CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

19982

STATISTICAL REVIEW(S)

Statistical Review and Evaluation

NDA #: 19-982 Date: JUN 26 1991

Applicant: Lederle Lab. Division, American Cyanamid Company

Name of Drug: Monocor (bisoprolol fumarate) Tablets

Documents Reviewed:

1. NDA submission volume 2.1, Date of Document, Dec. 27, 1989.
2. NDA submission volume 1.25-1.29, Date of Document, Dec. 27, 1989.

I. Background

Two animal carcinogenicity studies (one in rats, and one in mice) were included in this NDA submission. These two studies were intended to evaluate the oral carcinogenic potential of Monocor (bisoprolol fumarate) tablets in rats and mice for 26 months and 87 weeks, respectively. Dr. Ernest J. Belair, HFD-110, who is the reviewing pharmacologist of this NDA has requested the Division of Biometrics to perform the statistical review and evaluation of these two studies. The data submitted on computer floppy diskettes were used in the reviewer's independent analyses.

II. The Rat Study

II. a. Design

In this study, 250 male and 250 female Wistar rats were randomly assigned to five groups (50/sex/group). EMD 33 512 (bisoprolol) was administered daily to the rats in their feed for 26 months at dose levels of 5, 25, and 125 mg/kg/day. Additional two groups of 50/sex/group rats received untreated diet and were designated as controls. At the beginning of the study, the body weights were determined once a week, later on at longer intervals. The food consumption was determined once a week. The rats surviving at the end of the study were examined hematologically. Moribund animals were killed. After 26 months of treatment, all surviving rats were sacrificed, necropsies were performed and gross observations recorded. Histological examinations of all rats were carried out, in which particular attention was paid to tumors and organs suspected of being afflicted by tumors, although nonneoplastic lesions were recorded also.

II. b. Sponsor's Analyses

Cox's logrank procedure (Cox, D.R., "Regression Models and Life Tables", Journal of the Royal Statistical Society, Series B, 34, 187-220, 1972) was used for testing equality of survival distributions for each sex. In addition, dose-response was evaluated using the trend test of Tarone ("Tests for Trend in Life-table Analysis", Biometrika, 62, 679-682, 1975) with an ordinal dose scaling. Figures 1 and 2 show the survival curves

for male and female rats respectively. No significant differences (at 0.05 level) in mortality between the control groups were found either in males or females ($p > 0.3$ in both cases). Accordingly, the control groups were combined for each sex and analysis of mortality differences among the combined controls and three treated groups was performed. No significant difference was found in either sex.

The methods given in the paper of Peto et al. ("Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments", In Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research on Cancer Monographs, Annex to Supplement 2, World Health Organization, 311-426, 1980) were used to test the positive dose-response relationship in the tumor data. Analysis of the tumor data was carried out separately by organ, for each of 26 organs in males and 26 in females. To test for a dose-related increase in tumor prevalence, a modification of the Tarone trend test was used. Two-sided tests for heterogeneity were performed, while one-sided trend analyses were used. These analyses were implemented using a computer program developed by Kodell et al. ("CHRONIC: A SAS procedure for statistical analysis of carcinogenesis studies", Journal of Statistical Computation and Simulation, 16, 287-310). If five or fewer animals were observed to have a tumor in a particular organ, no significance test was performed. The sponsor also stated that "in presenting p-values associated with these tests no adjustment has been made for the multiplicity of testing."

In this review, the phrase "positive dose-response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing mortality or tumor rate as dose increases.

Tables 1 and 2 summarize the results of the analysis of tumor data by organ for males and females respectively. The results of the above analyses showed that there was a significant (at 0.05 level) positive dose-response relationship in pancreatic islet in female rats ($p = 0.03$). However, the sponsor claimed that "since a total of 21 trend tests were performed at different organs in this study, this apparent significant trend in females is most likely to be due solely to the large number of significance tests and not to a tumorigenic effect of treatment, particularly since no corresponding trend was observed in males."

Table 3 contains the results of another tumor data analysis by tumor type with more than five tumor-bearing animals in the study. Apart from the significant trend in islet cell adenomas in females discussed above, no other statistically significant dose-related trend in tumor prevalence was observed by the sponsor.

Based on the above analyses, the sponsor concluded that "overall, the data analyzed in this study do not provide evidence for an increase in tumor prevalence due to treatment with Bisoprolol."

II. c. Reviewer's Analyses and Comments

The Cox test and the generalized Wilcoxon test described in the paper of Thomas, Breslow, and Gart ("Trend and Homogeneity Analyses of Proportions and Life Table Data", Computers and Biomedical Research, 10, 373-381, 1977) were used to test for heterogeneity in survival distributions. Since there were two control groups in this study, two separate sets of analyses were applied to these data sets. In the first set of analyses (called S), the tests were applied to five groups of data (control 1, control 2, low, medium, and high dose groups). In the second set of analyses (called C), the tests were applied to four groups of data (both controls combined, low, medium, and high dose groups). The test results revealed that there was no statistically significant difference (at 0.05 level) in the survival distribution in either sex from Cox test (S: male: $p = 0.4432$, female: $p = 0.4924$; C: male: $p = 0.4548$, female: $p = 0.3373$) or from generalized Wilcoxon test (S: male: $p = 0.5789$, female: $p = 0.8046$; C: male: $p = 0.6199$, female: $p = 0.6979$).

The intercurrent mortality rates for both male and female rats (see Table 4) were tested for the dose-response relationship according to the methods given in the paper of Peto et al. (1980) using time intervals 0-50, 51-80, and 81-109 (male)/81-111 (female) weeks. Three separate sets of analyses were applied to these data sets. In the first set of analyses (called C1), the data of control 1, low, medium, and high dose groups were used. In the second set of analyses (called C2), the data of control 2, low, medium, and high dose groups were used. In the third set of analyses (called C1+C2), the data of both controls, low, medium, and high dose groups were used. The actual dose levels 0, 5, 25 and 125 mg/kg/day were the scores assigned to the controls, low, medium, and high dose groups, respectively. The results of the above three sets of analyses showed no significant dose-response relationship in intercurrent mortality rate in male (C1: $p = 0.9150$, C2: $p = 0.8073$, C1+C2: $p = 0.8933$) and female rats (C1: $p = 0.7551$, C2: $p = 0.8356$, C1+C2: $p = 0.7883$).

