CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-087

STATISTICAL REVIEW(S)

US Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

Statistical Review and Evaluation

New Drug Application

Clinical Studies

NDA/Serial Number:	22-087
Drug Name:	Calcitriol Ointment
Indication(s):	Psoriasis
Applicant:	Galderma Laboratories, L.P.
Dates:	Submitted: 12/27/2007 PDUFA: 10/27/2008
Review Priority:	Standard Review
Biometrics Division:	Division of Biometrics III
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Keywords:	superiority

Contents

1 Executive Summary

1.1 Conclusions and Recommendations

Calcitriol Ointment has been subject to numerous clinical trials over the last fifteen years, many of which were conducted by the previous sponsor ——. The clinical development by —— established a dose for the treatment of psoriasis which was used in two vehicle controlled, confirmatory Phase 3 trials, Study 18053 and Study 18054, conducted by the current sponsor, Galderma. b(4)

Primary efficacy analysis was based on the proportion of subjects who had an end of treatment Investigator Global Assessment (IGA) score of 'clear' or 'minimal' which the Division agreed to at an End of Phase 2 meeting. In Study 18053 Calcitriol Ointment was statistically superior to vehicle ($p = 0.0047$) with response rates of 34.4% and 22.5% for Calcitriol Ointment and vehicle, respectively. In Study 18054, Calcitriol Ointment was statistically superior to vehicle ($p < 0.001$) with observed response rates of 33.3% and 12.3%, respectively.

In the assessment of short-term safety, event rates for AE's were quite similar between Calcitriol Ointment and vehicle. Study 2663 was an open-label long-term safety study to assess the local and systemic safety of Calcitriol Ointment when applied twice daily for up to 52 weeks. Safety results from this study showed a slight increase in the rate of AE's reported in the short-term assessment of safety.

1.2 Brief Overview of Clinical Studies

Study 18053 and Study 18054 were identically designed multi-center, randomized, double-blind, vehicle-controlled, parallel group comparison studies conducted in the United States. Subjects with mild to moderate chronic plaque psoriasis were randomized in 1:1 ratio to Calcitriol Ointment or vehicle. Subjects were to apply treatment twice daily for 8 weeks. Study 18053 enrolled 418 subjects from 25 U.S. centers. Study 18054 enrolled 421 subjects from 25 U.S. centers. The primary efficacy endpoint was the proportion of subjects with an IGA score of 'clear' or 'minimal' at week 8.

1.3 Statistical Issues and Findings

At the End of Phase 2 meeting held on 11/15/1999, the Division was in agreement with defining the primary endpoint as the proportion of subjects with an IGA score of 'clear' or 'minimal' at week 8. The primary analysis was conducted on the ITT population with missing data imputed using LOCF. Primary efficacy results are shown in Table 1. Both studies demonstrated that Calcitriol Ointment was statistically superior to vehicle.

Table 1: Investigator Global Results ('Clear' or 'Minimal'): ITT

	Study 18053		Study 18054	
	Calcitriol ($N = 209$)	Vehicle ($N = 209$)	Calcitriol ($N = 210$)	Vehicle ($N = 211$)
Success (%)	72 (34.4%)	47 (22.5%)	70 (33.3%)	26 (12.3%)
p-value†	-	0.0047	-	< .001

† p-values are based on CMH stratified by pooled site.

Source: Study Report Table 13; results reproduced by reviewer.

Safety assessment was based upon adverse events recorded by body system and COSTART term. In the two short-term Phase trials, Studies 18053 and 18054, reported AE's were similar between Calcitriol Ointment and vehicle. In the open-label long-term safety study, Study 2663, there was a slight increase in the percentage of subjects reporting AE's which were observed in the short-term Phase 3 trials.

2 INTRODUCTION

2.1 Overview

Calcitriol Ointment has been subject to numerous clinical trials over the last fifteen years, many of which were conducted by the previous sponsor. [redacted] The clinical development by [redacted] established a dose which was used in two vehicle controlled, confirmatory Phase 3 trials, Study 18053 and Study 18054, conducted by the current sponsor, Galderma. In addition Galderma conducted an uncontrolled, international long-term safety trial, Study 2663. A summary of the trials conducted by Galderma and submitted to the NDA are described below in Table 2. b(4)

The review of efficacy is based on the two vehicle-controlled trials, Study 18053 and Study 18054. The review of of short-term safety is based on Study 18053 and 18054. Assessment of long-term safety is based on Study 2663.

2.2 Data Sources

The analysis data sets submitted did not include detailed documentation of derived variables such as derived analysis visits. However, the raw data sets which included date of visit were used to create an efficacy data set used to reproduce the efficacy results as presented in the sponsor's study reports. The raw data sets used to assess the safety and efficacy of Calcitriol Ointment are located at //Cdsesub1/nonectd/N22087/N_000/2007-12-21/Silkis SAS Database (CTD Module 5)/datasets.

Table 2: Efficacy and Safety Studies Overview

Study	Development Objective	Drug Products	Number Subjects	Date†
RD.06.SRE.18053 (Study 18053)	Phase 3 Superiority	Calcitriol Vehicle	209 209	01/2002 – 07/2002
RD.06.SRE.18054 (Study 18054)	Phase 3 Superiority	Calcitriol Vehicle	210 211	01/2002 – 07/2002
RD.03.SRE.2663 (Study 2663)	Phase 3 Long-term Safety	Calcitriol	324 -	09/2001 – 03/2003

† Dates correspond to the start and end of the study.

3 Statistical Evaluation

3.1 Evaluation of Efficacy

The evaluation of efficacy relies on the two identically designed vehicle-controlled Phase 3 trials, Study 18053 and Study 18054.

3.1.1 Study Design

Study 18053 and Study 18054 were multi-center, randomized, double-blind, vehicle-controlled, parallel group comparison studies conducted in the United States. The identically designed trials planned to enroll a total of 400 subjects with mild to moderate chronic plaque psoriasis randomized in 1:1 ratio to Calcitriol Ointment or vehicle. Subjects were to apply treatment twice daily for 8 weeks.

At baseline to be eligible for randomization subjects had to have an investigator global score of 2 (mild) or 3 (moderate) and the body surface area could not exceed 35%. Enrolled subjects were planned to be evaluated at screening, baseline, week 2, week 4, week 6, and week 8. Subjects were dispensed medication at baseline, week 2, week 4, and week 6 where they were told to apply it twice daily, once in the morning and once in the evening. In addition to the baseline visit, all post-baseline visits assessed both the safety and efficacy with the primary time point for efficacy evaluation occurring at week 8.

3.1.2 Endpoints

An investigator global assessment (IGA) is considered to be the primary endpoint which was assessed at all visits. A description of this endpoint is provided in Table 3. This endpoint was dichotomized to success/failure where a success was defined as all subjects who reached an IGA

score of 0 or 1 (clear or minimal) at week 8[1]. In addition, this review will also assesses efficacy where success is defined as a two grade improvement of the IGA score.

Table 3: Investigator Global Severity Description

Score	Label	Description
0	Clear	Plaque Elevation: No elevation over normal skin. Scaling: No scaling. Erythema: hyperpigmentation, pigmented macules, diffuse faint pink or red coloration.
1	Minimal	Plaque Elevation: Possible but difficult to ascertain whether there is slight elevation above normal skin. Scaling: Surface dryness with some white coloration. Erythema: Up to definite red coloration.
2	Mild	Plaque Elevation: Slight but definite elevation, typically edges are indistinct or sloped. Scaling: Fine scale partially or mostly covering lesion. Erythema: Up to definite red discoloration.
3	Moderate	Plaque Elevation: Moderate elevation with rough or sloped edges. Scaling: Coarse scale covering most of all of the lesions. Erythema: Definite red discoloration.
4	Severe	Plaque Elevation: Marked elevation typically with hard or sharp edges. Scaling: Coarse, non-tenacious scale predominates covering most or all the lesions. Erythema: Very bright red coloration.
5	Very Severe	Plaque Elevation: Very marked elevation typically with hard sharp edges. Scaling: Coarse, thick tenacious scale over most of lesions; rough surface. Erythema: Extreme red discoloration, dusky to deep red coloration.

Source: sponsor's protocol

3.1.3 Patient Disposition and Baseline Characteristics

3.1.3.1 Patient Disposition Subject disposition and reason for drop-out for the two Phase 3 trials is provided in Table 4. In each study the percentage of subjects completing the trial was around 89% for subjects randomized to Calcitriol Ointment and 85% for subjects randomized to vehicle. The most common reason for study withdrawal was due to subject request though no specific reason why the subject would request to withdraw is provided in either the study reports or electronic data.

[1]This definition of success coincides with the Division's recommendation at the End of Phase 2 Meeting held on 11/15/1999.

Table 4: Primary Subject Disposition

	Study 53		Study 54	
	Calcitriol ($N = 209$)	Vehicle ($N = 209$)	Calcitriol ($N = 210$)	Vehicle ($N = 211$)
Completed Study	185 (88.5)	178 (85.2)	187 (89.0)	181 (85.8)
Drop Out	24 (11.5)	31 (14.8)	23 (11.0)	30 (14.2)
Reason				
Adverse Event	1 (0.5)	6 (2.9)	6 (2.9)	5 (2.4)
Subject Request	12 (5.7)	13 (6.2)	8 (3.8)	20 (9.5)
Protocol Violation	4 (1.9)	3 (1.4)	0 (0.0)	0 (0.0)
Lost to Follow-Up	6 (2.9)	8 (3.8)	9 (4.3)	4 (1.9)
Other	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)
Pregnancy	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)

Source: sponsor's study report Table 8; results reproduced by reviewer.

3.1.3.2 Baseline Characteristics

3.1.3.2.1 Demographics A listing of the baseline demographics is provided in the Appendix in Section A.1. In both studies the majority of subjects enrolled were listed as Caucasian with balanced enrollment between the treatment groups. The median age of enrolled subjects was 47 and 48 in Studies 18053 and 18054, respectively which was balanced between treatment groups. Approximately 66% and 60% of subjects enrolled in Study 18053 and Study 18054, respectively, were male. Of the females enrolled in both studies, a higher proportion were randomized to vehicle than Calcitriol Ointment (37% vs. 30% in Study 53 and 45% vs. 35% in Study 54). The impact of this imbalance is discussed in Section 4.1.1.

3.1.3.2.2 Prognostic Factors In addition to demographics, the baseline distribution of several prognostic factors with the potential to impact efficacy were also assessed. These were BSA, IGA score, pruritus, erythema, plaque elevation, and scaling where the latter three, which were assessed on both bony and non-bony regions, were converted to the mean value of the bony and non-bony region. Pruritus, erythema, plaque elevation, and scaling were all recorded on a five-point scale with 0='None' and 4='Very Severe'.

Table 13 located in the Appendix (Section A.2) contains the baseline values of the above prognostic factors. The majority of subjects enrolled with an IGA score of moderate which was balanced between the treatment arms. The active assessment of erythema, pruritus, scaling, and plaque elevation also had a majority of subjects enrolled with a score of 2 ('Moderate'). Overall, the distributions are quite similar across treatment groups.

3.1.4 Statistical Methodology

The following details pertain to the statistical analysis as listed in the protocol. Any deviations from protocol definitions are noted. The protocol defined primary endpoint is the percent of subjects with an IGA score of 0 ('clear') or 1 ('minimal') at week 8. As a sensitivity analysis, the review also defines success as a two grade improvement using the IGA scale which requires subjects enrolled with IGA scores of 'mild' to reach 'clear' to be defined as success.

The primary analysis population is the intent-to-treat (ITT) population which is defined as all subjects enrolled and randomized to treatment. The per-protocol (PP) population is included as supportive which excludes those subjects with major protocol violations. Efficacy results of the primary endpoint are provided for both the ITT and PP populations in the review.

The comparison of Calcitriol Ointment to vehicle is carried out at the two-sided $\alpha = 0.05$ level with a null hypothesis of IGA success rates are equal for Calcitriol Ointment and vehicle. Centers recruiting less than 10 subjects within either treatment group are combined for analysis by pooled visit. Missing data is imputed using LOCF with no protocol defined sensitivity analysis. The review will include a sensitivity analysis to the method of data imputation. The protocol defined primary analysis of the primary endpoint will test Calcitriol Ointment versus vehicle using CMH stratified by pooled center on the ITT population.

The protocol also lists several secondary endpoints, some such as erythema and pruritus, are considered related to safety assessment of the local skin reactions. The review will assess efficacy over time using a dichotomized value of the IGA scale. The protocol did not include a multiplicity adjustment for the multiple assessments and as such this analysis is considered exploratory and summarized graphically.

3.1.5 Investigator Global Assessment Results (Intent-to-Treat/LOCF)

3.1.5.1 Primary Analysis: Success = 'Clear' or 'Minimal' Table 5 provides the efficacy results for each of the two Phase 3 trials using the primary endpoint defined as the proportion of subjects with an IGA score of 'clear' or 'minimal' at week 8. The treatment effect in Study 18053 is near 12% which is less than the treatment effect of 20% as observed in Study 18054. This is due to an approximately 10% higher vehicle response in Study 18053 as the response rate of Calcitriol Ointment is quite consistent across the two studies. Overall, both studies demonstrate the statistical superiority of Calcitriol Ointment over vehicle.

Table 5: Investigator Global Results ('Clear' or 'Minimal'): ITT

	Study 18053		Study 18054	
	Calcitriol ($N = 209$)	Vehicle ($N = 209$)	Calcitriol ($N = 210$)	Vehicle ($N = 211$)
Success (%)	72 (34.4%)	47 (22.5%)	70 (33.3%)	26 (12.3%)
p-value†	-	0.0047	-	< .001

† p-values are based on CMH stratified by pooled site.

Source: Study Report Table 13; results reproduced by reviewer.

3.1.5.2 Sensitivity Analysis: Success = Two Grade Improvement Table 6 provides the efficacy results for each of the two Phase 3 trials using an endpoint that defines IGA success as the proportion of subjects with a two grade improvement by week 8. As this definition of success requires subjects with 'mild' disease to reach 'clear', response rates are less than the primary endpoint definition of treatment success. While treatment effects based upon this definition are slightly lower, both studies demonstrated the statistical superiority of Calcitriol Ointment over vehicle.

Table 6: Investigator Global Results (Two Grade Improvement): ITT

	Study 18053		Study 18054	
	Calcitriol ($N = 209$)	Vehicle ($N = 209$)	Calcitriol ($N = 210$)	Vehicle ($N = 211$)
Success (%)	49 (23.4%)	30 (14.4%)	43 (20.5%)	14 (6.6%)
p-value†	-	0.0142	-	< .001

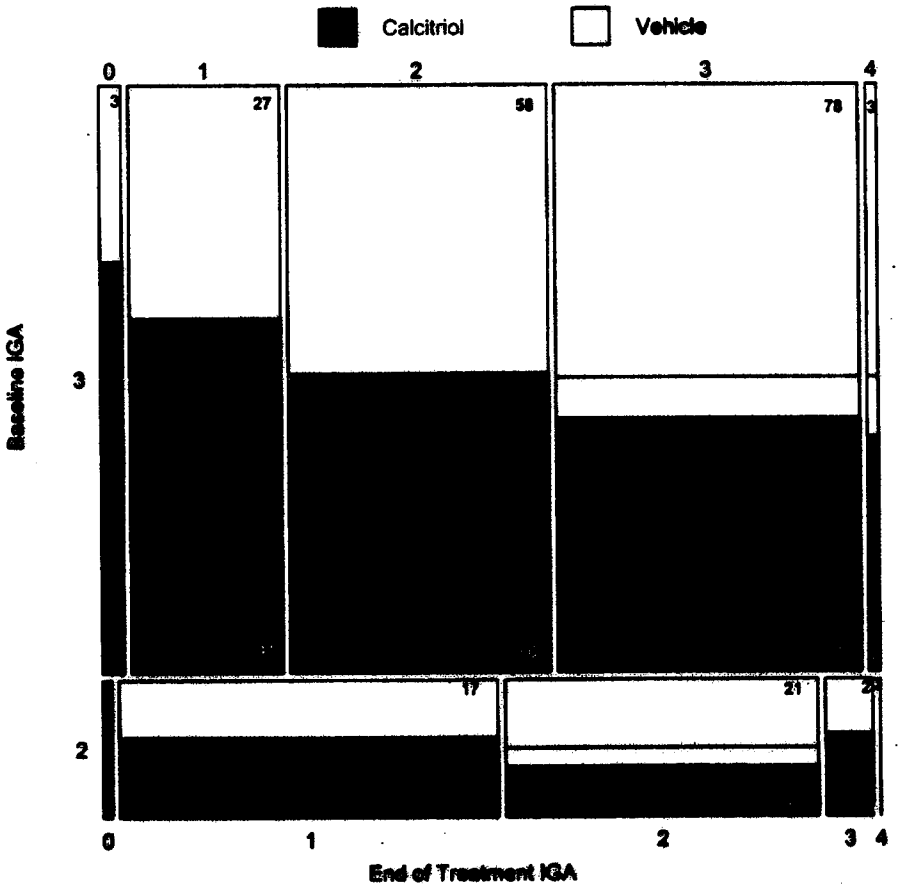
† p-values are based on CMH stratified by pooled site.

Source: Reviewer's analysis.

3.1.5.3 Efficacy by Baseline IGA Score In the following section a modified mosaic plot is used to assess efficacy by baseline IGA score. The modified mosaic plot is a visualization of the sample space, Ω, of which the size of each cell is represented by the proportion of subjects appearing within that cell. Then within each of the cells, the proportion attributed to each treatment groups is based upon the fraction of the marginal proportions for each treatment group. The end graphic is then a collection of tiles arranged as a mosaic plot. The Appendix Section A.3 provides further details on the derivation of the graphic.

3.1.5.3.1 Study 18053 Figure 1 depicts the modified mosaic plot for Study 18053[2]. Within each cell, when the shaded region for Calcitriol Ointment is above the horizontal line which corresponds to no effect, this implies a higher proportion of subjects treated with Calcitriol Ointment are represented in this cell than subjects treated with vehicle (i.e. there is a treatment effect favoring Calcitriol Ointment within this cell). If Calcitriol Ointment is more efficacious than vehicle, a downward staircase type of pattern across the end of treatment IGA scores would be seen for a given baseline IGA score. For the most part, this is the general trend seen in Figure 1.

Figure 1: Efficacy by Baseline IGA Score 18053



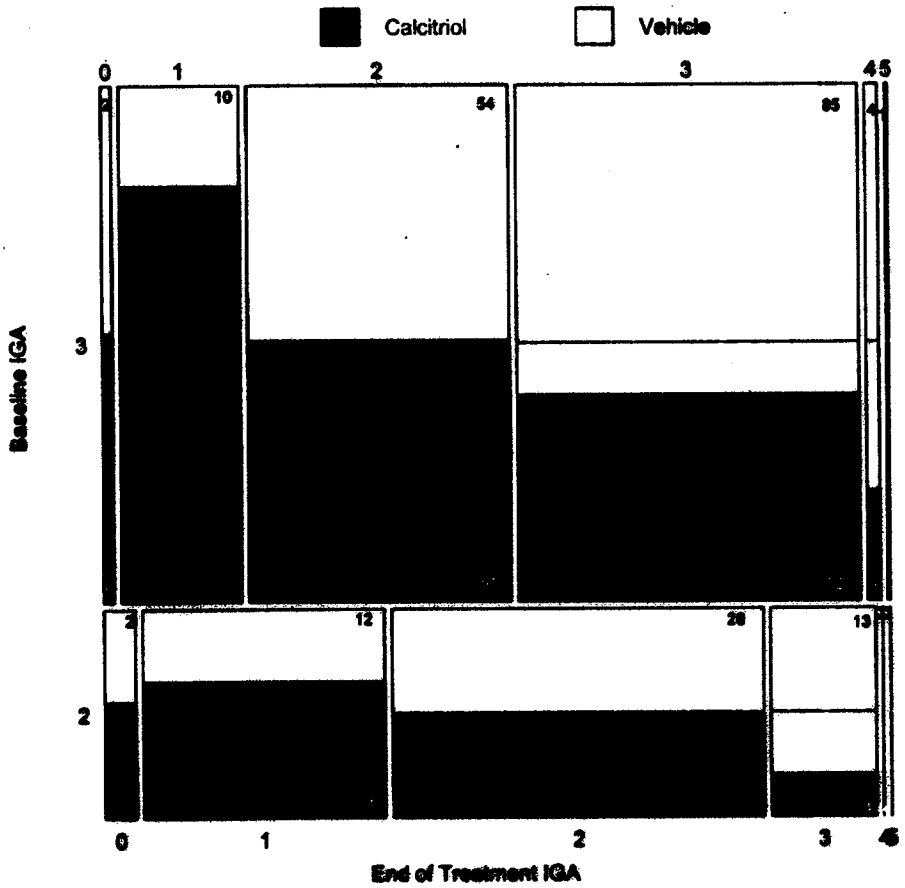
The number of subjects within a given cell and treatment group are also depicted in the plot, and as such the graphic can be used to derive the number of IGA successes as shown in Tables 5 and 6 in Study 18053. Using this information we can see that a total of 11 subjects had an

[2]The one subject with an IGA score of 4 at baseline was deleted prior to constructing the graphic for clarity.

end of treatment IGA score higher than the baseline IGA score: 5 (3 to Calcitriol Ointment and 2 to vehicle) entered with a baseline IGA of 2 and had an end of treatment score of 3; 5 (2 to Calcitriol Ointment and 3 to vehicle) entered with a baseline IGA score of 3 and had an end of treatment score of 4; and 1 subject treated with Calcitriol Ointment went from a baseline IGA score of 2 to an IGA score of 4 at the end of treatment.

3.1.5.3.2 Study 18054 Figure 2 depicts the modified mosaic plot for Study 18054. While Study 18054 does not have a clear downward staircase pattern as seen in Study 18053, there is still a treatment effect for an end of treatment score of 0 or 1 regardless of the baseline IGA score. In Study 18054 5 and 18 subjects randomized to Calcitriol Ointment and vehicle, respectively had an increase in their end of treatment IGA score.

Figure 2: Efficacy by Baseline IGA Score 18054



3.1.6 Investigator Global Assessment Results (Per Protocol/LOCF)

3.1.6.1 Success = 'Clear' or 'Minimal' Table 7 provides the efficacy results for each of the two Phase 3 trials using the primary endpoint defined as the proportion of subjects with an IGA score of 'clear' or 'minimal' at week 8 for the per protocol population. Treatment effects observed for Studies 18053 and 18054 were 12.7% and 22.6%, respectively, which are similar to those observed in the ITT population. The comparison of Calcitriol Ointment to vehicle reached statistical significance at the $\alpha = 0.05$ level in each study.

Table 7: Investigator Global Results ('None' or 'Minimal'): PP

	Study 18053		Study 18054	
	Calcitriol ($N = 188$)	Vehicle ($N = 179$)	Calcitriol ($N = 185$)	Vehicle ($N = 176$)
Success (%)	69 (36.7%)	43 (24.0%)	67 (36.2%)	24 (13.6%)
p-value†	-	0.0048	-	< .001

† p-values are based on CMH stratified by pooled site.

Source: Reviewer's analysis.

3.1.6.2 Success = Two Grade Improvement Table 8 provides the efficacy results for each of the two Phase 3 trials using an endpoint that defines IGA success as the proportion of subjects with a two grade improvement by week 8 for the per protocol population. Treatment effects observed for Studies 18053 and 18054 were 8.8% and 14.7%, respectively, which are similar to those observed in the ITT population. While treatment effects for a two grade IGA improvement are smaller than a definition defining success as an IGA score of 0 or 1, the comparison of Calcitriol Ointment to vehicle reached statistical significance at the $\alpha = 0.05$ level in each study.

Table 8: Investigator Global Results (Two Grade Improvement): PP

	Study 18053		Study 18054	
	Calcitriol ($N = 188$)	Vehicle ($N = 179$)	Calcitriol ($N = 185$)	Vehicle ($N = 176$)
Success (%)	47 (25.0%)	29 (16.2%)	42 (22.7%)	14 (8.0%)
p-value†	-	0.0269	-	< .001

† p-values are based on CMH stratified by pooled site.

Source: Reviewer's analysis.

3.1.7 Sensitivity Analysis to Method of Data Imputation

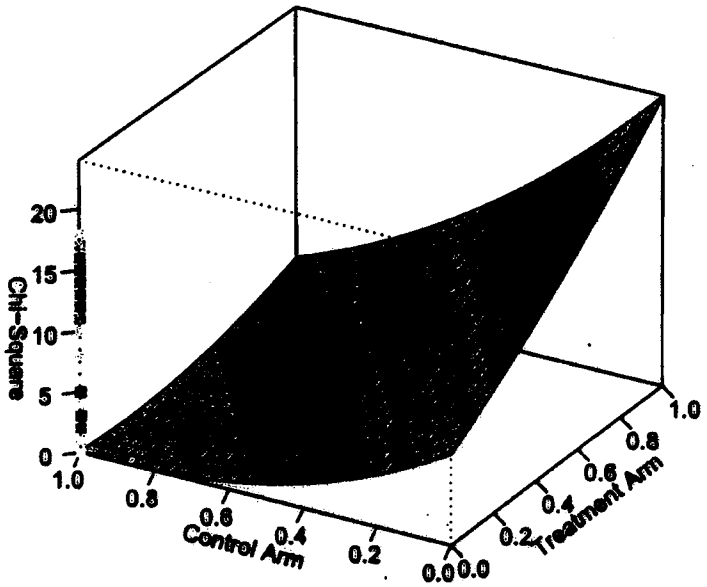
In the following sensitivity analysis to the method of data imputation, all missing data are imputed using various proportions of successes[3] for the missing data. This can vary from the extremes; all missing data for the control arm are imputed as successes and all missing data from the active arm are imputed as failures to the case where all missing controls are failures and all missing active are success. Everything in between the extremes is covered in this analysis. Once imputed these data are combined with the complete data and a Chi-square test is performed. The Chi-square test is performed for every possible proportion of imputed successes and the response surface of the Chi-Square statistic is plotted in a perspective plot. To reach statistical significance at the $\alpha = 0.05$ level (i.e. assuming no multiplicity adjustment), the value of the Chi-square statistic should be 3.84 or greater. This value is represented between blue ($\chi^2 = 3$) and cyan or light blue ($\chi^2 = 4$) in the perspective plot. Thus, for points falling in the cyan range, this area would correspond to statistical significance. Any range above this would also correspond to statistical significance.

