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0.1 Background

In this submission the sponsor included reports of five animal carcinogenicity studies, three in rats (a study of the prodrug TAK-491, a study of the metabolite TAK-536-MII, and a supplementary

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study of female rats treated with the metabolite) and two in Tg.ras.H2 transgenic mice (again, one study was of the prodrug, and one was of the metabolite). These studies were intended to assess the carcinogenic potential of azilsartan in rats and mice, administered at appropriate drug levels for about 104 weeks (in the case of the rats) or 26 weeks (in the case of the mice). Results of this review have been discussed with the reviewing pharmacologist Dr. Gatti.

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 incidence rate as dose increases.

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Chapter 1

Prodrug studies

1.1 Rat study

1.1.1 Overview

Two separate experiments were conducted, one in male and one in female rats. In each of these two experiments there were three treated groups and two control groups. Two hundred and fifty F344/Jcl rats of each sex were initially randomly allocated to three treatment groups (fifty animals of each sex per group), called the low dose group, the mid dose group, and the high dose group, and to two control groups (each control group consisted of fifty animals of each sex). The dose levels for the treatment groups were 60, 200, and 600 mg/kg/day respectively. The randomization used a block design to ensure homogeneity of group means of bodyweight.

The vehicle for the test was a solution of 0.5w/v% methylcellulose, with 0.005w/v% of citric acid per one mg/kg of the agent, administered orally by gavage as a dosage volume of 10 mL/kg, once per day. The control animals received this vehicle with 0.3w/v% citric acid, the same concentration of citric acid as the high dose group.

During the administration period animals were checked three times daily (twice daily on weekends and holidays) for clinical signs, including external appearance, nutritional condition, posture, behavior and excretions. A palpation exam was conducted every week, and animals found moribund were subject to necropsy, whereby all the organs/tissues in the cranial, thoracic and abdominal cavities were carefully examined and the results were recorded. Additionally hematological exams were conducted to the extent possible.

1.1.2 Sponsor's analysis

Survival analysis

Mortality data were plotted according to the Kaplan-Meier [5] product limit method, and then tested using Tarone's life table test [9] to test for trend in survival times across the four groups. Additionally, log rank tests were conducted to compare each treatment group with the combined control group. These tests were "conducted at the one-tailed significance level 0.05". The sponsor dose not specify the time intervals used in Tarone's test, but repeated references to the survival rate suggest that the sponsor has considered survival over the single interval consisting of the entire two years of the study.

No statistics or p-values are presented. However, significant increases in the survival rate are found in both sexes: in females the high dose group was found to significantly outperform the (combined) control group, and survival was found to improve with increases in dosage. Among males the mid dose group was found to have significantly better survival than the (combined) control. It is not clear how the sponsor found statistically significant *increases* in survival in a test designed to detect decreases in survival when the tests were "conducted at the one-tailed significance level 0.05".

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Tumor analysis

Each reported tumor type was assessed for signs that the incidence rate was associated with TAK-491. For tumor types with high incidence rates (at least 10 animals within one sex) a survival adjusted Peto test was used (see [7]) to test for a dose response relationship, and to test the incidence of each treatment group against the combined control group. In the case of less common tumors, an exact permutation test was used to assess dose response and to conduct pairwise comparisons.

Both trend tests and tests of comparisons were conducted at α levels of 0.01 and 0.05 for common and rare tumors respectively, where the determination of which tumors were common was based on the historical incidence in F344/Crlj rats at the (b) (4) (note that the study was conducted using F344/Jcl rats). An historical incidence rate above 1% qualified a tumor type to be considered common. In addition to individual tumor types, the sponsor also tested two combined endpoints; combined cortical cell adenomas and carcinomas and pancreatic islet cell adenomas and carcinomas.

Among the female animals, the only significant result found was for hemolymphoreticular histiocytic sarcomas; for such tumors both the test of trend and the comparison of the incidence rate between the high dose group and the controls were significant (the p-value for the test of trend was 0.0112, and the p-value for the comparison between the high dose group and the control was 0.0245). Note that the sponsor considered this to be a rare tumor.

For male rats, a significant dose response was found for pancreatic islet cell carcinomas; only two animals of either sex developed such tumors, both were high dose male animals. The p-value for the test was 0.0427. The results for pancreatic islet cell adenomas, and for the combined endpoint of pancreatic islet cell adenomas and carcinomas both yielded results that were not statistically significant.

The sponsor also found an increase in the incidence of adrenal cortical cell adenomas in the female group; the rate in the low dose female group was substantially higher than the rate in the control group. However this result was not significant at the 1% level ($p = 0.0148$), and there was no evidence of a dose-response relationship, so the sponsor concluded that the result was not the result of the treatment. Likewise, the test of adrenal cortical adenomas and carcinomas in combination yielded potentially significant results in the comparison of both the low dose group and the mid dose group to the control: the p-value of the test comparing the low dose group to the control is 0.0148, and the p-value of the test comparing the mid dose group to the control is 0.0465 (the comparison of the high dose group to the controls is not significant, but the p-value is still quite small: $p = 0.0705$. However, the spomsor designated these tumors as common, so none of these results are significant.

1.1.3 Reviewer's analysis

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

Control analysis

The sponsor divided the one hundred control animals (of each sex) into two separate control groups. Before recombining them, it is necessary to verify that the two control groups do indeed have comparable survival profiles. The survival plots for the control groups are shown in figures 1.1 and 1.2. A proportional hazards analysis has been done to assess whether the two groups do indeed have comparable risk profiles. The results of this test are shown in table 1.1.

In the case of both the male and the female rats, neither the survival plots nor the proportional hazards regression provide any reason to consider the two control groups differently, so all subsequent analyses are based on a combined control.

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Figure 1.1

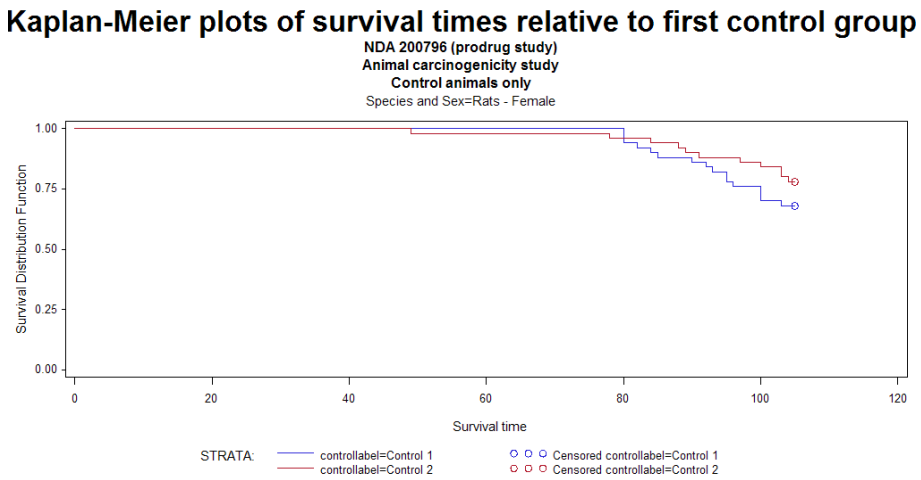

Figure 1.2

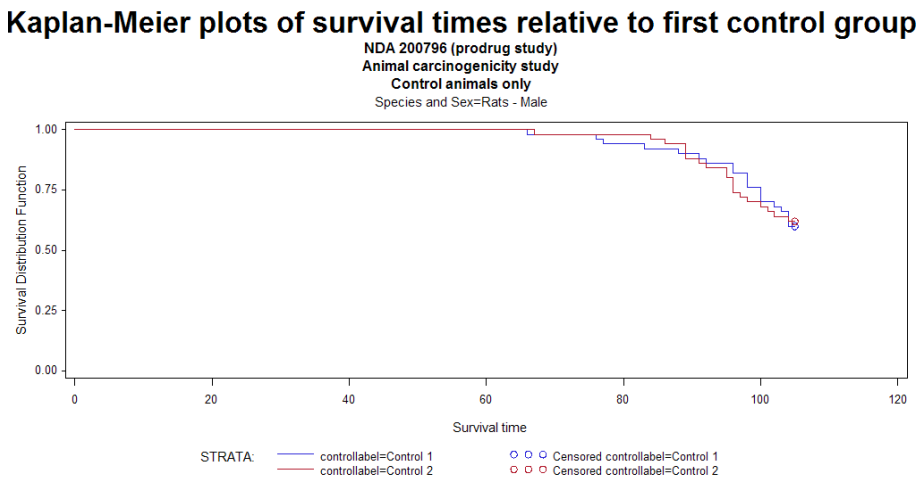

Table 1.1: Results of proportional hazards regression of control rats (prodrug study)

Species and Sex	p-value	Hazard Ratio	95% confidence interval for HR
Rats — Female	0.2569	0.641	$(0.2975, 1.3824)$
Rats — Male	0.9643	0.986	$(0.526, 1.8474)$

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Survival analysis

Kaplan-Meier plots of the survival rates for the two sexes are shown in figures 1.3 and 1.4. The results of log-rank tests for trend and homogeneity are presented in table 1.2 (on page 11). Intercurrent mortality data are presented in table 1.3 (on page 11).

Among the rats, there is no evidence for an decreasing trend in survival times as dosage is increased. There is some evidence of heterogeneity of survival times among the male rats, but from inspection of table 1.3, it is apparent that this is due to the slightly inferior survival rate of the control group.

Figure 1.3

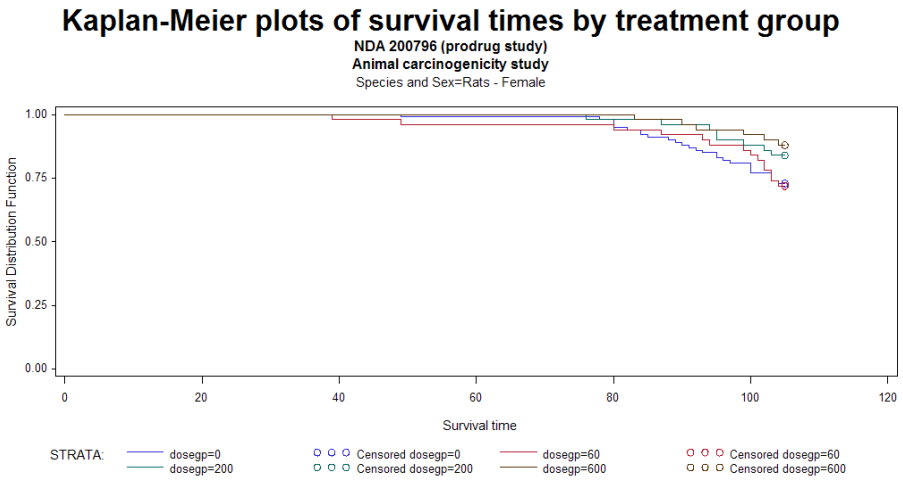
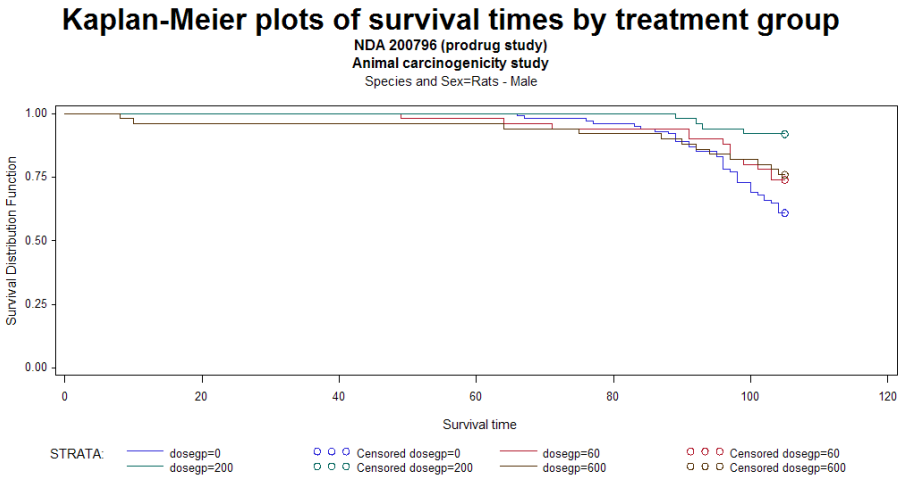


Figure 1.4



Tumor analysis

Theoretical underpinnings The tumor data were analyzed for dose response relationships and pairwise comparisons of tumor incidence of combined control with each of the treated groups. Both

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Table 1.2: Results of log-rank tests of survival in prodrug study on rats

Species and Sex	Number of dose groups	p-value (trend)	p-value (homogeneity)
Rats — Female	4	0.9893	0.0923
Rats — Male	4	0.9566	0.0016

Table 1.3: Intercurrent mortality for rats in prodrug study

Sex	Dose group	Dosage	At start	52 weeks		78 weeks		90 weeks		Termination	
		(mg/kg)	Number	Number	%	Number	%	Number	%	Number	%
Female	Control	0	100	99	99.0	99	99.0	89	89.0	74	74.0
	Low dose	60	50	48	96.0	48	96.0	46	92.0	37	74.0
	Mid dose	200	50	50	100	49	98.0	48	96.0	42	84.0
	High dose	600	50	50	100	50	100	49	98.0	45	90.0
Male	Control	0	100	100	100	96	96.0	89	89.0	65	65.0
	Low dose	60	50	49	98.0	47	94.0	47	94.0	37	74.0
	Mid dose	200	50	50	100	50	100	49	98.0	46	92.0
	High dose	600	50	48	96.0	46	92.0	45	90.0	39	78.0

the dose response relationship tests and pairwise comparisons were performed using the poly-k method described in the paper of Bailer and Portier[1] and developed in the paper of Bieler and Williams[2]. In this method, given a tumor type T, an animal h that lives the full study period (w_m) or dies before the terminal sacrifice with at least one tumor of type T gets a score of $s_h = 1$. An animal that dies at week w_h before the end of the study without such a tumor gets a score of

$$s_h = \left(\frac{w_h}{w_m}\right)^k < 1.$$

The adjusted group size is defined as $\sum_h s_h$. As an interpretation, an animal with score $s_h = 1$ can be considered as a whole animal while an animal with score $s_h < 1$ can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal develops at least one tumor of type T, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. The test is repeated for each tumor type of T.