The reviewer applied the prevalence method described in the paper of Peto et al. (1980) and the exact permutation trend test to test the positive dose-response relationship in the tumor data. Since there are no significant difference in mortality between two control groups for either sex, data of two control groups were combined for each sex in the following analysis. The time intervals 0-50, 51-80, 81-109 (male)/81-111 (female), and terminal sacrifice were used in those methods. The test results showed that there was a significant positive dose-response relationship in the adrenal cortical carcinoma ($p = 0.0449$) in male rats. The sponsor combined all of the adrenal tumors in male rats (cortical adenoma, cortical carcinoma, pheochromocytoma benign, pheochromocytoma (bilateral), pheochromocytoma malignant, ganglioneuroma, neuroblastoma, and infiltration by lymphoma) in the analysis and found no significant positive dose-response relationship in adrenal tumor incidence rate. The incidence rates of the above tumors are 8/100, 4/50, 4/50, and 4/50 calculated from the sponsor's submitted diskettes. However, the sponsor reported 7/100, 4/50, 4/50, and 4/50 in the document.

Although there was no statistically (at 0.05 level) significant dose-response relationship in the pancreatic islet cell adenoma ($p = 0.0607$) in female rats. The tumor incidence rates of 1/100, 1/50, 3/50, and 3/50 should be noticed. The sponsor's p-value ($p = 0.03$, see Table 2) and the reviewer's p-value ($p = 0.0607$) are different because the sponsor applied the Peto test and not the exact permutation trend test. The p-values from the Peto method are not stable and reliable for tumor types with small number of occurrences across treatment groups. The exact permutation trend test was applied for tumor types with total number of eight or less occurrences across treatment groups. The incidence rates of adrenal cortical carcinoma in male rats and pancreatic islet cell adenoma in female rats are given in Tables 5 and 6.

III. The Mouse Study

III. a. Design

In this study, 250 male and 250 female NMRI mice were randomly assigned to five groups (50/sex/group). EMD 33 512 (bisoprolol) was administered for a period of 87 weeks in daily doses of 10, 50, and 250 mg/kg body weight, mixed in with the feed, to the animals. Additional two groups of 50/sex/group mice received untreated diet and were designated as controls. The study was ended after 87 weeks of treatment when the survival rate in control group 1 was 22% (32% of the males and 12% of the females) still being alive. At the end of the study, the sponsor found that mouse 229, which had been thought to be a male, was in fact a female. Hence, only 49 males were included in group 5 in the analysis. The mice surviving at the end of the study were subjected to hematological examination. Any animals in a moribund state were sacrificed. All the mice were subjected to pathological-anatomical and histopathological examination. Particular attention was paid to tumors and organs suspected of being tumorous, although the non-tumorous lesions were also recorded.

III. b. Sponsor's Analyses

Cox's proportional hazard model (1972) was used for the test for uniformity of the survival time distributions in the various treated groups and in the control group. In addition, dose-response was evaluated using the Tarone trend test (1975) with an ordinal dose scaling. Survival in the two control groups was also compared by means of the logrank test. Two-tailed tests were performed in each case. Figures 3 and 4 show the survival curves for male and female mice respectively. In males, there was no significant difference in mortality between the control groups ($p = 0.68$). When both control groups were combined, no difference was found in male mortality among the control and treated groups. In females, mortality was significantly higher in control group 1 than in group 2 ($p = 0.01$). There was some evidence of longer survival in the Bisoprolol treated animals, particularly when compared to control group 1 (trend p-value < 0.05).

Analysis of the tumor data was carried out separately by organ, for each of 22 organs in males and 22 in females. For each organ, analysis is based on the number of animals observed to have a (primary) tumor in that organ. Tumor data were analyzed using the methods described by Peto et al. (1980). If five or fewer animals were observed to have a tumor in a particular organ, no significance test was performed. Tables 7 and 8 summarize the results of the analysis of the tumor data by organ for males and females respectively. The sponsor stated that "in no case was there a statistically significant dose-related trend in tumor prevalence by organ". Table 9 contains the results of another tumor data analysis by tumor type with more than five tumor-bearing animals in the study. In no case was there a statistically significant dose-related trend in tumor prevalence.

Based on the above analyses, the sponsor concluded that "overall, the data do not provide evidence for an increase in tumor prevalence due to treatment with Bisoprolol."

III. c. Reviewer's Analyses and Comments

The Cox test and the generalized Wilcoxon test described in the paper of Thomas, Breslow, and Gart (1977) were used to test for heterogeneity in survival distributions. Similar to the rats study, two separate sets of analyses (S and C) were applied to the data sets. The test results reveal that there was no statistically significant difference (at 0.05 level) in the survival distribution in either sex from Cox test (S: male: $p = 0.4733$, female: $p = 0.1492$; C: male: $p = 0.4567$, female: $p = 0.1062$) or from generalized Wilcoxon test (S: male: $p = 0.8240$, female: $p = 0.1376$; C: male: $p = 0.6888$, female: $p = 0.1316$).

The intercurrent mortality rates for both male and female mice (see Table 10) were tested for the dose-response relationship according to the methods given in the paper of Peto et al. (1980) using time intervals 0-50, 51-70, and 71-86 weeks. Similar to the rats study, three separate sets of analyses (C1, C2, and C1+C2) were applied to the data sets. The actual dose levels 0, 0, 10, 50, and 250 mg/kg/day were the scores assigned to the controls, low, medium, and high dose groups, respectively. The results of the analyses showed that there was no significant dose-response relationship in the intercurrent mortality rates in male mice (C1: $p = 0.7997$, C2: $p = 0.8528$, C1+C2: $p = 0.8497$). However, there were significant negative dose-response relationship in intercurrent mortality rate in female mice (C1: $p = 0.0013$, C2: $p = 0.0479$, C1+C2: $p = 0.0065$).