3.1.7.1 Study 18053 Twenty-two (10.5%) subjects treated with Calcitriol Ointment, and twenty-nine (13.9%) subjects treated with vehicle had missing week 8 data. Figure 3 depicts the full range of the percent imputed as successes and the corresponding χ^2 statistic. In the case where all missing data is imputed as failures, $\chi^2 > 5$, showing statistical significance in favor of Calcitriol Ointment over vehicle. When all missing data is imputed as successes, such a scenario increases the number of successes for the vehicle arm as there is a higher percentage of missing data for the vehicle arm than the Calcitriol Ointment arm. In this case, $\chi^2 \approx 3$, which fails to reach statistical significance. Even under the extreme scenario which is the least favorable to Calcitriol Ointment, which imputes all missing data for the Calcitriol Ointment arm as failures and all missing data for the vehicle arm as success did not result in a Chi-square value that would favor vehicle. As no imputation scenario shows a trend in favor of vehicle over Calcitriol Ointment, this suggests efficacy conclusions are not driven by the method of data imputation.

3.1.7.2 Study 18054 Twenty-three (11.0%) subjects treated with Calcitriol Ointment, and thirty (14.2%) subjects treated with vehicle had missing week 8 data. Recall that the treatment effect for Study 54 was 22.6%. In the extreme case all missing data for the vehicle arm is imputed as success and all missing data for the Calcitriol Ointment arm is imputed as failure. Even under such a scenario the treatment effect is 7.3% which favors Calcitriol Ointment over vehicle. Thus, the method of data imputation for Study 18054 does not impact efficacy conclusions.

[3]Success definition follows the protocol as an IGA score of 'none' or 'minimal'.

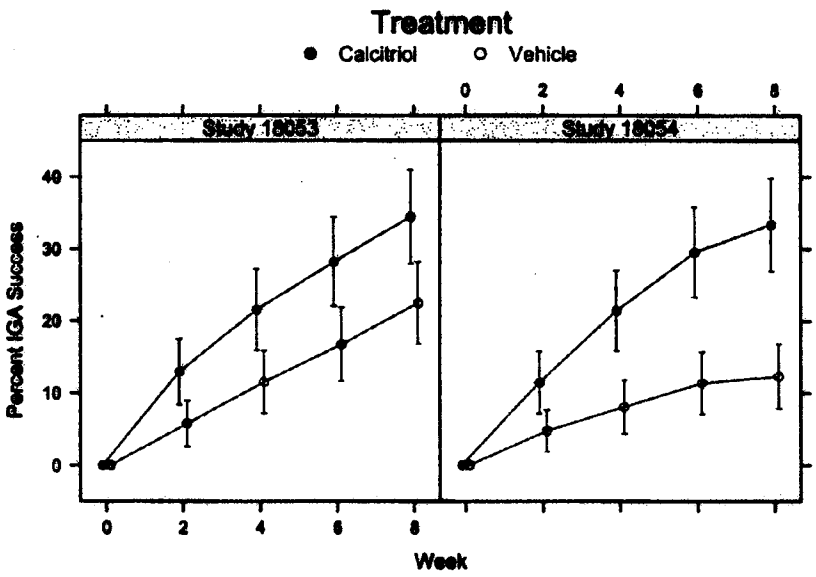
Figure 3: Sensitivity Analysis 18053



3.1.8 IGA Success Rate Over Time

Figure 4 depicts the percent of IGA successes ('clear' or 'minimal') at each week along with unadjusted 95% confidence intervals. In both studies the increase in the number of subjects with an IGA success was roughly linear with similar response rates for the two studies. The only difference was in the response of the vehicle in Study 18053 which also increased linearly over time.

Figure 4: Efficacy Across Time



3.2 Evaluation of Safety

Adverse events were recorded by body system and COSTART term. The frequency counts in the tables that follow reflect the number of subjects reporting one or more AE's that map to the body system and COSTART term. Note that subjects who report more than one event are counted only once in the following tables.

3.2.1 Study 18053 and Study 18054

In Study 18053 a total of 71 subjects (34.0%) treated with Calcitriol Ointment reported at least one AE whereas 63 subjects (30.1%) of subjects treated with vehicle reported at least one AE. In Study 18054 a total of 78 subjects (37.1%) treated with Calcitriol Ointment reported at least one AE. Similarly 78 subjects (37.9%) of subjects treated with vehicle reported at least one AE. Table 9 contains the adverse events reported in at least 3% of subjects who enrolled in the two Phase 3 trials. Event rates for these AE's were quite similar between Calcitriol Ointment and vehicle.

3.2.2 Study 2663

Study 2663 was an open-label long-term safety study to assess the local and systemic safety of Calcitriol Ointment when applied twice daily for up to 52 weeks. A total of 324 subjects

Table 9: Adverse Events (Study 18053 and Study 18054)

	Calcitriol ($N = 419$)	Vehicle ($N = 420$)
BODY AS A WHOLE		
LAB TEST ABNORMALITY	19 (4.5)	19 (4.5)
FLU SYNDROME	18 (4.3)	15 (3.6)
HEADACHE	11 (2.6)	11 (2.6)
INJURY ACCIDENT	9 (2.1)	10 (2.4)
RESPIRATORY SYSTEM		
PHARYNGITIS	9 (2.1)	12 (2.9)
SINUSITIS	6 (1.4)	12 (2.9)
SKIN AND APPENDAGES		
DISCOMFORT SKIN	13 (3.1)	9 (2.1)
PRURITUS	8 (1.9)	8 (1.9)
PSORIASIS	4 (1)	12 (2.9)

Source: Table SAF 4 of study report; results reproduced by reviewer.

with mild to moderate plaque psoriasis were enrolled into the trial. The median duration of treatment exposure was 191.5 days (see Appendix Section A.4 for a plot of the empirical cumulative distribution function of the days on treatment).

A total of 130 subjects (40.1%) reported at least one adverse event in Study 2663. Event rates for Calcitriol Ointment in Study 2663 are provided in Table 10 for those AE's that occurred in at least 3% of subjects. The AE most frequently reported was laboratory abnormalities which occurred in 7.7% of subjects.

Comparing the AE's reported in Study 2663 to those reported in the Phase 3 trials, there is an increase in the rates for lab test abnormalities, pharyngitis, psoriasis, and pruritus. In addition, the AE's infection of the skin, urine abnormality, and hypercalcinuria occurred at a rate greater than 3% in Study 2663 but were not observed at such a rate in the Phase 3 trials.

Table 10: Adverse Events (Study 2663)

	Calcitriol ($N = 324$)
BODY AS A WHOLE	
LAB TEST ABNORMALITY	25 (7.7)
FLU SYNDROME	12 (3.7)
RESPIRATORY SYSTEM	
PHARYNGITIS	12 (3.7)
SKIN AND APPENDAGES	
PSORIASIS	13 (4.0)
INFECTION OF SKIN	10 (3.1)
PRURITUS	10 (3.1)
UROGENITAL SYSTEM	
URINE ABNORMALITY	14 (4.3)
HYPERCALCINURIA	11 (3.4)

Source: Table 10 of Sponsor Study Report; results reproduced by reviewer.

4 Findings in Special/Subgroup Populations

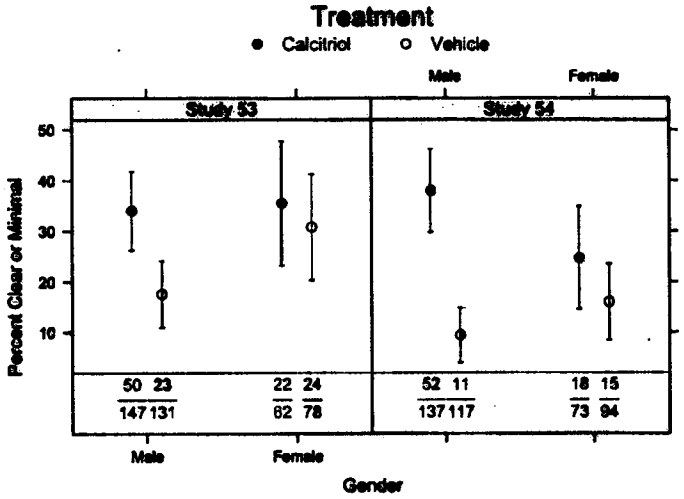
4.1 Gender, Race, and Age

In the following section assessing the efficacy in subgroups, the primary endpoint defined as the percent of subjects with an end of treatment IGA score of 'clear' or 'minimal' is used.

4.1.1 Primary Efficacy Results by Gender

Figure 5 depicts efficacy results according to gender along with unadjusted 95% confidence intervals. In Study 18053 the percent of subjects treated with Calcitriol Ointment with an IGA score of 'clear' or 'minimal' was similar for males and females though the response rate for subjects treated with vehicle was higher in females than males. In Study 18054 the response rate for males treated with Calcitriol Ointment was near 40% whereas the response rate for females treated with Calcitriol Ointment was around 25%. Recall that a higher proportion of females were randomized to vehicle than Calcitriol Ointment at baseline. As the vehicle response was highest in females, this baseline imbalance did not favor Calcitriol Ointment.

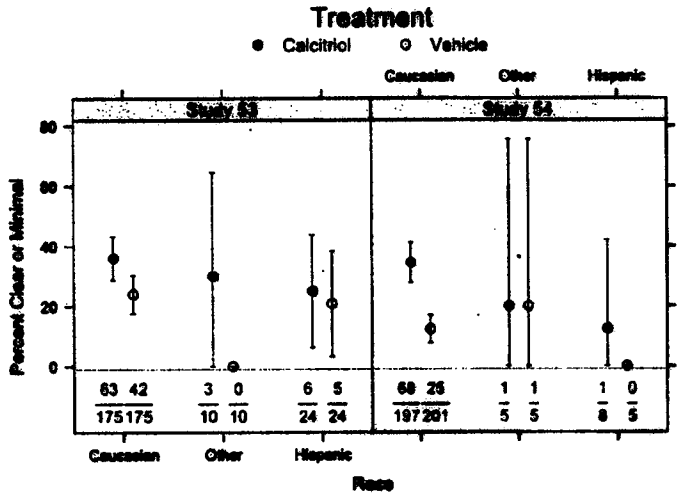
Figure 5: Percent IGA Success by Gender



4.1.2 Primary Efficacy Results by Race

Race was broken into three categories: Caucasian, Hispanic, and Other. Figure 6 depicts the mean response rates along with unadjusted 95% confidence intervals by race. The subjects enrolled were primarily listed as Caucasian and as such the estimates of response rates in Hispanic and Other subjects may not be reliable due to the limited sample size within each category.

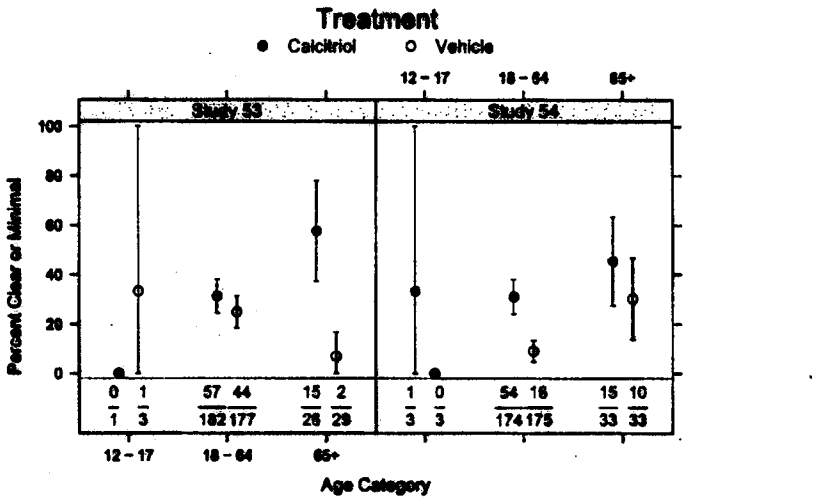
Figure 6: Percent IGA Success by Race



4.1.3 Efficacy by Age Group

Age was dichotomized into three groups: 12 to 17 years, 18 to 64 years, and 65 years and older. The choice of the age groups follow the study report. The majority of subjects enrolled were between the ages of 18 and 64. In the 18 to 64 years subgroup the response rate for Calcitriol Ointment was roughly 31% in both studies though the vehicle response rate was higher in Study 53 for this subgroup.

Figure 7: Percent IGA Success by Age Group



4.2 Other Special/Subgroup Populations

4.2.1 Efficacy By Site

Figures 8 and 9 depict the treatment effect for each study site (vertical gray dotted lines) as well as the overall percent of subjects with an IGA score of 'clear' or 'minimal' (horizontal solid lines). Sample size for a given treatment arm within a site is provided next to the plotting character of each treatment arm. Sites are listed according to the date the first subject enrolled at the site from earliest to latest. The graphic illustrates that the response rates are quite variable across sites and in some sites the vehicle has a higher response rate than Calcitriol Ointment.

Figures 8 and 9 were used to identify sites for inspection by the Division of Scientific Investigations (DSI). The following is the language issued in the DSI consult letter.

> "Please inspect sites 1170 and 2123 in study 18053. Site 2123 has a relatively large sample size and a high treatment effect (zero response for the vehicle and nearly 50%

Figure 8: Efficacy By Site (18053)

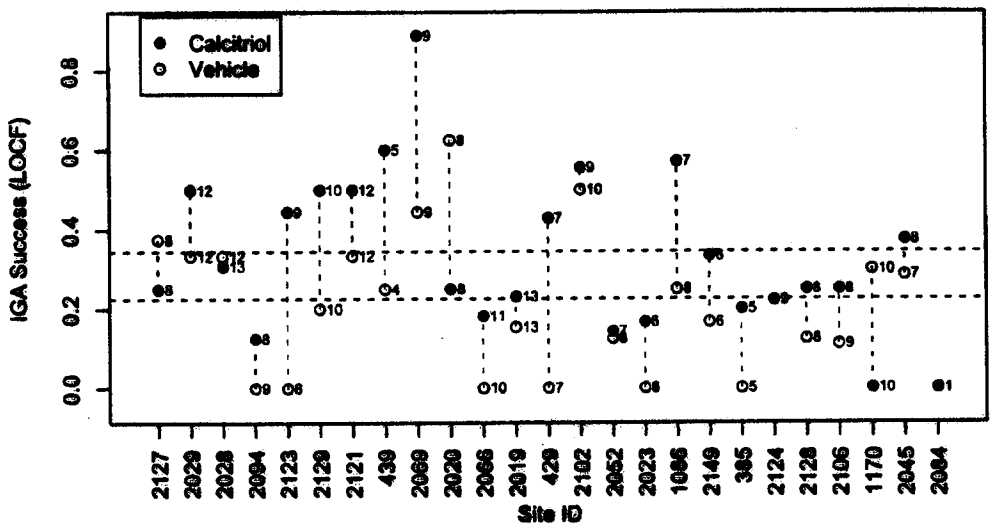
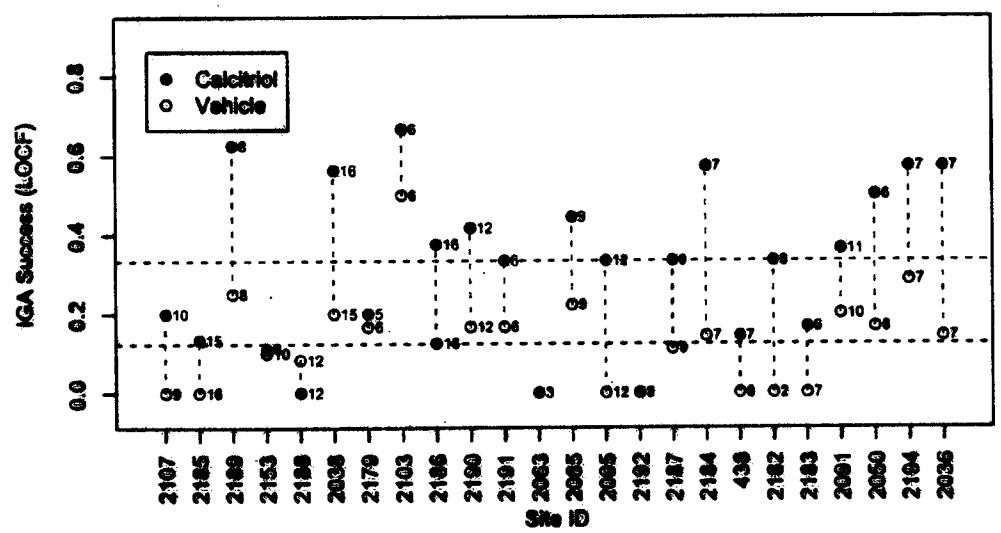


Figure 9: Efficacy By Site (18054)

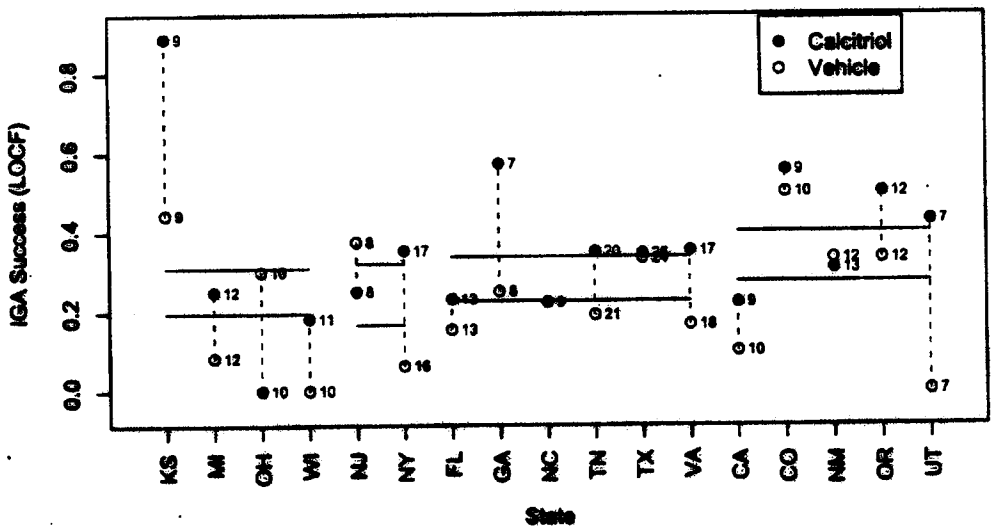


response for the active) which is considerably larger than the overall treatment effect of 12%. Site 1170 enrolled 20 subjects of which 0/10 subjects treated with active responded, whereas 3/10 treated with vehicle responded resulting in a treatment effect favoring vehicle. As this is not consistent with results from other centers, interest lies in how one might be able to explain such an extreme deviation from the overall study conclusions and if such results are due to study conduct at this site."

4.2.2 Efficacy By State/Region

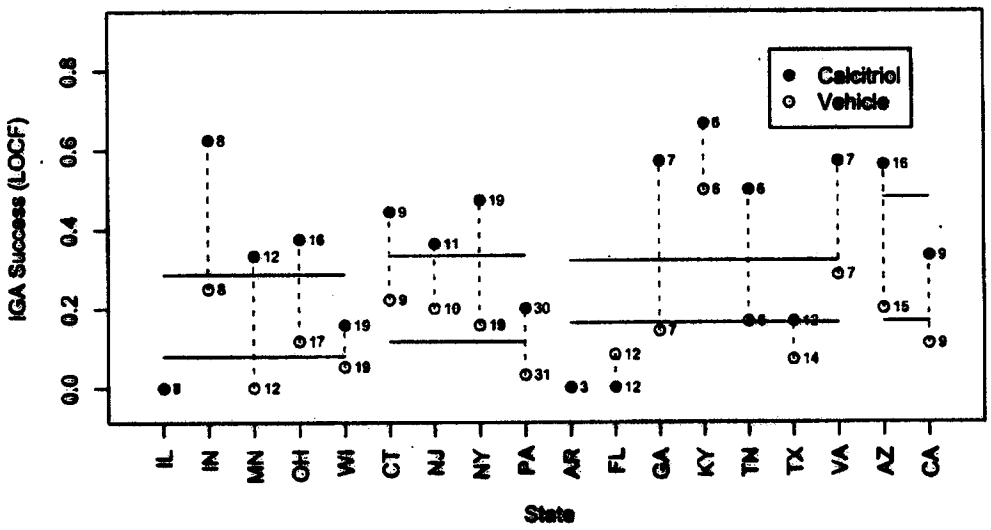
An additional analysis was conducted to determine if the efficacy results were impacted by the region in which the study was conducted. The regions were defined as West, South, Midwest, and Northeast based upon the U.S. Census Bureau designations. Specifically, the goal of this analysis was to see if region could explain the higher response rate for vehicle in Study 18053 than in Study 18054. Similar to Figures 8 and 9, graphical depictions across states grouped by region were constructed and are shown in Figures 10 and 11. The mean response within a region is shown using the horizontal lines.

Figure 10: Efficacy By State/Region (18053)



Comparing results between Study 18053 and Study 18054, even within the same region, the response rates for vehicle were consistently higher in Study 18053. Overall, there was not a large difference in the mean response rates between regions. In Study 18053 the response rates

Figure 11: Efficacy By State/Region (18054)



in Kansas were much higher than in other states though these subjects were enrolled at a single center (Center 2069 as shown in Figure 8). Thus, this analysis was not able to explain the difference in vehicle response rate between the two studies.

5 Summary and Conclusions

5.1 Statistical Issues and Collective Evidence

At the End of Phase 2 meeting held on 11/15/1999, the Division was in agreement with defining the primary endpoint as the proportion of subjects with an IGA score of 'clear' or 'minimal' at week 8. The primary analysis was conducted on the ITT population with missing data imputed using LOCF. Primary efficacy results are shown in Table 11. Both studies demonstrated that Calcitriol Ointment was statistically superior to vehicle.

Safety assessment was based upon adverse events recorded by body system and COSTART term. In the two short-term Phase trials, Studies 18053 and 18054, reported AE's were similar between Calcitriol Ointment and vehicle. In the open-label long-term safety study, Study 2663, there was a slight increase in the percentage of subjects reporting AE's which were observed in the short-term Phase 3 trials.

Table 11: Investigator Global Results ('Clear' or 'Minimal'): ITT

	Study 18053		Study 18054	
	Calcitriol ($N = 209$)	Vehicle ($N = 209$)	Calcitriol ($N = 210$)	Vehicle ($N = 211$)
Success (%)	72 (34.4%)	47 (22.5%)	70 (33.3%)	26 (12.3%)
p-value†	-	0.0047	-	< .001

† p-values are based on CMH stratified by pooled site.

Source: Study Report Table 13; results reproduced by reviewer.

5.2 Conclusions and Recommendations

Primary efficacy analysis was based on the proportion of subjects who had an end of treatment (week 8) Investigator Global Assessment (IGA) score of 'clear' or 'minimal' which the Division agreed to at an End of Phase 2 meeting. In Study 18053 Calcitriol Ointment was statistically superior to vehicle ($p = 0.0047$) with response rates of 34.4% and 22.5% for Calcitriol Ointment and vehicle, respectively. In Study 18054, Calcitriol Ointment was statistically superior to vehicle ($p < 0.001$) with observed response rates of 33.3% and 12.3%, respectively.

The following is a portion of the clinical studies section of the sponsor's proposed label as submitted to the NDA on 12/21/2007.

b(4)

The following are recommended changes to the label.

b(4)

b(5)

- Rather than listing efficacy in the text, a table depicting the response rate for each study and treatment arm should be included *without* p-values.

- b(4)

-

APPENDIX

A.1 Baseline Demographics

The demographics for each trial are provided in Table 12.

Table 12: Demographics by Treatment (Study 18053 and 18054)

	Study 18053		Study 18054	
	Calcitriol ($N = 209$)	Vehicle ($N = 209$)	Calcitriol ($N = 210$)	Vehicle ($N = 211$)
Age	36 46 56	39 47 55	39 47 59	40 48 59
Gender : Female	30% (62)	37% (78)	35% (73)	45% (94)
Race : Caucasian	84% (175)	84% (175)	94% (197)	95% (201)
Black	3% (6)	2% (5)	1% (2)	1% (2)
Asian	1% (2)	1% (3)	1% (3)	0% (1)
Hispanic	11% (24)	11% (24)	4% (8)	2% (5)
Other	1% (2)	1% (2)	0% (0)	1% (2)

a b c represent the lower quartile *a*, the median *b*, and the upper quartile *c* for continuous variables. Numbers after percents are frequencies. Source: Reviewer's Analysis.

A.2 Baseline Prognostic Factors

The baseline distribution of the prognostic factors for each trial is provided in Table 13.

Table 13: Prognostic Factors by Treatment (Study 18053 and 18054)

	Study 18053		Study 18054	
	Calcitriol ($N = 209$)	Vehicle ($N = 209$)	Calcitriol ($N = 210$)	Vehicle ($N = 211$)
Total Body Surface Area Involved	4 7 12	5 8 12	5.0 8.5 15.0	5.0 10.0 17.0
Global Severity : Mild	19% (39)	19% (40)	31% (66)	26% (55)
Moderate	81% (169)	81% (169)	69% (144)	74% (156)
Severe	0% (1)	0% (0)	0% (0)	0% (0)
Pruritus : None	8% (17)	4% (9)	13% (27)	11% (24)
Mild	32% (66)	28% (58)	36% (75)	29% (61)
Moderate	44% (91)	48% (100)	37% (78)	45% (94)
Severe	13% (28)	15% (32)	12% (26)	13% (27)
Very Severe	3% (7)	5% (10)	2% (4)	2% (5)
Erythema† : 0	0% (0)	0% (0)	0% (1)	0% (1)
0.5	0% (0)	0% (0)	0% (0)	0% (1)
1	6% (12)	7% (15)	11% (24)	9% (20)
1.5	9% (19)	10% (20)	13% (27)	12% (26)
2	60% (126)	59% (124)	57% (120)	59% (125)
2.5	11% (23)	11% (24)	6% (12)	8% (17)
3	12% (26)	11% (24)	12% (25)	10% (21)
3.5	1% (2)	0% (0)	0% (0)	0% (0)
4	0% (1)	1% (2)	0% (1)	0% (0)
Plaque Elevation† : 0.5	0% (0)	0% (0)	0% (0)	1% (2)
1	9% (18)	5% (11)	15% (31)	10% (21)
1.5	8% (17)	13% (27)	11% (24)	14% (29)
2	57% (120)	57% (120)	50% (104)	54% (113)
2.5	14% (30)	14% (29)	11% (24)	11% (24)
3	9% (19)	10% (21)	12% (25)	9% (20)
3.5	2% (5)	0% (1)	0% (0)	1% (2)
4	0% (0)	0% (0)	1% (2)	0% (0)
Scaling† : 0.5	0% (0)	0% (0)	0% (0)	1% (2)
1	2% (4)	3% (7)	10% (20)	7% (14)
1.5	8% (16)	7% (14)	13% (27)	10% (22)
2	54% (113)	59% (123)	46% (96)	50% (105)
2.5	15% (32)	14% (30)	13% (28)	15% (32)
3	19% (40)	14% (30)	16% (33)	16% (33)
3.5	0% (1)	2% (4)	1% (2)	1% (3)
4	1% (3)	0% (1)	2% (4)	0% (0)

a b c represent the lower quartile *a*, the median *b*, and the upper quartile *c* for continuous variables. Numbers after percents are frequencies. Source: Reviewer's Analysis.

