

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPROVAL PACKAGE FOR:**

**APPLICATION NUMBER**

**21-476**

**Statistical Review(s)**



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH  
OFFICE OF BIostatISTICS

**STATISTICAL REVIEW AND EVALUATION  
CARCINOGENICITY STUDIES**

**NDA /Serial Number:** NDA 21-476/N: 000  
**Name of Drug:** Estorra (eszopiclone), 2mg and 3mg Tablets  
**Applicant:** Sepracor Pharmaceuticals  
**Indication:** Hypnotic  
**Dates:** January 30, 2003 - October 14, 2003

**Biometrics Division:** Division of Biometrics 1 (HFD-710)  
**Statistical Reviewer:** Roswitha Kelly, MS (HFD-710)  
**Concurring Reviewers:** Kooros Mahjoob, Ph.D., Deputy Director  
(HFD-710)

**Medical Division:** Neuropharmacological Drug Products  
(HFD-120)  
**Pharmacologist:** Aisar Atrakchi, Ph.D. (HFD-120)  
**Pharm/Tox Team Leader:** Barry Rosloff, Ph.D. (HFD-120)  
**Project Manager:** Merril Mille, R.Ph. (HFD-120)

Keywords: Estorra, eszopiclone, carcinogenicity bioassay, intercurrent mortality, Kaplan- Meier estimates, exact permutation tests, multiplicity, validity of study.

## Table of Contents

<b>1 Executive Summary</b> .....	5
1.1 Conclusions .....	5
1.1.1 Rat Studies .....	5
1.1.2 Mouse Studies .....	6
1.2 Overview of Studies Reviewed.....	7
1.3 Principal Findings.....	8
<b>2 Introduction</b> .....	8
2.1 Overview .....	8
2.1.1 Background .....	9
2.1.2 Major Statistical Issues.....	9
2.2 Data Analyzed and Sources.....	11
<b>3 Statistical Evaluation</b> .....	12
3.1 Rat Carcinogenicity Study (Sepr. Doc. 190-833 and 190-874A1).....	12
3.1.1 Sponsor's Results and Conclusions .....	12
3.1.2 Statistical Methodologies .....	12
3.1.3 Detailed Review of Rat Carcinogenicity Study Nr. 190-833.....	13
3.1.4 Statistical Reviewer's Findings of Rat Study Nr. 190-833 .....	13
3.2 Rat Carcinogenicity Study 190-833 Appended (Sepr. Docs. 190-839, 190-877, 190-860, 190-874A1).....	20
3.2.1 Sponsor's Results and Conclusions .....	20
3.2.2 Statistical Methodologies .....	25
3.2.3 Detailed Review of Rat Carcinogenicity Study 190-833 Appended.....	25
3.2.4 Statistical Reviewer's Findings of Study 190-833 Appended.....	25
3.3 Rat Carcinogenicity Study (Sepr. Doc. 190-823F).....	28
3.3.1 Sponsor's Results and Conclusions .....	28
3.3.2 Statistical Methodologies .....	28
3.3.3 Detailed Review of Rat Carcinogenicity Study 190-823.....	29
3.3.4 Statistical Reviewer's Findings of Rat Data 190-823 .....	29
3.3.5 Validity of Male and Female Rat Study 190-823 .....	37
3.4 Mouse Carcinogenicity Study (Sepr. Doc. 190-834).....	38
3.4.1 Sponsor's Results and Conclusions .....	38
3.4.2 Statistical Methodologies .....	39
3.4.3 Detailed Review of Mouse Carcinogenicity Study Nr. 190-834 .....	39
3.4.4 Statistical Reviewer's Findings of Mouse Study Nr. 190-834 .....	39
3.5 Mouse Carcinogenicity Study 190-834 Appended (Sepr. Docs. 190-839, 190-873, 190-873A1).....	46
3.5.1 Sponsor's Results and Conclusions .....	46
3.5.2 Statistical Methods .....	48
3.5.3 Detailed Review of Mouse Study 190-834 Appended .....	48
3.5.4 Statistical Reviewer's Findings of Mouse Study 190-834 Appended.....	49
3.6 Mouse Carcinogenicity Study 190-844 .....	50
3.6.1 Sponsor's Results and Conclusions .....	50
3.6.2 Statistical Methodologies .....	51
3.6.3 Detailed Review of Mouse Carcinogenicity Study.....	51
3.6.4 Statistical Reviewer's Comments Based on Dr. Daphne Lin's Review.....	52
3.7 Mouse Carcinogenicity Study 190-830 .....	54
3.7.1 Sponsor's Results and Conclusions .....	54
3.7.2 Statistical Methodologies .....	54
3.7.3 Detailed Review of Mouse Carcinogenicity Study 190-830 .....	54
3.7.4 Statistical Reviewer's Findings of Study 190-830.....	55
3.7.5 Validity of Male and Female Mouse Study 190-830.....	62
<b>4 Conclusions</b> .....	63
4.1 Statistical Evaluation of Evidence .....	63
<b>5 Appendix</b> .....	64
5.1 Comments Regarding Sponsor's Report ST/CRV/TOX Nr. 198, (Mathematical Model, 1988).....	64

## Table of Tables

Table 1: Overview of Studies Reviewed.....	7
Table 2: Overview of Statistically Significant Findings from Carcinogenicity Studies .....	8
Table 3: Mortality by Time Interval for Female Rats, Study 190-833 .....	14
Table 4: Trend in Mortality for Female Rats, Study 190-833 .....	14
Table 5: Tumor Trend Tests for Female Rats, Study 190-833 .....	15
Table 6: Pair-wise and Trend Tests for Mammary Tumors Based on Time to Detection, Female Rats, Study 190-833 .....	16
Table 7: Mortality by Time Interval for Male Rats, Study 190-833.....	17
Table 8: Trend in Mortality for Male Rats, Study 190-833.....	17
Table 9: Tumor Trend Tests for Male Rats, Study 190-833.....	18
Table 10: Pair-wise and Trend Tests for Malignant Follicular Cell Carcinoma of the Thyroid, Male Rats, Study 190-833 .....	20
Table 11: Sponsor's Incidence of Mammary Tumors by PWG from Sepr. Doc. 190-877 .....	21
Table 12: Sponsor's Statistical Trend Tests for Mammary Tumors in Female Rats from Sepr. Doc. 190-874A1 .....	22
Table 13: Dr. Re-evaluation of Mammary Tumors in Female Rats of Study 190-833 Reported in Sepr. Doc. 190-860 .....	23
Table 14: Number of Thyroid Neoplasms before and after PWG Review, Sepr. Doc. 190-839 .....	24
Table 15: Sponsor's Statistical Trend Results for Thyroid Tumors in Male Rats before and after PWG Review, Sepr. Doc. 190-874A1 .....	24
Table 16: Reviewer's Analysis of Female Rat Mammary and Pituitary Tumors as Re-Evaluated by PWG Using Animal's Time to Death, Study 190-833A .....	26
Table 17: Time to Tumor Detection Analysis of PWG Mammary Tumors of Female Rats, Study 190-833A .....	27
Table 18: Reviewer's Trend Tests for Combined Left and Right Thyroid Tissues as PWG Re-Evaluated of Male Rats of Study 190-833 .....	27
Table 19: Mortality by Time Interval for Female Rats of Study 190-823 .....	30
Table 20: Mortality Trend for Female Rats of Study 190-823 .....	30
Table 21: Tumor Trends for Female Rats of Study 190-823.....	31
Table 22: Mortality by Time Interval for Male Rats of Study 190-823 .....	34
Table 23: Mortality Trend for Male Rats of Study 190-823 .....	34
Table 24: Tumor Trends for Male Rats of 190-823 .....	35
Table 25: Mortality by Time Interval for Female Mice, Study 190-834 .....	40
Table 26: Trend in Mortality for Female Mice, Study 190-834 .....	41
Table 27: Tumor Trend Test for Female Mice, Study 190-834.....	42
Table 28: Mortality by Time Interval for Male Mice, Study 190-834.....	43
Table 29: Trend in Mortality for Male Mice, Study 190-834.....	44
Table 30: Tumor Trend Tests for Male Mice, Study 190-834 .....	45
Table 31: Sponsor's Tables from Pharmsum.pdf of 06/30/03 Submission.....	47
Table 32: Sponsor's Results for Individual and Combined Pulmonary Tumors in Female Mice, Study 190-834A .....	48
Table 33: Reviewer's Results for Pulmonary Tumors among Female Mice in Study 190-834A .....	50
Table 34: Study Design for Mouse Study 190-844 .....	52
Table 35: Sponsor's Tumor Findings in Study 190-844.....	53
Table 36: Sponsor's Table on Weeks of Incrustations and Skin Tumors .....	53
Table 37: Mortality by Time Interval for Female Mice of Study 190-830.....	55
Table 38: Mortality Trend for Female Mice of Study 190-830.....	56
Table 39: Tumor Trends for Female Mice in Study 190-830.....	57
Table 40: Mortality by Time Interval for Male Mice of Study 190-830 .....	59
Table 41: Mortality Trend for Male Mice of Study 190-830 .....	60
Table 42: Tumor Trends for Male Mice of Study 190-830 .....	60

## Table of Figures

Figure 1: Kaplan Meier Survival Curves for Female Rats, Study 190-833.....	15
Figure 2: Kaplan-Meier Survival Curves for Male Rats, Study 190-833.....	18
Figure 3: Kaplan Meier Survival Curves for Female Rats of Study 190-823.....	31
Figure 4: Kaplan-Meier Survival Curves for Male Rats of Study 190-823.....	35
Figure 5: Kaplan Meier Survival Curves for Female Mice, Study 190-834.....	41
Figure 6: Kaplan Meier Survival Curves for Male Mice , Study 190-834.....	44
Figure 7: Kaplan Meier Survival Curves for Female Mice of Study 190-830.....	56
Figure 8: Kaplan Meier Survival Curves for Male Mice in Study 190-830.....	60

Appears This Way  
On Original

## **1 Executive Summary**

### **1.1 Conclusions**

The following comments are based on statistical findings and concerns only. It is recognized that not all tumor increases are of clinical importance.

There were six carcinogenicity studies addressing the oncogenic potential of (R,S) zopiclone or (S) zopiclone in rats and mice. The original rat and mouse studies were conducted with (R,S)-zopiclone. Each cell (each gender, each species) showed a statistically significant increase in a tumor. When these tissues were re-evaluated by a pathology-working group, the p-values changed to some degree, but the statistical significance remained at least for certain tumor/tissue combinations. The more recent rat study conducted with eszopiclone showed no statistically significant increases in any tumors. The study was judged valid for either gender. The recent mouse study conducted with eszopiclone showed one increased tumor finding among the females. The statistical significance of this finding depended on the test applied and is therefore borderline at best. There were no significant tumor increases among the males. As the tumor increase among the females is small and not clearly statistically significant, the validity of both sexes was addressed. Survival was judged adequate, but it appears that the MTD was not reached for either gender. The seventh study was not a regular carcinogenicity study, but investigated group housing and the resulting fighting behavior as the possible cause for skin sarcomas. In the opinion of the original statistical reviewers, with which this reviewer agrees, this study could not prove that group housing and fighting behavior consistently caused skin sarcomas or rule out an additional drug effect. The prominent findings of each study are summarized below.

#### **1.1.1 Rat Studies**

There are three carcinogenicity rat studies under consideration. The original study (190-833) was conducted in Sprague-Dawley CD rats for two years with doses of 0, 0, 1, 10, 100 mg/kg/day of (R,S) zopiclone in the diet. The animals were housed five to a cage. By both the sponsor's and this reviewer's analyses there were statistically significant increases in thyroid tumors among the males and in mammary tumors among the females. There was little effect on mortality for either gender.

The second study (190-833A) consisted of the re-evaluation by a pathology-working group (PWG) of the tissues with significant increases in the first study. The PWG determined different tumor types and different incidence rates at the mammary and the thyroid glands. However, some of the tumor findings were still statistically significant by both the sponsor's and this reviewer's approaches.

A third two-year study (190-823) was conducted again in Sprague-Dawley rats. It was an oral gavage study with eszopiclone at doses of 0, 0, 2, 4, 8, 16 mg/kg. The animals were housed individually. Some treatment arms were terminated early due to increased mortality. Neither the sponsor nor this reviewer observed any statistically significant

increases in tumor findings. Based on statistical criteria, this reviewer considered both the female and the male study valid, because there were sufficient numbers of animals exposed long enough and because the high-dose seemed close to the MTD.

### 1.1.2 Mouse Studies

There are four mouse studies under consideration. The original carcinogenicity study (190-834) was conducted in B6F3C1 mice. It was a feed study with 0, 0, 1, 10, 100 mg/kg/day of (R,S) zopiclone. The animals were housed four to a cage. By both the sponsor's and this reviewer's analyses, a statistically significant increase was observed in skin sarcoma among the males and in pulmonary carcinoma and (right) ovarian cystadenoma among the females.

The second study (190-834A) consisted of the findings of a pathology-working group, which re-evaluated the significant tumor findings of the first study. The PWG did not change the skin tumor findings. However, the PWG determined fewer pulmonary tumors among the females than had originally been observed. After several re-analyses of these data, the sponsor reported that only the pair-wise comparison between the high dose and controls of the combined pulmonary adenomas and carcinomas was statistically significant. This reviewer agreed with this finding. However, she could not reproduce the sponsor's non-significant trend. This reviewer's trend test for carcinoma of the lung, as determined by the PWG, was borderline significant. The trend test for the combined adenoma and carcinoma of the lung was clearly statistically significant.

The third mouse study (190-844) was a special study to compare skin tumors among singly housed and group housed male mice and attribute the increase in skin tumors to fighting behavior secondary to group housing. No skin tumors were observed among the singly-housed controls or the treated animals. For one set of gang-housed control and treated animals, the hypothesis was strongly supported. For the other set of gang-housed control and treated animals, the hypothesis was not clearly supported. The sponsor concluded that the effect of (R,S)-zopiclone on the incidence of subcutaneous tumors of gang-caged mice was related to an effect on fighting behavior rather than to any oncogenic potential of the compound. Dr. Daphne Lin (HFD-725) who had reviewed this study under the IND pointed out a series of short comings which shed doubt on the sponsor's conclusions. Dr. Karl Lin (HFD-715) had reviewed the sponsor's mathematical model relating the incrustations from biting to the onset of fibrosarcomas in control animals, also under the IND. He concluded that the sponsor's approach could not rule out at least a partial drug effect. This reviewer agrees with their conclusions.

The fourth mouse study (190-830) was conducted in CD mice. It was a gavage study with doses of eszopiclone similar to the early study (0, 0, 25, 50, 100 mg/kg/day). In particular, the high dose was again 100 mg/kg/day. The animals were housed one to a cage. The sponsor terminated the high dose males early, but concluded no effect on survival since at least 50 percent of all animals were alive at week 84. The sponsor found no statistically significant increase in any tumor. This reviewer agreed with the sponsor with respect to the male mice, but an increase in leiomyosarcoma of the uterus among the

female mice may be considered borderline statistically significant. In this reviewer's opinion, the male study had sufficient numbers of animals alive for a sufficient length of time, but based on statistical criteria of body weights and mortality, the high dose apparently did not reach the MTD. If the increase in leiomyosarcoma among the females is discounted, the same comments regarding the validity can be made for the female mice, in particular that the high dose may not have reached the MTD.

## 1.2 Overview of Studies Reviewed

This review addresses seven bioassays and thirteen study reports. Table 1 below details the type of bioassay, the compound used, and the relevant study reports. Five of studies were conducted with (R,S)-zopiclone (27 267 RP) administered in the diet. The most recent two studies were gavage studies with eszopiclone. The additional study reports refer to special topics or evaluations by experts convened by the sponsor. Study 190-844 is not a carcinogenicity study but an investigation to attribute the increase in sarcomas of the skin/subcutis to group caging. OB staff had previously reviewed this study and its mathematical model under the IND ( — ) These previous evaluations are briefly discussed as well.

**Table 1: Overview of Studies Reviewed**

DATE OF SUBMISSION	STUDY REPORT NUMBER	DATASET	STUDY DESCRIPTION	COMPOUND AND DOSES
Jan. 30, 2003 March 12, 2003	190-833.pdf	Tumor.xpt	2-year dietary study in Sprague-Dawley rats, 5 per cage	(R,S)-zopiclone doses: 0,0,1,10,100
Jan. 30, 2003 March 12, 2003	190-839.pdf 190-877.pdf 190-860.pdf 190-874A1.pdf*	Tumora.xpt	PWG re-evaluation of thyroid and mammary tumors observed in 190-833; — re-grading of mammary tumors;	(R,S)-zopiclone doses: 0,0,1,10,100
Aug.28, 2003 Oct. 14, 2003	190-823f.pdf	190823FT.xpt 190823MT.xpt	2-year gavage study in Sprague-Dawley rats, 1 per cage	Eszopiclone doses: 0,0,2,4,8,16
Jan. 30, 2003 March 12, 2003	190-834.pdf	Tumor.xpt	2-year dietary study in B6C3F1 mice, 4 per cage	(R,S)-zopiclone doses: 0,0,1,10,100
Jan. 30, 2003 March 12, 2003	190-834A.pdf 190-873.pdf 190-873A1.pdf	Tumora.xpt	PWG re-evaluation of skin and pulmonary tumors observed in 190-834	(R,S)-zopiclone doses: 0,0,1,10,100
Jan. 30, 2003 (IND — )	190-844.pdf	Not submitted	Special study to evaluate subcutis sarcomas and group housing in male B6C3F1 mice	(R,S)-zopiclone doses: 0,1,100 4 per cage vs. 1 per cage comparisons
Aug.28, 2003 Oct. 14, 2003	190-830f.pdf	190830FT.xpt 190830MT.xpt	2-year gavage study in CD-1 mice, 1 per cage	Eszopiclone doses: 0,0,25,50,100
April 4, 1988 (IND — )	ST/CRV/TOX Nr. 198	N/A	Mathematical model to attribute increase in skin sarcomas to group-caging	N/A

\*The cover page of this report lists both 190-874 and 190-874A1. There appears to be only one statistical report.

### 1.3 Principal Findings

The main statistical findings are tabulated below. In the individual review of each study, details as to pooling of tumors or tissues will be discussed. Table 2 serves only as a guide to statistically significant findings in tumor incidences.

**Table 2: Overview of Statistically Significant Findings from Carcinogenicity Studies**

SPECIES	STRAIN	SEX	STUDY	TISSUE	TUMOR	ORIGINAL	PWG	REVIEWER
Rat	Sprague Dawley	F	190-833	Mammary Gland	m-carcinoma	Trend and Pair-wise	N/A	Trend and Pair-wise
Rat	Sprague Dawley	M	190-833	L-Thyroid R-Thyroid C-Thyroid	m-follicular cell carcinoma	Trend and Pair-wise	N/A	Trend and Pair-wise
Rat	Sprague Dawley	F	190-833A	Mammary Gland	m-adenocarcinoma-m	N/A	Trend and Pair-wise	Trend and Pair-wise
Rat	Sprague Dawley	M	190-833A	L-Thyroid R-Thyroid C-Thyroid	Combinations of follicular cell adenoma and carcinoma	N/A	Trend and Pair-wise	Trend and Pair-wise
Rat	Sprague Dawley	F	190-823	None	None	None	N/A	None
Rat	Sprague Dawley	M	190-823	None	None	None	N/A	None
Mouse	B6F3C1	F	190-834	Lung// Right Ovary	Pulmonary Carcinoma// cystadenoma	Trend and Pair-wise for combinations // Trend	N/A	Trend and Pair-wise for combinations //Trend
Mouse	B6F3C1	M	190-834	Skin	Sarcoma	Trend	N/A	Trend
Mouse	B6F3C1	F	190-834A	Lung// Right Ovary	Pulmonary Adenoma or Carcinoma// Cyst-adenoma	N/A	Pair-wise for combined/ / Trend	Trend and Pair-wise for combined// Trend
Mouse	B6F3C1	M	190-834A	Skin	Sarcoma	N/A	Trend	Trend
Mouse	CD	F	190-830	Uterus	Leiomyosarcoma	None	N/A	Trend and Pair-wise*
Mouse	CD	M	190-830	None	None	None	N/A	None

\*Significance depends on test used

## 2 Introduction

### 2.1 Overview

This review addresses only findings that are statistical in nature. The mechanisms of action or the relevance of the findings to humans fall outside the range of statistical considerations and are not discussed by this reviewer except for Study 190-844 which specifically deals with the increase in skin sarcomas and their relation to group housing of the animals.

### 2.1.1 Background

The early studies were conducted by — The sponsor as well as the pathology-working group (PWG) which was convened to re-evaluated significant tumor findings in the early studies did not apply proper statistics to the data. — performed the early statistical analyses. When — acquired — additional data checking and analyses were performed, apparently according to the FDA draft guidance<sup>1</sup>. This reviewer will rely generally on the statistical findings and conclusions as presented in the reports by — It appears that the original statistical reports (190-873 and 190-874) have never been submitted. However, the final position seems to be reported in the appended versions of these reports, namely, 190873A1 and 190-874A1. — analyzed the most recent rat and mouse studies.

### 2.1.2 Major Statistical Issues

There are numerous statistical issues in the studies; however, not all are relevant to all studies. This reviewer will attempt to discuss all of them here, and note only special considerations with the individual studies.

To compensate for the multiplicity of testing, it is the practice of the Office of Biometrics (OB) to consider trends in tumor incidences as statistically significant when the p-values reach 0.025 for rare tumors and 0.005 for common tumors. A tumor finding is considered rare if it occurs in 1 percent or less among the concurrent controls and common otherwise. Pair-wise comparisons are considered statistically significant with p-values of 0.05 for rare tumors and 0.01 for common tumors. The sponsor appears to have used these methods in their final evaluation with the exception that their definition of rare tumors did not include 1 percent.

In this submission, there are more than twice the usual number of carcinogenicity studies being reviewed. However, no further adjustment for multiplicity of testing was undertaken by this reviewer or by the sponsor.

The sponsor, the laboratory and the pathology working group applied either no or only cursory statistical methods. When referring to the 'sponsor's analysis' this reviewer implies the results obtain by — for the early studies and by — for the later studies. This reviewer could not clarify what the sponsor meant by log dose scores of 0, 1, 2, and 3 for the trend tests, as  $\log(0)$  is minus infinity.

The sponsor noted that the consultants and the PWG used slightly different nomenclature when re-evaluating the tissues of concern. Therefore, results based on combined tumors may be of greater validity than those based on individual tumors. Of note is, that there were originally three mammary tumors among the female rats of the original study. Upon PWG re-evaluation, eleven different tumors were recorded. If the differentiation of

---

<sup>1</sup> Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceutical, January 1999.

tumors is done very finely, none may have sufficient power to show an increase as statistically significant, but a proper combination of tumors may.

None of the studies had the full battery of tissues examined for all animals. The terminally sacrificed mid doses usually had only target tissues and macroscopic abnormalities microscopically examined. Therefore, often the pair-wise comparison between controls and high dose animals is the most appropriate test. However, pair-wise comparisons have lower power than trend tests, and statistically significant findings may therefore not be detected. This reviewer opted to run trend tests on all tumor findings. As the tissues of all animals dying on study were microscopically examined, the trend test can be considered a rough approximation to the (unknown) results had all animals been fully examined. It needs to be pointed out, that it is possible to lose statistical significance when the microscopic examination of all data results in a very non-linear increase in tumor findings. In one such case (pulmonary adenomas and carcinomas among female mice re-evaluated by the PWG), the sponsor's trend test did not reach statistical significance but the pair-wise comparison did. Keeping this in mind, this reviewer used the trend test results as a signal only and followed up by a pair-wise comparison, where warranted.

Survival decreased in the later part of several of the studies. The sponsor terminated a treatment arm when the number of remaining animals approached 20. In one study each of the five treatment arms had a different termination date. This resulted in a problem with the statistical analysis as well as with the software used by OB statisticians. On one hand, allowing each treatment arm to terminate at a different time would result in different lengths of the time interval before the terminal sacrifice and affect the number of animals at risk during this time interval. Fatal tumors are not affected. On the other hand, if setting the terminal sacrifice to the earliest time any treatment arm has been terminated (within one species and gender) some treatment arms will have an artificially long terminal sacrifice period. Any modification to lengths of time intervals or the start of terminal sacrifice will affect the p-values of the trend test or pair-wise comparison. In general, the effects should be minor. However, for p-values close to the cut off for statistical significance, one cannot predict the direction the effect of modifying time intervals or start of terminal sacrifice may take.

Palpable tumors are often analyzed as incidental tumors observed at the time of the animal's death, which is not precise. As mammary carcinoma among female rats and sarcoma of the skin among male mice are findings of concern, this reviewer analyzed these tumors also using the time of tumor detection. It is noted that not all mortality independent tumors had time of detection recorded, which resulted in different incidence rates than those observed under the incidental/fatal context. It appears that the sponsor usually performed the onset rate method, except in the 'group-housing' study, where the skin tumors were treated as incidental or fatal.

With few exceptions, the sponsor's and this reviewer's numbers of tumor-bearing animals were identical. However, the p-values were usually not. The differences may be due to somewhat different scaling of the trend test, different time intervals, slightly different statistical methods, etc. Many times the sponsor and this reviewer arrive at the same

conclusions. The cases where this reviewer found statistical significance but the sponsor did not are discussed in detail.

In the original mouse and rat studies there were large numbers of autolyzed/unusable tissues. Some 76-90 percent of the records was coded as unusable. Most of these records were from terminally sacrificed mid dose animals. Generally, a study's validity would be in doubt when so many tissues are lost to examination. However, these studies showed statistically significant increases in some tumors. Therefore, one needs to consider that even more significant findings could have appeared, had all tissues been available, i.e. the observed findings may be an under-representation of the compound's oncogenic potential.

This reviewer used the OB standard software for all studies, which was developed based on Peto et al., (IARC, 1980)<sup>2</sup> paper. When the software tolerated it, actual dose levels were used as scores for the trend tests, otherwise 0, 0, 1, 2, 3 was applied. The terminal sacrifice for all animals was the earliest time of terminal sacrifice of any treatment arm within gender. This reviewer performed all tumor analysis against the combined control groups. On occasion the sponsor observed a significant difference in mortality among the two control groups and performed analyses against each of the controls as well as the combined groups. This reviewer relied on the sponsor not finding an explanation why the control groups differed and therefore presumed the difference to be due to random fluctuation. For tumor analysis the p-values from both the exact permutation trend test and the normal approximation are given, regardless of the number of tumor-bearing animals. Usually, the exact permutation trend test is more appropriate, except in cases where a tumor occurs both in the fatal and incidental context. When a fatal tumor occurs in a time interval an incidental tumor (of the same kind) occurs, the exact test may not be accurate. If the number of tumors is not small, the asymptotic results may be more appropriate. Otherwise, the true p-value lies somewhere between the ones given by the exact and the asymptotic test.

## **2.2 Data Analyzed and Sources**

The sponsor submitted the data of six of the seven studies (including the appended versions) in electronic format. The studies conducted with (R,S)-zopiclone (27 267 RP) were originally submitted January 30, 2003. Some errors were discovered in these data sets and they were re-submitted on March 12, 2003. This reviewer analyzes these latter data sets. Both the original and the appended (i.e. PWG) mouse and rat data sets are called tumor.xpt and tumora.xpt, and are found in \\Cdsesub1\21476\N\_000\2003-03-12\pharmtox\datasets\190-834 and \\Cdsesub1\21476\N\_000\2003-03-12\pharmtox\datasets\190-833, respectively. These studies were previously submitted under IND 19,258 and reviewed by Daphne Lin, Ph.D., now in HFD-725 (Feb. 15, 1991). However not all tissues information was available at that time. Therefore, this reviewer performed a full statistical review of these studies as submitted to the NDA.