The prevalence method described in the paper of Peto et al. (1980) and the exact permutation trend test were applied to test the positive dose-response relationship in the tumor data. The time intervals 0-50, 51-70, 71-86, and terminal sacrifice were used in those methods. The test results showed that there were statistically significant (at 0.05 level of significance) dose-response relationships in the lungs metastatic adenocarcinoma ($p = 0.03471$), ovaries cystadenoma ($p = 0.00893$) in female mice, and lymph nodes hemangioma-abdominal lymph node ($p = 0.0485$) in male

mice. The incidence rates of these three tumors are given in Tables 11 - 13. However, the dose-response relationships were not significant if some of the tumors in the same organ were combined. There was no significant dose-response relationship in the combined lung adenocarcinoma, metastatic adenocarcinoma, and metastatic adenocarcinoma B (incidence rates are 2/100, 0/50, 0/50, 2/50) in female mice. No significant dose-response relationship was detected in the combined ovaries cystadenoma; cystadenoma, bilateral; papillary cystadenoma; and papillary cystadenoma, bilateral (incidence rates are 8/100, 1/50, 3/50, 10/50) in female mice. No significant dose-response relationship was detected in the combined hemangioma-abdominal lymph node and malignant lymphoma (incidence rates are 0/100, 2/50, 0/50, 2/49) in male mice. The sponsor also submitted data of combined granulocytic leukemia for all organs (see Table 14). The exact permutation trend test showed that there was a statistically significant dose-response relationship ($p = 0.0397$) in granulocytic leukemia for all organs in female mice.

IV. Summary

In this review, the phrase "positive dose-response relationship" refers to the increasing linear component of the effect of treatment, and not necessarily to a strictly increasing tumor or mortality rate as dose increases.

IV. a. The Rat Study

The oncogenic potential of EMD 33 512 (bisoprolol) was evaluated in this rat study when administered continuously to the animals, via the diet, at dosage levels of 0, 5, 25, and 125 mg/kg/day for 26 months. The Cox and the generalized Wilcoxon methods were used to test the heterogeneity in survival distributions. The statistical methods given in the paper of Peto et al. (1980) and an exact permutation trend test were used to test the positive dose-response relationship in intercurrent mortality and incidental tumor rates.

The results of the Cox and the generalized Wilcoxon tests show that there is no significant difference in the survival distributions in either male or female rats. There are no significant dose-response relationship in the intercurrent mortality rates in either male or female rats. The test results also show that there is significant positive dose-response relationships in the adrenal cortical carcinoma ($p = 0.0449$) in male rats.

IV. b. The Mouse Study

The oncogenic potential of EMD 33 512 (bisoprolol) was evaluated in this mouse study when administered continuously to the animals, via the diet, at dosage levels of 0, 10, 50, and 250 mg/kg/day for 87 weeks. The Cox and the generalized Wilcoxon methods were used to test the heterogeneity in survival distributions. The statistical methods given in the paper of

Peto et al. (1980) and an exact permutation trend test were used to test the positive dose-response relationship in intercurrent mortality and incidental tumor rates.

Our analyses show that no significant difference in the survival distributions in either male or female mice. In addition, there was no significant positive dose-response relationship in intercurrent mortality rate in male mice. However, there is a significant dose-response relationship in the intercurrent mortality rates in the female mice. Although the test results show that there are statistically significant dose-response relationships in the lungs metastatic adenocarcinoma ($p = 0.03471$), ovaries cystadenoma ($p = 0.00893$) in female mice, and lymph nodes hemangioma-abdominal lymph node ($p = 0.0485$) in male mice. However, the dose-response relationships are not significant if some of the tumors in the same organ are combined. For example; there is no significant dose-response relationship in the combined lung adenocarcinoma, metastatic adenocarcinoma, and metastatic adenocarcinoma B (incidence rates are 2/100, 0/50, 0/50, 2/50) in female mice. No significant dose-response relationship is detected in the combined ovaries cystadenoma; cystadenoma, bilateral; papillary cystadenoma; and papillary cystadenoma, bilateral (incidence rates are 8/100, 1/50, 3/50, 10/50) in female mice. No significant dose-response relationship is detected in the combined hemangioma-abdominal lymph node and malignant lymphoma (incidence rates are 0/100, 2/50, 0/50, 2/49) in male mice. The sponsor also submitted data of combined granulocytic leukemia for all organs. The exact permutation trend test show that there is a statistically significant positive dose-response relationship ($p = 0.0397$) in granulocytic leukemia for all organs in female mice.

/S/

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Concur: /S/ 6/25/91

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HFD-110/Dr. Lipicky
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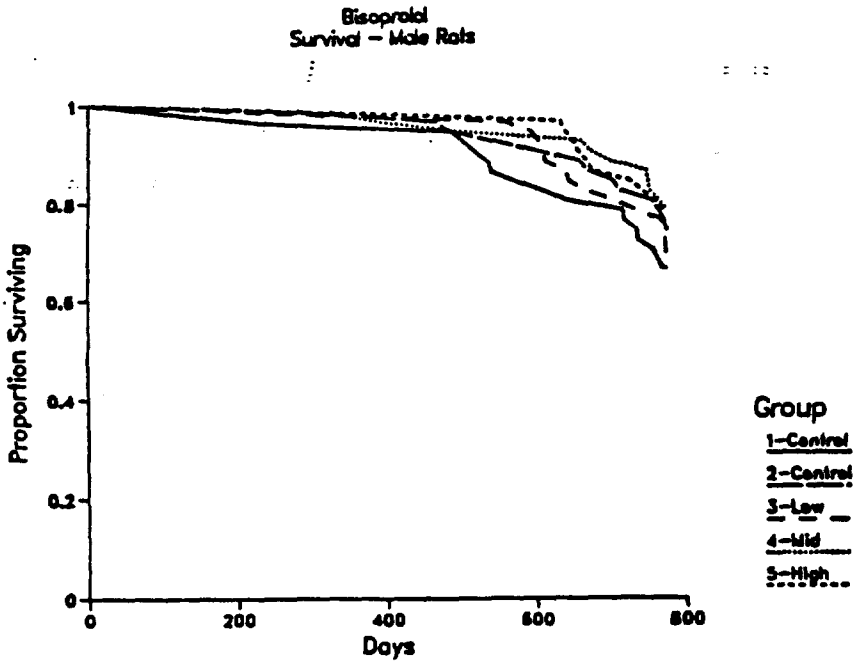
HFD-710/Chron
HFD-715/Dr. Karl Lin
HFD-715/Dr. Daphne Lin
HFD-715/Chron (SARB)
HFD-502/Dr. Weissinger
HFD-715/DRU 2.1.1, Monocor, American Cyanamid Company