† Mean score of the score from bony and non-bony areas.

A.3 Modified Mosaic Plot Details

The following are the details used to derive the modified mosaic plots shown in Figures 1 and 2.
Define S to represent a categorical r.v. corresponding to some subgroup of interest with a distinct levels s.t. $S = \{1, 2, \ldots, a\}$ (e.g. S = baseline IGA score).
Define Y to represent a r.v. corresponding to the response variable of interest with b distinct levels s.t. $Y = \{1, 2, \ldots, b\}$ (e.g. Y = end of treatment IGA score).
Define G to represent the treatment with 2 levels s.t. $G = \{A, B\}$

Define x_{ijg} = count corresponding to the i-th value of S $(i = 1, 2, \ldots, a)$, the j-th value of Y $(j = 1, 2, \ldots, b)$, and g-th treatment group $(G = A, B)$.

The observed total sample size is N s.t. $N = \sum_g n_{..g} = \sum_g x_{ijg}$.

The sample space for the r.v.'s S and Y is Ω. A total of $a \times b$ $(i,\ j)$ pairs exist where each can be thought to represent a 2-dimensional cell within the sample space.

The size of each $(i,\ j)$ cell in the sample space, Ω, is proportional to the number of observations within the $(i,\ j)$ cell which can be defined as
$w_{ij.} = \sum_g x_{ijg}/N$ s.t. $\sum_i \sum_j w_{ij.} = 1$.

Within each $(i,\ j)$ cell it must be determined what proportion of the cell space to designate for each treatment group. To derive this amount define the following.

- $N_{i.g}$ = marginal sample size of subjects treated with g in subgroup i.
- $p_{ijg}/n_{i.g}$ = marginal proportion of subjects in the i-th subgroup treated with g who have value j for variable Y.

The proportion of cell $(i,\ j)$ attributed to each treatment group can be defined as

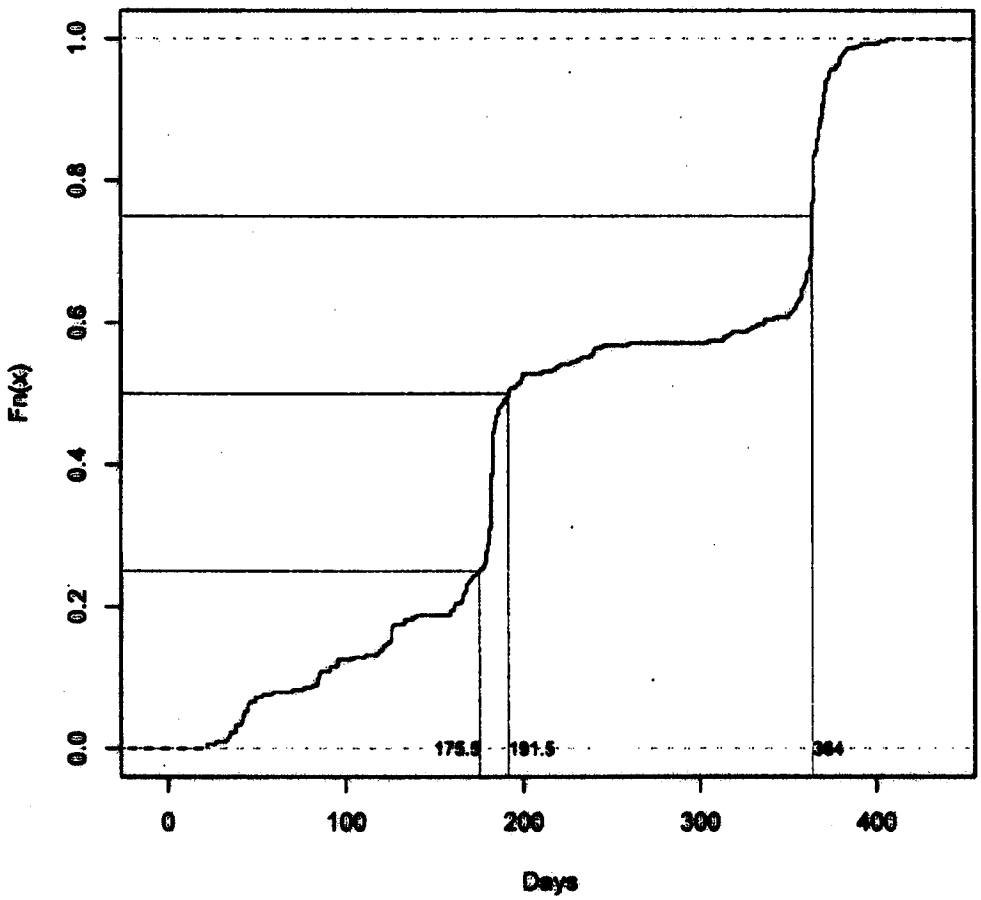
$$\lambda_{ijg} = \frac{p_{ijg}/n_{i.g}}{\sum_g (p_{ijg}/n_{i.g})} \tag{1}$$

Using such definitions it is possible to construct a visualization of the sample space, Ω of which the size of the (i, j) cells are represented by $w_{ij.}$. Then within each of the (i, j) cells, the proportion attributed to treatment group g is λ_{ijg}. The end graphic is then a collection of tiles arranged as a mosaic plot.

A.4 Treatment Duration in Study 2663

Figure 12 is a plot of empirical cumulative distribution function of the number of days on treatment. The median time on treatment was calculated to be 191.5 days and the 25th and 75th percentiles were 175.5 and 364 days, respectively. Note that the initial planned duration of treatment was 26 weeks, but a protocol amendment was made after study enrollment to extend the treatment period to 52 weeks.

Figure 12: Days on Treatment (2663)



SIGNATURES/DISTRIBUTION LIST

Primary Statistical Reviewer: Mat Soukup, Ph.D.
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Matt Soukup
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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Pharmacoepidemiology and Statistical Science
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CARCINOGENICITY STUDY

NDA Number:	22,087 / Serial 000
Drug Name:	Silkis Calcitriol ointment
Indication(s):	Treatment for plaque type psoriasis.
Applicant:	Galderma
Date(s):	Submitted 06/28/06
	Reports submitted 12/21/07
	Data submitted 02/20/08
Review Priority:	Standard
Biometrics Division:	Division 6
Statistical Reviewer:	Steve Thomson
Concurring Reviewer:	Team Leader: Karl Lin, Ph. D.
Medical Division:	Dermatological and Dental Drug Products
Toxicologist:	Reviewer: Norman See, Ph.D. Team Leader: Barbara Hill, Ph.D.
Project Manager:	Margo Owens Bronwyn Collier
Keywords:	Carcinogenicity, Cox regression, Kaplan-Meier product limit, Survival analysis, Trend test

Table of Contents

1. EXECUTIVE SUMMARY

According to the reports provided by the Contract Research Organization, this submission was intended to assess the carcinogenic potential of Calcitriol when administered orally to rats (gavage) and dermally as an ointment to mice for periods of up to 24 months. The sponsor was Galderma Research & Development in France. The studies were conducted by [redacted] Apparently no protocols for either study were included in the submission analyzed by this reviewer. The descriptions of the studies given below are taken from the corresponding Final Reports. b(4)

1.1. Conclusions and Recommendations

The submission summarizes the results of both an oral rat study and a dermal mouse study of the carcinogenic potential of Calcitriol when applied for up to two years. In the rat study there were five treatment groups per gender, numbered as groups 1-5, with group 1 denoting a water only control and group 5 a vehicle control, while the remaining groups 2-4 had oral (gavage) doses of 0.005, 0.03, and 0.1 µg/kg/day, respectively. The latter three treatment groups were labeled as Low, Medium, and High dose groups, respectively. In the mouse study there were four treatment groups per gender, numbered as groups 1 through 4, with dermally applied dose levels of 0.0, 0.03, 0.06, and 1 ppm. The dose groups in mice were also labeled as Vehicle Control, Low, Medium, and High, respectively. In both species and in each gender, each of the main treatment groups, including controls, had 60 animals per group. Note that while mice were housed singly, rats were housed together in groups of five. As noted in Section 1.3.1.5 below, this may cause problems with the analysis.

The statistical significances of the tests of differences in survival across treatment groups are given below (Table 1.). The test for homogeneity is a test that survival is equal across treatment groups, while the test of trend is a test of dose related trend. The Cox test is usually called the logrank test, while the K-W, i.e., Kruskal-Wallis test, is more commonly called the Wilcoxon test or the generalized Wilcoxon test. Note that the Wilcoxon test places more weight on earlier events than does the logrank test.

Table 1. Statistical Significances of Tests of Homogeneity and Trend in Survival

	Rats				Mice			
	Males		Females		Males		Females	
	Cox	K-W	Cox	K-W	Cox	K-W	Cox	K-W
Homogeneity over 5 groups (both controls)	0.3578	0.4830	0.1242	0.1745				
Homogeneity over 4 groups (with vehicle)	0.3594	0.4580	0.0739	0.1163	0.0305	0.0410	0.0057	0.0043
Trend over 4 groups	0.4438	0.6769	0.0098	0.0239	0.0033	0.0095	0.0006	0.0006
Departure from trend	0.2685	0.2977	0.8751	0.6685	0.8645	0.4652	0.6882	0.4938

For both genders in rats, the tests of homogeneity in survival over all five treatment groups, including the water control, and tests of homogeneity in survival in the group of four treatments defined by excluding the water control, were never rejected at the usual 0.05 level (all eight $p \geq 0.0739$), although significance levels were close in females. However, the more powerful test of no trend over dose levels was rejected in female rats (Cox p=0.0098, K-W p=0.0239), indicating there is a trend. Among the four treatment groups in mice there was fairly strong evidence of heterogeneity in survival, particularly in females, since the tests of homogeneity are all rejected (Males: Cox p=0.0305, K-W p=0.0410, Females: Cox p=0.0057, K-W p=0.0043). In both genders in mice there was even stronger evidence of a trend over dose (Males: Cox p=0.0033, K-W p=0.0095, Females: both Cox p = K-W p=0.0006). For neither gender in either species was there any strong evidence of treatment differences above those adequately modeled by simple trend in dose (all eight $p \geq 0.2685$).

From the mortality tables (tables 7, 8, 13, and 14 below) or the Kaplan-Meier curves in Appendix 1, one can see that in male rats there was no clear treatment related effect on survival. In female rats the vehicle treatment groups seemed to have the lowest mortality (i.e., highest survival). In both mouse genders there seemed to be a generally increasing mortality over dose, particularly later in the study. Again, further details are presented in Appendix 1.

The Sponsor notes that complete histopathological examinations were done for all treatment groups in rats only in the thyroid, stomach, kidneys, aorta, heart, and sternum, and in mice only at the administration site, duodenum, eyes, kidneys, aorta, and sternum. Otherwise complete examinations were performed only for the High dose groups and the control groups. In the Low and Medium dose groups histopathological examinations were performed only for all animals found dead, killed moribund, or showed macroscopic abnormalities, including masses or nodules during the study or at necropsy. This implies that, except for the organs cited above, in both studies the data generating processes for the Low and Medium dose groups was different from that for the Controls and the High dose group. In particular it could be expected to detect fewer tumors. Thus, except for the cited organs, tests of carcinogenicity that included these doses, such as the overall test of trend and the tests comparing these doses to the control were not strictly appropriate. However, results of such tests were included since they may be helpful.

To avoid confounding the effect of the vehicle with Calcitriol treatments, the carcinogenicity tests involving Calcitriol used the vehicle as the reference dose group to the Calcitriol treatment groups. In rats the water only control was used primarily to estimate background rate, and thus determine if the neoplasm could be classified as common (incidence ≥1%) or rare (incidence < 1%). A no-vehicle control group was not used in the mouse study, and the vehicle control was used to estimate the background rate to determine if the tumor was rare or not. The endpoint used in the FDA analyses of tumorigenicity is the minimum of the time of observation, time of death due to the tumor, or time of detection when the animal dies or is sacrificed. The Sponsor's analyses of tumorigenicity were apparently based only on the later two. This should have had little to no effect on actual tumor incidence, but could explain differences in the actual tests of tumorigenicity. Complete incidence tables and the results of the

FDA Peto tests and poly-3 tests of tumorigenicity are provided in Appendices 2 and 3, respectively. Statistically significant results are summarized in Table 2 below.

In female rats the Peto test of trend in pheochromocytomas in adrenal glands was highly statistically significant ($p=0.0001<0.025$) as was the Peto test comparing the high dose group and vehicle ($p=0.0036 < 0.05$). The corresponding poly-k tests were also statistically significant ($p=0.05$). In both male and female rats, the Peto tests of systemic hemangiomas would be classified as rare and the corresponding tests of trend were statistically significant ($p=0.0059$, $0.0198 <0.025$, respectively). However, the more appropriate (since trend tests may miss some tumors in the low and medium dose groups), but less powerful pairwise comparisons were only statistically significant in males ($p=0.0371<0.05$), not in females ($p=0.1274$). Systemic pooled hemangiomas and hemangiosarcomas were classified as common tumors in male rats and rare in female rats, and thus, adjusting for multiplicity, neither the tests of trend, nor the pairwise tests were not statistically significant in males, but the test of trend in female rats was very close to statistical significance ($p=0.0252$ versus 0.0250). In female rats the Peto test of trend in pooled C-cell adenoma and carcinoma in the thyroid was statistically significant ($p=0.0018<0.005$). Tests of pairwise differences between the high dose group and vehicle control in pooled follicular cell adenoma and carcinoma in male rats and tests of pars distalis adenoma of the pituitary in female rats were close to statistical significance ($p=0.0115$ and $p=0.0144$ versus 0.01, respectively). After adjusting for multiplicity none of the remaining tests were statistically significant. It may be noted that if the incidence in the vehicle group were used to determine whether or not a tumor is rare, the trend test in C-cell carcinoma of the thyroid in female rats would be statistically significant. No comparisons in mice even achieved the 0.05 level using the Peto tests. Overall, the results of the poly-k tests were generally consistent with the results of the Peto tests cited here (please see Appendix 3).

Table 2. Potentially Statistically Significant Trends and Comparisons

	Incidence:					p-values: High	
	Water	Veh	Low	Med	High	Trend	vs Veh
Rat Males							
Systemic							
Hemangioma,	0	1	0	3	6	0.0059	0.0371
Hemangioma/-sarcoma	4	2	5	10	8	0.0702	0.0251
THYROID GLANDS							
Foll. cell adenoma/carcinoma	2	3	5	7	11	0.0146	0.0115
Rat Females							
ADRENAL GLANDS							
Benign pheochromocytoma,	0	0	0	2	7	0.0001	0.0036
PITUITARY GLAND							
Adenoma of pars distalis,	40	29	32	37	39	0.1467	0.0144
SKIN/SUBCUTIS							
Basal cell carc/benign tmr	0	0	0	0	2	0.0291	0.1884
Systemic							
Hemangioma,	0	1	1	0	4	0.0198	0.1274
Hemangioma/-sarcoma	0	1	1	1	4	0.0252	0.1274

Table 2. (cont.) Potentially Statistically Significant Trends and Comparisons

	Incidence: Water	Veh	Low	Med	High	p-values: High Trend	High vs Veh
Rat Females (cont.)							
THYROID GLANDS							
C-cell adenoma/carcinoma	3	4	2	4	10	0.0018	0.0205
C-cell carcinoma	1	0	0	0	3	0.0263	0.1466

1.2. Brief Overview of the Studies

One mouse study and one rat study were submitted:

Study 12318: Calcitriol - 104 Week Oral (Gavage) Carcinogenicity Study in the Rat,

and

Study 12299: Calcitriol Ointment - 104-Week Dermal Carcinogenicity in the Mouse.

These studies were designed to assess the potential carcinogenic effect of Calcitriol when administered by daily oral (gavage) administration to the Wistar rat or by daily dermal application to the CD-1 (ICR) BR mouse. Both studies were planned to last for 104 weeks. The rat study included five treatment groups: 1. Water Control, 2. Low Dose (0.005 µg/kg/day), 3. Intermediate/Medium Dose (0.03 µg/kg/day), 4. High Dose (0.1 µg/kg/day), and the 5. Vehicle Control. The dermal mouse study had only four treatment groups: 1. Control (0 ppm), 2. Low Dose (0.3 ppm), 3. Intermediate/Medium Dose (0.6 ppm), 4. High Dose (1 ppm). Each treatment group in each gender in each species included 60 animals.

1.3. Statistical Issues and Findings

1.3.1. Statistical Issues

In this section, several issues, typical of statistical analyses of these studies, are considered. These issues include details of the survival analyses, tests on tumorigenicity, multiplicity of tests on neoplasms, and the validity of the designs.

1.3.1.1. Control Groups:

Since the group 1 water control in rats does not include the vehicle, its primary use was to determine the background rate, i.e., whether or not a certain neoplasm should be classified as common or rare (see Section 1.3.1.3 below). To make the effect of the Calcitriol dose clear, the primary dose groups should be compared to the Vehicle control (Group 5 in rats and Group 1 in mice). In the Sponsor's analyses for tests in rats, "unless major differences are evident between the water and vehicle control groups, statistical tests are carried out as if these animals formed a single control group. If major differences are seen, analyses of treatment effects are conducted based on data excluding the water control group." (page 14 of volume 3) Since a nonsignificant test of differences is not conclusive evidence of no effect this reviewer does not agree that this procedure was appropriate.

1.3.1.2. Survival Analysis:

Both the Cox logrank and Kruskal-Wallis-Wilcoxon tests were used to test homogeneity of survival among the treatment groups. Tests of dose related trend using a Cox proportional odds model were also performed. The number of such tests raises issues of multiple testing, but from the point of view of finding differences among treatment groups (i.e., reducing the probability of Type II error), this should be acceptable. Appendix 1 reviews the animal survival analyses in some detail. The Sponsor's analyses are summarized in Sections 3.2.1.1 and 3.2.2.1.

1.3.1.3. Tests on Neoplasms:

The FDA tumorigenicity analyses of fatal tumors are based on the time of death, and for observable tumors based on time of detection. Both are analyzed at the time of detection with an analysis equivalent to the death rate method. Non-fatal tumors found at the time of the animal's death were labeled as incidental, and were analyzed by the so-called prevalence method. For the FDA analyses all three results were pooled. The Sponsor notes that in both studies only the High dose group and the Control group or groups had complete histopathological examinations for all organs. In the Low and Medium dose groups histopathological examinations were performed only for all animals found dead, killed moribund, or showed macroscopic abnormalities, including masses or nodules during the study or at necropsy. However, the Sponsor also indicates that in rats the thyroid, stomach, kidneys, aorta, heart, and sternum were also examined, while in mice the administration site, duodenum, eyes, kidneys, aorta, and sternum were also examined. Note that this implies that, except for these organs, in both studies the data generating processes for the Low and Medium dose groups was different from that for the Controls and the High dose group. In particular it could be expected to detect fewer tumors. Then, except for the cited organs, tests of carcinogenicity that included these doses, such as the overall test of trend and the tests comparing these doses to the control were not strictly appropriate. However, since they may be somewhat informative, the tests of trend are included in both the FDA Peto analyses and the poly-3 analysis. In addition tests of differences between the medium and low dose group are included in the poly-3 tests of tumorigenicity. The primary analysis should be placed on the difference between the High dose group and the Vehicle Control. Note that had the animals in the Low and Medium dose group been chosen randomly, these tests would have been appropriate. In rats the number of tumors in the water only control group was used to determine if the tumor was classified as "rare" or as "common", while in mice the vehicle control was used to determine this classification. These had the effect on interpretation of results as outlined below.

1.3.1.4. Multiplicity of Tests on Neoplasms:

Testing the various neoplasms involved a large number of statistical tests, which in turn necessitated an adjustment in experiment-wise Type I error. Current FDA practice is based on the Haseman-Lin-Rahman rules. Namely, based on his extensive experience with such analyses, for pairwise tests comparing control to the high dose group, Haseman (1983) claimed that for a roughly 0.10 (10%) overall false positive error rate, rare tumors should be tested at a 0.05 (5%) level, and common tumors (with a historical control incidence greater than 1%) at a 0.01 level. For a standard chronic study in two species, i.e., rats and mice, based on simulations and their

experience, Lin & Rahman (1998) proposed a further p-value adjustment for tests of trend. That is, for a roughly 0.10 (10%) overall false positive error rate in tests of trend, rare tumors should be tested at a 0.025 (2.5%) level and common tumors at a 0.005 (0.5%) level. In this analysis in rats the observed incidence in the water only group control was used to decide if a tumor was rare or common (i.e., incidence < 1 or ≥1 in the appropriate controls), while in mice the vehicle group played a similar role. This approach was intended to balance both Type I error and Type II error (i.e., the error of concluding there was no evidence of a relation to tumorgenicity when there actually was such a relation). These rules seemed to apply to both the Peto tests and the poly-3 tests, however, it should be noted that including the tests comparing the Medium and Low dose to control (as is done in the poly-k tests) can be expected to increase the experiment-wise Type I error to above the rough nominal 10% level.

1.3.1.5. Housing of Animals:

The Sponsor states that rats were accommodated in groups of five, while mice were housed singly. Multiple housing of animals may cause statistical problems in the analysis. Even with gavage dosing proximity might induce correlations, positive or negative, in treatment response. Further, animals housed together might fight each other. The skins of some animals could be damaged, and this damage might be associated with skin and other tumors. Such effects may cause within treatment estimated variances to be too large or too small, resulting in conservative or liberal tests (in terms of Type I error). Thus, with this multiple housing, from a statistical design point of view, the appropriate treatment unit generally would be the group of five animals housed together.

Apparently these possible correlations were generally ignored, and even with multiple housing the treatment unit was assumed to be the individual animal. However, unless it has been clearly shown that tumor incidence was independent of cage, from a purely statistical point of view, this reviewer would generally recommend single housing of animals. Since cage identification was not included with the data, the impact of the between cage effects can not be assessed.

1.3.1.6. Validity of the Designs:

When determining the validity of designs there are two key points:

1) adequate drug exposure
2) tumor challenge to the tested animals.

1) is related to whether or not sufficient animals survived long enough to be at risk of forming late-developing tumors and 2) is related to the Maximum Tolerated Dose (MTD), designed to achieve the greatest likelihood of tumorigenicity.

Lin and Ali (1994), quoting work by Haseman, have suggested that a survival rate of about 25 animals, out of 50 or more animals, between weeks 80-90 of a two-year study may be considered a sufficient number of survivors as well as one measure of adequate exposure. Since this study involved more than 50 animals per treatment group, and except for the highest dose group in mice, there were around 25 animals that survived to the end of the study, this criterion

seems to have been satisfied. However, in male mice, from the survival plots in Appendix 1 or the incidence tables in Sections 3.2.1.2, the maximum dose (1 ppm) seems to be associated with a lower survival than implied by this criterion.

Chu, Ceuto, and Ward (1981), citing earlier work by Sontag et al. (1976) recommend that the MTD "is taken as 'the highest dose that causes no more than a 10% weight decrement as compared to the appropriate control groups, and does not produce mortality, clinical signs of toxicity, or pathologic lesions (other than those that may be related to a neoplastic response) that would be predicted to shorten the animal's natural life span' " The values in the following tables, Tables 3 and 4 are transcribed from the Sponsor's reports. Table 3 gives the final weight change from baseline and the final percent weight change relative to the water in rats and vehicle in mice in each study. Note that, roughly, the Chu, Ceuto, and Ward criterion seems to be only slightly exceeded in the high dose group in both genders in rats and in female mice (Recall that in the Sponsor's labeling in rats group 5 denoted the vehicle control).

Table 3: Relative Weight Change (compared to control)

Study 12318: Rats Group number & label	Dose Level (µg/kg/day)	Dose Conc. (µg/mL)	Change in Weight from Baseline To Day 728			
			Males (g)	% from Control	Females (g)	% from Control
1. Water Control	0	0	515.2		274.0	
2. Low	0.005	0.0025	498.8	-3.2%	294.4	7.4
3. Medium	0.03	0.015	479.1	-7.0%	272.5	-0.5
4. High	0.1	0.05	457.8	-11.1%	237.5	-13.3
5. Vehicle Control	0	0	498.1	-3.3%	311.6	13.7

Study 12299: Mice Group number & label	Dose Level (ppm)	Change in Weight from Baseline To Day 672			
		Males (g)	% from Control	Females(g)	% from control
1. Vehicle Control	0	13.6		12.1	
2. Low	0.3	13.4	-1.4%	12.2	0.8%
3. Medium	0.6	12.7	-6.6%	12.8	5.8%
4. High	1	12.3	-9.6%	10.5	-13.2%

Table 4 gives the mean food consumption at the end of the study, and percent change from the water control. Note that food consumption seemed to be lower in all treatment groups in rats. However, relative to the vehicle control the percent difference would be much smaller. In mice, there seems to be no simple strong dose related trend in food consumption.

Table 4: Food Consumption g/animal/day (compared to control)

Study 12318: Rats Group number & label	Dose Level (µg/kg/day)	Dose Conc. (µg/mL)	Consumption at day 728			
			Males (g)	% from Control	Females (g)	% from control
1. Water Control	0	0	26.8		24.0	
2. Low	0.005	0.0025	22.5	-16.0%	21.3	-11.3%
3. Medium	0.03	0.015	21.9	-18.3%	22.0	-8.3%
4. High	0.1	0.05	19.5	-27.2%	21.1	-16.3%
5 Vehicle Control	0	0	23.1	-13.8%	20.6	-14.2%

Study 12299: Mice Group number & label	Dose Level (ppm)	Consumption at day 672			
		Males (g)	% from Control	Females (g)	% from control
1. Vehicle Control	0	6.7		6.4	
2. Low	0.3	6.5	-3.0%	6.9	7.8%
3. Medium	0.6	6.8	1.5%	6.5	1.6%
4. High	1	6.3	-6.0%	6.3	-1.6%

Again from 2) above, excess mortality not associated with any tumor or sacrifice in the higher dose groups might have suggested that the MTD was exceeded. However, in both studies, all control animals and all high dose group animals (as well as any other animals that were histopathologically evaluated) had neoplasms, so this criterion does not seem to be useful. Modelling these as time to event, since all had neoplasms, all animals were censored. In rats the usual log rank and Wilcoxon tests showed no statistically significant differences. In mice, due to the early termination in the high dose groups, there were statistically significant differences, but since all animals developed tumors these do not necessarily reflect exceeding the MTD.

