobiology, and dermatology. The objective of these research studies was to determine whether benzoyl peroxide could be photo-activated and thereby enhance oxidative damage to DNA as determined in cell culture or in the skin of hairless mice. Further, the skin response in the hairless mouse to the combination of UVR and benzoyl peroxide was assessed and compared to the response of human skin. Finally, the ability of benzoyl peroxide carbopol gel to promote skin tumor formation after UV initiation was assessed.

The results from each of these studies is briefly summarized below. The final reports from these investigations are provided in **Attachments 5 and 6**.

6.1. Benzoyl Peroxide (BP): Species Comparisons of *In Vitro* Skin Penetration Following a Single Application to the Excised Skin of Rats, Mice, and Humans[30]

A study was conducted at Bushy Run Research Center to compare the *in vitro* percutaneous penetration of benzoyl peroxide suspended in carbopol gel between skin explants obtained from F344 rats, B6C3F1 and SKH1(hr/hr) albino hairless mice, and human cadaver skin and mounted in an infinite-dose, flow-through diffusion apparatus (Franz cell).[31] The objective of this study was to compare and contrast the rate of penetration of benzoyl peroxide through human skin versus the rate through skin of the animal species used for carcinogenicity testing, i.e., B6C3F1 mouse and F344 rat, and for photo co-carcinogenicity testing i.e., SKH1(hr/hr) albino hairless mouse. The complete report for the skin penetration study is provided in **Attachment 5** (two volumes—**5a** and **5b**).

Benzoyl peroxide at concentrations ranging from 2.5% to 25% w/v were applied to skin discs (1.77 cm^2), and effluent samples were collected at 30-minute intervals for 6 hours. The effluent, minimum essential medium (MEM), was analyzed for benzoyl peroxide and its primary metabolite, benzoic acid (BA) using HPLC with UV detection. Polyethylene glycol

[30] Nash *et al.* (1996), *The Toxicologist* 36:345.
[31] Frantz, *et al.* (1990), *J Toxicol: Cutaneous Ocular Toxicol* 9:277-299. Kao *et al.* (1983), *Toxicol Appl Pharmacol* 68:206-217. Holland *et al.* (1984), *Toxicol Appl Pharmacol* 72:272-280.

was added to the effluent to enhance the solubility of benzoyl peroxide and thereby greatly improve its measurement. However, no benzoyl peroxide was detected in the effluent in the presence or absence of polyethylene glycol, with an HPLC limit of detection of 50 ng/ml. Therefore, the concentration of BA was used to calculate indices of percutaneous penetration of benzoyl peroxide. This is consistent with methods in previous investigations of benzoyl peroxide skin penetration in which benzoic acid was measured as a surrogate.[32]

The penetration of 10% w/v benzoyl peroxide suspended in carbopol gel through skin explants obtained from the SKH1 hairless mice was 8.5 times greater than through human skin and 5 times greater than through F344 rat or B6C3F1 mouse skin. Penetration of benzoyl peroxide through F344 rat and B6C3F1 mouse skin was slightly greater (i.e., approximately 1.5 times) than through human cadaver skin in these studies. There was no difference in the rate of penetration among different concentrations of benzoyl peroxide gels for any of the species tested, and so the dissolution of particulate benzoyl peroxide was likely the rate limiting step for the percutaneous penetration in these studies.

These data suggest that the delivered dose of benzoyl peroxide to viable cells in the skin of SKH1(hr/hr) hairless mice is significantly greater than that in human skin. The penetration difference between SKH1(hr/hr) hairless mouse and human skin should be considered when comparing the doses used in photo co-carcinogenicity studies to human exposure.

6.2.   Effect of Benzoyl Peroxide on Photo-oxidative Damage to DNA

A study was conducted in collaboration with Gary Williams, M.D., formerly at the American Health Foundation, to assess whether benzoyl peroxide, in the presence or absence of UVB (290-320 nm) or UVA (320-400 nm) radiation, affects the formation of 8-oxo-deoxy-guanosine (8-oxo-dG), a measure of oxidative DNA damage. The rationale for this study was based on observations that benzoyl peroxide may produce DNA strand breaks by a copper-dependent, free radical mechanism.[33] Such an event might be exacerbated by UVR exposure. The study report is provided in **Attachment 6**.