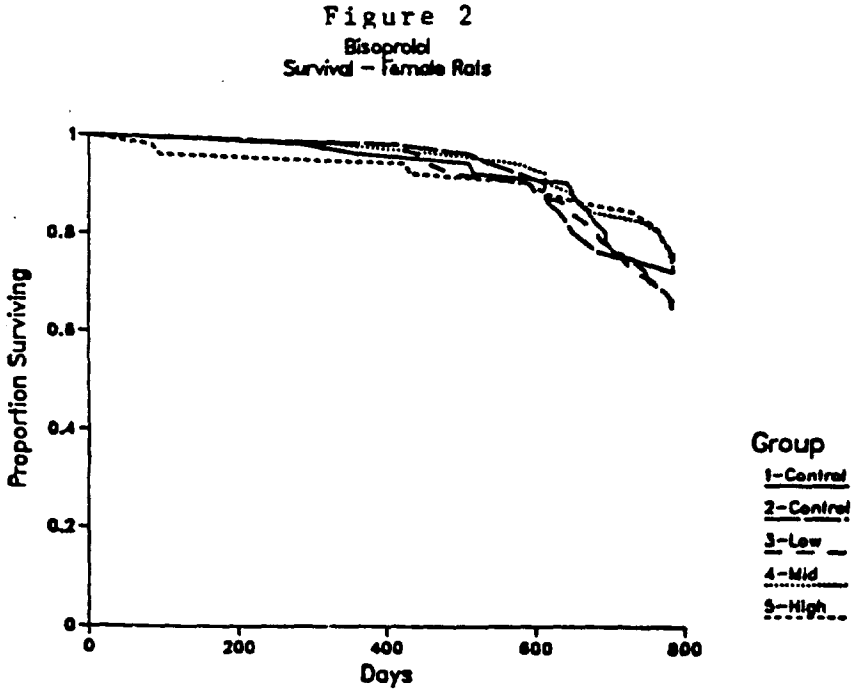
Figure 1



Statistical Analysis of Male Survival

Comparison	p-value
Controls - 1 vs 2	.35
Group Trend - combined controls, low, mid, high	.18

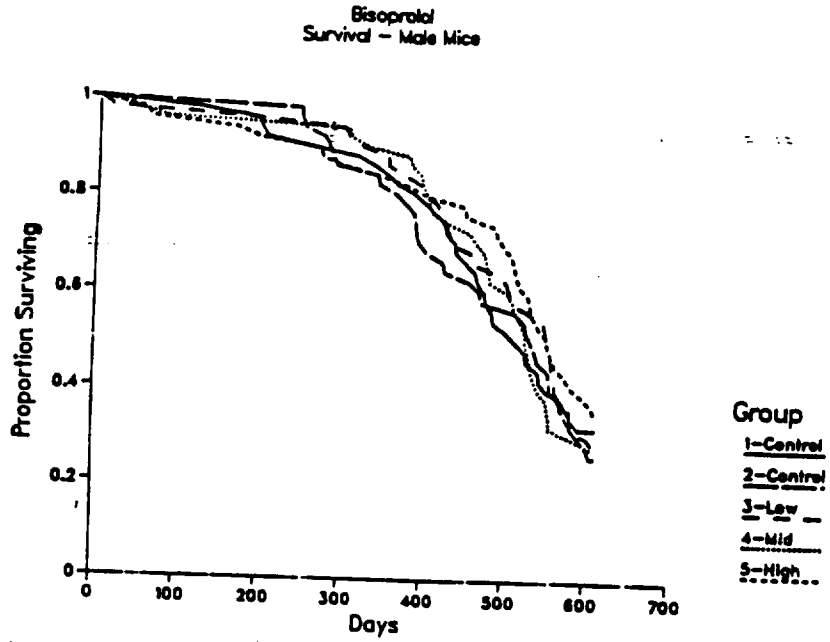
Figure 2



Statistical Analysis of Female Survival

Comparison	p-value
Controls - 1 vs 2	.52
Group Trend - combined controls, low, mid, high	.42

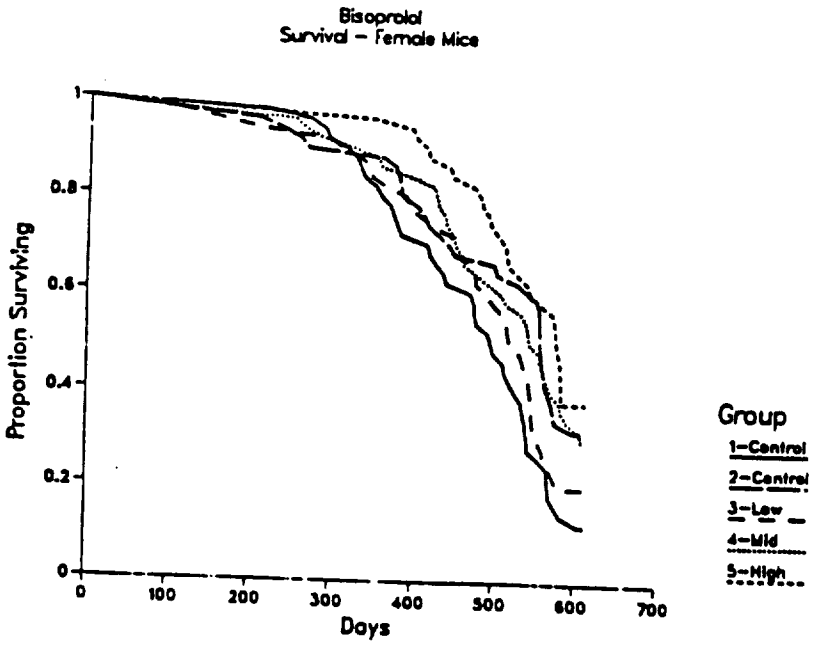
Figure 3



Statistical Analysis of Male Survival

Comparison	p-value
Controls - 1 vs 2	.68
Group Trend - combined controls, low, mid, high	.42

Figure 4



Statistical Analysis of Female Survival

Comparison	p-value
Controls - 1 vs 2	.01
Group Trend - combined controls, low, mid, high	.01

TABLE 1
BISOPROLOL: ONCOGENIC POTENTIAL IN RATS
FREQUENCY OF TUMOR-BEARING ANIMALS BY ORGAN (MALES): STATISTICAL ANALYSIS