The above evaluation of the validity of the study designs was based on body weight and mortality data. The pharm/tox reviewers should use their expertise and other information such as clinical signs or severe histopathologic toxic effects that are attributable to the dosed animals in their final evaluation of the appropriateness of the doses used.

1.3.2. Statistical Findings

Please see Section 1.1 above.

2. INTRODUCTION

2.1. Overview

This submission included results from both a study in Wistar —— WI (IOPS AF/Han) Rats with treatment administered orally (gavage) and a study in —CD-1® (ICR)BR Mice with dermal application of Calcitriol ointment. b(4)

2.2. Data Sources

Two SAS transport files, one for rats and the other for mice, were provided by the Sponsor and placed in the CDER electronic data room (edr). These files, each labeled tumor.xpt, each contained the single SAS data set tumor.sas7bdat. Several tumors appeared in a number of organs. Following the recommendation of the toxicologist, a number of these were combined for the report so that both the original incidences and the combined incidences are reported in the incidence tables in Appendices 2 and 3.

3. STATISTICAL EVALUATION

3.1. Evaluation of Efficacy

NA

3.2. Evaluation of Safety

More detailed results on the study are presented below.

3.2.1. Study 12318: Calcitriol - 104 Week Oral (Gavage) Carcinogenicity Study in the Rat.

RAT STUDY DURATION: Week 104.
DOSING STARTING DATE: 11 (Males) and 12 (Females) September 2003.
TERMINAL SACRIFICE: Final necropsies: Week 105, September 2005.
STUDY ENDING DATE (Final Report dated): June 15, 2006.
RAT STRAIN: Wistar——WI (IOPS AF/Han) Rats.
ROUTE: Daily Oral Gavage.

b(4)

Rats were randomized to the five treatment groups per gender, numbered by the Sponsor as groups 1-5, with group 1 denoting a water only control and group 5 a vehicle control, while the remaining groups had oral (gavage) doses of 0.005, 0.03, and 0.1 µg/kg/day. The latter three treatment groups were labeled as Low, Medium, and High dose groups, respectively. Dose volume was 2 mL/kg/day in each treatment, leading to dose concentrations of 0, 0.0025, 0.015, 0.05 and 0 1 µg/mL. The Sponsor states that "the dose levels were determined in agreement with the Study Sponsor on the basis of the FDA comments (IND 62,151; HFD-540) and on the basis of the results of a previous study in mice (RDS.03.SRE.12336,——study no. 913/093). In this study, toxicological endpoints induced by Calcitriol were clearly identified for dose levels of 0.01, 0.1 or 0.3 µg/kg/day. From those observations, the high dose for a carcinogenicity study should not exceed the medium dose of the previous study, namely 0.1 µg/kg/day." (page 39 of volume 1 of the rat report)

b(4)

In addition to the primary study animals there were 10 further animals per gender per treatment group serving as satellite toxicological groups.

Animals were approximately six weeks old at first dosing. During the study, animals were initially housed in groups of five of the same sex and dose group. Food and water were available ad libitum, except during procedures. The Sponsor states that detailed physical examinations were made on all animals each week. Body weights and overall food consumption were recorded weekly for the first 16 weeks, beginning approximately one week before initiation of dosing, and every 4 weeks thereafter.

3.2.1.1 Sponsor's Results and Conclusions

This section will present a summary of the Sponsor's analysis on survivability and tumorigencity in rats.

Survival analysis:

The Sponsor notes that: "During the 2-year treatment period a total of 104 males and 124 females were found dead or sacrificed moribund[1]. Deaths and mortality rate (%) were distributed as follows:"

Table 5: Sponsor's Summary Mortality Counts

Group number & label	Dose Level (µg/kg/day)	Survival			
		Males	%	Females	%
1. Water Control	0	17/60	28%	27/60	45%
2. Low	0.005	21/60	35%	21/60	35%
3. Medium	0.03	26/60	43%	24/60	40%
4. High	0.1	24/60	40%	33/60	55%
5. Vehicle Control	0	16/60	27%	19/60	32%

[1] excluding any animals found dead during the terminal period (week 105 to 107).

The Sponsor reports that: "Throughout the study, the mortality was similar between treated and both control groups except during the last 2 months where males receiving 0.03 and 0.1 µg/kg/day and females receiving 0.1 µg/kg/day had a slightly lower survival than both controls. This difference resulted in a significant dose trend ($p<0.05$) in females. This was mainly due to the high mortality of group 4 females (painvise analysis, $p<0.1$)." (page 54 of volume 1 of report)

Tumorigenicity analysis:

The Sponsor conclusions about the tumorigenicity are summarized as follows: "There was an increase in the incidence of total proliferative changes (hyperplasia of the adrenal medulla and pheochromocytoma) in both males and females treated at 0.03 µg/kg/day and 0.1 µg/kg/day. The incidence of these adrenal lesions is presented in the table [6] below.

Table [6] - Animal bearing hyperplasia and tumours of the adrenal medulla

Adrenal Group	Males					Females				
	1(*)	2	3	4	5(**)	1(*)	2	3	4	5(**)
Number examined	60	60	60	60	59	60	60	59	60	60
Benign Pheochromocytoma	1	1	4	5	3	0	0	2	7	0
Malignant Pheochromocytoma	1	0	2	0	0	0	0	1	0	0
Total tumours	2	1	6	5	3	0	0	3	7	0
Hyperplasia adrenal medulla	2	1	12	20	5	0	1	6	9	1
Total proliferative changes	4	2	18	25	8	0	1	9	16	1

(*) water controls (**) vehicle controls

"There was evidence of an increase in pheochromocytoma in females treated at 0.1 µg/kg/day, effect being less marked at 0.03 µg/kg/day. In males the incidence of these lesions at these . . . doses was slightly higher than in the control groups but the difference between the two doses was less marked. However an effect of the treatment was supported by the increase of the hyperplasia in both sexes at these two doses."

"Statistically . . .: there was evidence of an effect of Calcitriol on incidence of proliferative lesions for the adrenal medulla. A highly significant ($p<0.001$) trend and increase in incidence in females treated at 0.1 µg/kg/day was supported by evidence of an increase in proliferative changes at 0.03 µg/kg/day. Significant ($p<0.05$) pairwise differences from the combined controls for both groups and significant trend tests were generally evident except for the analysis of pheochromocytomas in males. There was a clear evidence of an increase in medullary hyperplasia incidence in both sexes at 0.03 and 0.1 µg/kg/day." (page 61 of volume 1)

"Statistically: The statistical report revealed also clear evidence that Calcitriol affected the thyroid C-cells at 0.1 µg/kg/day. The increase at 0.1 µg/kg/day was more clearly seen for hyperplasia ($p<0.01$) than for tumours ($p<0.05$) and more clearly seen for females ($p<0.01$ for tumours and hyperplasia) than for males ($p<0.1$). In addition, a significant trend was seen in females for both tumours ($p<0.01$) and focal hyperplasia." (page 63 of volume 1)

"Statistically: The positive trend in thyroid follicular tumour incidence in males ($p<0.01$) due to an increase at 0.1 µg/kg/day, provides less convincing evidence of an effect, as there is no trend for females or for hyperplasia in either sex.
Therefore, these results remain unclear, the incidences of findings suggest a possible effect of treatment only in males and only at 0.1 µg/kg/day." (page 64 of volume 1)

"Statistically: The positive trend in mesenteric lymph node haemangioma incidence in males ($p<0.01$) is not convincing, given the non-significant negative trend for haemangiosarcomas, and the lack of trend for combined incidence of the two tumour types in males, females or sexes combined. Therefore it is not clear whether there is any true treatment effect." (page 65 of volume 1)

3.2.1.2 FDA Reviewer's Results

This section will present the current Agency findings on survival and tumorigenicity in male and female rats.

Survival analysis:

The following tables (Table 7 for male rats, Table 8 for female rats) summarize the mortality results for the dose groups among rats. The data were grouped for the specified time period, and present the number of deaths during the time interval over the number at risk at the beginning of the interval. The percentage cited is the percent survived at the end of the interval.

Table 7. Summary of Male Rat Survival (dose/kg/day)

Period (Weeks)	Water Control	Vehicle Control	Low - 0.005 Mg/kg/day	Medium - 0.03 mg/kg/day	High - 0.1 mg/kg/day
0-50	2/60[1] 96.7%[2]	4/60 93.3%	1/60 98.3%	1/60 98.3%	0/60 100%
51-78	3/58 91.7%	1/56 91.7%	8/59 85%	4/56 91.7%	2/65 96.7%
79-91	6/55 81.7%	4/55 85%	5/51 76.7%	8/48 78.3%	5/54 88.3%
92-104	6/49 71.7%	7/51 73.3%	7/44 65.0%	13/40 56.7%	17/51 60%
Terminal 105-107	43	44	39	34	36

[1] number deaths / number at risk
[2] per cent survival to end of period.

Table 8. Summary of Female Rat Survival (dose/kg/day)

Period (Weeks)	Water Control	Vehicle Control	Low 0.1 mg/kg/day	Medium 0.2 mg/kg/day	High 0.5 mg/kg/day
0-50	0/60[1] 100%[2]	1/60 98.3%	1/60 98.3%	0/60 100%	0/60 100%
51-78	8/60 86.7%	2/59 95%	9/59 83.3%	6/60 90%	6/60 90%
79-91	9/52 71.7%	6/57 85%	8/50 70%	5/54 81.7%	11/54 71.7%
92-104	10/43 55.0%	10/51 68.3%	3/42 65.0%	13/49 60%	16/43 45%
Terminal 105-107	33	41	39	36	27

[1] number deaths / number at risk
[2] per cent survival to end of period.

Table 9 below presents the result of tests on survival over the dose groups. For both genders in rats the tests of homogeneity in survival over all five treatment groups including the water control, and tests of homogeneity in survival in the group of four treatments defined by

excluding the water control, never were rejected at the usual 0.05 level (all eight $p \geq 0.0739$), although significance levels were reasonably close in females. From Tables 7 and 8 above, or from the Kaplan-Meier survival curves in Appendix 1 it is evident that the survival curves for male rats are closely intertwined, consistent with the hypothesis of homogeneity in survival, while in female rats the vehicle control generally has the highest survival, with the other groups more or less intertwined. The more powerful test of no trend over dose levels is rejected in female rats (Cox $p = 0.0098$, K-W $p = 0.0239$), indicating there is a trend.

Table 9. Statistical Significances of Tests of Homogeneity and Trend in Survival in Rats

	Males		Females	
	Cox	K-W	Cox	K-W
Homogeneity over 5 groups (both controls)	0.3578	0.4830	0.1242	0.1745
Homogeneity over 4 groups (with vehicle)	0.3594	0.4580	0.0739	0.1163
Trend over all groups	0.4438	0.6769	0.0098	0.0239
Departure from trend	0.2685	0.2977	0.8751	0.6685

Tumorigenicity analysis:

The statistically significant Peto mortality adjusted tests of trend in the incidence of neoplasms over the vehicle control and the three Calcitriol treatment groups and the pairwise tests of differences between control and the high dose group are presented below. Appendix 3 includes the similar results from the poly-3 tests. Incidence tables and statistically nonsignificant results are displayed in more detail in Appendices 2 and 3.

Recall again that in rats the incidence in the water control group is only used to determine the rarity of the tumor, while tests of trend are based on the remaining groups. In female rats the test of trend in pheochromocytomas was highly statistically significant ($p = 0.0001 < 0.025$), as was the test comparing the high dose group and vehicle ($p = 0.0036 < 0.05$). In both male and female rats systemic hemangiomas were rare and the test of trend was statistically significant ($p = 0.0059$, $0.0198 < 0.025$, respectively). However, the more appropriate, but less powerful pairwise comparisons were only statistically significant in males ($p = 0.0371 < 0.05$), not in females ($p = 0.1274$). Systemic pooled hemangiomas and hemangiosarcomas were classified as common tumors in male rats and rare in female rats, and thus, adjusting for multiplicity neither the tests of trend nor the pairwise tests were not statistically significant in males, but the test of trend in female rats was very close to statistical significance ($p = 0.0252$ versus 0.0250). In female rats the test of trend in pooled C-cell adenoma and carcinoma in the thyroid was statistically significant ($p = 0.0018 < 0.005$). Tests of pairwise differences between the high dose group and vehicle control in pooled follicular cell adenoma and carcinoma in male rats and tests of pars distalis adenoma of the pituitary in female rats were close to statistical significance ($p = 0.0115$ and $p = 0.0144$ versus 0.01, respectively). After adjusting for multiplicity none of the remaining tests were statistically significant. It may be noted that if the incidence in the vehicle group were used to determine whether or not a tumor is rare, the trend test in C-cell carcinoma of the thyroid in female rats would be statistically significant. Please see the results of the corresponding poly-3 tests presented in Appendix 3.

Table 10. Peto Tests with Statistical Significances of 0.05 or Less

	Incidence: Water	Veh	Low	Med	High	p-values: High Trend	High vs Veh
Rat Males							
MESENT. LYMPH NODE							
Hemangioma,	0	1	0	2	5	0.0467	0.0918
Systemic							
Hemangioma,	0	1	0	3	6	0.0059	0.0371
Hemangioma/-sarcoma	4	2	5	10	8	0.0702	0.0251
THYROID GLANDS							
Foll. cell adenoma/carcinoma	2	3	5	7	11	0.0146	0.0115
Rat Females							
ADRENAL GLANDS							
Benign pheochromocytoma,	0	0	0	2	7	0.0001	0.0036
PITUITARY GLAND							
Adenoma of pars distalis,	40	29	32	37	39	0.1467	0.0144
SKIN/SUBCUTIS							
Basal cell carc/benign tmr	0	0	0	0	2	0.0291	0.1694
Systemic							
Hemangioma,	0	1	1	0	4	0.0198	0.1274
Hemangioma/-sarcoma	0	1	1	1	4	0.0252	0.1274
THYROID GLANDS							
C-cell adenoma,	2	4	2	4	7	0.0232	0.0820
C-cell adenoma/carcinoma	3	4	2	4	10	0.0018	0.0205
C-cell carcinoma	1	0	0	0	3	0.0263	0.1466

3.2.2. Study 12299: Calcitriol Ointment - 104-Week Dermal Carcinogenicity in the Mouse.

MOUSE STUDY DURATION: Up to 104 Weeks.
DOSING STARTING DATE: August 27, 2003 (Males) & August 28, 2003 (Females).
TERMINAL SACRIFICE: September 30 & October 1, 2004.
DOSING MODIFICATIONS: High Dose Group: Treatment stopped Week 23. Control only Weeks 25/26. Treatment resumed Week 29 but only three times/ week.
Medium Dose Group: Treatment stopped Week 29. Control only Week 29.Treatment resumed Week 33 but only three times/week.
Low and Control Dose Groups: Week 29 treatment three times/week
STUDY ENDING DATE (Final Report dated): June 7, 2006.
MOUSE STRAIN: —.CD-1® (ICR)BR Mice. b(4)
ROUTE: Daily Dermal Application.

Four treatment groups were formed for each of male and female CD-1 mice (each with 60 animals/gender), numbered by the Sponsor as groups 1 through 4, with dermally applied dose levels of 0.0, 0.03, 0.06, and 1 ppm. The dose groups in mice were also labeled as Control, Low, Medium, and High, respectively. Treatment was initially applied daily. The Sponsor

states that "the dose levels were determined in agreement with the Study Sponsor on the basis of the FDA comments (IND62, 151; HFD-540) and on the basis of the results of a previous 13-week dermal study in inice (RDS.03.SRE.12242——study no. 913/080). In this study, toxicological endpoints induced by Calcitriol were clearly identified for dose levels of 1, 2 or 3 µg/kg/day. From those observations, the high dose for a carcinogenicity study is the low dose of the previous study, namely 1 µg/kg/day." (page 40 of volume 1 of report)

b(4)

The Sponsor states that "On the day before the first application, the hair was clipped with an electric clipper, so as to expose the back from the scapular to the lumbar region. The clipped areas represented at least 10% of the total body surface. The application surface was approximately 10% of the body surface of the animal. . . . The animals were clipped again approximately once a week (as necessary). To avoid damage to the site, clipping was performed generally at least 2 hours prior to treatment. When animals were treated 3 times a week, clipping was performed on a day without treatment." (page 40 of volume 1 of report)

During the study animals were housed individually. Water was available ad libitum. The Sponsor states that detailed physical examinations were made on all animals each week. Body weights were recorded weekly for the first 13 weeks, beginning approximately one week before initiation of dosing, and every 4 weeks thereafter.

The Sponsor also notes that: "In addition to exposure to the test item via dermal absorption a significant, but unknown, exposure via the oral route occurred since it is not possible to prevent the mice from licking the application site. . . . In animals treated at 0.6 and 1 ppm increased serum calcium concentration, clinical signs (thin appearance), lower body weight (these findings being reversible during or after the wash out periods) and histopathological observations revealing widespread mineralisations in few decedents sacrificed in moribund condition were observed at the beginning of the study. It was concluded that the toxicity exceeded the maximal tolerated dose, and the study design was modified step wise from week 23 in each dose group.

"- For group 4 (1 ppm):
Treatment was stopped from week 23 and animals did not receive any administration for a 19-day wash out period. They received the control item from week 25/26 for 25 days. After this overall treatment-free period of 44 days the treatment at 1 ppm was restarted in week 29 but at a reduced frequency of three times a week.

"- For group 3 (0.6 ppm):
Treatment was stopped at week 29. These animals were kept for a 3-day wash out period without any administration and were treated with the control item three times a week until week 33. From this date and for the remaining part of the study, the frequency of treatment with the test item at 0.6 ppm was reduced to three times a week.

"- For group 2 (0.3 ppm) and group 1 (control):
From week 29, the frequency of treatment was reduced to three times a week in order to put all animals in the same treatment conditions." (page 17 of volume 1 report)

Note that analyses are based on the original nominal dosages, not adjusting for the changes in dosing cited above.

3.2.2.1 Sponsor's Results and Conclusions

This section will present a summary of the Sponsor's analysis on survivability and tumorigencity in mice.

Survival analysis:

The Sponsor notes that: "During the approximately 2 year treatment period a total of 160 males and 150 females were found dead or sacrificed moribund[1], distributed as follows:

Table 11: Sponsor's Summary Mortality Counts

Group number & label	Dose Level (ppm)	Mortality			
		Males	%	Females	%
1. Vehicle Control	0	34/60	57%	30/60	50%
2. Low	0.3	40/60	67%	34/60	57%
3. Medium	0.6	42/60	70%	42/60	70%
4. High	1	44/60	73%	44/60	73%

[1] excluding any animals found dead during the terminal period (week 101 for females and weeks 105/106 for males).

"Treated males had a slightly higher mortality than controls from about 14 months onwards, and mortality was markedly increased from 21 months onwards for males receiving 1 ppm until sacrifice at week 97 (see survival table below). A dose-related trend ($p<0.01$) in mortality was seen with a highly significant ($p<0.001$) increase at 1 ppm, a less significant increase at 0.6 ppm and a non-significant increase at 0.3 ppm.

Females receiving 0.6 and 1 ppm had a slightly higher mortality than controls from about 21 or 14 months onward, respectively (see survival table below). Females receiving the low dose (0.3 ppm) had a similar or lower mortality than controls during the study. A dose-related trend ($p<0.001$) in mortality was seen with a significant increase ($p<0.01$) at 1 ppm. At 0.6 ppm the increase was not quite significant ($0.05 < p < 0.1$) and no real increase was evident at 0.3 ppm. The combined sexes statistical analysis confirms the dose-related trend ($p<0.001$) and increases at 1 ppm ($p<0.001$) and 0.6 ppm ($p<0.01$)." (page 61 of volume 1 report)

Except for a single animal, these results agree with the corresponding tables 13 and 14 reported in the FDA analysis in Section 3.2.2.2, below.

Tumorigenicity analysis:

According to the Sponsor: "The most commonly occurring tumour types were as shown [in Table 12 below] . . . , which also gives information on the numbers that were malignant, the numbers contributing to the death of the animal and the numbers with an associated focal proliferative lesion. Other tumour types were seen in less than 10 animals."

Table 12. Incidence of most common tumour types

	Number of animals with				
	Any tumour	Malignant tumour	Fatal tumour	Focal prolif. lesion[a]	
Lungs – alveolar/bronchiolar	82	31[b]	15	37	(31)
Liver – hepatocellular	61	10	12	21[c]	(14)
Malignant lymphoma	45	45	30	-	
Harderian gland	25	3	1	10	(10)
Histiocytic sarcoma	19	19	16	-	
Uterus/cervix – stromal	17	1[d]	2	-	
Uterus/cervix - smooth muscle[e]	14	1	0	-	
Any site	244	134[f]	93	-	

[a] Bracketed numbers are numbers of animals with focal proliferative lesion and no tumour of type specified. Thus, for lungs alveolar/bronchiolar 37-31 = 6 animals had tumour and hyperplasia of the type specified.
[b] Three animals had a benign and a malignant alveolar/bronchiolar tumour.
[c] Basophilic, clear cell or eosinophilic focus.
[d] One animal had a stromal sarcoma of the cervix and a uterine endometrial stromal polyp.
[e] Leiomyomas or leiomyosarcomas.
[f] 42 animals had both a benign and a malignant tumour.
(page 20 of volume 3)

"*Systemic neoplasms*
The systemic neoplasms observed were malignant lymphoma, histiocytic sarcomas (mainly in females), and a malignant mast cell tumour in one control male.
There was no indication of any treatment-related increase in systemic neoplasm, but some evidence of a negative relationship with treatment for histiocytic sarcomas, due to a slightly reduced incidence in groups 3 and 4 ($0.05<p<0.1$ for trend).

"*Other tumors and proliferative changes*
. . . There was no evidence that the treatment affected the overall incidence of benign or malignant tumours or of tumours regardless of malignancy in males, females or sexes combined. There was some slight indication that the incidence of fatal malignant tumours was decreased in females given 1 ppm ($0.05<p<0.1$). However, the following changes were seen with a slightly greater or lower incidence or severity.

"Adrenal medulla: Benign phaeochromocytomas were seen in one male and one female given 1 ppm. In addition, hyperplasia was seen with a slightly greater incidence in females given 1 ppm than in controls (4/56 versus 1/60) giving some positive trend in the incidence of focal hyperplasia and combined incidence of tumour and hyperplasia.

"In males, marked or severe hyperplasia was seen in one animal given 0.6 ppm and one animal given 1 ppm only. Any relationship with the test item is unlikely.

"Uterus: there was a significant trend toward a lower incidence in the polyp and/or sarcoma incidence in females, any relationship with the test item is unclear.

"Harderian glands: Three adenocarcinomas were observed in females, two were in the 1 ppm group and one in the 0.6 ppm group with a statistically significant trend ($p<0.05$). The incidence of this change was at a low level. In addition, carcinomas could be seen in the ... mouse and published data give a range of 1.43 to 2.38% . . ., which would be equivalent to 1 or 2 cases for 60 animals. As a consequence, this slight increased incidence was considered to be unlikely related to the test item.

"Other malignant or benign neoplasms as well as main hyperplastic changes were observed sporadically, but without indication of a treatment-related change and were considered to be part of the normal background of changes in animals of this age." (pages 72-73 of volume 1)

3.2.2.2 FDA Reviewer's Results

This section will present the current Agency findings on survival and tumorigenicity in male and female mice.

Survival analysis:

Again, Kaplan-Meier plots comparing survival among treatment groups in both studies are given in Appendix 1, along with more details of the analysis. The following tables (Table 13 for male mice, Table 14 for female mice) summarize the mortality results for the dose groups. The data in the tables were grouped for each specified time period, and present the number of deaths during the time interval over the number at risk at the beginning of the interval. The percentage cited is the percent survived to the end of the interval.

Table 13. Summary of Male Mice Survival (dose/kg/day)

Period (Weeks)	Vehicle Control	Low 0.3 ppm	Medium 0.6 ppm	High 1.0 ppm
0-50	3/60[1] 95%[2]	4/60 93.3%	8/60 86.7%	3/60 95%
51-78	11/57 76.7%	18/56 63.3%	18/52 56.7%	19/57 63.3%
79-91	8/46 63.3%	9/38 48.3%	7/34 45.0%	16/38 36.7%
92-96	4/38 56.7%	3/29 43.3%	5/27 36.7%	6/22 26.7%
Terminal 97-106	34	26	22	16
97-105	8/34 43.3%	6/26 33.3%	5/22 28.3%	
Terminal 105-106	26	20	17	

[1] number deaths / number at risk

[2] per cent survival to end of period.

Table 14. Summary of Female Mice Survival (dose/kg/day)

Period (Weeks)	Vehicle Control	Low 0.3 ppm	Medium 0.6 ppm	High 1.0 ppm
0-50	3/60[1] 95%[2]	4/60 93.3%	6/60 90%	5/60 91.7%
51-78	12/57 75%	5/56 85%	10/54 73.3%	22/55 55.0%
79-91	8/45 61.7%	16/51 58.3%	15/44 48.3%	12/33 35.0%
92-99	7/37 50.0%	8/35 45.0%	10/29 31.7%	5/21 26.7%
Terminal 100-101	30	27	19	16

[1] number deaths / number at risk
[2] per cent survival to end of period.

Among the four treatment groups in mice there was fairly strong evidence of heterogeneity in survival, particularly in females, since the tests of homogeneity were all rejected (Males: Cox p = 0.0305, K-W p = 0.0410, Females: Cox p = 0.0057, K-W p = 0.0043). In both genders in mice there was even stronger evidence of a trend over dose (Males: Cox p = 0.0033, K-W p = 0.0095, Females: both p = 0.0006). From the incidence tables (tables 13, and 14) or the Kaplan-Meier survival curves in Appendix 1, one can see a general increase in mortality over dose, though with some intertwining, particularly at lower doses.

Table 15. Statistical Significances of Tests of Homogeneity and Trend in Survival

	Males		Females	
	Cox	K-W	Cox	K-W
Homogeneity over 4 groups (with vehicle)	0.0305	0.0410	0.0057	0.0043
Trend over all groups	0.0033	0.0095	0.0006	0.0006
Departure from trend	0.8645	0.4652	0.6882	0.4938

Although exact significance levels differ between this analysis and the Sponsor's analysis above, results are consistent. In female mice there is fairly strong evidence of heterogeneity in survival, since the tests of homogeneity are all rejected (Males: Cox p=0.0305, K-W p=0.0410, Females: Cox p=0.0057, K-W p=0.0043). In both genders in mice there is even stronger evidence of a trend over dose (Males: Cox p=0.0033, K-W p=0.0095, Females: both p = 0.0006). From the incidence tables in the report (tables 13 and 14) or the Kaplan-Meier survival curves below, one can see a general increase in mortality over dose, though with some intertwining, particularly at lower doses. Details are provided in Appendix 1.

