

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-275

STATISTICAL REVIEW(S)



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Drug
Office of Biostatistics

Statistical Review and Evaluation
CARCINOGENICITY STUDIES
(Addendum-1)

(Subject: Reanalysis of carcinogenicity data using the historical data and newly available software)

IND/NDA Number: NDA 22-275
Drug Name: OPC-156 (Tolvaptan)
Indication(s): 104 Week Carcinogenicity in Rats and Mice
Applicant: Sponsor: Otsuka Pharmaceutical Co., Ltd.



b(4)

Documents Reviewed: Electronic submission, Dated October 23, 2007, and data submitted electronically, received on October 23, 2007

Review Priority: Standard

Biometrics Division: Division of Biometrics -6
Statistical Reviewer: Mohammad Atiar Rahman, Ph.D.
Concurring Reviewer: Karl Lin, Ph.D.

Medical Division: Division of Cardiovascular and Renal Products
Reviewing Pharmacologist: Xavier Joseph Ph.D.
Project Manager: Daniel Brum

Keywords: Carcinogenicity, Dose-Response

Introduction: This reviewer performed a statistical review of this carcinogenicity study on Feb. 6, 2008. In that review, based on the concurrent control, the combined incidences of liver cholangiocellular adenoma and carcinoma in male rats was categorized as rare tumor and was found to have statistically significant dose response relationship. The agency requested the sponsor to submit historical data for this species of rats for further assessment of their spontaneous background rates. In this submission the sponsor submitted the historical background data from the laboratory involved this study. There were only two available studies for this particular species of rats in the involved laboratory. From these two studies, the sponsor reported the following historical incidence rates:

Cholangiocellular adenoma: Male 1.66% Female 0.83%
 Cholangiocellular carcinoma: Male 0% Female 0.83%

The data from these two studies show a background rate of >1.66% for the combined incidences of liver cholangiocellular adenoma and carcinoma in males and >0.83% for females. This indicates that the combined incidence rate of liver cholangiocellular adenoma and carcinoma is a common type for male rats.

Since no software for the calculation of exact dose response relationship p-value was available during the original review, at that time this reviewer calculated the asymptotic p-values. Now since the software for the calculation of the exact p-value became available, this reviewer re-analyzed the data using the new software. The following table shows the incidence rates and exact p-values for the combined incidences of liver cholangiocellular adenoma and carcinoma in male rats.

Organ Name	Tumor Name	Cont N=55	100mg N=55	300mg N=55	1000mg N=55	P_Value Dos Resp	P_Value C vs. M	P_Value C vs.MH	P_Value C vs. H
liver	CHOLANG ADENOMA+CARC	0	0	0	2	0.062	.	.	0.271

Conclusion: Based on the historical data, the combined incidences of liver cholangiocellular adenoma and carcinoma is considered to be a common type in male rats. Based on the adjustment method for multiple comparisons suggested by Lin and Rahman (see the original review for details), and the newly calculated exact p-value, the dose response relationship of the combined incidences of liver cholangiocellular adenoma and carcinoma in male rats is not considered to be statistically significant.

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Concur: Karl Lin, Ph.D.
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/s/

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5/6/2008 02:05:13 PM
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U.S. Department of Health and Human Services
Food and Drug Administration
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Statistical Review and Evaluation
CARCINOGENICITY STUDIES

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1. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of OPC-156 in rats and mice when administered orally by gavage at appropriate drug levels for about 104 weeks. Results of this review have been discussed with the reviewing pharmacologist Dr. Joseph.

2. Rat Study

Two separate experiments, one in males and one in females were conducted. The experiment with males had three treated groups and one control group, while the experiment with females had four treated groups and one control group. In experiment with males, two hundred and twenty CD(SD) rats were randomly allocated to treated and control groups in equal size of 55 animals. The dose levels for treated groups were 100, 300, and 1000 mg/kg/day of the study drug. In this review these treated groups will be referred to as medium, mid-high, and high dose groups. The controls received 1% hydroxypropyl methylcellulose (HPMC) by gavage. In experiment with females, two hundred and seventy five animals were randomly allocated to treated and control groups in equal size of 55 animals. The dose levels for treated groups were 30, 100, 300, and 1000 mg/kg/day of the study drug. In this review these treated groups will be referred to as low, medium, mid-high, and high dose group. The controls received 1% HPMC by gavage.

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Animals were checked at least twice daily for general condition, mortality and morbidity. In addition, palpation was performed once a week for the detection of neoplastic lesions and masses. Body weights were measured weekly and at the time of death. A complete histopathological examination was performed on all animals found dead, killed moribund, or sacrificed during or at the end of the experiment from control and high dose groups. Animals which died during the study (prior to terminal sacrifice) in other dose groups were also histopathologically examined.

2.1. Sponsor's analyses

2.1.1. Survival analysis

The sponsor calculated the percentage of survivor at termination and compared the mortality of animals in each treated groups with control using the Log rank test (Peto et al. 1977).

Sponsor's findings: The sponsor's analysis showed mortality rates of 58.2%, 34.5%, 45.5% and 38.2% at the end of the study in control, medium, mid-high, and high dose groups in males, and 45.5%, 38.2%, 30.9%, 30.9% and 32.7% in control, low, medium, mid-high, and high dose groups in females. The sponsor's analyses showed statistically significantly lower mortality in the medium and high dose group compared to the control in males. The sponsor's analysis showed no dose-related increase or decrease in mortality in females.

2.1.2. Tumor data analysis

The sponsor compared the incidence rate of tumors in each treated group with control using the Fisher exact test. In addition, they tested the dose response relationship¹ using the Cochran-Armitage test (Cochran 1954).

¹ In this review the phrase "dose-response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor rate as dose increases.

When the survival ratio was found to be significant between the groups, the sponsor analyzed the data for dose response relationship using the method suggested by Peto et al. (Peto et al. 1980). The numbers of benign, malignant and total tumor were analyzed by Dunnett's multiple comparison tests. The values were analyzed for significance of difference at the 5% and 1% levels of probability using the one-sided analysis.

Sponsor's findings: The sponsor's analysis did not show statistically significant increased incidence in any of the tested tumor types in the treated group compared to the control. The sponsor further stated that the neoplastic lesions did not show an earlier onset in the moribund or dead animals in the comparison between groups.

2.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analysis suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically.

2.2.1. Survival analysis

The survival distributions of animals in all treatment groups were estimated by the Kaplan-Meier product limit method. The homogeneity of survival distributions was tested using the Cox test (Cox, 1972) and the Generalized Wilcoxon test (Gehan, 1965). The intercurrent mortality data are given in Tables 1A and 1B in the appendix for males and females, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1A and 1B in the appendix for male and females, respectively. Results of the tests for homogeneity and dose response relationship in survivals are given in Tables 2A and 2B in the appendix for males and females, respectively.

Reviewer's findings: The tests showed no statistically significant dose response relationship or differences in survivals across treatment groups in females. In males the low dose group showed statistically significantly lower mortality compared to the control.

Reviewer's comment: The sponsor's analysis showed a significant difference in mortality between high dose group and the control. This reviewer's analysis showed a p-value of 0.058 for the comparison of control and high dose group, which is marginally not significant.

2.2.2. Tumor data analysis

Since all animals of only control and high dose group were completely histopathologically examined, a pairwise comparison of controls with high dose group would be statistically sound. Therefore, this reviewer primarily performed pairwise comparisons of control with high dose group. Additionally, this reviewer also performed dose response relationship tests in tumor incidences involving all treated groups. However it should be noted, since not all animals in the low, medium, and mid-high dose groups were histopathologically examined, any significant finding of dose response relationship should be interpreted carefully. In this review, the data for a tumor type with significant dose response relationship were explored with further investigations.

The tumor data were analyzed using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). One critical point for Poly-k test is the choice of the appropriate value of k. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. The tumor rates and the p-values of the tumor types tested for dose-response relationships and pairwise comparisons are listed in Tables 3A and 3B in the appendix for males and females, respectively.

Multiple testing adjustment: Since a huge number of simultaneous tests are involved in this tumor data analysis, an appropriate adjustment for multiple testing is necessary for appropriate interpretation of the statistical findings. In this analysis adjustment for the multiple dose-response relationship testing was done using the results of Lin and Rahman (1998), which recommends, to use a significance level $\alpha=0.025$ for rare tumors and $\alpha=0.005$ for common tumors for a submission with two studies, and a significance level $\alpha=0.05$ for rare tumors and $\alpha=0.01$ for common tumors for a submission with one study in order to keep the false-positive rate at the nominal level of approximately 10%. A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. Adjustment for multiple pairwise comparisons was done using the results of Haseman (1983), which recommends to use a significance level $\alpha=0.05$ for rare tumors and $\alpha=0.01$ for common tumors, in order to keep the false-positive rate at the nominal level of approximately 10%.

It should be noted that the recommended test levels by Lin and Rahman for the adjustment of multiple testing was originally based on the result of a simulation and an empirical study using the Peto method for dose response relationship analysis. However, more recent simulation results by the same authors (unpublished manuscript presented in 2006 BASS meeting in Savannah, Georgia) indicated similar usefulness of their recommendation for Poly-3 analysis also.

Reviewer's findings: The following tumor types showed p-values less than or equal to 0.05 for either the dose-response relationship or some pairwise comparisons of the control with the treated groups.