ORGAN	GROUP 1,2 (n=100) 0 mg/kg	GROUP 3 (n=50) 5 mg/kg	GROUP 4 (n=50) 25 mg/kg	GROUP 5 (n=50) 125 mg/kg	p-value for heterogeneity test	p-value for trend test
Adrenals	7	4	4	4	.99	.47
Bone, General	3/100	0/49	1/49	0/50	-	-
Cerebellum	0	0	0	0	-	-
Cerebrum	2	4	4	1	.18	.37
Eyes	0/99	0/50	2/50	1/50	-	-
Heart	0	0	0	0	-	-
Kidneys	0	1	0	0	-	-
Large Intestine	1	0	2	0	-	-
Liver	1	1	0	2	-	-
Lungs	1	0	0	0	-	-
Lymph Nodes	9	4	2	2	.50	.93
Pancreas	0/100	1/49	0/50	1/50	-	-
Pancreatic Islet	6	9	8	2	.03	.47
Parathyroid	1/95	0/44	0/49	0/47	-	-

TABLE 1 (Continued)
BISOPROLOL: ONCOGENIC POTENTIAL IN RATS
FREQUENCY OF TUMOR-BEARING ANIMALS BY ORGAN (MALES): STATISTICAL ANALYSIS

ORGAN	GROUP 1,2 (n=100) 0 mg/kg	GROUP 3 (n=50) 5 mg/kg	GROUP 4 (n=50) 25 mg/kg	GROUP 5 (n=50) 125 mg/kg	p-value for heterogeneity test	p-value for trend test
Pituitary Gland	29/96	19/48	16/48	8/47	.11	.91
Salivary Gland	1	0	0	0	-	-
Seminal Vesicle	1	0	0	0	-	-
Skin	2	1	3	1	.57	.34
Small Intestine	0	0	0	0	-	-
Spleen	0	0	0	0	-	-
Stomach	1	0	1	0	-	-
Testes	6	2	0	4	.26	.59
Thymus	0/98	0/45	1/49	0/48	-	-
Thyroid	15/100	8/50	6/49	9/50	.80	.52
Tongue	1	1	1	0	-	-

TABLE 2
BISOPROLOL: ONCOGENIC POTENTIAL IN RATS
FREQUENCY OF TUMOR-BEARING ANIMALS BY ORGAN (FEMALES): STATISTICAL ANALYSIS

ORGAN	GROUP 1,2 (n=100) 0 mg/kg	GROUP 3 (n=50) 5 mg/kg	GROUP 4 (n=50) 25 mg/kg	GROUP 5 (n=50) 125 mg/kg	p-value for heterogeneity test	p-value for trend test
Adrenals	7	2	4	3	.88	.55
Bone, General	3/98	1/49	0/50	3/50	.34	.32
Cerebellum	1	2	0	1	-	-
Cerebrum	5	1	0	2	.39	.78
Eyes	1	0	0	1	-	-
Heart	0	0	1	0	-	-
Kidneys	2	0	1	0	-	-
Large Intestine	2	0	0	0	-	-
Liver	1	1	1	0	-	-
Lungs	0	0	0	0	-	-
Lymph Nodes	1	3	1	1	.29	.40
Mammary Gland	16/98	5/49	5/46	3/49	.25	.97
Ovaries	2	0	2	1	.59	.35
Pancreas	0	0	0	2	-	-

TABLE 2 (continued)
BISOPROLOL: ONCOGENIC POTENTIAL IN RATS
FREQUENCY OF TUMOR-BEARING ANIMALS BY ORGAN (FEMALES): STATISTICAL ANALYSIS

ORGAN	GROUP 1,2 (n=100) 0 mg/kg	GROUP 3 (n=50) 5 mg/kg	GROUP 4 (n=50) 25 mg/kg	GROUP 5 (n=50) 125 mg/kg	p-value for heterogeneity test	p-value for trend test
Pancreatic Islet	1	1	3	3	.27	.03
Parathyroid	0/98	0/49	0/47	0/49	-	-
Pituitary Gland	69/99	32/49	34/50	36/50	.75	.32
Salivary Glands	1	1	0	0	-	-
Skin	3	3	4	0	.19	.59
Small Intestine	0	0	1	0	-	-
Spleen	1	0	0	0	-	-
Stomach	2	1	0	2	.55	.34
Thymus	6/100	3/47	8/46	2/49	.06	.37
Thyroid	11	8	8	8	.78	.24
Tongue	1	0	0	1	-	-
Uterus	4	2	3	0	.46	.78

TABLE 3
BISOPROLOL: ONCOGENIC POTENTIAL IN RATS
FREQUENCY OF TUMOR-BEARING ANIMALS FOR SELECTED TUMORS (MALES):STATISTICAL ANALYSIS

TUMOR TYPE	GROUP 1,2 0 mg/kg	GROUP 3 5 mg/kg	GROUP 4 25 mg/kg	GROUP 5 125 mg/kg	Heterogeneity p-value	Trend p-value
Cortical Adenoma	3/100	3/50	2/50	1/50	.72	.65
Cerebrum (Meningioma)	1/100	3/50	4/50	1/50	.17	.22
Lymph Node Hemangioma	9/100	4/50	1/50	2/50	.30	.95
Islet Cell Adenoma	6/100	9/50	8/50	2/50	.03	.47
Pituitary Adenoma	28/96	19/48	15/48	8/47	.11	.91
Testes, Leydig Cell Tumor	5/100	2/50	0/50	4/50	.27	.45
Thyroid Cystadenoma or Follicular Adenoma	9/100	4/50	4/49	6/50	.89	.40
Malignant Lymphoma or Myelosis	5/100	1/50	2/50	0/50	.38	.92

TABLE 3 (continued)
BISOPROLOL: ONCOGENIC POTENTIAL IN RATS
FREQUENCY OF TUMOR-BEARING ANIMALS FOR SELECTED TUMORS (FEMALES): STATISTICAL ANALYSIS

TUMOR TYPE	GROUP 1,2 0 mg/kg	GROUP 3 5mg/kg	GROUP 4 25mg/kg	GROUP 5 125 mg/kg	Heterogeneity p-value	Trend p-value
Cortical Adenoma	7/100	2/50	3/50	2/50	.83	.76
Cerebrum (Meningioma)	3/100	1/50	0/50	2/50	.57	.52
Mammary Gland Adenoma	11/98	4/49	0/46	2/49	.06	.99
Islet Cell Adenoma	1/100	1/50	3/50	3/50	.27	.03
Pituitary Adenoma	68/99	32/49	33/50	36/50	.70	.30
Skin, Squamous Carcinoma Cell	2/100	3/50	1/50	0/50	.25	.78
Benign Thymoma	4/100	1/47	5/46	2/49	.26	.30
Thyroid C-Cell Adenoma	8/100	6/50	6/50	6/50	.84	.27
Thyroid Cystadenoma or Follicular Adenoma	3/100	2/50	2/50	2/50	.99	.40
Malignant Lymphoma or Myelosis	5/100	3/50	2/50	0/50	.42	.91