One critical point for the application of a poly-k test is the choice of the appropriate value of k, which depends on the relationship between tumor onset time and increased dose. For long term 104 week standard rat and mouse studies, a value of $k = 3$ is suggested in the literature. Accordingly, the value $k = 3$ has been used in the analyses contained in this review. For the calculation of p-values the exact permutation method was used. The tumor rates and the p-values of the tested tumor types are listed in Table 1.7 on page 23.

For the adjustment of multiple testing of dose response relationship, the FDA guidance for the carcinogenicity study design and data analysis suggests the use of significance levels $\alpha = 0.005$ for common tumors and $\alpha = 0.025$ for rare tumors for a submission with two species, and a significance level $\alpha = 0.01$ for common tumors an $\alpha = 0.05$ for rare tumors for a submission with one species 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%. For pairwise comparisons, the FDA guidance the suggests the use of test levels $\alpha = 0.01$ for common tumors and $\alpha = 0.05$ for rare tumors. Reporting tumor types where either the test of trend or the pairwise test between the control group and the highest dose group yields a p-value that is significant according to these adjusted critical values has been shown (see the simulations of Lin and Rahman [6]) to yield a false-positive rate at the nominal level of approximately 10% for submissions with either

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two or one species.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman [6]. In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Rahman and Lin [8] showed that this rule for multiple testing for dose response relationship is also suitable for poly-k tests.

Rare and common tumors, and the use of historical controls The above mentioned methods depend on the ascertainment of which tumors are common (in that they have an underlying prevalence rate above 1%) and which are not. This information is often determined by consideration of the historical control data, that is, data from control animals of the same strain used in previous trials. Ideally, this data should be retricted to animals of the same substrain, used in studies at the same laboratory, and in orsder to minimize the effects of any genetic drift, from trials that were conducted within the ten years prior to the current study.

In this case, such historical control data are not available for either the prodrug rat study (TAK-491), or the metabolite study (TAK-536-MII). These studies were conducted at the (b) (4) , and were the first studies at that site to use F344/Jcl rats. The only available historical control data from this sponsor for F344/Jcl rats is from three studies initiated in 1990, 1992, and 1994 at Takeda Chemical Industries (two of these studies were by gavage, and the other was a dietary study). Historical rates for relevant tumor types based on this data are presented in Appendix B.

After consultation with the reviewing pharmacologist and the Executive Carcinogenesis Assessment Committee (ECAC), it was decided that it was not prudent to use the historical control data submitted by the sponsor. Instead, the ECAC suggested that the assessment of rare and common tumors be made based on the prevalence of such tumors in the control groups included in this submission (using control animals from both the prodrug and metabolite studies). This would potentially provide a surrogate historical control group of two hundred animals.

The ECAC also suggested the use of a point estimate rather than an interval estimate in order to make this determination, so that a tumor detected in at least two of the two hundred control animals would be considered common, whereas a tumor detected in no more than one animal would be considered rare.

However, before proceeding in this direction, it is necessary to verify that all four control groups are indeed compatible; if they are not even representative of one another, then we cannot assume that a conglomeration of the four groups are representative of the wider population of F344/Jcl rats. To this end, plots of survival and average weight are presented in figures 1.5, 1.6, 1.7, and 1.8. The results of the log-rank test of survival heterogeneity across the four control group are presented in table 1.4.

Table 1.4: Log-rank test of heterogeneity of survival across rat control groups

Species and Sex	Which groups	Number of groups	χ^2 statistic	Degrees of freedom	p-value
Rats — Female	All controls	4	7.9330	3	0.0474
Rats — Male	All controls	4	2.9423	3	0.4006
Rats — Female	All except metabolite vehicle	3	6.0461	2	0.0488
Rats — Male	All except metabolite vehicle	3	0.0346	2	0.9829

Note that these survival plots are just amalgamations of figures 1.1 and 2.1, and of figures 1.2 and 2.2, respectively. Likewise, the plots of the control animals' mean weights are amalgamations of figures 1.10 and 2.5 (for female rats) and of figures 1.11 and 2.6 (for the male rats).

From table 1.4, it appears that there is some significant heterogeneity of survival times across the female control groups. However, the most striking result is shown in figure 1.6; this plot clearly indicates that male animals in the the metabolite vehicle group experienced sharply increased mortality. Figure 1.8 suggests that the vehicle in this study (10 mL/kg/day of corn oil) led to increased weight gain, which in turn led to poorer health outcomes for the rats in this group. However, regardless of the veracity of this possible causal chain, it is clear that this group of

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Figure 1.5

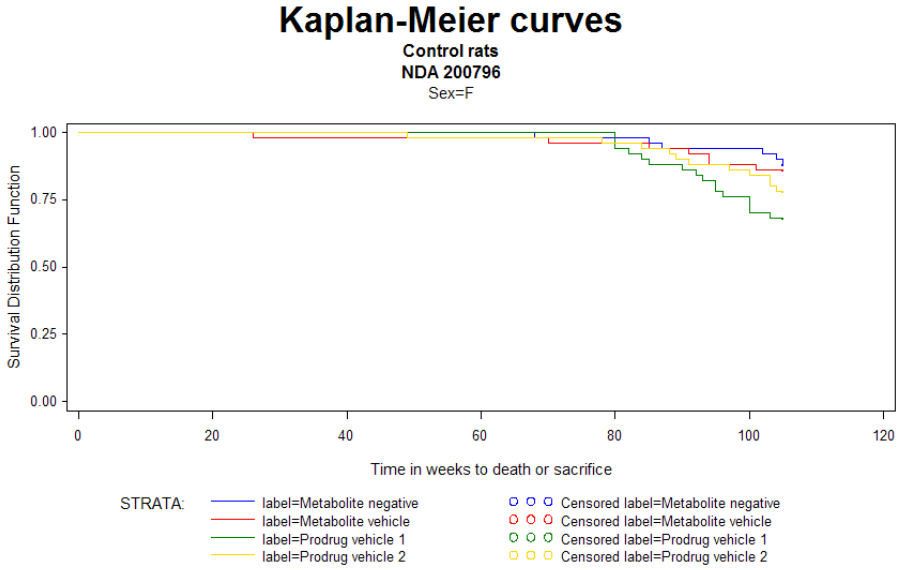

Figure 1.6

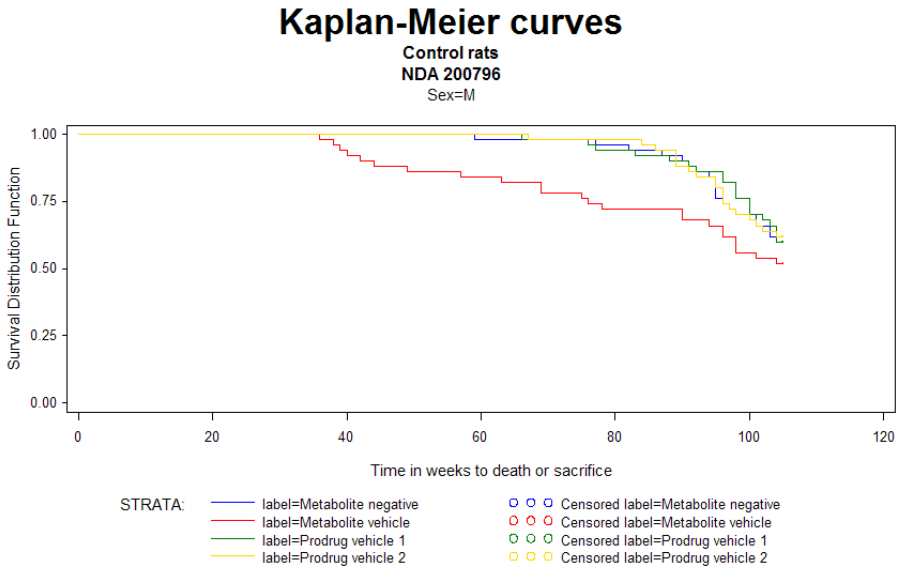

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Figure 1.7

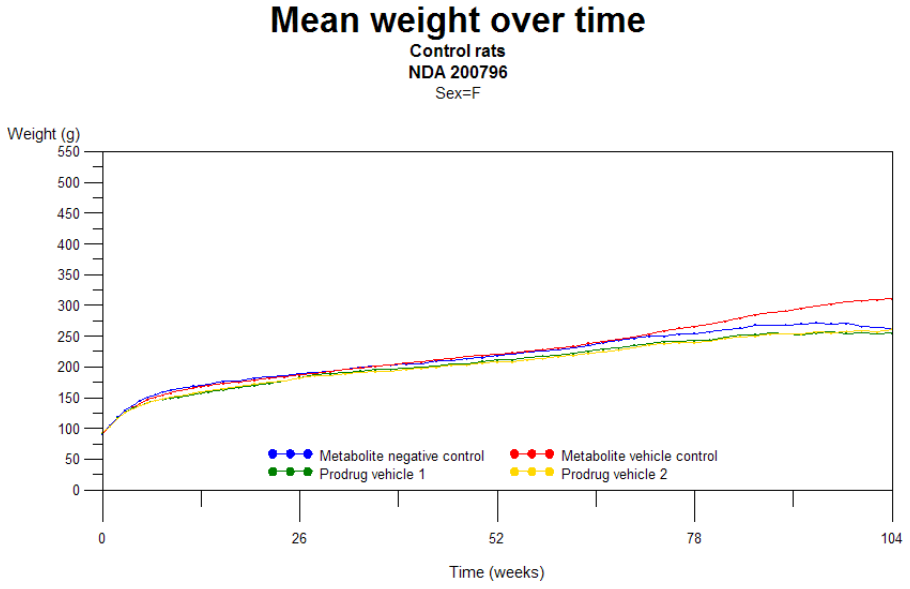
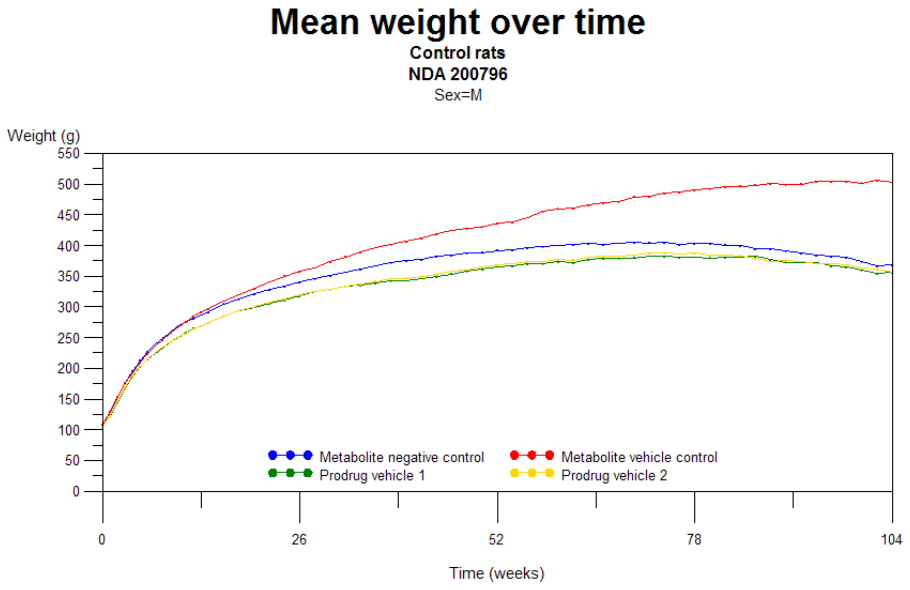


Figure 1.8



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animals experienced different outcomes during the course of the study, and therefore cannot be assumed to have come from the same population as the other rat control groups. Consequently, this group of animals has been left out of the surrogate historical control.