Tumor Types with Dose-Response Relationship Test or Pairwise Comparisons P-Values \leq 0.05

Sex: Male

Organ Name	Tumor Name	Cont N=55	100mg N=55	300mg N=55	1000mg N=55	P_Value Dos Resp	P_Value C vs. M	P_Value C vs.MH	P_Value C vs. H
liver	CHOLANG ADENOMA+CARC	0	0	0	2	0.022	1.000	1.000	0.087

Sex: Female

Organ Name	Tumor Name	Cont N=55	30mg N=55	100mg N=55	300mg N=55	1000mg N=55	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs.MH	P_Value C vs. H
Zymbal's gland	carcinoma	0	0	0	0	1	0.049	1.000	1.000	1.000	0.156
clitoral gland	squamous cell papill	0	0	0	0	1	0.049	1.000	1.000	1.000	0.155
liver	hepatocellular carci	0	0	0	0	1	0.049	1.000	1.000	1.000	0.155
mammary gland	adenocarcinoma	2	4	1	3	3	0.530	0.026	0.302	0.055	0.319
peritoneum	histiocytic sarcoma	0	0	0	0	1	0.050	1.000	1.000	1.000	0.157
stomach	adenoma	0	0	0	0	1	0.049	1.000	1.000	1.000	0.155
subcutaneous ti	fibrosarcoma	0	0	0	0	1	0.049	1.000	1.000	1.000	0.155
thyroid gland	follicular cell aden	0	0	0	0	1	0.049	1.000	1.000	1.000	0.155
uterus	adenoma	0	0	0	0	1	0.049	1.000	1.000	1.000	0.155

* There were 55 animals randomized in each of the treatment groups. However, all organs of 55, 19, 25, and 55 of males from control, medium, mid-high, and high dose groups, and 55, 21, 17, 17, and 55 females from control, low, medium, mid-high, and high dose groups were histopathologically examined. The statistical analyses were based on the actual number of animals histopathologically examined.

Based on the results of Lin and Rahman the dose-response relationship of the combined incidences of liver cholangiocellular adenoma and carcinoma in males was considered to be statistically significant. Based on the results of Haseman, none of the pairwise comparisons of control with high dose group or control with any other treated group was considered to be statistically significant.

3. Mouse Study

Two separate experiments, one in males and one in females were conducted. In each of these two experiments there were three treated groups and one control group. Two hundred and twenty B6C3F1 mice of each sex were randomly allocated to treated and control groups in equal size of 55 animals. For males the dose levels for treated groups were 10, 30, and 60 mg/kg/day. In this review these treated groups will be referred to as low, medium, and mid-high dose groups. For females the dose levels for treated groups were 10, 30, and 100 mg/kg/day. In this review these treated groups will be referred to as low, medium, and high dose groups. The controls received 1% hydroxypropyl methylcellulose (HPMC) by gavage.

Animals were checked for at least twice daily for general condition, mortality and morbidity. In addition, palpation was performed once a week for the detection of neoplastic lesions and masses. Body weights were measured weekly and at the time of death. A complete histopathological examination was performed on all animals found dead, killed moribund, or sacrificed during or at the end of the experiment from control and highest dose group. Animals which died during the study (prior to terminal sacrifice) in other dose groups were also histopathologically examined.

3.1. Sponsor's analyses

3.1.1. Survival analysis

The sponsor analyzed the survival data from the mouse study using the same statistical methodologies as they used to analyze the survival data from the rat study.

Sponsor's findings: Sponsor's analysis showed mortality rates of 12.7%, 10.9%, 10.9%, and 10.9% at the end of the study in control, low, medium, and mid-high dose groups in males, and 25.9%, 10.9%, 16.4%, and 27.3% in control, low, medium, and high dose groups in females. Sponsor's analyses showed no statistically significant difference in mortality due to treatment in either sex, except for a significant decreased mortality in low dose females compared to the control.

3.1.2. Tumor data analysis

The sponsor analyzed the tumor data from the mouse study using the same statistical methodologies as they used to analyze the tumor data from the rat study.

Sponsor's findings: Sponsor's analysis using the Fisher exact test did not show statistically significant increased incidence in the treated groups compared to the control in any of the tested tumor types. The Peto test showed a statistically significantly higher occurrence of lymph node malignant lymphoma in high dose group in males ($P=0.045$). The sponsor further stated that the neoplastic lesions did not show an earlier onset in the moribund or dead animals in the comparison between groups.

3.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analysis suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically.

3.2.1. Survival analysis

The reviewer analyzed the survival data from the mouse study using the same statistical methodologies as he used to analyze the survival data from the rat study. The intercurrent mortality data are given in Tables 4A and 4B in the appendix for males and females, respectively. The Kaplan-Meier curves for survival rate are given in Figures 2A and 2B in the appendix for male and females, respectively. Results of the tests for homogeneity and dose response relationship in survivals are given in Tables 5A and 5B in the appendix for males and females, respectively.

Reviewer's findings: The tests showed no statistically significant dose response relationship or differences in survivals across treatment groups in either sex.

3.2.2. Tumor data analysis

The reviewer analyzed the tumor data from the mouse study using the same statistical methodologies as he used to analyze the tumor data from the rat study i.e. primarily performed pairwise comparisons of control with high dose group and additional dose response relationship tests using the Poly-3 methods. Similar to the rat data analysis, any significant finding of dose response relationship should be interpreted carefully. The tumor rates and the p-values of the tumor types tested for dose-response relationships and pairwise comparisons are listed in Table 6A and 6B in the appendix for males and females, respectively. It should be noted that the submitted data showed, the sponsor examined 100% male mice in all treatment groups for at least one organ (coded as ANIMLXM=1).

Multiple testing adjustments: The reviewer used the same multiple testing adjustment rule for the mouse study as he used to adjust the multiple testing adjustment for the rat study.

Reviewer's findings: The following tumor types showed p-values less than or equal to 0.05 for either the dose-response relationship or some pairwise comparisons of the control with the treated groups.

Tumor Types with Dose-Response Relationship Test or Pairwise Comparisons P-Values ≤ 0.05

Sex: Male

Organ Name	Tumor Name	Cont N=55	10mg N=55	30mg N=55	60mg N=55	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. MH
lymph node	malignant lymphoma	1	4	1	5	0.053	0.004	0.176	0.051

Sex: Female

Organ Name	Tumor Name	Cont N=55	10mg N=55	30mg N=55	100mg N=55	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Harderian gland	adenoma	1	0	0	4	0.024	0.547	0.575	0.082

* There were 55 animals randomized in each of the treatment groups. However, all organs of 55, 6, 6, and 55 of males from control, low, medium, and mid-high dose groups, and 55, 6, 9, and 55 females from control, low, medium, and high dose groups were histopathologically examined. The statistical analyses were based on the actual number of animals histopathologically examined.

Based on the results of Lin and Rahman, the dose response relationship of none of the tested tumor types was considered to be statistically significant. Based on the results of Haseman, none the pairwise comparisons of control with high dose group was considered to be statistically significant. However, the increased incidence of malignant lymphoma in lymph node in male low dose group was considered to be statistically significant compared to the control.

4. Evaluation of validity of the design of the mouse study

As was seen, the tumor data from neither the rat nor the mouse studies showed statistically significant dose-response relationship or increased incidence in treated groups in any of the tested tumor types, except a dose response relationship in a combined tumor types in rats and a pairwise comparison of control and low dose group in mouse. Therefore, before drawing any conclusion regarding the carcinogenic or non-carcinogenic potential of OPC-156 in rats and mice, it is important to look into the following two issues, as have been pointed out in the paper by Haseman (1984).

- (i) Were enough animals exposed, for a sustained amount of time, to the risk of late developing tumors?
- (ii) Were dose levels high enough to pose a reasonable tumor challenge to the animals?

There is no consensus among experts regarding the number of animals and length of time at risk, although most carcinogenicity studies are designed with fifty animals per treatment group and run for two years. The following are some rules of thumb regarding these two issues as suggested by experts in this field:

Haseman (1985) has done an investigation on the first issue. He gathered data from 21 studies using Fischer 344 rats and B6C3F1 mice conducted at the National Toxicology Program (NTP). It was found that, on the average, approximately 50% of the animals in the high dose group survived the two-year study period. Also, in a personal communication with Dr. Karl Lin of Division of Biometrics-6, Haseman suggested that, as a rule of thumb, a 50% survival of 50 initial animals or 20 to 30 animals still alive in the high dose group, between weeks 80-90, would be consider as a sufficient number and adequate exposure. In addition Chu, Cueto and Ward (1981), suggested that "to be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have groups of animals with greater than 50% survival at one-year."

It appears, from these three sources that the proportions of survival at 52 weeks, 80-90 weeks, and two years are of interest in determining the adequacy of exposure and number of animals at risk.

Regarding the question of adequate dose levels, it is generally accepted that the high dose should be close to the maximum tolerated dose (MTD). In the paper of Chu, Cueto and Ward (1981), the following criteria are mentioned for dose adequacy. A high dose is considered as close to MTD if any of the criteria is met.

- (i) "A dose is considered adequate if there is a detectable loss in weight gain of up to 10% in a dosed group relative to the controls."
- (ii) "The administered dose is also considered as MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical."
- (iii) "In addition, doses are considered adequate if the dosed animals show a slight increased mortality compared to the controls."

We will now investigate the validity of the OPC-156 rat and mouse carcinogenicity study, in the light of the above guidelines.

4.1. Rat Study

The following is the summary of survival data of rats in the highest dose groups:

Percentage of survival in the highest dose group at the end of Weeks 52, 78, and 91

	Percentage of survival		
	End of 52 weeks	End of 78 weeks	End of 91 weeks
Male	96.4%	85.5%	74.5%
Female	90.9%	76.4%	70.9%

Based on the survival criterion Haseman proposed, it could be concluded that enough rats in both sexes were exposed to the highest dose for a sufficient amount of time.

The following table shows the percent difference in mean body weight gain from the concurrent control, defined as

$$\text{Percent difference} = \frac{(\text{Final BW} - \text{Baseline BW})_{\text{Treated}} - (\text{Final BW} - \text{Baseline BW})_{\text{Control}}}{(\text{Final BW} - \text{Baseline BW})_{\text{Control}}} \times 100$$

Percent Difference in Mean Body Weight Gain From Controls

Male			Female			
100mg	300mg	1000mg	30mg	100mg	300mg	1000mg
-51.06	-57.10	-57.70	-62.24	-65.03	-71.33	-66.43

Source: Table 3 of sponsor's submission

Therefore, relative to control, there had been more than 57% decrement in body weight gain in high dose group in both sexes. In fact, this loss of body weight gain actually seems to be quite excessive.

The mortality rates at the end of the experiment were as follows:

Mortality Rates at the End of the Experiment

	Cont.	30 mg	100 mg	300 mg	1000 mg
Male	58.2%		34.5%	45.5%	38.2%
Female	45.5%	38.2%	30.9%	30.9%	32.7%

Therefore, the mortality rate of in the high dose group in males is 20% lower than the control and also in females it is about 13% lower in high dose group than to the control.

