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CONSUMER HEALTHCARE PRODUCTS ASSOCIATION®

August 6, 2002

Dockets Management Branch
Food and Drug Administration
5630 Fishers Lane
Rockville, Maryland 20852

Re: Docket No. 81N-0114: Topical Acne Drug Products
for Over-the-Counter Human Use

Dear Madam or Sir:

This letter accompanies three boxes, each of which contains a complete seven-volume amended final report, "Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats." These reports should replace the rat study reports that were sent to FDA on December 27, 2001, and subsequently found to contain the wrong Individual Anatomical Pathology Data (Appendix 9), which are contained in Volumes 4, 5, 6, and 7. A copy of the memorandum from the performing contract organization for the study, Covance Laboratories, Inc., regarding the Amendment 1 to Final Report for Study No. 6711-101 is also enclosed in each box. These Final Report Amendments, the accompanying Amendment 1 memoranda, and the enclosed three copies of a letter to Tia M. Frazier, Project Manager, Division of Over-the-Counter Drug Products, Food and Drug Administration (with an attached letter to Charles J. Ganley, M.D., dated December 27, 2001, when it was previously submitted to the docket) are for submission to the docket regarding over-the-counter acne drug products. Three complete sets of this submission from the Consumer Healthcare Products Association Benzoyl Peroxide Study Group were sent directly to Ms. Frazier.

Sincerely,

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

LT/ict
BP/Docket 8-02

81N-0114

RPTS



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August 6, 2002

Tia M. Frazier
Project Manager
Division of Over-the-Counter
Drug Products (HFD-560)
Office of Drug Evaluation V
Center for Drug Evaluation
and Research
Food and Drug Administration
9201 Corporate Boulevard
Rockville, Maryland 20850

Re: Docket No. 81N-0114: Topical Acne Drug Products
for Over-the-Counter Human Use

Dear Ms. Frazier:

This letter accompanies three boxes, each of which contains a complete seven-volume final report "Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats." These reports should replace the rat study reports that were sent to FDA on December 27, 2001, and subsequently found to contain the wrong Individual Anatomical Pathology Data (Appendix 9), which are contained in Volumes 4, 5, 6, and 7. A copy of the memorandum from the performing contract organization for the study, Covance Laboratories, Inc., regarding the Amendment 1 to Final Report for Study No. 6711-101 is also enclosed in each box.

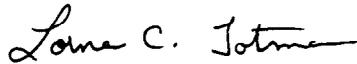
I regret that the error made by the contract laboratory in issuing the final report with the incorrect pathology data was not recognized before the report was sent to FDA. It is especially unfortunate that FDA reviewers were inconvenienced by not having complete and correct data. Results from the 2-year dermal carcinogenicity studies in F344 rats, as well as the results from a similar study in B6C3F1 mice, support the conclusion that benzoyl peroxide is not carcinogenic. Copies of the letter that was sent in December 2001 to Dr. Ganley with the reports are enclosed for the convenience of FDA reviewers. As stated in the letter, a careful consideration of the entire data base on benzoyl peroxide should lead to the decision to include benzoyl peroxide as a Category I ingredient in the Monograph for Topical Acne Drug Products.

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

August 6, 2002
Tia M. Frazier
Docket No. 81N-0114
Page 2 of 2

Please let me know if you or others at FDA have questions about the content of this submission or previous submissions by the association's Benzoyl Peroxide Study Group. The members of the study group would appreciate an opportunity to discuss the available data on benzoyl peroxide with FDA scientists. We look forward to full acceptance of benzoyl peroxide as a generally recognized safe and effective OTC drug ingredient.

Sincerely,



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

Enclosures: Final Report Amendment, "Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats" (7 volumes)

"Amendment 1 to Final Report" memorandum dated 06 May 2002

Letter to Ganley (FDA) from Totman (CHPA), December 27, 2001

LT/lct
BP/8-02



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CONSUMER HEALTHCARE PRODUCTS ASSOCIATION*

December 27, 2001

Charles J. Ganley, MD
Director, Division of Over-the Counter
Drug Products
Office of Drug Evaluation V
Center for Drug Evaluation and Research
9201 Corporate Boulevard
Rockville, MD 20850

Re: Docket No. 81N-0114

Dear Dr. Ganley:

Enclosed for FDA review are final reports on 2-year carcinogenicity studies with topically applied benzoyl peroxide gels in F344 rats and B6C3F1 mice submitted by the Benzoyl Peroxide Study Group of the Consumer Healthcare Products Association (CHPA; formerly the Nonprescription Drug Manufacturers Association). These studies show no evidence that topically applied benzoyl peroxide has any carcinogenic potential. These study results together with the extensive body of other data obtained over the past 30 years in humans and animals unequivocally support the safety of benzoyl peroxide as an active ingredient in acne treatments. We anticipate that benzoyl peroxide will be returned to Category I status, generally recognized as safe and effective, and included in the Monograph for Topical Acne Drug Products.

Background

Benzoyl peroxide had been included as a Category I ingredient in the Tentative Final Monograph (TFM) for Topical Acne Drug Products, which FDA published in January 1985.¹ In 1991, the agency changed the monograph status of benzoyl peroxide from Category I to Category III (more data needed). As stated in the preamble to the TFM amendment, which was published on August 7, 1991², FDA based this action on safety concerns regarding benzoyl peroxide as a tumor promoter in rodent studies and one study suggesting complete carcinogenicity in mice. 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.

¹ Food and Drug Administration, *Fed Reg* 50 2172-2182, Jan 15, 1985.

² Food and Drug Administration, *Fed Reg* 56.37622-37635, Aug 7, 1991

To address FDA's concerns, the CHPA Benzoyl Peroxide Study Group worked cooperatively with the agency and conducted 2-year dermal carcinogenicity studies in F344 rats and B6C3F1 mice, as well as a 1-year photo co-carcinogenicity study in SKH1(hr/hr) albino hairless mice. The final report on the photo co-carcinogenicity study was submitted to FDA on February 26, 1999.³ The key features of the 2-year dermal carcinogenicity studies and a summary of the results are presented in the following sections of this letter.

Conduct of Dermal Carcinogenicity Studies

Selection of Vehicle and Doses for 2-year Carcinogenicity Studies: The CHPA Benzoyl Peroxide Study Group selected the vehicle and doses of benzoyl peroxide on the basis of preliminary studies before conducting the 2-year chronic studies. Carbopol gel was selected as the most relevant and suitable vehicle for evaluation of benzoyl peroxide in the chronic studies based on consideration of human exposure and the results of several 14-day studies.⁴

The doses of benzoyl peroxide were selected for the 2-year carcinogenicity studies with F344 rats and B6C3F1 mice on the basis of results from 13-week studies.⁵ The doses selected for the 2-year studies were 25, 5, and 1 mg of benzoyl peroxide per day for B6C3F1 mice and 45, 15, and 3 mg of benzoyl peroxide per day for F344 rats. (The low dose for the rats was changed to 5 mg per day, as was recommended by FDA.) The criteria 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.⁶ 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. The top two doses represent the maximum (top dose) and near-maximum (mid-dose) skin effects observed in the 13-week studies. Moreover, the top two doses selected produced skin effects consistent with reaching but not exceeding the MTD and had no significant systemic effects. A full explanation of these data along with the study reports have been submitted to the agency.⁵ 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."⁷

³ NDMA "Update on Safety Studies with Benzoyl Peroxide," February 26, 1999, to FDA Docket No. 81N-0114.

⁴ 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.

⁵ Letter from NDMA, June 30, 1995, to FDA Docket No. 81N-0114. Nash *et al.* (1997), *The Toxicologist*

⁶ 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.

⁷ Letter from FDA (Dr. Gilbertson), Oct. 13, 1995, to NDMA

Study Design for 2-year Studies: The evaluation of the carcinogenic potential of benzoyl peroxide by the dermal route of exposure in male and female F344 rats and B6C3F1 mice was done with current state-of-the-art protocols. In brief, benzoyl peroxide carbopol gel was applied at doses of 5, 15, and 45 mg of benzoyl peroxide per rat and 1, 5, and 25 mg of benzoyl peroxide per mouse once daily for 104 weeks to a 3.5-x-5-cm and a 2-x-3-cm area on the dorsal skin of the rat and mouse, respectively. Discontinuous-treatment groups received the high dose of benzoyl peroxide, 45 mg per rat and 25 mg per mouse, for 52 weeks and the vehicle for the remainder of the study. Vehicle (i.e., carbopol gel) and no-treatment groups served as controls. The animals were sacrificed at 52 weeks (interim sacrifice) or 104 weeks, and complete necropsies were performed. A complete list of organs and tissues was collected for histopathologic evaluation.

Summary of Results

- In F344 rats, no findings indicative of oncogenicity resulted from daily topical application of benzoyl peroxide gel at doses up to 45 mg of benzoyl peroxide for 104 weeks.

Treatment with benzoyl peroxide gel had no effect on survival, body weights, food consumption or gross pathology, and produced no evidence of systemic toxicity. Survival at study termination in all groups ranged from 74% to 86% in males and from 72% to 88% in females, and was adequate to assess the carcinogenic potential of benzoyl peroxide. Microscopic evaluation revealed treatment-related findings confined to the site of application. Specific findings were mild-to-moderate degrees of hyperkeratosis, acanthosis, sebaceous gland hyperplasia, and chronic subepidermal inflammation in all treatment groups. These effects were observed in the interim-sacrifice groups and in the rats sacrificed at 104 weeks, and were consistent with reaching but not exceeding the MTD.

- In B6C3F1 mice, no findings indicative of oncogenicity resulted from topical application of benzoyl peroxide gel in any of the treatment groups.

The high dose (25 mg of benzoyl peroxide per day) exceeded the MTD, in that it caused treatment-site ulceration, and was lowered to 15 mg of benzoyl peroxide per day at week 57. Because ulceration also occurred in mice receiving 15 mg per day, treatment of the high-dose mice with benzoyl peroxide was suspended and vehicle was administered from week 93 until the end of the study, as agreed to by FDA.⁸ Benzoyl peroxide had no effect on survival, body

⁸ Letter from NDMA, April 23, 1997, to FDA Docket No. 81N-0114. Letter from FDA (Dr. Bowen), July 23, 1997, to NDMA. Letter from NDMA, Nov. 12, 1997, to FDA Docket No. 81N-0114. Letter from FDA (Dr. Bowen), Jan. 12, 1998, to NDMA. Letter from NDMA, Jan. 22, 1998, to FDA Docket No. 81N-0114. FDA memorandum to

weights, food consumption, or gross pathology, except for treatment-site ulceration in the high-dose group, and produced no evidence of systemic toxicity. Survival at study termination in all groups ranged from 71% to 88% in males and from 61% to 80% in females, and was adequate to assess the carcinogenic potential of benzoyl peroxide. Microscopic evaluation revealed treatment-related findings confined to the site of application. Specific findings were dose-dependent induction of acanthosis, hyperkeratosis, sebaceous gland hyperplasia and subepidermal inflammation. These findings were evident at the interim sacrifice as well as at the terminal sacrifice in groups continuously treated with benzoyl peroxide. Ulceration, which was observed grossly and confirmed microscopically, was consistent with the conclusion that the high dose exceeded the MTD. Gross and microscopic observations indicated that the mid-dose produced responses consistent with reaching but not exceeding the MTD.

Discussion of 2-year Studies with Benzoyl Peroxide Carbopol Gel

Based on these studies, it is concluded that benzoyl peroxide lacks carcinogenic potential. The absence of any skin tumors in F344 rats and B6C3F1 mice is particularly important considering the induction of chronic hyperplasia and other skin effects indicative of reaching the maximum tolerated dose in these studies. These data together with numerous published accounts of other chronic studies strongly support the conclusion that benzoyl peroxide is not carcinogenic.

The studies submitted herein, along with the results of a 12-month photo co-carcinogenicity study with albino hairless mice and several investigative studies that were previously submitted to FDA⁹, further support the safety of benzoyl peroxide. The results of the 12-month photo co-carcinogenicity study revealed no evidence for enhancement of photocarcinogenesis by benzoyl peroxide gels in SKH1(hr/hr) albino hairless mice. The highest dose of benzoyl peroxide gel (50 mg/ml) produced no enhancement, and there was a negative association between test article dose and UVR-induced skin tumor production. Under the conditions of this study, benzoyl peroxide in a carbopol gel vehicle is not a photocarcinogenic risk factor.

The photo co-carcinogenicity study results are supported by findings in several 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 along with the studies submitted herein

Dr. Bowen from Dr. Jacobs through Dr. Wilkin, Feb. 3, 1998, FDA Docket No. 81N-0114. Conference call between FDA Division of OTC Drug Products and NDMA, Feb. 18, 1998.

⁹ NDMA "Update on Safety Studies with Benzoyl Peroxide," February 26, 1999, to FDA Docket No. 81N-0114.