Tumorigenicity analysis:

The results of the Peto mortality adjusted tests of trend in the incidence of neoplasms over the vehicle control and the three Calcitriol treatment groups, the results of the pairwise tests of differences between the vehicle control and the high dose group, and the supporting incidence

tables are displayed in tables A.2.4 and A.2.5 in Appendix 2. Results for the poly-3 tests are given in tables A.3.2., A.3.5., and A.3.6. in Appendix 3. No results using the Peto tests achieved statistical significance. In the poly-k tests among mice, after adjusting for multiplicity using the Haseman-Lin-Rahman rules, no tests of trend that corresponded to increasing incidence over dose or tests comparing the vehicle group and High dose group were statistically significant.

4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

NA

5. SUMMARY AND CONCLUSIONS

5.1. Statistical Issues and Collective Evidence

Please see Section 1.3 above.

5.2. Conclusions and Recommendations

Please see section 1.1 above.

APPENDICES:

Appendix 1. Survival Analysis

The statistical significance of the tests of differences in survival across treatment groups are given below. The test for homogeneity is a test that survival is equal across treatment groups, while the test of trend is a test of dose related trend. Note that the Cox test is usually called the logrank test, while the K-W, i.e., Kruskal-Wallis test, is more commonly called the Wilcoxon test.

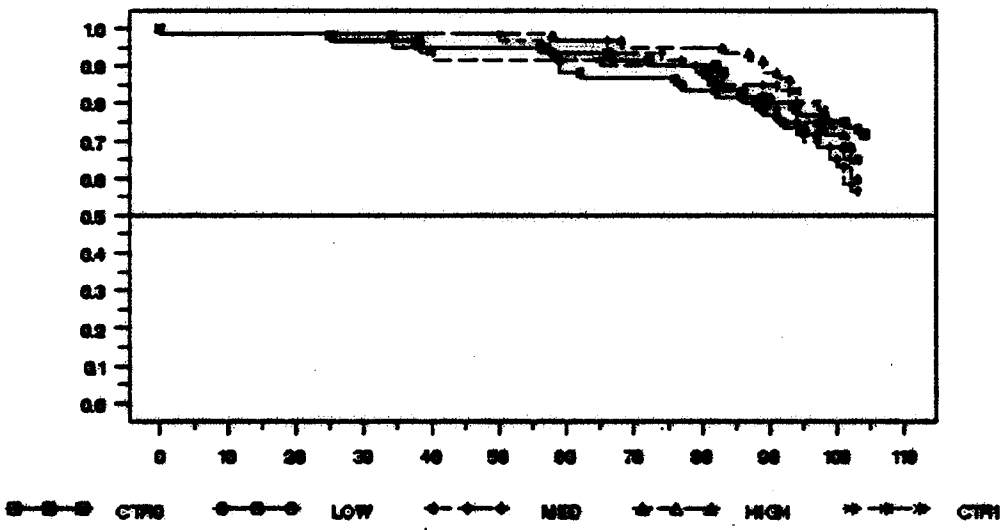
Table A.1.1 Statistical Significances of Tests of Homogeneity and Trend in Survival

	Rats				Mice			
	Males		Females		Males		Females	
	Cox	K-W	Cox	K-W	Cox	K-W	Cox	K-W
Homogeneity over 5 groups (both controls)	0.3578	0.4830	0.1242	0.1745				
Homogeneity over 4 groups (with vehicle)	0.3594	0.4580	0.0739	0.1163	0.0305	0.0410	0.0057	0.0043
Trend over all groups	0.4438	0.6769	0.0098	0.0239	0.0033	0.0095	0.0006	0.0006
Departure from trend	0.2685	0.2977	0.8751	0.6685	0.8645	0.4652	0.6882	0.4938

For both genders in rats the tests of homogeneity in survival over all five treatment groups including the water control, and as well as the tests of homogeneity in survival in the group of four treatments remaining after excluding the water control, were never rejected at the usual 0.05 level (all eight $p \geq 0.0739$). However, significance levels were close to significance in female rats. As can be seen from the Figure A.1.1, below, the Kaplan-Meier survival curves in male rats are closely intertwined, consistent with the hypothesis of homogeneity in survival. Descriptively, as seen in Figure A.1.2, in female rats the vehicle control generally has the highest survival, with the other groups more or less intertwined. However, the more powerful test of no trend over dose levels is rejected in female rats (Cox p=0.0098, K-W p=0.0239), indicating there is a trend. By comparison among the four treatment groups in mice there is fairly strong evidence of heterogeneity in survival, particularly in females, since the tests of homogeneity are all rejected (Males: Cox p=0.0305, K-W p=0.0410, Females: Cox p=0.0057, K-W p=0.0043). In both genders in mice there is even stronger evidence of a trend over dose (Males: Cox p=0.0033, K-W p=0.0095, Females: both p = 0.0006). From the incidence tables in the report (tables 13 and 14) or the Kaplan-Meier survival curves below, one can see a general increase in mortality over dose, though with some intertwining, particularly at lower doses. It should be noted that animals experiencing terminal sacrifice are counted as being censored.

The figures below display these Kaplan-Meier estimated survival curves for the two genders in each rodent species.

Figure A.1.1 Kaplan-Meier Survival Curves for Male Rats



For female mice the survival plots intertwine as depicted below:

Figure A.1.2 Kaplan-Meier Survival Curves for Female Rats

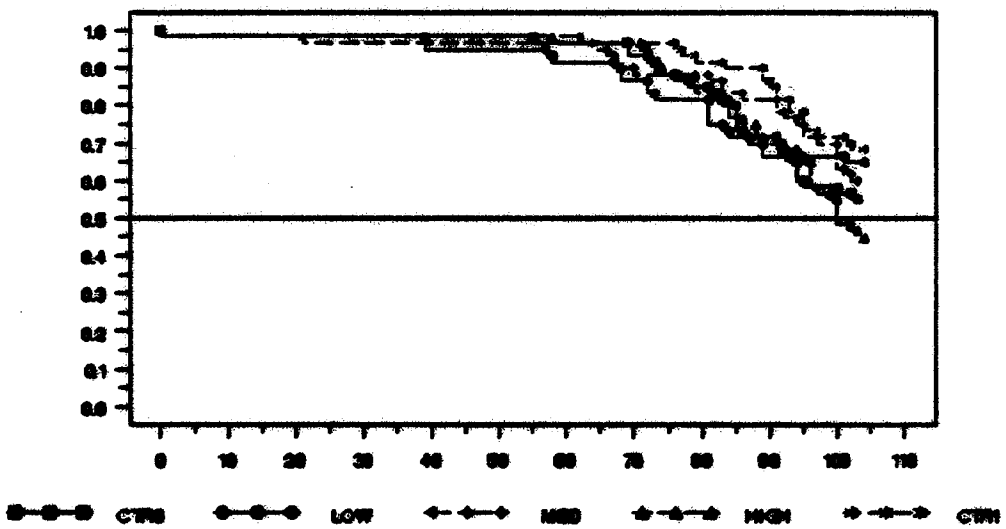


Figure A.1.3 Kaplan-Meier Survival Curves for Male Mice

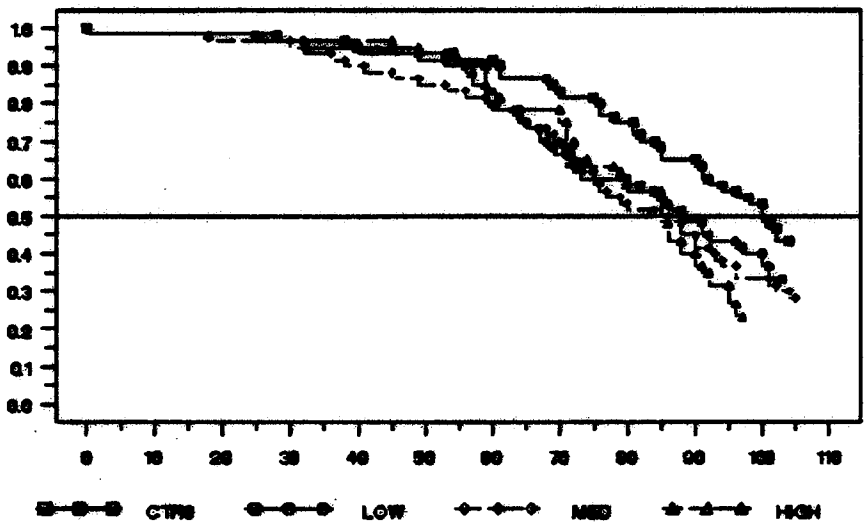
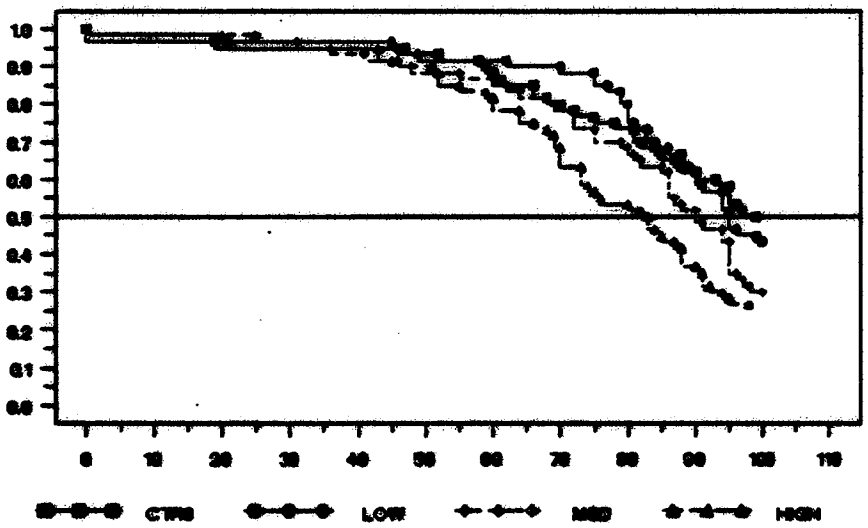


Figure A.1.4 Kaplan-Meier Survival Curves for Female Mice



Appendix 2. FDA Peto Tumorigenicity Analysis

Tables A.2.2 and A.2.3 below display the number of neoplasms in each organ and tumor combination in male and female rats, respectively, while tables A.2.4 and A.2.5 present similar results in male and female mice. Table A.2.1 includes all organ tumor combinations with a test of trend or comparison to vehicle that is statistically significant at least at 0.05 level. For each dose group, the tumor incidence is the number of animals where histopathological analysis detected a tumor. The column labeled "Trend" provides the observed p-values of the tests of trend over the vehicle control, and the low, medium, and high dose groups. The column labeled "High vs Veh" provides the significance levels of the tests comparing the high dose group to the vehicle control group. Note that in the low and medium dose groups not all animals were microscopically analyzed. The Sponsor states that in these dose groups histopathological examinations were only performed for animals found dead, killed moribund, or showed macroscopic abnormalities, including masses or nodules during the study or at necropsy. However, the Sponsor also indicates that in rats, the thyroid, stomach, kidneys, aorta, heart, and sternum were also examined, while in mice the administration site, duodenum, eyes, kidneys, aorta, and sternum were also examined. As noted earlier, this implies that, except for these organs, in both studies the data generating processes for the Low and Medium dose groups was different from that for the Controls and the High dose group. In particular it can be expected to detect a smaller proportion of tumors. Then, except for the cited organs, tests of trend in carcinogenicity over doses are not strictly appropriate and emphasis should be placed on the comparison of the high dose to the vehicle control. However, since the trend tests may be somewhat informative, the results from these usually strictly inappropriate tests are included in the analyses in this section. Note that in this report, when 10 or fewer animals are involved in the test, p-values are based on exact permutation tests, (i.e., assuming that the marginal totals for the number of animals with and without the neoplasm are fixed). When more than 10 animals were involved, the results of asymptotic tests are reported.

The Haseman-Lin-Rahman rules summarized below are designed to adjust for the multiplicity of tests over the organ by tumor combinations and to determine if the observed p-value is statistically significant. That is, to control the overall Type I error rate to roughly 10% for a standard two species, two sex study, one compares the unadjusted significance level to the appropriate bound below:

Haseman - Lin - Rahman Bounds: Comparison	Rare Tumor (Incidence ≤ 1%)	Common Tumor (Incidence > 1%)
Trend (over 3 or more groups)	0.025	0.005
Pairwise	0.05	0.01

So, for example, for a rare tumor (with incidence in the appropriate control groups < 1%, i.e., 0 tumors), a pairwise test between the high dose group and control would be considered statistically significant if the computed significance level was at or less than 0.05.

Recall again that in rats, the incidence in the water control group is only used to determine the rarity of the tumor, while tests of trend are based on the remaining groups. In female rats the test of trend in pheochromocytomas was highly statistically significant (p =

0.0001<0.025) as was the test comparing the high dose group and vehicle (p = 0.0036 < 0.05). In both male and female rats systemic hemangiomas were rare and the test of trend was statistically significant (p = 0.0059, 0.0198 <0.025, respectively). However, the more appropriate (since not all organs were examined in the Low and Medium dose groups), but less powerful pairwise comparisons between the Vehicle and the High dose group were only statistically significant in males (p = 0.0371<0.05), not in females (p = 0.1274). Systemic pooled hemangiomas and hemangiosarcomas were classified as common tumors in male rats and rare in female rats, and thus, adjusting for multiplicity the tests of trend nor the pairwise tests were not statistically significant in males, but the test of trend in female rats was very close to statistical significance (p=0.0252 versus 0.0250). In female rats the (here appropriate) test of trend in pooled C-cell adenoma and carcinoma in the thyroid was statistically significant (p = 0.0018<0.005). Tests of pairwise differences between the High dose group and Vehicle control in pooled follicular cell adenoma and carcinoma in male rats and tests of pars distalis adenoma of the pituitary in female rats were close to statistical significance (p = 0.0115 and p = 0.0144 versus 0.01, respectively). After adjusting for multiplicity, none of the remaining tests were statistically significant. It may be noted that if the incidence in the vehicle group were used to determine whether or not a tumor is rare, the trend test in C-cell carcinoma of the thyroid in female rats would be statistically significant. No comparisons in mice even achieved the 0.05 level using the Peto tests. (However, please see the results of the corresponding poly-3 tests in Appendix 3).

Table A.2.1. Peto Tests with Statistical Significances of 0.05 or Less

	Incidence:					p-values: High	
	Water	Veh	Low	Med	High	Trend	vs Veh
Rat Males							
MESENT. LYMPH NODE							
Hemangioma,	0	1	0	2	5	0.0467	0.0916
Systemic							
Hemangioma,	0	1	0	3	6	0.0059	0.0371
Hemangioma/-sarcoma	4	2	5	10	8	0.0702	0.0251
THYROID GLANDS							
Foll. cell adenoma/carcinoma	2	3	5	7	11	0.0146	0.0115
Rat Females							
ADRENAL GLANDS							
Benign pheochromocytoma,	0	0	0	2	7	0.0001	0.0036
PITUITARY GLAND							
Adenoma of pars distalis,	40	29	32	37	39	0.1467	0.0144
SKIN/SUBCUTIS							
Basal cell carc/benign tmr	0	0	0	0	2	0.0291	0.1694
Systemic							
Hemangioma,	0	1	1	0	4	0.0198	0.1274
Hemangioma/-sarcoma	0	1	1	1	4	0.0252	0.1274
THYROID GLANDS							
C-cell adenoma,	2	4	2	4	7	0.0232	0.0820
C-cell adenoma/carcinoma	3	4	2	4	10	0.0018	0.0205
C-cell carcinoma,	1	0	0	0	3	0.0263	0.1408

Table A.2.2. Peto Tests in Male Rats

	Incidence:					p-values:	High
	Water	Veh	Low	Med	High	Trend	vs Veh
ADRENAL GLANDS							
Adenoma,cortical	0	1	1	1	1	0.5847	0.7006
Adenoma/Carc. Cortical	0	1	1	1	2	0.2974	0.4243
Benign pheochromocytoma,	1	3	1	4	5	0.1596	0.3881
Benign/malig. Pheochromcytoma	1	3	1	4	5	0.1596	0.3881
Carcinoma,cortical	0	0	0	0	1	0.2353	0.4500
Ganglioneuroma,	0	0	0	1	0	0.6818	
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.6190	
Malig. lymphomatous infiltrat.	2	0	0	2	0	0.6153	
BONE MARROW, STERNUM							
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.6038	
BRAIN							
Astrocytoma,	0	1	0	0	0	1.0000	1.0000
Glioblastoma,	1	0	0	0	0		
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.3333	
BRONCHUS/BRONCHI							
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5909	
Malig. lymphomatous infiltrat.	1	0	1	0	0	0.3333	
DRAINING LYMPH NODES							
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5000	
DUODENUM							
Malig. lymphomatous infiltrat.	0	0	1	0	0	1.0000	
EPIDIDYMIDES							
Malig. lymphomatous infiltrat.	0	1	1	0	0	0.6589	1.0000
EYES							
Malig. lymphomatous infiltrat.	0	0	2	0	0	0.5889	
FEMUR							
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5909	
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.6038	
HEART							
Malig. lymphomatous infiltrat.	0	0	0	0	1	0.2353	0.4500
JEJUNUM							
Adenocarcinoma,	0	1	0	0	0	1.0000	1.0000
KIDNEYS							
Lipoma,	0	0	1	0	0	0.7124	
Liposarcoma,	0	1	0	0	0	1.0000	1.0000
Malig. lymphomatous infiltrat.	1	0	2	1	0	0.5092	
Tubular cell adenoma,	0	0	0	0	1	0.3864	0.7083
Tubular cell adenoma/carc.	0	0	0	1	1	0.2463	0.7083
Tubular cell carcinoma,	0	0	0	1	0	0.4575	
LIVER							
Cholangiocellular carcinoma,	0	0	0	1	0	0.4444	
Hepatocellular adenoma,	1	2	0	0	1	0.7079	0.8388
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5909	
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.6038	

Table A.2.2. (cont.) Peto Tests in Male Rats

	Incidence:					p-values:	High
	Water	Veh	Low	Med	High	Trend	vs Veh
LUNGS							
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5909	
Malig. lymphomatous infiltrat.	1	1	1	1	0	0.4756	
LYMPH NODES							
Histio. sarcomatous infiltrat.	0	0	0	1	0	1.0000	
Malig. lymphomatous infiltrat.	1	1	2	0	0	0.5000	
MAMMARY GLAND							
Fibroma,	1	0	1	0	0	0.4634	
Malig. lymphomatous infiltrat.	1	1	1	0	0	0.9556	
MANDIB. L.N/LEFT							
Hemangioma,	0	0	0	0	1	0.4268	0.4467
Malig. lymphomatous infiltrat.	1	1	2	0	0	0.7238	
MANDIB. L.N/RIGHT							
Hemangioma,	0	0	0	1	0		
Malig. lymphomatous infiltrat.	1	1	1	0	0	0.6667	
MANDIB.GLANDS, LEFT							
Malig. lymphomatous infiltrat.	0	0	2	0	0	0.5889	
MESENT. LYMPH NODE							
Hemangioma,	0	1	0	2	5	0.0467	0.0916
Hemangiosarcoma,	3	1	5	5	1	0.9347	0.6993
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.6542	
MESENTERY							
Schwannoma,	0	0	2	0	0	0.8750	
PANCREAS							
Acinar cell adenoma,	0	1	0	0	1	0.7006	0.7006
Malig. lymphomatous infiltrat.	0	0	1	1	0	0.3111	
PANCREAS ENDOCRINE							
Adenoma:islet cells,	1	1	0	0	2	0.4243	0.4243
Islet cell adenoma/-carc.	1	3	0	0	3	0.4752	0.6072
Islet cell carcinoma,	0	2	0	0	1	0.7566	0.8737
PARATHYROID GLANDS							
Adenoma,	0	0	1	0	0	0.6333	
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.9286	
PAROTID GLAND, LEFT							
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.9286	
PITUITARY GLAND							
Adenoma of pars distalis,	16	15	15	11	11	0.9828	0.7751
Adenoma of pars intermedia,	0	0	0	0	1	0.3684	0.4430
Malig. lymphomatous infiltrat.	1	0	1	0	0	0.3333	
PROSTATE GLAND							
Adenocarcinoma,	1	0	0	0	0		
Malig. lymphomatous infiltrat.	0	0	2	0	0	0.5889	
SCIATIC NERVES							
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.9333	

Table A.2.2. (cont.) Peto Tests in Male Rats

	Incidence:					p-values:	High
	Water	Veh	Low	Med	High	Trend	vs Veh
SKELETAL MUSCLE							
Hemangiosarcoma,	0	0	0	2	0	0.6886	
Malig. lymphomatous infiltrat.	1	0	0	0	0		
SKIN/SUBCUTIS							
Basal cell carc/benign tmr	0	2	0	0	1	0.5983	0.8751
Basal cell carcinoma,	0	2	0	0	0	1.0000	1.0000
Benign basal cell tumor,	0	0	0	0	1	0.2596	0.4909
Fibroma,	2	6	1	2	3	0.8576	0.9934
Fibrosarcoma,	0	1	2	1	2	0.4334	0.5923
Hair follicles tumour(s),	0	0	1	0	0	0.7308	
Hemangioma,	0	0	0	0	1	0.2596	0.4909
Hemangiosarcoma,	1	0	0	0	1	0.2596	0.4909
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.6429	
Keratoacanth./Sq. cell Carc.	2	2	4	4	1	0.8696	0.8751
Keratoacanthoma,	2	1	3	3	1	0.7197	0.7455
Lipoma,	1	0	0	1	2	0.1290	0.4444
Malig. lymphomatous infiltrat.	0	0	3	0	0	0.9138	
Malig.fibrous histiocytoma inf	0	0	0	2	0	0.5536	
Osteosarcoma,	0	0	0	1	0	0.5391	
Rhabdomyosarcoma,	0	0	0	1	1	0.2226	0.5362
Sarcoma (not otherwise specifi	0	1	0	0	0	1.0000	1.0000
Sebaceous cell adenoma,	1	0	1	0	0	0.7308	
Sebaceous cell carcinoma,	0	0	0	0	1	0.2596	0.4909
Sq. cell papilloma/-carc.	1	2	0	1	2	0.3970	0.6806
Squamous cell carcinoma,	0	1	1	1	0	0.8463	1.0000
Squamous cell papilloma,	1	2	0	1	2	0.3970	0.6806
SPINAL CORD, LUMBAR							
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.3333	
SPLEEN							
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5909	
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.6038	
STERNUM							
Malig. lymphomatous infiltrat.	1	0	1	0	0	0.3333	
STOMACH							
Malig. lymphomatous infiltrat.	0	0	1	0	0	1.0000	
Squamous cell carcinoma,	0	1	0	0	0	1.0000	1.0000
SYSTEMIC NEOPLASMS							
Histio. sarcomatous infiltrat.	0	0	0	1	0		
Malig. lymphomatous infiltrat.	1	1	2	3	0	0.9952	
Systemic							
Hemangioma,	0	1	0	3	6	0.0059	0.0371
Hemangioma/-sarcoma	4	2	5	10	8	0.0702	0.0251
Hemangiosarcoma,	4	1	5	7	2	0.6536	0.4243
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5909	
Malig. lymphomatous infiltrat.	2	2	5	5	1	0.7849	0.7006

Table A.2.2. (cont.) Peto Tests in Male Rats

	Incidence:					p-values:	High
	Water	Veh	Low	Med	High	Trend	vs Veh
TESTES							
Benign Leydig cell tumor,	0	2	2	0	0	0.9560	1.0000
THYMUS							
Benign thymoma,	3	1	1	0	0	0.7057	1.0000
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.7222	
Malig. lymphomatous infiltrat.	0	1	2	0	0	0.7238	
THYROID GLANDS							
C-cell adenoma,	1	3	2	3	2	0.7069	0.7515
C-cell adenoma/carcinoma	1	4	3	4	4	0.6333	0.6534
C-cell carcinoma,	0	1	1	1	2	0.4509	0.6443
Foll. cell adenoma/carcinoma	2	3	5	7	11	0.0146	0.0115
Follicular cell adenoma,	2	3	5	5	8	0.0846	0.0526
Follicular cell carcinoma,	0	1	0	3	3	0.0974	0.3259
Histiocytic sarcomatous infilt	0	0	0	1	0	0.6190	
Malignant lymphomatous infiltr	0	0	1	0	0	0.9333	
TOOTH/TEETH							
Odontoma,	1	0	0	0	0		
TRACHEA							
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.3333	
URETERS							
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.9167	
ZYMBAL'S GLANDS							
Sebaceous carcinoma,	0	0	0	1	0		

Table A.2.3. Peto Tests in Female Rats

	Incidence:					p-values:	High
	Water	Veh	Low	Med	High	Trend	vs Veh
ADRENAL GLANDS							
Adenoma,cortical	1	0	1	0	2	0.1557	0.3692
Adenoma/Carc. Cortical	1	0	2	0	2	0.2364	0.3692
Benign pheochromocytoma,	0	0	0	2	7	0.0001	0.0036
Benign/malig. Pheochromcytoma	0	0	0	2	7	0.0001	0.0036
Carcinoma,cortical	0	0	1	0	0	0.7113	
Malig. lymphomatous infiltrat.	0	1	0	1	0	0.8556	1.0000
BONE MARROW, STERNUM							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
BRAIN							
Mixed glioma,	0	0	0	0	1	0.3500	0.4712
BRONCHUS/BRONCHI							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
CECUM							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
CLITORAL GLANDS							
Squamous cell papilloma,	1	0	0	0	0		

Table A.2.3. (cont.) Peto Tests in Female Rats

	Incidence: Water	Veh	Low	Med	High	p-values: Trend	High vs Veh
COLON							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
DRAINING LYMPH NODES							
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5294	
DUODENUM							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
Myofibroma,	0	1	0	0	0	1.0000	1.0000
FEMUR							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
HARDERIAN GLANDS							
Squamous cell carcinoma,	0	1	0	1	0		
HEART							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
ILEUM							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
JEJUNUM							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
KIDNEYS							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
Nephroblastoma,	0	0	0	0	1	0.3667	0.6471
LARYNX							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
LIVER							
Hepatocellular adenoma,	0	2	0	1	1	0.5402	0.7873
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
LUNGS							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
LYMPH NODES							
Hemangioma,	0	0	0	0	1	0.5000	0.5000
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
MAMMARY GLAND							
Adenocarcinoma,	2	2	5	7	2	0.8375	0.6067
Adenoma,	0	2	1	2	0	0.9323	1.0000
Fibroadenoma,	22	21	15	25	16	0.9862	0.8026
Fibroadenoma/adenoma	22	23	15	26	16	0.9816	0.9049
Fibroma,	0	1	0	1	0	0.6811	1.0000
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
MANDIB. L.N/LEFT							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
MANDIB.GLANDS, LEFT							
Squamous cell carcinoma,	1	0	0	0	0		
MESENT. LYMPH NODE							
Hemangioma,	0	1	1	0	2	0.2143	0.3455
Hemangiosarcoma,	0	0	0	1	0	0.3919	
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000