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[32] Nacht et al. (1981), J Am Acad Dermatol 4:31-37. Yeung et al. (1983), J Am Acad Dermatol 9:920-924.
[33] Swauger et al. (1991), Chem Res Toxicol 4: 223-228. Kensler et al. (1991) in Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Radiation Injury. Nigam et al., eds. King et al. (1996), Carcinogenesis 17: 317-320.

The general design of these studies was based on previously conducted studies with hydrogen peroxide and fluoroquinolones.[34] ARL-18 cells, a continuously dividing rat liver epithelial cell line, were incubated in the dark for 2 hours with benzoyl peroxide at 0, 100, 200, 400, or 800 μM, dissolved in 0.4% DMSO. After the 2-hour incubation, the cells were irradiated with either UVB, 0.63 J/cm^2 (FS40 filtered through cellulose acetate, 290-320 nm), or UVA, 20 J/cm^2 (Spectroline Model UV-400 lamp, 320-400 nm) for 43 and 33 minutes, respectively. DNA from the cells was isolated and cleaved to nucleotides. The nucleotides were applied to an HPLC apparatus equipped with UV detection to quantitate deoxyguanosine (dG) and electrochemical detection to quantitate 8-oxo-dG. The ratio of 8-oxo-dG to dG provides a measure of oxidative damage to DNA. Separately, cell viability was determined by addition of trypan blue to the media and counting the number of live cells by microscope.

Benzoyl peroxide alone and in the presence of either UVB or UVA had a significant effect on cell viability. At the lowest concentration of benzoyl peroxide, 100 μM, cell viability was less than 2% with or without UVR. At the highest concentration of benzoyl peroxide, 800 μM, no cells survived. Cell death would not inhibit the formation of oxidative DNA damage, and might add to the formation of 8-oxo-dG through the breakdown of cellular compartments.

The ratio of 8-oxo-dG/dG was significantly ($p < 0.001$) increased after cells were exposed to the highest concentration of benzoyl peroxide (800 μM) alone or in the presence of UVB or UVA. Similarly, exposure to either UVA or UVB produced a statistically significant ($p < 0.012$) increase in the formation of 8-oxo-dG/dG. Importantly, there was no statistically significant interaction between benzoyl peroxide and UVR, as the effect of benzoyl peroxide on oxidative DNA damage was not dependent on exposure to UVB or UVA.

The effects observed with the highest concentration of benzoyl peroxide were less than with hydrogen peroxide and far less than with fluoroquinolone antibiotics.[34] In contrast to benzoyl peroxide, the fluoroquinolone lomefloxicin, which was found to increase 8-oxo-dG after UVA exposure, was previously shown to be phototoxic and enhance solar-simulated UV-induced skin tumor formation in the hairless mouse photo co-carcinogenicity model.[35]

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[34] Rosen *et al.* (1996), *Photochem Photobiol* 64:117-122. Rosen *et al.* (1997), *Photochem Photobiol* 65:990-996. Rosen *et al.* (1997), *Toxicol Appl Pharmacol* 145:381-387.
[35] Klecak *et al.* (1997), *J Photochem. Photobiol. B: Biol.* 37:174-181. Mäkinen *et al.* (1997), *J Photochem Photobiol B: Biol.* 37:182-187.

In conclusion, under the conditions of this study, benzoyl peroxide had a minimal effect on measures of oxidative DNA damage. The formation of 8-oxo-dG occurred at concentrations of benzoyl peroxide that were completely cytotoxic or in the absence of the protective mechanism(s) operative in intact cells.[36] Most important, there was no interaction between UVA or UVB irradiation and benzoyl peroxide on the formation of 8-oxo-dG. Thus, at a minimum these data support the lack of any photo-activation of benzoyl peroxide by UVR resulting in oxidative DNA damage.

6.3. Responses of SKH1(hr/hr) Albino Hairless Mouse Skin to Benzoyl Peroxide Alone, UVB Alone, and Benzoyl Peroxide Plus UVB: Chronic Studies

A study was conducted in collaboration with Irene Kochevar, Ph.D., Wellman Laboratories of Photomedicine, Harvard Medical School, to determine whether skin responses observed after chronic exposure to a combination of benzoyl peroxide and UVB radiation differ qualitatively or quantitatively from those produced by either agent alone. Since UVB radiation is the most tumorigenic form of UVR[37], any modulation by benzoyl peroxide carbopol gel would be expected to have the greatest and most relevant biological impact with respect to previously stated FDA concerns. Study reports are provided in **Attachment 6**.