December 27, 2001
Charles J. Ganley, M.D.
Docket No. 81N-0114
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support the position 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 CHPA, which is supported by a large body of existing data and the judgment of professional organizations including the American Academy of Dermatology¹⁰ and experts in the field of toxicology, photobiology and cancer research.¹¹

Summary and Conclusion

The 2-year dermal carcinogenicity studies conducted by the CHPA Benzoyl Peroxide Study Group found no evidence of oncogenicity resulting from daily topical application of benzoyl peroxide carbopol gel in F344 rats and B6C3F1 mice at doses that meet the MTD. The study results support the conclusion that benzoyl peroxide is not carcinogenic. In addition, the 12-month photo co-carcinogenicity study, which also was conducted by the study group, showed no evidence for enhancement of photocarcinogenesis by benzoyl peroxide carbopol gels in SKH1-(hr/hr) albino hairless mice after repeated topical administration for 40 weeks.

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 (see NDMA "Update on Safety Studies with Benzoyl Peroxide," February 26, 1999, in FDA Docket No. 81N-0114). And now the results from the 2-year dermal carcinogenicity studies in F344 rats and B6C3F1 mice submitted with this letter further confirm the conclusions from earlier studies.

It is clear from a careful consideration of the entire data base that acne treatments containing benzoyl peroxide pose no human health concerns above currently accepted standards for similar OTC drug products. Benzoyl peroxide should therefore be included in the Monograph for Topical Acne Drug Products as a Category I ingredient. Further, given the clinical and experimental data from humans and animals, benzoyl peroxide acne treatment products warrant no additional labeling warning against theoretical or rodent tumor promotion or phototoxicological concerns.

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 the content of this submission. The members of the CHPA Benzoyl Peroxide Study Group would appreciate an opportunity to

¹⁰ Letter from Stephen B. Webster, M.D., President, American Academy of Dermatology, September 25, 1991, to William Gilbertson, Pharm.D., FDA.

¹¹ Letter from NDMA, July 2, 1990, to FDA Docket No. 81N-0114

December 27, 2001
Charles J. Ganley, M.D.
Docket No. 81N-0114
Page 6 of 6

discuss the available data on benzoyl peroxide with FDA scientists. We look forward to full acceptance of benzoyl peroxide as a generally recognized safe and effective OTC drug ingredient.

On behalf of the CHPA Benzoyl Peroxide Study Group,

Sincerely yours,



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

Enclosures: Final Report, "Dermal Oncogenicity Study of Benzoyl Peroxide Gels
in Rats" (7 volumes)

Final Report, "Dermal Oncogenicity Study of Benzoyl Peroxide Gels
in Mice" (7 volumes)

LT:lct/FDA
BP:letter 12-01



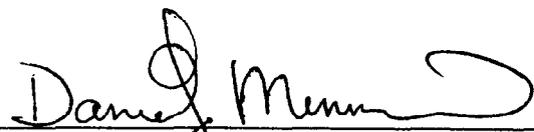
SPONSOR: Consumer Healthcare Products Association DATE: 06 May 2002

MATERIAL: Benzoyl peroxide gel

SUBJECT: AMENDMENT 1 TO FINAL REPORT
Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats
Study No. 6711-101

Page Nos. 1744-3795 are being submitted for incorporation into the final report dated 21 March 2000, to correct the Individual Anatomical Pathology Data (Appendix 9) due to the fact that the information from the benzoyl peroxide mouse study was inadvertently included in the rat study. In addition, the cover pages for Volumes 1-4 as well as the table of contents are being submitted to indicate correct pagination.

Study Director



Daniel J. Minnema, PhD
Department of Toxicology
Covance Laboratories Inc.

06 May 02
Date

Quality Assurance review of Amendment 1 to the final report was conducted on 01 May 2002, according to specified regulations, and findings were reported to the study director and management on 03 May 2002.



Gerald Faist
Quality Assurance Unit

06 May 02
Date



Sponsor:

Consumer Healthcare Products Association (CHPA)
1150 Connecticut Avenue, NW
Washington, D.C. 20036

FINAL REPORT

Study Title:

Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats

Author:

Daniel J. Minnema, PhD

Study Completion Date:

March 21, 2000

Final Report Amendment Completion Date:

May 6, 2002

Performing Laboratory:

Covance Laboratories Inc.
9200 Leesburg Pike
Vienna, Virginia 22182-1699

Laboratory Study Identification:

Covance 6711-101

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Sponsor:

Consumer Healthcare Products Association (CHPA)
1150 Connecticut Avenue, N.W.
Washington, D.C. 20036

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Daniel J. Minnema, PhD

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Covance Laboratories Inc.
9200 Leesburg Pike
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Volume 2 of 7

Page 460 of 3795

Sponsor:

Consumer Healthcare Products Association (CHPA)
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Volume 3 of 7

Page 1124 of 3795

Sponsor:

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Author:

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March 21, 2000

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May 6, 2002

Performing Laboratory:

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Vienna, Virginia 22182-1699

Laboratory Study Identification:

Covance 6711-101

Volume 4 of 7

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Final Report

Dermal Oncogenicity Study of Benzoyl
Peroxide Gels in Rats

PREPARED FOR:
Consumer Healthcare Products
Association (CHPA)

COVANCE STUDY NUMBER:
6711-101

VOLUME:
1 of 7



Sponsor:

Consumer Healthcare Products Association (CHPA)
1150 Connecticut Avenue, NW
Washington, D.C. 20036

FINAL REPORT

Study Title:

Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats

Author:

Daniel J. Minnema, PhD

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March 21, 2000

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Volume 1 of 7

Page 1 of 3795



Sponsor:

Consumer Healthcare Products Association (CHPA)
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Washington, D.C. 20036

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9200 Leesburg Pike
Vienna, Virginia 22182-1699

Laboratory Study Identification:

Covance 6711-101

Volume 1 of 7

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COMPLIANCE STATEMENT
Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats

This study, as performed by Covance Laboratories Inc. (Covance), was conducted in compliance with the Good Laboratory Practice Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58, issued December 22, 1978 (effective June 20, 1979), and with any applicable amendments, with the following exceptions:

Documentation of the first mortality check, morning temperature and humidity recordings, and cageside/postdose observation for February 27, 1998, was lost due to a computer systems failure. The loss of this information did not impact the integrity of the study.

Study Director:



Daniel J. Minnema, PhD
Department of Toxicology

March 21, 2000

Date

QUALITY ASSURANCE STATEMENT
Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats

Quality Assurance inspections and reviews of this study were conducted according to the standard operating procedures of the Quality Assurance Unit and according to the Food and Drug Administration Good Laboratory Practice Regulations as set forth in Title 21 of the United States Code of Federal Regulations, Part 58, issued December 22, 1978 (effective June 20, 1979) and with any applicable amendments. These inspections and reviews were performed and findings were reported to the Study Director and management as follows:

Dates of Inspection/Review	Dates Findings Reported	Inspector/Reviewer
Protocol Review: 03/11/96	03/11/96	Maloid/Grissinger
Inspection and/or Data Review: 03/12/96	03/13/96	Maloid/Grissinger
04/19,22-24/96	04/24/96	Cassell/Bland
06/12/96	06/12/96	Wingard
06/26-28/96	06/28/96	Mullet
09/25,26,30/96	09/26/96	Bland
9/26/96	9/26/96	Bland
12/9,12,13,17/96	12/24/96	Bland
03/12,27,28,31/97	03/12,31/97	Wingard
06/10,11,16/97	06/17/97	Wingard
09/18,30/97; 10/1-3/97	09/19/97; 10/3/97	Fitzpatrick
12/23,30,31/97; 01/02/98	01/02/98	Wingard
04/28-30/98; 05/01/98	05/01/98	Godfrey
Report and Data Review: 10/27-30;11/2-6,9-13,16-19/98	11/20/98	Godfrey
03/11-13,15,20/00	03/20/00	Bland
03/30,31/99; 04/3-6/99	03/21/00	Bland

Yois Cassell for _____ *3/21/00*
Desireé D. Bland Date Released
Quality Assurance Unit

STUDY IDENTIFICATION
Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats

Covance Study No.: 6711-101

Test Material: Benzoyl peroxide gel

Sponsor's Representative: Lorna C. Totman, PhD, DABT

Sponsor: Consumer Healthcare Products
Association (CHPA)
1150 Connecticut Avenue, NW
Washington, DC 20036
(202) 429-9260

Study Director: Daniel J. Minnema, PhD
Covance Laboratories Inc.
9200 Leesburg Pike
Vienna, Virginia 22182-1699
(703) 893-5400

Study Timetable

Study Initiation: March 5, 1996

Initiation of Dosing

 Males: March 12, 1996

 Females: March 13, 1996

Completion of Interim Necropsy: March 13, 1997

Completion of Terminal Necropsy: March 19, 1998

STUDY PERSONNEL
Dermal Oncogenicity Study of Benzoyl Peroxide Gels in Rats

Study Director:	Daniel J. Minnema, PhD
Toxicologist:	Marcia Rodwin, BA
Study Coordinator:	Amena S. Ali, BA
Veterinarian:	William E. Ridder, DVM, MS, PhD Robert L. Ridgway, DVM, Diplomate, ACLAM
Anatomical Pathologist:	Richard H. Cardy, DVM, DABT, Diplomate, ACVP
Biostatistician:	Ajit K. Thakur, PhD Cynthia Y. Liu, MA
Analytical Chemistry/ Dose Formulations Supervisor:	W. Mark Smyth, BS
Toxicology Operations Supervisor:	Nancy M. Centanni, MS, LATg Amy E. Wakefield, MS

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ABSTRACT

The purpose of this study was to evaluate the oncogenic potential of benzoyl peroxide gels when administered daily by topical application to the dorsal skin of rats for at least 104 weeks. Male and female CDF®(F-344)CrIBR rats were assigned to seven groups. Benzoyl peroxide in carbopol gel at concentrations of 1.67, 5.0, and 15.0% (5, 15, and 45 mg/rat/day) was applied topically once daily to a treatment area (approximately 3.5 x 5 cm) on the dorsal skin of rats in Groups 2, 3, and 4, respectively. Rats in Group 1 served as vehicle controls and received daily topical applications of the carbopol gel vehicle at a dose volume of 0.3 mL. Rats in Group 6 served as negative controls; the hair on the backs of these rats was clipped at the same intervals as the other rats on study; however, these rats were not treated. Sixty rats/sex were assigned to Groups 1, 2, 3, 4, and 6, with the first 10 rats/sex/group designated for interim sacrifice during Week 53 and the remaining 50 rats/sex/group designated for terminal sacrifice after 104 weeks of treatment. Fifty rats/sex in Group 5 served as recovery animals, in that they were treated with 15% benzoyl peroxide for 52 weeks, then treated with the vehicle for the remainder of the study. Twenty rats/sex in Group 7 served as sentinel animals for pathogen screening at Weeks 26, 52, 78, and 104.

Diet and water were provided *ad libitum*. Once weekly, each animal was removed from its cage and examined for abnormalities and signs of toxicity, specifically noting the location, size, and appearance of any grossly visible or palpable masses. The treated skin (or analogous site on the untreated control) was graded for irritation once weekly. Body weights were recorded weekly from Weeks 1 through 14 and every fourth week thereafter and at Weeks 53 and 105. Food consumption was measured and recorded weekly for Weeks 1 through 13 and every fourth week thereafter and at Weeks 52 and 104. Blood smears were prepared from all moribund-, interim-, and terminal-sacrifice animals for possible evaluation of hematopoietic neoplasia.

After 52 weeks (interim-sacrifice animals) or 104 weeks (terminal sacrifice animals) of treatment, the animals were anesthetized, weighed, exsanguinated, and necropsied. At necropsy, macroscopic observations were recorded, and selected tissues were collected and preserved. The liver, kidneys, and brain were weighed from all animals at interim sacrifice. Selected tissues (treated skin, untreated skin, and kidneys) were examined

microscopically from all interim-sacrifice rats (Groups 1, 2, 3, 4, and 6). All tissues were examined microscopically from all terminal-sacrifice rats in Groups 1, 2, 3, 4, and 6, whereas only treated and untreated skin was examined microscopically from the rats in Group 5. Tumors were statistically analyzed separately and combined for relationship to treatment.

Treatment had no effect on: survival, body weights, food consumption, clinical observations, application site findings, gross pathology findings and organ weight changes at the interim sacrifice and gross pathology findings at terminal sacrifice.