---

<sup>2</sup> Peto, et al (1980): "Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-term Animals Experiments", in Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal, WHO

A special study was conducted to investigate an association of increased incidence of subcutis sarcomas and the number of male mice per cage. This study was also previously submitted under IND [redacted] and reviewed by Dr. Daphne Lin (Feb. 15, 1991). No electronic data set has been submitted for this study. This reviewer will summarize Dr. Daphne Lin's findings.

The newer studies conducted with eszopiclone were first submitted on August 28, 2003 under 190-823f (rats) and 190-830f (mice) in \\Cdsub1\21476\N 000\2003-08-28\pharmtox\datasets. Due to difficulties in analyzing the data, the sponsor re-submitted newer versions of these data sets which followed the Guidance for electronic submission<sup>3</sup> more closely. The new data sets arrived via email on October 14, 2003 under 190-823 (rats) and 190-830 (mice). These latter data sets are reviewed here.

### 3 Statistical Evaluation

#### 3.1 Rat Carcinogenicity Study (Sepr. Doc. 190-833 and 190-874A1)

##### 3.1.1 Sponsor's Results and Conclusions

Sepr. Doc. 190-833 and 190-874A1 are relevant. The sponsor concluded that (R,S)-zopiclone (27 267 RP), when administered to Sprague-Dawley CD rats in the diet at doses up to 100 mg/kg/day for two years, had no effect on mortality. At the high dose group, the treatment caused oncogenic change in the (left, right, and combined) thyroid glands of the males (malignant follicular cell carcinoma, trend and pair-wise tests) and in the mammary glands of the females (malignant carcinoma, trend and pair-wise tests), as well as a number of toxic changes. According to the sponsor, subsequent studies demonstrated that neither lesion is considered clinically relevant.

##### 3.1.2 Statistical Methodologies

The sponsor assessed inter-group differences in mortality by the Cox's test, which is one of the methods used in the OB software. Pathological change was assessed using Fisher's Exact tests (two-tailed). For the thyroid neoplasms and the mammary carcinomas Peto's statistical tests were also applied. The sponsor and this reviewer have the same incidence numbers for thyroid carcinoma among the male rats and for mammary adenomas and carcinomas of the female rats.

For the tissues with statistically significant increases, all animals had been examined and the trend test is appropriate. For tissues where the terminally sacrificed low dose animals

<sup>3</sup> Guidance for Industry: Providing Regulatory Submission in Electronic Format - General Considerations (Jan 1999); Guidance for Industry: Providing Regulatory Submissions in Electronic Format - NDAs (Jan 1999).

were not examined, the trend test (reported by this reviewer) can be seen as an approximation. For the observed tumor incidence patterns, however, none appear that they would reach statistical significance had there been additional tumors among the low dose animals sacrificed at the end. The sponsor's pair-wise tests did not result in additional significant findings between the high dose and controls, other than those already observed by the trend test.

### 3.1.3 Detailed Review of Rat Carcinogenicity Study Nr. 190-833

This was a 105-week bioassay in Sprague-Dawley CD rats. Groups of 50 rats of either gender received (R,S)-zopiclone (27 267 RP) at doses of 0, 0, 1, 10, 100 mg/kg/day in the diet. Rats were housed five of one gender to a cage. The study was conducted by — , between April 1981 and May 1983. Microscopic examination of adrenal, thyroid, liver, macroscopically identifiable lesions, and skin and mammary glands were conducted for all rats. A battery of tissues was examined microscopically for all animals dying on study and for terminally sacrificed animals of the controls, the medium and high dose groups, i.e. not for the low dose group.

### 3.1.4 Statistical Reviewer's Findings of Rat Study Nr. 190-833

Tables 3 and 4 and Figure 1 demonstrate that there was no effect of treatment with (R,S)-zopiclone on the mortality experience of the female rats. As reported by the sponsor, there was a statistically significant increase in malignant mammary gland carcinomas ( $p=0.0019$ ,  $\alpha=0.005$ ) in the females (Table 5). This result was obtained when treating the tumors as incidental and using time of death rather than time of tumor detection. When analyzing the mammary tumors properly using time to detection of the tumor, essentially the same  $p$ -value was observed ( $p=0.0020$ ) (Table 6). The pair-wise comparison between high dose and controls for malignant carcinoma was also statistically significant ( $p=0.0027$ ,  $\alpha=0.01$ ). Other pair-wise comparisons, not reproduced here, did not show any statistical increase in any tumor. Combining left and right tissues of certain organs (not reproduced here) did not result in any statistically significant findings.

Tables 7 and 8 and Figure 2 demonstrate that there was no effect of treatment with (R,S)-zopiclone (27 267 RP) on the mortality experience of the male rats. Statistically significant increases in malignant follicular cell carcinoma of the thyroid were observed (Table 9). Several tissues were examined as right and left halves. This reviewer combined the right and left parts for a given tissue and a given tumor, but none reached statistical significance other than malignant follicular cell carcinoma for the combined left and right thyroid tissues (Table 10). Pair-wise comparisons for follicular cell carcinoma in the thyroid also reached statistical significance. These findings are consistent with the sponsor.

**Table 3: Mortality by Time Interval for Female Rats, Study 190-833**

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR1	53-78	50	4	46	92.0	8.0
	79-91	46	8	38	76.0	24.0
	92-104	38	13	25	50.0	50.0
	FINALKILL105-108	25	25	0		
CTR2	0-52	50	1	49	98.0	2.0
	53-78	49	6	43	86.0	14.0
	79-91	43	9	34	68.0	32.0
	92-104	34	10	24	48.0	52.0
	FINALKILL105-108	24	24	0		
LOW	0-52	50	1	49	98.0	2.0
	53-78	49	4	45	90.0	10.0
	79-91	45	9	36	72.0	28.0
	92-104	36	15	21	42.0	58.0
	FINALKILL105-108	21	21	0		
MED	0-52	50	3	47	94.0	6.0
	53-78	47	6	41	82.0	18.0
	79-91	41	8	33	66.0	34.0
	92-104	33	15	18	36.0	64.0
	FINALKILL105-108	18	18	0		
HIGH	0-52	50	4	46	92.0	8.0
	53-78	46	5	41	82.0	18.0
	79-91	41	6	35	70.0	30.0
	92-104	35	12	23	46.0	54.0
	FINALKILL105-108	23	23	0		

**Table 4: Trend in Mortality for Female Rats, Study 190-833**

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	2.4673	0.4812	1.8694	0.5999
Dose-Mortality Trend	1.3043	0.2534	1.6248	0.2024
Homogeneity	3.7717	0.4378	3.4942	0.4788

Figure 1: Kaplan Meier Survival Curves for Female Rats, Study 190-833

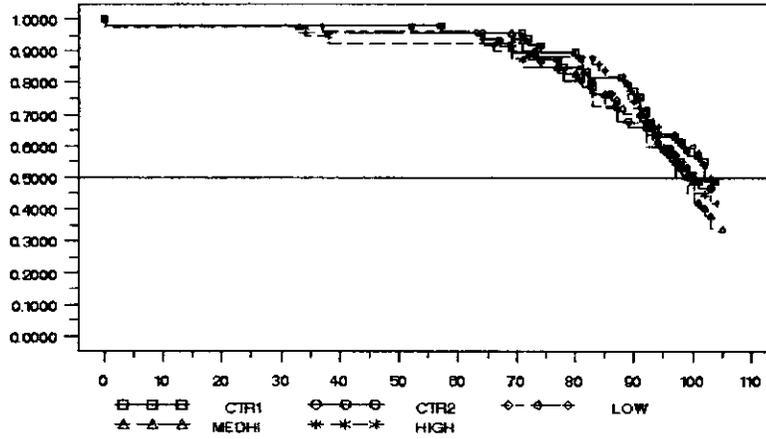


Table 5: Tumor Trend Tests for Female Rats, Study 190-833

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	MEDH	HIGH	P-value (Fisher's method)	P-value (Asymptotic method)
1	BONE	38	M-FIBROSARCOMA	0	0	0	1	0.2444	0.1493
14	H-POIETIC TISSUE	44	M-HISTIOCYTIC SARCOMA	2	1	0	0	1.0000	0.9737
14	H-POIETIC TISSUE	47	M-MALIGNANT LYMPHOMA	0	0	1	1	0.4419	0.4373
19	LEFT ADRENAL	36	M-CORTICAL CARCINOMA	0	0	1	1	0.4742	0.4726
22	LEFT OVARY	10	B-GRANULOSA-THECA CELL TUMOUR	0	1	0	0	1.0000	0.8978
22	LEFT OVARY	6	B-FALLOPIAN TUBE ADENOMA	0	1	0	0	1.0000	0.8978
25	LEFT THYROID	23	B-PARAFOLLICULAR CELL ADENOMA	2	5	0	3	0.9514	0.9467
25	LEFT THYROID	39	M-FOLLICULAR CELL CARCINOMA	1	0	0	1	0.4512	0.4548
25	LEFT THYROID	9	B-FOLLICULAR CELL ADENOMA	2	1	0	1	0.8170	0.8221
26	LIVER	43	M-HEPATOCELLULAR CARCINOMA	1	0	0	1	0.4458	0.4376
29	MAMMARY CRANIAL	7	B-FIBROEPITHELIAL TUMOUR	0	0	1	0	0.5750	0.6987
32	MAMMARY GLANDS	18	B-LIPOMA	1	0	0	0	1.0000	0.9231
32	MAMMARY GLANDS	34	M-CARCINOMA	4	9	6	18	0.0019	0.0017
32	MAMMARY GLANDS	7	B-FIBROEPITHELIAL TUMOUR	37	33	37	32	0.9600	0.9606
37	PANCREAS	15	B-ISLET CELL ADENOMA	2	1	0	2	0.3349	0.3384

37	PANCREAS	5	B-EXOCRINE CELL ADENOMA	0	0	0	1	0	0.4556	0.4002
38	PITUITARY	1	B-ADENOMA	32	28	27	27	19	0.9551	0.9545
38	PITUITARY	34	M-CARCINOMA	2	1	2	4	0	0.5825	0.5933
42	RIGHT KIDNEY	19	B-LIPOMA-RENAL-	0	0	1	0	0	0.4896	0.6963
43	RIGHT OVARY	10	B-GRANULOSA-THECA CELL TUMOUR	0	1	0	0	0	1.0000	0.8968
46	RIGHT THYROID	23	B-PARAFOLLICULAR CELL ADENOMA	1	0	5	3	2	0.1683	0.1665
46	RIGHT THYROID	39	M-FOLLICULAR CELL CARCINOMA	0	0	1	1	0	0.4731	0.4717
46	RIGHT THYROID	9	B-FOLLICULAR CELL ADENOMA	1	0	0	0	0	1.0000	0.9399
5	BRAIN-MID-BRAIN	49	M-MENINGIOMA-EPITHELIOID-	0	1	0	1	1	0.3553	0.3253
51	SKIN	18	B-LIPOMA	2	3	2	3	0	0.8693	0.8702
51	SKIN	28	B-SQUAMOUS CELL PAPILOMA	0	0	1	1	0	0.4385	0.4325
51	SKIN	33	M-BASAL CELL TUMOUR	0	0	1	0	0	0.5750	0.6987
51	SKIN	56	M-SARCOMA	1	1	1	0	1	0.8478	0.8545
51	SKIN	8	B-FIBROMA	2	7	9	2	2	0.9410	0.9390
57	THYMUS	56	M-SARCOMA	1	0	0	0	0	1.0000	0.8984
57	THYMUS	57	M-SQUAMMOUS CEL CARCINOMA OF T	0	0	0	0	1	0.1639	0.1170
59	URINARY BLADDER	62	M-TRANSITIONAL CELL CARCINOMA	0	1	0	0	0	1.0000	0.9096
61	UTERUS	37	M-ENDOMETRIAL CARCINOMA	0	0	1	0	1	0.2078	0.2594

**Table 6: Pair-wise and Trend Tests for Mammary Tumors Based on Time to Detection, Female Rats, Study 190-833**

Organ Code	Organ Name	Tumor Code	Tumor Name	C1	C2	L	M	H	p-value from Exact Test	p-value from Asymptotic Test
32	MAMMARY GLANDS	34	M-CARCINOMA	4	9			18	0.0027	0.0013
32	MAMMARY GLANDS	34	M-CARCINOMA	4	9	6	8	18	0.0020	0.0017

**Table 7: Mortality by Time Interval for Male Rats, Study 190-833**

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR1	0-52	50	4	46	92.0	8.0
	53-78	46	7	39	78.0	22.0
	79-91	39	8	31	62.0	38.0
	92-104	31	15	16	32.0	68.0
	FINALKILL105-108	16	16	0		
CTR2	0-52	50	1	49	98.0	2.0
	53-78	49	5	44	88.0	12.0
	79-91	44	12	32	64.0	36.0
	92-104	32	14	18	36.0	64.0
	FINALKILL105-108	18	18	0		
LOW	0-52	50	1	49	98.0	2.0
	53-78	49	8	41	82.0	18.0
	79-91	41	9	32	64.0	36.0
	92-104	32	9	23	46.0	54.0
	FINALKILL105-108	23	23	0		
MED	0-52	50	6	44	88.0	12.0
	53-78	44	4	40	80.0	20.0
	79-91	40	15	25	50.0	50.0
	92-104	25	8	17	34.0	66.0
	FINALKILL105-108	17	17	0		
HIGH	0-52	50	2	48	96.0	4.0
	53-78	48	14	34	68.0	32.0
	79-91	34	5	29	58.0	42.0
	92-104	29	11	18	36.0	64.0
	FINALKILL105-108	18	18	0		

**Table 8: Trend in Mortality for Male Rats, Study 190-833**

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	2.2255	0.5269	1.8790	0.5979
Depart from Trend				
Dose-Mortality Trend	0.2004	0.6544	0.8817	0.3477
Homogeneity	2.4259	0.6580	2.7607	0.5986

Figure 2: Kaplan-Meier Survival Curves for Male Rats, Study 190-833

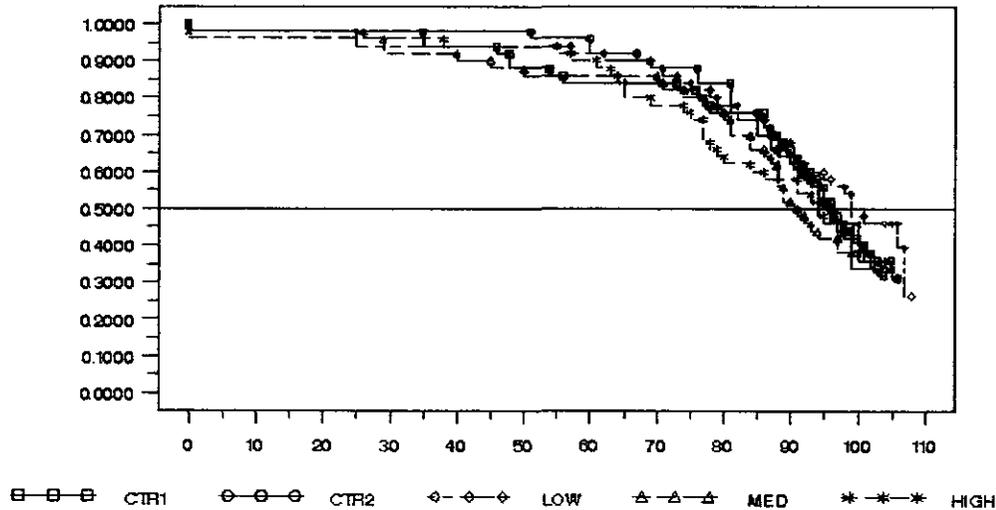


Table 9: Tumor Trend Tests for Male Rats, Study 190-833

Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	HIGH	Chi-Square	P-Value
1	BONE	50	M-OSTEOGENIC SARCOMA	0	1	0	0	0	1.0000	0.8948
14	H-POIETIC TISSUE	40	M-GRANULOCYTIC LEUKAEMIA	0	0	0	1	0	0.3627	0.3613
14	H-POIETIC TISSUE	47	M-MALIGNANT LYMPHOMA	3	2	1	2	0	0.9283	0.9248
16	JEJUNUM	45	M-INTESTINAL ADENOCARCINOMA	0	0	0	1	0	0.4091	0.3776
17	L.N. CERVICAL	12	B-HAEMANGIOMA	0	1	0	0	0	1.0000	0.8985
18	L.N. MESENTERIC	12	B-HAEMANGIOMA	0	2	0	1	1	0.4903	0.4981
19	LEFT ADRENAL	21	B-MEDULLARY ADENOMA	1	4	4	2	3	0.4356	0.4419
19	LEFT ADRENAL	3	B-CORTICAL ADENOMA	0	0	0	0	1	0.2093	0.1382
19	LEFT ADRENAL	36	M-CORTICAL CARCINOMA	1	0	0	0	0	1.0000	0.9386
19	LEFT ADRENAL	48	M-MEDULLARY CARCINOMA	0	1	0	0	1	0.4869	0.4801
21	LEFT KIDNEY	26	B-RENAL ADENOMA	0	0	0	1	0	0.4430	0.4161
23	LEFT PARATHYROID	1	B-ADENOMA	1	0	0	0	1	0.4853	0.4811
24	LEFT TESTIS	14	B-INTERSTITIAL CELL ADENOMA	3	3	3	2	2	0.7732	0.7770
24	LEFT TESTIS	56	M-SARCOMA	0	0	0	0	1	0.2500	0.1671
25	LEFT THYROID	23	B-PARAFOLLICULAR CELL ADENOMA	0	3	2	2	1	0.6001	0.6105

25	LEFT THYROID	39	M-FOLLICULAR CELL CARCINOMA	0	0	1	0	5	0.0013	0.0012
25	LEFT THYROID	52	M-PARAFOLLICULAR CELL CARCINOM	1	0	1	0	0	0.8120	0.8523
25	LEFT THYROID	9	B-FOLLICULAR CELL ADENOMA	0	1	3	0	1	0.8524	0.6611
26	LIVER	13	B-HEPATOCELLULAR ADENOMA	0	0	1	1	2	0.0373	0.0328
26	LIVER	43	M-HEPATOCELLULAR CARCINOMA	0	0	0	0	1	0.1957	0.1278
28	LUNGS/ALL LOBES	2	B-ALVEOLAR ADENOMA	0	1	0	0	0	1.0000	0.9259
32	MAMMARY GLANDS	7	B-FIBROEPITHELIAL TUMOUR	0	1	3	1	0	0.7119	0.7138
37	PANCREAS	15	B-ISLET CELL ADENOMA	5	3	0	5	2	0.7457	0.7493
37	PANCREAS	46	M-ISLET CELL CARCINOMA	0	0	0	1	0	0.4861	0.4268
37	PANCREAS	5	B-EXOCRINE CELL ADENOMA	1	2	0	1	0	0.8981	0.8940
38	PITUITARY	1	B-ADENOMA	21	21	18	14	10	0.9982	0.9979
4	BRAIN-CEREBRUM	32	M-ASTROCYTOMA	1	3	0	0	2	0.6916	0.7042
40	RIGHT ADRENAL	21	B-MEDULLARY ADENOMA	1	1	3	1	0	0.8171	0.8230
40	RIGHT ADRENAL	3	B-CORTICAL ADENOMA	1	0	0	0	1	0.4930	0.4860
40	RIGHT ADRENAL	36	M-CORTICAL CARCINOMA	0	0	1	0	0	0.5573	0.7012
40	RIGHT ADRENAL	48	M-MEDULLARY CARCINOMA	1	0	0	0	0	1.0000	0.9284
42	RIGHT KIDNEY	55	M-RENAL CARCINOMA	0	0	1	0	0	0.4912	0.6679
42	RIGHT KIDNEY	62	M-TRANSITIONAL CELL CARCINOMA	0	0	0	0	1	0.1958	0.1297
44	RIGHT PARATHYROID	1	B-ADENOMA	2	1	0	0	1	0.7735	0.7787
45	RIGHT TESTIS	14	B-INTERSTITIAL CELL ADENOMA	1	0	1	5	2	0.0767	0.0734
46	RIGHT THYROID	23	B-PARAFOLLICULAR CELL ADENOMA	1	1	1	0	0	0.9470	0.9436
46	RIGHT THYROID	39	M-FOLLICULAR CELL CARCINOMA	0	0	0	0	3	0.0067	0.0046
46	RIGHT THYROID	52	M-PARAFOLLICULAR CELL CARCINOM	0	1	0	0	0	1.0000	0.9332
46	RIGHT THYROID	9	B-FOLLICULAR CELL ADENOMA	2	1	2	0	3	0.3049	0.3094
51	SKIN	16	B-KERATOACANTHOMA	2	1	7	3	2	0.4322	0.4374
51	SKIN	17	B-LEIOMYOMA	1	0	0	0	1	0.4460	0.4531
51	SKIN	18	B-LIPOMA	6	6	6	1	3	0.9671	0.9645
51	SKIN	28	B-SQUAMOUS CELL PAPILLOMA	1	1	2	3	4	0.0454	0.0425
51	SKIN	33	M-BASAL CELL TUMOUR	2	1	1	1	2	0.3893	0.3979
51	SKIN	38	M-FIBROSARCOMA	1	0	0	0	0	1.0000	0.8985
51	SKIN	56	M-SARCOMA	2	0	3	2	2	0.3513	0.3536
51	SKIN	58	M-SQUAMO-SEBACEOUS CARCINOMA	0	1	0	0	0	1.0000	0.9444
51	SKIN	59	M-SQUAMOUS CELL CARCINOMA	0	2	0	0	0	1.0000	0.9501
51	SKIN	8	B-FIBROMA	7	9	8	6	9	0.4415	0.4441

**Table 10: Pair-wise and Trend Tests for Malignant Follicular Cell Carcinoma of the Thyroid, Male Rats, Study 190-833**

Organ Code	Organ Name	Tumor Code	Tumor Name	C 1	C 2	L	M	H	p-value from Exact Test	p-value from Asymptotic Test
25	LEFT THYROID	39	M-FOLLICULAR CELL CARCINOMA	0	0			5	0.0012	0.0002
46	RIGHT THYROID	39	M-FOLLICULAR CELL CARCINOMA	0	0			3	0.0244	0.0067
46	COMBINED THYROIDS	39	M-FOLLICULAR CELL CARCINOMA	0	0			6	0.0002	0.0000
25	LEFT THYROID	39	M-FOLLICULAR CELL CARCINOMA	0	0	1	0	5	0.0013	0.0012
46	RIGHT THYROID	39	M-FOLLICULAR CELL CARCINOMA	0	0	0	0	3	0.0067	0.0046
46	COMBINED THYROIDS	39	M-FOLLICULAR CELL CARCINOMA	0	0	1	0	6	0.0003	0.0003

### 3.2 Rat Carcinogenicity Study 190-833 Appended (Sepr. Docs. 190-839, 190-877, 190-860, 190-874A1)

#### 3.2.1 Sponsor's Results and Conclusions

Sepr. Doc. 190-867 contains evaluations by consultants of the original findings, their potential mechanisms of action, and their relevance to humans, and is not statistical in nature and therefore not discussed here.

In Sepr. Doc. 190-877 the sponsor reports the peer/PWG reviewed proliferative lesions found in the mammary and pituitary glands of the female rats of the (R,S)-zopiclone study 190-833. In Sepr. Doc. 190-839 the sponsor reports the peer/PWG reviewed proliferative lesions found in the thyroid of the male rats of the (R,S)-zopiclone study 190-833. In the original study there were three types of tumors found in the mammary glands. The PWG determined nine different tumor types in their reassessment of the original tissues (Table 11). For the mammary gland and cranial combined all adenocarcinomas resulted in a significant trend and pair-wise comparison. Malignant adenocarcinoma-multiple was also statistically significant for trend and pair-wise comparison between high dose and controls (Table 12). The PWG concluded that there was no increase in the combined (benign and malignant) mammary tumor rates in female rats treated with 1, 10, and 100 mg/kg/day of (R,S)-zopiclone when compared to untreated female control rats. There was an apparent shift from benign to malignant for mammary tumors in the high dose females. They considered this a gender-specific effect (i.e. related to early onset of reproductive senescence) not on tumor induction but rather on tumor distribution. The PWG did not perform any statistical analyses but described the

percentage of each group with respect to the controls. The quoted statistical results were done by on the PWG reviewed findings and are reported in Sepr. Doc.190-874A1.

Table 11: Sponsor's Incidence of Mammary Tumors by PWG from Sepr. Doc. 190-877

PROJECT NO. 492-001

TABLE 2: 105-Week Dietary Oncogenicity Study in CD Rats with Zopiclone

Comparison of the Incidence of Mammary Gland Neoplasms Reported by the Study Pathologist (SP) and Diagnosed by the Pathology Working Group (PWG)

MAMMARY GLAND (NO. EXAMINED)	Group 1		Group 2		Group 3		Group 4		Group 5	
	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
	SP	PWG								
Adenocarcinoma	2	2	5	9	4	6	3	7	12	9
Adenocarcinoma, Multiple	2	2	1	1	2	2	3	5	5	8
Adenocarcinoma Arising in a Fibroadenoma	0	0	0	1	0	1	0	3	0	1
Fibroadenoma	13	13	14	12	14	13	14	12	11	10
Fibroadenoma, Multiple	24	24	19	19	23	22	18	18	15	16
Adenoma	0	0	0	0	0	0	0	1	0	0
Adenopoma	0	0	0	0	0	1	0	0	0	0
No. of Animals with Benign Tumors	37	37	33	31	37	35	32	29	26	28
No. of Animals with Malignant Tumors	4	4	9	11	6	9	6	15	18	18
No. of Animals with Both Benign and Malignant Tumors	4	4	6	7	5	5	3	7	7	5
No. of Animals with Mammary Tumors (Benign or Malignant)*	37	37	39	38	38	38	37	36	37	37

\*Some effected had more than one tumor type.

APPEARS THIS WAY  
ON ORIGINAL

Table 12: Sponsor's Statistical Trend Tests for Mammary Tumors in Female Rats from Sepr. Doc. 190-874A1

Text Table B. Statistical Trend Test Results for Target Tumors of Mammary Gland in Females			
	Vs. Group 1 Only	Vs. Group 2 Only	Vs. Groups 1 and 2 Combined
P-Values from Peto Fixed Interval (P) or Exact Permutation Analyses (E)			
<b>Female Mammary Tumors</b>			
Pre-PWG			
Fibroepithelial Tumor	0.935 P	0.892 P	0.868 P
Carcinoma	<0.001 P #	0.010 P	0.001 P #
Combined	0.315 P	0.374 P	0.217 P
Post-PWG			
Adenoma-Single	0.495 E	0.495 E	0.392 E
Fibroadenoma-Multiple	0.935 P	0.757 P	0.881 P
Fibroadenoma-Single	0.654 P	0.658 P	0.599 P
All Fibroadenomas and Adenomas	0.954 P	0.820 P	0.881 P
Adenocarcinoma Arising in Fibroadenoma-Single	0.191 E	0.410 E	0.187 E
Adenocarcinoma-Multiple	0.007 P	0.002 P #	0.001 P #
Adenocarcinoma-Single	0.014 P	0.424 P	0.092 P
All Adenocarcinomas	<0.001 P #	0.031 P	0.001 P #
All Above Tumors Combined	0.376 P	0.328 P	0.226 P

Significant differences are flagged with regard to the appropriate common (#) and rare (\*) cutoffs (common  $p \leq 0.005$  and rare  $p \leq 0.025$ ).

also re-examined the mammary tumor slides of the female rats and reported the findings in Sepr. Doc.190-860. Dr. read the slides in blinded fashion and employed a grading scale to reflect the extent of tumor progression. The results of his review are reproduced below (Table 13). He concluded that there were no compound-related differences among groups in any parameter except in the numbers of animals with grade 4 neoplasms. This comparison with controls did not reach statistical significance. The statistical method was not identified. Also, it was pointed out that the increase in grade 4 tumors in the high dose is considered small and without an increase in other parameters does not provide adequate evidence for carcinogenicity.