Thus, from the body weight gain data it may be concluded that the used highest dose level might have reached or exceeded the MTD in both sexes. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

4.2. Mouse Study

The following is the summary of survival data of mice in the highest dose groups:

Percentage of survival in the highest dose group at the end of Weeks 52, 78, and 91

	Percentage of survival		
	End of 52 weeks	End of 78 weeks	End of 91 weeks
Male	100%	100%	98.2%
Female	96.4%	94.5%	83.6%

Based on the survival criterion Haseman proposed, it could be concluded that enough mice in both sexes were exposed to the highest dose for a sufficient amount of time.

The following table shows the percent difference in mean body weight gain from the concurrent control (Calculated using the formula given in section 4.1 for Rat study)

Percent Difference in Mean body Weight Gain From Control

Male			Female		
10 mg	30 mg	60 mg	10 mg	30 mg	100 mg
-12.56	-15.82	-25.12	0.00	-12.82	-5.13

Source: Table 3 of sponsor's submission

Therefore, relative to control, there had been more than 25% decrement in body weight gain in highest dose group in males, and more than 5% decrement in body weight gain in highest dose group in females.

The mortality rates at the end of the experiment were as follows:

Mortality Rates at the End of the Experiment

	Cont.	10 mg	30 mg	60mg	100 mg
Male	12.7%	10.9%	10.9%	10.9%	
Female	25.5%	10.9%	16.4%		27.3%

This shows that the mortality rate of in the mid-high dose group (the highest dose for males) in males is 2% lower than the control, while in female it is about 2% higher in high dose group compared to the control.

Thus, from the body weight gain data it may be concluded that the used highest dose levels might have reached or exceeded the MTD in males but at MTD in females. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

5. Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of OPC-156 in rats and mice when administered orally by gavage at appropriate drug levels for about 104 weeks.

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

Rat Study: Two separate experiments, one in males and one in females were conducted. The experiment with males had three treated groups and one control, while the experiment with females had four treated groups and one control. The group size in both sexes was 55. For males, the dose levels for treated groups were 100, 300, and 1000 mg/kg/day of the study drug. For females the dose levels for treated groups were 30, 100, 300, and 1000 mg/kg/day of the study drug. The controls received 1% hydroxypropyl methylcellulose (HPMC) by gavage.

The tests showed no statistically significant dose response relationship or differences in survivals across treatment groups in females. In males the low dose group showed statistically significantly lower mortality compared to the control. Tests showed statistically significant dose-response relationship in the combined incidences of liver cholangiocellular adenoma and carcinoma in males. None of the pairwise comparisons of control with high dose group or with any other treated group was considered to be statistically significant.

Mouse Study: Two separate experiments, one in males and one in females were conducted. In each of these two experiments there were three treated groups and one control group. The group size in both sexes was 55. For males the dose levels for treated groups were 10, 30, and 60 mg/kg/day. For females the dose levels for treated groups were 10, 30, and 100 mg/kg/day. The controls received 1% HPMC by gavage.

The tests showed no statistically significant dose response relationship or differences in survivals across treatment groups in either sex. Test showed statistically significant dose-response relationship in none of the tested tumor types in either sex. None of the pairwise comparisons of control with highest dose group was considered to be statistically significant in either sex. However, male low dose group showed statistically significant increased incidence of malignant lymphoma in lymph nodes compared to the control.

From the body weight gain data it may be concluded that the individual highest dose used in males and females in rats and mice might have reached or exceeded the MTD for male and female rats, and male mice. The high dose used for female mice might be at MTD. However, for a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

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6. Appendix

**Table 1A: Intercurrent Mortality Rate
Male Rats**

Week	CTR0		MEDIUM		MEDHI		HIGH	
	No. of Death	Cum. %						
0-52	1	1.8	2	3.6
53-78	10	18.2	3	5.5	8	16.4	6	14.5
79-91	10	36.4	2	9.1	5	25.5	6	25.5
92-104	12	58.2	14	34.5	11	45.5	7	38.2
Term. Sac.	23	41.8	36	65.5	30	54.5	34	61.8

**Table 1B: Intercurrent Mortality Rate
Female Rats**

Week	CTR0		LOW		MEDIUM		MEDHI		HIGH	
	No. of Death	Cum. %								
0-52	2	3.6	.	.	3	5.5	.	.	5	9.1
53-78	7	16.4	6	10.9	5	14.5	4	7.3	8	23.6
79-91	8	30.9	4	18.2	2	18.2	6	18.2	3	29.1
92-104	8	45.5	11	38.2	7	30.9	7	30.9	2	32.7
Term. Sac.	30	54.5	34	61.8	38	69.1	38	69.1	37	67.3

**Table 2A: Intercurrent Mortality Comparison
Male Rats**

Method	Test	Statistic	P-Value
Cox	Dose response	1.1963	0.2741
	Homogeneity	8.9134	0.0305
Kruskal-Wallis	Dose response	0.8660	0.3521
	Homogeneity	9.6772	0.0215

**Table 2B: Intercurrent Mortality Comparison
Female Rats**

Method	Test	Statistic	P-Value
Cox	Dose response	0.2426	0.6223
	Homogeneity	3.1995	0.5250
Kruskal-Wallis	Dose response	0.0073	0.9319
	Homogeneity	2.9196	0.5714

Table 3A

Tumor Rates, Dose-Response Relationship, and Pairwise Comparisons p-values of Tested Tumors Types
Male Rats - Fed Over 104 Weeks

Organ Name	Tumor Name	Cont	100mg	300mg	1000mg	P_Value	P_Value	P_Value	P_Value
		N=55	N=55	N=55	N=55	Dos Resp	C vs. M	C vs. MH	C vs. H
adrenal gland	adenocarcinoma	0	0	0	1	0.080	.	.	0.170
	adenoma	4	0	0	0	0.998	0.820	0.820	0.985
	malignant pheochromo	0	0	1	0	0.579	.	0.088	.
	pheochromocytoma	11	1	1	1	1.000	0.876	0.874	1.000
bone	osteochondroma	1	0	1	0	0.865	0.671	0.260	0.852
bone marrow	myelogenous leukemia	0	0	0	1	0.080	.	.	0.171
brain	astrocytoma	1	1	0	1	0.568	0.258	0.669	0.522
	granular cell tumor	0	1	0	0	0.644	0.087	.	.
	malignant meningioma	2	0	0	0	0.973	0.731	0.731	0.930
coagulation gla	adenocarcinoma	0	1	0	0	0.645	0.088	.	.
ear	squamous cell papill	1	0	0	0	0.914	0.671	0.671	0.852
heart	hemangioma	1	0	0	0	0.914	0.671	0.671	0.852
kidney	adenoma	1	0	0	0	0.914	0.671	0.671	0.852
	angiomyolipoma	1	0	0	0	0.913	0.670	0.670	0.851
	liposarcoma	0	0	1	0	0.576	.	0.087	.
liver	CHOLANG ADENOMA+CARC	0	0	0	2	0.022	.	.	0.087
	cholangiocellular ad	0	0	0	1	0.080	.	.	0.170
	cholangiocellular ca	0	0	0	1	0.080	.	.	0.170
	hepatocellular adeno	2	0	0	3	0.249	0.738	0.738	0.364
	hepatocellular carci	3	0	0	1	0.910	0.779	0.779	0.862
lung	alveolar/bronchiolar	1	0	0	0	0.913	0.670	0.670	0.852
mammary gland	adenocarcinoma	1	0	0	1	0.488	0.672	0.672	0.527
	fibroadenoma	1	0	0	0	0.913	0.670	0.670	0.852
nasal cavity	sebaceous adenoma	1	0	0	0	0.913	0.669	0.669	0.851
other	carcinoma	0	1	0	0	0.644	0.087	.	.
pancreatic isle	adenocarcinoma	2	0	0	2	0.479	0.736	0.736	0.535
	adenoma	14	2	4	10	0.900	0.853	0.637	0.875
pituitary gland	adenocarcinoma	1	0	2	0	0.849	0.670	0.096	0.852
	adenoma	32	10	11	17	1.000	0.584	0.596	0.999
skin	keratoacanthoma	0	1	0	2	0.076	0.087	.	0.087
	squamous cell papill	1	0	1	1	0.533	0.671	0.263	0.526
spinal cord	malignant meningioma	0	0	0	1	0.081	.	.	0.172

(Continued)

Table 3A (Continued)

Tumor Rates, Dose-Response Relationship, and Pairwise Comparisons p-values of Tested Tumors Types
Male Rats - Fed Over 104 Weeks

Organ Name	Tumor Name	Cont N=55	100mg N=55	300mg N=55	1000mg N=55	P_value Dos Resp	P_value C vs. M	P_value C vs.MH	P_value C vs. H
spleen	hemangioma	1	1	0	0	0.891	0.262	0.672	0.853
	hemangiosarcoma	0	0	0	1	0.080	.	.	0.170
stomach	adenoma	0	1	0	0	0.643	0.087	.	.
subcutaneous ti	fibroma	1	0	0	0	0.914	0.672	0.672	0.853
	fibrosarcoma	1	0	1	0	0.867	0.672	0.268	0.853
	lipoma	1	1	0	1	0.576	0.261	0.672	0.528
	sarcoma, NOS	1	0	0	0	0.913	0.670	0.670	0.852
tail	fibrosarcoma	1	0	0	0	0.914	0.672	0.672	0.853
testis	interstitial cell tu	5	1	0	7	0.220	0.650	0.852	0.333
	mesothelioma	0	0	1	0	0.576	.	0.087	.
thyroid gland	C-cell adenoma	4	3	1	3	0.785	0.184	0.579	0.696
	C-cell carcinoma	1	0	0	1	0.488	0.672	0.672	0.527
	follicular cell aden	3	2	0	1	0.922	0.266	0.782	0.865
thyroid gland	follicular cell carc	2	0	0	1	0.779	0.738	0.738	0.748
trigeminal nerv	malignant schwannoma	1	0	0	0	0.913	0.670	0.670	0.852
urinary bladder	transitional cell pa	1	1	0	1	0.574	0.265	0.671	0.526
adrenal gland	adenocarcinoma	0	0	0	1	0.080	.	.	0.170
	adenoma	4	0	0	0	0.998	0.820	0.820	0.985
	malignant pheochromo	0	0	1	0	0.579	.	0.088	.
	pheochromocytoma	11	1	1	1	1.000	0.876	0.874	1.000
bone	osteochondroma	1	0	1	0	0.865	0.671	0.260	0.852
bone marrow	myelogenous leukemia	0	0	0	1	0.080	.	.	0.171
brain	astrocytoma	1	1	0	1	0.568	0.258	0.669	0.522
	granular cell tumor	0	1	0	0	0.644	0.087	.	.
	malignant meningioma	2	0	0	0	0.973	0.731	0.731	0.930
coagulation gla	adenocarcinoma	0	1	0	0	0.645	0.088	.	.
ear	squamous cell papill	1	0	0	0	0.914	0.671	0.671	0.852
heart	hemangioma	1	0	0	0	0.914	0.671	0.671	0.852
kidney	adenoma	1	0	0	0	0.914	0.671	0.671	0.852
	angiomyolipoma	1	0	0	0	0.913	0.670	0.670	0.851
	liposarcoma	0	0	1	0	0.576	.	0.087	.