Microscopic evaluation revealed that the only treatment-related histopathologic findings noted at both the interim and terminal sacrifices were those seen at the site of topical dermal exposure. These consisted of hyperkeratosis, acanthosis, sebaceous gland hyperplasia, and chronic subepidermal inflammation. These changes occurred in all treatment groups (1.67, 5, and 15% benzoyl peroxide) and were mostly of a minimal to mild degree of severity. They progressed only slightly between 52 and 104 weeks of exposure. For the recovery animals (52 weeks of treatment with 15% benzoyl peroxide followed by 52 weeks of vehicle), hyperkeratosis was present approximately twice as frequently as it was in either the vehicle or negative control group, but less than half as frequently as in any other treatment group. Acanthosis was not appreciably increased in the recovery animals as compared to controls, and sebaceous gland hyperplasia was only equivocally present. These findings indicated good, although not complete, recovery from the effects of 52 weeks of treatment with 15% benzoyl peroxide, with remnants of hyperkeratosis remaining after 52 weeks of recovery.

In conclusion, under the conditions of the study, there were no findings indicative of oncogenicity resulting from daily topical exposure of rats to benzoyl peroxide gels at concentrations up to 15% (dose level of 45 mg/rat/day) for 104 consecutive weeks.

PURPOSE

This study was designed to evaluate the oncogenic potential of benzoyl peroxide gels when administered daily by topical application to the dorsal skin of rats for at least 104 weeks.

REGULATORY COMPLIANCE

The study, as performed by Covance, was conducted in compliance with the Good Laboratory Practice Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58, issued December 22, 1978 (effective June 20, 1979), and with any applicable amendments, and with the following exception:

Documentation of the first mortality check, morning temperature and humidity recordings, and cageside/postdose observation for February 27, 1998, was lost due to a computer systems failure. The loss of this information did not impact the integrity of the study.

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Covance.

TEST AND VEHICLE/CONTROL MATERIALS

Test and Control Articles

The test material, Benzoyl peroxide gel [prepared with hydrous benzoyl peroxide USP grade (BP-USP), CAS 94-36-0, and aqueous carbopol gel], as well as the control material, aqueous carbopol gel, were received from MPI Research, Mattawan, Michigan, in several shipments and stored frozen at -20°C. The benzoyl peroxide test articles were described as smooth white gels and the control article was described as smooth clear gel.

Concentrations of 0, 1.67, 5.0, and 15.0% BP were received from the supplier. The concentration of the benzoyl peroxide in the gels takes into account the 30% water

content of the neat (BP-USP) material. Information on the methods of preparation and stability, as well as data on composition or other characteristics which define the test material, is on file with the Sponsor.

Reserve (Archive) Samples

Reserve samples of the test and control articles (two jars at each BP concentration) were taken from each batch used on study and stored frozen at -20°C. The samples were subsequently discarded. The residual test material was stored frozen at -20°C.

TEST ANIMALS AND HUSBANDRY

Eight hundred forty (420/sex), approximately 4-week-old, CDF@(F-344)CrIBR rats were received on February 27, 1996, from Charles River Laboratories, Inc., Raleigh, North Carolina. They were assigned temporary numbers, acclimated to laboratory conditions for approximately 2 weeks, and released for study use by a staff veterinarian.

During the acclimation period, five rats/sex were randomly selected and screened for viral and bacterial pathogens. Rats were examined for the presence of ectoparasites and pinworms. Serology tests (as indicated in the protocol) were performed by Microbiological Associates, Inc., Rockville, Maryland. The following organs were collected and preserved in neutral-buffered formalin: trachea, lung, heart, liver, kidneys, stomach, skin, intestines, spleen, brain, salivary glands, eyes, and pancreas.

Housing. Upon receipt, animals of the same sex were housed two/cage in stainless-steel, hanging, wire-mesh cages measuring 24.2 x 22.0 x 17.3 cm (d x w x h) as specified in the raw data. Following assignment to study, each animal was individually housed in one of two animal rooms (males in one room, females in one room). To allow for homogeneous exposure to the room environment, racks were rotated in a clockwise fashion around the room once every 2 weeks. In addition, cages were rotated vertically once every 2 weeks, and racks were rotated 180° once every week.

Diet. PMI® Certified Rodent Diet® #5002 was available *ad libitum* during the acclimation and study periods, unless otherwise noted. The diet was analyzed by the manufacturer for nutritional components and environmental contaminants.

Water. Tap water, via an automatic watering system, was available *ad libitum*. Samples of the water were routinely analyzed for specified microorganisms and environmental contaminants.

Contaminants. Results of the diet and water analyses are reviewed for compliance and are on file at Covance-Vienna.

No contaminants were known to be present in the diet or water at levels which might interfere with this study.

Environmental Conditions. The temperature and relative humidity in the animal rooms were monitored at least once daily and set to maintain temperatures at $22 \pm 3^{\circ}\text{C}$ with a relative humidity of $55 \pm 15\%$. Ten or greater air changes/hour and a 12-hour light/12-hour dark cycle (lights on approximately 0600 to 1800 hours) were maintained. Variations from these conditions are documented in the data and are considered to have had no effect on the outcome of the study.

Justification of Species. The rat historically has been used in safety evaluation studies and is recommended by appropriate regulatory agencies.

METHODS

This study was conducted in accordance with Covance Protocol 6711-101 and any protocol amendments. The protocol and protocol amendments are presented in Appendix 1. The protocol deviations are presented in Appendix 2.

Group Designations and Dose Levels

Animals were initially accepted into the randomization pool based upon physical examinations; animals with findings were eliminated from the randomization pool. The

animals were randomized into eight groups/sex using a computerized weight-randomization program, with the eighth group serving as possible replacement animals. The computerized weight-randomization program first eliminated the animals with extreme body weights, then selected the random assignment that produced homogeneity of variance and means by Bartlett's Test (1937) and One-Way Analysis of Variance (ANOVA). At randomization, the weight variation of the animals selected did not exceed ± 2 standard deviations of the mean body weight for each sex, and the mean body weight for each group of each sex was not statistically different. During the randomization process, each study animal was assigned a unique number and individually housed. A microidentification device implanted subcutaneously was used to identify each animal. Animals not used on study were removed from the study room. Animals were assigned to study groups as follows:

Group	Dosage mg/rat/day	Concentration % BP ^b	Number of Animals		Animal Numbers	
			Male	Female	Male	Female
1 (Vehicle/Control)	0	0	60 ^a	60 ^a	B73500-B73559	B73560-B73619
2 (Low)	5	1.67	60 ^a	60 ^a	B73620-B73679	B73680-B73739
3 (Mid)	15	5.0	60 ^a	60 ^a	B73740-B73799	B73800-B73859
4 (High)	45	15.0	60 ^a	60 ^a	B73860-B73919	B73920-B73979
5 (High-Discontinued)	45 ^c	15.0	50	50	B73980-B74029	B74030-B74079
6 (Untreated controls)	-	-	60 ^a	60 ^a	B74080-B74139	B74140-B74199
7 (Sentinels) ^d	-	-	20	20	B74200-B74219	B74220-B74239

^a The first 10 animals/sex were designated as Interim-Sacrifice rats and sacrificed after 52 weeks of treatment.

^b Percent benzoyl peroxide in carbopol gel, corrected for water content.

^c Animals were treated with the high-dose test article for 52 weeks and treated with vehicle for the following 52 weeks.

^d Sentinel animals did not receive treatment. Blood was collected from five predesignated animals/sex during Weeks 26, 52, 78, and 104. Sera were used for pathogen screening.

At initiation of dosing, the animals were approximately 6 weeks of age with body weights ranging from 94 to 130 g for the males and 80 to 106 g for the females.

Reason for Dosage Design

The dermal route is the intended route of human exposure.

DOSING PROCEDURES

Dose Preparation

Gel suspensions were provided by the Sponsor. The frozen test article and vehicle/control were thawed in a water bath at approximately 25°C and used within 8 hours after removal from the freezer. Any test article remaining after dosing each day was discarded.

Method of Administration

The test or control material was topically administered to the dorsal skin of animals in Groups 1-4, 7 days a week, for at least 104 weeks until the day before necropsy. The test material was topically administered for 52 weeks to animals in Group 5, followed by topical administration of the vehicle for the remaining 52 weeks. The appropriate test material or vehicle/control was applied to a standard area (approximately 3.5 x 5 cm) of dorsal skin at the intrascapular region. At least 24 hours prior to the first dose and weekly thereafter (as needed), an area of dorsal skin, which was larger than but included the application site, was clipped free of hair to allow uniform application of doses and clear observation of the application site. Group 6 rats (untreated controls) were also clipped, but not treated. The location of any skin nicks were noted and mapped on a diagram. The skin was carefully examined before clipping to detect and avoid removing tumors.

Dosing suspensions were applied at fixed volumes of 0.3 mL per application. Templates were used to aid in defining the application site and to help ensure that the test article was reproducibly applied to approximately the same area of skin. Positive displacement pipettes (Eppendorf Repeater Pipette 4780), one dedicated for each dose level, were used to deliver the dose, and the doses were evenly distributed over the application site with a glass rod (one rod dedicated for each group and wiped between each animal).

Dose Analyses and Stability Testing

The concentration of the test material was determined throughout the course of the study. The methods used to select the test material for analysis and the procedures used to

measure the concentration of benzoyl peroxide in the cabopol gel are outlined in Attachment 4 in the protocol.

Routine Concentration. Routine analyses were conducted by Covance using the U.S.P. Official Monographs XXII method validated at Covance as described in Attachment #4 of the protocol (and as amended in Amendment 2 to the protocol). Two jars were taken from each concentration batch that was used on study. Every 6 months, based on the study timeline, analyses were conducted on each batch of each concentration of test and control material used or in use during the previous 6 months. These analyses were performed in conjunction with analyses from Covance Study 6711-100 in the mouse.

Observation of Animals (Groups 1-6)

Clinical Observations. The rats were observed twice daily (a.m. and p.m., at least 6 hours between observations) for evidence of mortality and moribundity.

Daily cageside observations for obvious indications of a toxic effect were recorded as they were observed, noting only those animals for which an observation was made.

Once before treatment and weekly thereafter, each animal was removed from its cage and examined. The time of onset, location, size (small or large), appearance, and progression of each grossly visible or palpable mass occurring at sites other than the application site were recorded. Additionally, the treated skin or analogous site on the untreated control was graded (prior to dosing) for irritation according to the scale in Attachment #2 of the protocol. Special attention was paid to mass development and to skin conditions indicative of exceeding the maximum tolerated dose (see Attachment #3 of the protocol). The following information on each grossly visible skin lesion or mass within the application site was recorded: time of onset, location, size (to the nearest mm), appearance, and progression.

Body Weights. Body weights were recorded at randomization, weekly from Weeks 1 through 14 and every fourth week thereafter, and at Weeks 53 and 105.

Food Consumption. Food consumption was measured and recorded weekly for Weeks 1 through 13 and every fourth week thereafter and at Weeks 52 and 104. When obvious spillage was recorded for an animal during the detailed physical examination, the estimate of the food consumed by the animal was excluded from the group mean calculation for that particular interval.

Procedures for Animal Disease Screening - Sentinel Animals (Group 7)

Blood was collected during Weeks 26, 52, 78, and 104 from five surviving predesignated animals per sex under carbon dioxide/oxygen inhalation via the orbital plexus.

The only procedures performed on Group 7 animals were the collection of body weight data to be used for assignment to group, twice-daily observation for mortality and moribundity, and a complete necropsy on unscheduled deaths.

POSTMORTEM PROCEDURES

Clinical Pathology

Blood smears were prepared from all moribund-, interim-, and terminal-sacrifice animals. Blood samples were collected by puncture of the orbital plexus, using carbon dioxide/oxygen inhalation for anesthesia and EDTA as anticoagulant, air-dried, fixed in methanol, and stained with Wright-Giemsa.

Necropsy

All animals which were found dead or sacrificed *in extremis* during the study were subjected to a gross postmortem examination. The first 10 surviving predesignated rats/sex in Groups 1, 2, 3, 4, and 6 were sacrificed following 52 weeks of treatment. The remaining animals were sacrificed during Weeks 105 and 106. Each animal was weighed on the day of scheduled necropsy, anesthetized with sodium pentobarbital, and exsanguinated. A necropsy was performed on each animal by appropriately trained personnel using procedures approved by board-certified pathologists, and all findings were recorded. Each scheduled necropsy was performed under the direct supervision of a veterinary pathologist. The necropsy included examination of the following:

all orifices

carcass

cranial cavity

external surface of the brain; the external surface of the spinal cord and cut surfaces of the brain and spinal cord were examined whenever tissue trimming was performed.

cervical tissues and organs

thoracic, abdominal and pelvic cavities and their viscera

external body surface

nasal cavity and paranasal sinuses

Organ Weights

The liver, kidneys, and brain were weighed after careful dissection and trimming of fat and other contiguous tissue from all animals at interim sacrifice. Organ weight, organ/body weight, and organ/brain weight data were analyzed.