**Table 13: Dr. — Re-evaluation of Mammary Tumors in Female Rats of Study 190-833  
Reported in Sepr. Doc. 190-860**

**Mammary tumors in female rats receiving (RS)-zopiclone  
in the diet for 105 weeks  
-Dr — Diagnosis\*-**

Mammary Tumors	Dose (mg/kg/day) of (RS)-Zopiclone				
	0	0	1	10	100
N° of Tumors/Group	37/50	36/50	38/50	37/50	37/50
Tumor Classification					
Grade 2-3	10	18	18	20	12
Grade 4	3	6	1	4	11
Mean N° Tumors/Rat	2.6	2.3	2.5	2.4	2.1
Mean Histological Grade	1.6	2.2	1.7	2.2	2.4

\* Grading scale: 1-adenoma/fibroadenoma with atypia;  
2-well-differentiated carcinoma with slight atypia;  
3-moderately differentiated carcinoma with moderate atypia; and  
4-poorly differentiated carcinoma with severe atypia.

The PWG re-evaluation of the thyroid tissues is presented in Sepr. Doc.190-839. Originally, a total of 12 follicular cell adenomas and 7 follicular cell carcinomas had been observed among the male rats. Upon the PWG evaluation, there were a total of 7 follicular cell adenomas and 3 follicular cell carcinomas (Table 14). The statistical evaluation of the PWG findings considered the trend in benign follicular cell adenoma in the left thyroid and in the combined left and right thyroid statistically significant. The corresponding pair-wise comparison between the high dose and controls was also statistically significant (Table 15). Malignant follicular cell carcinoma was statistically significant by trend and pair-wise comparison for the combined left and right thyroid tissues. Combining follicular cell adenomas and carcinomas for the combined left and right thyroid tissues also reached statistical significance by trend and pair-wise comparisons. The PWG did not attribute the increase in thyroid neoplasms in the male rats to any mechanism recognized to be clinically relevant to humans.

**APPEARS THIS WAY  
ON ORIGINAL**

Table 14: Number of Thyroid Neoplasms before and after PWG Review, Sepr. Doc. 190-839

**Comparative Diagnoses: Incidence of Thyroid Follicular Cell Neoplasms in Sprague Dawley Rats**

	Follicular cell adenoma		Follicular cell carcinoma		Total animals with neoplasms	
	SP	PWG	SP	PWG	SP	PWG
Group 1	2	0	0	0	2	0
Group 2	2	0	0	0	2	0
Group 3	5	3*	1	0	6	3
Group 4	0	0	0	0	0	0
Group 5	3	4	6	3	9	7

SP, diagnosis of original study pathologist;

PWG, consensus diagnosis of pathology working group

\*The thyroid from animal 107 was missing from the archives; this adenoma was diagnosed by the SP and was counted in the PWG total (for comparative purposes only) even though it was not examined by the PWG

Legend: Animal numbers of rats with neoplasms

Gp 1 (SP-Adenoma-31, 50)

Gp 2 (SP-Adenoma-62, 80)

Gp 3 (SP-Carcinoma-111; Adenoma-107, 117, 129, 133, 137)

(PWG-Adenoma-107, 111, 117)

Gp 5 (SP-Carcinoma-212, 225, 227, 238, 240 bilateral, 246 bilateral; Adenoma-205

-bilateral, 214, 247)

(PWG-Carcinoma-212, 240 bilateral, 238; Adenoma-205, 225, 227, 246 bilateral)

Table 15: Sponsor's Statistical Trend Results for Thyroid Tumors in Male Rats before and after PWG Review, Sepr. Doc. 190-874A1

**Text Table A. Statistical Trend Test Results for Target Tumors of Thyroid Gland in Males**

	Vs. Group 1 Only	Vs. Group 2 Only	Vs. Groups 1 and 2 Combined
	P-Values from Peto Fixed Interval (P) or Exact Permutation Analyses (E)		
<b>Male Thyroid Tumors</b>			
<b>Pre-PWG</b>			
Follicular Cell Adenoma	0.633 E	0.629 E	0.481 P
Follicular Cell Carcinoma	0.002 E *	0.001 E *	<0.001 E *
Follicular Cell Tumors Combined	0.034 P	0.031 P	0.009 P
Parafollicular Cell Adenoma	0.628 E	0.931 E	0.768 P
Parafollicular Cell Carcinoma	0.925 E	0.922 E	0.932 E
Parafollicular Cell Tumors Combined	0.808 E	0.957 P	0.903 P
All Tumors Combined	0.136 P	0.268 P	0.121 P
<b>Post-PWG</b>			
Follicular Cell Adenoma	0.085 E	0.080 E	0.024 E *
Follicular Cell Carcinoma	0.015 E *	0.014 E *	0.006 E *
Follicular Cell Tumors Combined	0.005 E *	0.004 E *	0.001 E *
Parafollicular Cell Adenoma	0.628 E	0.931 E	0.786 P
Parafollicular Cell Carcinoma	0.925 E	0.922 E	0.932 E
Parafollicular Cell Tumors Combined	0.808 E	0.957 P	0.903 P
All Above Tumors Combined	0.063 P	0.256 P	0.088 P

Significant differences are flagged with regard to the appropriate common (#) and rare (\*) cutoffs (common  $p \leq 0.005$  and rare  $p \leq 0.025$ ).

### 3.2.2 Statistical Methodologies

The findings of the PWG were reported in Sepr. Doc. 190-839 (male rat thyroid gland findings) and 190-877 (female rat mammary gland and pituitary findings) and analyzed by [redacted]. When [redacted] acquired [redacted], they performed further control checks and data analyses of the tissues and tumors in question. [redacted] used the FDA draft guidance on statistical analysis of carcinogenicity studies.

The statistical methods applied by Dr. [redacted] were not stated. This reviewer does not consider the increase from 9 percent among controls to 22 percent among high dose female rats 'small', as noted by Dr. [redacted]. However, the unadjusted trend test did not reach statistical significance. The more appropriate time to tumor detection analysis could not be performed because these data (evaluation by Dr. [redacted]) were not electronically submitted.

### 3.2.3 Detailed Review of Rat Carcinogenicity Study 190-833 Appended

The PWG worked by consensus and without knowledge of the treatment groups. They concluded that their review generally confirmed the diagnoses initially reported by the original study pathologist. In most reports, the sponsor only discussed the increases in thyroid tumors among the male rats and in mammary tumor findings among the female rats, without formal statistical analysis. In Sepr. Doc. 190-874 and 190-874A1 [redacted] presents the statistical evaluation of thyroid and mammary tumor findings of rat study 190-833 data, as appended by the PWG. (The title page uses both Sepracor document numbers. However, there appears to be only one statistical report by [redacted] for the appended rat study).

The tumor data were provided in tumora.xpt of the March 12, 2003 submission. Only the thyroid tumors among the male rats and the mammary tumors among the female rats are analyzed using the PWG evaluations. The pituitary findings showed no increase in tumors with dose and are not further discussed.

### 3.2.4 Statistical Reviewer's Findings of Study 190-833 Appended

As expected, there were no changes in mortality between the original and the appended rat study data. Table 16 gives this reviewer's results when analyzing the mammary and the pituitary tumors as 'incidental', i.e. using the animals' time of death. There is no increase in pituitary tumors and these are not further discussed. In the original study only three tumor types were identified in mammary glands. The PWG identified nine different tumor types. There is a concern in principle that by differentiating findings too finely,

there may be insufficient numbers for each to show a reliable trend. However, as can be seen from Tables 16 and 17, malignant adenocarcinoma-multiple and combined adenoma and adenocarcinoma reached statistical significance. The significance was observed using both the animals' time of death and the more appropriate time to tumor detection analyses. These findings are consistent with the sponsor's but differ somewhat in the actual p-values. This reviewer did not combine findings for mammary gland and cranial.

A consultant, \_\_\_\_\_, re-graded the original mammary tumor findings (Sepr. Doc. 190-860). This reviewer does not agree with Dr. \_\_\_\_\_ conclusion that an increase in grade 4 carcinomas from 9 percent among the controls to 22 percent among the high dose females is 'small', even if it does not reach statistical significance (Cochran-Armitage trend test,  $p=0.022$  (exact),  $0.019$  (asymptotic),  $\alpha=0.005$ ). An adjusted analysis using time of detection was not possible, as these data were not electronically submitted.

**Table 16: Reviewer's Analysis of Female Rat Mammary and Pituitary Tumors as Re-Evaluated by PWG Using Animal's Time to Death, Study 190-833A**

Organ Code	Organ Name	Tumor Code	Tumor Name	C 1	C 2	L	M	H	p-value from Exact Test	p-value from Asymptotic Test
32	MAMMARY GLANDS	101	M-ADENOCARCINOMA ARISING IN FI	0	1	1	3	1	0.1931	0.1909
32	MAMMARY GLANDS	103	M-ADENOCARCINOMA-MULTIPLE	2	1	2	5	8	0.0004	0.0003
32	MAMMARY GLANDS	104	M-ADENOCARCINOMA-SINGLE	2	9	6	7	9	0.0890	0.0875
32	MAMMARY GLANDS	105	B-ADENOLIPOMA	0	0	1	0	0	0.5534	0.6928
32	MAMMARY GLANDS	106	B-ADENOMA-SINGLE	0	0	0	1	0	0.3421	0.3521
32	MAMMARY GLANDS	107	B-FIBROADENOMA-MULTIPLE	25	19	22	16	17	0.7699	0.7703
32	MAMMARY GLANDS	108	B-FIBROADENOMA SINGLE	12	12	13	12	9	0.6053	0.6075
32	MAMMARY GLANDS	109	B-FIBROMA-SINGLE	0	0	0	0	2	0.0291	0.0191
32	MAMMARY GLANDS	111	B-LIPOMA-SINGLE	1	0	0	0	0	1.0000	0.9227
38	PITUITARY	1	B-ADENOMA	32	29	27	30	19	0.7805	0.7811
38	PITUITARY	34	M-CARCINOMA	2	0	2	1	0	0.7664	0.7792

**APPEARS THIS WAY  
ON ORIGINAL**

**Table 17: Time to Tumor Detection Analysis of PWG Mammary Tumors of Female Rats, Study 190-833A**

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR 1	CTR 2	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
32	MAMMARY GLANDS	101	M-ADENOCARCINOMA ARISING IN FI	0	1	1	3	1	0.1659	0.1626
32	MAMMARY GLANDS	103	M-ADENOCARCINOMA-MULTIPLE	2	1	2	5	8	<b>0.0026</b>	<b>0.0022</b>
32	MAMMARY GLANDS	104	M-ADENOCARCINOMA-SINGLE	2	9	6	7	9	0.1088	0.1071
32	MAMMARY GLANDS	105	B-ADENOLIPOMA	0	0	1	0	0	0.5500	0.6987
32	MAMMARY GLANDS	106	B-ADENOMA-SINGLE	0	0	0	1	0	0.3925	0.3944
32	MAMMARY GLANDS	107	B-FIBROADENOMA-MULTIPLE	25	19	22	16	17	0.7458	0.7475
32	MAMMARY GLANDS	108	B-FIBROADENOMA-SINGLE	12	12	13	12	9	0.5860	0.5900
32	MAMMARY GLANDS	109	B-FIBROMA-SINGLE	0	0	0	0	2	0.0445	0.0289
32	MAMMARY GLANDS	111	B-LIPOMA-SINGLE	1	0	0	0	0	1.0000	0.9161
32	MAMMARY GLANDS	C	COMBINED ADENOMA AND ADENOCARINOMA	4	11	8	16	18	<b>0.0010</b>	<b>0.0008</b>
32	MAMMARY GLANDS	C	COMBINED FIBROMA AND FRIBROADENOMA	37	31	35	28	26	0.7438	0.7451

The PWG also reassessed the thyroid findings in the male rats of the original study. Malignant follicular cell carcinoma, benign follicular cell adenoma, and combined follicular cell adenoma and carcinoma for the combined left and right thyroid tissues all reached statistical significance (Table 18). The incidence rates and the conclusion are consistent with the sponsor, even though the actual p-values are somewhat different.

**Table 18: Reviewer's Trend Tests for Combined Left and Right Thyroid Tissues as PWG Re-evaluated of Male Rats of Study 190-833**

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR 1	CTR 2	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
25	[C]LEFT THYROID	23	B-PARAFOLLICULAR CELL ADENOMA	1	4	3	2	1	0.7984	0.7948
25	[C]LEFT AND RIGHT THYROID	39	M-FOLLICULAR CELL CARCINOMA	0	0	0	0	3	<b>0.0067</b>	<b>0.0002</b>
25	[C]LEFT THYROID	52	M-PARAFOLLICULAR CELL CARCINOMA	1	1	1	0	0	0.9318	0.8291
25	[C]LEFT AND RIGHT THYROID	9	B-FOLLICULAR CELL ADENOMA	0	0	3	0	4	<b>0.0205</b>	<b>0.0086</b>
25	[C]LEFT AND RIGHT THYROID	9	B-FOLLICULAR CELL ADENOMA AND CARCINOMA	0	0	3	0	7	<b>0.0003</b>	<b>0.0000</b>

### **3.3 Rat Carcinogenicity Study (Sepr.Doc. 190-823F)**

#### **3.3.1 Sponsor's Results and Conclusions**

In this oncogenicity study in Sprague-Dawley rats, eszopiclone had an effect on the survival of both males and females. The treatment arms of the males were terminated early between weeks 89 and 95, the controls on weeks 95 and 97. The female high dose was terminated during week 97, while the other groups were dosed till week 104. However, all groups had at least 50 percent survival at study week 83. Among the males, the Kaplan-Meier analysis indicated a statistically significant difference between the male control groups 1 and 2. Control group 2 was considered the more appropriate one based on historical control data. Compared to control group 2, there were statistically significant decreases in survival of the male rats in the 4, 8, and 16 mg/kg/day. The 4 and 16 mg/kg/day groups also had statistically significant decreased survival against the combined male controls. Among the females, there was no difference between the concurrent controls. The decrease in survival of the high dose females was considered test article related. The sponsor observed no increased incidence of neoplastic findings at any dose level, in particular not for mammary gland tumors among the females or thyroid tumors among the males.

#### **3.3.2 Statistical Methodologies**

— conducted the bioassay and some preliminary statistics.  
— performed the statistical evaluation of survival and tumor incidence data. — tested for similarity between the control groups per sex and analyzed tumor data against each and the combined control groups for the males and against the combined controls for the females. A log-rank dose response test for survival was performed using the doses as coefficients. Peto's methods for incidental or fatal tumors were employed. Trend or pair-wise tests were one-sided and depended on whether all animals were examined. Exact permutation tests were used for tumors with less than five animals. The levels of significance outlined in the FDA draft guidance were followed.

This reviewer used only the combined controls in all analyses, on the premise, that no explanation was found by the sponsor why the two male controls differed statistically in survival. As the two control groups present only a random allocation of one major group, any observed difference between the two controls are expected to be due to random fluctuation. All tumor analyses are survival adjusted.

The issues resulting from early termination of treatment arms is discussed in detail under 2.1.2 Major Statistical Issues. Suffice it to say that this reviewer used the earliest termination of any treatment arm as start of the terminal sacrifice for the whole gender.

For the females, only the high dose was terminated early. This reviewer performed a mortality and tumor trend analysis excluding the high dose, in case it would be argued that the high dose was terminated too early. The findings are not reported in detail, but

the trend in mortality, the deviation from linear trend as well as heterogeneity were all still significant, but to a lesser degree. There were no statistically significant increases in any tumors with this approach.

The submitted data files were 190823FT and 190823MT.

### 3.3.3 Detailed Review of Rat Carcinogenicity Study 190-823

The oncogenic potential of eszopiclone [(S)-zopiclone] was assessed in a two-year gavage study in Sprague-Dawley rats. The dose levels were 0, 0, 2, 4, 8, and 16 mg/kg/day. The controls received the vehicle, 0.5% CMC at 10mL/kg. After one month of dosing the number of animals/sex/group was culled to 70 for all groups. Due to increased mortality all surviving males in the 4, 16, 8 and 2 mg/kg/day groups and in the control groups 1 and 2 were euthanized during study weeks 89, 91, 93, 95, 95, and 97, respectively. The sponsor observed a statistically significant difference in survival between the two controls groups. Compared to control group 2, the treated groups showed significant decreases in survival. Against control group 1 there was no compound related difference in survival. Compared to the combined control groups, the survival of the treated groups was generally statistically significantly lower. All surviving females in the 16mg/kg/day group were euthanized during study week 97. The remaining female groups were dosed for 104 weeks. The two controls groups did not differ significantly in survival. The high dose group had consistent lower survival compared to the pooled or individual control groups.

### 3.3.4 Statistical Reviewer's Findings of Rat Data 190-823

This reviewer agrees with the sponsor that survival was significantly different between the treated and control groups, but not strictly with dose (tables 20, 21, 23, 24, Figures 3 and 4). However, the high dose for both the males and females experienced the poorest survival. This reviewer used only the combined controls in all analyses.

This reviewer performed onset-rate analysis for mammary tumors among the female rats. The results are given in bold in Table 22. Neither the individual tumors nor the combined adenomas and adenocarcinomas, or the combined single and multiple fibroadenomas approached statistical significance. For the male rats, the combined c-cell adenomas and carcinomas of the thyroid did also not approach statistical significance (Table 25). Follicular cell tumors of the thyroid appeared only as adenomas. This reviewer agrees with the sponsor that there are no statistically significant increases in any tumors recorded.

**Table 19: Mortality by Time Interval for Female Rats of Study 190-823**

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR1	53-78	70	7	63	90.0	10.0
	79-91	63	14	49	70.0	30.0
	92-96	49	10	39	55.7	44.3
	FINALKILL 97-104	39	39	0		
CTR2	0-52	70	2	68	97.1	2.9
	53-78	68	10	58	82.9	17.1
	79-91	58	12	46	65.7	34.3
	92-96	46	6	40	57.1	42.9
	FINALKILL 97-104	40	40	0		
LOW	0-52	70	3	67	95.7	4.3
	53-78	67	21	46	65.7	34.3
	79-91	46	7	39	55.7	44.3
	92-96	39	11	28	40.0	60.0
	FINALKILL 97-104	28	28	0		
MED	0-52	70	1	69	98.6	1.4
	53-78	69	7	62	88.6	11.4
	79-91	62	13	49	70.0	30.0
	92-96	49	8	41	58.6	41.4
	FINALKILL 97-104	41	41	0		
MEDHI	0-52	70	4	66	94.3	5.7
	53-78	66	15	51	72.9	27.1
	79-91	51	17	34	48.6	51.4
	92-96	34	6	28	40.0	60.0
	FINALKILL 97-104	28	28	0		
HIGH	0-52	70	6	64	91.4	8.6
	53-78	64	16	48	68.6	31.4
	79-91	48	16	32	45.7	54.3
	92-96	32	10	22	31.4	68.6
	FINALKILL 97-104	22	22	0		

**Table 20: Mortality Trend for Female Rats of Study 190-823**

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	8.0909	0.0883	10.0677	0.0393
Dose-Mortality Trend	15.1352	0.0001	15.6792	0.0001
Homogeneity	23.2261	0.0003	25.7469	0.0001

Figure 3: Kaplan Meier Survival Curves for Female Rats of Study 190-823

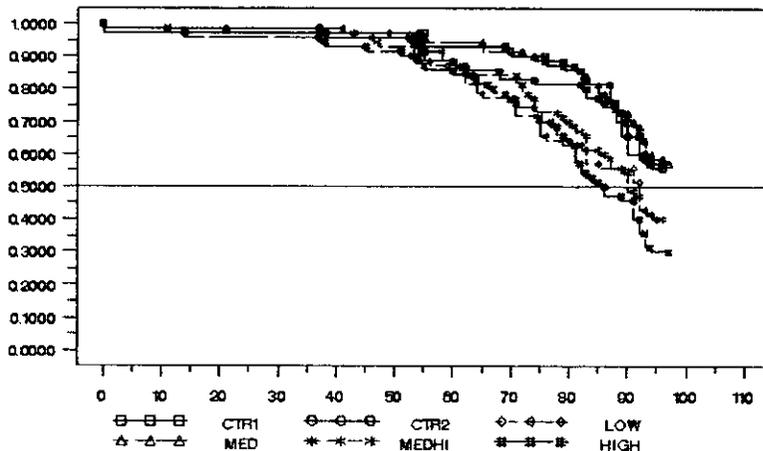


Table 21: Tumor Trends for Female Rats of Study 190-823

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	LOW	MED	MEDHI	MEDH	Survival	Survival
AC	ADRENAL CORTEX	HP002009	#B ADENOMA	2	2	3	0	1	2	0.5653	0.5758
AM	ADRENAL MEDULLA	HP003002	#B PHEOCHROMOCYTOMA, BENIGN	3	4	1	2	0	1	0.9110	0.8989
AM	ADRENAL MEDULLA	HP003004	#B PHEOCHROMOCYTOMA, COMPLEX,	1	0	0	0	0	0	1.0000	0.8160
BR	BRAIN	HP007005	#B GLIOMA, BENIGN	1	0	0	0	0	0	1.0000	0.8160
BR	BRAIN	HP007007	#B ASTROCYTOMA, BENIGN	0	1	0	0	1	0	0.5173	0.5613
CE	CECUM	HP008010	#M SARCOMA, UNDIFFERENTIATED	1	0	0	0	0	0	1.0000	0.8160
CL	CLITORAL GL	HP042005	#B ADENOMA	1	0	0	0	1	0	0.5657	0.5648
CL	CLITORAL GL	HP042007	#M CARCINOMA	0	0	1	0	0	0	0.6757	0.7730
CX	CERVIX	HP063005	#M SCHWANNOMA, MALIGNANT	0	3	1	1	0	1	0.6471	0.6634
CX	CERVIX	HP063010	#B POLYP	1	0	1	0	1	0	0.6302	0.6905
CX	CERVIX	HP063013	#M CARCINOMA	0	1	0	1	0	0	0.6955	0.7026
CX	CERVIX	HP063014	#M CARCINOMA, SQUAMOUS CELL	0	0	0	1	0	0	0.3987	0.3811

EA	EAR(S)	HP02800 4	#B PILOMATRICOMA	0	1	0	0	0	0	1.0000	0.8603
EA	EAR(S)	HP02800 5	#M MELANOMA, MALIGNANT	0	0	1	0	0	0	0.6863	0.7315
EE	TEETH	HP05500 1	#B ODONTOMA	0	0	0	1	0	0	0.4938	0.5962
EY	EYES/OPTIC N.	HP01400 5	#B MELANOMA, BENIGN	0	1	0	0	0	0	1.0000	0.8160
HE	HEART	HP01701 4	#B SCHWANNOMA, ENDOCARDIAL, BE	1	0	0	0	0	0	1.0000	0.8307
KI	KIDNEYS	HP02000 6	#M NEPHROBLASTOMA	0	0	1	0	0	0	0.6593	0.7270
KI	KIDNEYS	HP02001 0	#B ADENOMA, RENAL TUBULE	0	1	1	1	0	0	0.7996	0.8169
KI	KIDNEYS	HP02001 5	#B LIPOMA	1	0	0	0	0	1	0.3216	0.2320
LI	LIVER	HP02101 3	#B ADENOMA, HEPATOCELLULAR	2	0	1	1	1	0	0.7454	0.7651
LU	LUNGS	HP02602 3	#M CARCINOMA, SQUAMOUS CELL	0	0	0	0	1	0	0.2525	0.2441
LU	LUNGS	HP02602 4	#B ADENOMA, BRONCHIOLO-ALVEOLA	0	1	0	0	0	0	1.0000	0.8160
ME	MESENTERY	HP06600 7	#M SCHWANNOMA, MALIGNANT	0	0	1	0	0	0	0.7763	0.7709
MG	MAMMARY GLAND	HP02700 1	#B FIBROADENOMA	29	31	24	26	26	23	0.3599 0.3290*	0.3603 0.3306
MG	MAMMARY GLAND	HP02700 2	#B FIBROADENOMA, MULTIPLE	8	8	13	8	10	9	0.1487 0.1536	0.1447 0.1499
MG	MAMMARY GLAND	HP02700 3	#M ADENOCARCINOMA	3	3	7	6	6	3	0.3678 0.2316	0.3720 0.2300
MG	MAMMARY GLAND	HP02700 8	#B ADENOMA	2	2	2	2	0	2	0.5190 0.2922	0.5308 0.2986
MG	MAMMARY GLAND	HP02701 0	#B ADENOLIPOMA	1	0	0	0	1	0	0.6002 0.3069	0.6169 0.2982
OV	OVARIES	HP03300 5	#M CYSTADENOCARCINOMA	1	0	0	0	0	0	1.0000	0.8160
OV	OVARIES	HP03300 8	#B GRANULOSA CELL TUMOR, BENIG	0	0	2	0	1	1	0.2561	0.2599
OV	OVARIES	HP03301 0	#B ADENOMA, SEX CORD STROMAL	0	1	0	0	0	0	1.0000	0.8160
OV	OVARIES	HP03301 1	#B LUTEOMA	0	0	1	0	0	0	0.6863	0.7315
PA	PANCREAS	HP03400 8	#M ADENOCARCINOMA	0	1	0	0	0	1	0.2430	0.1397
PA	PANCREAS	HP03401 0	#B ADENOMA, ISLET CELL	0	1	0	0	0	0	1.0000	0.8160
PA	PANCREAS	HP03401 1	#M CARCINOMA	0	1	0	0	0	0	1.0000	0.8160
PI	PITUITARY	HP04000 2	#B ADENOMA, PARS DISTALIS	37	32	24	32	19	26	0.7294	0.7274
PW	PAW(S)	HP02500 3	#M FIBROSARCOMA	0	1	0	0	0	0	1.0000	0.8350
SA	SOFT TISSUE- ABD	HP05400 1	#M SCHWANNOMA, MALIGNANT	0	0	1	1	0	0	0.6770	0.7413
SH	SOFT TISSUE- THO	HP06700 1	#B MAST CELL TUMOR, BENIGN	0	1	0	0	0	0	1.0000	0.8603
SK	SKIN	HP04600 7	#M SARCOMA, UNDIFFERENTIATED	1	0	0	0	0	0	1.0000	0.8329
SK	SKIN	HP04601 3	#M SCHWANNOMA, MALIGNANT	0	0	0	0	0	1	0.1437	0.0218
SK	SKIN	HP04601 4	#B PILOMATRICOMA	0	1	2	0	0	1	0.5644	0.5734

SK	SKIN	HP04601 5	#B FIBROMA	2	0	0	0	0	0	1.0000	0.9079
SK	SKIN	HP04601 6	#M FIBROSARCOMA	0	1	0	0	0	0	1.0000	0.8184
SK	SKIN	HP04601 9	#B LIPOMA	0	1	0	0	0	0	1.0000	0.8160
SL	STOMACH, GLAN	HP05100 9	#M CARCINOMA	1	0	0	0	0	0	1.0000	0.8542
SN	STOMACH, NON	HP05201 3	#B PAPILLOMA, SQUAMOUS CELL	0	0	0	0	1	0	0.4079	0.3908
SN	STOMACH, NON	HP05201 4	#M SARCOMA, UNDIFFERENTIATED	0	0	0	1	0	0	0.5823	0.6511
SY	SYSTEMIC TUMOR	HP07200 1	#M SARCOMA, HISTIOCYTIC	0	0	0	0	0	1	0.1541	0.0257
SY	SYSTEMIC TUMOR	HP07200 2	#M LYMPHOMA, MALIGNANT	1	1	2	3	1	0	0.7489	0.7544
SY	SYSTEMIC TUMOR	HP07200 3	#B HEMANGIOMA	0	1	0	0	0	1	0.3216	0.2320
SY	SYSTEMIC TUMOR	HP07200 4	#M HEMANGIOSARCOMA	2	0	0	1	0	0	0.8976	0.8712
TG	THYROID GLANDS	HP05300 4	#B ADENOMA, C-CELL	13	13	6	9	5	2	0.9995	0.9988
TG	THYROID GLANDS	HP05300 8	#B ADENOMA, C-CELL, MULTIPLE	1	1	0	0	0	0	1.0000	0.8860
TG	THYROID GLANDS	HP05300 9	#M CARCINOMA, C-CELL	0	1	0	2	0	1	0.3182	0.3167
TG	THYROID GLANDS	HP05301 0	#B ADENOMA, FOLLICULAR CELL	0	0	1	0	0	0	0.7867	0.7750
UT	UTERUS	HP06000 6	#B POLYP, ENDOMETRIAL STROMAL	13	13	7	14	12	7	0.8221	0.8199
UT	UTERUS	HP06000 7	#B POLYP, ENDOMETRIAL STROMAL,	9	8	2	3	1	2	0.9874	0.9799
UT	UTERUS	HP06001 6	#M CARCINOMA	0	1	0	0	0	1	0.2298	0.1461
UT	UTERUS	HP06001 7	#M SARCOMA, UNDIFFERENTIATED	0	0	1	0	0	0	0.6453	0.7226
UT	UTERUS	HP06001 8	#M CARCINOMA, SQUAMOUS CELL	0	0	2	1	0	0	0.7066	0.7295
UT	UTERUS	HP06002 2	#B LEIOMYOMA	0	0	1	0	0	0	0.6863	0.7315
UT	UTERUS	HP06002 4	#B PAPILLOMA, SQUAMOUS CELL	0	0	1	0	0	0	0.6863	0.7315
UT	UTERUS	HP06002 5	#B ADENOMA, ENDOMETRIAL	0	0	1	0	0	0	0.6010	0.6921
UT	UTERUS	HP06002 6	#M SCHWANNOMA, MALIGNANT	0	0	0	2	0	0	0.5765	0.6212
VA	VAGINA	HP06500 1	#M SCHWANNOMA, MALIGNANT	1	1	0	0	0	0	1.0000	0.9105
VA	VAGINA	HP06500 7	#B POLYP	0	1	0	1	1	1	0.3337	0.3403
MG	MAMMARY GLAND	HP0270 03	COMBINED ADENOMA AND ADENOCARCINOMA	5	5	9	8	6	5	0.3964 0.1747*	0.4001 0.1704
MG	MAMMARY GLAND	HP027 001	COMBINED SINGLE AND MULTIPLE FIBROADENOMA	37	39	37	34	36	32	0.1884 0.1897*	0.1868 0.1886

\* Bolded entries are for time to tumor detection. Not all mammary tumors had time to detection recorded and the incidences are somewhat different.