UVB and benzoyl peroxide differ in the primary molecular processes they initiate and in their site of action in the skin. UVB radiation is absorbed by DNA and produces photo-products that are believed to initiate the process leading to, for example, tumor formation.[38] Benzoyl peroxide applied to the skin is converted to benzoic acid via a nonenzymatic process. The primary intermediate in this pathway is the benzoyloxyl radical. Benzoyl peroxide can also decompose into benzoyloxyl radicals as a result of UVB radiation, although the overlap between the absorption spectrum of benzoyl peroxide and the solar UVB spectrum is low, if not negligible[39] (**Attachment 6, Kochevar report**). Regardless, free radicals formed by metabolism of benzoyl peroxide, UVB radiation, or the theoretical interaction between them could produce cellular damage and may be responsible for benzoyl peroxide's weak tumor promotion ability.

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[36] Zhang et al. (1997), Photochem Photobiol 65:119-124.

[37] de Gruijl and Forbes (1995), Bioessays 17:651-660. Black et al. (1997), J Photochem. Photobiol. B: Biol. 40: 29-48.

[38] Cadet et al. (1992), J Photchem Photobiol 15:277-298. Tornaletti et al. (1993), Oncogene 8:2051-2057.

[39] Ibbotson et al. (1998), J Invest Dermatol 110:79-83.

In this study, female SKH1(hr/hr) albino hairless mice, eight per group, were treated topically with benzoyl peroxide carbopol gel, 0, 1, 15 or 50 mg/ml, the same doses used in the 12-month photo co-carcinogenicity study (**Attachment 3**). Each dose of benzoyl peroxide carbopol gel was interacted with three fluences of UVB (290-320 nm), delivered from Kodacel filtered fluorescent tubes (Ho-90°, Elder Pharmaceuticals Inc.). The 12-week cumulative doses of UVB were 5.1 J/cm^2, 2.2 J/cm^2, and 0.9 J/cm^2. Animals were treated with benzoyl peroxide carbopol gel followed by UVB exposure, 5 days/week for 12 weeks.

At the end of the study, 24 hours after the last UVR-benzoyl peroxide carbopol gel exposure, mice were killed, and the treated skin was removed. Punch biopsies were taken, fixed in formalin, imbedded in paraffin, and sectioned. Sections of tissue were stained with hemotoxylin and eosin for counting epidermal layers (i.e., hyperplasia) and dyskaryotic (sunburn) cells in the epidermis. Additionally, sections were stained with resorcin-fuschin to count mast cells and evaluate elastosis in the dermis. The effect of benzoyl peroxide and UVR on collagen (measured as hydroxyproline using a colorometric assay), elastin (as desmosine using a radioimmunoasay), and glycosamino-glycans (GAGs) (as uronic acid by colorimetric assay) were evaluated.

Except for collagen, all epidermal and dermal markers of damage were increased by UVB exposure in a fluence (i.e., dose)-dependent manner. The skin damage produced by topical application of benzoyl peroxide was considerably less compared to the effects elicited by UVB. Specifically, treatment with benzoyl peroxide had no effect on measures of collagen, dermal thickness, mast cells, or sunburn cells. There was a modest, dose-dependent increase in epidermal thickness, consistent with previous studies in SKH1(hr/hr) albino hairless mice.[29] Elastin and GAGs were increased at only the highest dose, 50 mg/ml, of benzoyl peroxide carbopol gel.

In general, chronic treatment with benzoyl peroxide did not affect UVB-induced skin damage. Likewise, UVB exposure did not enhance the effects of benzoyl peroxide on measures of epidermal and dermal damage. This includes the number of sunburn cells, an indicator of UVR-induced DNA damage[40], which would most likely be increased if benzoyl peroxide and UVR were interacting. Treatment with benzoyl peroxide did have a modest inhibitory effect on the UVB-induced increase in elastin. The apparent lack of an interaction

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[40] Young (1986), *Photodermatol* 4:127-134. Bayerl *et al.* (1995), *Photodermatol Photoimmunol Photomed* 11: 149-154.

between UVB and benzoyl peroxide as evidenced by these studies may be related to the lack of absorption of these wavelengths by benzoyl peroxide.[39]

In conclusion, there was little or no evidence for an interaction between UVB radiation and benzoyl peroxide under the conditions of this study. Benzoyl peroxide in carbopol gel apparently is not photo-activated by UVB and does not affect UVB dose delivery in SKH1(hr/hr) albino hairless mice.