Tissue Preservation

The following tissues (when present) from all animals were preserved in 10% neutral-buffered formalin:

aorta	mammary gland with skin (males and females) ^b
adrenals	mid-thoracic spinal cord
bone marrow (femur, sternum)	nasal cavity and nasal turbinates
brain with brainstem (medulla/pons, cerebellar cortex, and cerebral cortex)	ovaries
cervical spinal cord	pancreas
clitoral gland	pituitary
colon, cecum, rectum	preputial gland
duodenum, jejunum, ileum	prostate
esophagus	salivary glands (mandibular)
eyes including optic nerve with contiguous Harderian gland	sciatic nerve
femur including articular surface	seminal vesicles
heart	skeletal muscle (thigh)
kidneys	skin [treated and untreated (left hip)]
lacrimal gland (exorbital) (2)	spleen
larynx	sternum
lesions	stomach (glandular and nonglandular)
liver (collected whole; left lateral, right lateral and median lobes examined microscopically)	testes with epididymides
lumbar spinal cord	thymus ^b
lungs with bronchi	thyroid (parathyroids ^b)
lymph nodes; mandibular, mesenteric and regional when applicable ^a	tissue masses
	tongue
	trachea
	ureters
	urinary bladder
	uterus with vagina and cervix
	Zymbal's gland (auditory sebaceous gland) (2)

^a When a tissue mass was present, the lymph node draining the region of the mass was examined.

^b At times, these tissues cannot be identified with the unaided eye because of physiological variation in size. However, tissues from the region were fixed.

Skin specimens were free of artifacts and oriented to permit evaluations of epidermal, dermal, and folliculosebaceous units. Sections of skin were taken from the site of application with respect to the longitudinal axis of the animal and included subcutis and muscular layer for complete examination.

Samples of skin taken at necropsy were flattened on a piece of paper board, gently stretched to remove wrinkles, and adequately fixed in formalin before trimming. Skin for sectioning was trimmed from the center of the application site or from the analogous site on untreated controls, maintaining the orientation to the longitudinal axis. Skin masses were trimmed with surrounding normal skin.

Photographs (using color-slide film) were taken of gross skin lesions that were representative of the findings and were archived with the raw data and other study-related records.

Histopathology

At the interim sacrifice, all preserved tissues were collected from rats designated for interim sacrifice and processed. Skin from the application site, untreated skin, and kidneys were processed and examined microscopically from all rats in Groups 1 through 4 and Group 6. At the terminal sacrifice, all tissues were processed and examined microscopically from all rats in Groups 1, 2, 3, 4, and 6. All tissues were collected from Group 5 animals at terminal sacrifice, but only treated and untreated skin was examined microscopically. Microscopic examinations were conducted by a board-certified veterinary pathologist. Also, tissues from animals found dead or sacrificed moribund were also examined microscopically.

Statistical Analyses

In-life and Organ Weight Data. Body weight, body weight gain, food consumption, and organ weight, organ/body weight, and organ/brain weight data were analyzed separately for each sex. Statistical analyses included both normal distribution and distribution-free techniques. The treated groups (Groups 2, 3, 4, and 5) were compared only to the vehicle/control group (Group 1). The vehicle control group was compared to

the untreated control group (Group 6). If Levene's test (Levene, 1960) for homogeneity of variance was not significant, comparisons with the control groups were based on Dunnett's t-test. If Levene's test was significant, these comparisons were based on the Wilcoxon rank sum test.

Histopathology Data. Skin histopathology responses that were binary or graded were analyzed by nonparametric methods for each sex separately. When skin responses were binary, a Fisher's exact test was used to compare each treated group to controls. Dose response was evaluated with an exact form of the Cochran-Armitage test, in which scores were equal to treatment group dose. In the case of graded response data, comparisons were based upon tests of linear-by-linear association (LxL test; see Agresti, 1990). The graded responses were assigned equally spaced scores. The groups were given scores equal to their administered dose. A dose-response test is obtained when all groups are included in the LxL test. Pairwise comparisons to control were conducted by including only the appropriate groups in the LxL test. All statistical tests were conducted at a 5% two-sided risk level. Significance at the 1% P values will also be reported.

Analysis of tumor incidence data was performed mostly as described by Huff et al. (1988), with the exception that more recently the NTP has adopted the logistic regression procedure of Dinse and Lagakos (1983) for the analysis of incidental tumors. The procedures to be used thus included life table tests, logistic regression tests, Fisher's exact tests, and Cochran-Armitage trend tests. These are described below in detail.

Survival Analysis. The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and presented in graphical form. Animals were censored from the survival analyses at the time they are found to be missing or dead from other than natural causes; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences are detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves can be first detected. All reported P values for the survival analysis were two-sided.

Calculation of Incidence. The incidence of neoplastic or nonneoplastic lesions was given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators included only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) before histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consisted of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence. Three statistical methods were used to analyze tumor incidence data: life table tests, incidental tumor analyses, and Fisher exact/Cochran-Armitage trend analyses. Tests of significance included pairwise comparisons of dosed groups with vehicle controls and tests for overall dose-response trends. If administration of the study compound had little effect on survival, the results of the three alternative analyses were generally similar. If differing results were obtained by the three methods, the final interpretation of the data depended on the extent to which the tumor under consideration was regarded as being the cause of death.

Life Table Analyses: This method of analysis assumes that all tumors of a given type observed in animals dying before the end of the studies were fatal, i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and vehicle control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were combined by the Mantel-Haenszel (1959) method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time of death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analyses: This method assumes that the neoplasms observed in animals dying before the end of the studies were incidental, that is, merely observed at necropsy in animals dying of an unrelated cause.

In the logistic regression approach, as described in Dinse and Lagakos (1983) and in Dinse and Haseman (1986), the proportions of tumor-bearing animals are modeled as a function of the age at which the animals died, and the dose to which it was exposed. The final model used may be a polynomial in age, although Dinse and Haseman (1986) states that when the tumor is not reversible and not fatal, the linear in age model should be adequate.

Fisher Exact/Cochran-Armitage Trend Analyses: In addition to survival-adjusted methods, the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage 1971, Gart et al. 1979) were used. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

Tests of significance included pairwise comparisons of each dosed group with vehicle controls and a test for an overall dose-response trend. Continuity corrected tests were used in the analysis of tumor incidence, when appropriate, and reported P values were one-sided.

Record Retention

All raw data, documentation, records, and reports generated by the Sponsor or other designees (i.e., for work not performed by Covance-Vienna) were the responsibility of the Sponsor. All paper raw data, documentation, records, protocol, specimens, and the final report generated as a result of this study will be archived in the storage facilities of Covance-Vienna for a period of 1 year following submission of the final report. One year after submission of the final report, all of the aforementioned materials will be sent to the Sponsor. The Sponsor may elect to have the materials retained in the Covance Archives for an additional period of time. All raw data stored on magnetic media will be retained by Covance.

The examination of sera was performed by Microbiological Associates, Inc., of Rockville, Maryland. The test results (in tabular form) were provided to Covance by Microbiological Associates; the raw data will be maintained at Microbiological Associates. Microbiological Associates provided Covance with documentation of GLP compliance.

RESULTS

Dose Analyses and Stability Testing

Results of routine concentration analyses are presented in Table 1.

Results of routine concentration analyses indicated that all values for benzoyl peroxide concentration were within 10% of target for all dose levels.

Disease Screening - Sentinel Animals (Group 7)

Blood was collected during Weeks 26, 52, 78, and 104 from five surviving predesignated animals per sex under carbon dioxide/oxygen inhalation via the orbital plexus. Rats were examined for the presence of ectoparasites and pinworms. Serology tests were performed by the same laboratory and in the same method as outlined in the quarantine section. With the exception of the presence of pinworms, there was no evidence of infection from the repeated serological testing

In-life Observations

Survival. Adjusted survival through Week 104 is presented in Table 2 and depicted graphically in Figure 1; individual animal disposition is presented in Appendix 3. Results of the survival analysis are further discussed in the Statistical Report.

There were no treatment-related effects on survival. Statistical evaluation of survival data revealed no significant positive or negative trend in the male or female mortality, nor were there significant group comparisons observed in either sex. Survival rates through Week 52 (prior to the Week 53 interim sacrifice) were 100% for Group 1, 3, 4, and 5

males, 98% for Group 2 and 6 males, 100% for Group 1-4 and 6 females, and 94% for Group 5 females. Group 1-6 survival rates through Week 104 were 74, 75, 78, 80, 86, and 74% for the males and 84, 78, 88, 88, 72, and 78% for the females, respectively.

Clinical Observations. Daily cageside and weekly physical observations are summarized in Table 3 and presented individually in Appendix 4. (Observations at the dermal application site are presented below.)

There were no treatment-related clinical observations. In general, the findings observed occurred sporadically and/or were of the type commonly seen in this species at this laboratory. There were no distinct or pronounced test material-related differences between the vehicle control (Group 1) and test groups (Groups 2, 3, 4, and 5) or between the untreated group (Group 6) and the test material groups.

Application Site Observations. Dermal observations based on the scale for evaluating skin reactions are presented in Table 4 and individually in Appendix 5. Individual tracking and sizing of application site masses are presented in Text Table 1.

Dermal Irritation: Incidences of slight erythema were seen in both treated and vehicle control animals. Moderate erythema was noted only in two rats (one male and one female) that received 15% benzoyl peroxide. However, due to the low incidence (2 of 120 rats), this finding cannot be definitely attributed to treatment. The incidence and extent of test material accumulation at the application site was dose-related.

Dermal Masses: Masses at the application site were observed in males only. As summarized in Text Table 1, masses were noted only in the Group 4 (continuous treatment with 15% benzoyl peroxide), Group 5 (discontinuous 15% benzoyl peroxide), and Group 6 (negative control) animals. These findings are not considered treatment-related since the negative control (untreated) animals exhibited the greatest incidence of findings.

Text Table 1
Summary of Masses at the Application Site

Group	Males											
	1	2	3	4			5		6			
Dose Level (%)	0	1.67	5	15			15/0		-			
Number of animals	60	60	60	60			50		60			
Animals with masses	0	0	0	2			1		5			
				<u>Rat No.</u>	<u>Mass</u>	<u>Week</u>	<u>Rat No.</u>	<u>Mass</u>	<u>Week</u>	<u>Rat No.</u>	<u>Mass</u>	<u>Week</u>
				B73902	1	91	B74017	1	57	B74093	1	57
				B73917	1	99		2	99	B74094	1	81
										B74121	1	77
										B74126	1	86
										B74133	1	90

This table identifies those rats exhibiting masses at the application site (Rat No.) and the week (Week) during which the mass (Mass) was initially observed. All animals with masses at the application site survived to terminal sacrifice except for Rat No. B74126.

Body Weights. Mean body weight data are presented in Table 5 and body weight change data are presented in Table 6. Individual body weight and body weight change data are presented in Appendices 6 and 7, respectively.

There were no treatment-related differences in body weights and/or body weight changes. Mean body weight change values fluctuated (sometimes significantly) from the corresponding control values, with no apparent pattern. The mean overall (Weeks 1-104) body weight change values were similar among the groups, with the exception of the value for Group 4 females which was significantly decreased versus vehicle control. Although the Group 4 value was approximately 6% lower than the control (Group 1) value, this finding is not considered treatment-related.

Food Consumption. Mean food consumption data are presented in Table 7. Individual food consumption data are presented in Appendix 8.

There were no treatment-related changes in food consumption. Mean food consumption values for treated males and females were occasionally significantly increased from the control (Group 1) values. The only significant decreases were noted at Week 2 for Group 4 and 5 males. The overall mean food consumption values for Group 3 and 4 females

were significantly increased from the control value. When no significant differences occurred, the mean food consumption values were generally similar among the groups.

Anatomical Pathology

Mean organ weights from the interim sacrifice are summarized in Table 8. Gross pathology findings are summarized in Tables 9A (unscheduled deaths), 9B (interim sacrifice), 9C (terminal sacrifice), and 9D (all deaths). Microscopic findings are summarized in Tables 10A (unscheduled deaths), 10B (interim sacrifice), 10C (terminal sacrifice), 10D (all deaths), and 10E (neoplasms), and expanded findings for treated and untreated skin are found in corresponding Tables 11A-11D. All individual data are presented in Appendix 9. Findings are further discussed in the Pathology Report and mathematically analyzed in the Statistical Report.

Organ Weights. Mean terminal body weight, absolute organ weight, organ-to-body weight percentage, and organ-to-brain weight ratio values for the interim-sacrifice animals were similar among all treatment groups.

Gross Pathology - Unscheduled Deaths. Gross pathology findings for animals with unscheduled deaths included but were not limited to, indented ventral surface of the brain, enlarged pituitary gland, enlarged spleen, granular/pitted/rough liver, dark kidneys, and dark areas on the stomach for both males and females. Gross pathology findings for male animals also included unequally sized, small, soft testis filled with dark or pale material, and small seminal vesicles. None of these findings occurred with any apparent pattern and are not considered treatment-related.