**Table 22: Mortality by Time Interval for Male Rats of Study 190-823**

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR1	0-52	70	2	68	97.1	2.9
	53-78	68	23	45	64.3	35.7
	79-89	45	18	27	38.6	61.4
	FINALKILL 90-97	27	27	0		
CTR2	0-52	70	3	67	95.7	4.3
	53-78	67	11	56	80.0	20.0
	79-89	56	12	44	62.9	37.1
	FINALKILL 90-97	44	44	0		
LOW	0-52	70	6	64	91.4	8.6
	53-78	64	17	47	67.1	32.9
	79-89	47	20	27	38.6	61.4
	FINALKILL 90-97	27	27	0		
MED	0-52	70	4	66	94.3	5.7
	53-78	66	24	42	60.0	40.0
	79-89	42	21	21	30.0	70.0
	FINALKILL 90-97	21	21	0		
MEDHI	0-52	70	4	66	94.3	5.7
	53-78	66	20	46	65.7	34.3
	79-89	46	23	23	32.9	67.1
	FINALKILL 90-97	23	23	0		
HIGH	0-52	70	15	55	78.6	21.4
	53-78	55	21	34	48.6	51.4
	79-89	34	12	22	31.4	68.6
	FINALKILL 90-97	22	22	0		

**Table 23: Mortality Trend for Male Rats of Study 190-823**

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	10.2432	0.0365	8.3385	0.0799
Dose-Mortality Trend	10.9027	0.0010	13.7990	0.0002
Homogeneity	21.1459	0.0008	22.1376	0.0005

Figure 4: Kaplan-Meier Survival Curves for Male Rats of Study 190-823

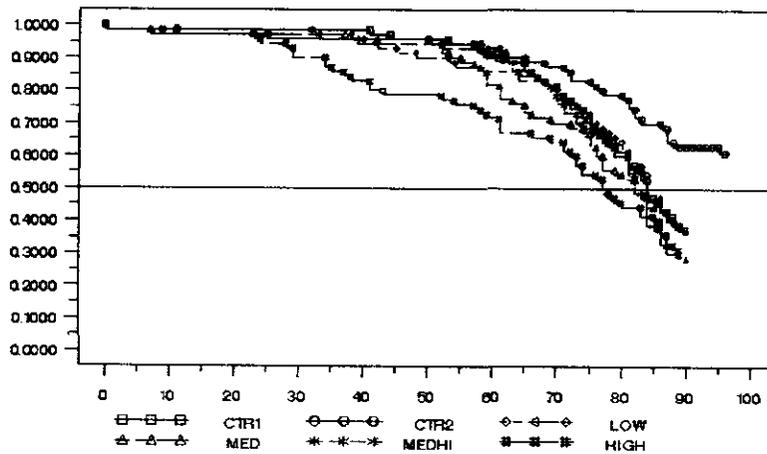


Table 24: Tumor Trends for Male Rats of 190-823

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR1	CTR2	MED	MEDHI	HIGH	P-Value (Exact Method)	Fisher's P-Value
AC	ADRENAL CORTEX	HP002008	#M CARCINOMA	1	0	0	0	0	1.0000	0.8508
AC	ADRENAL CORTEX	HP002009	#B ADENOMA	2	0	0	1	0	0.9048	0.8708
AM	ADRENAL MEDULLA	HP003002	#B PHEOCHROMOCYTOMA, BENIGN	7	12	3	4	5	0.2369	0.2350
AM	ADRENAL MEDULLA	HP003003	#B PHEOCHROMOCYTOMA, BENIGN, M	0	2	0	1	0	0.4025	0.4113
AM	ADRENAL MEDULLA	HP003004	#B PHEOCHROMOCYTOMA, COMPLEX,	0	0	0	0	1	0.5588	0.5780
AM	ADRENAL MEDULLA	HP003005	#M PHEOCHROMOCYTOMA, MALIGNANT	0	0	2	0	0	0.7484	0.7660
AT	ADIPOSE TISSUE	HP064003	#M SCHWANNOMA, MALIGNANT	0	0	0	1	0	0.4684	0.5826
BR	BRAIN	HP007004	#B MENINGIOMA, BENIGN	0	0	1	0	0	0.7069	0.7540
BR	BRAIN	HP007006	#M ASTROCYTOMA, MALIGNANT	0	1	0	1	0	0.6948	0.7417
DU	DUODENUM	HP010002	#M CARCINOMA	0	0	1	0	0	0.1767	0.1514
EA	EAR(S)	HP065003	#B PAPILLOMA, SQUAMOUS CELL	1	0	0	0	0	1.0000	0.8035
EA	EAR(S)	HP065004	#M FIBROSARCOMA	0	1	0	0	0	1.0000	0.8538

EY	EYES/OPTIC N.	HP014005	#B MELANOMA, BENIGN	0	0	0	0	1	1	0.0626	0.0226
EY	EYES/OPTIC N.	HP014010	#M MELANOMA, MALIGNANT	0	1	0	0	0	0	1.0000	0.8035
KI	KIDNEYS	HP020006	#M NEPHROBLASTOMA	0	0	0	0	0	1	0.1472	0.0238
KI	KIDNEYS	HP020010	#B ADENOMA, RENAL TUBULE	1	0	1	0	1	1	0.2537	0.2485
KI	KIDNEYS	HP020019	#M RENAL MESENCHYMAL TUMOR, MA	0	0	0	0	1	0	0.2893	0.2759
LD	LYMPH NODE, MAN	HP060006	#M CARCINOMA, SQUAMOUS CELL; U	1	0	0	0	0	0	1.0000	0.8538
LI	LIVER	HP021013	#B ADENOMA, HEPATOCELLULAR	0	0	1	0	2	1	0.0836	0.0624
LI	LIVER	HP021015	#B CHOLANGIOMA	1	0	0	0	0	0	1.0000	0.8538
LU	LUNGS	HP026015	#M CARCINOMA, BRONCHIOLO-ALVEO	1	0	0	0	0	0	1.0000	0.8035
LU	LUNGS	HP026016	#B ADENOMA, BRONCHIOLO-ALVEOLA	1	0	1	0	0	0	0.8141	0.8114
MG	MAMMARY GLAND	HP027001	#B FIBROADENOMA	0	0	0	1	0	0	0.5041	0.6012
MG	MAMMARY GLAND	HP027006	#B ADENOLIPOMA	0	1	0	0	0	0	1.0000	0.8441
PA	PANCREAS	HP034007	#B ADENOMA, ISLET CELL	1	3	0	0	0	0	1.0000	0.9527
PA	PANCREAS	HP034008	#B ADENOMA, ACINAR CELL	0	1	0	0	0	1	0.2716	0.1713
PA	PANCREAS	HP034011	#M CARCINOMA, ISLET CELL	0	0	1	1	0	0	0.6667	0.7137
PI	PITUITARY	HP040002	#B ADENOMA, PARS DISTALIS	12	12	4	16	8	10	0.3632	0.3640
PI	PITUITARY	HP040005	#B ADENOMA, PARS INTERMEDIA	0	0	0	0	0	1	0.1358	0.0181
PR	PROSTATE	HP042006	#M SCHWANNOMA, MALIGNANT	0	0	0	0	0	1	0.1548	0.0264
PR	PROSTATE	HP042008	#B ADENOMA	2	1	1	1	1	0	0.8318	0.8338
PT	PARATHYROID	HP035002	#B ADENOMA	0	0	0	0	1	1	0.0595	0.0216
SA	SOFT TISSUE- ABD	HP057001	#M SCHWANNOMA, MALIGNANT	0	0	0	1	1	0	0.3392	0.3568
SG	SAL. GLAND MAND	HP043007	#B ADENOMA	0	0	1	0	0	0	0.7170	0.7419
SK	SKIN	HP046002	#B BASAL CELL TUMOR, BENIGN	0	0	2	0	0	1	0.2795	0.2712
SK	SKIN	HP046005	#M CARCINOMA, SQUAMOUS CELL	0	0	0	0	0	1	0.1486	0.0243
SK	SKIN	HP046007	#M SARCOMA, UNDIFFERENTIATED	0	1	1	0	0	0	0.8635	0.8380
SK	SKIN	HP046010	#B PILOMATRICOMA	3	7	2	3	3	1	0.8834	0.8762
SK	SKIN	HP046016	#B FIBROMA	0	3	0	0	1	0	0.8001	0.7998
SK	SKIN	HP046017	#M FIBROSARCOMA	0	0	1	0	0	0	0.6383	0.7160
SK	SKIN	HP046020	#M SCHWANNOMA, MALIGNANT	0	0	0	1	0	0	0.4059	0.5455
SK	SKIN	HP046021	#B LIPOMA	0	0	2	0	1	0	0.4854	0.5387
SK	SKIN	HP046022	#B PAPHILOMA,	0	0	0	0	1	0	0.2761	0.2668

		3	SQUAMOUS CELL									
SL	STOMACH, GLAN	HP051009	#B GRANULAR CELL TUMOR, BENIGN	0	0	1	0	0	0	0.8529	0.8620	
SM	SKELETAL MUSCLE	HP045005	#M SCHWANNOMA, MALIGNANT	0	0	0	0	0	1	0.1635	0.0299	
SN	STOMACH, NON	HP052009	#M SCHWANNOMA, MALIGNANT	1	0	0	0	0	0	1.0000	0.8196	
SN	STOMACH, NON	HP052012	#M CARCINOMA, SQUAMOUS CELL	0	0	0	0	1	0	0.2744	0.2650	
SP	SPLEEN	HP048009	#M LEIOMYOSARCOMA	0	0	1	0	0	0	0.7170	0.7419	
SY	SYSTEMIC TUMOR	HP078001	#M LYMPHOMA, MALIGNANT	2	1	0	1	2	1	0.2288	0.2211	
SY	SYSTEMIC TUMOR	HP078002	#B HEMANGIOMA	0	0	0	0	0	1	0.1132	0.0148	
SY	SYSTEMIC TUMOR	HP078003	#M HEMANGIOSARCOMA	0	2	0	0	0	0	1.0000	0.8858	
SY	SYSTEMIC TUMOR	HP078004	#B MESOTHELIOMA, BENIGN	1	0	1	0	1	0	0.5511	0.6196	
TG	THYROID GLANDS	HP053004	#B ADENOMA, C-CELL	7	8	5	5	7	3	0.7710	0.7709	
TG	THYROID GLANDS	HP053007	#B ADENOMA, FOLLICULAR CELL	1	0	1	0	0	1	0.2963	0.2889	
TG	THYROID GLANDS	HP053009	#M CARCINOMA, C-CELL	0	0	1	1	0	1	0.1649	0.1618	
TG	THYROID GLANDS	HP053012	#B ADENOMA, C-CELL, MULTIPLE	0	1	2	0	0	0	0.8279	0.8290	
TH	THYMUS	HP063006	#B THYMOMA, BENIGN	1	0	0	0	0	1	0.2570	0.1906	
VO	VERTEBRAL COLUMN	HP077001	#M SCHWANNOMA, MALIGNANT	1	0	0	0	0	0	1.0000	0.8158	
TG	THYROID GLANDS		COMBINED C-CELL ADENOMA AND CARCINOMA	7	9	8	6	7	4	0.7431	0.7436	

### 3.3.5 Validity of Male and Female Rat Study 190-823

As there were no statistically significant tumor trends among the male or female rats in Study 190-823, the validity of both gender sub-studies needs to be assessed. Two criteria are set up for this purpose (Haseman<sup>45</sup>, Chu et al.<sup>6</sup>, and Bart et al.<sup>7</sup>):

<sup>4</sup> Haseman: Statistical Issues in the Design, Analysis and Interpretation of Animal Carcinogenicity Studies, *Environmental Health Perspectives*, Vol. 58, pp 385-392, 1984.

<sup>5</sup> Haseman: Issues in Carcinogenicity Testing: Dose Selection, *Fundamental and Applied Toxicology*, Vol. 5, pp. 66-78, 1985.

<sup>6</sup> Chu, Cueto, Ward: Factors in the Evaluation of 200 National Cancer Institute Carcinogenicity Bioassays, *Journal of Toxicology and Environmental Health*, Vol. 8, pp 251-280, 1981.

<sup>7</sup> Bart, Chu, Tarone: Statistical Issues in Interpretation of Chronic Bioassay Tests for Carcinogenicity, *Journal of the National Cancer Institute*, pp. 957-974, 1979.

- i) Were sufficient numbers of animals exposed long enough to allow for late-developing tumors?
- ii) Did the high dose provide a sufficient tumor challenge?

The number of animals and length of exposure can be assessed at weeks 52, 80-90, and at termination, but are generally considered satisfied if 20-30 animals survive through weeks 80-90. Though there was a significant effect of the compound on survival, all groups for both the male and female rats had at least 50 percent survival at week 83 and this reviewer agrees with the sponsor that a sufficient number of animals were exposed for a sufficient length of time. In determining whether the high dose provided an adequate tumor challenge, one expects the high dose to be close to the MTD. The following criteria are employed in this assessment:

- i) A dose is considered adequate if there is a detectable reduction in average body weight of up to 10 % in a dosed group relative to the controls.
- ii) A dose is considered adequate if the dosed animals show a slightly increased mortality compared to the controls.
- iii) A dose is considered an MTD if the dosed animals exhibit severe toxic effects attributed to the chemical. This latter evaluation is performed by the pharmacologist/toxicologist.

The cycling nature of increased and decreased bodyweights of the treated animals with respect to the controls makes it difficult to use the bodyweight as a criterion. In particular as these differences leveled out after 86 weeks. However, survival for both the high dose male and the high dose female rats was significantly lower than their controls. On this basis, the high dose may actually have exceeded the MTD.

In this reviewer's opinion, Study 190-823 in Sprague-Dawley rats appears valid using the above criteria. There were also no significant increases in tumor findings among the female rats when this reviewer excluded the high dose from analysis.

### **3.4 Mouse Carcinogenicity Study (Sepr. Doc. 190-834)**

#### **3.4.1 Sponsor's Results and Conclusions**

The sponsor's report Sepr. Doc. 190-834 and the data set tumor.xpt are relevant.

Intercurrent mortality was not affected by treatment. On page 032 of the 190-834.pdf report, the sponsor had concluded that the treatment with (R,S)-zopiclone at 100 mg/kg/day was associated with an increased incidence of neoplastic changes in the lungs

of female and in the subcutis of the male mice. Specifically, the sponsor observed a higher incidence of pulmonary adenoma or carcinoma ( $p < 0.01$  for combined tumors) among high dose females. The sponsor also stated that the observed incidences were outside the historical range. There was also an increase in ovarian cyst-adenomas in high dose females. Among the male mice, the sponsor observed an increase in sarcomas of the skin with a p-value of  $< 0.001$ . The sponsor again stated that the observed incidences among high dose males were outside the historical range. The sponsor noted on page 031 of the 190-834.pdf file, that the results indicated that there was strong evidence of an effect of treatment in the highest dose group.

In the Report Summary, the sponsor considered the increases in tumor incidences of no clinical relevance. Based on subsequent studies, sarcomas of the skin/subcutis among the male mice were considered secondary to fighting behavior (Sepr. Doc. 190-844). The increase in pulmonary tumors among the females was not statistically significant when 'contemporary statistical methods' were employed (Sepr. Doc. 190-873A1). These reports are addressed below.

### 3.4.2 Statistical Methodologies

Any statistical issues relevant to this study have been discussed already under 2.1.2.

### 3.4.3 Detailed Review of Mouse Carcinogenicity Study Nr. 190-834

The study was conducted by \_\_\_\_\_ on behalf of Rhone-Poulenc Recherches (France) between 1981 and 1985. The statistical analysis of the original study data was performed by \_\_\_\_\_ (Sepr. Doc. 190-834). This review utilizes the data submitted March 12, 2003.

(R,S)-zopiclone (27 267 RP) was administered to B6C3F1 mice in the diet at doses of 0, 0, 1, 10, and 100 mg/kg/day for 24 months. There were 52 animals per gender per treatment group. The animals were housed four to a cage. A full range of tissues were examined for all animals of the control 1, control 2 and high dose groups and for all animals of the low and mid doses that died before termination of the study. All tissues showing macroscopic abnormality and abnormal lymph nodes were examined. Lungs of males and females, thyroid glands and livers of females and testes of males were also examined for all treatment groups.

### 3.4.4 Statistical Reviewer's Findings of Mouse Study Nr. 190-834

Tables 25 and 26, and Figure 5 demonstrate that there was no effect of treatment with (R,S)-zopiclone on the mortality experience of the female mice. Increases in tumor incidence rates reached statistical significance for malignant pulmonary carcinoma ( $p=0.0055$ , asymptotic trend;  $p=0.0034$ , asymptotic pair-wise) and benign cystadenoma of the right ovary ( $p=0.0178$ , exact trend;  $p=0.0293$ , exact pair-wise) (Table 27). Among

the male mice, there was also no effect of the treatment on mortality (Tables 28, 29, Figure 6). Tumor incidences were highly increased for malignant sarcoma of the skin/subcutis ( $p=0.0000$ , exact trend) (Table 30). The same level of significance was observed when the skin sarcomas were analyzed using time to tumor detection. These findings are consistent with the sponsor's original report.

Combinations of tissues, such as left and right organ parts, or tumors did not result in any significant findings. In particular, benign cystadenoma of the left and right ovaries did not reach statistical significance ( $p(\text{combined})=0.1383$ , exact trend;  $p=0.1351$  exact pair-wise).

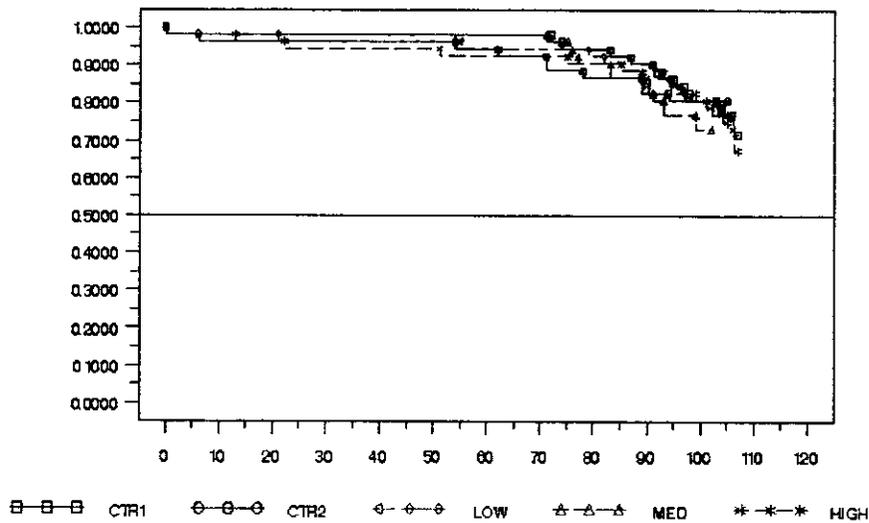
**Table 25: Mortality by Time Interval for Female Mice, Study 190-834**

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR1	53-78	52	2	50	96.2	3.8
	79-91	50	3	47	90.4	9.6
	92-105	47	6	41	78.8	21.2
	FINALKILL106-115	41	41	0		
CTR2	0-52	52	1	51	98.1	1.9
	53-78	51	5	46	88.5	11.5
	79-91	46	1	45	86.5	13.5
	92-105	45	3	42	80.8	19.2
	FINALKILL106-115	42	42	0		
LOW	0-52	52	1	51	98.1	1.9
	53-78	51	1	50	96.2	3.8
	79-91	50	3	47	90.4	9.6
	92-105	47	7	40	76.9	23.1
	FINALKILL106-115	40	40	0		
MED	53-78	52	4	48	92.3	7.7
	79-91	48	5	43	82.7	17.3
	92-105	43	5	38	73.1	26.9
	FINALKILL106-115	38	38	0		
HIGH	0-52	52	3	49	94.2	5.8
	53-78	49	1	48	92.3	7.7
	79-91	48	4	44	84.6	15.4
	92-105	44	5	39	75.0	25.0
	FINALKILL106-115	39	39	0		

Table 26: Trend in Mortality for Female Mice, Study 190-834

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	0.1244	0.9888	0.1152	0.9899
Dose-Mortality Trend	1.1092	0.2923	1.0153	0.3136
Homogeneity	1.2336	0.8725	1.1305	0.8894

Figure 5: Kaplan Meier Survival Curves for Female Mice, Study 190-834



APPEARS THIS WAY  
ON ORIGINAL



52	SKIN+SUBCUTIS	56	M-SARCOMA	3	1	1	3	2	0.3942	0.3996
53	SPLEEN	41	M-HAEMANGIOSARCOMA	1	0	0	2	0	0.5457	0.5722
54	STOMACH KERAT.	28	B-SQUAMOUS CELL PAPILLOMA	0	2	0	1	1	0.3974	0.4140
61	UTERUS	12	B-HAEMANGIOMA	1	0	0	0	0	1.0000	0.9079
61	UTERUS	30	B-STROMAL POLYP	3	0	0	0	0	1.0000	0.9794
61	UTERUS	41	M-HAEMANGIOSARCOMA	0	1	0	0	0	1.0000	0.9235
61	UTERUS	60	M-STROMAL SARCOMA	2	0	0	0	0	1.0000	0.9574
8	CERVICAL L.N.	41	M-HAEMANGIOSARCOMA	0	1	0	0	0	1.0000	0.9372

Table 28: Mortality by Time Interval for Male Mice, Study 190-834

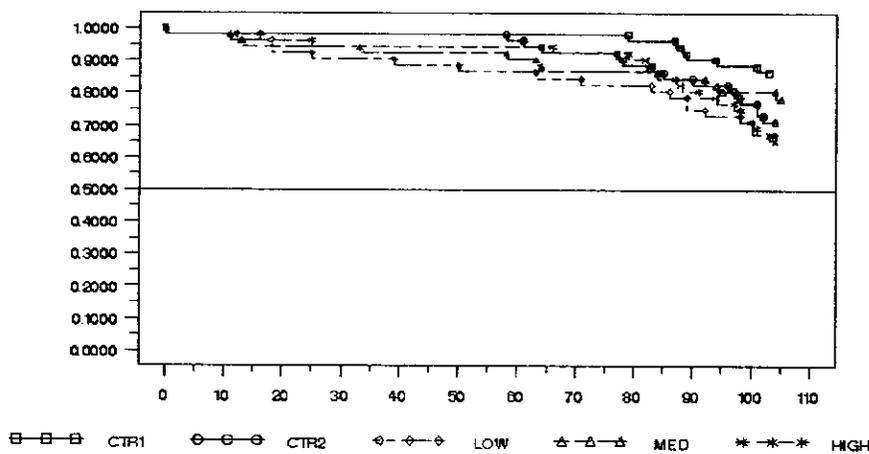
Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR1	79-91	52	4	48	92.3	7.7
	92-103	48	3	45	86.5	13.5
	FINALKILL104-106	45	45	0		
CTR2	53-78	52	5	47	90.4	9.6
	79-91	47	3	44	84.6	15.4
	92-103	44	6	38	73.1	26.9
	FINALKILL104-106	38	38	0		
LOW	0-52	52	6	46	88.5	11.5
	53-78	46	2	44	84.6	15.4
	79-91	44	3	41	78.8	21.2
	92-103	41	4	37	71.2	28.8
	FINALKILL104-106	37	37	0		
MED	0-52	52	3	49	94.2	5.8
	53-78	49	3	46	88.5	11.5
	79-91	46	1	45	86.5	13.5
	92-103	45	3	42	80.8	19.2
	FINALKILL104-106	42	42	0		
HIGH	0-52	52	2	50	96.2	3.8
	53-78	50	1	49	94.2	5.8
	79-91	49	7	42	80.8	19.2
	92-103	42	7	35	67.3	32.7
	FINALKILL104-106	35	35	0		