(Continued)

Table 3A (Continued)

Tumor Rates, Dose-Response Relationship, and Pairwise Comparisons p-values of Tested Tumors Types
Male Rats - Fed Over 104 Weeks

Organ Name	Tumor Name	Cont N=55	100mg N=55	300mg N=55	1000mg N=55	P_Value Dos Resp	P_Value C vs. M	P_Value C vs.MH	P_Value C vs. H
liver	CHOLANG ADENOMA+CARC	0	0	0	2	0.022	.	.	0.087
	cholangiocellular ad	0	0	0	1	0.080	.	.	0.170
	cholangiocellular ca	0	0	0	1	0.080	.	.	0.170
	hepatocellular adeno	2	0	0	3	0.249	0.738	0.738	0.364
	hepatocellular carci	3	0	0	1	0.910	0.779	0.779	0.862
lung	alveolar/bronchiolar	1	0	0	0	0.913	0.670	0.670	0.852
mammary gland	adenocarcinoma	1	0	0	1	0.488	0.672	0.672	0.527
	fibroadenoma	1	0	0	0	0.913	0.670	0.670	0.852
nasal cavity	sebaceous adenoma	1	0	0	0	0.913	0.669	0.669	0.851
other	carcinoma	0	1	0	0	0.644	0.087	.	.
pancreatic isle	adenocarcinoma	2	0	0	2	0.479	0.736	0.736	0.535
	adenoma	14	2	4	10	0.900	0.853	0.637	0.875
pituitary gland	adenocarcinoma	1	0	2	0	0.849	0.670	0.096	0.852
	adenoma	32	10	11	17	1.000	0.584	0.596	0.999
skin	keratoacanthoma	0	1	0	2	0.076	0.087	.	0.087
	squamous cell papill	1	0	1	1	0.533	0.671	0.263	0.526
spinal cord	malignant meningioma	0	0	0	1	0.081	.	.	0.172
spleen	hemangioma	1	1	0	0	0.891	0.262	0.672	0.853
	hemangiosarcoma	0	0	0	1	0.080	.	.	0.170
stomach	adenoma	0	1	0	0	0.643	0.087	.	.
subcutaneous ti	fibroma	1	0	0	0	0.914	0.672	0.672	0.853
	fibrosarcoma	1	0	1	0	0.867	0.672	0.268	0.853
	lipoma	1	1	0	1	0.576	0.261	0.672	0.528
	sarcoma, NOS	1	0	0	0	0.913	0.670	0.670	0.852
tail	fibrosarcoma	1	0	0	0	0.914	0.672	0.672	0.853
testis	interstitial cell tu	5	1	0	7	0.220	0.650	0.852	0.333
	mesothelioma	0	0	1	0	0.576	.	0.087	.
thyroid gland	C-cell adenoma	4	3	1	3	0.785	0.184	0.579	0.696
	C-cell carcinoma	1	0	0	1	0.488	0.672	0.672	0.527
	follicular cell aden	3	2	0	1	0.922	0.266	0.782	0.865
thyroid gland	follicular cell carc	2	0	0	1	0.779	0.738	0.738	0.748
trigeminal nerv	malignant schwannoma	1	0	0	0	0.913	0.670	0.670	0.852
urinary bladder	transitional cell pa	1	1	0	1	0.574	0.265	0.671	0.526

Table 3B

Tumor Rates, Dose-Response Relationship, and Pairwise Comparisons p-values of Tested Tumors Types
Female Rats - Fed Over 104 Weeks

Organ Name	Tumor Name	Cont	30mg	100mg	300mg	1000mg	P_Value	P_Value	P_Value	P_Value	P_Value
		N=55	N=55	N=55	N=55	N=55	Dos Resp	C vs. L	C vs. M	C vs. MH	C vs. H
adrenal gland	adenoma	1	0	1	0	0	0.879	0.658	0.211	0.622	0.839
bone marrow	myelogenous leukemia	1	0	1	0	0	0.878	0.655	0.212	0.619	0.836
brain	astrocytoma	1	0	1	0	1	0.493	0.656	0.210	0.620	0.489
	granular cell tumor	1	0	0	0	0	0.923	0.656	0.599	0.621	0.838
clitoral gland	adenoma	1	0	0	0	0	0.924	0.657	0.599	0.621	0.838
	squamous cell papill	0	0	0	0	1	0.049	.	.	.	0.155
ear	neural crest tumor	0	1	0	0	0	0.659	0.086	.	.	.
exocrine pancre	adenoma	1	0	0	0	0	0.924	0.658	0.600	0.622	0.839
kidney	adenoma	0	0	0	1	0	0.546	.	.	0.081	.
	nephroblastoma	0	0	1	0	0	0.591	.	0.080	.	.
liver	hepatocellular adeno	4	1	1	0	5	0.263	0.546	0.439	0.736	0.343
	hepatocellular carci	0	0	0	0	1	0.049	.	.	.	0.155
lymph node	malignant lymphoma	0	1	0	0	0	0.662	0.086	.	.	.
mammary gland	adenocarcinoma	2	4	1	3	3	0.530	0.026	0.302	0.055	0.319
	adenoma	0	0	1	0	0	0.584	.	0.079	.	.
	fibroadenoma	16	5	1	3	9	0.976	0.480	0.774	0.638	0.928
ovary	malignant granulosa-	0	0	0	1	0	0.547	.	.	0.082	.
	yolk sac tumor	0	0	0	1	0	0.547	.	.	0.082	.
pancreatic isle	adenocarcinoma	1	0	0	0	0	0.924	0.658	0.600	0.622	0.839
pancreatic isle	adenoma	7	2	1	2	4	0.885	0.532	0.558	0.419	0.814
peritoneum	histiocytic sarcoma	0	0	0	0	1	0.050	.	.	.	0.157
pituitary gland	adenocarcinoma	6	4	1	1	1	0.994	0.160	0.526	0.575	0.970
	adenoma	44	9	8	13	22	1.000	0.991	0.696	0.368	1.000
rectum	squamous cell papill	0	0	0	1	0	0.546	.	.	0.081	.
stomach	adenoma	0	0	0	0	1	0.049	.	.	.	0.155
subcutaneous ti	fibroma	1	0	0	0	0	0.924	0.658	0.600	0.622	0.839
	fibrosarcoma	0	0	0	0	1	0.049	.	.	.	0.155
	hemangioma	1	0	0	0	0	0.924	0.658	0.600	0.622	0.839
	hemangiosarcoma	1	0	0	0	0	0.923	0.655	0.598	0.619	0.836
	histiocytic sarcoma	1	0	0	0	0	0.924	0.657	0.600	0.621	0.838
	lipoma	1	0	0	1	1	0.470	0.656	0.599	0.220	0.490
	sarcoma, NOS	0	0	0	1	0	0.546	.	.	0.081	.

(Continued)

Table 3B (Continued)

Tumor Rates, Dose-Response Relationship, and Pairwise Comparisons p-values of Tested Tumors Types
Female Rats - Fed Over 104 Weeks

Organ Name	Tumor Name	Cont	30mg	100mg	300mg	1000mg	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. MH	P_Value C vs. H
		N=55	N=55	N=55	N=55	N=55					
thyroid gland	C-cell adenoma	4	3	2	0	4	0.566	0.163	0.225	0.735	0.471
	follicular cell aden	0	0	0	0	1	0.049	.	.	.	0.155
urinary bladder	transitional cell pa	0	0	1	0	0	0.584	.	0.079	.	.
uterus	adenocarcinoma	1	0	0	0	0	0.924	0.658	0.600	0.622	0.839
	adenoma	0	0	0	0	1	0.049	.	.	.	0.155
	deciduoma	1	0	0	0	0	0.923	0.655	0.598	0.619	0.836
	endometrial stromal	7	6	0	1	8	0.433	0.056	0.764	0.643	0.366
	granular cell tumor	1	0	0	0	1	0.437	0.658	0.600	0.622	0.493
	hemangioma	0	1	0	0	0	0.657	0.085	.	.	.
	leiomyoma	0	1	0	0	0	0.660	0.086	.	.	.
uterus	leiomyosarcoma	1	0	0	0	0	0.924	0.658	0.600	0.622	0.839
	stromal sarcoma	0	1	0	0	0	0.657	0.085	.	.	.
vagina	granular cell tumor	5	3	0	2	8	0.108	0.235	0.722	0.340	0.165
	squamous cell carcin	0	0	0	1	0	0.544	.	.	0.081	.
	squamous cell papill	2	0	0	0	0	0.980	0.719	0.641	0.671	0.921
Zymbal's gland	carcinoma	0	0	0	0	1	0.049	.	.	.	0.156

Appears This Way
On Original

**Table 4A: Intercurrent Mortality Rate
Male Mice**

Week	CTRO		LOW		MEDIUM		HIGH	
	No. of Death	Cum. %						
53-78	3	5.5	2	3.6	1	1.8	.	.
79-91	1	7.3	2	7.3	2	5.5	1	1.8
92-104	3	12.7	2	10.9	3	10.9	5	10.9
Term. Sac.	48	87.3	49	89.1	49	89.1	49	89.1