Although the data only show inferior outcomes for the male rats in the metabolite vehicle group, it does not seem reasonable to conclude that female vehicle control rats from the metabolite are somehow comparable to their peers in the other female control groups. Therefore, the surrogate historical controls for both sexes have been constructed using the two vehicle groups from the prodrug study, and the negative control group only from the metabolite study.

According to table 1.4, the heterogeneity among this smaller collection of female control groups does not appear to have been reduced. However, inspection of the Kaplan-Meier plot (figure 1.5) suggests that there is no pattern to this heterogeneity, and that it is reasonable to combine these animals.

It should be noted that by using the same control group to both determine the rarity of a tumor type (and so by extension the α-levels of the tests for that tumor type) and also to conduct the hypothesis tests for that tumor type creates the potential for confounding. This effect will be somewhat diluted by the inclusion of the negative control group from the metabolite study, but it is still the case that two thirds of the animals used to determine the rarity of a tumor type are also used in the tests of possible tumorigenic effects of the agent.

The determination of a tumor type as rare or not on the basis of a sample size of just one hundred and fifty animals is something of a hit-or-miss proposition. A tumor type with a true incidence rate of 1% has a 55.2% probability of being adjudged as rare, and even a tumor type with a true incidence rate of 0.5% has a 17.8% probability of being incorrectly adjudged common. On the other hand, a tumor type with a true incidence rate of 2% has an 19.4% probability of being misclassified as rare. The exact probabilities of being adjudged rare on the basis of a representative sample of one hundred and fifty are shown in figure 1.9.

It is interesting to note that due to the interplay of discrete and continuous variables, concluding rarity or commonness from a sample of one hundred and fifty animals is not greatly inferior (and in fact, often superior) to making the same judgment on the basis of two hundred animals. Thus excluding the metabolite vehicle control animals from our surrogate historical control does not greatly reduce the accuracy of our assessments.

The list of all reported tumor types, and their classification as either rare or common is presented in table 1.6.

Analysis of single tumor types In accordance with the methods described above, a poly-3 analysis of each reported tumor type has been conducted. The complete results are presented in table 1.7. An excerpt of this table, consisting of those results for which a p-value of less than 0.05 was detected at least one test, in at least one sex is presented as table 1.9.

Significant results are noted for cortical cell adenomas of the adrenal gland in female rats, hemolymphoreticular histiocystic sarcomas in the female rats, pancreatic islet cell carcinomas in male rats, and uterine leiomyosarcomas in female rats.

In the case of the hemolymphoreticular histiocystic sarcomas in female rats, there is some evidence of an increasing trend ($p = 0.0168$) and a significant difference ($p = 0.0483$) between the incidence rates in the high dose group (8.2%) and the control group (1.1%). Based on the historical control data, these tumors are considered rare[1], and so these results are both still significant after adjusting for multiplicity. This is therefore considered a positive result. There is, however, no evidence for a corresponding tumorigenic effect among male rats.

In the case of the adrenal cortical cell adenomas, the significant p-value is for the comparison between the control group and the low dose group. The p-value is 0.0116, driven by the fact that

[1] (b) (4)

(b) (4) the ECAC assessed them to be common tumors. This conclusion was arrived at by using all four available control groups to construct a surrogate control, rather than the three groups who were not treated with corn oil. Since one animal out of fifty in the corn oil group was found to have a histiocytic sarcoma, this difference is sufficient to raise the observed incidence to 1%, and therefore to identify these tumors as common. This is important within the context of this discussion as the multiplicity adjustments for common tumors are stricter than those for rare tumors, and the p-values reported for the tests of incidence for histiocytic sarcomas fail to meet this higher standard for significance.

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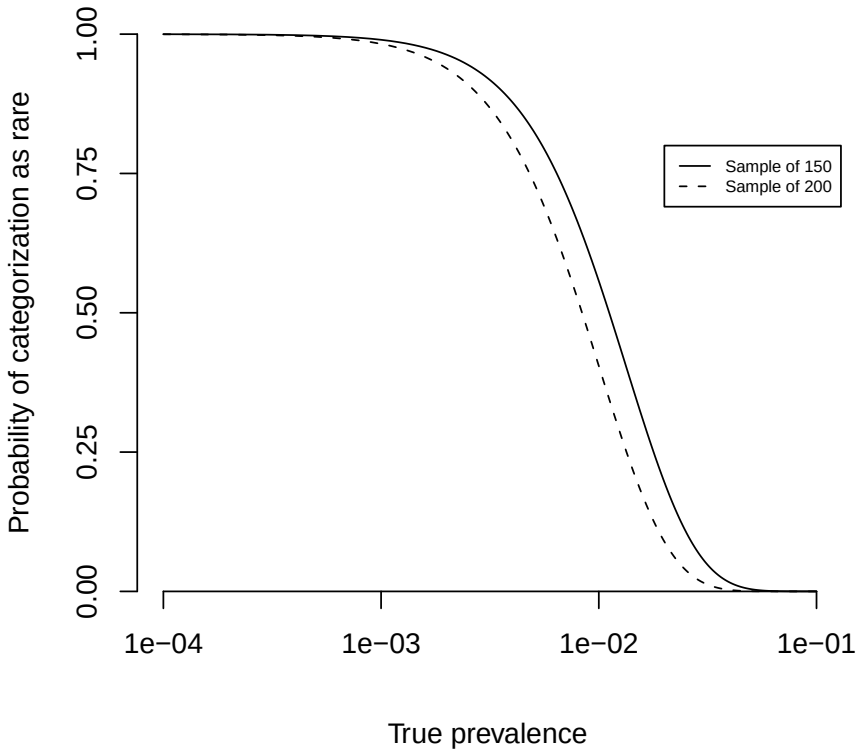
Figure 1.9: Probability of a tumor being adjudged rare



Reference ID: 2868673

adjusted incidence rate in the low dose group is 8.7%, and the incidence rate in the control group is 0%. The p-values for the contrasts with the mid and high dose groups are both 0.1176. These results are not significant, and neither is the test of trend ($p = 0.2533$). However, as these tumors are considered rare (see table 1.6, although the sponsor considers them to be common tumors), the elevated rate in the low dose group is high enough to cause some concern. Nonetheless, despite the significant result for the pairwise comparison between the control group and the low dose group, the draft guidance suggests that the analysis only be considered as yielding a positive result in the case that either the control-high dose comparison or the test of trend gives a significant result (after the multiplicity adjustment). Therefore we must conclude that this is not a positive result. It must also be observed that there is no evidence of any corresponding tumorigenic effect among the male rats, where only two animals (one control animal and one mid dose animal) developed adrenal cortical cell tumors.

For the pancreatic islet cell carcinomas, the significant result ($p = 0.0369$) is the result for the test of trend. This is driven by the fact that two high dose male rats developed these tumors, but no animals in any of the lower dose groups did. However, even allowing for the fact that these are considered rare tumors (see table 1.6), this p-value does not remain significant after adjusting for multiplicity (the threshold for a significant trend in a rare tumor type is 0.025). Furthermore, the contrast between the control group and the high dose group is not significant, even before the multiplicity adjustment is made ($p = 0.1062$).

The situation with the uterine leiomyosarcomas is similar; only two rats developed such tumors, but both were in the high dose group. The test for trend is just significant at the 5% level ($p = 0.0417$), but after adjusting for multiplicity, this result loses its significance, even when we consider uterine leiomyosarcomas to be rare tumors. Likewise, the pairwise test between the control group and the high dose group does not yield a significant result ($p = 0.1176$).

Analysis of composite endpoints At the request of the pharmacological reviewer, an analysis was conducted of all hemangiosarcomas, regardless of their site, and of uterine adenomas and adenocarcinomas combined. The results are presented in table 1.8. In the case of the hemangiosarcomas, no male rats, and only one control female rat developed such tumors (the female rat developed the tumor in the spleen) so these results do not suggest any further cause for concern.

Similarly, in the case of the uterine adenomas and adenocarcinomas, neither the trend nor the comparison between the any treatment group and the control is found to be significant.

Analysis of autolytic and unexamined organs

Autolysis The only noteworthy observation is that the number of cases in which the mesothelium was reported as being autolytic - it was reported as being autolyic in every single female rat, and in every single male rat, except for the six (two control animals, one low dose animal, one mid dose animal, and two high dose animals) who developed mesothelioma. Additionally, one animal (number 2036, a male control animal) was reported as having had its mesothelium unexamined, but also as having had mesothelioma.

Unexamined A similar situation applies to the reports of unexamined organs. Ten different organs/sites are listed as being missing in all but a few animals: Abdominal cavity, clitoral gland, cranial bone, ear, nasal cavity, oral cavity, preputial gland, thoracic cavity, vertebra, Zymbal's gland. The presumption made by the ECAC was that these organs were not subjected to routine histopathologic analysis, and that the tumors that were reported at these sites were detected by gross inspection. Regardless, we must conclude that the study provides no evidence either for or against possible tumorigenic effects of TAK-491 at these sites. In particular, negative results from these organs should not be viewed as providing any evidence of safety.

Table 1.10 lists the animals for which these organs *are* reported as being examined, together with the all the tumors with which they were diagnosed.

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Suitability of dose levels

Issues of concern when selecting dose levels The determination of an appropriate dose level for the high dose group is made difficult by the need to satisfy two competing imperatives: on the one hand, if the dose level is insufficiently high, then genuine carcinogenicity effects may not be apparent, but on the other hand, if the dose level is too high, then there is a risk of non-carcinogenic toxic effects killing the animals before they have a chance to demonstrate a carcinogenicity effect.

Haseman [4] suggested that a satisfactory balance between these two imperatives has been found when the following two conditions are both satisfied:

1. Were enough animals exposed, for a sustained amount of time, to the risk of late developing tumors?

2. 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 to run for two years with fifty animals per treatment group. The following are some rules of thumb regarding these two issues as suggested by experts in this field:

Haseman [4] has done an investigation on the first issue. He gathered data from 21 studies using Fischer 344 rats and B6C3Fl 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 considered as a sufficient number and adequate exposure. In addition Chu, Cueto and Ward [3], 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 [3], the following criteria are mentioned for dose adequacy. A high dose is considered as close to MTD if any of the criteria is met:

1. 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.

2. The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical.

3. In addition, doses are considered adequate if the dosed animals show a slight increased mortality compared to the controls.

Dose levels in rat prodrug study Since there are no strong carcinogenicity signals present in the data for male rats, and only one result of borderline significance in the data for female rats (the histiocytic sarcomas), it is appropriate to ask whether the dose level assigned as the high dose was adequate. As observed in the analysis of survival data, there is no evidence of any increased mortality in the high dose group compared to the control.

Table 1.5 shows the diminished weight gain in the treatment groups. There does appear to be some sign that the treated animals experienced less weight gain than the control animals. The effect is modest, well under the 10% threshold mentioned in [3], but doses appear to be a genuine effect. It seems therefore reasonable to conclude that the mid and high level dose levels were suitable in both the male and female rat studies. Plots of mean weight for male and female rats over time are presented in figures 1.12 and 1.13. Furthermore, the mortality data presented in table 1.3 indicate that enough animals were exposed to these dose levels for sufficient time to allow for an increase in incidence of late developing tumors to be detected.