Table 4
Intercurrent Mortality Rates
Male Rats

Weeks	Control 1			Control 2			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	2	4	50	1	2	50	1	2	50	1	2	50	0	0
51-80	48	5	10	49	3	6	49	1	2	49	2	4	50	1	2
81-109	43	10	23	46	9	20	48	14	29	47	8	17	49	10	20
Term.	33			37			34			39			39		

Female Rats

Weeks	Control 1			Control 2			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	1	2	50	0	0	50	0	0	50	1	2	50	2	4
51-80	49	3	6	50	3	6	50	4	8	49	1	2	48	2	4
81-111	46	10	22	47	14	30	46	15	33	48	12	25	46	9	20
Term.	36			33			31			36			37		

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

Table 5
Tumor Incidence Rates
Male Rats, Adrenal Cortical Carcinoma

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	3	0	1	0	1	0	0
51-80	0	8	0	1	0	2	0	1
81-109	0	19	0	14	0	8	0	10
Terminal	0	70	0	34	0	39	2	39
Total	0	100	0	50	0	50	2	50

Table 6
Tumor Incidence Rates
Female Rats, Pancreatic Islet Cell Adenoma

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	1	0	2
51-80	0	6	0	4	0	1	0	2
81-111	1	24	0	15	1	12	0	9
Terminal	0	69	1	31	2	36	3	37
Total	1	100	1	50	3	50	3	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 7

BISOPROLOL: ONCOGENIC POTENTIAL IN MICE
FREQUENCY OF TUMOR-BEARING ANIMALS BY ORGAN (MALES): STATISTICAL ANALYSIS

ORGAN	GROUP 1,2 (n=100) 0 mg/kg	GROUP 3 (n=50) 10 mg/kg	GROUP 4 (n=50) 50 mg/kg	GROUP 5 (n=49) 250 mg/kg	p-value for heterogeneity test	p-value for trend test
Adrenals	2	0	0	0	-	-
Bone, General	0	0	1	0	-	-
Cerebrum	0	1	0	0	-	-
Epididymis	1	0	1	0	-	-
Eyes	1	4	1	1	.10	.45
Gall Bladder	0/94	0/49	0/50	0/47	-	-
Kidneys	1	1	0	0	-	-
Large Intestine	2	0	1	1	-	-
Liver	3	2	2	1	.89	.63
Lungs	18	13	12	10	.62	.49
Lymph Nodes	0/100	2/50	0/50	2/49	-	-

Table 7 (continued)

BISOPROLOL: ONCOGENIC POTENTIAL IN MICE
FREQUENCY OF TUMOR-BEARING ANIMALS BY ORGAN (MALES): STATISTICAL ANALYSIS

ORGAN	GROUP 1,2 (n=100) 0 mg/kg	GROUP 3 (n=50) 10 mg/kg	GROUP 4 (n=50) 50 mg/kg	GROUP 5 (n=49) 250 mg/kg	p-value for heterogeneity test	p-value for trend test
Pancreatic Islet	0	1	0	0	-	-
Pituitary Gland	0/97	0/48	0/48	0/44	-	-
Seminal Vesicle	2	1	0	0	-	-
Skeletal Muscle	2	0	0	0	-	-
Skin	0	0	0	1	-	-
Spleen	0/100	1/50	0/50	0/48	-	-
Stomach	0	1	0	1	-	-
Testes	1	4	3	2	.17	.17
Thymus	0/87	0/40	0/38	0/37	-	-
Thyroids	0/97	0/49	0/47	1/48	-	-
Urinary Bladder	1/100	1/49	2/49	0/46	-	-

BISOPROLOL: ONCOGENIC POTENTIAL IN MICE
FREQUENCY OF TUMOR-BEARING ANIMALS BY ORGAN (FEMALES): STATISTICAL ANALYSIS

ORGAN	GROUP 1,2 (n=100) 0 mg/kg	GROUP 3 (n=50) 10 mg/kg	GROUP 4 (n=50) 50 mg/kg	GROUP 5 (n=50) 250 mg/kg	p-value for heterogeneity test	p-value for trend test
Adrenals	1	2	1	0	-	-
Bone, General	2	1	2	1	.89	.58
Cerebrum	0	0	0	0	-	-
Eyes	2	1	2	0	-	-
Gall Bladder	0/94	0/48	0/47	1/46	-	-
Kidneys	0	0	0	0	-	-
Large Intestine	0	0	0	0	-	-
Liver	4	0	0	1	-	-
Lungs	21	8	5	10	.26	.91
Lymph Nodes	0	1	0	1	-	-
Mammary Gland	8/94	6/50	2/48	7/49	.44	.40
Ovaries	19/100	7/50	7/50	16/49	.19	.18

Table 8 (Continued)

BISOPROLOL: ONCOGENIC POTENTIAL IN MICE
FREQUENCY OF TUMOR-BEARING ANIMALS BY ORGAN (FEMALES): STATISTICAL ANALYSIS

ORGAN	GROUP 1,2 (n=100) 0 mg/kg	GROUP 3 (n=50) 10 mg/kg	GROUP 4 (n=50) 50 mg/kg	GROUP 5 (n=49) 250 mg/kg	p-value for heterogeneity test	p-value for trend test
Pancreatic Islet	0	0	0	-	-	-
Pituitary Gland	4/96	3/49	4/50	3/48	.86	.36
Skeletal Muscle	0	0	0	0	-	-
Skin	1	0	0	0	-	-
Spleen	0	0	0	0	-	-
Stomach	1	1	1	0	-	-
Thymus	2/94	1/47	0/47	0/45	-	-
Thyroids	2/97	2/50	3/50	2/50	.72	.27
Urinary Bladder	1/98	0/50	1/50	0/50	-	-
Uterus	10	1	3	3	.22	.91

Table 9

BISOPROLOL: ONCOGENIC POTENTIAL IN MICE
FREQUENCY OF TUMOR-BEARING ANIMALS FOR SELECTED TUMORS: STATISTICAL ANALYSIS

TUMOR TYPE	GROUP 1,2 0 mg/kg	GROUP 3 10 mg/kg	GROUP 4 50 mg/kg	GROUP 5 250 mg/kg	Heterogeneity p-value	Trend p-value
MALES						
Liver Cell Adenoma	2/100	2/50	1/50	1/49	.87	.59
Adenoma of the Lung	16/100	11/50	12/50	10/49	.62	.33
Testes: Leydig Cell Tumor	1/100	3/50	3/50	2/49	.28	.14
Malignant Lymphoma or Myelosis	30/100	10/50	14/50	10/49	.48	.82