**Table 4B: Intercurrent Mortality Rate
Female Mice**

Week	CTRO		LOW		MEDIUM		HIGH	
	No. of Death	Cum. %						
0-52	2	3.6
53-78	3	5.5	1	1.8	1	1.8	1	5.5
79-91	6	16.4	1	3.6	2	5.5	6	16.4
92-104	5	25.5	4	10.9	6	16.4	6	27.3
Term. Sac.	41	74.5	49	89.1	46	83.6	40	72.7

**Table 5A: Intercurrent Mortality Comparison
Male Mice**

Method	Test	Statistic	P-Value
Cox	Dose response	0.0854	0.7701
	Homogeneity	0.1642	0.9832
Kruskal-Wallis	Dose response	0.1229	0.7260
	Homogeneity	0.1998	0.9776

**Table 5B: Intercurrent Mortality Comparison
Female Mice**

Method	Test	Statistic	P-Value
Cox	Dose response	0.68	0.4093
	Homogeneity	0.73	0.8661
Kruskal-Wallis	Dose response	0.51	0.4732
	Homogeneity	0.56	0.9055

Table 6A

Tumor Rates, Dose-Response Relationship, and Pairwise Comparisons p-values of Tested Tumors Types
Male Mice - Fed Over 104 Weeks

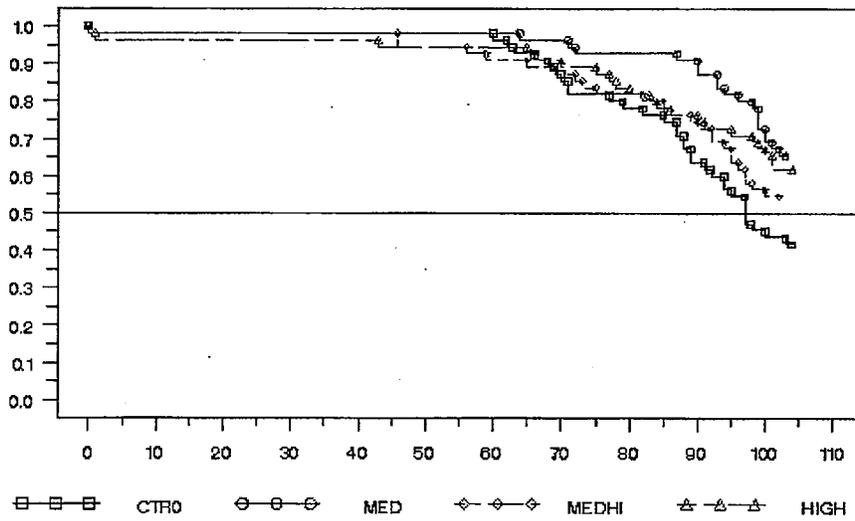
Organ Name	Tumor Name	Cont N=55	10mg N=55	30mg N=55	60mg N=55	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P-value C vs. MH
bone marrow	hemangioma	1	1	1	2	0.276	0.169	0.177	0.289
duodenum	adenocarcinoma	0	0	0	1	0.081	.	.	0.162
jejunum	malignant lymphoma	0	0	0	1	0.081	.	.	0.162
kidney	adenocarcinoma	1	0	0	0	0.924	0.532	0.546	0.845
liver	hemangiosarcoma	0	0	1	0	0.501	.	0.076	.
	hepatocellular adeno	18	1	0	13	0.944	0.503	0.723	0.873
	hepatocellular carci	8	3	2	7	0.668	0.137	0.283	0.621
	histiocytic sarcoma	1	1	0	0	0.857	0.172	0.546	0.845
lung	ALVIO_BRONC ADENOMA+	8	0	3	11	0.193	0.596	0.154	0.258
	alveolar/bronchiolar	2	0	1	3	0.297	0.545	0.248	0.341
		6	0	2	8	0.251	0.581	0.224	0.308
lymph node	hemangiosarcoma	1	0	0	0	0.924	0.532	0.546	0.845
	malignant lymphoma	1	4	1	5	0.053	0.004	0.176	0.051
mandibular glan	mastocytoma	1	0	0	0	0.924	0.532	0.546	0.845
pancreatic isle	adenoma	0	0	0	1	0.081	.	.	0.162
pituitary gland	adenocarcinoma	0	0	1	0	0.501	.	0.076	.
spleen	hemangioma	0	0	0	1	0.081	.	.	0.162
	hemangiosarcoma	3	1	0	1	0.911	0.273	0.582	0.852
stomach	squamous cell papill	1	0	0	0	0.924	0.532	0.546	0.845
subcutaneous ti	hemangioma	1	0	0	0	0.924	0.532	0.546	0.845
testis	interstitial cell tu	0	0	0	1	0.081	.	.	0.162
thyroid gland	follicular cell aden	3	0	0	0	0.994	0.556	0.582	0.963
Harderian gland	adenoma	4	0	0	1	0.976	0.565	0.595	0.920

Table 6B

Tumor Rates, Dose-Response Relationship, and Pairwise Comparisons p-values of Tested Tumors Types
Female Mice - Fed Over 104 Weeks

Organ Name	Tumor Name	Cont N=55	10mg N=55	30mg N=55	100mg N=55	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
bone marrow	hemangioma	1	0	0	1	0.486	0.547	0.575	0.493
jejunum	adenoma	0	0	1	0	0.531	.	0.078	.
liver	hemangioma	0	0	0	1	0.075	.	.	0.156
	hemangiosarcoma	1	0	0	0	0.916	0.547	0.574	0.837
	hepatocellular adeno	10	1	0	3	0.997	0.475	0.743	0.980
	hepatocellular carci	3	0	0	2	0.721	0.583	0.630	0.666
	histiocytic sarcoma	0	0	1	2	0.052	.	0.078	0.076
Lung	alveolar/bronchiolar	4	0	0	6	0.152	0.596	0.650	0.238
lymph node	malignant lymphoma	8	3	3	4	0.929	0.150	0.191	0.874
mammary gland	adenocarcinoma	0	0	1	1	0.165	.	0.077	0.156
ovary	cystadenoma	2	0	0	1	0.781	0.568	0.606	0.713
pituitary gland	adenocarcinoma	0	0	0	1	0.076	.	.	0.157
	adenoma	4	0	0	0	0.998	0.595	0.648	0.978
spleen	hemangioma	3	0	0	0	0.993	0.583	0.629	0.959
	hemangiosarcoma	0	0	0	1	0.076	.	.	0.156
	histiocytic sarcoma	1	0	0	0	0.917	0.547	0.575	0.839
	malignant lymphoma	0	0	0	1	0.075	.	.	0.156
stomach	leiomyosarcoma	0	0	0	1	0.077	.	.	0.158
	squamous cell papill	1	0	0	2	0.193	0.547	0.575	0.273
thyroid gland	follicular cell aden	3	0	0	0	0.993	0.583	0.630	0.960
uterus	endometrial sarcoma	2	0	0	0	0.976	0.568	0.606	0.921
	endometrial stromal	1	0	0	1	0.486	0.547	0.575	0.493
	histiocytic sarcoma	3	2	1	4	0.338	0.129	0.342	0.342
vagina	histiocytic sarcoma	0	0	0	1	0.075	.	.	0.156
Harderian gland	adenoma	1	0	0	4	0.024	0.547	0.575	0.082

Figure 1A: Kaplan-Meier Survival Functions for Male Rats

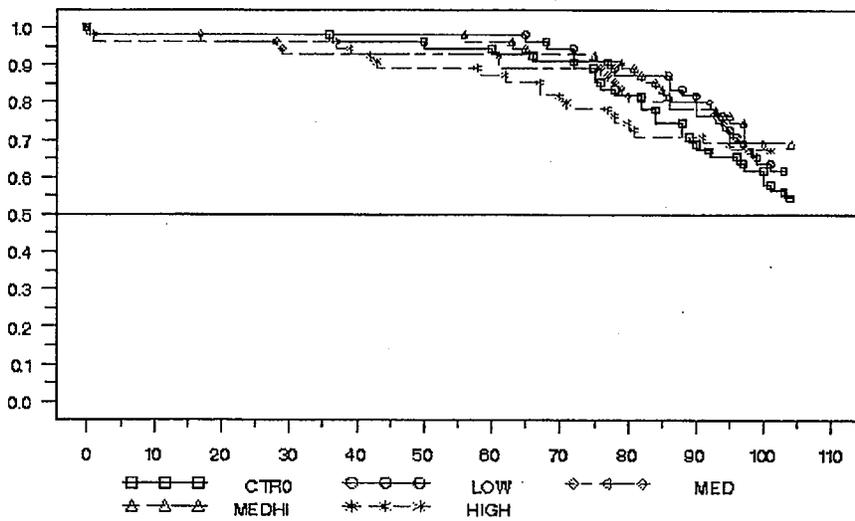


X-Axis: Weeks, Y-Axis: Survival rates

Figure 1B: Kaplan-Meier Survival Functions for Female Rats

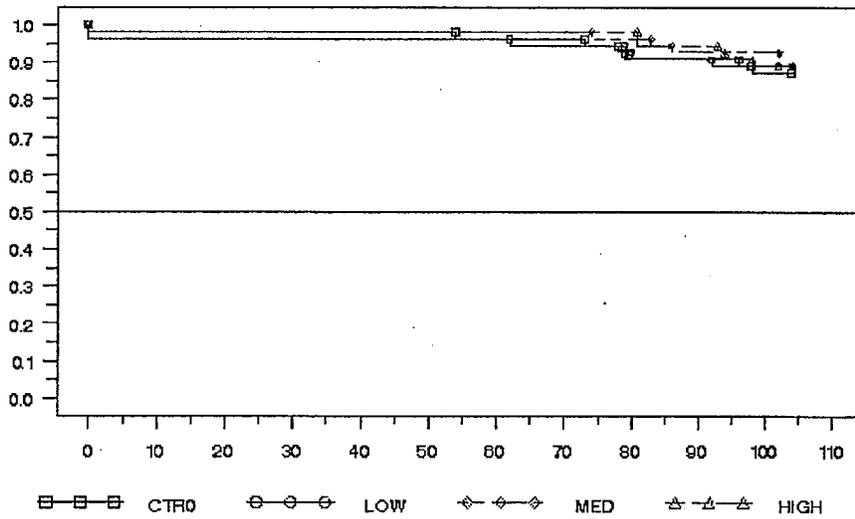
Species: Rat, Sex: Female, ██████████

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X-Axis: Weeks, Y-Axis: Survival rates