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Figure 1.10

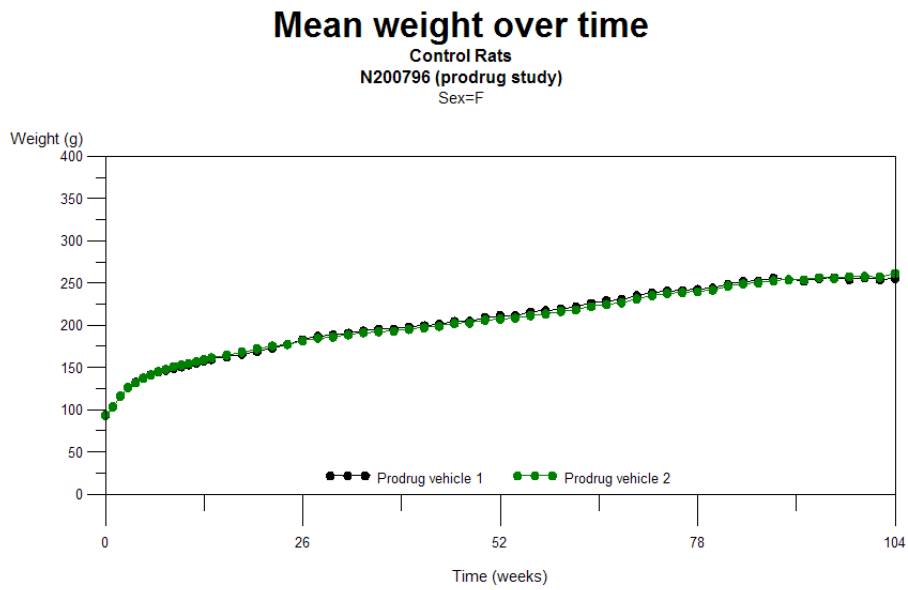

Figure 1.11

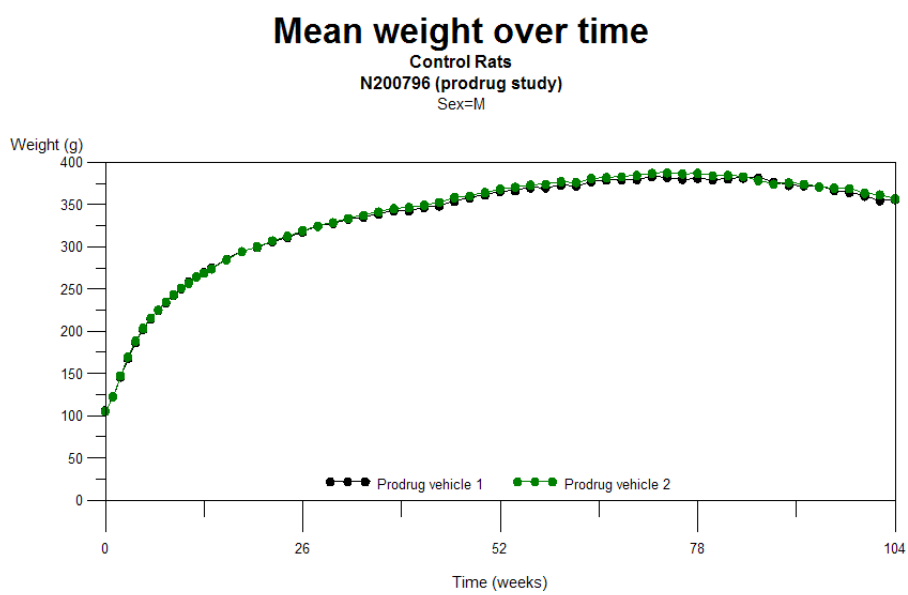

Table 1.5: Weight change by group — rat prodrug study

Species and Sex	Δ_C	Δ_L	$\frac{\Delta_L}{\Delta_C} - 1$	Δ_M	$\frac{\Delta_M}{\Delta_C} - 1$	Δ_H	$\frac{\Delta_H}{\Delta_C} - 1$
Rats - Female	161.6	167.9	+3.9%	160.4	−0.7%	150.6	−6.8%
Rats - Male	251.1	251.6	+0.2%	252.0	+0.4%	236.8	−5.7%

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Figure 1.12

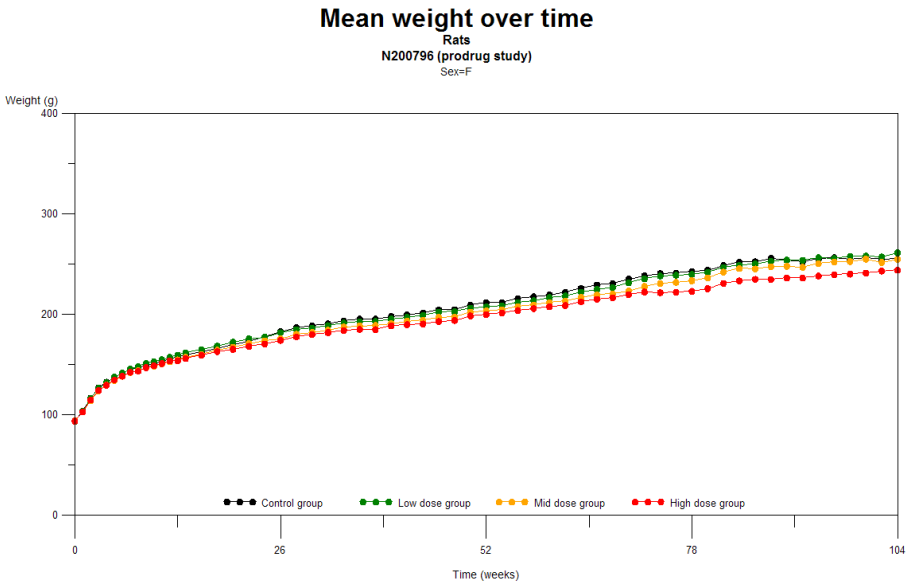

Figure 1.13

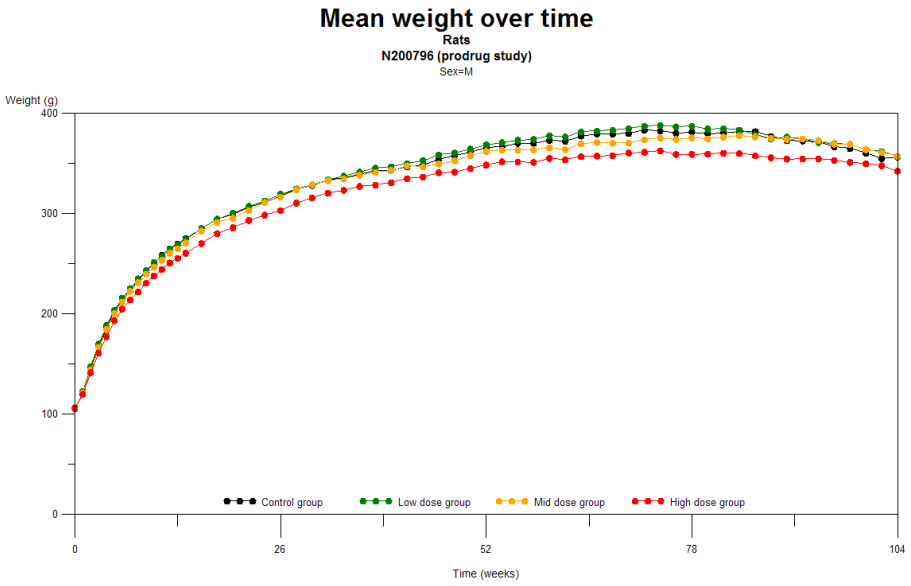

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Table 1.6: Tumors reported in rat studies, and their classification as rare or common

Organ/Tissue name	Tumor name	Female rats		Male rats	
		Incidence	Classification	Incidence	Classification
Abdominal cavity	PARAGANGLIOMA,MALIGNANT	0.7%	Rare	.	Rare
Adrenal	ADENOMA,CORTICAL CELL	2.0%	Common	1.3%	Common
	PHEOCHROMOCYTOMA	2.0%	Common	9.3%	Common
	PHEOCHROMOCYTOMA,MALIGNANT	0.7%	Rare	0.7%	Rare
Bone(marrow),sternum	GRANULAR CELL TUMOR	.	Rare	0.7%	Rare
Bone,cranial	OSTEOSARCOMA	.	Rare	0.7%	Rare
Cerebellum	MENINGIOMA,MALIGNANT	.	Rare	0.7%	Rare
Cerebrum	ASTROCYTOMA,MALIGNANT	0.7%	Rare	0.7%	Rare
	OLIGODENDROGLIOMA,MALIGNANT	1.3%	Common	.	Rare
	RETICULOSIS,MALIGNANT	0.7%	Rare	.	Rare
Clitoral gland	ADENOCARCINOMA	2.0%	Common	.	Rare
	ADENOMA	2.0%	Common	.	Rare
Eye	LEIOMYOMA	.	Rare	0.7%	Rare
Heart	SCHWANNOMA	1.3%	Common	0.7%	Rare
Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC	28%	Common	40%	Common
	SARCOMA,HISTIOCYTIC	1.3%	Common	2.7%	Common
Intestine,duodenum	LEIOMYOMA	0.7%	Rare	.	Rare
Intestine,ileum	ADENOMA	.	Rare	0.7%	Rare
Kidney	ADENOMA,RENAL CELL	0.7%	Rare	.	Rare
	CARCINOMA,TRANSITIONAL CELL	.	Rare	0.7%	Rare
	RENAL MESENCHYMAL TUMOR	.	Rare	0.7%	Rare
Liver	ADENOMA,HEPATOCELLULAR	0.7%	Rare	4.7%	Common
Lung(bronchus)	ADENOCARCINOMA,BRONCHIOLO−ALVEOLAR	0.7%	Rare	1.3%	Common
	ADENOMA,BRONCHIOLO−ALVEOLAR	3.3%	Common	4.0%	Common
Mammary gland	ADENOCARCINOMA	1.3%	Common	.	Rare
	ADENOMA	0.7%	Rare	.	Rare
	FIBROADENOMA	4.0%	Common	.	Rare
	TUMOR,MIXED,MALIGNANT	0.7%	Rare	.	Rare
Mesothelium	MESOTHELIOMA,MALIGNANT	.	Rare	2.7%	Common
Oral cavity	CARCINOMA,SQUAMOUS CELL	1.3%	Common	.	Rare
	PAPILLOMA,SQUAMOUS CELL	.	Rare	0.7%	Rare
Ovary	GRANULOSA CELL TUMOR,MALIGNANT	1.3%	Common	.	Rare
Pancreas	ADENOMA,ACINAR CELL	0.7%	Rare	0.7%	Rare

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Table 1.6 (continued) — Rare and common tumors in rat study

Organ/Tissue name	Tumor name	Female rats		Male rats	
		Incidence	Classification	Incidence	Classification
	ADENOMA,ACINAR−ISLET CELL	1.3%	Common	1.3%	Common
	ADENOMA,ISLET CELL	4.0%	Common	25%	Common
	CARCINOMA,ISLET CELL	0.7%	Rare	.	Rare
Parathyroid	ADENOMA	0.7%	Rare	.	Rare
Pituitary	ADENOMA,ANTERIOR	41%	Common	44%	Common
	ADENOMA,INTERMEDIATED	0.7%	Rare	.	Rare
	CARCINOMA,ANTERIOR	1.3%	Common	0.7%	Rare
Preputial gland	ADENOCARCINOMA	.	Rare	2.0%	Common
	ADENOMA	.	Rare	1.3%	Common
Prostate	ADENOMA	.	Rare	20%	Common
Skin+Subcutis	ADENOMA,SEBACEOUS CELL	0.7%	Rare	.	Rare
	CARCINOMA,SQUAMOUS CELL	.	Rare	0.7%	Rare
	FIBROMA	.	Rare	0.7%	Rare
	FIBROSARCOMA	.	Rare	0.7%	Rare
	HAIR FOLLICLE TUMOR,BENIGN	.	Rare	0.7%	Rare
	KERATOACANTHOMA	.	Rare	4.0%	Common
	LIPOMA	0.7%	Rare	1.3%	Common
	SCHWANNOMA,MALIGNANT	0.7%	Rare	0.7%	Rare
Spinal cord	SCHWANNOMA,MALIGNANT	.	Rare	2.0%	Common
Spleen	HEMANGIOSARCOMA	0.7%	Rare	0.7%	Rare
	OSTEOSARCOMA	.	Rare	0.7%	Rare
Stomach	CARCINOMA,SQUAMOUS CELL	0.7%	Rare	.	Rare
Testis	LEYDIG CELL TUMOR	.	Rare	104%	Common
Thyroid	ADENOMA,C CELL	15%	Common	13%	Common
	ADENOMA,FOLLICULAR CELL	0.7%	Rare	2.0%	Common
	CARCINOMA,C CELL	2.0%	Common	1.3%	Common
	CARCINOMA,FOLLICULAR CELL	.	Rare	0.7%	Rare
Tongue	PAPILLOMA,SQUAMOUS CELL	.	Rare	0.7%	Rare
Urinary bladder	CARCINOMA,TRANSITIONAL CELL	.	Rare	0.7%	Rare
	PAPILLOMA,TRANSITIONAL CELL	1.3%	Common	1.3%	Common
Uterus	ADENOCARCINOMA	2.0%	Common	.	Rare
	GRANULAR CELL TUMOR	2.7%	Common	.	Rare
	LEIOMYOMA	0.7%	Rare	.	Rare
	POLYP,ENDOMETRIAL STROMAL	41%	Common	.	Rare

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Table 1.6 (continued) — Rare and common tumors in rat study

Organ/Tissue name	Tumor name	Female rats		Male rats	
		Incidence	Classification	Incidence	Classification
	SARCOMA,NOS	0.7%	Rare	.	Rare
	SCHWANNOMA,MALIGNANT	0.7%	Rare	.	Rare
Vagina	GRANULAR CELL TUMOR	0.7%	Rare	.	Rare
	POLYP,VAGINAL STROMAL	0.7%	Rare	.	Rare
Vertebra	CHORDOMA	0.7%	Rare	0.7%	Rare
Zymbal gland	ADENOCARCINOMA	0.7%	Rare	2.7%	Common
	CARCINOMA,SQUAMOUS CELL	0.7%	Rare	.	Rare

Table 1.7: All reported neoplastic tumors in rat prodrug study

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Abdominal cavity	PARAGANGLIOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.
Adrenal	ADENOMA,CORTICAL CELL	Female	*p*-value of pairwise and trend tests	.2533	.0116	.1176	.1176
			Observed number of cases	0	4	2	2
			Poly-3 adjusted incidence rate	.00%	8.7%	4.2%	4.1%
		Male	*p*-value of pairwise and trend tests	.6474	1.000	.5763	1.000
			Observed number of cases	1	0	1	0
			Poly-3 adjusted incidence rate	1.1%	.00%	2.0%	.00%
	CARCINOMA,CORTICAL CELL	Female	*p*-value of pairwise and trend tests	.4120		.3453	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.1%	.00%
	PHEOCHROMOCYTOMA	Female	*p*-value of pairwise and trend tests	.4120		.3453	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.1%	.00%
		Male	*p*-value of pairwise and trend tests	.1780	.5452	.7277	.2510
			Observed number of cases	9	5	4	7
			Poly-3 adjusted incidence rate	10%	11%	8.2%	15%
	PHEOCHROMOCYTOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	.6474	1.000	.5763	1.000
			Observed number of cases	1	0	1	0
			Poly-3 adjusted incidence rate	1.1%	.00%	2.0%	.00%
Bone(marrow),sternum	GRANULAR CELL TUMOR	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0