Histopathology - Unscheduled Deaths. The most commonly cited causes of early death, including animals that were sacrificed *in extremis*, were hematopoietic neoplasia (mostly mononuclear cell leukemia) and pituitary neoplasia. Both findings are prevalent in normal populations of F-344 rats as they age, and both were randomly scattered among exposure groups within this study.

Gross Pathology - Interim Sacrifice. There were no treatment-related necropsy findings noted at the interim sacrifice. Several females in Groups 1,2,3 and 6 were noted with ovarian cysts, fluid in the uterus, and/or distended uteri; these findings occurred with no

apparent pattern. Other observations for the males and females were generally unremarkable.

Histopathology - Interim Sacrifice. Microscopic evaluation revealed that the only treatment-related histopathologic findings noted were those observed at the application site. These treatment-related skin findings consisted of hyperkeratosis, acanthosis, sebaceous gland hyperplasia, and chronic subepidermal inflammation. These findings were observed in male and female test article treatment groups (Groups 2, 3 and 4) with the severity of findings increased at the higher dose levels. Changes were mostly of a minimal to mild degree of severity. Evidence of hyperkeratosis was observed in a few vehicle control (Group 1) and untreated control (Group 6) animals. Histopathologic changes observed in the kidneys were all of the kinds usually encountered in normal populations of rats of this strain and age and were not related to treatment.

Gross Pathology - Terminal Sacrifice. There were no treatment-related necropsy findings noted at the terminal sacrifice. The most prevalent findings among terminal-sacrifice animals included enlarged spleen, enlarged, mottled, and/or darkened pituitary, distended colon, and/or distended cecum among both males and females. Males also showed a high prevalence of small prostate, small seminal vesicles, and unequally sized or enlarged testes commonly filled with dark or pale material. These findings were comparable across all treatment groups and are not considered treatment-related.

Histopathology - Terminal Sacrifice. Aside from expected increases in normally occurring age-related background changes, the findings after 104 weeks of exposure were similar to those that occurred after 52 weeks. Microscopic evaluation revealed that the only treatment-related histopathologic findings noted were those seen at the application site. These consisted of hyperkeratosis, acanthosis, sebaceous gland hyperplasia, and chronic subepidermal inflammation. As with the interim-sacrifice animals, these changes occurred in male and female test article treatment groups (Groups 2, 3 and 4) and were mostly of a minimal to mild degree of severity. Also consistent with the interim-sacrifice animals, hyperkeratosis was noted in a few vehicle control (Group 1) and untreated control (Group 6) animals. These changes progressed only slightly between 52 and 104 weeks of exposure.

For the Group 5 animals (52 weeks of treatment with 15% benzoyl peroxide followed by 52 weeks of recovery/vehicle), hyperkeratosis was present approximately twice as frequently as it was in either control group (Groups 1 and 6), but less than half as frequently as in any other group, including that group exposed to the lowest level (1.67% benzoyl peroxide) for either 52 or 104 weeks. Acanthosis was not appreciably increased in the recovery group compared to controls, and the finding of sebaceous gland hyperplasia in Group 5 was equivocal. These results, in general, imply good, although not complete, recovery from the effects of 52 weeks of treatment at the high-dose level, with remnants of hyperkeratosis remaining after 52 weeks of recovery.

There were no histologic findings suggestive of any toxic or oncogenic effects from exposure to benzoyl peroxide gel. Although some of the neoplastic findings (e.g. large granular lymphocytic leukemia in females) showed borderline significance at the $p \leq 0.05$ level, they were of inconsistent nature (i.e., nonmonotonic) characteristic of aging F-344 rats, and do not suggest a carcinogenic response. Also, in the specific case of large granular lymphocytic leukemia, the highest incidence in the treated animals was similar to that in untreated controls.

DISCUSSION AND CONCLUSION

The purpose of this study was to evaluate the oncogenic potential of benzoyl peroxide gels when administered daily by topical application to the dorsal skin of rats for at least 104 weeks. Male and female CDF®(F-344)Cr1BR rats were assigned to seven groups. Benzoyl peroxide in carbopol gel at concentrations of 1.67, 5.0, and 15.0% (5, 15, and 45 mg/rat/day) was applied topically once daily to a treatment area (approximately 3.5 x 5 cm) on the dorsal skin of rats in Groups 2, 3, and 4, respectively. Rats in Group 1 served as vehicle controls and received daily topical applications of the carbopol gel vehicle at a dose volume of 0.3 mL. Rats in Group 6 served as negative controls; the hair on the backs of these rats was clipped at the same intervals as the other rats on study; however, these rats were not treated. Sixty rats/sex were assigned to Groups 1, 2, 3, 4, and 6, with the first 10 rats/sex/group designated for interim sacrifice during Week 53 and the remaining 50 rats/sex/group designated for terminal sacrifice after 104 weeks of treatment. Fifty rats/sex in Group 5 served as recovery animals, in that they were treated with 15% benzoyl peroxide for 52 weeks, then treated with the vehicle for the remainder of the study. Twenty rats/sex in Group 7 served as sentinel animals for pathogen screening at Weeks 26, 52, 78, and 104. Assessment of toxicologic and oncogenic potential was based on mortality (survival), body weights, food consumption, clinical signs (including all grossly visible/palpable masses), dermal irritation, organ weight data (interim-sacrifice rats), and macroscopic (gross pathology) and microscopic examinations of tissues.

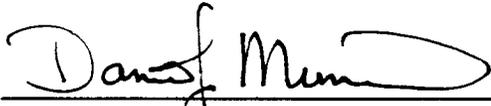
Treatment had no effect on survival; body weights, food consumption, or clinical observations; application site findings; gross pathology findings and organ weight changes at the interim sacrifice; or gross pathology findings at terminal sacrifice.

Microscopic evaluation revealed that the only treatment-related histopathologic findings noted at both the interim and terminal sacrifices were those seen at the site of topical dermal application. These consisted of hyperkeratosis, acanthosis, sebaceous gland hyperplasia, and chronic subepidermal inflammation. The changes occurred in all treatment groups (1.67, 5, and 15% benzoyl peroxide) and were mostly of a minimal to mild degree of severity. They progressed only slightly between 52 and 104 weeks of exposure. For the recovery animals (52 weeks of treatment with 15% benzoyl peroxide

followed by 52 weeks of vehicle), hyperkeratosis was present approximately twice as frequently as it was in either the vehicle or negative control group, but less than half as frequently as in any other treatment group. Acanthosis was not appreciably increased in the recovery animals as compared to controls, and sebaceous gland hyperplasia was only equivocally present. These findings indicated good, although not complete, recovery from the effects of 52 weeks of treatment with 15% benzoyl peroxide, with remnants of hyperkeratosis remaining after 52 weeks of recovery.

In conclusion, under the conditions of this study, there were no findings indicative of oncogenicity resulting from daily topical exposure of rats to benzoyl peroxide gels at concentrations up to 15% (dose level of 45 mg/rat/day) for 104 consecutive weeks.

Study Director:



Daniel J. Minnema, PhD
Department of Toxicology

March 21, 2000

Date

Study Coordinator:

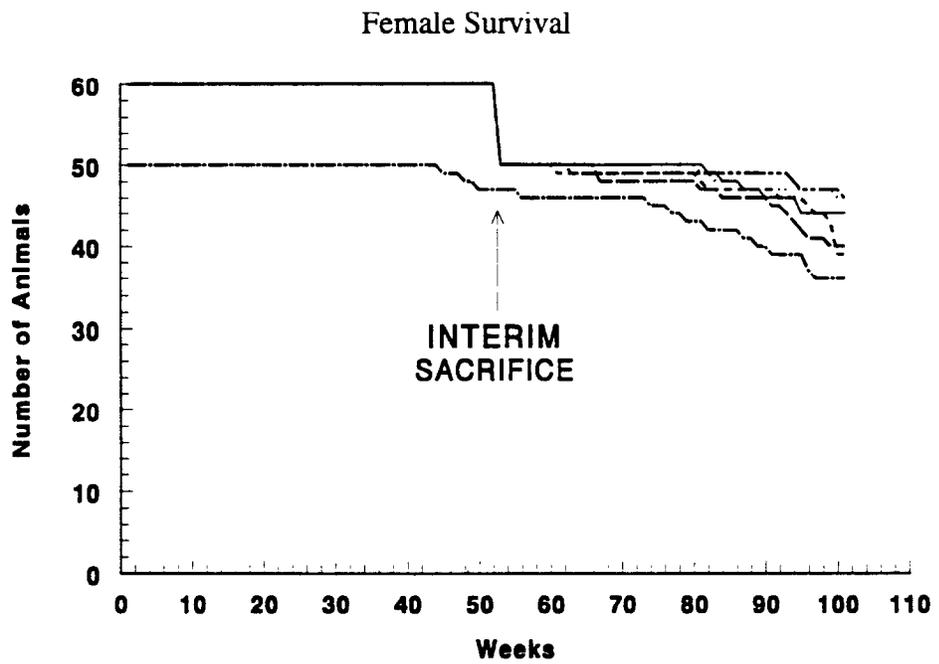
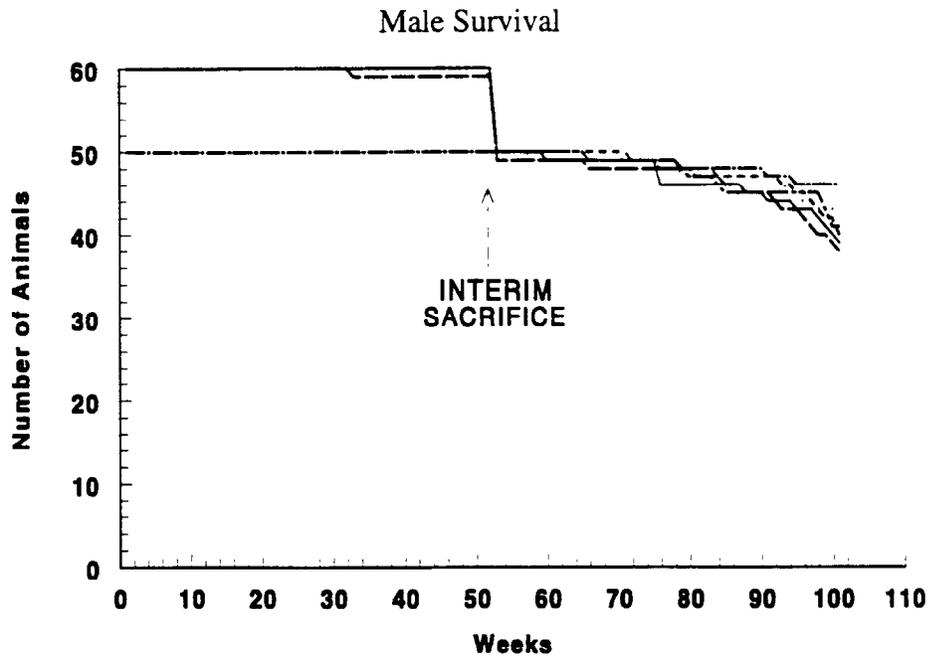


Amena S. Ali, BA
Department of Toxicology

3/21/2000

Date

Figure 1 - Animal Survival



Key:

1
 2
 3
 4
 5
 6
 VEHICLE 1.67% 5% 15% 15%/0% UNTREATED

PATHOLOGY REPORT

SUMMARY

There were no gross or histologic findings suggestive of any oncogenic or systemic toxic effect following 52 or 104 weeks of daily topical dermal exposure to benzoyl peroxide gel at any of the dose levels employed in this study (5, 15, or 45 mg/rat/day). In general the animals remained in good health for the duration of the study, with no apparent effects on mortality or growth. The only treatment-related histopathologic findings noted were those seen at the site of topical dermal exposure. Consisting of hyperkeratosis, acanthosis, sebaceous gland hyperplasia, and chronic subepidermal inflammation, the changes were mostly of a minimal to mild degree of severity and progressed only slightly between 52 and 104 weeks of exposure. Recovery was good following a 52-week recovery period, during which one group was exposed to the vehicle alone after having been treated with benzoyl peroxide gel daily at the highest dose level (45 mg/rat/day) for the previous 52 weeks. Traces of the described changes remained following the recovery period.

METHODS

Five groups of CDF®(F-344)CrIBR/Charles River rats were treated with benzoyl peroxide gel (prepared from USP grade benzoyl peroxide with aqueous carbopol gel as a vehicle) or aqueous carbopol gel as a vehicle control. A sixth group served as an untreated control.

Group	Number of Animals		Dose Level
	Male	Female	mg/rat/day
1 (Vehicle control)	60	60	0
2 (Low)	60	60	5
3 (Mid)	60	60	15
4 (High)	60	60	45
5 (High discontinued)	50	50	45
6 (Untreated control)	60	60	0

Note: A seventh group of untreated *sentinel animals* (20 male and 20 females) was also maintained under identical conditions and used for pathogen screening. Group 7 animals are of no relevance to this portion of the study and will not be discussed further in the pathology report.