APPEARS THIS WAY  
ON ORIGINAL

**Table 29: Trend in Mortality for Male Mice, Study 190-834**

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	6.2215	0.1013	6.1312	0.1054
Dose-Mortality Trend	1.5920	0.2070	1.6806	0.1948
Homogeneity	7.8135	0.0987	7.8118	0.0987

**Figure 6: Kaplan Meier Survival Curves for Male Mice , Study 190-834**



**APPEARS THIS WAY  
ON ORIGINAL**

Table 30: Tumor Trend Tests for Male Mice, Study 190-834

Organ Code	Organ Name	Number	Tumor Type	7	9	12	16	6	0.3142	0.3159
14	H-POIETIC TISSUE	47	M-MALIGNANT LYMPHOMA	7	9	12	16	6	0.3142	0.3159
15	ILEUM	31	M-ADENOCARCINOMA	0	1	0	0	0	1.0000	0.8524
19	LEFT ADRENAL	3	B-CORTICAL ADENOMA	3	0	0	0	0	1.0000	0.9106
19	LEFT ADRENAL	53	M-PHAECHROMOCYTOMA	1	1	0	0	0	1.0000	0.8816
20	LEFT HARDERIAN GLAND	1	B-ADENOMA	1	3	2	2	3	0.2131	0.2051
26	LIVER	12	B-HAEMANGIOMA	1	0	0	0	0	1.0000	0.8952
26	LIVER	13	B-HEPATOCELLULAR ADENOMA	15	17	8	17	11	0.4319	0.4329
26	LIVER	35	M-CHOLANGIOPHYSIOMA	0	1	0	0	0	1.0000	0.9268
26	LIVER	41	M-HAEMANGIOSARCOMA	1	1	1	1	0	0.8424	0.8367
26	LIVER	42	M-HEPATOBLASTOMA	0	0	0	0	1	0.2215	0.1245
26	LIVER	43	M-HEPATOCELLULAR CARCINOMA	9	10	5	3	11	0.6422	0.6456
27	LUNGS	25	B-PULMONARY ADENOMA	13	12	14	9	7	0.9529	0.9519
27	LUNGS	54	M-PULMONARY CARCINOMA	1	2	4	1	3	0.3031	0.3058
37	PANCREAS	15	B-ISLET CELL ADENOMA	1	0	0	0	0	1.0000	0.8639
37	PANCREAS	46	M-ISLET CELL CARCINOMA	1	0	0	0	0	1.0000	0.8639
38	PITUITARY	1	B-ADENOMA	0	1	0	0	0	1.0000	0.9245
40	RIGHT ADRENAL	3	B-CORTICAL ADENOMA	0	1	0	0	0	1.0000	0.8535
40	RIGHT ADRENAL	53	M-PHAECHROMOCYTOMA	0	2	0	0	0	1.0000	0.8909
45	RIGHT TESTIS	14	B-INTERSTITIAL CELL ADENOMA	0	0	1	0	0	0.5765	0.7116
46	RIGHT THYROID	9	B-FOLLICULAR CELL ADENOMA	0	0	0	0	1	0.2893	0.1193
52	SKIN+SUBCUTIS	29	B-SQUAMOUS PAPILOMA	0	0	1	0	0	0.5389	0.6980
52	SKIN+SUBCUTIS	56	M-SARCOMA	1	1	2	0	13	0.0000	0.0000
52	SKIN+SUBCUTIS	59	M-SQUAMOUS CELL CARCINOMA	0	0	0	1	0	0.3636	0.3161
52	SKIN+SUBCUTIS	8	B-FIBROMA	1	1	0	0	2	0.4076	0.4071
53	SPLEEN	12	B-HAEMANGIOMA	0	0	0	1	0	0.3357	0.3456
53	SPLEEN	41	M-HAEMANGIOSARCOMA	1	2	1	0	0	0.9679	0.9652
54	STOMACH KERAT.	28	B-SQUAMOUS CELL PAPILOMA	0	1	1	0	1	0.4661	0.5204

### **3.5 Mouse Carcinogenicity Study 190-834 Appended (Sepr. Docs. 190-839, 190-873, 190-873A1)**

#### **3.5.1 Sponsor's Results and Conclusions**

The relevant reports are Sepr. Doc. 190-839, 190-873 and 190-873A1. In addition, Sepr. Doc. 190-867 contains evaluations by consultants of the original findings, their potential mechanisms of action, and their relevance to humans, but is not statistical in nature and therefore not discussed here. The relevant data file is tumora.xpt of the 03/12/03 submission.

The sponsor had convened a Pathology Working Group which re-evaluated tumor data of Study 190-834. Their report uses little statistics (Sepr. Doc.190-839). The statistical analyses of the re-evaluated tumors are in reports 190-873 and 190-873A1. The sponsor notes in the 06/30/03 submission, that Sepr. Doc. 190-873 had not been previously submitted. It appears it has not been submitted hence either. Briefly, the sponsor stated that in the 190-873 report, the statistical conclusion still considered the increase in lung carcinomas among female mice statistically significant. However, once reanalyzed by [redacted], see below, this finding did no longer reach statistical significance.

The sponsor's final results of the re-analysis by [redacted] are in report 190-873A1. [redacted] concluded that no statistically significant findings were detected in the survival of either sex and that an adequate number of mice survived to study termination (2 years). Using the levels of significance outlined in the FDA draft Guidance, [redacted] found the trend test and the pair-wise comparison of high dose against combined controls statistically significant for the incidence of sarcoma in the skin and subcutis in male mice. However, the sponsor attributed the increase in skin sarcomas to group-housing (Sepr. Doc. 190-844) and not to the test article. Pulmonary adenomas among the female mice were not statistically significant in either the original or the PWG data. Pulmonary carcinoma among the female mice was statistically significant in the original study data for trend and pair-wise comparison. However, for the PWG numbers of pulmonary carcinoma, statistical significance was not achieved (Table 31). **Combining pulmonary adenomas and carcinomas, [redacted] reported the trend as not statistically significant (p=0.019 Peto), but the pair-wise comparison between the combined controls and the high dose as statistically significant (p=0.005 Peto) (Table 32).** They also reported a statistically significant trend in cystadenoma in the right ovary (p=0.017).

Table 31: Sponsor's Tables from Pharmsum.pdf of 06/30/03 Submission

Table 9.2.3-6 Incidence of Target Tumors with (R,S)-Zopiclone in Mice

(R,S)-Zopiclone (mg/kg/day)	Incidence per Treatment (%)			
	0	1	10	100
<b>Male Skin/Subcutis (Study Report Data)</b>				
Sarcoma	2 / 104 (1.9)	2 / 43 (4.7)	0 / 44 (0)	13 / 52 (25)
<b>Female Lung Pre-PWG (Study Report Data)</b>				
Adenoma	11 / 104 (11)	5 / 52 (9.6)	2 / 52 (3.8)	11 / 52 (21)
Carcinoma	0 / 104 (0)	1 / 52 (1.9)	1 / 52 (1.9)	4 / 52 (7.7)
<b>Female Lung Post-PWG</b>				
Adenoma	9 / 104 (8.7)	5 / 52 (9.6)	3 / 52 (5.8)	10 / 52 (19)
Carcinoma	0 / 104 (0)	1 / 52 (1.9)	0 / 52 (0)	2 / 52 (3.8)

Table 9.2.3-7 Statistical Decision Rules for Controlling the Overall False-Positive Rates Associated with Tests for Positive Trend or with Control-High Pair-Wise Comparisons in Tumor Incidences for the Male Skin/Subcutis and Female Lung

Categories / Tumor Types	Tests for Positive Trends	Control-High Dose Pair-Wise Comparisons
Significance p values from a 2-year study with 2 species and 2 sexes *	Common tumors $p \leq 0.005$ Rare tumors $p \leq 0.025$	Common tumors $p \leq 0.01$ Rare tumors $p \leq 0.05$
<b>Male skin &amp; subcutis - RPR data</b>		
Sarcoma (common tumor)	$p < 0.001$ Peto Fixed Interval *	$p < 0.001$ Peto Fixed Interval *
<b>Female lungs - RPR data</b>		
Pulmonary adenoma (common tumor)	$p = 0.102$ Peto Fixed Interval	$p = 0.036$ Peto Fixed Interval
Pulmonary carcinoma (rare tumor)	$p = 0.005$ Exact Permutation *	$p = 0.011$ Exact Permutation *
<b>Female lungs - PWG data</b>		
Pulmonary adenoma (common tumor)	$p = 0.069$ Peto Fixed Interval	$p = 0.030$ Peto Fixed Interval
Pulmonary carcinoma (rare tumor)	$p = 0.075$ Exact Permutation	$p = 0.109$ Exact Permutation

\* From FDA's Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (May 2001, Draft Guidance). The statistical significance (p) values from the classification of rare and common tumors. For each tumor type, classifications for common and rare tumors were based on the incidence in the study controls; tumors of less than 1% in the study control groups were classified as a rare tumor, and those that were more than 1% in the study control groups were classified as a common tumor.

\* Statistically significant. The Peto method with standard fixed intervals (weeks 0-52, 53-78, 79-92, 93-104, and termination) was performed. When the tumor incidence across groups for each sex was small ( $\leq 10$ ), an Exact Permutation test was performed.

RPR: Rhône-Poulenc Recherches. PWG: Pathology Working Group

APPEARS THIS WAY  
ON ORIGINAL

**Table 32: Sponsor's Results for Individual and Combined Pulmonary Tumors in Female Mice, Study 190-834A**

**Table 5**  
Analyses of Histopathology Pathology Working Group Review of Female Lungs

Organ/Tissue and Tumor	Control	1 mg/kg/day	10 mg/kg/day	100 mg/kg/day	Rare/Common
<b>LUNGS</b>					
<b>B-PULMONARY ADENOMA</b>					Common
Overall Rate	9/104( 9%)	5/52( 10%)	3/52( 6%)	10/52( 19%)	
Peto Fixed Interval	P=0.069	P=0.451	P=0.774	P=0.030	
<b>M-PULMONARY CARCINOMA</b>					Rare
Overall Rate	0/104( 0%)	1/52( 2%)	0/52( 0%)	2/52( 4%)	
Exact Permutation	P=0.075	P=0.331	P=1.000	P=0.109	
<b>PULMONARY ADENOMAS AND CARCINOMAS</b>					Common
Overall Rate	9/104( 9%)	6/52( 12%)	3/52( 6%)	12/52( 23%)	
Peto Fixed Interval	P=0.019	P=0.308	P=0.774	P=0.005*	

### 3.5.2. Statistical Methods

Only the two tissues re-evaluated by the PWG, namely the skin for the male mice and the lungs for the female mice, are of concern here. As the lungs and gross lesions (e.g. skin tumors) were examined for all animals, trend tests are appropriate.

There is a difference between the sponsor's and this reviewer's conclusion regarding the trend test for combined pulmonary tumors. The numbers of tumors reported by the sponsor and found in the submitted data sets are identical. However, the numbers of animals at risk differ occasionally. Though the statistical methods used appear similar, if not identical, the sponsor's analysis appears more sensitive to the linearity component of the trend test. As the mid-dose group had the lowest incidence in pulmonary tumors, the increase from controls to high dose is not strictly linear. This may be the reason why the sponsor found the less-powerful pair-wise comparison between the high dose and controls highly statistically significant (0.005 versus  $\alpha=0.01$ ) whereas the trend test did not reach statistical significance. It appears, that a combination of generally small differences between the sponsor's and this reviewer's approaches was sufficient to affect the p-values and resulting conclusion regarding the trend test.

### 3.5.3 Detailed Review of Mouse Study 190-834 Appended

A pathology-working group (PWG) peer-reviewed the original findings of skin tumors among the male mice and pulmonary neoplasms among the female mice from study 190-834 and reported their findings in Sepr. Doc.190-839. \_\_\_\_\_ evaluated the survival and pertinent tumor data, including the modifications by the PWG. Their report, 190-873, was apparently not submitted with the NDA. However, the sponsor

states, that the increase in pulmonary tumors was considered statistically significant. — was acquired by — , which performed additional data quality, program evaluation, and statistical re-analysis according to the FDA draft guidance. Sepr. Doc. 190-873A1 is — re-evaluation and re-assessment of report 190-873 and the PWG data. The sponsor's — Report (Sepr. Doc. 190-844) addresses the issue whether the increase in skin tumors among the male mice is attributable to group housing. This is discussed in a separate section below.

#### 3.5.4 Statistical Reviewer's Findings of Mouse Study 190-834 Appended

As expected, the mortality findings were identical as those of mouse study 190-834. The intercurrent mortality was not statistically significant among either the female or the male mice. The statistics and p-values were somewhat different from the sponsor's, but led to the same conclusions. Tumor analyses are adjusted for survival differences.

For the female mice, the review by the PWG resulted in fewer pulmonary tumors. The original study contained a total of 29 animals with pulmonary adenoma and 6 animals with pulmonary carcinoma. Table 33 summarizes the results for pulmonary adenomas and carcinomas as determined by the PWG. Pulmonary adenomas were observed as common tumors and the increase did not reach statistical significance. Pulmonary carcinomas were observed as rare tumors. The fatal carcinomas occurred during time intervals the incidental tumors were observed. Therefore, the p-value of the asymptotic test is more relevant. However, since there are only three tumors now, the asymptotic test may be unstable and the result may be only approaching statistical significance. The combined pulmonary adenomas and carcinomas, however, resulted in a statistically significant finding for common tumors, based on both the exact and asymptotic tests. The asymptotic test is considered the more correct p-value ( $p=0.0016$ ). The pair-wise comparison between high dose and controls for the combined pulmonary tumors also reached statistical significance ( $p=0.0063$ ).

This reviewer concludes that there are increases in pulmonary adenomas and carcinomas with dose among the female mice. These increases are not equally judged statistically significant by the sponsor's latest evaluation and by this reviewer. However, at a minimum, both recognize the increase in the high dose compared to the controls as statistically significant.

**Table 33: Reviewer's Results for Pulmonary Tumors among Female Mice in Study 190-834A**

Organ Code	Organ Name	Tumor Code	Tumor Name	C1	C2	Low	Med	HD	P-Value Exact	P-Value Asymp
27	LUNGS	25	B-PULMORNAY ADENOMA	5	4	5	3	10	0.0202	0.0124
27	LUNGS	54	M-PULMONARY CARCINOMA	0	0	1	0	2	0.0542	0.0232*
27	LUNGS	C**	ADENOMA OR CARCINOMA	5	4	6	3	12	0.0037	0.0016***
27	LUNGS	C**	ADENOMA OR CARCINOMA	5	4			12	0.01397	0.0063***

\* The p-value from the asymptotic test is relevant and statistically significant for  $\alpha=0.025$

\*\* For the combined tumors

\*\*\* The p-value from the asymptotic test is relevant and statistically significant for  $\alpha=0.005$

This reviewer agrees with the sponsor that there was a statistically significant increase in cystadenomas in the right ovary, but not in the left ovary, nor when the ovaries were combined.

The PWG did not change the number of skin tumors among the male mice from the original data set. Both the sponsor and this reviewer agree that the increase is statistically significant. The sponsor addressed the issue whether the occurrence of these skin tumors may be attributed to group housing in report Sepr. Doc.190-844.

### **3.6 Mouse Carcinogenicity Study 190-844**

#### **3.6.1 Sponsor's Results and Conclusions**

This was a special study to confirm the assumption that the increased incidence of tumors of the subcutis found in study 190-834 was due to fighting of gang-caged male B6C3F1 mice and not due to the administration of (R,S)-zopiclone.

The sponsor found that the study compound had an effect on intercurrent mortality (positive and negative) and performed age-adjusted tumor analysis. The study design is represented in Table 34. The tumor findings are given in Table 35. The study lasted for 107 weeks. Among the individually housed animals (groups 1 and 2) only a sebaceous adenoma in the control group and a malignant lymphoma among the treated group, i.e. no fibromas or sarcomas of the skin, were observed. The incidence of skin tumors was increased among animals housed four per cage compared to the singly housed animals. One zopiclone treated group (group 4) exhibited increased mortality and a statistically significant increase in non-fatal skin neoplasms. No significant difference was observed for fatal skin tumors. Between groups 5 and 7 there was no statistical difference in benign and/or malignant skin tumors. Among the animals housed four per cage, the incidences of

lesions associated with fighting were higher for treated mice than for their controls. These changes were most pronounced in mice of group 4.

The sponsor concluded that the apparent effect of (R,S)-zopiclone, when administered at 100 mg/kg/day, on the incidence of subcutaneous tumors of gang-caged mice was related to an effect on fighting behavior rather than to any oncogenic potential of (R,S)-zopiclone.

### 3.6.2 Statistical Methodologies

The sponsor used the Cochran-Armitage trend test to investigate the association between the number of weeks with dorsal incrustations and dorsal subcutis tumors. Cox's test was used for pair-wise comparison of mortality. Tumor incidence analysis was performed using the approach of Peto et al. (IARC, 1980) and the age of the animal at sacrifice. Therefore, the sponsor treated the skin tumors as incidental or fatal, not as mortality independent. The sponsor refers to 'trend' in mortality when only pair-wise comparisons are made.

The data for this study were not electronically submitted with the NDA. The study was previously reviewed under IND — by Dr. Daphne Lin. Dr. Daphne Lin performed her own evaluation of intercurrent mortality for a positive increase in mortality with dose. The tumor findings are evaluated based on the sponsor's results. This reviewer did not perform any statistical analysis of the data from this study.

### 3.6.3 Detailed Review of Mouse Carcinogenicity Study

In Study 190-834 a highly statistically significant increase in sarcomas of the subcutis had been observed among the male B6C3F1 mice. Study 190-844 was conducted to address the hypothesis that lesions from fighting of gang caged animals were responsible for the increase in subcutaneous sarcomas. Therefore, Study 190-844 does not directly address the carcinogenic potential of zopiclone. The study had been previously submitted to IND — and was reviewed by Dr. Daphne Lin, now with HFD-725 (Feb. 1991) and this reviewer will only summarize her findings. The data were not electronically available under the NDA.

**Table 34: Study Design for Mouse Study 190-844**

Study Number	Group Number	Treatment	Dosage	No. of mice per group	No. of mice per cage
RHS/017/ZOP	1	Control	0	100	1
	2	Zopiclone	100	100	1
	3	Control	0	100	4
	4	Zopiclone	100	100	4
RHS/016/ZOP	5	Control	0	52	4
	6	Zopiclone	1	52	4
	7	Zopiclone	100	52	4

Animals receiving 1 mg/kg/day were sacrificed after 26 weeks. All other animals were sacrificed after 106 weeks of treatment. Only skin and subcutis tumors were evaluated.

### 3.6.4 Statistical Reviewer's Comments Based on Dr. Daphne Lin's Review

This reviewer is summarizing Dr. Lin's original review and takes responsibility for any inadvertent misrepresentation. According to Dr. Daphne Lin's calculations, there were no statistically significant increases in mortality between groups 1 and 2, and groups 5 and 7. There was a statistically significant higher mortality among group 4 (100mg/kg/day) than group 3 (control). The sponsor's significant difference in mortality between groups 1 and 2 showed the increase in mortality among the controls not among the treated group, i.e. a negative relationship with dose. Similarly, the sponsor observed a borderline negative relationship with dose for differences in mortality between groups 5 and 7. Dr. Daphne Lin agreed with the sponsor about the increase in skin tumors between groups 3 and 4, but pointed out that there was no statistically significant difference between tumor rates of groups 5 and 7 (Table 35). Therefore, the sponsor's conclusion that the incidence of skin tumors was increased in animals, which were housed 4 per cage as compared to singly housed animals, are based only on the findings of groups 1-4. The sponsor's conclusion is not justified when considering the results from groups 5 and 7. In addition, she pointed out, that the combined analysis of groups 3 and 5 (controls) versus groups 4 and 7 (100mg/kg/day) was not appropriate because the underlying intercurrent mortality went in opposite directions for groups 3 and 4 (positive with dose) and groups 5 and 7 (negative with dose).

As Dr. Lin pointed out, the results of Study 190-844 are not consistent and therefore cannot provide complete evidence in this reviewer's opinion, that fighting due to group-housing is the sole cause for the observed increase in skin tumors in Study 190-834. The sponsor's Test-table 4 (Table 36 below) shows the association between weeks of dorsal incrustations and incidence of skin tumors. This reviewer notes, that though the findings

show an increase in the percent of tumors with increased length of incrustations, an analysis showing that tumor development was subsequent to the presence of incrustation would also be necessary.

Table 35: Sponsor's Tumor Findings in Study 190-844

Study Group	Male mice with fibroma or sarcoma on the dorsal surface					
	RHS/O17/ZOP				RHS/O16/ZOP	
Treatment	1	2	3	4	5	7
Dosage (mg/kg/day)	Control	Zopiclone	Control	Zopiclone	Control	Zopiclone
No. mice per cage	1	1	4	4	4	4
No. mice per group	100	100	100	100	52	52
<b>Fibroma:</b>						
No. tumours found	0	0	0	6	0	2
No. mice affected	0	0	0	6	0	2
% mice affected	0	0	0	6	0	4
<b>Sarcoma:</b>						
No. tumours found	0	0	3*	14*	0	2*
No. mice affected	0	0	3*	13*	0	2*
% mice affected	0	0	3*	13*	0	4*
<b>Fibroma and Sarcoma:</b>						
No. tumours found	0	0	3*	20*	0	4*
No. mice affected	0	0	3*	19*	0	4*
% mice affected	0	0	3*	19*	0	8*

\* Includes one animal with a sarcoma on the dorsal surface of a hind limb  
+ Includes one animal with a sarcoma on the cranium and one animal with a sarcoma on the hind limb  
x Includes one animal with a sarcoma on the cranium

Table 36: Sponsor's Table on Weeks of Incrustations and Skin Tumors

Text-table 4

Association between the number of weeks dorsal encrustations were observed and dorsal tumours (fibroma and sarcoma)

	Weeks dorsal encrustations observed				
	0	1-5	6-10	11-20	21-52*
Incidence (%) of mice with tumours among mice bearing encrustation for the specified period.					
RHS/O16/ZOP Groups 5+7	0/69 (0%)	1/10 (10%)	0/5 (0%)	1/15 (7%)	2/5 <sup>c</sup> (40%)
RHS/O17/ZOP Groups 3+4	5/101 (5%)	1/32 (3%)	3/15 (20%)	5/25 (20%)	8/27 <sup>c</sup> (30%)
Total Groups 3+4 + 5+7	5/170 (3%)	2/42 (5%)	3/20 (15%)	6/40 (15%)	10/32 <sup>c</sup> (31%)

\* Encrustations observed after Week 52 were not considered as it was thought that tumour development might possibly cause encrustations  
c Significant trend, P < 0.001 (Cochrane Armitage)

### **3.7 Mouse Carcinogenicity Study 190-830**

#### **3.7.1 Sponsor's Results and Conclusions**

Though the high dose (week 97) males and control group 1 males (week 102) were terminated early, the sponsor considers the effect not due to the test article and concluded that survival was unaffected by oral gavage administration of eszopiclone. At week 84, at least 50 percent of all groups were alive. All remaining male groups and all female groups were dosed for 104 weeks. The sponsor observed no evidence of increased incidence of neoplastic findings at any dose.

#### **3.7.2 Statistical Methodologies**

\_\_\_\_\_ performed the statistical analysis for the sponsor. Palpable masses were analyzed by two-tailed Fisher's Exact test, comparing each group to control group 1. Survival data were analyzed via Kaplan-Meier estimates, generalized Wilcoxon test and the log-rank trend test. For tissues where all animals were examined, tumor incidences were analyzed by trend according to Peto, using the actual doses as scales. Otherwise, pair-wise comparisons were performed. For low tumor incidences, an exact permutation test was used.

This reviewer employed the usual methods of the OB software. As two arms of the male mice were terminated early, she used the time of the earliest termination as the terminal sacrifice time for all male mice. This approach resulted in different numbers of animals at risk at the later time intervals. However, fatal tumors are not affected by this change. As there were only isolated cases of palpable masses in the treated groups this reviewer did not perform an onset rate type of analysis.

#### **3.7.3 Detailed Review of Mouse Carcinogenicity Study 190-830**

This was a 24-months oral gavage oncogenicity study of eszopiclone in CD-1 mice [CD-1 (ICR)BR]. The doses administered were 0, 0, 25, 50, 100 mg/kg. The controls received the vehicle only (0.5% CMC). The animals were housed individually. After one month, the toxicology groups were culled to 70 animals/sex/dose group. Due to increased mortality, all surviving high dose males were euthanized at week 97 and all surviving control group 1 males were euthanized at week 102 respectively. The remaining males and all female groups were dosed for 104 weeks. All tissues from animals dying on study were microscopically examined. In addition, heart, liver, kidneys, gross lesions for both sexes and urinary bladder from males and lungs from females were microscopically examined for all animals.

### 3.7.4 Statistical Reviewer's Findings of Study 190-830

Among the female mice, the high dose animals experienced the poorest survival, but not to a significant degree (Tables 37, 38, Figure 7). Departure from linearity and heterogeneity showed that the compound had an effect on survival, but not strictly increasing with dose. This reviewer agrees with the sponsor that at least 50 percent of the animals were alive at week 84. This reviewer observed a statistically significant increase in leiomyosarcoma of uterus by the asymptotic trend ( $p=0.0035$ ) (Table 39). There were only two tumors, both among HD females. One was fatal and one incidental, but not in the same time intervals. The exact trend test approached statistical significance for a rare tumor ( $p=0.0272$ ). The pair-wise comparison was also significant for the asymptotic test ( $p=0.0151$ ) but not for the exact test ( $p=0.0883$ ). Combining leiomyoma and leiomyosarcoma of the uterus resulted in a non-significant asymptotic trend test ( $p=0.1029$ ). The corresponding pair-wise comparison was also not statistically significant ( $p=0.1086$ ). As not all uterine tissues were microscopically evaluated, the increase of leiomyosarcoma in the uterus can represent a statistical signal, which needs to be evaluated for any potential biological concern.

Among the male mice there was no statistical effect of eszopocline on survival (Tables 40, 41, Figure 8). This reviewer agrees with the sponsor that there were no statistically significant increases in tumor findings among the male mice of this study (Table 42).