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Table 1.7: All reported neoplastic tumors in rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Bone,cranial	OSTEOSARCOMA	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.	.	.	100%
		Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.
Cerebellum	ASTROCYTOMA	Male	*p*-value of pairwise and trend tests	.4053		.3478	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.0%	.00%
	MENINGIOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Cerebrum	ASTROCYTOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
	OLIGODENDROGLIOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	.6111	.3406		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.1%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.4053		.3478	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.0%	.00%
	RETICULOSIS,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.4053		.3478	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.0%	.00%
Clitoral gland	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.2619	1.000	.5000	.6286
			Observed number of cases	1	0	1	2
			Poly-3 adjusted incidence rate	25%	.00%	50%	50%
	ADENOMA	Female	*p*-value of pairwise and trend tests	1.000			1.000
			Observed number of cases	3	2	1	2
			Poly-3 adjusted incidence rate	77%	100%	58%	50%
Ear	NEURAL CREST TUMOR	Male	*p*-value of pairwise and trend tests	1.000			

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Table 1.7: All reported neoplastic tumors in rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
Eye	LEIOMYOMA	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Heart	SCHWANNOMA	Female	*p*-value of pairwise and trend tests	.8485	.5604	1.000	1.000
			Observed number of cases	1	1	0	0
			Poly-3 adjusted incidence rate	1.1%	2.2%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.6474	1.000	.5763	1.000
			Observed number of cases	1	0	1	0
			Poly-3 adjusted incidence rate	1.1%	.00%	2.0%	.00%
Hemolymphoreticular(all	LEUKEMIA,LARGE GRANULAR LYMPHO	Female	*p*-value of pairwise and trend tests	.5075	.8847	.8291	.6674
			Observed number of cases	23	8	9	11
			Poly-3 adjusted incidence rate	24%	17%	18%	22%
		Male	*p*-value of pairwise and trend tests	.9996	.9999	.9985	1.000
			Observed number of cases	41	7	10	6
			Poly-3 adjusted incidence rate	43%	15%	20%	13%
	SARCOMA,HISTIOCYTIC	Female	*p*-value of pairwise and trend tests	.0168	.5604	.5730	.0483
			Observed number of cases	1	1	1	4
			Poly-3 adjusted incidence rate	1.1%	2.2%	2.1%	8.2%
		Male	*p*-value of pairwise and trend tests	.5234	.4162	.4334	.7004
			Observed number of cases	2	2	2	1
			Poly-3 adjusted incidence rate	2.2%	4.3%	4.1%	2.2%
Intestine,duodenum	ADENOMA	Male	*p*-value of pairwise and trend tests	.6035	.3333		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
	LEIOMYOMA	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Intestine,ileum	ADENOMA	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Liver	ADENOMA,HEPATOCELLULAR	Female	*p*-value of pairwise and trend tests	.7041	.5604	.5730	1.000
			Observed number of cases	1	1	1	0
			Poly-3 adjusted incidence rate	1.1%	2.2%	2.1%	.00%

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Table 1.7: All reported neoplastic tumors in rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
		Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	5	0	0	0
			Poly-3 adjusted incidence rate	5.5%	.00%	.00%	.00%
	CARCINOMA,HEPATOCELLULAR	Male	*p*-value of pairwise and trend tests	.4053		.3478	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.0%	.00%
Lung(bronchus)	ADENOMA,BRONCHIOLO−ALVEOLAR	Female	*p*-value of pairwise and trend tests	.9915	.8755	1.000	1.000
			Observed number of cases	4	1	0	0
			Poly-3 adjusted incidence rate	4.4%	2.2%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.9026	.8069	.8233	1.000
			Observed number of cases	3	1	1	0
			Poly-3 adjusted incidence rate	3.3%	2.2%	2.0%	.00%
Mammary gland	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.7041	.5604	.5730	1.000
			Observed number of cases	1	1	1	0
			Poly-3 adjusted incidence rate	1.1%	2.2%	2.1%	.00%
	ADENOMA	Female	*p*-value of pairwise and trend tests	.4120		.3453	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.1%	.00%
	FIBROADENOMA	Female	*p*-value of pairwise and trend tests	.3582	.5604	.5730	.5730
			Observed number of cases	1	1	1	1
			Poly-3 adjusted incidence rate	1.1%	2.2%	2.1%	2.1%
Mesothelium	MESOTHELIOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	2	1	1	2
			Poly-3 adjusted incidence rate	200%	100%	100%	100%
Nasal cavity	CARCINOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.	100%	.	.
Oral cavity	CARCINOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	2	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.
		Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	1
			Poly-3 adjusted incidence rate	.	100%	.	100%
Ovary	GRANULOSA CELL TUMOR,MALIGNANT	Female	*p*-value of pairwise and trend tests	.2842	1.000	.5698	.5698
			Observed number of cases	1	0	1	1

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Table 1.7: All reported neoplastic tumors in rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	1.1%	.00%	2.1%	2.1%
Pancreas	ADENOMA,ACINAR CELL	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
	ADENOMA,ACINAR−ISLET CELL	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	2	0	0	0
			Poly-3 adjusted incidence rate	2.2%	.00%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.3861	.5572	1.000	.5506
			Observed number of cases	1	1	0	1
			Poly-3 adjusted incidence rate	1.1%	2.2%	.00%	2.2%
	ADENOMA,ISLET CELL	Female	*p*-value of pairwise and trend tests	.0598	.3358	.3453	.1176
			Observed number of cases	0	1	1	2
			Poly-3 adjusted incidence rate	.00%	2.2%	2.1%	4.1%
		Male	*p*-value of pairwise and trend tests	.8843	.5488	.8788	.9020
			Observed number of cases	19	10	7	6
			Poly-3 adjusted incidence rate	21%	22%	14%	13%
	CARCINOMA,ISLET CELL	Male	*p*-value of pairwise and trend tests	.0369			.1062
			Observed number of cases	0	0	0	2
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	4.4%
Parathyroid	ADENOMA	Male	*p*-value of pairwise and trend tests	.1734	.3409	.3556	.3308
			Observed number of cases	0	1	1	1
			Poly-3 adjusted incidence rate	.00%	2.2%	2.0%	2.3%
Pituitary	ADENOMA,ANTERIOR	Female	*p*-value of pairwise and trend tests	.7081	.5588	.9174	.7377
			Observed number of cases	28	14	10	13
			Poly-3 adjusted incidence rate	30%	30%	21%	26%
		Male	*p*-value of pairwise and trend tests	.9936	.1164	.7902	.9883
			Observed number of cases	29	20	13	7
			Poly-3 adjusted incidence rate	31%	43%	26%	16%
	CARCINOMA,ANTERIOR	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	2	0	0	0
			Poly-3 adjusted incidence rate	2.2%	.00%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Preputial gland	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	.9000		.8333	1.000

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Table 1.7: All reported neoplastic tumors in rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	1	0	1	0
			Poly-3 adjusted incidence rate	50%	.	50%	.00%
	ADENOMA	Male	*p*-value of pairwise and trend tests	.8000		1.000	
			Observed number of cases	2	0	1	1
			Poly-3 adjusted incidence rate	95%	.	50%	100%
Prostate	ADENOMA	Male	*p*-value of pairwise and trend tests	.3770	.1724	.3169	.3678
			Observed number of cases	11	9	8	7
			Poly-3 adjusted incidence rate	12%	20%	16%	16%
Skin+Subcutis	ADENOMA,SEBACEOUS CELL	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
	CARCINOMA,BASAL CELL	Female	*p*-value of pairwise and trend tests	.4120		.3453	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.1%	.00%
	CARCINOMA,SQUAMOUS CELL	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
	FIBROMA	Male	*p*-value of pairwise and trend tests	.6053	.3382		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
	FIBROSARCOMA	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
	HAIR FOLLICLE TUMOR,BENIGN	Female	*p*-value of pairwise and trend tests	.2087	.3358		.3453
			Observed number of cases	0	1	0	1
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	2.1%
		Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
	KERATOACANTHOMA	Male	*p*-value of pairwise and trend tests	.9309	1.000	.8866	1.000
			Observed number of cases	4	0	1	0
			Poly-3 adjusted incidence rate	4.4%	.00%	2.0%	.00%
	LIPOMA	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%

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Table 1.7: All reported neoplastic tumors in rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
		Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	2	0	0	0
			Poly-3 adjusted incidence rate	2.2%	.00%	.00%	.00%
	SCHWANNOMA	Male	*p*-value of pairwise and trend tests	.1974			.3333
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
	SCHWANNOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	.8485	.5604	1.000	1.000
			Observed number of cases	1	1	0	0
			Poly-3 adjusted incidence rate	1.1%	2.2%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.1974			.3333
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
Spinal cord	SCHWANNOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Spleen	HEMANGIOSARCOMA	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
	OSTEOSARCOMA	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Stomach	LIPOMA	Male	*p*-value of pairwise and trend tests	.6035	.3333		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
	PAPILLOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	.2060			.3453
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.1%
Testis	LEYDIG CELL TUMOR	Male	*p*-value of pairwise and trend tests	.3997	.8149	.6759	.5751
			Observed number of cases	86	41	44	42
			Poly-3 adjusted incidence rate	90%	87%	89%	91%
Thoracic cavity	OSTEOSARCOMA	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
Thyroid	ADENOMA,C CELL	Female	*p*-value of pairwise and trend tests	.5081	.2026	.6561	.4948
			Observed number of cases	8	7	4	5

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Table 1.7: All reported neoplastic tumors in rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	8.8%	15%	8.3%	10%
		Male	*p*-value of pairwise and trend tests	.9047	.8903	.9641	.9493
			Observed number of cases	13	4	3	3
			Poly-3 adjusted incidence rate	14%	8.9%	6.1%	6.8%
	ADENOMA,FOLLICULAR CELL	Female	*p*-value of pairwise and trend tests	.2883	1.000	.5763	.5763
			Observed number of cases	1	0	1	1
			Poly-3 adjusted incidence rate	1.1%	.00%	2.1%	2.1%
		Male	*p*-value of pairwise and trend tests	.6664	.5605	.1235	1.000
			Observed number of cases	1	1	3	0
			Poly-3 adjusted incidence rate	1.1%	2.2%	6.1%	.00%
	CARCINOMA,C CELL	Female	*p*-value of pairwise and trend tests	.1094	1.000	1.000	.2773
			Observed number of cases	1	0	0	2
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	4.1%
		Male	*p*-value of pairwise and trend tests	.8167	.4119	.4380	1.000
			Observed number of cases	2	2	2	0
			Poly-3 adjusted incidence rate	2.2%	4.4%	4.1%	.00%
	CARCINOMA,FOLLICULAR CELL	Female	*p*-value of pairwise and trend tests	.4138		.3478	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.1%	.00%
		Male	*p*-value of pairwise and trend tests	.4815	.3358	.3504	
			Observed number of cases	0	1	1	0
			Poly-3 adjusted incidence rate	.00%	2.2%	2.0%	.00%
Urinary bladder	CARCINOMA,TRANSITIONAL CELL	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Uterus	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.7584	.2609	.5730	1.000
			Observed number of cases	1	2	1	0
			Poly-3 adjusted incidence rate	1.1%	4.3%	2.1%	.00%
	ADENOMA	Female	*p*-value of pairwise and trend tests	.1270		.3453	.3453
			Observed number of cases	0	0	1	1
			Poly-3 adjusted incidence rate	.00%	.00%	2.1%	2.1%
	FIBROMA	Female	*p*-value of pairwise and trend tests	.6094	.3358		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
	GRANULAR CELL TUMOR	Female	*p*-value of pairwise and trend tests	.6444	.8097	.8205	.8205

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Table 1.7: All reported neoplastic tumors in rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	3	1	1	1
			Poly-3 adjusted incidence rate	3.3%	2.2%	2.1%	2.1%
	LEIOMYOMA	Female	*p*-value of pairwise and trend tests	.4937	.3358	.3453	
			Observed number of cases	0	1	1	0
			Poly-3 adjusted incidence rate	.00%	2.2%	2.1%	.00%
	LEIOMYOSARCOMA	Female	*p*-value of pairwise and trend tests	.0417			.1176
			Observed number of cases	0	0	0	2
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	4.1%
	POLYP,ENDOMETRIAL STROMAL	Female	*p*-value of pairwise and trend tests	.1592	.9231	.0603	.3515
			Observed number of cases	32	11	24	19
			Poly-3 adjusted incidence rate	34%	24%	49%	39%
	SCHWANNOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Vagina	POLYP,VAGINAL STROMAL	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	1.1%	.00%	.00%	.00%
Vertebra	CHORDOMA	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.
		Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.
Zymbal gland	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	1
			Poly-3 adjusted incidence rate	.	100%	.	100%
	CARCINOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.