Exposure of Groups 1-4 was by topical application to the dorsal skin, 7 days per week, for at least 104 weeks, except that after 52 weeks of exposure, the first 10 rats/sex/group of Groups 1-4 and Group 6, predesignated as *interim-sacrifice animals*, were sacrificed for pathologic evaluation. All surviving animals of those groups, designated as *terminal-sacrifice animals*, were sacrificed after 104 weeks of exposure. Animals of Group 5, which constitute a *recovery group* were exposed to benzoyl peroxide gel at the high-dose level for 52 weeks after which they were treated with the vehicle alone for the following 52 weeks and sacrificed along with the terminal-sacrifice animals.

All animals were weighed once prior to the initiation of treatment, weekly on the first day of Weeks 1-14 and once every fourth week thereafter, at Week 53 (following discontinuation of treatment in Group 5 animals), and at termination. Blood smears for possible future evaluation were prepared from each animal sacrificed at the scheduled interval or in a moribund state during the course of the study. Samples were collected by puncture of the orbital plexus using carbon dioxide/oxygen for anesthesia.

All surviving animals were sacrificed, at the appropriate time, by exsanguination under barbiturate anesthesia, and all animals were subjected to a necropsy examination. Clinical observations were reviewed at necropsy, and all grossly observed abnormalities were entered directly into the computerized data collection system. Liver, kidneys, and brain were weighed from each animal sacrificed after 52 weeks of treatment. After gross examination, appropriate samples of each of the following organs/tissues were preserved in 10% neutral-buffered formalin:

adrenals	mid-thoracic spinal cord
aorta (thoracic)	nasal cavity and nasal turbinates
bone with marrow (sternum, femur)	ovaries
brain with brainstem (medulla/pons, cerebellar cortex, and cerebral cortex)	pancreas
cervical spinal cord	pituitary
clitoral gland	preputial gland
cecum, colon, rectum	salivary glands (mandibular)
duodenum, jejunum, ileum	sciatic nerve
esophagus	seminal vesicles
eyes (with optic nerve and contiguous Harderian gland))	skeletal muscle (thigh)
femur including articular surface	skin (treated and untreated)
heart	spleen
	sternum
	stomach (glandular and nonglandular)

kidneys	testes with epididymides
lacrimal gland (exorbital)	thymus
larynx	thyroid with parathyroids
lesions	tissue masses
liver	tongue
lumbar spinal cord	trachea
lungs with bronchi	ureters
lymph nodes (mandibular, mesenteric, and regional when present in area draining a mass)	urinary bladder
mammary gland with skin	uterus with vagina and cervix
prostate	Zymbal's gland (auditory sebaceous gland)

All bony tissues were decalcified prior to processing. Tissues to be examined histologically were embedded in paraffin, sectioned at approximately 5 μm , and stained with hematoxylin and eosin.

Histologic evaluations were conducted on kidneys and skin (treated and untreated) from all animals sacrificed after 52 weeks of exposure. All tissues listed above were evaluated histologically from animals of Groups 1-4 and Group 6 sacrificed after 104 weeks; only treated and untreated skin sites were evaluated from animals of Group 5. All tissues listed were evaluated from any animal dying or sacrificed *in extremis* during the course of the study.

All histologic findings were entered directly into the computerized data capture system. Most lesions were graded as to relative severity or degree of involvement (1 = minimal, 2 = mild, 3 = moderate, 4 = marked). The grades are subjective, comparative evaluations, based on morphology alone, and are not intended by themselves to imply any degree of functional impairment; however, predetermined criteria were applied to the grading of acanthosis and hyperkeratosis within the specific "treated" and "untreated" skin sections:

Normal - One to two layers of epithelial cells (continuous stratum basale and an occasional cell in the stratum spinosum) and an interrupted stratum granulosum. The stratum lucidum is not distinguishable and the stratum corneum consists of a few curled, mostly basophilic layers of keratin.

Minimal acanthosis (Grade 1) - Two layers of epithelial cells prominent (single layer stratum basale and single layer in stratum spinosum) and one to two layers in the stratum granulosum.

Mild acanthosis (Grade 2) - Two to six layers of epithelial cells, including the stratum granulosum, which may be two to four layers.

Moderate acanthosis (Grade 3) - Seven to nine layers of epithelial cells, including the stratum granulosum, which may be four or more layers.

Marked acanthosis (Grade 4) - Nine or more layers of epithelial cells, including the stratum granulosum.

Minimal hyperkeratosis (Grade 1) - The stratum corneum stains more eosinophilic and has a few thin layers of keratin that are more compact than normal and more adherent to the epithelial surface.

Mild hyperkeratosis (Grade 2) - The stratum corneum consists of several layers of compact eosinophilic keratin with the more superficial layers beginning to have sloughed.

Moderate hyperkeratosis (Grade 3) - The stratum corneum consists of many layers of compact eosinophilic keratin and several of the superficial layers have sloughed.

Marked hyperkeratosis (Grade 4) - The stratum corneum consists of many layers of compact eosinophilic keratin and many of the superficial layers have sloughed. There may be irregularity in the sloughing, with some areas appearing as loose *stacks* of keratin.

One-way analysis of variance [ANOVA (Winter, 1971)] was used to analyze the terminal body weights, organ weights, organ-to-body weight percentages, and organ-to-brain weight ratios. Levene's test (Levene, 1960) was performed to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance. ANOVA was done on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's multiple comparison t-test (Dunnett, 1964) was used for pairwise comparisons between treated and control groups. Group comparisons were evaluated at the 5.0% two-tailed probability level.

RESULTS AND DISCUSSION

Mortality

All *interim-sacrifice animals* survived until the 52-week sacrifice interval, except for one Group 2 male that died during Week 52 with a pituitary adenoma and bronchopneumonia. Mortality between 52 and 104 weeks was essentially distributed among the groups without relation to dose. The most commonly cited causes of early death, including animals that were sacrificed *in extremis*, were hematopoietic neoplasia (mostly mononuclear cell leukemia) and pituitary neoplasia. Both are very prevalent in normal populations of F-344 rats as they age, and both were randomly scattered among exposure groups within this study.

Body and Organ Weights

No consistent, dose-related differences in mean group body weight values were observed during the course of the study. Statistically significant variations were seen sporadically throughout the study, but not in any pattern that would suggest that exposure to the test material had any overall effect on body weight gain.

Organ weights were unaffected by exposure to the test material after 52 weeks; they were not measured at the Week 104 terminal sacrifice.

Macroscopic Observations

Week 52 - There were few grossly noted abnormalities; none were suggestive of any effect of treatment with any of the test formulations. The frequently noted clinical observation, *test material at the application site*, was not repeated at necropsy.

Week 104 - Gross abnormalities noted at the Week 104 sacrifice (including animals that died or were sacrificed *in extremis* during the course of the study) were generally typical of groups of equivalently aged normal F-344 rats at this laboratory. None were suggestive of any effect of treatment.

Five males, all sacrificed after 104 weeks, had lesions within the treated skin area described grossly as "mass." They were not related to treatment. Of the five, three occurred in Group 6 (untreated control), and of these, two were shown histologically to be nonneoplastic in nature -- one of these was focal, nonneoplastic dermal thickening, and the other a focal area of mineralization within the dermis. A similar area of dermal mineralization was seen in one Group 5 male. The other skin "mass" present in a Group 6 male proved to be a keratoacanthoma, a common benign neoplasm that commonly occurs in the skin of older rats. A keratoacanthoma was also seen in a single Group 4 male.

Microscopic Observations

Week 52 - An expanded summary of incidence and severity of selected histopathologic findings in treated and untreated skin in animals sacrificed after 52 weeks of treatment is presented in Text Table 1.

The treated skin from animals of Groups 2, 3, and 4 (those groups exposed to the benzoyl peroxide gel test formulation) consistently exhibited increased keratin production (hyperkeratosis) compared to either Group 1 (vehicle control) or Group 6 (untreated control). This hyperkeratosis was minimal to mild in severity and was spotty to diffuse along the examined sections, sometimes extending down into hair follicles or adnexal ducts. Frequently associated with the hyperkeratosis was a minimal to mild degree of epithelial thickening (acanthosis), the result of increased numbers of epithelial layers comprising the epidermis from 1-2, which seemed to be the norm (excluding the normal Haarscheibe structures located near hair follicles), to as many as 6-7. The stratum spinosum and stratum granulosa were variably increased in affected skin sections, and the basal cell layer was also more prominent, with slightly increased mitotic activity. Also seen in the skin of animals of both sexes exposed to benzoyl peroxide gel was increased prominence of the sebaceous glands. Diagnosed as hyperplasia, this change was characterized by an increase in both the size and apparent visible number of the glands. Subtle chronic subepidermal inflammation was associated with the changes described. It was minimal in degree, observable as a slight increase in cellularity just below the epidermal/dermal junction, and was the result of a barely perceptible increase in fibroplasia and infiltration by a few scattered chronic inflammatory cells.

The incidence of hyperkeratosis was increased in untreated skin sections from animals (males and females) of Groups 2, 3, and 4 to levels comparable to those seen in the treated skin sections from those same groups, suggesting that there was some contamination of untreated skin sites with test material during or after the treatment process. Hyperkeratosis was also present in a number of animals of both sexes from both the vehicle and untreated control groups, while acanthosis, sebaceous gland hyperplasia, and subepidermal inflammation were not seen in any control animals at this time interval. This suggests a nominal background level of "hyperkeratosis," as defined for this study, against which any treatment-related expressions of that reaction must be compared.

A shallow ulcer was encountered in the treated skin of one male each from Groups 2 (low dose) and 6 (untreated control). There was little inflammation associated with either of these ulcers, which seemed to have occurred without relation to any other precursor changes. There is no reason to suspect that either of these ulcerative lesions was related to the test material.

Text Table 1
Incidence of Selected Lesions in Treated and Untreated Skin from Animals Sacrificed after 52 Weeks

GROUP	MALE					FEMALE				
	1	2	3	4	6	1	2	3	4	6
TREATED SKIN										
NO. EXAMINED	10	9	10	10	10	10	10	10	10	10
NORMAL	6	0	0	0	7	7	2	0	0	6
--Hyperkeratosis										
Minimal (Grade 1)	4	6	2	0	2	3	8	5	0	4
Mild (Grade 2)	0	3	8	10	0	0	0	5	8	0
Moderate (Grade 3)	0	0	0	0	0	0	0	0	2	0
Total Affected	4	9	10	10	2	3	8	10	10	4
Mean Lesion Grade	1.0	1.3	1.8	2.0	1.0	1.0	1.0	1.5	2.2	1.0
Group Mean	0.4	1.3	1.8	2.0	0.2	0.3	0.8	1.5	2.2	0.4
--Acanthosis										
Minimal (Grade 1)	0	7	3	1	0	0	7	8	3	0
Mild (Grade 2)	0	2	6	9	0	0	0	2	7	0
Total Affected	0	9	9	10	0	0	7	10	10	0
Mean Lesion Grade	0.0	1.2	1.7	1.9	0.0	0.0	1.0	1.2	1.7	0.0
Group Mean	0.0	1.2	1.5	1.9	0.0	0.0	0.7	1.2	1.7	0.0
--Chronic subepidermal inflammation										
Minimal (Grade 1)	0	6	10	10	0	0	8	10	10	0
Total Affected	0	6	10	10	0	0	8	10	10	0
Mean Lesion Grade	0	1.0	1.0	1.0	0.0	0.0	1.0	1.0	1.0	0.0
Group Mean	0.0	0.7	1.0	1.0	0.0	0.0	0.8	1.0	1.0	0.0
--Sebaceous gland hyperplasia										
Minimal (Grade 1)	0	4	3	0	0	0	4	6	0	0
Mild (Grade 2)	0	5	7	5	0	0	0	0	8	0
Moderate (Grade 3)	0	0	0	5	0	0	0	0	2	0
Total Affected	0	9	10	10	0	0	4	6	10	0
Mean Lesion Grade	0.0	1.6	1.7	2.5	0.0	0.0	1.0	1.0	2.2	0.0
Group Mean	0.0	1.6	1.7	2.5	0.0	0.0	0.4	0.6	2.2	0.0
--Ulcer										
Mild (Grade 2)	0	1	0	0	1	0	0	0	0	0
Total Affected	0	1	0	0	1	0	0	0	0	0
Mean Lesion Grade	0.0	2.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0
Group Mean	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
UNTREATED SKIN										
NO. EXAMINED	10	9	10	10	10	10	10	10	10	10
NO. NORMAL	7	4	3	3	8	7	2	0	0	6
--Hyperkeratosis										
Minimal (Grade 1)	3	5	6	6	2	3	8	10	10	4
Mild (Grade 2)	0	0	1	1	0	0	0	0	0	0
Total Affected	3	5	7	7	2	3	8	10	10	4
Mean Lesion Grade	1.0	1.0	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.0
Group Mean	0.3	0.6	0.8	0.8	0.2	0.3	0.8	1.0	1.0	0.4
--Sebaceous gland hyperplasia										
Minimal (Grade 1)	0	1	0	0	0	0	0	0	0	0
Total Affected	0	1	0	0	0	0	0	0	0	0
Mean Lesion Grade	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Group Mean	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Note: Mean Lesion Grade excludes normals in calculations; Group Mean includes normals as the value 0.