6.4. The Effects of Benzoyl Peroxide Carbopol Gel on Skin Tumor Formation in UVB-Initiated SKH1(hr/hr) Hairless Mice

A study was conducted in collaboration with Thomas Slaga, Ph.D., University of Texas, M.D. Anderson Cancer Center, to determine whether benzoyl peroxide in carbopol gel was a tumor promoter after initiation with UVB radiation in SKH1(hr/hr) albino hairless mice. In addition, a short-term marker of promotion, sustained epidermal hyperplasia, was measured following 8 weeks of treatment with benzoyl peroxide carbopol gel as a potential predictive measure of the outcome after 40 weeks of treatment. The study report is provided in **Attachment 6**.

There has been significant progress in understanding the multistage nature of carcinogenesis. The mouse skin model, which represents one of the best understood experimental models of multistage carcinogenesis, has permitted the resolution of three distinct stages: initiation, promotion, and progression.[41] Skin tumor promotion is characterized by selective and sustained hyperplasia, alterations in cellular differentiation, and genetic instability leading to the expansion of the initiated cells into skin papillomas and carcinomas. Histologic observations of skin from mice treated with tumor promoters have demonstrated that sustained cellular hyperplasia plays a critical role in promotion.[42] For organic peroxides, the induction of sustained hyperplasia is a necessary event for promotion of skin tumors in the mouse skin model of multistage carcinogenesis.[43]

In the present study, female SKH1(hr/hr) albino hairless mice were randomly assigned to one of the following treatment groups: (1) UVB/carbopol gel, (2) no UVB/carbopol gel, (3) UVB/1 mg/ml benzoyl peroxide, (4) no UVB/1 mg/ml benzoyl peroxide, (5) UVB/

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[41] Yuspa and Porkier (1988), *Adv Cancer Res* 50:25-69.

[42] Slaga *et al.* (1976), *JNCI* 57:1145-1149. Klein-Szanto and Slaga (1981), *Cancer Res* 41:4437-4440. Klein-Szanto and Slaga (1982), *J Invest Dermatol* 79:30-34.

[43] Gimenez-Conti *et al.* (1998), *Toxicol Appl Pharmacol* 149:73-79.

15 mg/ml benzoyl peroxide, (6) no UVB/15 mg/ml benzoyl peroxide, (7) UVB/50 mg/ml benzoyl peroxide, (8) no UVB/50 mg/ml benzoyl peroxide, (9) UVB/acetone, (10) no UVB/ acetone, (11) UVB/12-O-tetradecanoylphorbol-13-acetate (TPA), and (12) no UVB/TPA. The light source for UVB was a FS40 bulb filtered through cellulose acetate to yield a spectrum from 290 to 320 nm. In the initiation phase of this study, mice were exposed to a cumulative dose of 6 J/cm^2 of UVB divided evenly Monday through Friday for 6 weeks. Promotion with benzoyl peroxide or TPA was initiated following a 1-week rest period. Animals were administered benzoyl peroxide daily (Monday through Friday) for 40 weeks followed by a 12-week observation period. At 8 weeks of promotion, 10 mice per group were sacrificed for analysis of epidermal hyperplasia as a short-term marker of promotion.

In this study, the highest dose of benzoyl peroxide, 50 mg/ml, showed a slight trend for promotion of UV-initiated skin tumors compared to the carbopol vehicle treatment. However, the 50 mg/ml benzoyl peroxide response was equivalent to that with acetone and much less than with TPA. The number of tumors per mouse in each of the UVB/benzoyl peroxide treatment groups was not different from that in UVB/vehicle-treated mice. There were substantially more tumors in the UVB/TPA treatment group. After 8 weeks of treatment with benzoyl peroxide carbopol gel, there was only a very slight increase in measures of epidermal thickness. The slight increase observed in UVB/benzoyl peroxide-treated mice was equivalent to that observed in the UVB/acetone treatment group. In contrast, there was evidence of sustained hyperplasia in the UVB/TPA treatment group.