Table 1.8: Composite endpoint analyses for rat prodrug study

Endpoint	Sex	Quantity	Control	Low dose	Mid dose	High dose
Hemangiosarcoma, all sites	Female	*p*-value of pairwise and trend tests	1	1	1	1
		Poly-3 adjusted incidence rate	1.1%	0%	0%	0%

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Table 1.8: Composite endpoint analyses from rat prodrug study (continued)

Endpoint	Sex	Quantity	Control	Low dose	Mid dose	High dose
		Observed number of cases	1	0	0	0
		Adjusted number at risk	91.53	46.15	48.02	48.65
	Male	*p*-value of pairwise and trend tests	1	.	.	.
		Poly-3 adjusted incidence rate	0%	0%	0%	0%
		Observed number of cases	0	0	0	0
		Adjusted number at risk	90.08	45.94	48.90	44.97
Uterine adenoma & adenocarcinoma	Female	*p*-value of pairwise and trend tests	0.4350	0.2609	0.5730	0.5730
		Poly-3 adjusted incidence rate	1.1%	4.3%	2.1%	2.1 %
		Observed number of cases	1	2	1	1
		Adjusted number at risk	91.53	46.15	48.02	48.65

Table 1.9: Neoplastic tumors in rat prodrug study with at least one significant result

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Adrenal	ADENOMA,CORTICAL CELL	Female	*p*-value of pairwise and trend tests	.2533	.0116	.1176	.1176
			Observed number of cases	0	4	2	2
			Poly-3 adjusted incidence rate	.00%	8.7%	4.2%	4.1%
			Adjusted number at risk	91.53	46.21	48.02	48.71
		Male	*p*-value of pairwise and trend tests	.6474	1.000	.5763	1.000
			Observed number of cases	1	0	1	0
			Poly-3 adjusted incidence rate	1.1%	.00%	2.0%	.00%
			Adjusted number at risk	90.08	45.94	48.90	44.97
Hemolymphoreticular(all	SARCOMA,HISTIOCYTIC	Female	*p*-value of pairwise and trend tests	.0168	.5604	.5730	.0483
			Observed number of cases	1	1	1	4
			Poly-3 adjusted incidence rate	1.1%	2.2%	2.1%	8.2%
			Adjusted number at risk	91.53	46.15	48.02	48.85
		Male	*p*-value of pairwise and trend tests	.5234	.4162	.4334	.7004
			Observed number of cases	2	2	2	1
			Poly-3 adjusted incidence rate	2.2%	4.3%	4.1%	2.2%
			Adjusted number at risk	90.08	46.02	48.90	44.97
Pancreas	CARCINOMA,ISLET CELL	Male	*p*-value of pairwise and trend tests	.0369			.1062

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Table 1.9: Significant results from rat prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	0	0	0	2
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	4.4%
			Adjusted number at risk	90.08	45.94	48.90	44.97
Uterus	LEIOMYOSARCOMA	Female	*p*-value of pairwise and trend tests	.0417			.1176
			Observed number of cases	0	0	0	2
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	4.1%
			Adjusted number at risk	91.53	46.15	48.02	48.65

Table 1.10: Animals for which commonly unexamined organs were examined (rat prodrug study)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
Abdominal cavity	Female	Control	1115	Abdominal cavity	Autolytic
				Mesothelium	Autolytic
				Thyroid	Autolytic
				Ovary	GRANULOSA CELL TUMOR,MALIGNANT
				Vagina	POLYP,VAGINAL STROMAL
			1122	Mesothelium	Autolytic
				Abdominal cavity	PARAGANGLIOMA,MALIGNANT
		Low	3124	Abdominal cavity	Autolytic
				Mesothelium	Autolytic
			3139	Abdominal cavity	Autolytic
				Mesothelium	Autolytic
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Uterus	POLYP,ENDOMETRIAL STROMAL
	Male	Control	2015	Abdominal cavity	Autolytic
				Mesothelium	Autolytic
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Pancreas	ADENOMA,ISLET CELL
				Skin+Subcutis	CARCINOMA,SQUAMOUS CELL
				Testis	LEYDIG CELL TUMOR

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Table 1.10: Animals for which commonly unexamined organs were examined (rat prodrug study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
			2032	Abdominal cavity	Autolytic
				Mesothelium	Autolytic
				Tail	Autolytic
				Cerebrum	ASTROCYTOMA,MALIGNANT
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Testis	LEYDIG CELL TUMOR
		Low	3013	Abdominal cavity	Autolytic
				Mesothelium	Autolytic
			3044	Abdominal cavity	Autolytic
				Mesothelium	MESOTHELIOMA,MALIGNANT
				Parathyroid	ADENOMA
				Testis	LEYDIG CELL TUMOR
		Mid	4013	Abdominal cavity	Autolytic
				Adrenal	ADENOMA,CORTICAL CELL
				Mesothelium	MESOTHELIOMA,MALIGNANT
				Testis	LEYDIG CELL TUMOR
		High	5001	Abdominal cavity	Autolytic
				Mesothelium	MESOTHELIOMA,MALIGNANT
				Pancreas	ADENOMA,ACINAR−ISLET CELL
				Prostate	ADENOMA
				Testis	LEYDIG CELL TUMOR
Bone,cranial	Female	Control	2126	Bone,cranial	Autolytic
				Mesothelium	Autolytic
		High	5109	Mesothelium	Autolytic
				Bone,cranial	OSTEOSARCOMA
	Male	Control	1044	Mesothelium	Autolytic
				Bone,cranial	OSTEOSARCOMA
				Testis	LEYDIG CELL TUMOR
Clitoral gland	Female	Control	1110	Mesothelium	Autolytic

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Table 1.10: Animals for which commonly unexamined organs were examined (rat prodrug study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
				Clitoral gland	ADENOCARCINOMA
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
			1134	Clitoral gland	Autolytic
				Mesothelium	Autolytic
				Pituitary	ADENOMA,ANTERIOR
			1135	Mesothelium	Autolytic
				Clitoral gland	ADENOMA
				Lung(bronchus)	ADENOMA,BRONCHIOLO-ALVEOLAR
				Uterus	POLYP,ENDOMETRIAL STROMAL
			1141	Mesothelium	Autolytic
				Planta	Autolytic
				Cerebrum	RETICULOSIS,MALIGNANT
				Clitoral gland	ADENOMA
				Pituitary	ADENOMA,ANTERIOR
				Uterus	POLYP,ENDOMETRIAL STROMAL
			1145	Clitoral gland	Autolytic
				Mesothelium	Autolytic
		Control	2134	Clitoral gland	Autolytic
				Mesothelium	Autolytic
				Pituitary	ADENOMA,ANTERIOR
			2142	Mesothelium	Autolytic
				Clitoral gland	ADENOMA
			2146	Clitoral gland	Autolytic
				Mesothelium	Autolytic
				Thyroid	ADENOMA,C CELL
					ADENOMA,FOLLICULAR CELL
		Low	3141	Mesothelium	Autolytic
				Clitoral gland	ADENOMA
				Thyroid	ADENOMA,C CELL

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Table 1.10: Animals for which commonly unexamined organs were examined (rat prodrug study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
			3142	Mesothelium	Autolytic
				Clitoral gland	ADENOMA
				Pituitary	ADENOMA,ANTERIOR
		Mid	4111	Mesothelium	Autolytic
				Clitoral gland	ADENOCARCINOMA
			4113	Mesothelium	Autolytic
				Clitoral gland	ADENOMA
				Uterus	POLYP,ENDOMETRIAL STROMAL
		High	5118	Mesothelium	Autolytic
				Clitoral gland	ADENOMA
			5124	Mesothelium	Autolytic
				Clitoral gland	ADENOCARCINOMA
				Pituitary	ADENOMA,ANTERIOR
			5127	Mesothelium	Autolytic
				Clitoral gland	ADENOCARCINOMA
				Stomach	PAPILLOMA,SQUAMOUS CELL
			5128	Clitoral gland	Autolytic
				Mesothelium	Autolytic
				Uterus	POLYP,ENDOMETRIAL STROMAL
			5136	Mesothelium	Autolytic
				Clitoral gland	ADENOMA
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
Ear	Male	Mid	4026	Mesothelium	Autolytic
				Ear	NEURAL CREST TUMOR
				Pancreas	ADENOMA,ISLET CELL
				Pituitary	ADENOMA,ANTERIOR
				Testis	LEYDIG CELL TUMOR

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Table 1.10: Animals for which commonly unexamined organs were examined (rat prodrug study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
Nasal cavity	Female	Low	3118	Mesothelium	Autolytic
				Tail	Autolytic
				Nasal cavity	CARCINOMA,SQUAMOUS CELL
				Thyroid	ADENOMA,C CELL
Oral cavity	Female	Control	1125	Mesothelium	Autolytic
				Oral cavity	CARCINOMA,SQUAMOUS CELL
			1129	Mesothelium	Autolytic
				Intestine,duodenum	LEIOMYOMA
				Oral cavity	CARCINOMA,SQUAMOUS CELL
	Male	Low	3008	Mesothelium	Autolytic
				Intestine,duodenum	ADENOMA
				Oral cavity	CARCINOMA,SQUAMOUS CELL
				Pituitary	ADENOMA,ANTERIOR
				Testis	LEYDIG CELL TUMOR
		High	5046	Mesothelium	Autolytic
				Oral cavity	CARCINOMA,SQUAMOUS CELL
				Skin+Subcutis	SCHWANNOMA
				Testis	LEYDIG CELL TUMOR
Preputial gland	Male	Control	1013	Mesothelium	Autolytic
				Preputial gland	ADENOMA
				Testis	LEYDIG CELL TUMOR
			1020	Mesothelium	Autolytic
				Planta	Autolytic
				Adrenal	PHEOCHROMOCYTOMA
				Pancreas	ADENOMA,ISLET CELL
				Pituitary	ADENOMA,ANTERIOR
				Preputial gland	ADENOCARCINOMA
				Prostate	ADENOMA
				Testis	LEYDIG CELL TUMOR

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Table 1.10: Animals for which commonly unexamined organs were examined (rat prodrug study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
		Mid	4028	Mesothelium	Autolytic
				Planta	Autolytic
				Preputial gland	ADENOCARCINOMA
				Prostate	ADENOMA
				Testis	LEYDIG CELL TUMOR
			4039	Mesothelium	Autolytic
				Preputial gland	ADENOMA
				Prostate	ADENOMA
				Testis	LEYDIG CELL TUMOR
				Thyroid	ADENOMA,C CELL
		High	5041	Mesothelium	Autolytic
				Pancreas	ADENOMA,ISLET CELL
				Preputial gland	ADENOMA
				Prostate	ADENOMA
				Testis	LEYDIG CELL TUMOR
Thoracic cavity	Female	Mid	4106	Mesothelium	Autolytic
				Thoracic cavity	OSTEOSARCOMA
				Uterus	POLYP,ENDOMETRIAL STROMAL
Vertebra	Female	Control	1126	Mesothelium	Autolytic
				Tail	Autolytic
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Pituitary	ADENOMA,ANTERIOR
				Vertebra	CHORDOMA
	Male	Control	1040	Mesothelium	Autolytic
				Pancreas	ADENOMA,ISLET CELL
				Testis	LEYDIG CELL TUMOR
				Vertebra	CHORDOMA
Zymbal gland	Female	Control	1132	Mesothelium	Autolytic
				Zymbal gland	CARCINOMA,SQUAMOUS CELL
	Male	Low	3037	Mesothelium	Autolytic

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Table 1.10: Animals for which commonly unexamined organs were examined (rat prodrug study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
				Pancreas	ADENOMA,ISLET CELL
				Testis	LEYDIG CELL TUMOR
				Zymbal gland	ADENOCARCINOMA
		High	5030	Mesothelium	Autolytic
				Adrenal	PHEOCHROMOCYTOMA
				Zymbal gland	ADENOCARCINOMA

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1.2 Mouse study

1.2.1 Overview

Two separate experiments were conducted, one in male and one in female mice.

In each of these two experiments there were three treated groups and one control group. One hundred and twenty five hemizygous Tg.rasH2 mice of each sex were initially randomly allocated to three treatment groups (twenty five animals of each sex per group), called the low dose group, the mid dose group, and the high dose group, to one vehicle control group (twenty five animals of each sex), and a positive control group (twenty five animals of each sex) who received urethane. The dose levels for the treatment groups were 50, 150, and 450 mg/kg/day respectively. The positive control group received three doses of urethane, on days 1, 3, and 5 of the study, for a total dose of 1000 mg/kg.

The vehicle for the test was a solution of 0.5 w/v% methylcellulose, with 1 w/v% of citric acid, administered orally by gavage as a dosage volume of 10 mL/kg, once per day. The vehicle for the positive control animals was saline, administered by three intraperitoneal injections, with a total dosage volume of 10 mL/kg.