Histopathologic changes observed in the kidneys after 52 weeks were all of the kinds usually encountered in normal populations of rats of this strain and age. None suggested any relation to exposure to the test material.

Week 104 - An expanded summary of incidence and severity of selected histopathologic findings in treated and untreated skin of animals sacrificed after 104 weeks of treatment (including those that died or were sacrificed in extremis) is presented in Text Table 2.

Aside from expected increases in normally occurring age-related background changes, the findings after 104 weeks of exposure were similar to those that occurred after 52 weeks. There were no histologic findings suggestive of any toxic or oncogenic effects from exposure to benzoyl peroxide gel. After 104 weeks of exposure, the treated skin from *terminal-sacrifice animals* of Groups 2, 3, and 4 (those groups exposed to the benzoyl peroxide gel test formulation for the entire 104 week exposure period) showed hyperkeratosis, acanthosis, sebaceous gland hyperplasia, and chronic subepidermal inflammation that were morphologically indistinguishable from those same changes seen after 52 weeks. These alterations were present in a high percentage of animals of those groups at both sacrifice intervals, but with relatively little progression in severity between 52 and 104 weeks. Hyperkeratosis (mostly minimal) was diagnosed in treated skin sections from some animals of both the vehicle and untreated control groups after 104 weeks to a degree that was comparable to what was seen in the control groups after 52 weeks, which defines background levels of this change, as graded according to the predetermined criteria. Furthermore, as was also the case after 52 weeks, the other salient changes were mostly absent from both the vehicle and untreated control groups at the 104 week interval.

Hyperkeratosis was present in untreated skin sections from animals of all groups, including controls, without relation to the dose applied to the treatment site, and while the incidence within untreated skin sections was inconsistent among dose groups, overall it was not very different from what was seen in the untreated skin sections after 52 weeks, except that after 104 weeks untreated skin sections from animals of Groups 2, 3, and 4 were not obviously affected at a level above what was seen in untreated skin sections from the control groups.

Recovery - Within *recovery animals* (Group 5) of both sexes, hyperkeratosis was present approximately twice as frequently as it was in either control group, but less than half as frequently as in any other group, including that group exposed at the lowest level for either 52 or 104 weeks. Acanthosis was not appreciably increased in the recovery group compared to controls, and sebaceous gland hyperplasia was only equivocally present in Group 5. These results in general imply good, though not complete recovery from the effects of 52 weeks of treatment at the high-dose level, with remnants of hyperkeratosis remaining after 52 weeks of recovery.

The occurrence of other skin lesions, including a few ulcers, was independent of exposure to the test material.

Text Table 2
Incidence of Selected Lesions in Treated and Untreated Skin after 104 Weeks of Treatment (exclusive of interim sacrifice)

	GROUP	MALE						FEMALE					
		1	2	3	4	5	6	1	2	3	4	5	6
TREATED SKIN	NO. EXAMINED	50	51	50	50	50	50	50	50	50	50	50	50
	NORMAL	32	0	0	0	21	32	38	0	0	0	21	36
--Hyperkeratosis	Minimal (Grade 1)	13	12	5	4	22	10	12	13	2	0	18	12
	Mild (Grade 2)	1	38	38	37	0	1	0	34	45	28	2	0
	Moderate (grade 3)	0	1	7	9	0	0	0	3	3	22	1	0
	Total Affected	14	51	50	50	22	11	12	50	50	50	21	12
	Mean Lesion Grade	1.1	1.8	2.0	2.1	1.0	1.1	1.0	1.8	2.0	2.4	1.2	1.0
	Group Mean	0.3	1.8	2.0	2.1	0.4	0.2	0.2	1.8	2.0	2.4	0.5	0.2
--Acanthosis	Minimal (Grade 1)	0	24	28	23	2	1	0	30	10	1	5	1
	Mild (Grade 2)	0	9	17	23	1	0	0	11	40	45	0	0
	Total Affected	0	33	45	46	3	1	0	41	50	46	5	1
	Mean Lesion Grade	0.0	1.3	1.4	1.5	1.3	1.0	0.0	1.3	1.8	2.0	1.0	1.0
	Group Mean	0.0	0.8	1.2	1.4	0.1	0.0	0.0	1.0	1.8	1.8	0.1	0.0
--Chronic subepidermal inflammation	Minimal (Grade 1)	0	33	41	46	14	0	0	42	41	39	11	1
	Mild (Grade 2)	0	0	1	0	0	0	0	1	6	7	0	0
	Total Affected	0	33	42	46	14	0	0	43	47	46	11	1
	Group Mean	0.0	0.6	0.9	0.9	0.3	0.0	0.0	0.9	1.1	1.1	0.2	0.0
--Sebaceous gland hyperplasia	Minimal (Grade 1)	0	23	0	1	0	0	0	25	0	1	2	0
	Mild (Grade 2)	0	13	36	25	0	0	0	16	34	22	0	0
	Moderate (Grade 3)	0	0	8	23	0	0	0	0	9	26	0	0
	Total Affected	0	36	44	49	0	0	0	41	43	49	2	0
	Group Mean	0.0	1.0	1.9	2.4	0.0	0.0	0.0	1.1	1.9	2.5	0.0	0.0
--Ulcer	Minimal (Grade 1)	1	1	0	0	0	0	0	1	0	0	0	0
	Mild (Grade 2)	3	0	1	1	1	2	0	1	0	0	0	0
	Moderate (Grade 3)	0	0	0	0	0	0	0	0	0	1	0	0
	Total Affected	4	1	1	1	1	2	0	2	0	1	0	0
	Group Mean	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0
UNTREATED SKIN	NO. EXAMINED	50	51	50	50	50	50	50	50	50	50	50	
	NORMAL	36	41	38	34	37	39	40	43	39	33	36	33
--Hyperkeratosis	Minimal (Grade 1)	12	8	7	5	12	7	10	6	8	8	12	17
	Mild (Grade 2)	1	1	4	6	1	4	0	0	2	5	0	0
	Moderate (grade 3)	0	0	0	5	0	0	0	0	0	3	0	0
	Total Affected	13	9	11	16	13	11	10	6	10	16	12	17
	Group Mean	0.3	0.2	0.3	0.6	0.3	0.3	0.2	0.1	0.2	0.5	0.2	0.3
--Acanthosis	Minimal (Grade 1)	0	3	1	2	0	1	0	1	0	1	0	1
	Mild (Grade 2)	0	0	1	0	0	0	0	0	0	1	0	0
	Total Affected	0	3	2	2	0	1	0	1	0	2	0	1
	Group Mean	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0

Note: Mean Lesion Grade excludes normals in calculations; Group Mean includes normals as the value 0.

A wide variety of other histologic changes were seen -- some in a high percentage of animals and some sporadically, all without relation to exposure level. Most of these findings were characteristic of those usually encountered in aging F-344 rats at this and other laboratories; however, those lesions not typical for normal populations of rats were of either singular or random occurrence, with no suggestion that they were influenced by exposure to the test material.

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STATISTICAL REPORT OF SURVIVAL, NON-NEOPLASTIC, AND NEOPLASTIC LESIONS

Methods

Survival. Survival was analyzed by life table techniques consisting of Kaplan-Meier product limit estimates, Cox-Tarone binary regression on life tables, and Gehan-Breslow nonparametric methods (Thomas, Breslow, and Gart, 1977). Cox-Tarone methods are weighted heavier toward late incidences and Gehan-Breslow methods are weighted toward early incidences due to treatment. As a result, they are both valuable tools for incidence data with onset times. Week 106 was treated as the end of study (EOS) in the National Cancer Institute package (Thomas *et al.*, 1977) for both sexes. Ordinal dose levels 1, 2, 3, and 4 corresponding to Groups 1, 2, 3, and 4 were used in the analysis. Two-sided tail probabilities for trend and group comparisons are reported in Text Tables 1 and 2. Figures 1 and 2 represent Kaplan-Meier product limit survival curves for the males and females, respectively.

Non-neoplastic Lesions. Non-neoplastic lesions were chosen for statistical analyses if the incidence in at least one treated group (Groups 2, 3, or 4) was increased or decreased by at least two over the vehicle control (Group 1), and approximately 75% of animals per group were examined. The selected lesions were analyzed by Cochran-Armitage method for trend and Fisher-Irwin exact test for control versus treatment comparisons (Thakur, Berry, and Mielke, 1985). Ordinal dose levels 1, 2, 3, and 4 corresponding to Groups 1, 2, 3, and 4 were used in the analysis. Continuity corrected one-sided tail probabilities for trend and exact one-sided tail probabilities for group comparisons are presented in Text Tables 3 and 4 for the males and females, respectively.

Neoplastic Lesions. Neoplastic lesions were chosen for statistical analyses if the incidence in at least one treated group (Groups 2, 3, and 4) was increased or decreased by at least two over the vehicle control (Group 1), and approximately 75% of animals per group were examined. All the selected lesions were analyzed by survival-adjusted analyses and unadjusted analyses. The adjusted-analyses are Cox-Tarone binary regression test (Thomas *et al.*, 1977), as in the case of survival, assuming all the tumors are fatal observations, and logistic regression of tumor prevalences (Dinse and Lagakos, 1983) assuming all the tumors are incidental observations. The unadjusted analyses consist of Cochran-Armitage method for trend and Fisher-Irwin exact test for control

versus treatment comparisons (Thakur, Berry, and Mielke, 1985) and do not adjust for survival differences. In the case of palpable tumors, the first palpation time (if applicable) was used in the Cox-Tarone test as the surrogate for tumor onset time.

The benign and malignant neoplastic incidences were evaluated separately as well as combined, where appropriate. The criteria for combination were based on the work of McConnell *et. al.* (1986). In addition, the incidences of hemangioma or hemangiosarcoma, or both; endometrial stromal polyp or endometrial stromal sarcoma, or both; leiomyoma or leiomyosarcoma, or both were also counted by animal. Since their incidences did not meet the selection criterion for tumor analyses, they were not statistically analyzed.

Ordinal dose levels were used in all the tumor analyses described above. Continuity correction was used for all the asymptotic tests. One-sided tail probabilities for trend analysis and group comparisons are shown in Text Tables 5 and 6 for the males and females, respectively.

Graded Histopathology of Skin

Skin histopathology graded responses were analyzed using categorical data analysis techniques (Agresti, 1990). In the cases where the response types have natural ordering (e.g., the levels of severity), an overall trend test using uncorrected asymptotic linear-by-linear association method was conducted, followed by pairwise comparisons of treated groups with the vehicle control using the same method. The actual dose levels 0-, 5-, 15-, and 45-mg/rat/day were assigned as the scores for Groups 1, 2, 3, and 4, respectively, according to protocol specifications. 0, 1, 2, 3, etc. were used for the levels (grades or scores) of the responses. The statistical results were obtained by StatXact-Turbo (1992) and were evaluated at a 5% two-sided significance level. They are presented in Text Tables 7 and 8 for the male and females, respectively. No analyses were performed on tables which did not exhibit any visual increase or decrease in comparison to the vehicle control. There were no binary type (e.g., present or absent) of responses in this category requiring exact form of Cochran-Armitage test for trend and Fisher-Irwin test for group comparisons.

Comparisons between the vehicle control (Group 1) and high-discontinued-dose (Group 5) groups; the vehicle (Group 1) and untreated (Group 6) control groups were also

performed for survival, non-neoplastic, neoplastic, graded histopathology responses when appropriate. Comparisons between the high- (Group 4) and high-discontinued-dose (Group 5) groups were only performed on the treated and untreated skin tissue when appropriate.

Results and Discussion

Survival. As Text Tables 1 and 2 indicate, there was no significant positive or negative trend in the male or female mortality, nor were there significant group comparisons observed in either sex. In other words, there was no survival difference between the vehicle control (Group 1) and high-discontinued-dose (Group 5) groups. The vehicle (Group 1) and untreated (Group 6) controls in each sex showed statistically similar survival distributions and the treated groups (Groups 2-4) also showed similar survival distributions as the vehicle control.