Figure 2A: Kaplan-Meier Survival Functions for Male Mice

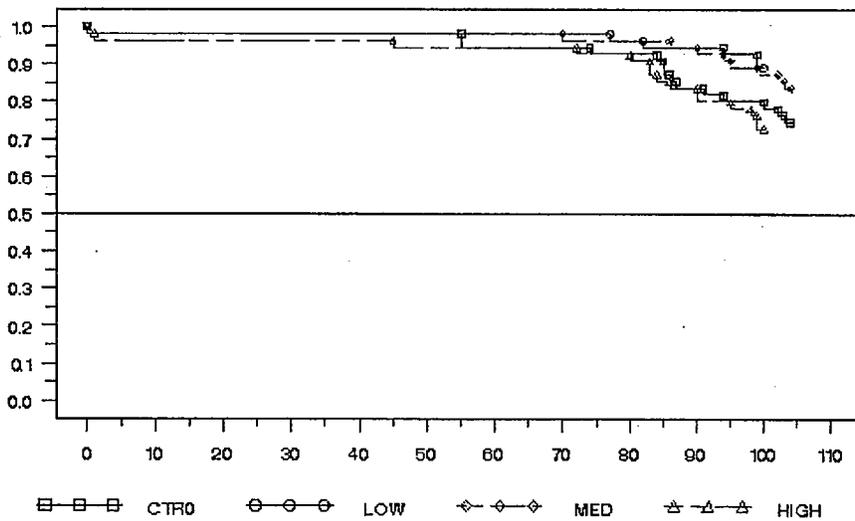


X-Axis: Weeks, Y-Axis: Survival rates

Figure 2B: Kaplan-Meier Survival Functions for Female Mice

Species: Rat, Sex: Female, ~~_____~~

b(4)



X-Axis: Weeks, Y-Axis: Survival rates

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PHARMACOMETRIC REVIEW

NDA:	22275
Drug name:	Tolvaptan
Indication:	Treatment of Hyponatremia and Worsening Heart Failure
Proposed Regimen (Sponsor):	15 mg QD with titration up to 60 mg QD
Applicant:	Otsuka Pharmaceutical Development & Commercialization, Inc.
Clinical Pharmacology Reviewer	Peter Hinderling, M.D.
Clinical Pharmacology Team Leader	Patrick J Marroum, Ph.D.
Pharmacometrics Reviewer:	Justin C. Earp, Ph.D.
Pharmacometrics Team Leader:	Yaning Wang, Ph.D.
Type of Submission:	NDA
Submission Date:	October 22, 2007
PDUFA Date:	August 22, 2008

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1 INTRODUCTION

Samska (Tolvaptan) is a small-molecule vasopressin antagonist designed to increase water removal from patients with fluid-overload and reduced serum sodium concentrations. This submission is a New Drug Application for the treatment of hyponatremia and worsening heart failure.

2 AIMS OF THE ANALYSIS

There are four main objectives of the following review:

1. The sponsors constructed three population pharmacokinetic models depending on disease status. What are the major covariates affecting pharmacokinetic parameters based on population pharmacokinetic analysis?
2. Is it necessary to adjust dose based on identified pharmacokinetic covariates?
3. Is tolvaptan effective for patients with lower baseline serum sodium concentrations?
4. Tolvaptan acts by increasing free water clearance, indirectly causing an increase in serum sodium concentrations. Do patients with impaired renal function yield less responsiveness to Tolvaptan and require dose-adjustment?
5. Does Tolvaptan prolong the QT-interval?

3 QUESTION BASED REVIEW

1. What are the major covariates affecting pharmacokinetic parameters?

Population pharmacokinetic (PK) models were developed for three different disease scenarios: hyponatremia of any origin, hyponatremia with CHF, and CHF. The sponsor's population pharmacokinetic models identified several covariates affecting clearance and volume of distribution (Table 1 to Table 3). The effect of disease status is listed in Table 1.

Table 1. Typical values for apparent clearance, volume of distribution, and half-life as reported from the final population PK model parameter estimates.

	Hyponatremia	CHF
CL/F (L/hr)	10	7
V/F (L)	143	84
T1/2 (hr)	10	8

Table 2. Values for apparent clearance, volume of distribution, and half-life after correction for the influence of body weight in patients with hyponatremia of any origin.

	35 kg	70 kg	150kg
CL/F (L/hr)	8	10	13
V/F (L)	74	143	276
T1/2 (hr)	7	10	14

Table 3. Values for apparent clearance, volume of distribution, and half-life after correction for the influence of hepatic impairment (Child-Pugh scores B=moderate or C=severe) in patients with hyponatremia of any origin.

	Normal	Moderate/Severe
CL/F (L/hr)	10	8
V/F (L)	143	213
T1/2 (hr)	10	18

Creatinine clearance had no significant impact on clearance of Tolvaptan. The range of creatinine clearance values from patients with hyponatremia or hyponatremia and CHF were between 9.5 and 150 ml/min. There were 198 patients with normal, 169 with mild, 100 with moderate, and 33 with severe renal impairment. For the CHF data set there were 345 patients with severe, 957 with moderate, 961 with mild renal impairment and 363 individuals with normal renal function.

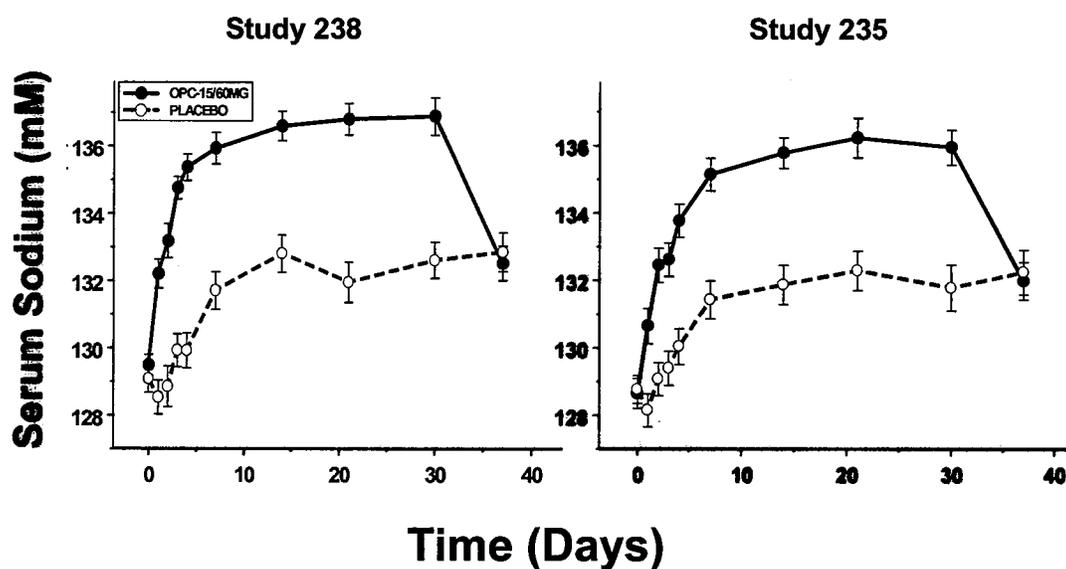
2. Is it necessary to adjust dose based on identified covariates?

Given the relatively small magnitude of covariate effect on apparent clearance (<30%, Table 1 to Table 3) and efficacy (serum sodium level)-based dose titration, adjusting dose based on these PK covariates is not necessary.

3. Is the proposed dosing of Tolvaptan effective for Hyponatremia?

There appears to be a clear increase in serum sodium concentrations following 15-60 mg Tolvaptan QD, when compared to placebo response in phase 3 studies 238 and 235 (Figure 1). Treatment is once daily for 30 days with dose titration increasing dose if the change in serum sodium from baseline was less than 5 mM and the serum sodium concentrations were below 135 mM. After day 30 when treatment stops, the effect is confirmed by a drop in serum sodium concentration to a level that is similar to what was observed in placebo group. The rise in placebo is attributed to restricted fluid intake to no more than one liter per day. This restriction was not done for the first study day to prevent too rapid a rise in serum sodium concentrations.

Figure 1. Time course of tolvaptan response in studies 238 and 235 (mean \pm SE).



It is evident from Figure 1 that Tolvaptan effectively increases serum sodium concentrations to the normal range of health individuals (135-145 mM).

4. Is tolvaptan effective for patients with lower baseline serum sodium concentrations?

All longitudinal data from study 235 and 238 up to day 30 were fitted with sigmoid E_{\max} model (Equation 1). Response was the change from baseline of serum sodium concentrations. The base structural model independent of error was parameterized as:

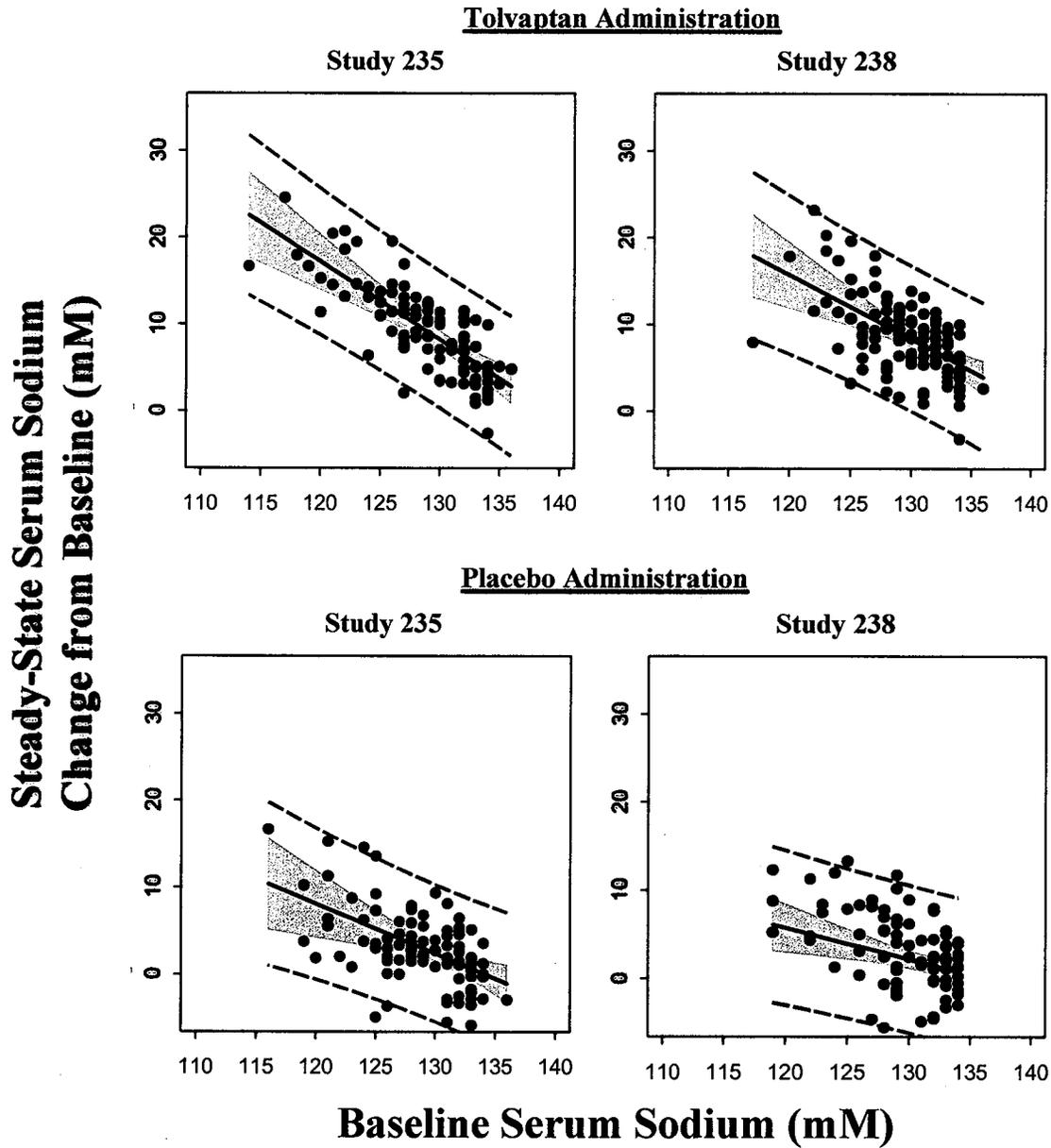
$$\text{Serum Sodium Change from Baseline} = \begin{cases} \frac{E_{\max, \text{placebo}} \cdot \text{Time}}{ET_{50} + \text{Time}}, & \text{Placebo} \\ \frac{E_{\max, \text{treatment}} \cdot \text{Time}}{ET_{50} + \text{Time}}, & \text{Treatment} \end{cases} \quad (\text{Equation 1})$$

where Time is the time after dosing in days and ET_{50} is the time at which half the maximal response for placebo ($E_{\max, \text{placebo}}$) or for treatment ($E_{\max, \text{treatment}}$) is reached. Since the doses were adjusted to achieve a target level of serum sodium concentration (between 135 and 145 mM), the maximum response corresponds to a steady state response. The objective of this analysis was to determine whether a larger change in serum sodium concentration can be achieved for a patient with lower baseline serum sodium concentration in order to reach the target. Exploratory analysis indicates a greater responsiveness in individuals with a lower baseline, which is consistent with the experimental design. The model parameter E_{\max} was used as the individuals measure of responsiveness to tolvaptan and was modeled as a function of baseline serum sodium (BSLN, Equation 2).

$$E_{\max} = \text{INT} + \text{SL} \cdot (\text{BSLN} - \text{BSLN}_{\text{median}}) \quad (\text{Equation 2})$$

The intercept is defined by INT and slope by SL. The median of the baseline values is indicated by $\text{BSLN}_{\text{median}}$ and was 130 mM and fixed during the model fitting. Model fitting was performed using the FOCE algorithm of NONMEM VI (Globomax, San Francisco, CA). Inter-individual variation was modeled on ET_{50} and E_{\max} parameters by an exponential relationship. Residual error was modeled using an additive relationship. The final model fitting relationship for E_{\max} and BSLN is shown in Figure 2. The final model parameters are presented in Table 4.

Figure 2. Final model fitting of E_{max} dependent on baseline serum sodium for studies 235 and 238, with Tolvaptan (top row) and with placebo (bottom row).



In the final model, both $E_{max, placebo}$ and $E_{max, treatment}$ were modeled as a linear function of baseline serum sodium concentration (

Figure 2). Figure 2 shows that for individuals with lower baselines a greater response is observed. The modeling results indicate that, by average, a patient with lower baseline serum sodium will have a larger response. For example, a patient with a baseline of 120 mM is expected to have a response of 16 mM, reaching a steady state serum sodium concentration of 136 mM.

Table 4. Parameter Estimates for Studies 235 and 238.

Study	235		238	
Number of Subjects	198		240	
Number of Observations	1341		1555	
Parameter	Estimate (%RSE)		Estimate (%RSE)	
INT _{placebo} (mM)	2.37	24.3	2.37	21.4
INT _{tolvaptan} (mM)	8.22	6.3	8.37	5.4
SL _{placebo}	0.57	31.3	0.39	30.3
SL _{tolvaptan}	0.90	16.7	0.74	23.3
BSV E _{max} (CV%)	3.99	2.8	4.24	4.0
ET ₅₀ (hr)	4.17	16.3	2.70	14.9
BSV ET ₅₀ (CV%)	0.76	1.98	0.73	1.6
Residual Variability (mM)	8.34	6.70	8.22	7.0

BSV: Between Subject Variability

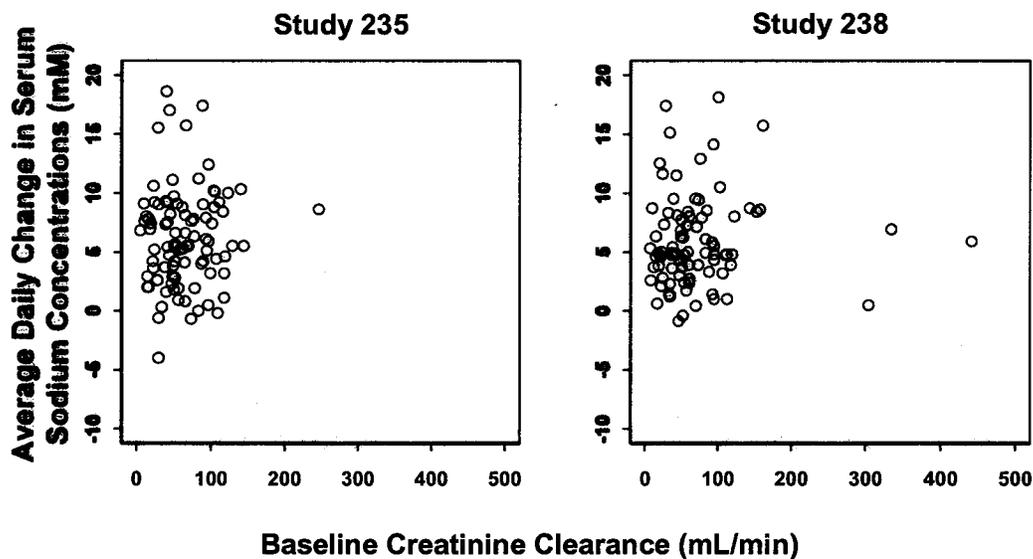
RSE: Relative Standard Error

INT: Intercept

SL: Slope

Is dose-adjustment required for patients with impaired renal function?

It was hypothesized that reduced renal function as indicated by creatinine clearance might mean less aquaretic effect and less water removal, which may lead to less effect on serum sodium. The average change of serum sodium concentration at day 30 from baseline was plotted against baseline creatinine clearance for each individual (Figure 3). Figure 3 did not indicate any apparent relationship between serum sodium response following treatment and baseline creatinine clearance. Therefore dose-adjustment is not required for patients with renal impairment.

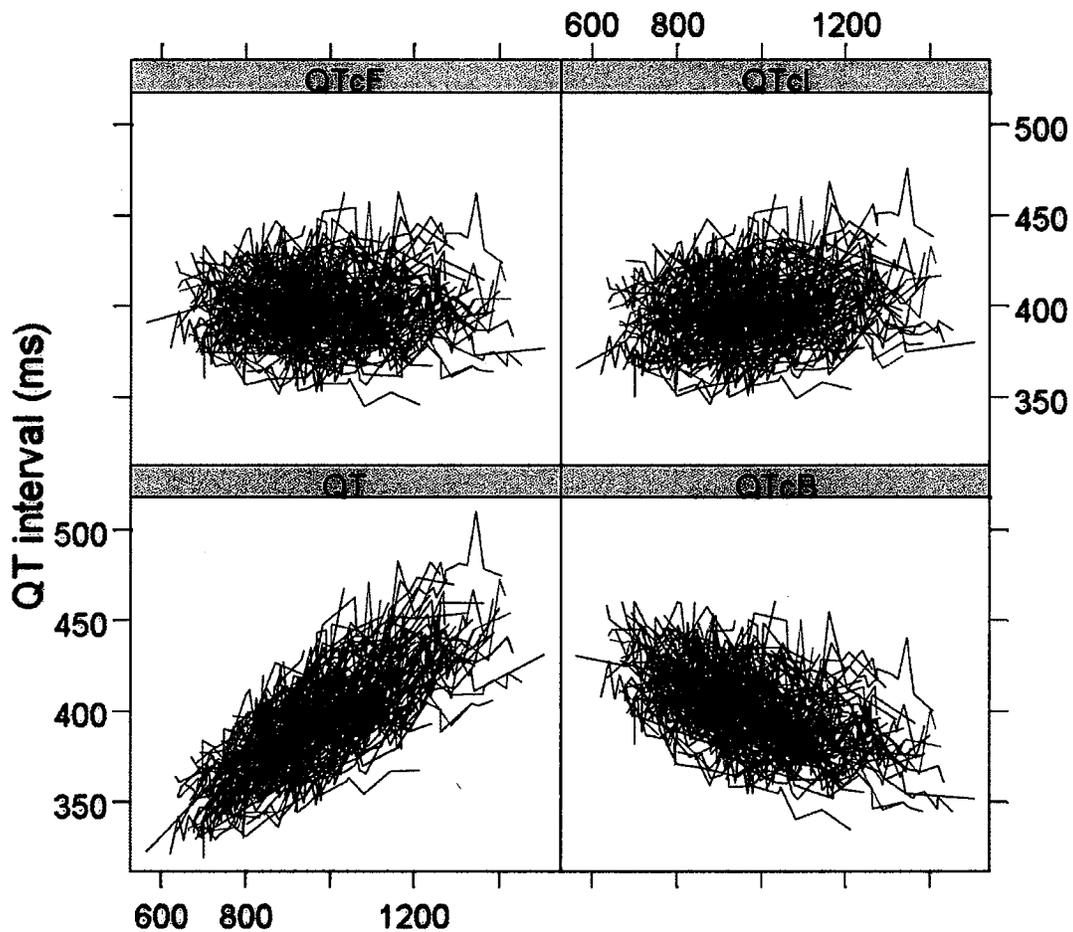
Figure 3: Serum Sodium Response – Baseline Creatinine Clearance Relationship

Does Tolvaptan prolong the QT interval?