During the administration period, animals were checked twice daily for moribundity and mortality. Each animal received a weekly hands-on exam and a weekly exam for clinical signs of toxicity. After death, all animals were necropsied as soon as possible.

1.2.2 Sponsor's analysis

Survival analysis

The sponsor analyzed survival data using Kaplan-Meier product-limit estimates and Cox-Tarone binary regression on life tables (see [9]), using a twenty six week window. Continuity-corrected one-sided tail probabilities for trend, and group comparisons were measured against a 5% significance level. Analyses were also conducted using the Gehan-Breslow method.

No significant results were found using the Cox-Tarone method. When the Gehan-Breslow test(see [10]) was conducted, the sponsor reports a statistically significant increase in survival rates as the dose is increased ($p = 0.0353$), with survival significantly improved, when compared with the control group, in both the low dose ($p = 0.0396$) and high dose ($p = 0.0396$) male groups. No significant results were reported in the study of female mice, regardless of the test used. It is not clear how significant improvements in survival were found when the sponsor planned a one tailed test to investigate possible deterioration in survival times.

Tumor analysis

Neoplasm incidence data were analyzed only in those cases when the incidence count in at least one treatment group exceeded the count on the corresponding control group by at least two. In these cases, either Peto's method [7], or an exact method (when the incidence table was sparse) were used. Trend and pairwise comparisons were conducted at the 5% significance level; all tests were one tailed.

In the female mouse study, the only significant result reported is that the rate of alveolar bronchiolar adenomas was increased in the low dose group compared with the control group ($p = 0.0455$). There was no evidence of a dose-response relationship, and no evidence that the higher dose levels were associated with a higher incidence rate of this type of tumor.

No significant results were reported in the study of male mice.

Urethane study

In both male and female mice, the sponsor found that the mice treated with urethane had significantly worse survival times than the control group. The p-values for these tests are shown in table 1.11.

The sponsor also found strong evidence in both male and female mice of increased rates of alveolar bronchiolar adenomas ($p = 0.0001$ for females, $p < 0.0001$ for males), carcinomas ($p =$

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Table 1.11: p-values from sponsor's survival analyses: urethane treated mice against control (prodrug study)

	Cox-Tarone test	Gehan-Breslow test
Female	0.0003	0.0001
Male	0.0072	0.0034

0.0049 for females, $p = 0.0148$ for males), and adenomas and carcinomas when combined ($p = 0.0001$ for females, $p < 0.0001$ for males), and of hemangiosarcomas, both when reported as "whole body" ($p = 0.0002$ for females and $p < 0.0001$ for males) and splenetic ($p = 0.0002$ for females, $p < 0.0001$ for males), in the urethane treated group, compared with the control group.

1.2.3 Reviewer's analysis

Survival analysis

Kaplan-Meier plots of the survival rates for the two sexes are shown in figures 1.14 and 1.15. The results of log-rank tests for trend and homogeneity are presented in table 1.12.

There is no evidence for a decreasing trend in survival times as dosage is increased ($p = 0.4116$ for female mice, $p = 0.9260$ for male mice). There is some evidence of heterogeneity of survival times among the male mice ($p = 0.1021$ — the p-value for the test of homogeneity among female mice is 0.2947), but given the very small number of premature deaths in the study, it is not possible to make any conclusions about the impact of TAK-491 on the mortality of mice.

Table 1.12: Results of log-rank tests of survival in prodrug study on mice

Species and Sex	Number of dose groups	p-value (trend)	p-value (homogeneity)
Mice — Female	4	0.4116	0.2947
Mice — Male	4	0.9260	0.1021

Figure 1.14

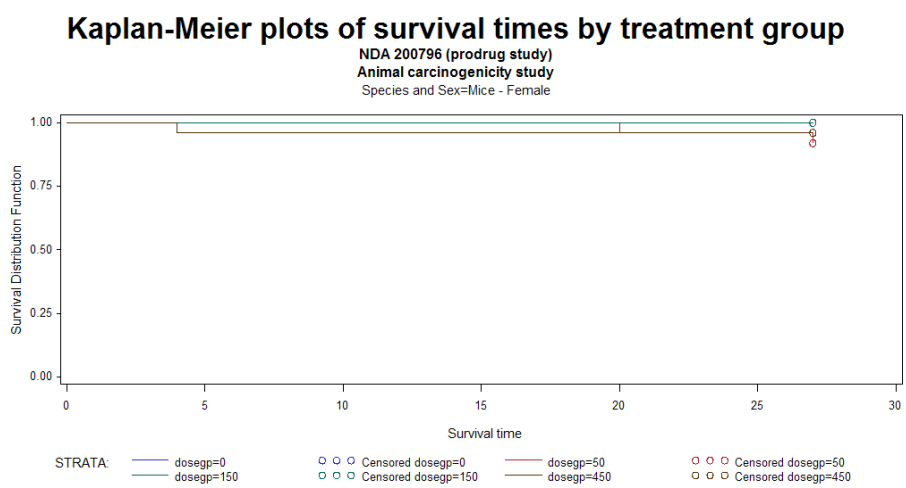

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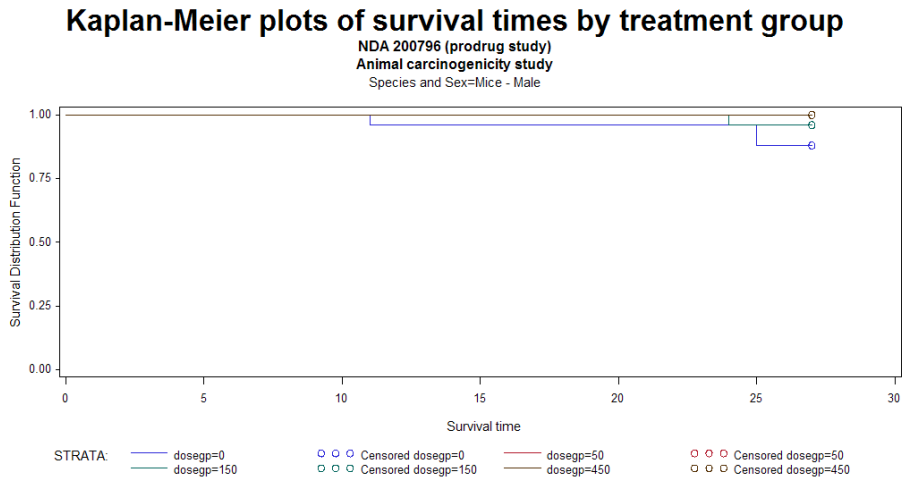
Figure 1.15



Tumor analysis

A poly-k analysis has been conducted for each reported tumor type. The literature dose not provide conclusive evidence for a suitable value of k to use with transgenic mice in a twenty six month study, so it has been decided to use a value of $k = 1$. An animal's membership in the risk set is therefore proportional to its lifespan, unless it is recorded as having developed the tumor of interest[2].

The results of the poly-1 analyses are presented in table 1.14. A composite analysis of all hemangiosarcomas is presented in table 1.15. No animals in this study developed either uterine adenomas or uterine adenocarcinomas, so this composite endpoint cannot be analyzed. Ordinarily, an abridged version of table 1.14, consisting of those tumor types for which at least one test, in at least one sex yielded a p-value below 0.05 would also be presented, but in this study there are no such tumor types.

There are no noteworthy results to discuss.

Autolytic and unexamined organs

Autolytic organs No animals in this study were reported as having any organs autolyzed to the extent that no usable tissue sample could be obtained.

Unexamined organs Six organs (ears, perinium, scrotal sac, skeletal muscle, skin (leg) and skin (nasal))are reported as being unexamined in the vast majority of animals. In the few animals where these organs were examined, a high proportion were found to have tumors. As in the case of the rat study, it is assumed here that these organs were not included as part of a standard histopathological exam, and that those tumors reported were detected by gross exam, or incidentally. Irrespective of the explanation, the study can not be viewed as providing any information, positive or negative, regarding the incidence of tumors in these sites. Table 1.16 lists those animals whose ears etc. were examined, together with all tumors found in those animals.

Note that the one animal reported as having its ears examined (a low dose female animal, number 5593) was not reported as having a tumor in its ears. It was, however, found to have a "hyperactive mass", variously described as a "pustule" and a "nodule" on the right ear.

[2]Note that the very low mortality rate in this study, a rate that is typical in studies of transgenic mice, suggests that the results will not be very sensitive to changes in the value of k.

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Urethane study

The purpose of the urethane study was to verify that the study could indeed detect the impact of a known toxin. However thehistopathological exams conducted on these animals were incomplete; the organs listed in table 1.13 were all left unexamined in all animals.

Table 1.13: Organs unexamined in mice from active control group (prodrug study)

adrenal glands	intestine, ileum	seminal vesicles
aorta	intestine, jejunum	skeletal muscle
bone marrow, femur	intestine, rectum	skeletal muscle (thigh)
bone marrow, sternum	kidneys	skin
bone, femur	liver	skin, leg
bone, sternum	lymph node, mandibular	skin, nasal
brain	lymph node, mediastinal	spinal cord, cervical
cavity, nasal	lymph node, mesenteric	spinal cord, lumbar
ears	mammary gland	spinal cord, thoracic
epididymides	nerve, sciatic	stomach
esophagus	ovaries	testes
eyes	pancreas	thymus
gall bladder	parathyroid glands	thyroid glands
harderian glands	perinium	tongue
heart	pituitary gland	trachea
intestine, cecum	prostate gland	urinary bladder
intestine, colon	salivary glands	uterus
intestine, duodenum	scrotal sac	vagina

Thus a full comparison of tumor incidence rates between the urethane group and the control is not possible. The only organs in which tumors were reported to have been found in the urethane group are the spleen (hemangiosarcomas), the lungs (hemangiosarcomas, alveolar bronchiolar adenomas and carcinomas), and "multicentric" (lymphoma).

The results of these tumor analyses are presented in table 1.17. Note that tumors found in the control group but in organs not examined in any of the urethane-treated animals are not included. There is clearly very strong evidence of urethane's carcinogenic effect; the p-value for the test of comparison between the urethane group and the control group was less than 0.0001 for both alveolar bronchiolar carcinomas and adenomas, and for splenetic hemangiosarcomas, in both male and female mice.

Survival plots for the urethane treated animals, compared with the controls, are shown as figures 1.16 and 1.17. As can be seen from these plots, the survival profiles of the urethane-treated animals is clearly worse than the control group, in both sexes. It is no surprise that the log-rank test finds strongly significant differences for both males and females ($p < 0.0001$ in both cases). Taken together, we can conclude that the study has successfully verified the toxic and carcinogenic effects of urethane.

1.2.4 Suitability of dose level

There are currently no accepted statistical guidelines to determine whether a dose level is or was appropriate in a transgenic mouse study. The retrospective determination of suitability of the dose level therefore depends on the interpretation of clinical signs, and so falls outside the scope of this review.

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Figure 1.16

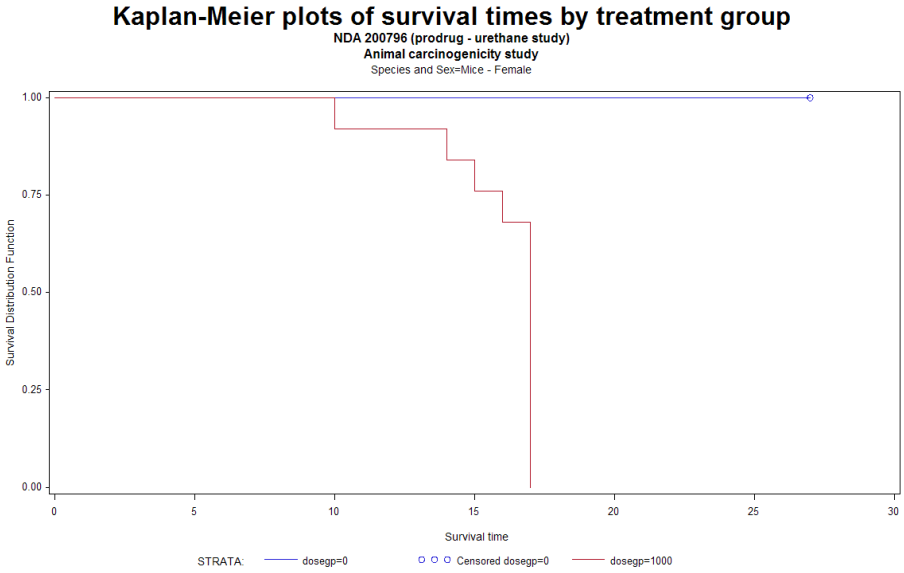

Figure 1.17

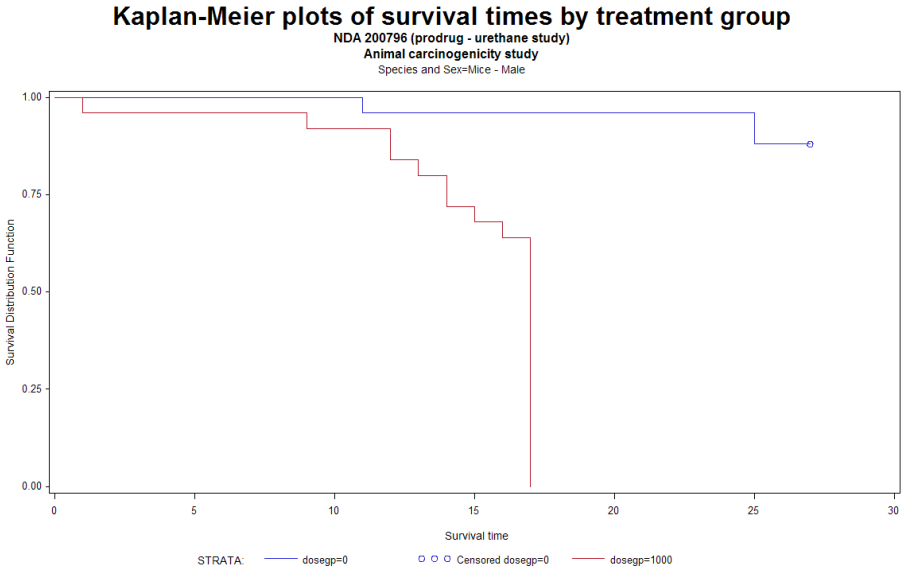

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Table 1.14: All reported neoplastic tumors in mouse prodrug study