**Table 37: Mortality by Time Interval for Female Mice of Study 190-830**

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR1	0-52	70	7	63	90.0	10.0
	53-78	63	11	52	74.3	25.7
	79-91	52	18	34	48.6	51.4
	92-103	34	9	25	35.7	64.3
	FINALKILL 104-104	25	25	0		
CTR2	0-52	70	5	65	92.9	7.1
	53-78	65	13	52	74.3	25.7
	79-91	52	9	43	61.4	38.6
	92-103	43	16	27	38.6	61.4
	FINALKILL 104-104	27	27	0		
LOW	0-52	70	3	67	95.7	4.3
	53-78	67	8	59	84.3	15.7
	79-91	59	11	48	68.6	31.4
	92-103	48	12	36	51.4	48.6
	FINALKILL 104-104	36	36	0		
MED	53-78	70	15	55	78.6	21.4
	79-91	55	10	45	64.3	35.7
	92-103	45	18	27	38.6	61.4
	FINALKILL 104-104	27	27	0		

HIGH	0-52	70	10	60	85.7	14.3
	53-78	60	16	44	62.9	37.1
	79-91	44	12	32	45.7	54.3
	92-103	32	10	22	31.4	68.6
	FINALKILL 104-104	22	22	0		

Table 38: Mortality Trend for Female Mice of Study 190-830

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	7.4938	0.0577	9.2910	0.0257
Dose-Mortality Trend	1.6323	0.2014	2.1554	0.1421
Homogeneity	9.1260	0.0580	11.4464	0.0220

Figure 7: Kaplan Meier Survival Curves for Female Mice of Study 190-830

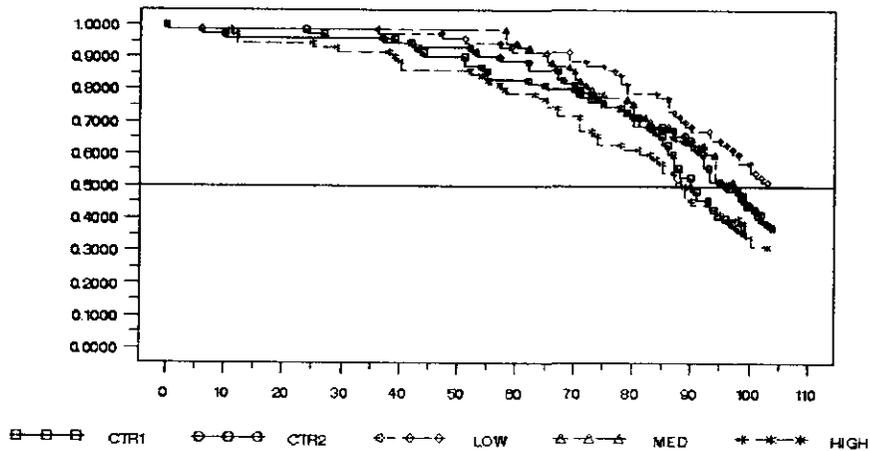


Table 39: Tumor Trends for Female Mice in Study 190-830

AC	ADRENAL CORTEX	HP002007	#M CARCINOMA, A CELL	1	0	0	0	0	1.0000	0.8240
AC	ADRENAL CORTEX	HP002017	#B ADENOMA, A CELL	1	0	0	0	0	1.0000	0.8287
AC	ADRENAL CORTEX	HP002018	#B ADENOMA	0	1	0	0	0	1.0000	0.8287
AM	ADRENAL MEDULLA	HP003002	#B PHEOCHROMOCYTOMA, BENIGN	1	2	1	2	1	0.6129	0.5831
BR	BRAIN	HP007002	#B ASTROCYTOMA, BENIGN	1	0	0	0	0	1.0000	0.8299
BR	BRAIN	HP007004	#M OLIGODENDROGLIOMA, MALIGNANT	0	1	0	0	0	1.0000	0.8273
BR	BRAIN	HP007008	#M GLIOMA, ANAPLASTIC, MALIGNANT	0	0	1	0	0	0.6316	0.5962
CX	CERVIX	HP044002	#B LEIOMYOMA	1	0	0	0	0	1.0000	0.8451
CX	CERVIX	HP044007	#M SCHWANNOMA, MALIGNANT	1	0	2	0	0	0.8395	0.7961
CX	CERVIX	HP044010	#B PAPILOMA, SQUAMOUS CELL	0	0	0	0	1	0.2632	0.0707
CX	CERVIX	HP044016	#M SARCOMA, ENDOMETRIAL STROMA	0	2	0	1	1	0.4502	0.4043
CX	CERVIX	HP044018	#B FIBROMA	0	0	1	0	0	0.6343	0.5988
HE	HEART	HP017013	#B MESOTHELIOMA, PARIETAL, B	0	1	0	0	0	1.0000	0.8101
HG	HARDERIAN GLANDS	HP015004	#B ADENOMA	1	3	1	0	6	0.0252	0.0158
HG	HARDERIAN GLANDS	HP015005	#M CARCINOMA	0	1	0	0	0	1.0000	0.8490
KI	KIDNEYS	HP020021	#B ADENOMA, RENAL TUBULE	1	0	0	0	0	1.0000	0.8392
KI	KIDNEYS	HP020022	#M CARCINOMA, RENAL TUBULE	1	0	0	0	0	1.0000	0.8287
LI	LIVER	HP021012	#M CHOLANGIOCARCINOMA	0	0	1	0	0	0.6012	0.6064
LI	LIVER	HP021018	#M CARCINOMA, HEPATOCELLULAR	1	2	0	1	1	0.6003	0.5671
LI	LIVER	HP021026	#B ADENOMA, HEPATOCELLULAR	1	1	2	2	2	0.2168	0.1843
LU	LUNGS	HP026010	#B ADENOMA, BRONCHIOLO-ALVEOLA	5	5	7	4	4	0.6805	0.6632
LU	LUNGS	HP026012	#B ADENOMA, BRONCHIOLO-ALVEOLA	1	0	1	0	1	0.4302	0.3873
LU	LUNGS	HP026014	#M CARCINOMA, BRONCHIOLO-ALVEOLA	1	3	3	2	5	0.0745	0.0576
LU	LUNGS	HP026023	#M OSTEOSARCOMA; UNKNOWN	0	1	0	0	0	1.0000	0.8298
MG	MAMMARY GLAND	HP027003	#M ADENOCARCINOMA	0	4	2	1	1	0.8107	0.7828
MG	MAMMARY GLAND	HP027007	#M CARCINOSARCOMA	0	0	0	0	1	0.1588	0.0277
MG	MAMMARY	HP027008	#B ADENOMA	0	0	0	1	0	0.3966	0.3009

	GLAND	8									
OV	OVARIES	HP042006	#M GRANULOSA CELL TUMOR, MALIG	0	0	0	0	1	0.1698	0.0304	
OV	OVARIES	HP042017	#B CYSTADENOMA	4	3	7	2	2	0.8284	0.8095	
OV	OVARIES	HP042022	#B CYSTADENOMA, MULTIPLE	0	0	1	0	0	0.6190	0.6563	
OV	OVARIES	HP042029	#M CYSTADENOCARCINOMA	1	0	0	0	0	1.0000	0.8321	
OV	OVARIES	HP042034	#B LUTEOMA	1	1	0	1	0	0.8417	0.7945	
PI	PITUITARY	HP040005	#B ADENOMA, PARS DISTALIS	1	4	1	1	2	0.6141	0.5874	
PI	PITUITARY	HP040008	#B ADENOMA, PARS INTERMEDIA	0	0	1	1	0	0.4341	0.4166	
RE	RECTUM	HP039005	#M ADENOCARCINOMA	0	0	0	0	1	0.1606	0.0269	
SK	SKIN	HP046003	#M OSTEOSARCOMA	0	1	0	0	0	1.0000	0.8279	
SK	SKIN	HP046015	#B TRICHOEPITHELIOMA	1	0	0	0	0	1.0000	0.8101	
SK	SKIN	HP046017	#M SCHWANNOMA, MALIGNANT	0	1	0	0	0	1.0000	0.8271	
SK	SKIN	HP046021	#M FIBROSARCOMA	1	1	0	0	2	0.2140	0.1673	
SK	SKIN	HP046024	#M SARCOMA, UNDIFFERENTIATED	0	0	0	1	0	0.3780	0.3283	
SK	SKIN	HP046025	#B BASAL CELL TUMOR, BENIGN	1	0	0	0	0	1.0000	0.8287	
SK	SKIN	HP046026	#M LIPOSARCOMA	0	1	0	0	0	1.0000	0.8287	
SK	SKIN	HP046027	#M MYXOSARCOMA	0	0	1	0	0	0.6204	0.5908	
SL	STOMACH, GLD	HP051005	#M OSTEOSARCOMA	0	1	0	0	0	1.0000	0.8277	
SL	STOMACH, GLD	HP051015	#M LEIOMYOSARCOMA	0	0	0	0	1	0.1720	0.0313	
SM	SKELETAL MUSCLE	HP045007	#M FIBROSARCOMA	1	0	0	0	0	1.0000	0.8269	
SY	SYSTEMIC TUMORS	HP068001	#M SARCOMA, HISTIOCYTIC	6	5	6	6	5	0.4684	0.4532	
SY	SYSTEMIC TUMORS	HP068002	#M LYMPHOMA, MALIGNANT	17	15	7	12	7	0.9648	0.9576	
SY	SYSTEMIC TUMORS	HP068003	#M FIBROUS HISTIOCYTOMA, MALIG	1	0	1	1	0	0.6782	0.6587	
SY	SYSTEMIC TUMORS	HP068004	#M HEMANGIOSARCOMA	4	5	3	7	2	0.7176	0.7005	
SY	SYSTEMIC TUMORS	HP068005	#B HEMANGIOMA	3	7	1	3	2	0.9063	0.8888	
TG	THYROID GLANDS	HP053005	#B ADENOMA, FOLLICULAR CELL, M	1	0	0	0	0	1.0000	0.8064	
TH	THYMUS GLAND	HP061009	#M THYMOMA, MALIGNANT	0	0	0	1	0	0.3868	0.3288	
TO	TONGUE	HP023002	#B PAPILOMA, SQUAMOUS CELL	1	0	0	0	0	1.0000	0.8261	
UT	UTERUS	HP060002	#M SARCOMA, ENDOMETRIAL STROMA	4	2	3	3	3	0.4904	0.4670	
UT	UTERUS	HP060009	#B POLYP, ENDOMETRIAL STROMAL	4	13	8	6	5	0.8631	0.8496	
UT	UTERUS	HP060012	#B LEIOMYOMA	0	1	1	1	0	0.7081	0.6928	
UT	UTERUS	HP06001	#M LEIOMYOSARCOMA	0	0	0	0	2	0.0272	0.0035	

		7								
UT	UTERUS	HP06001 8	#M CARCINOMA	0	0	1	0	1	0.1744	0.1169
UT	UTERUS	HP06002 4	#M OSTEOSARCOMA	0	1	0	0	0	1.0000	0.8266
VA	VAGINA	HP06300 7	#B PAPILLOMA, SQUAMOUS CELL	0	0	0	0	1	0.2000	0.0401
VA	VAGINA	HP06301 2	#M LEIOMYOSARCOMA	0	1	0	0	0	1.0000	0.8392
UT	UTERUS		#B LEIOMA AND #M LEIOMYOSARCOMA	0	1	1	1	2	0.1346	0.1029

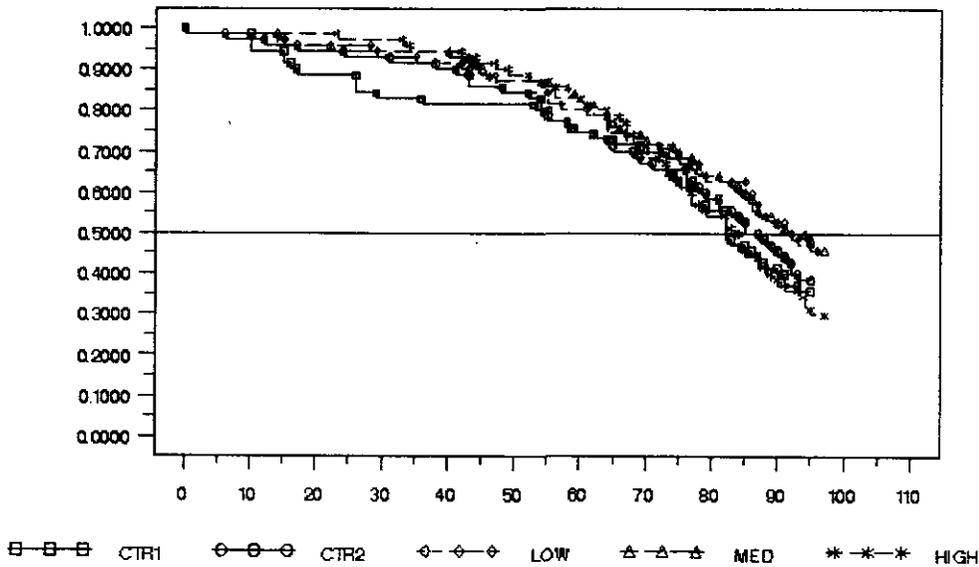
Table 40: Mortality by Time Interval for Male Mice of Study 190-830

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR1	0-52	70	12	58	82.9	17.1
	53-78	58	15	43	61.4	38.6
	79-91	43	15	28	40.0	60.0
	92-96	28	3	25	35.7	64.3
	FINALKILL 97-104	25	25	0		
CTR2	0-52	70	11	59	84.3	15.7
	53-78	59	16	43	61.4	38.6
	79-91	43	12	31	44.3	55.7
	92-96	31	4	27	38.6	61.4
	FINALKILL 97-104	27	27	0		
LOW	0-52	70	10	60	85.7	14.3
	53-78	60	13	47	67.1	32.9
	79-91	47	10	37	52.9	47.1
	92-96	37	5	32	45.7	54.3
	FINALKILL 97-104	32	32	0		
MED	0-52	70	8	62	88.6	11.4
	53-78	62	16	46	65.7	34.3
	79-91	46	10	36	51.4	48.6
	92-96	36	2	34	48.6	51.4
	FINALKILL 97-104	34	34	0		
HIGH	0-52	70	8	62	88.6	11.4
	53-78	62	22	40	57.1	42.9
	79-91	40	14	26	37.1	62.9
	92-96	26	4	22	31.4	68.6
	FINALKILL 97-104	22	22	0		

**Table 41: Mortality Trend for Male Mice of Study 190-830**

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	4.2213	0.2385	3.7279	0.2924
Dose-Mortality Trend	0.1357	0.7126	0.0016	0.9677
Homogeneity	4.3570	0.3598	3.7296	0.4438

**Figure 8: Kaplan Meier Survival Curves for Male Mice in Study 190-830**



**Table 42: Tumor Trends for Male Mice of Study 190-830**

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR 1	CTR 2	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
AC	ADRENAL CORTEX	HP002015	#B ADENOMA, A CELL	0	1	0	1	0	0.6610	0.6400
AC	ADRENAL CORTEX	HP002016	#B ADENOMA	1	0	1	0	1	0.3849	0.3367
AC	ADRENAL CORTEX	HP002017	#B ADENOMA, MULTIPLE	1	0	0	0	0	1.0000	0.8370

AM	ADRENAL MEDULLA	HP00300 3	#B PHEOCHROMOCYTOMA, BENIGN	0	0	0	0	1	0.2625	0.0696
BR	BRAIN	HP00700 2	#M ASTROCYTOMA, MALIGNANT	1	0	0	0	0	1.0000	0.8353
DU	DUODENUM	HP01000 3	#M CARCINOMA	0	0	0	0	2	0.0361	0.0064
EP	EPIDIDYMIDES	HP01201 2	#B ADENOMA, INTERSTITIAL CELL	1	0	0	0	0	1.0000	0.8379
HG	HARDERIAN GLANDS	HP01500 7	#B ADENOMA	5	3	4	2	3	0.7730	0.7540
HG	HARDERIAN GLANDS	HP01501 3	#M CARCINOMA	0	0	0	2	0	0.3714	0.2727
HG	HARDERIAN GLANDS	HP01501 4	#B ADENOMA, MULTIPLE	0	0	0	1	0	0.4000	0.3224
KI	KIDNEYS	HP02002 0	#B ADENOMA, RENAL TUBULE	1	1	0	1	1	0.4035	0.3484
KI	KIDNEYS	HP02002 1	#M CARCINOMA, RENAL TUBULE	0	0	0	0	1	0.1795	0.0353
KI	KIDNEYS	HP02002 2	#B ADENOMA, RENAL TUBULE, MULT	0	0	0	0	1	0.2295	0.0522
KI	KIDNEYS	HP02002 9	#M RENAL MESENCHYMAL TUMOR, MA	0	0	1	0	0	0.6286	0.6035
LI	LIVER	HP02101 9	#B ADENOMA, HEPATOCELLULAR	2	9	9	6	1	0.9675	0.9579
LI	LIVER	HP02102 2	#M CARCINOMA, HEPATOCELLULAR	4	7	3	6	1	0.9445	0.9298
LI	LIVER	HP02102 3	#M CARCINOMA, HEPATOCELLULAR,	2	0	1	0	0	0.9476	0.8981
LI	LIVER	HP02102 4	#B ADENOMA, HEPATOCELLULAR, MU	0	0	1	0	0	0.5574	0.6070
LU	LUNGS	HP02601 5	#B ADENOMA, BRONCHIOLO-ALVEOLA	1	2	0	2	3	0.1295	0.1017
LU	LUNGS	HP02601 7	#B ADENOMA, BRONCHIOLO-ALVEOLA	6	7	9	10	7	0.4078	0.3916
LU	LUNGS	HP02601 9	#M CARCINOMA, BRONCHIOLO-ALVEO	4	2	3	4	2	0.5467	0.5229
PA	PANCREAS	HP03401 1	#B ADENOMA, ISLET CELL	0	2	0	0	0	1.0000	0.9088
SK	SKIN	HP04600 9	#B PAPILLOMA, SQUAMOUS CELL	0	1	1	0	0	0.8225	0.8009
SK	SKIN	HP04602 0	#B KERATOACANTHOMA, BENIGN, MU	0	0	0	1	0	0.4634	0.4122
SK	SKIN	HP04602 2	#M LIPOSARCOMA	0	0	1	0	0	0.6258	0.6039
SM	SKELETAL MUSCLE	HP04500 5	#M CARCINOMA, SQUAMOUS; UNKNOW	1	0	0	0	0	1.0000	0.8297
SM	SKELETAL MUSCLE	HP04500 9	#M OSTEOSARCOMA; UNKNOWN	1	0	0	0	0	1.0000	0.8329
SN	STOMACH, NONGLD	HP05200 5	#M CARCINOMA, SQUAMOUS CELL	0	0	1	0	0	0.6258	0.6066
SY	SYSTEMIC TUMORS	HP06900 1	#M LYMPHOMA, MALIGNANT	1	6	2	2	7	0.0945	0.0784
SY	SYSTEMIC TUMORS	HP06900 2	#M LEUKEMIA	1	0	0	0	0	1.0000	0.8348
SY	SYSTEMIC TUMORS	HP06900 3	#M SARCOMA, HISTIOCYTIC	2	0	3	3	0	0.7165	0.6897
SY	SYSTEMIC TUMORS	HP06900 4	#M HEMANGIOSARCOMA	1	2	6	2	1	0.6112	0.5856
SY	SYSTEMIC TUMORS	HP06900 5	#M LEUKEMIA, GRANULOCYTIC	0	0	1	0	0	0.6174	0.6209

SY	SYSTEMIC TUMORS	HP06900 6	#B HEMANGIOMA	2	2	1	2	2	0.4839	0.4573
TE	TESTES	HP06001 0	#B ADENOMA, INTERSTITIAL CELL	0	0	1	1	0	0.4600	0.4400
TG	THYROID GLANDS	HP05301 1	#B ADENOMA, FOLLICULAR CELL	0	0	1	1	0	0.5095	0.4614
ZG	ZYMBAL'S GLAND	HP02500 9	#M CARCINOMA, SQUAMOUS CELL	0	0	0	1	0	0.4250	0.3683

### 3.7.5 Validity of Male and Female Mouse Study 190-830

There was no statistically significant tumor finding among the male mice of study 190-830 and therefore, its validity needs to be evaluated. Recognizing that the number of leiomyosarcoma in the uterus was small and not clearly statistically significant, this reviewer also evaluated the validity of the female mouse study 190-830. As noted above for the 190-823 rat study, two criteria are set up for this purpose:

- i) Were sufficient numbers of animals exposed long enough to allow for late-developing tumors?
- ii) Did the high dose provide a sufficient tumor challenge?

The number of animals and length of exposure can be assessed at weeks 52, 80-90, and at termination, but are generally considered satisfied if 20-30 animals survive through weeks 80-90. At least 50 percent of the original 70 animals were alive at week 84, providing a sufficient number of animals, which were exposed long enough. In determining whether the high dose provided an adequate tumor challenge, one expects the high dose to be close to the MTD. The following criteria are employed in this assessment:

- iii) A dose is considered adequate if there is a detectable reduction in average body weight of up to 10 % in a dosed group relative to the controls.
- iv) A dose is considered adequate if the dosed animals show a slightly increased mortality compared to the controls.
- v) A dose is considered an MTD if the dosed animals exhibit severe toxic effects attributed to the chemical. This latter evaluation is performed by the pharmacologist/toxicologist.

There was slight cycling of increased and decreased bodyweights by the treated animals with respect to the controls. However, the mean bodyweights were almost indistinguishable from the controls throughout the study. For the high dose male mice, 22/70 (31.4%) lived till terminal sacrifice at week 96. For the combined controls 52/140 (37.1%) lived till this terminal sacrifice. This minor difference in mortality may not be sufficient to indicate that the high dose was close to the MTD. For the females, the same percentages of survival occurred at the end of study, namely week 103. Therefore, it appears that for the female mice of study 190-830 the high dose did not reach the MTD either.

## **4 Conclusions**

### **4.1 Statistical Evaluation of Evidence**

As discussed under 2.1.2 Major Statistical Issues, many of the studies had shortcomings. Therefore, the statistical results may have a wider margin of error than usual. In addition, no further correction of multiplicity was undertaken, though more than twice the usual number of carcinogenicity studies was reviewed.

In the opinion of this reviewer,

Study 190-833 in rats with (R,S)-zopiclone clearly showed statistically significant findings in both sexes (mammary gland carcinomas among the females and follicular cell carcinomas of the thyroid among the males).

Study 190-833A is not a new study but the re-evaluation by a pathology-working group of the findings that reached statistical significance in study 190-833. Though the tumor incidences were changed for both tumor types, the results remained statistically significant.

Study 190-823 was a gavage study with eszopiclone in rats. It did not show any significant increases in tumor findings. The study is considered valid for both genders.

Study 190-834 in mice with (R,S)-zopiclone clearly showed statistically significant findings in both sexes (malignant pulmonary carcinoma and benign cyst-adenoma among females and malignant sarcoma of the skin among males).

Study 190-834A is not a new study but the re-evaluation by a pathology-working group of the findings that reached statistical significance in study 190-834. Though the tumor incidences were changed for pulmonary tumors, the results remained statistically significant for some statistical tests, if not for all. The incidence numbers for skin sarcomas were not changed.

Study 190-844 was a special study, which was to show that fighting due to group caging was responsible for the increase in subcutis sarcomas among the male mice. Based on a previous OB review, the study had deficiencies and inconsistencies, which could affect the conclusions. In this reviewer's opinion, at least a partial effect of the compound on the increase in skin tumors among group-housed animals cannot be ruled out.

Study 190-830 was a gavage study with eszopiclone in mice. It showed an increase in leiomyosarcoma in the uterus among the females, which reached statistical significance by the asymptotic tests but not by the more appropriate exact tests. There were no significant increases in tumor findings among the males. There were sufficient numbers of animals living long enough, but it is questionable for either gender whether the high dose reached the MTD.

The sponsor developed a mathematical model from the data of control B6C3F1 male mice about the relationship of incrustations resulting from biting and the onset of fibrosarcomas. Applying this model to mice treated with zopiclone may not be valid, however, because a treatment effect on mortality, body weight, or drug toxicities may have changed the treated animals such that they are no longer from the same distribution as the untreated animals were.

## **5 Appendix**

### ***5.1 Comments Regarding Sponsor's Report ST/CRV/TOX Nr. 198, (Mathematical Model, 1988)***

The sponsor's mathematical model has been previously submitted to IND and was reviewed by Dr. Karl Lin (HFD-715) in August of 1988. This reviewer accepts full responsibility of inadvertently misrepresenting Dr. Karl Lin's evaluation. The sponsor postulated that there was an association between fighting lesions and tumors of the dorsal subcutis. Based on the data from twelve different gang-housed control groups, a mathematical model was developed for the expected percentage of fibrosarcomas as a function of the mean percentage of animals with incrustations, the mean duration of incrustations, and the onset time of fibrosarcomas in male B6C3F1 mice. The sponsor then concluded that the high expected fibrosarcomas in the treated mice could be fully accounted for by the high incidence of incrustations and the duration of the incrustations. Dr. Karl Lin found that the sponsor failed to rule out a possibly significant direct or indirect drug effect. Further, the approach did not address the tumorigenic potential of zopiclone. He suggested different statistical methods to test for the carcinogenic potential of zopiclone in the male mice of Study 190-834 while adjusting for factors such as mortality differences, incrustation occurrence, onset time of fibrosarcomas, etc. This reviewer agrees with Dr. Karl Lin's comments and conclusions.

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

/s/

-----  
Roswitha Kelly  
12/4/03 09:41:15 AM  
BIOMETRICS

Kooros Mahjoob  
12/4/03 04:30:46 PM  
BIOMETRICS



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

Clinical Studies

**NDA #** 21-476  
**Drug Name** ESTORRA (Esopiclone) tablets  
**Indication** — insomnia  
**Sponsor** SEPRACOR INC.  
**Filing Date** March 17, 2003  
**Review Priority** Standard

**Biometrics Division:** Biometrics I (HFD 710)  
**Statistical Reviewer:** Ohidul Siddiqui  
**Concurring Reviewers:** Kun Jin, , Ph.D., Team Leader  
Kooros Mahjoob, Deputy Director

**Medical Division:** Neuropharmacological Drug Products (HFD 120)  
**Clinical Team:** Karen Brugee, M.D., Clinical Reviewer  
Paul Andreason, M.D., Team Leader  
Russell Katz, M.D., Director  
**Project Manager:** Merril Mille

**Keywords:** Transient and chronic insomnia, objective latency to persistent sleep (LPS)

## TABLE OF CONTENTS

<b>1. EXECUTIVE SUMMARY OF STATISTICAL FINDINGS .....</b>	<b>3</b>
1.1 STATISTICAL ISSUES.....	3
<b>2. INTRODUCTION .....</b>	<b>4</b>
2.1 DESIGN AND KEY EFFICACY ENDPOINTS OF EACH OF THE SIX STUDIES:.....	7
<b>3. EFFICACY EVALUATION .....</b>	<b>13</b>
3.1 PRIMARY MEASURES.....	13
3.2 KEY SECONDARY MEASURE.....	13
3.3 HANDLING DROPOUTS AND MISSING DATA .....	13
3.4 HANDLING SMALL CENTERS.....	13
3.5 PRIMARY ANALYSIS .....	14
3.6 KEY SECONDARY ANALYSES.....	14
3.7 MULTIPLE COMPARISONS/MULTIPLICITY .....	15
3.8 INTERIM ANALYSES AND DATA MONITORING .....	17
3.9 SUBGROUP ANALYSES .....	17
<b>4. SPONSOR' FINDINGS .....</b>	<b>17</b>
4.1 SUBJECT DISPOSITION.....	17
4.2 SUBJECT DEMOGRAPHICS.....	18
4.3 EFFICACY FINDINGS OF STUDY 190-026.....	22
4.4 EFFICACY FINDINGS OF STUDY 190-046.....	22
4.5 EFFICACY FINDINGS OF STUDY 190-045.....	22
4.6 EFFICACY FINDINGS OF STUDY 190-049.....	23
4.7 EFFICACY FINDINGS OF STUDY 190-047.....	23
4.8 EFFICACY FINDINGS OF STUDY 190-048.....	23
4.9 SUBGROUP ANALYSES FINDINGS .....	23
<b>5. SPONSOR'S FINAL CONCLUSION .....</b>	<b>25</b>
<b>6. REVIEWER'S ANALYSES AND COMMENTS .....</b>	<b>25</b>
<b>7. PATIENT DROPOUT RATES.....</b>	<b>25</b>
<b>8. ISSUES RELATED TO THIS NDA.....</b>	<b>25</b>
<b>9. REVIEWER'S OVERALL CONCLUSION.....</b>	<b>26</b>

## 1. EXECUTIVE SUMMARY OF STATISTICAL FINDINGS

The sponsor submitted results of six randomized, double-blinded, placebo controlled studies to demonstrate the efficacy and safety of ESTORRA (Esopiclone) for the treatment of transient and chronic insomnia. Among the six studies, four studies were conducted on adult subjects (21-64 years ages), and two studies were conducted on elderly (64-86 years ages) subjects.