Table A.2.3. (cont.) Peto Tests in Female Rats

	Incidence:					p-values: High	
	Water	Veh	Low	Med	High	Trend	vs Veh
OVARIES							
Benign Sertoli cell tumor,	0	0	1	0	1	0.4472	0.6154
Benign granulosa-theca cell tu	0	0	0	0	1	0.3418	0.3971
Benign luteoma,	0	0	0	1	1	0.3810	0.6154
Benign thecoma,	1	0	0	0	0		
Benign undifferentiated stroma	0	0	1	0	0	0.4810	
Fibroma,	0	0	1	0	0	0.4810	
Malig. lymphomatous infiltrat.	0	2	0	0	0	1.0000	1.0000
Yolk sac carcinoma,	1	0	0	1	0	0.5041	
PANCREAS							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
PEYER'S PATCHES							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
PITUITARY GLAND							
Adenoma of pars distalis,	40	29	32	37	39	0.1467	0.0144
Adenoma of pars intermedia,	0	0	0	1	0	0.4348	
Ganglioneuroma (pars nervosa),	0	0	0	1	0	0.4348	
SKIN/SUBCUTIS							
Basal cell carc/benign tmr	0	0	0	0	2	0.0291	0.1694
Basal cell carcinoma,	0	0	0	0	1	0.1748	0.4186
Benign basal cell tumor,	0	0	0	0	1	0.1748	0.4186
Fibroma,	0	1	0	0	0	1.0000	1.0000
Fibrosarcoma,	0	0	0	1	0	0.4486	
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.6000	
Keratoacanth./Sq. cell Carc.	1	1	1	0	0	0.9393	1.0000
Keratoacanthoma,	0	0	1	0	0	0.7573	
Leiomyosarcoma,	0	0	0	0	1	0.1827	0.4318
Lipoma,	0	0	0	2	0	0.3923	
Rhabdomyosarcoma,	1	2	0	0	0	1.0000	1.0000
Squamous cell carcinoma,	1	1	0	0	0	1.0000	1.0000
SPLEEN							
Hemangioma,	0	0	0	0	1	0.4024	0.4342
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
STOMACH							
Fibrosarcoma,	0	0	1	0	0	0.7183	
Squamous cell carcinoma,	0	1	0	0	0	1.0000	1.0000
SYSTEMIC NEOPLASMS							
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5000	
Malig. lymphomatous infiltrat.	0	1	0	0	0		
Systemic							
Hemangioma,	0	1	1	0	4	0.0196	0.1274
Hemangioma/-sarcoma	0	1	1	1	4	0.0252	0.1274
Hemangiosarcoma,	0	0	0	1	0	0.4406	
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.5085	
Malig. lymphomatous infiltrat.	2	2	1	3	0	0.9000	1.0000

Table A.2.3. (cont.) Peto Tests in Female Rats

	Incidence:					p-values: High	
	Water	Veh	Low	Med	High	Trend	vs Veh
THYMUS							
Benign thymoma,	1	2	1	1	1	0.6469	0.7841
Malig. lymphomatous infiltrat.	1	0	0	0	0		
THYROID GLANDS							
C-cell adenoma,	2	4	2	4	7	0.0232	0.0820
C-cell adenoma/carcinoma	3	4	2	4	10	0.0018	0.0205
C-cell carcinoma,	1	0	0	0	3	0.0263	0.1466
Foll. cell adenoma/carcinoma	3	0	2	2	3	0.1421	0.1466
Follicular cell adenoma,	3	0	1	2	3	0.0951	0.1466
Follicular cell carcinoma,	0	0	1	0	0	0.7133	
UTERUS							
Adenocarcinoma,	0	0	0	1	0	0.4568	
Adenoma,	0	0	0	1	0	0.4568	
Adenoma/-carcinoma	0	0	0	2	0	0.5722	
Malig. lymphomatous infiltrat.	1	0	1	2	0	0.7144	
Stromal polyp,	3	2	1	7	0	0.8999	1.0000
VAGINA							
Malig. lymphomatous infiltrat.	0	1	0	0	0	1.0000	1.0000
ZYMBAL'S GLANDS							
Sebaceous carcinoma,	1	0	0	1	1	0.5000	

Table A.2.4. Peto Tests in Male Mice

	Incidence:				p-values: High	
	Veh	Low	Med	High	Trend	vs Veh
ADRENAL GLANDS						
B subcapsular adenoma,	0	1	0	0	0.4603	
Malig. lymphoma/-infiltrat.	0	1	1	0	0.7222	
ADRENAL MEDULLAS						
Benign pheochromocytoma,	0	0	0	1	0.2923	0.6333
APPLICATION SITE 1						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.8333	
BONE MARROW, STERNUM						
Malig. lymphoma/-infiltrat.	0	1	1	0	0.7222	
Mast cell tumor infiltration,	1	0	0	0	1.0000	1.0000
BRAIN						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.8333	
CECUM						
Malig. lymphoma/-infiltrat.	0	1	1	0	0.7154	
COLON						
Malig. lymphoma/-infiltrat.	0	1	1	0	0.7135	
DRAINING LYMPH NODES						
Malig. lymphoma/-infiltrat.	1	0	0	0	1.0000	1.0000
DUODENUM						
Adenoma,	0	1	0	0	0.8495	
Malig. lymphoma/-infiltrat.	0	1	1	0	0.7222	

Table A.2.4. (cont.) Peto Tests in Male Mice

	Incidence: Veh	Low	Med	High	p-values: High Trend	vs Veh
EPIDIDYMIDES						
Histio. sarcomatous infiltrat.	0	1	0	0	0.8333	
Malig. lymphoma/-infiltrat.	0	0	1	0	0.6111	
EYES						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.8333	
FEMUR						
Malig. lymphoma/-infiltrat.	1	0	1	0	0.8347	1.0000
Mast cell tumor infiltration,	1	0	0	0	1.0000	1.0000
GALL BLADDER						
Malig. lymphoma/-infiltrat.	0	0	1	1	0.2857	0.5556
HARDERIAN GLANDS						
Adenoma,	3	4	2	3	0.5356	0.5340
Malig. lymphoma/-infiltrat.	0	1	1	0	0.7222	
HEART						
Malig. lymphoma/-infiltrat.	0	0	1	1	0.2941	0.6000
JOINT,KNEE,LEFT						
Malig. lymphoma/-infiltrat.	0	0	1	0	0.6111	
KIDNEYS						
Malig. lymphoma/-infiltrat.	1	1	1	3	0.3107	0.5314
Tubular cell adenoma,	1	0	3	0	0.3479	1.0000
Tubular cell carc./adenoma	1	1	4	0	0.3471	1.0000
Tubular cell carcinoma,	0	1	1	0	0.4702	
LARYNX						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.8333	
LIVER						
Hemangioma,	1	1	0	1	0.5793	0.7733
Hemangiosarcoma,	1	0	0	2	0.1569	0.2671
Hepato. carcinoma/adenoma	18	12	17	11	0.6161	0.6264
Hepatocellular adenoma,	14	11	15	9	0.6247	0.6301
Hepatocellular carcinoma,	4	1	2	2	0.4448	0.6335
Histio. sarcomatous infiltrat.	1	1	0	0	0.9244	1.0000
Malig. lymphoma/-infiltrat.	1	1	2	1	0.5502	0.8667
Mast cell tumor infiltration,	1	0	0	0	1.0000	1.0000
LUNGS						
Alveo./bronch. adenoma, carc.	21	8	8	14	0.4974	0.5597
Alveolar/bronchiolar adenoma,	13	5	4	8	0.7296	0.7836
Alveolar/bronchiolar carc.	9	3	4	7	0.2286	0.2308
Malig. lymphoma/-infiltrat.	1	1	1	1	0.7080	0.8667
LYMPH NODES						
Malig. lymphoma/-infiltrat.	2	1	1	1	1.0000	1.0000
MANDIB. L.N/LEFT						
Malig. lymphoma/-infiltrat.	1	1	1	0	0.8902	1.0000
MANDIB. L.N/RIGHT						
Malig. lymphoma/-infiltrat.	1	1	0	0	0.6667	
MANDIB.GLANDS, LEFT						
Malig. lymphoma/-infiltrat.	1	1	1	1	0.7049	0.8667

Table A.2.4. (cont.) Peto Tests in Male Mice

	Incidence: Veh	Low	Med	High	p-values: High Trend	vs Veh
MESENT. LYMPH NODE						
Hemangioma,	0	1	0	0	0.7778	
Malig. lymphoma/-infiltrat.	1	1	2	2	0.4425	0.7043
OPTIC NERVES						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.8182	
PANCREAS						
Islet cell adenoma,	1	0	0	0	1.0000	1.0000
Malig. lymphoma/-infiltrat.	0	1	2	0	0.5951	
PAROTID GLAND, LEFT						
Malig. lymphoma/-infiltrat.	0	1	0	1	0.4769	0.6000
PEYER'S PATCHES						
Malig. lymphoma/-infiltrat.	1	0	1	0	0.8295	1.0000
PREPUTIAL GLANDS						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.7895	
PROSTATE GLAND						
Malig. lymphoma/-infiltrat.	0	1	1	1	0.4419	0.6000
RECTUM						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.8281	
SCIATIC NERVES						
Malig. lymphoma/-infiltrat.	0	0	1	0	0.6111	
SEMINAL VESICLES						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.8333	
SKIN UNTREATED						
Malig. lymphoma/-infiltrat.	0	1	0	1	0.4815	0.6000
SKIN/SUBCUTIS						
Hemangiosarcoma,	1	0	0	0	1.0000	1.0000
Malig. lymphoma/-infiltrat.	0	0	0	1	0.5000	0.8000
SPINAL CORD, LUMBAR						
Malig. lymphoma/-infiltrat.	1	0	0	0	1.0000	1.0000
Mast cell tumor infiltration,	1	0	0	0	1.0000	1.0000
SPINAL CORD, THORAC.						
Malig. lymphoma/-infiltrat.	1	0	1	0	0.8347	1.0000
Mast cell tumor infiltration,	1	0	0	0	1.0000	1.0000
SPLEEN						
Hemangiosarcoma,	0	0	0	2	0.0656	0.0980
Histio. sarcomatous infiltrat.	1	0	0	0	1.0000	1.0000
Malig. lymphoma/-infiltrat.	2	1	1	2	0.8100	0.7742
Mast cell tumor infiltration,	1	0	0	0	1.0000	1.0000
STERNUM						
Malig. lymphoma/-infiltrat.	1	1	0	0	0.9667	1.0000
STOMACH						
Malig. lymphoma/-infiltrat.	0	1	0	0	0.8308	
SUBLING.GLAND, LEFT						
Malig. lymphoma/-infiltrat.	0	1	0	1	0.4769	0.6000

Table A.2.4. (cont.) Peto Tests in Male Mice

	Incidence: Veh	Low	Med	High	p-values: High Trend	vs Veh
SYSTEMIC NEOPLASMS						
Histiocytic sarcoma,	1	1	0	0	0.8190	1.0000
Malig. lymphoma/-infiltrat.	3	1	3	3	0.2045	0.4534
Malignant mast cell tumor,	1	0	0	0	1.0000	1.0000
Systemic						
Hemagioma/-sarcoma	3	3	0	3	0.5697	0.5004
Hemangioma,	1	3	0	1	0.7921	0.7733
Hemangiosarcoma,	2	0	0	2	0.2579	0.4873
Histio. sarcomatous infiltrat.	1	1	0	0	0.9422	1.0000
Malig. lymphoma/-infiltrat.	3	1	3	3	0.4168	0.7266
Mast cell tumor infiltration,	1	0	0	0	1.0000	1.0000
TAIL						
Hemangioma,	0	1	0	0	0.9130	
TESTES						
Benign Leydig cell tumor,	3	0	0	0	1.0000	1.0000
THYMUS						
Malig. lymphoma/-infiltrat.	1	1	0	1	0.7853	0.9028
THYROID GLANDS						
Follicular cell adenoma,	1	0	0	0	1.0000	1.0000
Malig. lymphoma/-infiltrat.	0	1	1	0	0.7222	
URETERS						
Malig. lymphoma/-infiltrat.	1	1	0	0	0.9632	1.0000
URINARY BLADDER						
Malig. lymphoma/-infiltrat.	0	0	1	0	0.6111	
Transitional cell papilloma,	0	0	0	1	0.2462	0.3200

Table A.2.5. Peto Tests in Female Mice

	Incidence: Veh	Low	Med	High	p-values: High Trend	vs Veh
ADRENAL GLANDS						
B subcapsular adenoma,	0	0	1	0	0.5294	
Malig. lymphoma/-infiltrat.	2	0	2	1	0.7529	0.9176
ADRENAL MEDULLAS						
Benign pheochromocytoma,	0	0	0	1	0.3333	0.3478
APPLICATION SITE 1						
Malig. lymphoma/-infiltrat.	3	0	1	2	0.7719	0.9158
Sarcoma (not otherwise specifi	0	0	1	0	0.5000	
BONE MARROW, STERNUM						
Malig. lymphoma/-infiltrat.	3	1	7	0	0.8871	1.0000
BRAIN						
Malig. lymphoma/-infiltrat.	2	0	2	0	0.9400	1.0000
Meningeal sarcoma,	0	0	1	0	0.4510	

Table A.2.5. (cont.) Peto Tests in Female Mice

	Incidence: Veh	Low	Med	High	p-values: High Trend	vs Veh
CERVIX						
Endo. stromal polyp tumor	1	1	1	0	0.7734	1.0000
Endo.strom.pol./strom.sarc	2	1	1	0	0.8969	1.0000
Histio. sarcomatous infiltrat.	3	5	1	0	0.9947	1.0000
Leiomyoma,	3	0	3	1	0.8480	0.9697
Malig. lymphoma/-infiltrat.	2	1	2	0	0.9477	1.0000
Squamous cell carcinoma,	0	0	1	0	0.3704	
Stromal cell sarcoma,	1	0	0	0	1.0000	1.0000
COLON						
Malig. lymphoma/-infiltrat.	0	0	0	1	0.2449	0.6000
DRAINING LYMPH NODES						
Histio. sarcomatous infiltrat.	2	6	1	0	0.8541	1.0000
Malig. lymphoma/-infiltrat.	3	2	2	3	0.1803	0.2857
DUODENUM						
Adenoma,	0	1	0	0	0.6739	
Malig. lymphoma/-infiltrat.	0	0	2	0	0.5057	
EARS						
Malig. lymphoma/-infiltrat.	0	0	1	1	0.8667	
ESOPHAGUS						
Malig. lymphoma/-infiltrat.	0	0	1	0	0.5882	
EYES						
Malig. lymphoma/-infiltrat.	3	0	0	0	1.0000	1.0000
FEMUR						
Hemangiosarcoma,	0	0	1	0	0.3673	
Histio. sarcomatous infiltrat.	0	3	0	0	0.9070	
Malig. lymphoma/-infiltrat.	2	2	4	0	0.9172	1.0000
GALL BLADDER						
Malig. lymphoma/-infiltrat.	3	0	1	1	0.8955	0.9676
HARDERIAN GLANDS						
Adenocarcinoma,	0	0	1	2	0.0538	0.1449
Adenoma,	5	0	3	2	0.7219	0.9092
Histio. sarcomatous infiltrat.	0	1	0	0	0.7447	
Malig. lymphoma/-infiltrat.	3	0	3	1	0.8777	0.9804
HEART						
Histio. sarcomatous infiltrat.	0	1	0	1	0.4506	0.6471
Malig. lymphoma/-infiltrat.	6	1	3	2	0.9831	0.9952
JOINT,KNEE,LEFT						
Malig. lymphoma/-infiltrat.	2	0	1	1	0.8472	0.9529
KIDNEYS						
Histio. sarcomatous infiltrat.	2	5	1	0	0.9688	1.0000
Malig. lymphoma/-infiltrat.	8	2	6	2	0.9848	0.9983
LARYNX						
Malig. lymphoma/-infiltrat.	4	0	2	2	0.8770	0.9628

Table A.2.5. (cont.) Peto Tests in Female Mice

	Incidence:				p-values: High	
	Veh	Low	Med	High	Trend	vs Veh
LIVER						
Hepato. carcinoma/adenoma	1	0	1	1	0.4237	0.7688
Hepatocellular adenoma,	0	0	1	1	0.2226	0.6471
Hepatocellular carcinoma,	1	0	0	0	1.0000	1.0000
Histio. sarcomatous infiltrat.	3	8	2	1	0.9619	0.9693
Malig. lymphoma/-infiltrat.	9	4	7	5	0.9501	0.9655
LUNGS						
Alveo./bronch. adenoma, carc.	13	7	5	6	0.9049	0.9086
Alveolar/bronchiolar adenoma,	9	5	5	5	0.7733	0.8420
Alveolar/bronchiolar carc.	4	2	1	1	0.8252	0.8967
Histio. sarcomatous infiltrat.	1	3	0	1	0.8808	0.8824
Malig. lymphoma/-infiltrat.	9	2	5	4	0.9770	0.9835
LYMPH NODES						
Histio. sarcomatous infiltrat.	1	4	0	0	0.9632	1.0000
Malig. lymphoma/-infiltrat.	5	2	6	4	0.3696	0.8800
MAMMARY GLAND						
Adenocarcinoma,	3	0	1	0	0.9005	1.0000
Adenoma,	0	0	1	0	0.3542	
Adenosq. Carc./Adenocarc.	3	1	2	0	0.7979	1.0000
Adenosquamous carcinoma,	0	1	1	0	0.5742	
Malig. lymphoma/-infiltrat.	2	0	1	1	0.8123	0.9464
MANDIB. L.N/LEFT						
Histio. sarcomatous infiltrat.	0	1	0	0	0.8222	
Malig. lymphoma/-infiltrat.	6	2	5	2	0.9729	0.9951
MANDIB. L.N/RIGHT						
Malig. lymphoma/-infiltrat.	2	0	2	1	0.6000	
MANDIB.GLANDS, LEFT						
Malig. lymphoma/-infiltrat.	4	0	4	3	0.6337	0.8707
MANDIBULAR GLANDS						
Malig. lymphoma/-infiltrat.	0	0	0	1	0.3333	
MESENT. LYMPH NODE						
Hemangioma,	0	0	0	1	0.2909	0.3478
Histio. sarcomatous infiltrat.	1	0	0	0	1.0000	1.0000
Malig. lymphoma/-infiltrat.	9	3	4	2	0.9893	0.9880
OVARIES						
Benign luteoma,	0	0	0	1	0.1975	0.3478
Cystadenoma,	3	1	1	0	0.9435	1.0000
Histio. sarcomatous infiltrat.	0	5	0	0	0.9463	
Malig. lymphoma/-infiltrat.	8	2	5	3	0.9633	0.9929
OVIDUCTS						
Histio. sarcomatous infiltrat.	0	1	0	0	0.7708	
Malig. lymphoma/-infiltrat.	5	0	2	0	0.9889	1.0000
PANCREAS						
Histio. sarcomatous infiltrat.	1	2	0	0	0.9081	1.0000
Malig. lymphoma/-infiltrat.	5	2	2	2	0.9068	0.9849

Table A.2.5. (cont.) Peto Tests in Female Mice

	Incidence:				p-values:	
	Veh	Low	Med	High	Trend	High vs Veh
PAROTID GLAND, LEFT						
Malig. lymphoma/-infiltrat.	5	1	3	2	0.9486	0.9849
PEYER'S PATCHES						
Malig. lymphoma/-infiltrat.	1	1	3	1	0.6384	0.8632
PITUITARY GLAND						
Adenoma of pars distalis,	0	3	0	0	0.6981	
Adenoma of pars intermedia,	0	1	1	0	0.6044	
Malig. lymphoma/-infiltrat.	3	0	4	0	0.9491	1.0000
SCIATIC NERVES						
Histio. sarcomatous infiltrat.	1	0	0	0	1.0000	1.0000
Malig. lymphoma/-infiltrat.	0	0	1	0	0.5294	
SKELETAL MUSCLE						
Malig. lymphoma/-infiltrat.	2	0	1	1	0.7607	0.9176
SKIN UNTREATED						
Malig. lymphoma/-infiltrat.	3	1	1	2	0.8212	0.9158
SKIN/SUBCUTIS						
Malig. lymphoma/-infiltrat.	3	0	2	2	0.9223	0.9905
Sarcoma (not otherwise specifi	1	1	1	0	0.7192	1.0000
Squamous cell carcinoma,	0	1	0	0	0.6667	
SPINAL CORD, LUMBAR						
Malig. lymphoma/-infiltrat.	4	1	3	0	0.9924	1.0000
SPINAL CORD, THORAC.						
Malig. lymphoma/-infiltrat.	7	1	3	0	0.9998	1.0000
SPLEEN						
Hemangioma,	0	1	0	0	0.8431	
Histio. sarcomatous infiltrat.	0	1	0	0	0.7551	
Malig. lymphoma/-infiltrat.	6	4	8	5	0.6746	0.7834
STERNUM						
Malig. lymphoma/-infiltrat.	3	1	0	2	0.8564	0.9317
STOMACH						
Malig. lymphoma/-infiltrat.	7	0	3	3	0.9670	0.9906
SUBLING.GLAND, LEFT						
Malig. lymphoma/-infiltrat.	3	0	2	0	0.9827	1.0000
SYSTEMIC NEOPLASMS						
Histiocytic sarcoma,	5	9	2	1	0.9089	0.9478
Malig. lymphoma/-infiltrat.	12	5	11	7	0.5352	0.7422
Systemic						
Hemagioma/-sarcoma	1	1	1	2	0.1636	0.2740
Hemangioma,	0	1	0	2	0.0655	0.1159
Hemangiosarcoma,	1	0	1	0	0.7026	1.0000
Histio. sarcomatous infiltrat.	5	9	2	1	0.9949	0.9902
Malig. lymphoma/-infiltrat.	12	5	12	7	0.8659	0.9752
TAIL						
Sarcoma (not otherwise specifi	0	0	0	1	0.1282	0.2174

Table A.2.5. (cont.) Peto Tests in Female Mice

	Incidence:				p-values: High	
	Veh	Low	Med	High	Trend	vs Veh
THYMUS						
Histio. sarcomatous infiltrat.	1	1	0	0	0.8679	1.0000
Malig. lymphoma/-infiltrat.	8	2	6	5	0.8343	0.9182
THYROID GLANDS						
Malig. lymphoma/-infiltrat.	4	0	0	2	0.9085	0.9590
URETERS						
Malig. lymphoma/-infiltrat.	2	0	1	3	0.3214	0.7557
URINARY BLADDER						
Histio. sarcomatous infiltrat.	1	1	0	0	0.8920	1.0000
Malig. lymphoma/-infiltrat.	5	0	3	1	0.9849	0.9978
UTERUS						
Adenocarcinoma,	0	0	1	0	0.4035	
Endo. stromal polyp tumor	6	5	2	1	0.9669	0.9850
Hemangioma,	0	0	0	1	0.1839	0.3478
Hemangiosarcoma,	1	0	0	0	1.0000	1.0000
Histio. sarcomatous infiltrat.	2	3	1	0	0.9484	1.0000
Leiomyoma,	3	1	2	0	0.9055	1.0000
Leiomyosarcoma,	0	0	0	1	0.2353	0.6000
Malig. lymphoma/-infiltrat.	6	1	1	1	0.9974	0.9991
VAGINA						
Histio. sarcomatous infiltrat.	0	2	0	0	0.8678	
Malig. lymphoma/-infiltrat.	3	0	1	0	0.9699	1.0000

Appendix 3. FDA Poly-k Tumorigenicity Analysis

The tables below display the tumor incidence and the p-values using the poly-k adjustment to the Cochran-Armitage test of trend in dose. The first p-value provides the results of the poly-k test of trend, here with k=3. The remaining p-values correspond to the tests of differences between the vehicle control and, in order, the low, medium, and high dose groups, respectively. In the report of the Society of Toxicological Pathology "town hall" meeting in June 2001 the poly-k modification of the Cochran-Armitage test of trend was generally recommended over use of the Peto tests presented in the preceding Appendix 2.

As has been noted several times earlier, in the low and medium dose groups not all animals were microscopically analyzed. The Sponsor states that in these dose groups histopathological examinations were only performed for animals found dead, killed moribund, or showed macroscopic abnormalities, including masses or nodules during the study or at necropsy. However, the Sponsor also indicates that in rats the thyroid, stomach, kidneys, aorta, heart, and sternum were also examined, while in mice the administration site, duodenum, eyes, kidneys, aorta, and sternum were also examined. Again this implies that, except for these organs, in both studies the data generating processes for the low and medium dose groups is fundamentally different from that for the Control and the High dose group, so that tests of trend and pairwise comparisons of the low and medium groups to the vehicle control are not strictly appropriate. Emphasis should be placed on the comparison of the high dose to the vehicle control. However, since the trend tests may be somewhat informative, the results from these usually strictly inappropriate tests are included in the analyses in this section. All p-values are based on exact permutation tests, (i.e., assuming that the marginal totals for the number of animals with and without the neoplasm are fixed).

Preliminary studies suggest that to adjust for multiplicity in testing, the Haseman-Lin-Rahman rules discussed in Section 1.3.1.3. of the report may be applied. That is, for a roughly 0.10 (10%) overall false positive error rate in tests of trend, rare tumors should be tested at a 0.025 (2.5%) level and common tumors at a 0.005 (0.5%) level, while the test comparing the high dose group to the control should be tested at a 0.05 (5%) level for rare tumors and 0.01 (1%) for common tumors. In this analysis in rats the observed incidence in the water only group control is used to decide if a tumor is rare or common (i.e., incidence <1 or ≥ 1 in the appropriate controls), while in mice the vehicle group plays a similar role. Note, however, strictly speaking, those rules only apply to the tests of trend and the comparison of the high dose group to control. Incorporating lower dose comparisons, as is done here, can be expected to increase the overall error rate to above the nominal roughly 10% rate associated with the Haseman-Lin-Rahman rules.