Non-neoplastic Lesions. In the males, as shown in Text Table 3, the most notable findings were hyperkeratosis, acanthosis, subepidermal chronic inflammation, and sebaceous gland hyperplasia of the treated skin which showed strongly significant positive trends ($p = .0000$ in each case) associated with highly elevated increased incidences in all the treated groups (Groups 2-4) over that of the vehicle control ($p = .0000$ in each case). No such significant findings were observed for the untreated skin. Eosinophilic cellular alteration of the liver and retrobulbar arteriole degeneration/mineralization of the eyes also showed significant positive trends ($p = .0076$ and $.0329$, respectively) in the incidences. The former was associated with a significant increase in Group 4 ($p = .0281$); the latter did not show any significant group comparisons. There were some cases showing significantly higher incidences in Groups 2 and/or 4 compared to that of the vehicle control, but no significant positive trends were associated. They are lipoidosis of the adrenal cortex ($p = .0229$ for Group 2 and $p = .0188$ for Group 4); focal chronic inflammation of the lung ($p = .0297$ for Group 2 and $p = .0281$ for Group 4); degenerative cardiomyopathy ($p = .0458$ for Group 4) and great and/or coronary vessels mineralization ($p = .0008$ for Group 2 and $p = .0269$ for Group 4) of the heart; and hyperkeratosis of the skin excluding the treated and untreated areas ($p = .0141$ for Group 4). In the cases of focal hyperplasia of the pituitary and focal chronic inflammation of the liver, there were significant negative trends ($p = .0086$ and $.0076$, respectively) associated with significantly lower incidences in the mid- and high-dose groups ($p < .05$) compared to that of the vehicle control. The low-dose focal

hyperplasia of the pituitary also showed a significantly lower incidence over the vehicle control ($p = .0162$). Significant negative trends were observed for angiectasis of the adrenal cortex ($p = .0066$) and hyperkeratosis of the nonglandular stomach ($p = .0193$), but no significant group comparisons were noted. The significant negative trends in these two cases were due to no finding (zero incidence) in any of the treated groups. Although Group 2 increased pigment of the spleen ($p = .0219$) and Group 3 tunica media mineralization of the thoracic aorta ($p = .0281$) incidences were significantly lower than that of the vehicle control, no linear (monotone) trends in the incidences across Groups 1-4 were indicated.

In the females, as shown in Text Table 4, the most notable findings were, as in the males, hyperkeratosis, acanthosis, subepidermal chronic inflammation, and sebaceous gland hyperplasia of the treated skin which showed strongly significant positive trends ($p = .0000$ in each case) associated with highly elevated increased incidences in all the treated groups (Groups 2-4) over that of the vehicle control ($p = .0000$ in each case). Hyperkeratosis of the untreated skin also showed a significant positive trend ($p = .0029$) with a significant two-fold increase in the high-dose group incidence ($p = .0094$) when compared to the vehicle control. There were significant positive trends in the incidences of acinar atrophy of the pancreas ($p = .0352$), endometrial fibrosis/cystic hyperplasia of the uterus ($p = .0087$), and hyperkeratosis of the skin excluding the treated and untreated areas ($p = .0083$). Each of the three cases was associated with a significantly increased incidence in the high-dose group ($p = .0458$, $.0278$, and $.0281$, respectively). In addition, the mid-dose endometrial fibrosis/cystic hyperplasia incidence in the uterus was also significantly larger than that of the vehicle control ($p = .0058$). Alveolar histiocytosis of the lung, hepatodiaphragmatic nodule of the liver, and dysplasia of the mammary also showed marginally significant positive trends in the incidences ($p = .0318$, $.0430$, and $.0444$, respectively) with no significant group comparisons observed. In the cases of axonal degeneration of the sciatic nerve and arteriole degeneration/mineralization of the tongue, although the mid-dose group incidences were significantly higher than that of the vehicle control ($p = .0098$ and $.0204$, respectively), no linear (monotone) trends in the incidences across Groups 1-4 were indicated. There were significant negative trends in the incidences of pelvis calculi of the kidney ($p = .0014$) and lactation of the mammary gland ($p = .0038$), which were associated with significantly decreased incidences in the high-dose group ($p = .0023$ and $.0102$, respectively) over the vehicle control. Endometrial fibrosis of the uterus also showed a significant negative trend ($p = .0004$)

with significantly lower incidences in the mid- and high-dose groups ($p = .0058$ for both cases) when compared to that of the vehicle control. In fact, there were no findings (zero incidence) in both groups in this case. A significant negative trend was observed for pigmented macrophages of the liver ($p = .0480$), but no significant group comparisons were noted. Although Group 3 angiectasis of the adrenal cortex ($p = .0013$), Groups 2 and 3 increased pigment of the spleen ($p = .0464$ and $.0088$, respectively), Group 3 eosinophilic cellular alteration of the liver ($p = .0281$), and Group 3 osteopetrosis of the sternum bone ($p = .0297$) incidences were significantly lower than that of the vehicle control, no linear (monotone) trends in the incidences across Groups 1-4 were indicated.

In general, the vehicle (Group 1) and untreated (Group 6) controls were not statistically significantly different from each other in both sexes, except in the cases of focal hyperplasia of the pituitary ($p = .0098$, incidence of untreated decreased over vehicle), lipoidosis of the adrenal cortex ($p = .0392$, increased over vehicle), and focal chronic inflammation of the liver ($p = .0047$, decreased over vehicle) of the males; and eosinophilic cellular alteration of the liver ($p = .0281$, decreased over vehicle), mucosa erosion of the glandular stomach ($p = .0281$, increased over vehicle), and parasitism of the rectum ($p = .0495$, increased over vehicle) of the females.

The male high-discontinued-dose group (Group 5) showed larger hyperkeratosis ($p = .0933$), acanthosis ($p = .0908$), and subepidermal chronic inflammation ($p = .0001$) incidences in the treated skin than those of the vehicle control (Group 1). Likewise, the female high-discontinued-dose group also showed larger hyperkeratosis ($p = .0457$), acanthosis ($p = .0173$), subepidermal chronic inflammation ($p = .0001$), and sebaceous gland hyperplasia ($p = .2043$) incidences in the treated skin than those of the vehicle control. The high-discontinued-dose group incidences of the above cases were, however, all significantly much lower ($p = .0000$ for each case) than those of the high-dose group (Group 4), as indicated in Text Tables 3 and 4. Except for a marginally significantly decreased incidence in the female high-discontinued-dose hyperkeratosis of the untreated skin ($p = .0266$) when compared to that of the high-dose group, there were no other significant findings observed for the untreated skin in each sex when the vehicle and high-discontinued-dose groups (Groups 1 vs. 5) or the high- and high-discontinued-dose groups (Groups 4 vs. 5) were compared.

Neoplastic Lesions. In the males, as Text Table 5 indicates, the only significant finding was observed in the case of combined c-cell adenoma/carcinoma of the thyroid, which showed a significant negative trend in the incidences of 6/50, 13/51, 4/49, and 2/49 for Groups 1, 2, 3, and 4, respectively, by all the three tests ($p < .05$ for each test). No significant group comparisons were associated with this significant trend.

In the females, as Text Table 6 indicates, there were significant negative trends in the incidences of pituitary anterior lobe adenoma ($p < .005$ for all three tests) and combined anterior lobe adenoma/carcinoma ($p < .005$ for all three tests). Both significant negative trends were associated with significantly decreased incidences in the mid- and high-dose groups when compared to that of the vehicle control ($p < .05$ for all cases). The untreated control incidences in these two cases were also significantly lower than that of the vehicle control ($p < .05$ for all cases). No such significant findings were observed when anterior lobe carcinoma was analyzed alone. There was a significant positive trend in the incidences of large granular lymphocytic leukemia (5/60, 8/60, 12/60, and 8/60 for Groups 1, 2, 3, and 4, respectively) by logistic regression of tumor prevalence test ($p = .0285$), which was associated with a significantly increased incidence in the mid-dose group over that of the vehicle control ($p = .0062$). Because of the apparent nonmonotonicity of the rates, the significant positive trend is of questionable biological implication. Also, the highest incidence of large granular lymphocytic leukemia in the treated animals was similar to that in the untreated control animals.

Graded Histopathology of Skin. As indicated in Text Tables 7 and 8, hyperkeratosis, acanthosis, subepidermal chronic inflammation, and sebaceous gland hyperplasia of the treated skin in both sexes showed significant positive trends ($p = .0000$ for each case) implying that more animals were noted with higher grades (or scores) as the doses increased. In fact, the low-, mid-, and high-dose groups of both sexes all showed significant increases in the number of animals in the higher grades when compared with that of the vehicle control ($p = .0000$ for each case). There was also a significant positive trend observed in the case of hyperkeratosis of the untreated skin in both sexes ($p = .0003$ and $.0000$ for the males and females, respectively). However, only the high-dose was noted with significantly larger incidences in the higher grades when compared to that of the vehicle control ($p = .0086$ and $.0016$ for the males and females, respectively).

No significant findings were observed in comparing the vehicle (Group 1) and untreated (Group 6) controls of the graded responses of treated and untreated skin in both sexes.

The male high-discontinued-dose group (Group 5) showed larger hyperkeratosis ($p = .2015$), acanthosis ($p = .0714$), and subepidermal chronic inflammation ($p = .0000$) incidences in the higher grades in the treated skin than those of the vehicle control (Group 1). Likewise, the female high-discontinued-dose group also showed larger hyperkeratosis ($p = .0221$), acanthosis ($p = .0126$), subepidermal chronic inflammation ($p = .0001$), and sebaceous gland hyperplasia ($p = .1196$) incidences in the higher grades in the treated skin than those of the vehicle control. The high-discontinued-dose group incidences in the higher grades of the above cases were, however, all significantly much lower ($p = .0000$ for each case) than those of the high-dose group (Group 4), as indicated in Text Tables 7 and 8. In addition, the high-discontinued-dose group also showed significantly decreased incidences in the higher grades of hyperkeratosis of the untreated skin in either sex ($p = .0139$ and $.0057$ for the males and females, respectively) when compared to those of the high-dose group. There were no other significant findings observed for the untreated skin in each sex when the vehicle and high-discontinued-dose groups (Groups 1 vs. 5) or the high- and high-discontinued-dose groups (Groups 4 vs. 5) were compared.

Male untreated skin hyperkeratosis did not show any significances when overall proportions were analyzed by Cochran-Armitage and Fisher-Irwin tests (see Text Table 3). All other significant findings of the graded histopathology responses of the skin are consistent with the results of those cases under non-neoplastic lesions when overall proportions of tumor-bearing animals were analyzed.

Conclusion. In conclusion, there was no significant effect on mortality in the male or female treated animals (including high-discontinued group) compared to the vehicle control. The untreated control animals also showed similar survival pattern as the vehicle control animals in both sexes.

Significantly increased incidences were observed in hyperkeratosis, acanthosis, subepidermal chronic inflammation, and sebaceous gland hyperplasia of the treated skin in all treated groups over those of the vehicle control in both sexes. Except for female hyperkeratosis of the untreated skin which showed significantly higher incidence in the high-dose group, no other significant findings in comparing treated groups versus the

vehicle control were noted for the untreated skin in either sex. Similar conclusions can be made when those non-neoplastic lesions were graded. In other words, there were significantly larger number of animals noted with higher grades as the doses increased in the above cases. In addition, although the high-dose male hyperkeratosis incidence (23/60) in the untreated skin was not statistically significantly larger than that of the vehicle control (16/60), there were significantly more animals in the high-dose group noted with higher grades compared to the vehicle control, based on the statistical analysis. Except for a significantly increased incidence in the high-dose hyperkeratosis of the skin excluding the treated and untreated areas in both sexes, the rest of the significant positive findings in the non-neoplastic lesions were sporadic and not consistent between the two sexes.

There were no treatment related effects on any of the neoplastic lesions of the skin in both sexes. The significant positive trend in the female incidences of large granular lymphocytic leukemia was borderline at the 5% significance level. No such significant finding was observed in the males. In fact, the trend was of questionable relevance due to nonmonotonicity because the high-dose incidence in this case was equal to that of the low-dose group. Also, the highest incidence of large granular lymphocytic leukemia in the treated animals was similar to that in untreated control animals. In summary, although some of the neoplastic findings, as noted above, showed borderline significance at $p \leq 0.05$ level, they were of inconsistent nature and do not indicate any true carcinogenic response in either sex.

In general, the vehicle and untreated controls were comparable to each other in the non-neoplastic, neoplastic, and graded skin histopathology analyses. The high-discontinued group showed larger incidences in hyperkeratosis, acanthosis, subepidermal chronic inflammation, and sebaceous gland hyperplasia of the treated skin than those of the vehicle control, but much smaller incidences than the high-dose group in both sexes.

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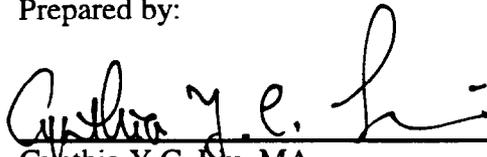
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