In adult subjects (for both transient and chronic insomnia), Esopiclone at 2.0 mg and above consistently produced statistically significant effective as compared to placebo based on the primary measures- objective latency to persistent sleep (LPS) and subjective sleep latency. The 3.0 mg dose of Esopiclone consistently produced numerically better improvements than the 2.0 mg dose.

In elderly subjects, the 2.0 mg dose of Esopiclone similarly appeared statistically significant effective as compared to placebo based on the primary measures- LPS and subjective sleep latency.

The results of the six controlled randomized studies demonstrate that the nighttime administration of Esopiclone 2.0 mg will provide effective treatment of transient and chronic insomnia for both adult and elderly subjects.

### 1.1 STATISTICAL ISSUES

In study 190-049, the dropout rates were 43%, 39% in placebo and Esopiclone 3.0 mg arms, respectively. As the dropout rates are higher, the findings from LOCF analysis as the primary statistical analysis method are questionable.

The sponsor reported several errors in the study reports of the three studies (190-026, 190-045, and 190-048). The case report forms (CRFs) of the patients were not submitted with this NDA. Therefore, submitted patients' data were not validated through the individual patient's CRF.

## 2. INTRODUCTION

The sponsor has submitted results of six randomized, double-blinded, placebo controlled studies to demonstrate the efficacy and safety of ESTORRA (Esopiclone) for the treatment of transient and chronic insomnia. All of the studies were conducted in the U.S. and Canada.

Table 1 lists an overview of the designs of the studies. Table 2 lists the inclusion criteria of patients in each of the six studies. Table 3 lists the primary objectives of each of the studies. Tables 4, 5, and 6 list the patient disposition, primary efficacy results, and key secondary efficacy results of the studies.

Table 1: Overview of Designs of the six Primary Placebo Controlled Studies.

Study	Indication (Population)	Design	Esopiclone Doses (mg)	Duration	Total Randomized	Primary and Key Secondary Efficacy Endpoints
<b>ADULT SUBJECTS</b>						
<b>190-026</b> Phase II/III, pivotal for transient insomnia	Transient Insomnia (non-elderly adults aged 25 to 50)	Randomized, double-blind, placebo-controlled, parallel group, multi-center	1.0*, 2.0*, 3.0, 3.5	1 night	436*	<b>Primary:</b> objective latency to persistent sleep (LPS) <b>Key secondary:</b> objective sleep efficiency
<b>190-046</b> Phase III, pivotal for chronic insomnia	Chronic Insomnia (non-elderly adults aged 21 to 64)	Randomized, double-blind, placebo-controlled, parallel group, multi-center	2.0, 3.0	44 nights	308	<b>Primary:</b> objective LPS <b>Key secondary:</b> objective sleep efficiency, objective wake time after sleep onset (WASO)
<b>190-045</b> Phase II/III, supportive for chronic insomnia	Chronic Insomnia (non-elderly adults aged 21 to 64)	Randomized, double-blind, placebo- and active-controlled, 6-way cross-over, multi-center	1.0, 2.0, 2.5, 3.0	2 nights on each treatment (12 nights total)	65	<b>Primary:</b> objective LPS <b>Key secondary:</b> objective sleep efficiency, objective WASO
<b>190-049</b> Phase III, long-term study, supportive for chronic insomnia	Chronic Insomnia (non-elderly adults aged 21 to 64)	Randomized, double-blind and open-label, multi-center, outpatient study.	3.0	12 months (6 months double-blind and 6 months open-label)	791	<b>Primary:</b> subjective sleep latency <b>Key secondary:</b> subjective total sleep time

<b>ELDERLY SUBJECTS</b>						
<b>190-047</b> Phase III, pivotal for chronic insomnia in elderly	Chronic Insomnia (elderly aged 65 to 86)	Randomized, double-blind, placebo- controlled, parallel group, multi-center	1.5*, 2.0	14 nights	292**	<b>Co-primary:</b> objective LPS and objective sleep efficiency <b>Key secondary:</b> objective WASO
<b>190-048</b> Phase III, pivotal For chronic Insomnia in Elderly	Chronic Insomnia (elderly aged 64 to 85)	Randomized, double-blind, placebo- controlled, parallel group, multi-center	1.0, 2.0	14 nights	234	<b>Primary:</b> subjective sleep latency <b>Key secondary:</b> subjective total sleep time

\*The Esopiclone 1.0 mg and 2.0mg treatment arms were dropped per Protocol Amendment 1. The total number randomized subjects (in Placebo, 3.0 and 3.5 mg) for this study is 292.

\*\*The Esopiclone 1.5 mg treatment arm was dropped per Protocol Amendment 2. The total number randomized for this study, 292 subjects, includes the 28 subjects who were randomized to the Esopiclone 1.5 mg arm.

Table 2: Overview of major Inclusion Criteria of each of the studies.

Study#	Inclusion Criteria
<b>Studies in Adult subjects</b>	
190-026	Healthy males or females between 25 and 50 years of age and willing to remain in the sleep center overnight for approximately 12 to 14 hours. Subjects with evidence of any clinically significant unstable medical abnormality, chronic disease, or a history of a clinically significant abnormality of the cardiovascular, respiratory, hepatic, or renal systems were Excluded.
190-046	Males or females between 21 and 64 (inclusive) years of age; met DSM-IV criteria for primary insomnia and reported sleeping no more than 6.5 hours per night and taking More than 30 minutes each night to fall asleep for at least one month; during screening polysomnography (PSG), latency to persistent sleep (LPS) mean of two nights $\geq 20$ minutes with neither night $< 15$ minutes, plus either a total sleep time mean of two nights $\leq 420$ minutes or a wake time after sleep onset (WASO) mean of two nights $\geq 20$ minutes with neither night $< 15$ minutes.
190-049	Males or females between 21 and 64 years of age who report sleeping no more than 6.5 hours per night and/or taking more than 30 minutes each night to fall asleep for at least one month prior to screening.
190-045	Males or females between 21 and 64 years of age; met DSM-IV criteria for primary insomnia and reported sleeping no more than 6.5 hours per night and taking more than 30 minutes each night to fall asleep for at least one month; during screening polysomnography (PSG), latency to persistent sleep (LPS) of at least two nights $\geq 20$ minutes with none of three nights $< 15$ minutes, plus either a total sleep time (TST) of at least two nights $\leq 420$ minutes or a wake time after sleep onset (WASO) of at least two nights $\geq 20$ minutes with none of three nights $< 15$ minutes.

<b>Studies in Elderly subjects</b>	
190-047	Males or females between 65 and 85 years of age; met DSM-IV criteria for primary insomnia and reported sleeping no more than 6.5 hours per night and taking more than 30 minutes each night to fall asleep for at least one month; during screening polysomnography (PSG), latency to persistent sleep (LPS) mean of two nights $\geq 20$ minutes with neither night $< 15$ minutes and a wake time after sleep onset (WASO) mean of two nights $\geq 20$ minutes with neither night $< 15$ minutes.
190-048	Males or females between 65 and 85 years of age; met DSM-IV criteria for primary insomnia and reported sleeping no more than 6.5 hours per night and taking more than 30 minutes each night to fall asleep for at least one month.

Table 3: Primary objectives of each of the studies.

Study#	Primary Objectives
<b>Studies in Adult subjects</b>	
190-026	The primary objective was to determine the hypnotic efficacy (as measured by latency to persistent sleep), safety, and tolerability of Esopiclone 3.0 mg and 3.5 mg compared with placebo in healthy adults using a model of transient insomnia (First Night Effect Model).
190-046	The primary objective was to evaluate the hypnotic efficacy and safety of two doses of Esopiclone (2mg, 3mg) compared to placebo in adult subjects with primary insomnia.
190-049	The primary objective was to evaluate the safety of Esopiclone 3 mg administered for 12 months in subjects with insomnia.
190-045	The primary objective was to evaluate the hypnotic efficacy of three doses of Esopiclone (2.0 mg, 2.5 mg, and 3.0 mg) compared with placebo in adult subjects with primary insomnia.
<b>Studies in Elderly subjects</b>	
190-047	The primary objective was to evaluate objectively the hypnotic efficacy and safety of Esopiclone 2.0 mg administered for two weeks in elderly subjects with primary insomnia.
190-048	The primary objective was to evaluate the hypnotic efficacy and safety of Esopiclone 1.0 and 2.0 mg administered for two weeks in elderly subjects with primary insomnia.

Table 4: Subject Disposition

Study #	Disposition	Placebo n (%)	Esopiclone 2.0 mg n (%)	Esopiclone 3.0 mg n (%)
190-046	Randomized	99(100.0)	104 (100.0)	105 (100.0)
	Completed	94 (94.9)	97 (93.3)	101 (96.2)
	Discontinued	5 (5.1)	7 (6.7)	4 (3.8)
	AE	0 (0.0)	3 (2.9)	0 (0.0)
	Protocol violation	2 (2.0)	2 (1.9)	0 (0.0)
	Voluntary withdrawal	2 (2.0)	2 (1.9)	2 (1.9)
	Did not meet entry Criteria	0 (0.0)	0 (0.0)	1 (1.0)
	Other	1 (1.0)	0 (0.0)	1 (1.0)
190-049		Placebo n (%)		Esopiclone 3.0 mg n (%)
	Randomized	196		595
	Received treatment	195 (100)		593 (100)
	Completed double blind phase	111 (57)		360 (61)
	Discontinued	84 (43)		233 (39)
	AE	14 (7)		76 (13)
	Protocol violation	7 (4)		17 (3)
	Voluntary withdrawal	50 (26)		81 (14)
	Lost to follow-up	8 (4)		52 (9)
	Did not meet entry Criteria	1 (1)		0 (0)
Other	4 (2)		7 (1)	

		Placebo n (%)	Esopiclone 1.5 mg n (%)	Esopiclone 2.0 mg n (%)
190-047	Randomized	128	28	136
	Completed	122 (95.3)	28 (100.0)	133 (97.8)
	Discontinued	6 (4.7)	0 (0.0)	3 (2.2)
	AE	3 (2.3)	0 (0.0)	2 (1.5)
	Protocol violation	1 (0.8)	0 (0.0)	0 (0.0)
	Voluntary withdrawal	2 (1.6)	0 (0.0)	0 (0.0)
	Did not meet entry Criteria	0 (0.0)	0 (0.0)	1 (0.7)
		Placebo n (%)	Esopiclone 1.0 mg n (%)	Esopiclone 2.0 mg n (%)
190-048	Randomized	81	74	79
	Received treatment	80 (100)	72 (100)	79 (100)
	Completed	73 (91.3)	67 (93.1)	70 (88.6)
	Discontinued	7 (8.8)	5 (6.9)	9 (11.4)
	AE	5 (6.3)	1 (1.4)	2 (2.5)
	Voluntary withdrawal	2 (2.5)	2 (2.8)	7 (8.9)
	Did not meet entry Criteria	0 (0.0)	1 (1.4)	0 (0.0)
	Other	0 (0.0)	1 (1.4)	0 (0.0)

## 2.1 DESIGN AND KEY EFFICACY ENDPOINTS OF EACH OF THE SIX STUDIES:

### STUDY 190-026: AN EFFICACY, SAFETY AND TOLERABILITY STUDY OF (S)-ZOPICLONE IN SUBJECTS WITH TRANSIENT INSOMNIA

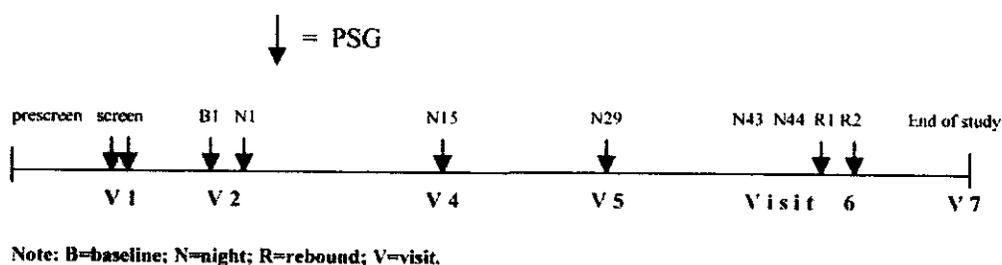
Study 190-026 was a prospective, randomized, multi-center, double-blind, placebo-controlled, five-arm (2:1:2:2:2) parallel-group, night-time efficacy and safety study in healthy male and female subjects with transient insomnia. The first night in the sleep-disturbing environment of a sleep laboratory for otherwise normal sleepers is a well-validated model of transient insomnia (First Night Effect model), and was used in Study 190-026. Following the screening phase and pre-dose assessments, 436 subjects were randomized: 98 subjects to the placebo, 47 subjects to Esopiclone 1.0 mg, 97 subjects to Esopiclone 2.0 mg, 98 subjects to Esopiclone 3.0 mg arms, and 96 subjects to Esopiclone 3.5 mg. The Esopiclone 1.0 mg and 2.0mg treatment arms were dropped per Protocol Amendment 1. Each subject received a single dose of study medication at one 12-14 hour overnight dosing visit. Hypnotic efficacy was measured objectively [polysomnographic (PSG) recording] and subjectively (morning questionnaires). Centralized scoring was used for evaluation of the PSG recordings.

The primary efficacy endpoint was objective latency to persistent sleep (LPS), defined as the time from lights out to the first of 20 consecutive 30-second epochs (10 minutes) of sleep. Objective sleep efficiency, defined as the total sleep time expressed as a percent of the total recording time, was the key secondary endpoint.

**STUDY 190-046: A RANDOMIZED DOUBLE-BLIND, PLACEBO-CONTROLLED PARALLEL GROUP STUDY OF THE EFFICACY AND SAFETY OF ESOPICLONE IN THE TREATMENT OF ADULT SUBJECTS WITH PRIMARY INSOMNIA**

Study 190-046 was a prospective, randomized, multi-center, double-blind, placebo-controlled, three-arm (1:1:1) parallel-group, hypnotic efficacy and safety study in adult subjects with primary insomnia as defined by DSM-IV criteria. Following the screening phase and pre-dose assessments, 308 subjects were randomized: 99 subjects to placebo, 104 subjects to Esopiclone 2.0 mg, and 105 subjects to Esopiclone 3.0 mg.

Hypnotic efficacy was measured objectively (via PSGs at Nights 1, 15, 29, 45, and 46) and subjectively (via questionnaires the mornings after Nights 1, 15, 29, 43, 44, 45, and 46). A study schematic is provided below.



Each subject was to receive 44 days of treatment with 2.0 mg Esopiclone, 3.0 mg Esopiclone, or placebo. All subjects received single-blind placebo during screening, on the baseline night, and on Days 45 (Rebound 1 [R1]) and 46 (Rebound 2 [R2]) to evaluate rebound effect. All PSG nights were conducted in the same manner. Each night prior to the PSG, subjects received study drug (single-blind placebo or double-blind treatment). Each morning following the PSG, subjects completed a morning questionnaire. On study days between visits, subjects took daily study medication at home.

Study participation was approximately ten weeks and involved six visits (three multi-night and three single-night visits): a screening visit, four sleep lab visits (baseline and Night 1; Night 15; Night 29; and Nights 45 and 46), and an end-of-study visit.

**Screening (Visit 1):** Two nights of PSGs were performed to assess sleep and physiological measurements and to rule out the presence of other sleep disorders. Eligible subjects returned within 2-5 days to be admitted to the sleep lab for two nights and to be randomized to one of three treatment arms.

**Visits 2 and 6:** Subjects were admitted to the sleep lab for two consecutive nights. At Visit 2, subjects received single-blind placebo and had a one-night baseline PSG. Subjects then began dosing with assigned treatment, and PSG recordings were obtained on Night 1. At Visit 6, subjects were admitted to the sleep lab for single-blind placebo administration and PSG recordings to evaluate rebound effect on Nights 45 (R1) and 46 (R2).

Visits 4 and 5: Subjects were admitted to the sleep lab for PSGs on Nights 15 (Visit 4) and 29 (Visit 5).

Visit 7: Subjects returned 5-7 days after their last PSG night (Rebound night 2) or upon early discontinuation for an end of study visit.

The primary efficacy measure was objective LPS. The key secondary endpoints were objective sleep efficiency and objective WASO. Objective WASO was defined as the total wake time after the onset of persistent sleep to the end of the recording. Each of the measures LPS, Sleep efficiency, and WASO was averaged over the double-blind period.

**STUDY 190-045: A DOUBLE- BLIND, PLACEBO-CONTROLLED RANDOMIZED SIX-WAY CROSS-OVER STUDY OF THE EFFICACY AND SAFETY OF (S)-ZOPICLONE IN THE TREATMENT OF ADULT SUBJECTS WITH PRIMARY INSOMNIA**

Study 190-045 was a prospective, randomized, multi-center, sleep laboratory/outpatient, double-blind, placebo-controlled, six-way crossover, hypnotic efficacy and safety study in adult subjects with primary insomnia as defined by DSM-IV criteria. Sixty-five (65) subjects who met the inclusion criteria were randomized to one of six treatment sequences. Centralized scoring was used for evaluation of the PSG recordings. Study participation was approximately eight weeks and involved eight visits: a screening multi-night visit, six dosing multi-night visits, and an end-of-study visit.

Screening (Visit 1): Up to three nights of PSGs were performed to assess sleep and physiological measurements and to rule out the presence of other sleep disorders. Each night prior to PSG, subjects received single-blind placebo. Each morning following PSG, subjects completed a morning questionnaire. Eligible subjects returned within 2-5 days to be admitted to the sleep lab and to be randomized to one of six treatment sequences.

Visits 2, 3, 4, 5, 6 and 7: Each dose (Esopiclone 1.0, 2.0, 2.5, or 3.0 mg; placebo; or zolpidem 10 mg) was given as a night-time dose for two consecutive days with a 3-7 day washout period between visits, during which no treatment was given. Polysomnographic measurements were done on both nights. Each morning following PSG, subjects completed a morning questionnaire.

Visit 8: Subjects returned within 5-7 days after their last dose or upon early discontinuation for an end of study visit.

The primary efficacy measure was objective LPS. Objective sleep efficiency and objective WASO were the key secondary endpoints.

**STUDY 190-049: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED AND OPEN-LABEL TWELVE MONTH STUDY OF THE SAFETY OF (S)-ZOPICLONE IN ADULT SUBJECTS WITH INSOMNIA**

Study 190-049 was a twelve-month, randomized, double-blind and open-label, multi-center, outpatient study to determine the safety of Esopiclone in the treatment of adult subjects with primary insomnia. Seven hundred ninety-one subjects were randomized using a 3:1 ratio to receive one of the two double-blind treatments, Esopiclone 3.0 mg or placebo, for six months. All subjects who completed six months of treatment (471 subjects) were eligible to receive open-label Esopiclone 3.0 mg for an additional six months.

Visit 1: Screening period (maximum 14 days) during which eligibility was to be assessed.  
Visit 2: Baseline, subjects were to return to the clinic to confirm eligibility, enroll and begin double-blind dosing.

Visit 3-8 (double-blind): Subjects were to return for safety and compliance assessments and medication refills monthly (every 30 days  $\pm$  5 days). Once a week, subjects were to utilize an interactive voice response system (IVRS) to assess subjective sleep quality, quality of life and subjective work/school/home productivity for the preceding week.  
Visit 9-14 (open-label): Subjects were to return for safety and compliance assessments and medication refills monthly (every 30 days  $\pm$  5 days). Once a week, subjects were to utilize an interactive voice response system (IVRS) to assess subjective sleep quality, quality of life and subjective work/school/home productivity.

Visit 15: Regardless of time on study, all subjects were to return for an end-of-study visit approximately one week (5-7 days) after final dosing.

The primary efficacy measure was subjective sleep latency, defined as the time in minutes after lights out until sleep onset. Subjective total sleep time (total minutes of sleep) was the key secondary efficacy variable. The primary and key secondary efficacy endpoints were the last-three-month average subjective sleep latency during the double-blind treatment period and the last-three-month average subjective total sleep time during the double-blind treatment period, respectively.

For each subject, "Month 1-3 Average" represents the average over Months 1-3 of the double-blind period, and "Month 4-6 Average" represents the average over Months 4-6 of the double-blind period based on the last observation carried forward algorithm. For each subject, each month represents the average of all weekly data collected during that month. In the event that no data were available for a month, the previous month average was imputed. In addition, if only one value was available for a month, then the mean of that value and the previous month average was used.

**STUDY 190-047: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PARALLEL, TWO-WEEK OBJECTIVE EFFICACY AND SAFETY STUDY OF ESOPICLONE IN ELDERLY SUBJECTS WITH PRIMARY INSOMNIA**

Study 190-047 was a randomized, double-blind, placebo-controlled, two-arm (1:1) parallel-group, objective efficacy and safety study in elderly adult subjects with primary insomnia as defined by DSM-IV criteria. Following a screening phase, 292 subjects were randomized to double-blind treatment. Subjects received 14 bedtime doses of one of the following treatments: Esopiclone 2.0 mg or placebo. Hypnotic efficacy was measured objectively by PSG recordings and subjectively by morning and evening questionnaires using an Interactive Voice Response System (IVRS).

A subject's study participation involved four visits, a screening visit (Visit 1), the first two dosing nights (Visit 2), an end of treatment visit including the last two dosing nights (Visit 3), and a follow-up visit (Visit 4). All subjects received fourteen days of treatment with either Esopiclone 2.0 mg or placebo.

Visit 1: All screening assessments occurred within 21 days of signing informed consent.

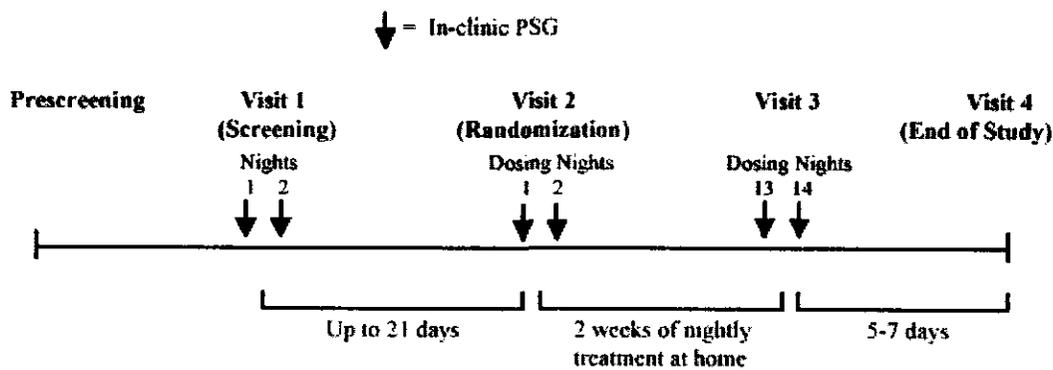
Visit 2 (Dosing Nights 1 and 2): Subjects were admitted to the sleep lab for two consecutive nights. Randomization and double-blind dosing began on the first night. Subjects received double-blind dosing on the second night of dosing in the sleep lab and continued to take study medication once daily at home until returning to the sleep lab for Visit 3.

Visit 3 (Dosing Nights 13 and 14): Subjects were admitted to the sleep lab for two consecutive nights of PSGs and last two days of double-blind dosing.

Visit 4 (5-7 days post last dose): End-of-study assessments were performed at Visit 4.

A Subject's participation was complete at this visit. Each night, prior to PSGs in Visits 1, 2 and 3, subjects completed an evening questionnaire by IVRS and received drug treatment (single-blind placebo or double-blind treatment). Each morning following PSGs, subject completed a morning questionnaire by IVRS.

On study days between Visits 2 and 3, and between Visits 3 and 4, subjects completed a morning questionnaire and an evening questionnaire by IVRS. Subjects took the double-blind treatment at home nightly at bedtime between Visit 2 and 3. A subject's total time on study was approximately six weeks (three weeks screening, two weeks double-blind treatment, and one-week follow-up visit). A study timeline is presented below. Objective LPS and objective sleep efficiency were co-primary measures. Objective WASO was designated as a key secondary measure. The average of all PSG results during the double-blind period was used for the analyses of each variable. A study schematic is provided below.



**STUDY 190-048: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PARALLEL, TWO-WEEK EFFICACY AND SAFETY STUDY OF (S)-ZOPICLONE IN ELDERLY SUBJECTS WITH PRIMARY INSOMNIA**

Study 190-048 was a randomized, double-blind, placebo-controlled, three-arm (1:1:1) parallel-group, hypnotic efficacy and safety study in elderly subjects (64 to 85 years, inclusive) with primary insomnia. Subjects met DSM-IV criteria for primary insomnia, reported sleeping no more than 6.5 hours per night and taking more than 30 minutes each night to fall asleep for at least one month. Following the screening phase and baseline assessments, 234 subjects were randomized: 81 subjects to placebo, 74 subjects to Esopiclone 1.0 mg, and 79 subjects to Esopiclone 2.0 mg. Subjects were to receive 14 bedtime doses of one of the following three treatments: Esopiclone 1.0 mg, Esopiclone 2.0 mg, or placebo. Subjective hypnotic efficacy was measured daily with morning and evening questionnaires via an interactive voice response system (IVRS).

Study participation was approximately four weeks (two weeks screening, two weeks double-blind treatment) and involved four visits.

**Screening (Visit 1):** The screening visit included a brief neurological examination, sleep history, and a medical/psychiatric history. Eligible subjects returned for the baseline visit within fourteen days for randomization to one of three treatment arms.

**Baseline (Visit 2):** Subjects returned to the clinic to confirm eligibility. Subjects returned home and provided baseline efficacy measurements via IVRS and began dosing in the evening. Between visits at home, subjects utilized an IVRS each morning to record subjective sleep quality and subjective residual effects.

**Visits 3 and 4:** Subjects returned to the clinic after one week (Visit 3) and two weeks (Visit 4) for safety assessments.

The primary endpoint was subjective sleep latency, defined as the time in minutes after lights out until sleep onset. Subjective total sleep time (total minutes of sleep) was the key secondary variable. For each efficacy parameter, the average of the results over the two-week double-blind (DB) period for each individual was used for the treatment comparisons. For subjects with missing data, the average of the nights for which data were present was used in the analysis.

### 3. EFFICACY EVALUATION

#### 3.1 PRIMARY MEASURES

For the studies with objective measures (190-026, 190-045, and 190-046), objective LPS was the primary efficacy measure. Objective LPS and objective sleep efficiency were co-primary measures in Study 190-047. For Studies 190-048 and 190-049, subjective sleep latency was the primary efficacy measure.

#### 3.2 KEY SECONDARY MEASURE

Secondary endpoints of key importance were identified in each protocol. For Studies 190-026, 190-046, and 190-045, objective sleep efficiency was a key secondary efficacy measure. Objective WASO was designated as a key secondary measure for Studies 190-045, 190-046, and 190-047. For the entirely subjective studies, 190-048 and 190-049, subjective total sleep time was designated as the key secondary efficacy measure.