Tables A.3.1 in rats and A.3.2 in mice present the incidence and p-values for those neoplasms with at least one comparison with a p-value statistically significant at the usual 0.05 level. Note that the Peto tests are sensitive to deviations from no trend that correspond to an increasing linear trend over dose, while, as currently implemented, the corresponding poly-k

tests are sensitive to either a decreasing or increasing trend. That is, a decreasing trend in tumorgenicity over increasing dose would likely be statistically significant. Tables A.3.3 and A.3.4 present the complete incidence and results of tests for male and female rats, while Tables A.3.5 and A.3.6 present similar results for male and female mice.

In this table, as in the Peto tests, in female rats the test of trend in pheochromocytomas was highly statistically significant ($p < 0.0005 < 0.025$) as was the more appropriate test comparing the high dose group and vehicle ($p = 0.005$). In both male and female rats systemic hemangiomas would be classified as rare tumors, however now the test of trend would only be assessed as statistically significant in males ($p = 0.006 < 0.025$) but not quite statistically significant in females ($p = 0.027$). However, the more appropriate, but less powerful, pairwise comparison in systemic hemangiomas was close to statistical significant in males ($p = 0.062$ versus 0.05), but not in females ($p = 0.152$). Again, systemic pooled hemangiomas and hemangiosarcomas were classified as common tumors in male rats and rare in female rats, and thus, after adjusting for multiplicity, neither the tests of trend nor the pairwise tests were statistically significant in males, but the test of trend in female rats was very close to statistical significance ($p = 0.0252$ versus 0.0250). The more specific test of trend in hemangiomas in the mesentery lymph node of male rats was statistically significant ($p = 0.011 < 0.025$). The test of trend in pooled thyroid C-cell adenoma/carcinoma was exactly statistically significant at the rough 10% level (i.e. $p = 0.005$). Note that if one had used the vehicle group to determine if the tumor was rare or not, the test of trend in C-cell carcinoma would also have been statistically significant at the rough 10% level ($p = 0.013$). The remaining statistical tests, after adjusting for multiplicity using the Haseman-Lin-Rahman rules were no longer statistically significant, corresponded to decreasing incidence over dose, or were for tests comparing either the Low or the Medium dose group to the Vehicle control. That was true for all the neoplasms in mice.

Table A.3.1. Results of Poly-k tests in Rats for Neoplasms with at Least One P-value ≤ 0.05

	Incidence:					p-values:			
	Water	Veh	Low	Med	High	Trend	Low vs Veh	Med vs Veh	High vs Veh
Male Rats									
MESENT. LYMPH NODE									
Hemangioma,	0	1	0	2	5	0.011	0.510	0.493	0.112
Systemic									
Hemangioma,	0	1	0	3	6	0.006	0.510	0.302	0.062
Hemangioma/-sarcoma	4	2	5	10	8	0.093	0.202	0.013	0.053
Hemangiosarcoma,	4	1	5	7	2	0.301	0.094	0.028	0.514
TESTES									
Benign Leydig cell tumor,	0	2	2	0	0	0.040	0.676	0.252	0.238
THYROID GLANDS									
Foll. cell adenoma/carcinoma	2	3	5	7	11	0.016	0.336	0.152	0.025

Table A.3.1. (cont.) Results of Poly-k tests in Rats for Neoplasms with at Least One P-value ≤ 0.05

	Incidence:					p-values:			
	Water	Veh	Low	Med	High	Trend	Low vs Veh	Med vs Veh	High vs Veh
Female Rats									
ADRENAL GLANDS									
Benign pheochromocytoma,	0	0	0	2	7	0.000	.	0.238	0.005
PITUITARY GLAND									
Adenoma of pars distalis,	40	29	32	37	39	0.040	0.279	0.136	0.043
Systemic									
Hemangioma,	0	1	1	0	4	0.027	0.727	0.510	0.152
Hemangioma/-sarcoma	0	1	1	1	4	0.038	0.727	0.743	0.152
THYROID GLANDS									
C-cell adenoma,	2	4	2	4	7	0.049	0.387	0.620	0.208
C-cell adenoma/carcinoma	3	4	2	4	10	0.005	0.387	0.620	0.054
C-cell carcinoma,	1	0	0	0	3	0.013	.	.	0.104
Follicular cell adenoma,	3	0	1	2	3	0.047	0.475	0.243	0.104

Table A.3.2. Results of Poly-k tests in Mice for Neoplasms with at Least One P-value ≤ 0.05

	Incidence:				p-values:			
	Veh	Low	Med	High	Trend	Low vs Veh	Med vs Veh	High vs Veh
Male Mice								
LUNGS								
Alveo./bronch. adenoma, carc.	21	8	8	14	0.176	0.016	0.023	0.212
Alveolar/bronchiolar adenoma,	13	5	4	8	0.187	0.078	0.050	0.277
TESTES								
Benign Leydig cell tumor,	3	0	0	0	0.023	0.155	0.174	0.158
Female Mice								
CERVIX								
Histio. sarcomatous infiltrat.	3	5	1	0	0.045	0.381	0.354	0.179
EYES								
Malig. lymphoma/-infiltrat.	3	0	0	0	0.021	0.125	0.148	0.185
HARDERIAN GLANDS								
Adenocarcinoma,	0	0	1	2	0.041	.	0.476	0.187
Adenoma,	5	0	3	2	0.323	0.028	0.411	0.333
LUNGS								
Malig. lymphoma/-infiltrat.	9	2	5	4	0.266	0.033	0.288	0.266
MESENT. LYMPH NODE								
Malig. lymphoma/-infiltrat.	9	3	4	2	0.049	0.070	0.184	0.083
OVARIES								
Histio. sarcomatous infiltrat.	0	5	0	0	0.226	0.035	.	.
OVIDUCTS								
Malig. lymphoma/-infiltrat.	5	0	2	0	0.030	0.031	0.266	0.060
SPINAL CORD, THORAC.								
Malig. lymphoma/-infiltrat.	7	1	3	0	0.012	0.036	0.229	0.021

Table A.3.2. (cont.) Results of Poly-k tests in Mice for Neoplasms with at Least One P-value ≤ 0.05

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
STOMACH								
Malig. lymphoma/-infiltrat.	7	0	3	3	0.258	0.007	0.218	0.301
SYSTEMIC NEOPLASMS								
Histiocytic sarcoma,	5	9	2	1	0.037	0.243	0.279	0.177
Systemic								
Histio. sarcomatous infiltrat.	5	9	2	1	0.037	0.243	0.279	0.177
URINARY BLADDER								
Malig. lymphoma/-infiltrat.	5	0	3	1	0.167	0.030	0.422	0.184
UTERUS								
Endo. stromal polyp tumor	6	5	2	1	0.034	0.485	0.174	0.104
Malig. lymphoma/-infiltrat.	6	1	1	1	0.033	0.059	0.084	0.121

Table A.3.3. Overall Results of Poly-k tests in Male Rats

	Incidence: Water	Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
ADRENAL GLANDS									
Adenoma,cortical	0	1	1	1	1	0.566	0.743	0.748	0.743
Adenoma/Carc. Cortical	0	1	1	1	2	0.277	0.743	0.748	0.514
Benign pheochromocytoma,	1	3	1	4	5	0.120	0.324	0.489	0.379
Benign/malig. Pheochromcytoma	1	3	1	4	5	0.120	0.324	0.489	0.379
Carcinoma,cortical	0	0	0	0	1	0.261	.	.	0.509
Ganglioneuroma,	0	0	0	1	0	0.739	.	0.495	.
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	2	0	0	2	0	0.545	.	0.243	.
BONE MARROW, STERNUM									
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.166	0.485	0.743	0.495
BRAIN									
Astrocytoma,	0	1	0	0	0	0.255	0.515	0.510	0.495
Glioblastoma,	1	0	0	0	0	.	.	.	.
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.495	0.495	.	.
BRONCHUS/BRONCHI									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	1	0	1	0	0	0.495	0.495	.	.
DRAINING LYMPH NODES									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
DUODENUM									
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.493	0.490	.	.
EPIDIDYMIDES									
Malig. lymphomatous infiltrat.	0	1	1	0	0	0.185	0.748	0.506	0.491
EYES									
Malig. lymphomatous infiltrat.	0	0	2	0	0	0.244	0.243	.	.

Table A.3.3. (cont.) Overall Results of Poly-k tests in Male Rats

	Incidence: Water	Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
FEMUR									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.166	0.485	0.743	0.495
HEART									
Malig. lymphomatous infiltrat.	0	0	0	0	1	0.261	.	.	0.509
JEJUNUM									
Adenocarcinoma,	0	1	0	0	0	0.255	0.515	0.510	0.495
KIDNEYS									
Lipoma,	0	0	1	0	0	0.493	0.490	.	.
Liposarcoma,	0	1	0	0	0	0.251	0.510	0.505	0.491
Malig. lymphomatous infiltrat.	1	0	2	1	0	0.301	0.243	0.495	.
Tubular cell adenoma,	0	0	0	0	1	0.261	.	.	0.509
Tubular cell adenoma/carc.	0	0	0	1	1	0.196	.	0.495	0.509
Tubular cell carcinoma,	0	0	0	1	0	0.739	.	0.495	.
LIVER									
Cholangiocellular carcinoma,	0	0	0	1	0	0.739	.	0.495	.
Hepatocellular adenoma,	1	2	0	0	1	0.451	0.257	0.252	0.486
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.166	0.485	0.743	0.495
LUNGS									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	1	1	1	1	0	0.259	0.743	0.748	0.495
LYMPH NODES									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	1	1	2	0	0	0.108	0.485	0.510	0.495
MAMMARY GLAND									
Fibroma,	1	0	1	0	0	0.493	0.490	.	.
Malig. lymphomatous infiltrat.	1	1	1	0	0	0.187	0.738	0.510	0.495
MANDIB. L.N/LEFT									
Hemangioma,	0	0	0	0	1	0.261	.	.	0.509
Malig. lymphomatous infiltrat.	1	1	2	0	0	0.108	0.485	0.510	0.495
MANDIB. L.N/RIGHT									
Hemangioma,	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	1	1	1	0	0	0.188	0.743	0.510	0.495
MANDIB.GLANDS, LEFT									
Malig. lymphomatous infiltrat.	0	0	2	0	0	0.244	0.243	.	.
MESENT. LYMPH NODE									
Hemangioma,	0	1	0	2	5	0.011	0.510	0.493	0.112
Hemangiosarcoma,	3	1	5	5	1	0.156	0.084	0.098	0.743
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.166	0.485	0.743	0.495
MESENTERY									
Schwannoma,	0	0	2	0	0	0.242	0.238	.	.

Table A.3.3. (cont.) Overall Results of Poly-k tests in Male Rats

	Incidence:					p-values:			
	Water	Veh	Low	Med	High	Trend	Low vs Veh	Med vs Veh	High vs Veh
PANCREAS									
Acinar cell adenoma,	0	1	0	0	1	0.455	0.510	0.505	0.743
Malig. lymphomatous infiltrat.	0	0	1	1	0	0.488	0.495	0.495	.
PANCREAS ENDOCRINE									
Adenoma:islet cells,	1	1	0	0	2	0.167	0.510	0.505	0.514
Islet cell adenoma/-carc.	1	3	0	0	3	0.188	0.129	0.125	0.643
Islet cell carcinoma,	0	2	0	0	1	0.451	0.257	0.252	0.486
PARATHYROID GLANDS									
Adenoma,	0	0	1	0	0	0.493	0.490	.	.
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.493	0.490	.	.
PAROTID GLAND, LEFT									
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.493	0.490	.	.
PITUITARY GLAND									
Adenoma of pars distalis,	16	15	15	11	11	0.162	0.585	0.286	0.250
Adenoma of pars intermedia,	0	0	0	0	1	0.261	.	.	0.509
Malig. lymphomatous infiltrat.	1	0	1	0	0	0.495	0.495	.	.
PROSTATE GLAND									
Adenocarcinoma,	1	0	0	0	0	.	.	.	.
Malig. lymphomatous infiltrat.	0	0	2	0	0	0.244	0.243	.	.
SCIATIC NERVES									
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.493	0.490	.	.
SKELETAL MUSCLE									
Hemangiosarcoma,	0	0	0	2	0	0.545	.	0.243	.
Malig. lymphomatous infiltrat.	1	0	0	0	0	.	.	.	.
SKIN/SUBCUTIS									
Basal cell carc/benign tmr	0	2	0	0	1	0.451	0.257	0.252	0.486
Basal cell carcinoma,	0	2	0	0	0	0.062	0.257	0.252	0.238
Benign basal cell tumor,	0	0	0	0	1	0.261	.	.	0.509
Fibroma,	2	6	1	2	3	0.376	0.066	0.148	0.235
Fibrosarcoma,	0	1	2	1	2	0.367	0.478	0.743	0.507
Hair follicles tumour(s),	0	0	1	0	0	0.493	0.490	.	.
Hemangioma,	0	0	0	0	1	0.261	.	.	0.509
Hemangiosarcoma,	1	0	0	0	1	0.261	.	.	0.509
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Keratoacanth./Sq. cell Carc.	2	2	4	4	1	0.147	0.320	0.339	0.486
Keratoacanthoma,	2	1	3	3	1	0.293	0.294	0.302	0.743
Lipoma,	1	0	0	1	2	0.067	.	0.495	0.257
Malig. lymphomatous infiltrat.	0	0	3	0	0	0.120	0.118	.	.
Malig.fibrous histiocytoma infil	0	0	0	2	0	0.547	.	0.248	.
Osteosarcoma,	0	0	0	1	0	0.739	.	0.495	.
Rhabdomyosarcoma,	0	0	0	1	1	0.196	.	0.495	0.508
Sarcoma (not otherwise specified	0	1	0	0	0	0.255	0.515	0.510	0.495

Table A.3.3. (cont.) Overall Results of Poly-k tests in Male Rats

	Incidence:					p-values:	Low	Med	High
	Water	Veh	Low	Med	High	Trend	vs Veh	vs Veh	vs Veh
Sebaceous cell adenoma,	1	0	1	0	0	0.493	0.490	.	.
Sebaceous cell carcinoma,	0	0	0	0	1	0.261	.	.	0.509
Sq. cell papilloma/-carc.	1	2	0	1	2	0.309	0.257	0.507	0.677
Squamous cell carcinoma,	0	1	1	1	0	0.254	0.743	0.752	0.491
Squamous cell papilloma,	1	2	0	1	2	0.309	0.257	0.507	0.677
SPINAL CORD, LUMBAR									
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.495	0.495	.	.
SPLEEN									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	1	1	2	1	0	0.166	0.485	0.743	0.495
STERNUM									
Malig. lymphomatous infiltrat.	1	0	1	0	0	0.495	0.495	.	.
STOMACH									
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.493	0.490	.	.
Squamous cell carcinoma,	0	1	0	0	0	0.251	0.510	0.505	0.491
SYSTEMIC NEOPLASMS									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	1	1	2	3	0	0.146	0.485	0.309	0.495
Systemic									
Hemangioma,	0	1	0	3	6	0.006	0.510	0.302	0.062
Hemangioma/-sarcoma	4	2	5	10	8	0.003	0.202	0.013	0.053
Hemangiosarcoma,	4	1	5	7	2	0.301	0.084	0.028	0.514
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	2	2	5	5	1	0.115	0.211	0.219	0.493
TESTES									
Benign Leydig cell tumor,	0	2	2	0	0	0.040	0.676	0.252	0.238
THYMUS									
Benign thymoma,	3	1	1	0	0	0.184	0.743	0.505	0.491
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.739	.	0.495	.
Malig. lymphomatous infiltrat.	0	1	2	0	0	0.108	0.485	0.510	0.495
THYROID GLANDS									
C-cell adenoma,	1	3	2	3	2	0.382	0.519	0.652	0.482
C-cell adenoma/carcinoma	1	4	3	4	4	0.460	0.522	0.631	0.621
C-cell carcinoma,	0	1	1	1	2	0.277	0.743	0.748	0.514
Foll. cell adenoma/carcinoma	2	3	5	7	11	0.016	0.336	0.152	0.026
Follicular cell adenoma,	2	3	5	5	8	0.096	0.336	0.347	0.113
Follicular cell carcinoma,	0	1	0	3	3	0.090	0.510	0.302	0.323
Histiocytic sarcomatous infiltra	0	0	0	1	0	0.739	.	0.495	.
Malignant lymphomatous infiltrat	0	0	1	0	0	0.493	0.490	.	.
TOOTH/TEETH									
Odontoma,	1	0	0	0	0	.	.	.	.

Table A.3.3. (cont.) Overall Results of Poly-k tests in Male Rats

	Incidence: Water	Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
TRACHEA									
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.495	0.495	.	.
URETERS									
Malig. lymphomatous infiltrat.	0	0	1	0	0	0.493	0.490	.	.
ZYMBAL'S GLANDS									
Sebaceous carcinoma,	0	0	0	1	0	0.739	.	0.495	.

Table A.3.4. Overall Results of Poly-k tests in Female Rats

	Incidence: Water	Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
ADRENAL GLANDS									
Adenoma,cortical	1	0	1	0	2	0.098	0.475	.	0.223
Adenoma/Carc. Cortical	1	0	2	0	2	0.175	0.223	.	0.223
Benign pheochromocytoma,	0	0	0	2	7	0.000	.	0.238	0.005
Benign/malig. Pheochromcytoma	0	0	0	2	7	0.000	.	0.238	0.005
Carcinoma,cortical	0	0	1	0	0	0.505	0.475	.	.
Malig. lymphomatous infiltrat.	0	1	0	1	0	0.393	0.529	0.738	0.529
BONE MARROW, STERNUM									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
BRAIN									
Mixed glioma,	0	0	0	0	1	0.240	.	.	0.475
BRONCHUS/BRONCHI									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
CECUM									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
CLITORAL GLANDS									
Squamous cell papilloma,	1	0	0	0	0	.	.	.	.
COLON									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
DRAINING LYMPH NODES									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.761	.	0.495	.
DUODENUM									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
Myofibroma,	0	1	0	0	0	0.265	0.525	0.510	0.525
FEMUR									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
HARDERIAN GLANDS									
Squamous cell carcinoma,	0	1	0	1	0	0.393	0.529	0.738	0.529
HEART									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529

Table A.3.4. (cont.) Overall Results of Poly-k tests in Female Rats

	Incidence: Water	Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
ILEUM									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
JEJUNUM									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
KIDNEYS									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
Nephroblastoma,	0	0	0	0	1	0.240	.	.	0.475
LARYNX									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
LIVER									
Hepatocellular adenoma,	0	2	0	1	1	0.506	0.273	0.507	0.538
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
LUNGS									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
LYMPH NODES									
Hemangioma,	0	0	0	0	1	0.240	.	.	0.475
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
MAMMARY GLAND									
Adenocarcinoma,	2	2	5	7	2	0.278	0.179	0.076	0.661
Adenoma,	0	2	1	2	0	0.161	0.545	0.677	0.278
Fibroadenoma,	22	21	15	25	16	0.351	0.207	0.254	0.298
Fibroadenoma/adenoma	22	23	15	26	16	0.281	0.130	0.321	0.201
Fibroma,	0	1	0	1	0	0.390	0.525	0.743	0.525
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
MANDIB. L.N/LEFT									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
MANDIB.GLANDS, LEFT									
Squamous cell carcinoma,	1	0	0	0	0	.	.	.	.
MESENT. LYMPH NODE									
Hemangioma,	0	1	1	0	2	0.220	0.727	0.510	0.463
Hemangiosarcoma,	0	0	0	1	0	0.760	.	0.490	.
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
OVARIES									
Benign Sertoli cell tumor,	0	0	1	0	1	0.295	0.480	.	0.475
Benign granulosa-theca cell tumo	0	0	0	0	1	0.240	.	.	0.475
Benign luteoma,	0	0	0	1	1	0.180	.	0.490	0.475
Benign thecoma,	1	0	0	0	0	.	.	.	.
Benign undifferentiated stromal	0	0	1	0	0	0.505	0.475	.	.
Fibroma,	0	0	1	0	0	0.505	0.475	.	.
Malig. lymphomatous infiltrat.	0	2	0	0	0	0.071	0.278	0.282	0.278
Yolk sac carcinoma,	1	0	0	1	0	0.760	.	0.490	.

Table A.3.4. (cont.) Overall Results of Poly-k tests in Female Rats

	Incidence:					p-values:	Low	Med	High
	Water	Veh	Low	Med	High	Trend	vs Veh	vs Veh	vs Veh
PANCREAS									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
PEYER'S PATCHES									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
PITUITARY GLAND									
Adenoma of pars distalis,	40	29	32	37	39	0.040	0.279	0.138	0.043
Adenoma of pars intermedia,	0	0	0	1	0	0.760	.	0.490	.
Ganglioneuroma (pars nervosa),	0	0	0	1	0	0.760	.	0.490	.
SKIN/SUBCUTIS									
Basal cell carc/benign tmr	0	0	0	0	2	0.057	.	.	0.223
Basal cell carcinoma,	0	0	0	0	1	0.240	.	.	0.475
Benign basal cell tumor,	0	0	0	0	1	0.240	.	.	0.475
Fibroma,	0	1	0	0	0	0.265	0.525	0.510	0.525
Fibrosarcoma,	0	0	0	1	0	0.760	.	0.490	.
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.761	.	0.495	.
Keratoacanth./Sq. cell Carc.	1	1	1	0	0	0.200	0.722	0.514	0.529
Keratoacanthoma,	0	0	1	0	0	0.505	0.475	.	.
Leiomyosarcoma,	0	0	0	0	1	0.240	.	.	0.475
Lipoma,	0	0	0	2	0	0.577	.	0.238	.
Rhabdomyosarcoma,	1	2	0	0	0	0.071	0.278	0.262	0.278
Squamous cell carcinoma,	1	1	0	0	0	0.269	0.529	0.514	0.529
SPLEEN									
Hemangioma,	0	0	0	0	1	0.240	.	.	0.475
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
STOMACH									
Fibrosarcoma,	0	0	1	0	0	0.505	0.475	.	.
Squamous cell carcinoma,	0	1	0	0	0	0.265	0.525	0.510	0.525
SYSTEMIC NEOPLASMS									
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.761	.	0.495	.
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
Systemic									
Hemangioma,	0	1	1	0	4	0.027	0.727	0.510	0.152
Hemangioma/-sarcoma	0	1	1	1	4	0.038	0.727	0.743	0.152
Hemangiosarcoma,	0	0	0	1	0	0.760	.	0.490	.
Histio. sarcomatous infiltrat.	0	0	0	1	0	0.761	.	0.495	.
Malig. lymphomatous infiltrat.	2	2	1	3	0	0.154	0.537	0.473	0.278
THYMUS									
Benign thymoma,	1	2	1	1	1	0.412	0.538	0.515	0.538
Malig. lymphomatous infiltrat.	1	0	0	0	0	.	.	.	.

Table A.3.4. (cont.) Overall Results of Poly-k tests in Female Rats

	Incidence: Water	Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
THYROID GLANDS									
C-cell adenoma,	2	4	2	4	7	0.049	0.387	0.620	0.208
C-cell adenoma/carcinoma	3	4	2	4	10	0.005	0.387	0.620	0.054
C-cell carcinoma,	1	0	0	0	3	0.013	.	.	0.104
Foll. cell adenoma/carcinoma	3	0	2	2	3	0.087	0.223	0.243	0.104
Follicular cell adenoma,	3	0	1	2	3	0.047	0.475	0.243	0.104
Follicular cell carcinoma,	0	0	1	0	0	0.505	0.475	.	.
UTERUS									
Adenocarcinoma,	0	0	0	1	0	0.760	.	0.490	.
Adenoma,	0	0	0	1	0	0.760	.	0.490	.
Adenoma/-carcinoma	0	0	0	2	0	0.577	.	0.238	.
Malig. lymphomatous infiltrat.	1	0	1	2	0	0.423	0.480	0.238	.
Stromal polyp,	3	2	1	7	0	0.192	0.538	0.076	0.273
VAGINA									
Malig. lymphomatous infiltrat.	0	1	0	0	0	0.269	0.529	0.514	0.529
ZYMBAL'S GLANDS									
Sebaceous carcinoma,	1	0	0	1	1	0.180	.	0.490	0.475

Table A.3.5. Overall Results of Poly-k tests in Male Mice

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
ADRENAL GLANDS								
B subcapsular adenoma,	0	1	0	0	0.531	0.457	.	.
Malig. lymphoma/-infiltrat.	0	1	1	0	0.585	0.463	0.443	.
ADRENAL MEDULLAS								
Benign pheochromocytoma,	0	0	0	1	0.242	.	.	0.460
APPLICATION SITE 1								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
BONE (OTHER)								
Osteosarcoma,	0	1	0	0	0.533	0.463	.	.
BONE (SKULL)								
Osteosarcoma,	0	0	1	0	0.470	.	0.443	.
BONE MARROW, STERNUM								
Malig. lymphoma/-infiltrat.	0	1	1	0	0.585	0.463	0.443	.
Mast cell tumor infiltration,	1	0	0	0	0.287	0.543	0.564	0.547
BRAIN								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
CECUM								
Malig. lymphoma/-infiltrat.	0	1	1	0	0.585	0.463	0.443	.
COLON								
Malig. lymphoma/-infiltrat.	0	1	1	0	0.585	0.463	0.443	.