Table 9 (continued)

BISOPROLOL: ONCOGENIC POTENTIAL IN MICE
FREQUENCY OF TUMOR-BEARING ANIMALS FOR SELECTED TUMORS: STATISTICAL ANALYSIS

TUMOR	GROUP 1,2 0 mg/kg	GROUP 3 10 mg/kg	GROUP 4 50 mg/kg	GROUP 5 250 mg/kg	Heterogeneity p-value	Trend p-value
FEMALES						
Adenoma of the Lung	19/100	8/50	5/50	10/49	.37	.86
Mammary Gland Adenocarcinoma	7/94	6/50	0/48	5/49	.12	.66
Cystadenoma of the Ovaries	8/100	1/50	3/50	10/49	.05	.07
Ovaries: Granulosa Theca Cell Tumor	7/100	5/50	4/50	4/49	.88	.57
Pituitary Adenoma	4/96	3/49	4/50	3/48	.86	.36
Thyroid: Cystadenoma or Follicular Adenoma	2/97	2/50	3/50	2/50	.72	.27
Uterus: Leiomyoma	7/100	1/50	2/50	1/50	.27	.95
Malignant Lymphoma or Myelosis	45/100	21/50	21/50	24/50	.93	.48

Table 10
Intercurrent Mortality Rates
Male Mice

Weeks	Control 1			Control 2			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	8	16	50	9	18	50	7	14	50	5	10	49	8	16
51-70	42	15	36	41	12	29	43	11	29	45	14	31	41	7	17
71-86	27	11	41	29	16	55	32	18	56	31	17	55	34	17	50
Term.	16			13			14			14			17		

Female Mice

Weeks	Control 1			Control 2			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	50	9	18	50	5	10	50	8	16	50	6	12	50	2	4
51-70	41	16	39	45	11	24	42	12	29	44	12	27	48	11	23
71-86	25	19	76	34	18	53	30	20	67	32	17	53	37	18	49
Term.	6			16			10			15			19		

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

Table 11
Tumor Incidence Rates
Female Mice, Lungs Metastatic Adenocarcinoma

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	14	0	8	0	6	0	2
51-70	0	27	0	12	0	12	1	11
71-86	0	37	0	20	0	17	1	18
Terminal	0	22	0	10	0	15	0	19
Total	0	100	0	50	0	50	2	50

Table 12
Tumor Incidence Rates
Female Mice, Ovaries Cystadenoma

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	14	0	8	1	6	0	2
51-70	0	27	0	12	1	12	0	11
71-86	2	37	0	20	0	17	1	18
Terminal	1	22	0	10	1	15	5	19
Total	3	100	0	50	3	50	6	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 13
Tumor Incidence Rates
Male Mice, Lymph Nodes Hemangioma-abdominal Lymph Node

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	17	0	7	0	5	0	8
51-70	0	27	0	11	0	14	1	7
71-86	0	27	1	18	0	17	0	17
Terminal	0	29	0	14	0	14	1	17
Total	0	100	1	50	0	50	2	49

Table 14
Tumor Incidence Rates
Female Mice, All Organs, Granulocytic Leukemia

Weeks	Control		Low		Medium		High	
	T	N	T	N	T	N	T	N
0-50	0	14	0	8	1	6	0	2
51-70	1	27	0	12	0	12	2	11
71-86	1	37	0	20	2	17	1	18
Terminal	0	22	0	10	0	15	0	19
Total	2	100	0	50	3	50	3	50

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

AUG - 6 1998

STATISTICAL REVIEW AND EVALUATION
(Review of Protocols)

NDA: [redacted] and 20-186
Applicant: Wyeth-Ayerst Research
Name of Drug: Zinc (bisoprolol fumarate and hydrochlorothiazide) Tablets
Indication: Pediatric hypertension
Document Reviewed: Protocols of two studies.
Received 06/11/98.

1. INTRODUCTION

The sponsor has submitted the protocols of two studies, studies 0896A2-903 and 0896A2-904, of bisoprolol (Zebeta) and bisoprolol/HCTZ combination (Ziac) in hypertensive children between 8 and 18 years of age. Pediatric hypertension is characterized by having an average sitting diastolic BP (SiDBP) and/or sitting systolic BP (SiSBP) above the 95th percentile of BP distribution according to age, sex and height. The sponsor has provided tables for the 95th percentile of BP distribution for different height percentiles by age and sex.

The sponsor is seeking an additional six months of market exclusivity (which expires on March 24, 2000) for the pediatric use of both Zebeta and Ziac after the completion of these studies.

2. STUDY 0896A2-903

This is a randomized, multicenter, double-blind, placebo-controlled and titration study for hypertensive children between 8 and 18 years of age. The sponsor is expecting to screen 200 patients in order that at least 105 patients would be eligible for randomization to double-blind treatment. After a 2-week placebo run-in period patients will be randomized to receive either bisoprolol 2.5/HCTZ 6.25 mg (Q) or matching placebo for 12 weeks. Patients dose level will be titrated sequentially to bisoprolol 5/HCTZ 6.25 mg and bisoprolol 10/HCTZ 6.25 mg to control their BP (SiDBP or SiSBP $\leq$95th percentile) at two occasions: two weeks and four weeks after randomization. The primary endpoint is a multiple endpoint consisting of the change from baseline in SiDBP and SiSBP.

3. STUDY 0896A2-904

This is a randomized, single-center, single-dose, open-label crossover study to determine the pharmacokinetics profiles of bisoprolol (Zebeta) and bisoprolol/HCTZ combination (Ziac) in hypertensive children between 8 and 18 years of age.

4. REVIEWER'S COMMENTS

In study 0896A2-903 the sponsor is proposing to have a multiple primary endpoint consisting of the change from baseline in SiDBP and SiSBP. This is based on the sponsor's characterization of pediatric hypertension (page 9 of the protocol) as having an average SiDBP and/or SiSBP above the 95th percentile of BP distribution according to age, sex and height. Thus, this characterization implies that a drug applied to pediatric hypertensive patients would be considered effective if it significantly reduces both their SiDBP and SiSBP. But, on page 19 of the protocol it is stated that "*patients dose level will be titrated sequentially to bisoprolol 5/HCTZ 6.25 mg and bisoprolol 10/HCTZ 6.25 mg to control their BP (SiDBP or SiSBP ≤95th percentile) at two occasions . . .*". This last statement could mean that the study drug will be effective if it reduces **either** the SiDBP **or** the SiSBP.