The text and figures relevant to question 1 are taken from the IRT-QT group's assessment of Tolvaptan.

The relationship between QT (raw and different correction methods) and RR interval at baseline is illustrated in the Figure 4. The Federicia's and Individual correction seem to be reasonable.

Figure 4: QT (Raw QT measurements, Bazett's, Fridericia's and Individual corrected QT)-RR interval relationship



The comparative time course for mean $\Delta\Delta\text{QTcF}$ on Day 1 and 5 are illustrated in Figure 5. It appears that tolvaptan does not prolong the QT interval at both the studied doses.

Figure 5: Time course of mean $\Delta\Delta Q_{TcF}$

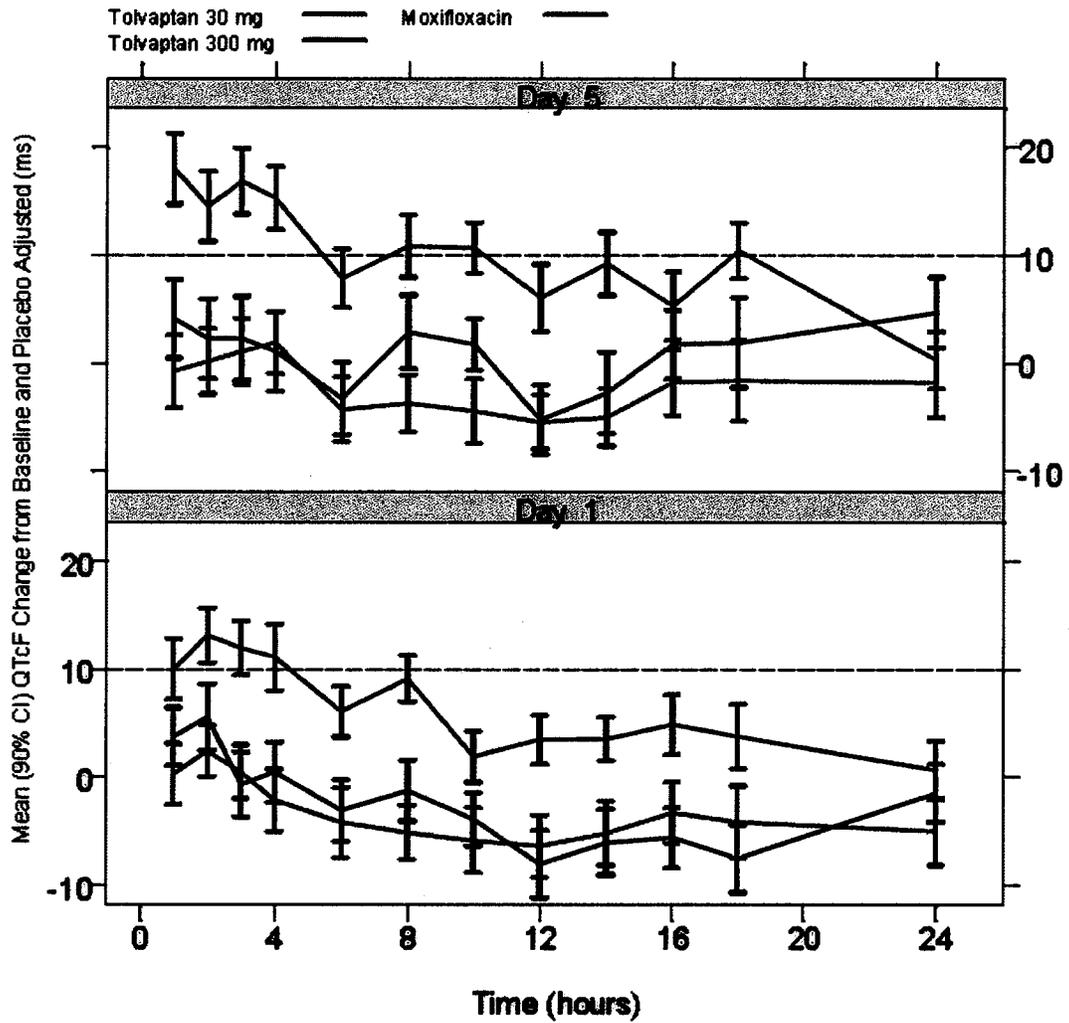
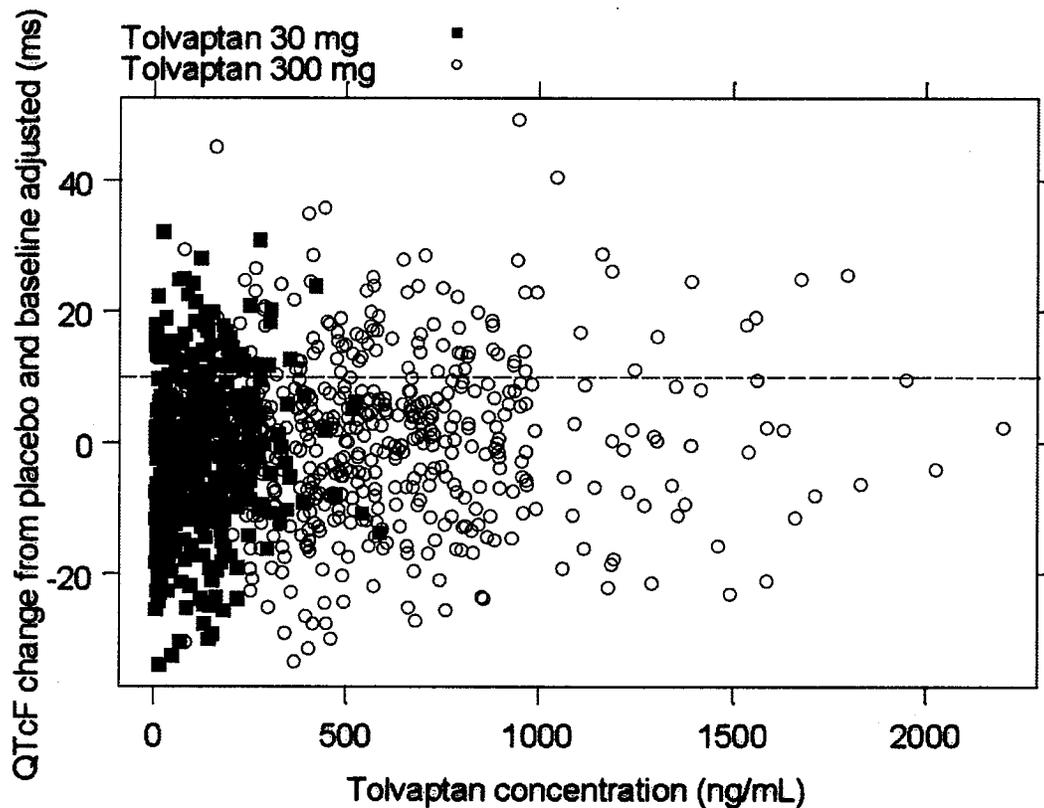


Figure 6 illustrates no relationship between Tolvaptan concentrations and $\Delta\Delta Q_{TcF}$. The mean (upper CI) effect at mean C_{max} (982.5 ng/mL) after supratherapeutic dose is 3.1 (5.6) ms.

Figure 6: Concentration- $\Delta\Delta$ QTcF relationship

Moxifloxacin increased the $\Delta\Delta$ QTcI interval by 12.3 ms with lower bound of 95% CI of 6.1 ms at 2 hours after dosing on Day 1. At steady-state on Day 5 moxifloxacin increased the $\Delta\Delta$ QTcI interval by 16.7 ms with lower bound of 95% CI of 9.4 ms at 1 hour after dosing on Day 5. These results are consistent for moxifloxacin following a single dose as well as at steady-state indicating that the study was adequately designed and conducted to detect an effect on the QT interval.

4 CONCLUSION

1. A clear increase is observed on serum sodium concentrations after treatment with tolvaptan when compared to placebo.
2. Major covariates affecting pharmacokinetics are body weight, liver function (indicated by the Child-Pugh score) and disease status. Typical values of clearance were higher at 10 L/hr for individuals with hyponatremia when compared to patients with CHF (CL = 7 L/hr). Values for the volume of distribution were primarily dependent on liver impairment (1.5-fold increase) and proportional to body weight.
3. The individual responsiveness to tolvaptan was correlated with baseline serum sodium concentrations. At lower concentrations a greater response was observed and sufficient to return the patient to normal serum sodium concentrations within the studied patient population. For all baseline serum sodium levels observed in the studies, tolvaptan is generally effective in returning the serum sodium to between 135 and 145 mM.
4. No clear relationship between baseline renal function as indicated by creatinine clearance and effect on serum sodium concentrations was observed. Dose adjustment based on renal-impairment is not necessary.
5. There does not appear to be an increase in prolongation of the QT interval after tolvaptan administration at 5 times the maximum recommended therapeutic dose (60 mg).

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