Table A.3.5. (cont.) Overall Results of Poly-k tests in Male Mice

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
DRAINING LYMPH NODES								
Malig. lymphoma/-infiltrat.	1	0	0	0	0.291	0.543	0.564	0.552
DUODENUM								
Adenoma,	0	1	0	0	0.531	0.457	.	.
Malig. lymphoma/-infiltrat.	0	1	1	0	0.585	0.463	0.443	.
EPIDIDYMIDES								
Histio. sarcomatous infiltrat.	0	1	0	0	0.533	0.463	.	.
Malig. lymphoma/-infiltrat.	0	0	1	0	0.470	.	0.443	.
EYES								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
FEMUR								
Malig. lymphoma/-infiltrat.	1	0	1	0	0.418	0.543	0.693	0.552
Mast cell tumor infiltration,	1	0	0	0	0.287	0.543	0.564	0.547
GALL BLADDER								
Malig. lymphoma/-infiltrat.	0	0	1	1	0.166	.	0.443	0.454
HARDERIAN GLANDS								
Adenoma,	3	4	2	3	0.519	0.403	0.609	0.582
Malig. lymphoma/-infiltrat.	0	1	1	0	0.585	0.463	0.443	.
HEART								
Malig. lymphoma/-infiltrat.	0	0	1	1	0.166	.	0.443	0.454
JOINT,KNEE,LEFT								
Malig. lymphoma/-infiltrat.	0	0	1	0	0.470	.	0.443	.
KIDNEYS								
Malig. lymphoma/-infiltrat.	1	1	1	3	0.110	0.715	0.693	0.243
Tubular cell adenoma,	1	0	3	0	0.523	0.543	0.217	0.552
Tubular cell carc./adenoma	1	1	4	0	0.493	0.708	0.110	0.552
Tubular cell carcinoma,	0	1	1	0	0.587	0.457	0.436	.
LARYNX								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
LIVER								
Hemangioma,	1	1	0	1	0.550	0.708	0.564	0.704
Hemangiosarcoma,	1	0	0	2	0.179	0.543	0.564	0.430
Hepato. carcinoma/adenoma	18	12	17	11	0.294	0.230	0.383	0.220
Hepatocellular adenoma,	14	11	15	9	0.353	0.422	0.256	0.298
Hepatocellular carcinoma,	4	1	2	2	0.352	0.247	0.464	0.442
Histio. sarcomatous infiltrat.	1	1	0	0	0.222	0.715	0.564	0.547
Malig. lymphoma/-infiltrat.	1	1	2	1	0.417	0.715	0.414	0.699
Mast cell tumor infiltration,	1	0	0	0	0.287	0.543	0.564	0.547

Table A.3.5. (cont.) Overall Results of Poly-k tests in Male Mice

	Incidence:				p-values:			
	Veh	Low	Med	High	Trend	Low vs Veh	Med vs Veh	High vs Veh
LUNGS								
Alveo./bronch. adenoma, carc.	21	8	8	14	0.176	0.016	0.023	0.212
Alveolar/bronchiolar adenoma,	13	5	4	8	0.187	0.078	0.050	0.277
Alveolar/bronchiolar carc.	9	3	4	7	0.498	0.105	0.220	0.552
Malig. lymphoma/-infiltrat.	1	1	1	1	0.489	0.715	0.693	0.699
LYMPH NODES								
Malig. lymphoma/-infiltrat.	2	1	1	1	0.406	0.556	0.586	0.578
MANDIB. L.N/LEFT								
Malig. lymphoma/-infiltrat.	1	1	1	0	0.309	0.715	0.693	0.552
MANDIB. L.N/RIGHT								
Malig. lymphoma/-infiltrat.	1	1	0	0	0.226	0.715	0.564	0.552
MANDIB.GLANDS, LEFT								
Malig. lymphoma/-infiltrat.	1	1	1	1	0.489	0.715	0.693	0.699
MESENT. LYMPH NODE								
Hemangioma,	0	1	0	0	0.531	0.457	.	.
Malig. lymphoma/-infiltrat.	1	1	2	2	0.215	0.715	0.414	0.422
OPTIC NERVES								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
PANCREAS								
Islet cell adenoma,	1	0	0	0	0.287	0.543	0.564	0.547
Malig. lymphoma/-infiltrat.	0	1	2	0	0.448	0.463	0.193	.
PAROTID GLAND, LEFT								
Malig. lymphoma/-infiltrat.	0	1	0	1	0.283	0.463	.	0.454
PEYER'S PATCHES								
Malig. lymphoma/-infiltrat.	1	0	1	0	0.418	0.543	0.693	0.552
PREPUTIAL GLANDS								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
PROSTATE GLAND								
Malig. lymphoma/-infiltrat.	0	1	1	1	0.259	0.463	0.443	0.454
RECTUM								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
SCIATIC NERVES								
Malig. lymphoma/-infiltrat.	0	0	1	0	0.470	.	0.443	.
SEMINAL VESICLES								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
SKIN UNTREATED								
Malig. lymphoma/-infiltrat.	0	1	0	1	0.283	0.463	.	0.454
SKIN/SUBCUTIS								
Hemangiosarcoma,	1	0	0	0	0.291	0.543	0.564	0.552
Malig. lymphoma/-infiltrat.	0	0	0	1	0.238	.	.	0.454

Table A.3.5. (cont.) Overall Results of Poly-k tests in Male Mice

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
SPINAL CORD, LUMBAR								
Malig. lymphoma/-infiltrat.	1	0	0	0	0.291	0.543	0.564	0.552
Mast cell tumor infiltration,	1	0	0	0	0.287	0.543	0.564	0.547
SPINAL CORD, THORAC.								
Malig. lymphoma/-infiltrat.	1	0	1	0	0.418	0.543	0.693	0.552
Mast cell tumor infiltration,	1	0	0	0	0.287	0.543	0.564	0.547
SPLEEN								
Hemangiosarcoma,	0	0	0	2	0.055	.	.	0.203
Histio. sarcomatous infiltrat.	1	0	0	0	0.287	0.543	0.564	0.547
Malig. lymphoma/-infiltrat.	2	1	1	2	0.422	0.556	0.586	0.610
Mast cell tumor infiltration,	1	0	0	0	0.287	0.543	0.564	0.547
STERNUM								
Malig. lymphoma/-infiltrat.	1	1	0	0	0.226	0.715	0.564	0.552
STOMACH								
Malig. lymphoma/-infiltrat.	0	1	0	0	0.533	0.463	.	.
SUBLING.GLAND, LEFT								
Malig. lymphoma/-infiltrat.	0	1	0	1	0.283	0.463	.	0.454
SYSTEMIC NEOPLASMS								
Histiocytic sarcoma,	1	1	0	0	0.222	0.715	0.564	0.547
Malig. lymphoma/-infiltrat.	3	1	3	3	0.323	0.365	0.547	0.571
Malignant mast cell tumor,	1	0	0	0	0.287	0.543	0.564	0.547
Systemic								
Hemagioma/-sarcoma	3	3	0	3	0.479	0.564	0.179	0.558
Hemangioma,	1	3	0	1	0.398	0.245	0.564	0.704
Hemangiosarcoma,	2	0	0	2	0.382	0.298	0.321	0.610
Histio. sarcomatous infiltrat.	1	1	0	0	0.222	0.715	0.564	0.547
Malig. lymphoma/-infiltrat.	3	1	3	3	0.323	0.365	0.547	0.571
Mast cell tumor infiltration,	1	0	0	0	0.287	0.543	0.564	0.547
TAIL								
Hemangioma,	0	1	0	0	0.531	0.457	.	.
TESTES								
Benign Leydig cell tumor,	3	0	0	0	0.023	0.155	0.174	0.158
THYMUS								
Malig. lymphoma/-infiltrat.	1	1	0	1	0.557	0.715	0.564	0.699
THYROID GLANDS								
Follicular cell adenoma,	1	0	0	0	0.287	0.543	0.564	0.547
Malig. lymphoma/-infiltrat.	0	1	1	0	0.585	0.463	0.443	.
URETERS								
Malig. lymphoma/-infiltrat.	1	1	0	0	0.226	0.715	0.564	0.552
URINARY BLADDER								
Malig. lymphoma/-infiltrat.	0	0	1	0	0.470	.	0.443	.
Transitional cell papilloma,	0	0	0	1	0.238	.	.	0.454

Table A.3.6. Overall Results of Poly-k tests in Female Mice

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
ABDOMINAL CAVITY								
Malig. lymphoma/-infiltrat.	0	0	0	1	0.210	.	.	0.443
ADIPOSE TISSUE								
Malig. lymphoma/-infiltrat.	1	0	0	0	0.270	0.495	0.529	0.570
ADRENAL GLANDS								
B subcapsular adenoma,	0	0	1	0	0.455	.	0.482	.
Malig. lymphoma/-infiltrat.	2	0	2	1	0.534	0.247	0.656	0.594
ADRENAL MEDULLAS								
Benign pheochromocytoma,	0	0	0	1	0.210	.	.	0.436
APPLICATION SITE 1								
Malig. lymphoma/-infiltrat.	3	0	1	2	0.521	0.121	0.353	0.629
Sarcoma (not otherwise specified)	0	0	1	0	0.455	.	0.476	.
BONE MARROW, STERNUM								
Malig. lymphoma/-infiltrat.	3	1	7	0	0.419	0.308	0.124	0.190
BRAIN								
Malig. lymphoma/-infiltrat.	2	0	2	0	0.270	0.247	0.657	0.328
Meningeal sarcoma,	0	0	1	0	0.455	.	0.476	.
CERVIX								
Endo. stromal polyp tumor	1	1	1	0	0.326	0.742	0.729	0.564
Endo.strom.pol./strom.sarc	2	1	1	0	0.159	0.484	0.545	0.321
Histio. sarcomatous infiltrat.	3	5	1	0	0.045	0.381	0.354	0.179
Leiomyoma,	3	0	3	1	0.431	0.121	0.606	0.417
Malig. lymphoma/-infiltrat.	2	1	2	0	0.227	0.492	0.656	0.321
Squamous cell carcinoma,	0	0	1	0	0.455	.	0.476	.
Stromal cell sarcoma,	1	0	0	0	0.270	0.495	0.529	0.570
COLON								
Malig. lymphoma/-infiltrat.	0	0	0	1	0.210	.	.	0.443
DRAINING LYMPH NODES								
Histio. sarcomatous infiltrat.	2	6	1	0	0.079	0.155	0.545	0.321
Malig. lymphoma/-infiltrat.	3	2	2	3	0.364	0.500	0.565	0.528
DUODENUM								
Adenoma,	0	1	0	0	0.545	0.506	.	.
Malig. lymphoma/-infiltrat.	0	0	2	0	0.323	.	0.230	.
EARS								
Malig. lymphoma/-infiltrat.	0	0	1	1	0.151	.	0.482	0.443
ESOPHAGUS								
Malig. lymphoma/-infiltrat.	0	0	1	0	0.458	.	0.482	.
EYES								
Malig. lymphoma/-infiltrat.	3	0	0	0	0.021	0.125	0.148	0.185
FEMUR								
Hemangiosarcoma,	0	0	1	0	0.455	.	0.476	.
Histio. sarcomatous infiltrat.	0	3	0	0	0.326	0.133	.	.
Malig. lymphoma/-infiltrat.	2	2	4	0	0.316	0.683	0.296	0.328

Table A.3.6. (cont.) Overall Results of Poly-k tests in Female Mice

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
GALL BLADDER								
Malig. lymphoma/-infiltrat.	3	0	1	1	0.281	0.121	0.344	0.408
HARDERIAN GLANDS								
Adenocarcinoma,	0	0	1	2	0.041	.	0.476	0.187
Adenoma,	5	0	3	2	0.323	0.028	0.411	0.333
Histio. sarcomatous infiltrat.	0	1	0	0	0.548	0.511	.	.
Malig. lymphoma/-infiltrat.	3	0	3	1	0.428	0.121	0.617	0.417
HEART								
Histio. sarcomatous infiltrat.	0	1	0	1	0.268	0.511	.	0.443
Malig. lymphoma/-infiltrat.	6	1	3	2	0.201	0.062	0.326	0.270
JOINT,KNEE,LEFT								
Malig. lymphoma/-infiltrat.	2	0	1	1	0.493	0.247	0.544	0.603
KIDNEYS								
Histio. sarcomatous infiltrat.	2	5	1	0	0.086	0.245	0.545	0.321
Malig. lymphoma/-infiltrat.	8	2	6	2	0.152	0.056	0.511	0.126
LARYNX								
Malig. lymphoma/-infiltrat.	4	0	2	2	0.418	0.061	0.395	0.476
LIVER								
Hepato. carcinoma/adenoma	1	0	1	1	0.389	0.495	0.729	0.693
Hepatocellular adenoma,	0	0	1	1	0.150	.	0.476	0.443
Hepatocellular carcinoma,	1	0	0	0	0.270	0.495	0.524	0.564
Histio. sarcomatous infiltrat.	3	8	2	1	0.127	0.128	0.556	0.408
Malig. lymphoma/-infiltrat.	9	4	7	5	0.440	0.141	0.504	0.420
LUNGS								
Alveo./bronch. adenoma, carc.	13	7	5	6	0.078	0.095	0.056	0.150
Alveolar/bronchiolar adenoma,	9	5	5	5	0.246	0.170	0.262	0.334
Alveolar/bronchiolar carc.	4	2	1	1	0.112	0.318	0.219	0.279
Histio. sarcomatous infiltrat.	1	3	0	1	0.422	0.325	0.529	0.687
Malig. lymphoma/-infiltrat.	9	2	5	4	0.266	0.033	0.288	0.286
LYMPH NODES								
Histio. sarcomatous infiltrat.	1	4	0	0	0.137	0.208	0.524	0.564
Malig. lymphoma/-infiltrat.	5	2	6	4	0.307	0.226	0.422	0.577
MAMMARY GLAND								
Adenocarcinoma,	3	0	1	0	0.076	0.117	0.344	0.179
Adenoma,	0	0	1	0	0.455	.	0.476	.
Adenosq. Carc./Adenocarc.	3	1	2	0	0.114	0.282	0.545	0.179
Adenosquamous carcinoma,	0	1	1	0	0.570	0.511	0.476	.
Malig. lymphoma/-infiltrat.	2	0	1	1	0.493	0.247	0.535	0.594
MANDIB. L.N/LEFT								
Histio. sarcomatous infiltrat.	0	1	0	0	0.548	0.511	.	.
Malig. lymphoma/-infiltrat.	6	2	5	2	0.259	0.141	0.578	0.269

Table A.3.6. (cont.) Overall Results of Poly-k tests in Female Mice

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
MANDIB. L.N/RIGHT								
Malig. lymphoma/-infiltrat.	2	0	2	1	0.534	0.247	0.647	0.603
MANDIB.GLANDS, LEFT								
Malig. lymphoma/-infiltrat.	4	0	4	3	0.331	0.061	0.591	0.651
MANDIBULAR GLANDS								
Malig. lymphoma/-infiltrat.	0	0	0	1	0.214	.	.	0.443
MESENT. LYMPH NODE								
Hemangioma,	0	0	0	1	0.210	.	.	0.436
Histio. sarcomatous infiltrat.	1	0	0	0	0.270	0.495	0.524	0.564
Malig. lymphoma/-infiltrat.	9	3	4	2	0.049	0.070	0.184	0.083
OVARIES								
Benign luteoma,	0	0	0	1	0.210	.	.	0.436
Cystadenoma,	3	1	1	0	0.069	0.300	0.345	0.174
Histio. sarcomatous infiltrat.	0	5	0	0	0.226	0.035	.	.
Malig. lymphoma/-infiltrat.	8	2	5	3	0.225	0.056	0.382	0.253
OVIDUCTS								
Histio. sarcomatous infiltrat.	0	1	0	0	0.548	0.511	.	.
Malig. lymphoma/-infiltrat.	5	0	2	0	0.030	0.031	0.266	0.080
PANCREAS								
Histio. sarcomatous infiltrat.	1	2	0	0	0.193	0.517	0.524	0.564
Malig. lymphoma/-infiltrat.	5	2	2	2	0.213	0.217	0.277	0.356
PAROTID GLAND, LEFT								
Malig. lymphoma/-infiltrat.	5	1	3	2	0.295	0.102	0.422	0.356
PEYER'S PATCHES								
Malig. lymphoma/-infiltrat.	1	1	3	1	0.332	0.742	0.282	0.687
PITUITARY GLAND								
Adenoma of pars distalis,	0	3	0	0	0.326	0.129	.	.
Adenoma of pars intermedia,	0	1	1	0	0.570	0.511	0.476	.
Malig. lymphoma/-infiltrat.	3	0	4	0	0.255	0.125	0.437	0.190
SCIATIC NERVES								
Histio. sarcomatous infiltrat.	1	0	0	0	0.270	0.495	0.524	0.564
Malig. lymphoma/-infiltrat.	0	0	1	0	0.455	.	0.482	.
SKELETAL MUSCL OTHER								
Sarcoma (not otherwise specified)	0	1	0	0	0.548	0.511	.	.
SKELETAL MUSCLE								
Malig. lymphoma/-infiltrat.	2	0	1	1	0.496	0.247	0.535	0.594
SKIN UNTREATED								
Malig. lymphoma/-infiltrat.	3	1	1	2	0.466	0.300	0.353	0.629
SKIN/SUBCUTIS								
Malig. lymphoma/-infiltrat.	3	0	2	2	0.459	0.125	0.585	0.629
Sarcoma (not otherwise specified)	1	1	1	0	0.324	0.747	0.729	0.564
Squamous cell carcinoma,	0	1	0	0	0.548	0.506	.	.

Table A.3.6. (cont.) Overall Results of Poly-k tests in Female Mice

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
SPINAL CORD, LUMBAR								
Malig. lymphoma/-infiltrat.	4	1	3	0	0.090	0.181	0.563	0.107
SPINAL CORD, THORAC.								
Malig. lymphoma/-infiltrat.	7	1	3	0	0.012	0.036	0.229	0.021
SPLEEN								
Hemangioma,	0	1	0	0	0.548	0.511	.	.
Histio. sarcomatous infiltrat.	0	1	0	0	0.548	0.511	.	.
Malig. lymphoma/-infiltrat.	6	4	8	5	0.285	0.370	0.303	0.551
STERNUM								
Malig. lymphoma/-infiltrat.	3	1	0	2	0.385	0.308	0.148	0.629
STOMACH								
Malig. lymphoma/-infiltrat.	7	0	3	3	0.258	0.007	0.218	0.301
SUBLING.GLAND, LEFT								
Malig. lymphoma/-infiltrat.	3	0	2	0	0.141	0.125	0.544	0.185
SYSTEMIC NEOPLASMS								
Histiocytic sarcoma,	5	9	2	1	0.037	0.243	0.279	0.177
Malig. lymphoma/-infiltrat.	12	5	11	7	0.497	0.077	0.528	0.402
Systemic								
Hemagioma/-sarcoma	1	1	1	2	0.215	0.742	0.729	0.403
Hemangioma,	0	1	0	2	0.076	0.511	.	0.187
Hemangiosarcoma,	1	0	1	0	0.429	0.495	0.729	0.564
Histio. sarcomatous infiltrat.	5	9	2	1	0.037	0.243	0.279	0.177
Malig. lymphoma/-infiltrat.	12	5	12	7	0.469	0.077	0.427	0.402
TAIL								
Sarcoma (not otherwise specified)	0	0	0	1	0.210	.	.	0.443
THORACIC CAVITY								
Malig. lymphoma/-infiltrat.	0	0	0	1	0.210	.	.	0.443
THYMUS								
Histio. sarcomatous infiltrat.	1	1	0	0	0.221	0.742	0.524	0.564
Malig. lymphoma/-infiltrat.	8	2	6	5	0.472	0.058	0.508	0.519
THYROID GLANDS								
Malig. lymphoma/-infiltrat.	4	0	0	2	0.251	0.061	0.077	0.476
URETERS								
Malig. lymphoma/-infiltrat.	2	0	1	3	0.145	0.247	0.535	0.381
URINARY BLADDER								
Histio. sarcomatous infiltrat.	1	1	0	0	0.221	0.742	0.524	0.564
Malig. lymphoma/-infiltrat.	5	0	3	1	0.167	0.030	0.422	0.184

Table A.3.6. (cont.) Overall Results of Poly-k tests in Female Mice

	Incidence: Veh	Low	Med	High	p-values: Trend	Low vs Veh	Med vs Veh	High vs Veh
UTERUS								
Adenocarcinoma,	0	0	1	0	0.455	.	0.476	.
Endo. stromal polyp tumor	6	5	2	1	0.034	0.485	0.174	0.104
Hemangioma,	0	0	0	1	0.210	.	.	0.436
Hemangiosarcoma,	1	0	0	0	0.270	0.495	0.524	0.564
Histio. sarcomatous infiltrat.	2	3	1	0	0.116	0.531	0.536	0.315
Leiomyoma,	3	1	2	0	0.114	0.300	0.546	0.174
Leiomyosarcoma,	0	0	0	1	0.210	.	.	0.443
Malig. lymphoma/-infiltrat.	6	1	1	1	0.033	0.059	0.084	0.121
VAGINA								
Histio. sarcomatous infiltrat.	0	2	0	0	0.431	0.264	.	.
Malig. lymphoma/-infiltrat.	3	0	1	0	0.078	0.121	0.353	0.185

Appendix 4. References

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Steven Thomson
5/19/2008 04:54:56 PM
BIOMETRICS

Karl Lin
5/20/2008 08:55:55 AM
BIOMETRICS
Concur with review

Statistical Review and Evaluation
Fileability Review

NDA Number:	22-087
Drug Name:	Silkis (calcitriol) ointment
Applicant:	Galderma
Indication:	Plaque Psoriasis
Filing Date:	11/26/2006
Fileability Meeting Date:	11/13/2006
User Fee Date:	07/27/2007
Received for Stat Review:	10/03/2006
Statistical Reviewer:	Mat Soukup, Ph.D., DBIII
Medical Officer:	Brenda Carr, M.D., DDDP
Project Manager:	Margo Owens, DDDP

1 Background and Summary

This is a paper CTD NDA submission. Studies 18053 and 18054 are Phase 3 trials with the objective of establishing the superiority of Silkis ointment over vehicle in the treatment of mild to moderate plaque psoriasis. In addition to the two Phase 3 trials, the sponsor has submitted data and study reports for Study 2663 which is an open-label 52 week study of Silkis ointment.

2 Organization and Data Representation

1. Is there a comprehensive table of contents with adequate indexing and pagination? *Yes*
2. Are the original protocols, protocol amendments, and proposed label provided? *Yes. Protocols are located in each study report, and the label is available in EDR.*
3. Based on either the electronic data sets or the study reports can the following information be reviewed?
   (a) Patient profile listings by center for all enrolled subjects. *Yes, this will be possible with the requested electronic data sets.*
   (b) Discontinued subject tables by center (includes reason and time of loss). *Yes, the information is available in the data set SUB1308x.*
   (c) Subgroup analysis summary tables (gender, race, age, etc.). *Yes, both study reports and electronic data: SUB1308x.*
   (d) Adverse event listings by center and time of occurrence. *Yes, this is available in the AEF1308x data sets; note that AE events are reported using COSTART terminology.*
4. Information specific to the electronically submitted data.

(a) Has adequate documentation of the data sets been provided? *Yes, all data sets include a define file for variable description.*

(b) Do the data appear to accurately represent the data described in the study reports? *The recording of visit in the derived EFF_OC efficacy data set resulted in success rates that differed from study reports. The raw efficacy data set, VIS1308x, also resulted in counts of success that differed from the study reports. As a result, the reviewer requests the sponsor resubmit the data according to the example provided as an attachment.*

(c) Can the data be easily merged across studies and indications? *Yes, however, most data sets appear to be self-contained as they include a treatment variable.*

3 Statistical Methodology

1. Are all primary efficacy studies of appropriate design to meet basic approvability requirements within current Division policy or to the extent agreed upon previously with the sponsor by the Division. *Yes, the primary analysis is CMH stratified by site for the ITT analysis population imputing missing data by LOCF.*

2. For each study, is there a comprehensive statistical summary of the efficacy which covers the intent-to-treat population and per protocol population? *Yes.*

3. Based on the summary analyses of each study:

   (a) Are the analyses appropriate for the type of data collected, the study design, and the study objectives (based on protocol objectives and proposed labeling claims)? *Yes, although efficacy claims are proposed in the label for the open-label long term study. This will be a review issue.*

   (b) Are the intent-to-treat and per protocol patient analyses properly performed? *Yes.*

   (c) Has missing data been appropriately handled? *Yes, this is LOCF - no sensitivity analyses to method of data imputation are provided in the study reports. This will be assessed in the review.*

   (d) Have multiplicity issues (regarding endpoints, timepoints, or dose groups) been adequately addressed? *N/A*

   (e) If interim analyses were performed, were they planned in the protocol and appropriate significance level adjustments made? *N/A*

4. Were sufficient and appropriate references included for novel statistical approaches? *N/A*

5. Are all pivotal studies complete? *Yes.*

6. Has the safety data been comprehensively and adequately summarized? *Yes, this appears to be the case based upon the study reports.*

4 FILEABILITY CONCLUSIONS

From a statistical perspective this submission, or indications therein, *is* reviewable with further input from the sponsor.

5 74-DAY LETTER COMMENTS

1. Filing Issues: The statistical reviewer was not able to reproduce the counts for IGA success as those included in the study reports for Studies 18053 and 18054 based on the derived data set EFF_OC nor the raw data set VIS1804x.

2. Request for Information: To facilitate the statistical review the Agency requests the sponsor to submit an efficacy data set which clearly defines visit. For example, if a subject attended a visit this should be recorded with the appropriate visit number and time of visit. If the subject did not attend the visit then the visit number may be recorded with no time of visit (or the visit excluded altogether). The attached example is provided as one method of constructing an efficacy data set which includes one record per subject per visit per analysis visit type (Observed and LOCF for your data).

Mat Soukup, Ph.D.
Mathematical Statistician, Biometrics 3

Concur: Mohamed Alosh, Ph.D.
Team Leader, Biometrics 3

Cc:
Orig. NDA 22,087/SN000
DDDP/Walker
DDDP/Lindstrom
DDDP/Carr
DDDP/Owens
OBIO/O'Neill
OBIO/Patrician
DBIII/Wilson
DBIII/Alosh
DBIII/Soukup
November 13, 2008

Example of a data set for a study with 3 planned visits and two treatment arms and two efficacy endpoints (note that the notation uses subscripts *i* and *j* which correspond to the value for the *i*-th visit and the *j*-th subject). In this example Observed and LOCF analysis visit types (AVISFLG variable) were defined. Note that in the following example: Subject 0001 attended all visits, Subject 0002 missed visit 2, and Subject 0003 missed visit 3 and the endpoint X was not collected at visit 2 ('-' denoting missing in this example).

Table 2. Example Efficacy Data Set

PATNO	INVNUM	AVISIT	AVISFLG	VISITDT	STUDYDAY	EFFICACY ENDPOINTS		INVGRP	AGE	GENDER	RACE	ITT	PP
0001	1	1	Observed		1	X_{11}	Y_{11}	88					
0001	1	1	LOCF		1	X_{11}	Y_{11}	88					
0001	1	2	Observed		1	X_{21}	Y_{21}	88					
0001	1	2	LOCF		1	X_{21}	Y_{21}	88					
0001	1	3	Observed		1	X_{31}	Y_{31}	88					
0001	1	3	LOCF		1	X_{31}	Y_{31}	88					
0002	2	1	Observed		0	X_{12}	Y_{12}	88					
0002	2	1	LOCF		0	X_{12}	Y_{12}	88					
0002	2	2	LOCF		0	X_{12}	Y_{12}	88					
0002	2	3	Observed		0	X_{32}	Y_{32}	88					
0002	2	3	LOCF		0	X_{32}	Y_{32}	88					
0003	1	1	Observed		1	X_{13}	Y_{13}	88					
0003	1	1	LOCF		1	X_{13}	Y_{13}	88					
0003	1	2	Observed		1	-	Y_{23}	88					
0003	1	2	LOCF		1	X_{13}	Y_{23}	88					
0003	1	3	LOCF		1	X_{13}	Y_{23}	88					
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/s/

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