Tthe above statements do not make it clear how would one considers a drug to be effective in pediatric hypertensive patients. Should this effectiveness be evaluated by the reduction of both or either one of the SiDBP and the SiSBP? The protocol of this study should answer this question clearly.

To seek FDA's position on the matter of pediatric hypertension assessment, this reviewer has consulted Dr. Robert Fenichel (Deputy Director of the Division of Cardio-Renal Drugs). Dr. Fenichel's answer was that, since no information is available about pediatric hypertension, the sponsor should provide a convincing argument to support the sponsor's decision in including SiDBP and/or SiSBP for the efficacy assessment.

In any case, we are dealing with a primary endpoint consisting of two components that are correlated. Therefore, an appropriate method should be applied for adjusting the alpha level (α_1, say) under which each component is to be tested so that the overall type I error would be controlled. Bonferroni approach can be used for such an adjustment but, it is to the sponsor's disadvantage using this approach because of its conservativeness.

Here, it should be noted that, if the efficacy of a study drug is to be assessed based on the significance of all components of a multiple endpoint, then in the FDA's view no adjustment is necessary for the above mentioned alpha level. The rationale behind this view is that the chance of winning in all components is very small which makes it difficult to demonstrate the efficacy. Table 1 of the appendix shows, as an example, the probability of winning in both of the two components of an endpoint. Thus, if the efficacy is to be determined based on the significance of both the SiDBP and the SiSBP then, no need to adjust the above mentioned alpha level; otherwise, if the efficacy is to be determined based on the significance of either the SiDBP or the SiSBP then, the sponsor needs to look into finding an appropriate adjustment for the alpha level.

This reviewer has gone through a theoretical investigation (see the appendix of this review for detail) to find values of α_1 for a set of values of the correlation between SiDBP and SiSBP, assuming an overall type I error α=0.05 and two-sided tests. The results are shown in Table 2 of

the appendix.

To use the results of Table 2, the sponsor needs to investigate an appropriate value for the correlation between the SiDBP and the SiSBP for the population of patients described above. This correlation may be estimated by using historical data.

In this reviewer's opinion the design and method of analysis proposed for study 0896A2-903 should serve the goals of the investigation, provided that the sponsor makes it clear how the efficacy of the study drug is going to be assessed.

Concerning the protocol of the open-label study 0896A2-904, this reviewer has no comment.

/S/

Walid A. Nuri, Ph.D.
Mathematical Statistician

This review consists of three pages and an appendix.

Concur:
Dr. Mahjoob 08/06/98
Dr. Chi 8/6/98

cc: Orig. NDA 19-982 and 20-186
HFD-110/Dr. Gordon
HFD-110/Ms. McDonald
HFD-344/Dr. Barton
HFD-710/Dr. Chi
HFD-710/Dr. Mahjoob
HFD-710/Dr. Nuri
Chron: W A Nuri: 594-5303 DB I: 07-23-98: DISC11/ziac.wpd.

APPENDIX

Suppose that we are testing simultaneously the following two hypotheses.

$$H_{01}: \Delta_1 = 0, \qquad H_{02}: \Delta_2 = 0,$$
$$\text{vs. } H_{a1}: \Delta_1 \neq 0. \qquad \text{vs. } H_{a2}: \Delta_2 \neq 0.$$

Then, it is not unrealistic, using many practical examples, to assume that each of the corresponding test statistics (Z_1 , Z_2 , say) for testing the two hypotheses is normally distributed with mean 0 and variance 1. If these test statistics are correlated with correlation equals to ρ, then Z_1 , Z_2 have the bivariate normal distribution with means 0 , variances 1 and correlation coefficient ρ.

Let α , c be the overall alpha level and a two-sided critical value, respectively, for testing the above hypotheses simultaneously. Under the corresponding null hypotheses, let the events A_i (i=1,2) be defined as

$$A_i=\{\omega : |Z_i| \geq c\}, \quad (i=1,2).$$

Then, the type I error α will be defined by the following formula.

$$\alpha = Pr[A_1 \cup A_2] = Pr[A_1] + Pr[A_2] - Pr[A_1 A_2], \quad (A_1 A_2=A_1 \cap A_2) . \qquad (1)$$

Assume that each individual hypothesis is to be tested under a level of significance α_1 , such that.
$Pr[A_1]= Pr[A_2]= \alpha_1$. Thus, formula (1) becomes

$$\alpha = 2\,\alpha_1 - Pr[A_1 A_2], \text{ or}$$

$$\alpha_1 = (\alpha + Pr[A_1 A_2])/2 . \qquad (2)$$

A numerical integration approach was used to calculate $Pr[A_1 A_2]$ and then the value of α_1 , for different values of ρ, by assuming $\alpha = 0.05$ and the corresponding critical value c=1.96, for a two-sided test for each hypothesis.

The results of calculation are shown in Tables 1 and 2 below.

Table 1. Probability of rejecting both of two correlated hypotheses (with correlation ρ) and an overall type I error α=0.05 in two-sided tests.

ρ	$Pr[A_1 A_2]$
0.10	0.0020
0.20	0.0031
0.30	0.0047
0.40	0.0067
0.50	0.0092
0.60	0.0124
0.70	0.0165
0.80	0.0219
0.90	0.0296

Table 2. The alpha level (α_1) under which each one of two correlated hypotheses (with correlation ρ) is to be tested to control an overall type I error α=0.05 in two-sided tests.

$0.1 \le \rho \le 0.9$	α_1	$\rho < 0.1$	α_1	$\rho > 0.9$	α_1
0.10	0.0260	0.01	0.0256	0.91	0.0403
0.20	0.0266	0.02	0.0257	0.92	0.0408
0.30	0.0273	0.03	0.0257	0.93	0.0414
0.40	0.0283	0.04	0.0257	0.94	0.0421
0.50	0.0296	0.05	0.0258	0.95	0.0427
0.60	0.0312	0.06	0.0258	0.96	0.0435
0.70	0.0332	0.07	0.0259	0.97	0.0444
0.80	0.0359	0.08	0.0259	0.98	0.0455
0.90	0.0398	0.09	0.0259	0.99	0.0470