#### 3.3 HANDLING DROPOUTS AND MISSING DATA

Subjects who dropped out before study completion were not replaced. Dropouts were included in all analyses as data allowed. There were several subjective values in excess of 540 minutes, however, subjects were allowed to sleep for only eight hours. These values were for subjective sleep latency, subjective WASO, and subjective total sleep time in Study 190-046, and for subjective sleep latency and subjective WASO in Study 190-048. These values were examined in conjunction with other responses to the Morning Questionnaire items and were found to be an error or misunderstanding in subjects<sup>TM</sup> reporting. Consequently, the results presented are based on analyses without reported values greater than 540 minutes. Additional analyses that included those values were also done; those results do not substantively alter the findings and do not affect any conclusions.

For Study 190-049, a Last Observation Carried Forward (LOCF) approach was used in the derivation of monthly averages to account for dropouts during the double-blind treatment period.

#### 3.4 HANDLING SMALL CENTERS

For each study that included site in the ANOVA model, sites with fewer than three subjects per treatment group were pooled for analysis purposes. For Study 190-026, all sites with fewer than three subjects were pooled into one virtual site. For Studies 190-046, 190-049, 190-047, and 190-048, sites with fewer than three subjects per

treatment group were ranked according to the number of subjects randomized. Sites were pooled together, starting with the site that had the fewest number of subjects (lowest rank), until a pseudo-site was formed that met the three subjects per treatment group requirement. Then, a second pseudo-site was formed by combining the sites with the next lowest ranks until the three subjects per treatment group requirement was met, and so on. If the final pseudo-site did not have at least three subjects per treatment group, then it was combined with the previous pseudo-site created.

For Study 190-045, no adjustments for site effects were made in the analysis of the efficacy variables. For the primary and two key secondary efficacy variables, an assessment of site effect and site by treatment interaction was conducted as an exploratory analysis.

### 3.5 PRIMARY ANALYSIS

For each study, the primary analysis was conducted using rank-transformed data for the study-defined primary efficacy variable. All efficacy analyses were conducted on the Intent-to-Treat (ITT) population. The ITT population was defined as all randomized subjects who received at least one dose of study medication.

For Study 190-026, the overnight results were used for the primary and key secondary analyses. For the studies 190-046, 190-047, and 190-048, the average of the results over the double-blind period, compared with placebo, were used for the primary and the key secondary analyses. For the six-way cross-over study (190-045), the mean of two nights on each treatment was used for the primary and key secondary analyses. For Study 190-049, the last-three-month average (i.e., the average of Months 4, 5, and 6) was used for the primary (sleep latency) and key secondary analyses.

For the parallel group studies (190-026, 190-046, 190-047, 190-048, and 190-049), an ANOVA model with treatment and site as fixed effects was utilized. For the six-way cross-over study (190-045), the ANOVA model included treatment, sequence, and visit as fixed effects, and subject nested within sequence as a random effect. The primary analysis compared the highest Esopiclone dose group (3.0 mg for 190-046 and 190-049; 2.0 mg for 190-047 and 190-048) with the placebo group using the appropriate contrast from the ANOVA model.

### 3.6 KEY SECONDARY ANALYSES

Key secondary analyses were performed based on the key secondary endpoints using the same method as for the primary analysis.

### 3.7 MULTIPLE COMPARISONS/MULTIPLICITY

All statistical tests were two-sided and conducted at the 5% significance level, unless specified otherwise. Several strategies were employed to adjust for multiple comparisons; the strategies differed from study to study due to differences in study design and/or objectives.

#### Study 190-026

Interpretation of the results for the primary and the key secondary variables was done using Fisher's protected approach. For each measure, the two highest Esopiclone doses (3.0 and 3.5 mg) were combined and compared with placebo. If the test was significant at the two-sided 5% significance level, then the significance of the pair-wise comparisons of each of these doses with placebo was assessed. If the test was not significant, then the pair-wise comparisons were to be considered not significant.

#### Study 190-046

A structured approach to hypothesis testing was employed. Specifically, a sequential approach across endpoints was used within the 3.0 mg Esopiclone dose group. The same sequential approach was used within the 2.0 mg Esopiclone dose group, but only for those endpoints where the 3.0 mg dose group was significantly different from placebo. The details of this structured approach are described below.

The primary null hypothesis was:

H01: 3.0 mg Esopiclone and placebo result in the same objective LPS averaged over all double-blind assessments.

If the primary null hypothesis was not rejected, then the study was to be considered negative regarding the efficacy of both 3.0 mg and 2.0 mg Esopiclone. If the primary null hypothesis was rejected, then it was to be concluded that 3.0 mg Esopiclone was efficacious for the primary endpoint, and testing of the second null hypothesis proceeded:

H02: 3.0 mg Esopiclone and placebo result in the same objective sleep efficiency averaged over all double-blind assessments.

If the second null hypothesis was not rejected, then it was to be concluded that neither the 3.0 mg nor the 2.0 mg Esopiclone dose had a significant effect on objective sleep efficiency. If the second null hypothesis was rejected, then it was to be concluded that 3.0 mg Esopiclone was efficacious for objective sleep efficiency over a four-week period, and testing of the third null hypothesis proceeded:

H03: 3.0 mg Esopiclone and placebo result in the same objective WASO averaged over all double-blind assessments.

If the third null hypothesis was not rejected, then it was to be concluded that neither the 3.0 mg nor the 2.0 mg Esopiclone dose had a significant effect on WASO. If the third null hypothesis was rejected, then it was to be concluded that 3.0 mg Esopiclone decreased objective WASO over a four-week period.

Assessment of the 2.0 mg Esopiclone dose group proceeded as above, using the same testing order, but only for the endpoints where the 3.0 mg dose was effective.

**Specifically:**

- If H01 was not rejected, then the comparison of the 2.0 mg dose versus placebo for all three endpoints was to be considered not significant.

- If H01 was rejected, but H02 was not, then the 2.0 mg Esopiclone dose group was to be assessed to make inferences regarding the comparison of the 2.0 mg dose with placebo for the primary endpoint, but the differences were to be considered not significant for the two key secondary endpoints.

- If H01 and H02 were rejected, but H03 was not, then the sequential testing approach was to be applied for objective LPS and objective sleep efficiency, but not for objective WASO.

- If H01, H02, and H03 were all rejected, then the sequential testing approach was to be applied to all three endpoints.

In summary, first the hypotheses regarding the comparison of the 3.0 mg Esopiclone dose with placebo for the primary and two key secondary endpoints were interpreted. Then, the corresponding tests were performed for the comparison of the 2.0 mg Esopiclone dose with placebo guided by the findings at the higher dose. This structured approach was partially based on the assumption that the 3.0 mg dose would have equal or greater efficacy than the 2.0 mg dose for the selected endpoints.

**Study 190-045**

Fisher's protected approach was used for the interpretation of the primary and key secondary efficacy parameter results. For each measure, the primary test compared the higher esopiclone doses (2.0, 2.5, and 3.0 mg) combined with placebo. If the primary test was significant at the two-sided 5% significance level, then the significance of the pair-wise comparison of each esopiclone dose with placebo was assessed. If the primary test was not significant, then the pair-wise comparisons were to be considered not significant.

**Study 190-049**

No adjustments for multiple comparisons were made.

**Study 190-047**

A structured approach to hypothesis testing to control for multiple comparisons was utilized in the analysis of the co-primary and the key secondary efficacy endpoints. The analysis first compared the esopiclone 2.0 mg group to the placebo group for the two co-primary efficacy parameters, objective LPS and objective sleep efficiency, using the double-blind period averages. If either test was not significant at the 5% significance level, no further inferential statements regarding superior efficacy of the study drug were to be made. If the tests were significant, the esopiclone 2.0 mg group was to be compared to the placebo group for the key secondary efficacy parameter, WASO, using the average of the results over the double-blind period.

### Study 190-048

A structured approach to hypothesis testing to control for multiple comparisons, similar to that for study 190-046, was utilized for interpretation of the results. That is, the primary analysis compared the esopiclone 2.0 mg group to the placebo group for subjective sleep latency. If the test was not significant at the 5% significance level, then the comparison of the 2.0 mg dose with placebo for subjective total sleep time was to be considered not significant. If the test was significant, then the 2.0 mg group was compared with placebo for subjective total sleep time and the 1.0 mg group was compared with placebo for subjective sleep latency using the same analysis method. The comparison of the 1.0 mg dose with placebo for subjective total sleep time was to be considered significant only if all four tests yielded significant results.

### 3.8 INTERIM ANALYSES AND DATA MONITORING

No interim analyses were conducted for any of the efficacy studies.

### 3.9 SUBGROUP ANALYSES

The following subgroup analyses were performed on the primary and key secondary efficacy variables based on Gender (male, female); and Race category (Caucasian and Non-Caucasian in Studies 190-046, 190-047, and 190-048; Caucasian, Black, Other in Studies 190-045 and 190-049; no race analysis was conducted in Study 190-026).

## 4. SPONSOR' FINDINGS

### 4.1 SUBJECT DISPOSITION

In study 190-026, a total of 292 subjects were randomized to three treatment groups (Placebo: 98 subjects; Esopiclone 3.0 mg: 98 subjects; Esopiclone 3.5 mg: 96 subjects). Only one subject discontinued; one placebo-treated subject left during the study visit because of a family emergency.

In study 190-045, a total of 65 subjects were randomized to one of six treatment sequences, and 63 (96.9%) subjects completed. Two (3.1%) subjects discontinued: one subject voluntarily withdrew during the washout following zolpidem 10.0 mg; one subject was discontinued during the washout following Esopiclone 2.5 mg due to disallowed medications given after a car accident in which she was a passenger.

Table 2 lists the reasons for patients' discontinuation in the remaining four studies. In the studies of 6-week duration or less, there was a high rate of subjects who completed (over 90% in each study). There were no deaths or treatment-emergent serious AEs, and a few subjects discontinued due to AEs while on double-blind treatment.

In Study 190-049 (6 months double-blind duration), 60% of all subjects completed six months of dosing, and discontinuation rates were similar between the placebo (43%) and Esomeprazole 3.0 mg (39%) groups.

#### 4.2 SUBJECT DEMOGRAPHICS

STUDY 190-026: The overall age range was 20-54 years. There were slightly more females than males, and there were more Caucasians than any other racial group. Demographics were well-balanced across treatment groups.

STUDY 190-046: The overall mean age was 39.8 years and the age range was 21-64 years. Overall, there were more Caucasians (66.2%) than any other racial group, and more females (64.6%) than males (35.4%).

STUDY 190-045: The overall mean age was 40.6 years and the age range was 22-63 years. There were more females (73.8%) than males (26.2%), and there were more Caucasians (67.7%) than any other racial group. Age, height, weight, and BMI data were well balanced across treatment sequences.

STUDY 190-049: The overall mean age was 44 years and the age range was 21-69 years. There were more females (63.2%) than males (36.8%), and there were more Caucasians (78.9%) than any other racial group. Age, height, weight, and BMI data were well balanced across treatment sequences.

STUDY 190-047: The age range was 64-86 years. In each of the above treatment groups, there were more females (60.7% to 71.1%) than males (28.9 to 39.3%), and there were more Caucasians (88.2% to 96.4%) than any other racial group.

STUDY 190-048: The overall mean age was 72.3 years and the age range was 64-85 years. There were more females (57.7%) than males (42.3%), and there were more Caucasians (96.6%) than any other racial group.

In each study, demographics were generally well balanced among treatment groups. More female than male subjects participated, and the most common racial group was Caucasian.

Table 5: Findings based on the Primary measures

Study#	Objective Latency to Persistent Sleep[LPS] (minutes)	Placebo	Esopiclone		
			3.0mg	3.5 mg	
190-026	N	98	98*	96	
	Mean	17.90	9.10	6.62	
	Median	12.5	5.5	5.0	
	Overall treatment effect**		≤0.0001		
	Pairwise p-value vs. placebo		≤0.0001	≤0.0001	
*One subject from 3.0mg arm did not have analyzable objective PSG data but was included in the efficacy analysis for subjective measures.					
**The overall treatment effect and pairwise comparisons were tested using an ANOVA model on rank-transformed data with effects for site and treatment, using only subjects in the placebo, Esopiclone 3.0 mg, and Esopiclone 3.5 mg groups.					
190-046	Objective LPS	Placebo	Esopiclone		
			2.0 mg	3.0 mg	
	N	99	104	105	
	Mean	33.0	23.0	18.0	
	Median	29.0	15.0	13.1	
Overall treatment effect*					
Pairwise p-value vs. placebo			≤0.0001	≤0.0001	
* The pairwise comparison was performed using the appropriate contrast within an ANOVA model on the rank-transformed data with treatment and site as fixed effects.					
190-045	Objective LPS	Placebo	Esopiclone		
			2.0mg	2.5mg	3.0mg
	N	63	63	65	64
	Mean	37.8	20.1	18.6	18.3
	Median	29.0	15.5	13.8	13.1
Overall treatment effect*			≤0.0001		
Pairwise p-value vs. placebo			≤0.0001	≤0.0001	≤0.0001
* The overall treatment effect and pairwise comparisons were tested using an ANOVA model on rank-transformed data with treatment, sequence, and visit as fixed effects and subject nested within sequence as a random effect. The analysis compared the three highest Esopiclone dose groups combined (2.0, 2.5, and 3.0 mg) with the placebo.					
190-049	Subjective Sleep Latency (Month 4-6 Average)	Placebo	Esopiclone 3.0 mg		
	N	195	593		
	Mean	64.7	46.7		
	Median	44.8	31.7		
	P-value (Vs. Placebo) *			<0.0001	
* The treatment comparison was performed using an ANOVA model on the rank-transformed data with treatment and site as fixed effects.					
<b>ELDERLY STUDIES</b>					
190-047	Objective LPS (min) [Entire Double-Blind Period]	Placebo	Esopiclone 2.0 mg		
	N	128	136		
	Mean	40.8	19.3		
	Median	30.4	14.8		
	P-value (Vs. Placebo) *			<0.0001	

Objective Sleep Efficiency (%) [Entire Double-Blind Period]		Placebo	Esopiclone 2.0 mg	
N		128	136	
Mean		73.3	79.3	
Median		74.6	80.4	
P-value (Vs. Placebo) *			<0.0001	
*The treatment comparison was performed using an ANOVA model on the rank-transformed data with treatment and site as fixed effects. Note: "Entire double-blind period" is the average of "Night 1" and "Night 14" results. "Night 1" represents the average of Nights 1 and 2 of Visit 2, and "Night 14" represents the average of Nights 1 and 2 of Visit 3.				
190-048	Subjective Sleep Latency [Entire Double-Blind Period]	Placebo	Esopiclone	
			1.0 mg	2.0 mg
N		79	70	79
Mean		87.6	54.7	50.7
Median		52.7	35.9	36.2
Overall treatment effect*				
Pairwise p-value vs. placebo			0.0091	0.0029
* The pairwise comparison was performed using the appropriate contrast within an ANOVA model on the rank-transformed data with treatment and site as fixed effects.				

Table 6: Key secondary measures:

Study#	Objective Sleep Efficiency (%)	Placebo	Esopiclone	
			3.0mg	3.5 mg
190-026	N	98	98*	96
	Mean	87.88	93.02	94.01
	Median	90.3	94.6	95.1
	Pairwise p-value vs. placebo*		≤0.0001	≤0.0001
*One subject from 3.0mg arm did not have analyzable objective PSG data but was included in the efficacy analysis for subjective measures.  ** The overall treatment effect and pairwise comparisons were tested using an ANOVA model on rank-transformed data with effects for site and treatment, using only subjects in the placebo, Esopiclone 3.0 mg, and Esopiclone 3.5 mg groups.				
190-046 (Two key secondary measures)	Objective Sleep Efficiency (%)	Placebo	Esopiclone	
	N		2.0 mg	3.0 mg
	Mean	99	104	105
	Median	83.5	86.5	88.8
	Pairwise p-value vs. placebo	85.7	88.1	90.1
			0.0059	<0.0001

	Objective Wake Time After Sleep Onset (minutes)	Placebo	Esopiclone		
			2.0 mg	3.0 mg	
	N	99	104	105	
	Mean	50.0	44.5	38.0	
	Median	44.1	37.1	33.8	
	Pairwise p-value vs. placebo		0.2564	0.0055	
* The pairwise comparison was performed using the appropriate contrast within an ANOVA model on the rank-transformed data with treatment and site as fixed effects.					
190-045	Objective Sleep Efficiency (%)	Placebo	Esopiclone		
			2.0mg	2.5mg	3.0mg
	N	63	63	65	64
	Mean	83.9	88.9	89.7	89.2
	Median	86.0	89.6	90.4	92.0
	Pairwise p-value vs. placebo		≤0.0001	≤0.0001	≤0.0001
	Objective Wake Time After Sleep Onset (minutes)	Placebo	Esopiclone		
			2.0mg	2.5mg	3.0mg
	N	63	63	65	64
	Mean	43.1	36.0	33.1	35.9
Median	39.0	30.5	29.5	31.7	
Pairwise p-value vs. placebo		0.018	0.012	0.328	
* The overall treatment effect and pairwise comparisons were tested using an ANOVA model on rank-transformed data with treatment, sequence, and visit as fixed effects and subject nested within sequence as a random effect. The analysis compared the three highest Esopiclone dose groups combined (2.0, 2.5, and 3.0 mg) with the placebo.					
190-049	Subjective Total Sleep Time (Month 4-6 Average)	Placebo	Esopiclone 3.0 mg		
	N	195	593		
	Mean	341.1	377.3		
	Median	345.1	381.7		
	P-value (Vs. Placebo) *		<0.0001		
* The treatment comparison was performed using an ANOVA model on the rank-transformed data with treatment and site as fixed effects.					
ELDERLY STUDIES					
190-047	Objective Wake Time After Sleep Onset (minutes) [Entire Double-Blind Period]	Placebo	Esopiclone 2.0 mg		
	N	128	136		
	Mean	94.2	83.6		
	Median	91.2	81.7		
	P-value (Vs. Placebo) *		0.0345		
*The treatment comparison was performed using an ANOVA model on the rank-transformed data with treatment and site as fixed effects. Note: "Entire double-blind period" is the average of "Night 1" and "Night 14" results. "Night 1" represents the average of Nights 1 and 2 of Visit 2, and "Night 14" represents the average of Nights 1 and 2 of Visit 3.					
190-048	Subjective Total Sleep Time [Entire Double-Blind Period]	Placebo	Esopiclone		
			1.0 mg	2.0 mg	
	N	79	70	79	
	Mean	328.2	349.8	372.3	

	<b>Median</b>	345.0	352.1	383.2
	<b>Pairwise p-value vs. placebo</b>		0.2682	0.0003
	* The pairwise comparison was performed using the appropriate contrast within an ANOVA model on the rank-transformed data with treatment and site as fixed effects.			

#### 4.3 EFFICACY FINDINGS OF STUDY 190-026

Results for the objective LPS, the primary efficacy measure for Study 190-026, are Summarized in Table 5. Esopiclone 3.0 mg, and 3.5 mg significantly decreased the objective LPS, relative to placebo ( $p \leq 0.0001$  for each dose). At the two doses (3.0 and 3.5 mg), the median LPS was less than half the time observed with placebo.

Results for objective sleep efficiency, a key secondary efficacy measure in Study 190-026, are summarized in Table 6. Esopiclone 3.0 mg and 3.5 mg doses significantly increased objective sleep efficiency relative to placebo ( $p \leq 0.0001$  for each dose).

#### 4.4 EFFICACY FINDINGS OF STUDY 190-046

Results for the objective LPS, the primary efficacy measure for Study 190-046, are Summarized in Table 5. Both Esopiclone 2.0 mg and 3.0 mg doses significantly reduced objective LPS relative to placebo over the entire double-blind period (for each pairwise test,  $p \leq 0.0001$ ).

Results for objective sleep efficiency, a key secondary efficacy measure in Study 190-046, are summarized in Table 6. Esopiclone 2.0 mg and 3.0 mg doses significantly increased objective sleep efficiency relative to placebo ( $p \leq 0.0001$  for each dose) over the entire double-blind period. Esopiclone 3.0 mg significantly decreased objective WASO relative to placebo for the double-blind period ( $P \leq 0.005$ ).

#### 4.5 EFFICACY FINDINGS OF STUDY 190-045

Results for the objective LPS, the primary efficacy measure for Study 190-045, are summarized in Table 5. All doses of Esopiclone (2.0 mg, 2.5mg and 3.0 mg) significantly reduced objective LPS relative to placebo over the entire double-blind period (each pairwise test,  $p \leq 0.0001$ ).

Results for objective sleep efficiency, a key secondary efficacy measure in Study 190-045, are summarized in Table 6. All Esopiclone (2.0 mg, 2.5 mg and 3.0 mg) doses significantly increased objective sleep efficiency relative to placebo ( $p \leq 0.006$  for each dose) over the entire double-blind period. Esopiclone 2.5 mg and 3.0 mg significantly decreased objective WASO relative to placebo ( $P \leq 0.018$  for each dose).

#### 4.6 EFFICACY FINDINGS OF STUDY 190-049

Results for the objective LPS, the primary efficacy measure for Study 190-049, are summarized in Table 5. Esopiclone 3 mg significantly reduced subjective sleep latency relative to placebo over the Month 4-6 averages in the double blind period ( $p \leq 0.0001$ ).

Results for objective sleep efficiency, a key secondary efficacy measure in Study 190-049, are summarized in Table 6. Esopiclone 3 mg significantly increased subjective total sleep time relative to placebo for the Month 4-6 averages in the double blind period ( $p < 0.0001$ ).

#### 4.7 EFFICACY FINDINGS OF STUDY 190-047

Results for the objective LPS, the primary efficacy measure for Study 190-047, are summarized in Table 5. Esopiclone 2.0 mg significantly reduced objective LPS (co-primary measure) relative to placebo over the double-blind period ( $p < 0.0001$ ). Esopiclone 2.0 mg also significantly increased objective sleep efficiency (co-primary) relative to placebo for the double-blind period ( $p \leq 0.0001$ ).

Results for objective sleep efficiency, a key secondary efficacy measure in Study 190-047, are summarized in Table 6. Esopiclone 2.0 mg significantly decreased objective WASO relative to placebo for the entire double-blind period ( $P \leq 0.034$ ).

#### 4.8 EFFICACY FINDINGS OF STUDY 190-048

Results for the objective LPS, the primary efficacy measure for Study 190-048, are summarized in Table 5. Both Esopiclone (1.0 mg and 2.0 mg) doses significantly decreased subjective sleep latency relative to placebo during the double blind period (each pairwise test,  $P \leq 0.009$ ).

Results for objective sleep efficiency, a key secondary efficacy measure in Study 190-048, are summarized in Table 6. Esopiclone 2.0 mg significantly increased subjective total sleep time compared with placebo during the double blind period ( $P \leq 0.0003$ ).

#### 4.9 SUBGROUP ANALYSES FINDINGS

In each study, the efficacy results in the subgroup analyses (based on gender and race) demonstrated the same trends found in the overall sample. There were similar trends between adult and elderly subjects for subjective sleep latency, subjective total sleep time, and subjective WASO.

Table 7: Summarized primary efficacy measures results of the six studies.

Study#	Double blind duration	Primary measure	P-values				
			Esopiclone				
			1.0 mg vs. Placebo	2.0 mg vs. Placebo	2.5 mg vs. Placebo	3.0 mg vs. Placebo	3.5 mg vs. Placebo
<b>Adults studies</b>							
190-026	1 night	Objective LPS				≤0.0001	≤0.0001
190-046	44 nights	Objective LPS		≤0.0001		≤0.0001	
190-045	2 nights	Objective LPS		≤0.0001	≤0.0001	≤0.0001	
190-049	6 months	Subjective sleep latency		≤0.0001			
<b>Elderly studies</b>							
190-047	14 nights	Objective LPS		≤0.0001			
		Objective sleep efficacy		≤0.0001			
190-048	14 nights	Subjective sleep latency	0.009	0.0029			

Table 8: Summarized Key Secondary efficacy measures results of the six studies.

Study#	Double blind duration	Key Secondary measure	P-values				
			Esopiclone				
			1.0 mg vs. Placebo	2.0 mg vs. Placebo	2.5 mg vs. Placebo	3.0 mg vs. Placebo	3.5 mg vs. Placebo
<b>Adults studies</b>							
190-026	1 night	Objective sleep efficacy				≤0.0001	≤0.0001
190-046	44 nights	Objective sleep efficacy		0.0059		≤0.0001	
		Objective WASO		0.2564		0.0055	
190-045	2 nights	Objective sleep efficacy		≤0.0001	≤0.0001	≤0.0001	
		Objective WASO		0.018	0.012	0.328	
190-049	6 months	Subjective				≤0.0001	

		total sleep time				
<b>Elderly studies</b>						
190-047	14 nights	Objective WASO		0.0345		
190-048	14 nights	Subjective total sleep time	0.2682	0.0003		

## 5. SPONSOR'S FINAL CONCLUSION

There were statistically significant increases in objective sleep efficiency with doses 2.0 mg, 2.5 mg, 3.0mg, and 3.5 mg, and statistically significant increases in subjective total sleep time with doses 2.0 mg and 3.0 mg.

Esopiclone (3.0 mg and 3.5 mg in Studies 190-026, and 3.0 mg in 190-046 and 190-045) was statistically significant effective than placebo for WASO, number of awakenings, and/or wake time during sleep.

## 6. REVIEWER'S ANALYSES AND COMMENTS

This reviewer was able to reproduce the sponsor's results in each of the six studies. This reviewer also did some exploratory analyses on each of the studies. The conclusion on the treatment efficacy remained consistent with the sponsor's conclusion. Tables 7 and 8 list the summarized results of the primary and key secondary efficacy measures. Each of the study is positive based on the defined primary efficacy measures. However, all of the studies were not positive for the individual study defined key secondary measures.

## 7. PATIENT DROPOUT RATES

In study 190-049, the dropout rates were 43%, 39% in placebo and Esopiclone 3.0 mg arms, respectively. As the dropout rates were higher, the findings from LOCF analysis as the primary statistical analysis method are questionable.

## 8. ISSUES RELATED TO THIS NDA

The sponsor reported several errors in the study reports of the three studies (190-026, 190-045, and 190-048). The case report forms (CRFs) of the patients were not submitted with this NDA. Therefore, submitted patients' data were not validated through the individual patient's CRF.

## 9. REVIEWER'S OVERALL CONCLUSION

In adults (both transient and chronic insomnia), Esopiclone at 2.0 mg and above consistently produced statistically significant improvements in sleep induction and quality of sleep measures. Esopiclone at 3.0 mg consistently produced significant improvements in sleep maintenance parameters. For the primary and key secondary measures in adult studies, the 3.0 mg dose of Esopiclone consistently produced numerically better improvements than the 2.0 mg dose.

In elderly subjects, the 2.0 mg dose of Esopiclone similarly appeared more effective than the 1.0 mg dose, often producing numerically better improvements and achieving statistical significance on a greater number of measures.

It is demonstrated that the nighttime administration of Esopiclone 2.0 mg will provide effective treatment of transient and chronic insomnia in the adult and elderly subjects.

**APPEARS THIS WAY  
ON ORIGINAL**

---

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

---

/s/

-----  
Ohidul Siddiqui  
11/14/03 11:25:09 AM  
BIOMETRICS

Kun Jin  
11/14/03 01:15:14 PM  
BIOMETRICS

Kooros Mahjoob  
11/14/03 04:00:54 PM  
BIOMETRICS