In conclusion, benzoyl peroxide had a very weak tumor promoting effect on UVB-initiated skin of SKH1(hr/hr) mice. These data are consistent with previously published studies investigating the tumor promoting effects of benzoyl peroxide in chemical- or UV-initiated mouse skin.

6.5.   The Effect of Repeated Application of 10% Benzoyl Peroxide Lotion in the Presence and Absence of Solar-Simulated Radiation on Human Skin

A clinical study was conducted at TKL Research, Inc., under the supervision of Alan R. Shalita, MD., State University of New York, to determine the effect of repeated application of 10% benzoyl peroxide lotion or its vehicle in the presence or absence of solar-simulated radiation on short-term markers of skin damage in human volunteers. The study report is provided in **Attachment 6**.

As noted previously, mechanistic studies of skin carcinogenesis have operationally identified multiple stages in the transformation of normal cells to tumors. These stages are initiation, irreversible damage to the gene; promotion, a reversible clonal expansion of initiated cells; and progression, transformation from a benign to a malignant tumor. Mouse skin carcinogenesis has been studied extensively in the context of a multistage model of cancer.[11] In this regard, skin tumor formation has been found to be dependent on dose and duration of exposure to the initiating and promoting agents. Repeated, topical application of acetone solutions of benzoyl peroxide have been extensively used to promote tumor formation in sensitive strains of mice after treatment with potent chemical initiators. The tumor promoting properties of benzoyl peroxide in rodents are thought to be mediated by oxidative damage produced from the formation of free radicals.

Short-term markers of skin tumor promotion have been the focus of numerous investigations. Several acute and subacute biological markers have been reported to correlate with tumor formation after repeated administration of a promoter.[44] Among short-term endpoints for benzoyl peroxide-induced tumor promotion, epidermal hyperplasia has been shown to be necessary in strains of mice responsive to benzoyl peroxide-induced tumor formation. Importantly, in species or strains of rodents resistant to skin tumor promotion, epidermal hyperplasia produced by promoters is not sustained.[45]

Although benzoyl peroxide and other organic peroxides have been found to be skin tumor promoters in select strains of mice, to date, the relevance of these findings with respect to humans is unclear. Because epidermal hyperplasia is necessary for skin tumor promotion in rodents, the present study was conducted to determine if a similar hyperplasia occurs in human skin after repeated exposure to benzoyl peroxide. Further, given the concern regarding application of benzoyl peroxide on sun-exposed skin, the effect of simultaneous exposure to benzoyl peroxide and solar-simulated radiation (SSR) was also investigated.

Fifteen healthy volunteers, ranging in age from 18 to 33 years, were recruited. Benzoyl peroxide (10%) or vehicle, carbopol gel, was applied to the lower back at 2 mg/cm^2 before or after exposure to half a minimal erythema dose (MED) of SSR daily, for 5 weeks. A punch biopsy was obtained 24 hours after the last treatment. Epidermal thickness and proliferating

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[44] Naito *et al.* (1987), *Carcinogenesis* 8:1807-1815. Gimenez-Conti *et al.* (1991), *Carcinogenesis* 12:563-569.
[45] Slaga *et al.* (1981), in Reviews in Biochemical Toxicology, Hodgson *et al.*, eds., Vol 3, pp 321-381. Slaga *et al.* (1982), *J Cell Biochem* 18:99-119.

cell nuclear antigen (PCNA) immunostaining were measured as markers of epidermal hyperplasia and cell hyperproliferation.

The incidence and severity of erythema were not affected by benzoyl peroxide or vehicle, in the presence or absence of SSR. Similarly, neither treatment with or without SSR affected any measure of epidermal hyperplasia or cell proliferation.

Repeated exposure to 10% benzoyl peroxide was found not to produce hyperplasia in human skin, consistent with earlier reports[46]. These data suggest that epidermal hyperplasia, a biological response necessary for skin tumor promotion in rodents, does not occur in humans treated repeatedly with benzoyl peroxide. Thus, under the conditions of this study, it appears that human skin responses are qualitatively different from those of strains of mice sensitive to benzoyl peroxide-induced skin tumor promotion.

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[46] Vasarinsh (1968), *Arch Dermatol* <u>98</u>:183-187. Plewig and Braun-Falco (1975), *Acta Dermatol (Stockholm)* <u>Suppl. 74</u>:7-98.

7. Summary and Conclusion

In 1991, the FDA changed the monograph status of benzoyl peroxide from Category I (generally recognized as safe and effective) to Category III (more data needed). This action was based on safety concerns regarding benzoyl peroxide as a tumor promoter in mice and a singular study suggesting complete carcinogenicity in mice. To address these concerns, the NDMA Benzoyl Peroxide Study Group worked cooperatively with FDA and conducted 2-year dermal carcinogenicity studies in B6C3F1 mice and F344 rats and a 12-month photo co-carcinogenicity study in SKH1(hr/hr) albino hairless mice.

To date, these studies have found:

- no evidence of cutaneous toxicity or carcinogenicity after 1 year of daily application of benzoyl peroxide carbopol gel in B6C3F1 mice and F344 rats at doses that meet the MTD;

- no evidence for enhancement of photocarcinogenesis by benzoyl peroxide carbopol gels in the SKH1(hr/hr) albino hairless mouse after repeated topical administration for 40 weeks; and

- collective support for the absence of an interaction between UVR and benzoyl peroxide in data from several investigative research studies.

Although benzoyl peroxide has been shown to be a skin tumor promoter in certain strains of mice, there is no scientifically established basis to use data from such studies for human risk assessment. Nonetheless, the FDA raised the theoretical concern that benzoyl peroxide may act as a tumor promoter or co-carcinogen in human skin exposed to UVR, a known carcinogen. Importantly, the photo co-carcinogenicity study, conducted at the request of the agency, showed no evidence for exacerbation by benzoyl peroxide of solar-simulated UVR-induced skin tumor formation in a highly sensitive mouse strain, a result that is consistent with previously published studies. Thus, even under exaggerated test conditions, no evidence of co-carcinogenicity was observed. These findings are consistent with investigative research studies conducted with leading authorities in photobiology, carcinogenicity and dermatology, and most important, with epidemiological evidence. Collectively, all these data fail to demonstrate a biologically significant interaction between UVR and benzoyl peroxide.

Topical benzoyl peroxide has been used for over 30 years in the treatment of acne with no reports of adverse effects that could be related to skin cancer. This positive clinical experience is supported by the results of epidemiological studies and chronic animal carcinogenicity studies.

And now the interim results from the 2-year dermal carcinogenicity studies in B6C3F1 mice and F344 rats presented in this update further uphold the conclusions from earlier studies.

In conclusion, acne treatments containing benzoyl peroxide pose no human health concerns above currently accepted standards for similar OTC drug products and should therefore be included in the Monograph as a Category I ingredient. Further, these products warrant no additional labeling warning against theoretical or rodent tumor promotion concerns.

BPrev.LT

2-26-99

List of Attachments

Attachment 1: Pathology Report, Dermal Oncogenicity Study of Benzoyl Peroxide in Rats (52-Week Interim Sacrifice), Covance Study No. 6711-101

Attachment 2: Pathology Report, Dermal Oncogenicity Study of Benzoyl Peroxide in Mice (52-week Interim Sacrifice), Covance Study No. 6711-100 [in same volume as Attachment 1]

Attachment 3: Final Report, 12-Month Topical Study to Determine the Influences of Benzoyl Peroxide on Photocarcinogenesis in Albino Hairless Mice Crl: SKH1(hr/hr)BR, Argus C-314-001 [in three volumes, which include Appendices A, B, and C to Attachment 3]

Attachment 4: Report on "Preparation of Gels Containing 0, 0.1, 1.5, and 5% (w/w) Benzoyl Peroxide for Use in a Photocarcinogenicity Study," MPI Research Study 657-012

Attachment 5a and b: Report on "Benzoyl Peroxide (BP): Species Comparisons of In Vitro Skin Penetration Following a Single Application to the Excised Skin of Rats, Mice and Humans," Bushy Run Research Center 92N1070 [in two volumes]

Attachment 6: Reports of Investigative Studies