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
cavity, nasal	adenocarcinoma	Female	*p*-value of pairwise and trend tests	.5000		.5000	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
		Male	*p*-value of pairwise and trend tests	.6314	.5102	.5000	
			Observed number of cases	0	1	1	0
			Poly-1 adjusted incidence rate	.00%	4.0%	4.0%	.00%
	adenoma	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	4.1%	.00%	.00%	.00%
harderian glands	adenoma	Male	*p*-value of pairwise and trend tests	.5000		.5000	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
lungs with bronchi	alveolar bronchiolar adenoma	Female	*p*-value of pairwise and trend tests	.8877	.0502	.5000	
			Observed number of cases	0	4	1	0
			Poly-1 adjusted incidence rate	.00%	16%	4.0%	.00%
		Male	*p*-value of pairwise and trend tests	.3273	.7653	.1738	.5156
			Observed number of cases	1	1	4	2
			Poly-1 adjusted incidence rate	4.1%	4.0%	16%	8.0%
	alveolar bronchiolar carcinoma	Male	*p*-value of pairwise and trend tests	.9419	.7653	1.000	1.000
			Observed number of cases	1	1	0	0
			Poly-1 adjusted incidence rate	4.1%	4.0%	.00%	.00%
	mesothelioma	Female	*p*-value of pairwise and trend tests	.7475	.5000		
			Observed number of cases	0	1	0	0
			Poly-1 adjusted incidence rate	.00%	4.0%	.00%	.00%
lymph node, mesenteric	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	.1843		.5000	.4898
			Observed number of cases	0	0	1	1
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	4.1%
multicentric	mesothelioma	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	4.0%	.00%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	4.0%	.00%	.00%	.00%
perinium	squamous cell carcinoma	Female	*p*-value of pairwise and trend tests	1.000			

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Table 1.14: All reported neoplastic tumors in mouse prodrug study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	0	0	0	1
			Poly-1 adjusted incidence rate	.	.	.	100%
prostate gland	hemangiosarcoma	Male	*p*-value of pairwise and trend tests	.5000		.5000	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
	transitional cell carcinoma	Male	*p*-value of pairwise and trend tests	.7551	.5102		
			Observed number of cases	0	1	0	0
			Poly-1 adjusted incidence rate	.00%	4.0%	.00%	.00%
skeletal muscle	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	0
			Poly-1 adjusted incidence rate	.	100%	.	.
skin, leg	hemangiosarcoma	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	0
			Poly-1 adjusted incidence rate	.	100%	.	.
skin, nasal	sarcoma	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	0
			Poly-1 adjusted incidence rate	.	100%	.	.
spleen	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	.1618	1.000	.5000	.4844
			Observed number of cases	1	0	2	2
			Poly-1 adjusted incidence rate	4.0%	.00%	8.0%	8.3%
		Male	*p*-value of pairwise and trend tests	.3119	1.000	.6957	.7110
			Observed number of cases	2	0	2	2
			Poly-1 adjusted incidence rate	8.2%	.00%	8.0%	8.0%
stomach	papilloma	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	4.0%	.00%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.2551			.5102
			Observed number of cases	0	0	0	1
			Poly-1 adjusted incidence rate	.00%	.00%	.00%	4.0%
	squamous cell carcinoma	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	4.1%	.00%	.00%	.00%
testes	hemangiosarcoma	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	4.1%	.00%	.00%	.00%

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Table 1.15: Composite endpoint analyses for mouse prodrug study

Endpoint	Sex	Quantity	Control	Low dose	Mid dose	High dose
All hemangiosarcomas	Female	p-value of pairwise and trend tests	.1314	.7449	.3046	.2890
		Poly-1 adjusted incidence rate	4.0%	4.0%	12%	12%
		Observed number of cases	1	1	3	3
		Adjusted number at risk	25.00	24.77	25.00	24.15
	Male	p-value of pairwise and trend tests	.5607	.9498	.6669	.8384
		Poly-1 adjusted incidence rate	12%	4.0%	12%	8.0%
		Observed number of cases	3	1	3	2
		Adjusted number at risk	24.35	25.00	24.92	25.00

Table 1.16: Animals for which commonly unexamined organs were examined (mouse prodrug study)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
ears	Female	Low	5593	lungs with bronchi	alveolar bronchiolar adenoma
perinium	Female	High	5639	perinium spleen	squamous cell carcinoma hemangiosarcoma
scrotal sac	Male	Control	5402	testes	hemangiosarcoma
skeletal muscle	Female	Low	5587	skeletal muscle	hemangiosarcoma
skin, leg	Male	Low	5463	skin, leg	hemangiosarcoma
skin, nasal	Female	Low	5590	lungs with bronchi skin, nasal	alveolar bronchiolar adenoma sarcoma

Table 1.17: All tumors reported in urethane study (prodrug study)

Organ	Tumor type	Sex	Quantity	Control	Urethane
lungs with bronchi	alveolar bronchiolar adenoma	Female	p-value of pairwise test		< 0.0001
			Observed number of cases	0	25
			Poly-1 adjusted incidence rate	.00%	100%

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Table 1.17: All reported neoplastic tumors in urethane study (prodrug study) (continued)

Organ	Tumor type	Sex	Quantity	Control	Urethane
		Male	p-value of pairwise test		< 0.0001
			Observed number of cases	1	24
			Poly-1 adjusted incidence rate	4.1%	100%
	alveolar bronchiolar carcinoma	Female	p-value of pairwise test		< 0.0001
			Observed number of cases	0	20
			Poly-1 adjusted incidence rate	.00%	89%
		Male	p-value of pairwise test		< 0.0001
			Observed number of cases	1	12
			Poly-1 adjusted incidence rate	4.1%	64%
	hemangiosarcoma	Male	p-value of pairwise test		.1417
			Observed number of cases	0	2
			Poly-1 adjusted incidence rate	.00%	13%
multicentric	lymphoma	Female	p-value of pairwise test		.3750
			Observed number of cases	0	1
			Poly-1 adjusted incidence rate	.00%	6.3%
		Male	p-value of pairwise test		.3684
			Observed number of cases	0	1
			Poly-1 adjusted incidence rate	.00%	6.7%
	mesothelioma	Female	p-value of pairwise test		1.000
			Observed number of cases	1	0
			Poly-1 adjusted incidence rate	4.0%	.00%
		Male	p-value of pairwise test		1.000
			Observed number of cases	1	0
			Poly-1 adjusted incidence rate	4.0%	.00%
spleen	hemangiosarcoma	Female	p-value of pairwise test		< 0.0001
			Observed number of cases	1	24
			Poly-1 adjusted incidence rate	4.0%	98%
		Male	p-value of pairwise test		< 0.0001

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Table 1.17: All reported neoplastic tumors in urethane study (prodrug study) (continued)

Organ	Tumor type	Sex	Quantity	Control	Urethane
			Observed number of cases	2	24
			Poly-1 adjusted incidence rate	8.2%	100%

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Chapter 2

Metabolite studies

2.1 Main rat study

2.1.1 Overview

Two separate experiments were conducted, one in male and one in female rats. In each of these two experiments there were three treated groups (called the low dose group, the mid dose group, and the high dose group respectively), one vehicle control group, and one negative control group. Two hundred and fifty F344/Jcl rats of each sex were initially randomly allocated to each of these five treatment groups (fifty animals of each sex per group). The randomization used a block design to ensure homogeneity of group means of bodyweight. In the study of male rats, the daily dose levels of the treated groups were 100mg/kg, 300mg/kg and 1000/mg/kg for the low, mid and high dose groups respectively. In the case of the female rats, the dose levels were 300mg/kg, 1000mg/kg and 3000mg/kg. Note that this means that the low dose female rats received the same dose as the mid dose male rats, and that the mid dose female rats received the same dose as the high dose male rats. For this reason, the sponsor refers to the 300mg/kg level as the *mid dose* level, the 1000mg/kg level as the *high dose* dose level, and the 3000mg/kg level as the *highest dose* dose level, regardless of the sex of the animal.

The vehicle for the test was a suspension of TAK-536-MII in corn oil (10mL per kilogram of bodyweight), administered orally by gavage once per day. The vehicle control animals received corn oil only, and the negative control animals received an injection of water.

During the administration period animals were checked three times daily (twice daily on weekends and holidays) for clinical signs, including external appearance, nutritional condition, posture, behavior and excretions. A palpation exam was conducted every week, and animals found moribund were subject to necropsy, whereby all the organs/tissues in the cranial, thoracic and abdominal cavities were carefully examined and the results were recorded. Additionally, hematological exams were conducted to the extent possible.

2.1.2 Sponsor's analysis

Survival analysis

Mortality data were plotted according to the Kaplan-Meier [5] product limit method, and then tested using Tarone's life table test [9] to test for trend in survival times across the four groups. Additionally, log rank tests were conducted to compare each treatment group with the combined control group. These tests were "conducted at the one-tailed significance level 0.05". The sponsor dose not specify the time intervals used in Tarone's test, but repeated references to the survival rate suggest that the sponsor has considered survival over the single interval consisting of the entire two years of the study.

No statistics or p-values are presented. However, a significant increases in the survival rate is found in the male rats in the low dose group compared with the vehicle control. As in the analysis of TAK-491, it is not clear how the sponsor found statistically significant *increases* in survival in

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a test designed to detect decreases in survival when the tests were "conducted at the one-tailed significance level 0.05".

Tumor analysis

Each reported tumor type was assessed for signs that the incidence rate was associated with TAK-536-MII. For tumor types with high incidence rates (at least 10 animals within one sex) a survival adjusted Peto test was used (see [7]) to test for a dose response relationship, and to test the incidence of each treatment group against the vehicle control group. In the case of less common tumors, an exact permutation test was used to assess dose response and to conduct pairwise comparisons.

Both trend tests and tests of proportion were conducted at α levels of 0.01 and 0.05 for common and rare tumors respectively, where the determination of which tumors were common was based on the historical incidence of F344/Crlj rats at the (b) (4). An historical incidence rate above 1% qualified a tumor type to be considered common[1]. In addition to individual tumor types, the sponsor also tested two combined endpoints; combined thyroidal C-cell adenomas and carcinomas, and combined uterine adenomas and adenocarcinomas.

No tumor type was found to have a significant dose response. However, the combined incidence of thyroidal C-cell adenomas and carcinomas in the low dose male animals was found to be significantly higher than the rate in the corresponding vehicle control group. The sponsor concludes that the absence of a dose response relationship, and the lack of any impact on rates focal C-cell hyperplasia lead to the conclusion that this is not a drug related effect.

Several other tumors were observed to have an increased incidence rate in one or more of the treated groups compared to the vehicle control group. In each case this increase is not significant after adjusting for multiplicity of endpoints. In none of these cases is there statistically significant evidence of a dose response relationship, and the rate in the treatment group is comparable to that in the negative control. Consequently, the sponsor has concluded in each case that the increased incidence is not related to TAK-536-MII. Additionally, in two cases, the sponsor has cited the fact that certain other tumors have not increased in incidence as further evidence that the increase is not drug related. Assessment of the relevance of these claims is outside the scope of this review. These tumors types are presented in table 2.1.

The sponsor also noted that large granular lymphocytic leukemia, anterior adenoma of the pituitary gland, and Leydig cell tumors of the testis were all less common in the male vehicle control group than in the male negative control group. No statistics are presented.

2.1.3 Reviewer's analysis

Control analysis

For each sex, there were two separate control groups; a vehicle control group, whose members received the corn oil vehicle, and the negative control group, whose members received an injection of water. Kaplan-Meier plots of the survival rates of these two groups of rats are presented in figures 2.1 and 2.2. For each sex, a proportional hazards analysis has been conducted to test whether the two groups experiences the same survival outcomes. The results of this test are presented in table 2.2.

Although the tests for heterogeneity do not yield significant p-values, it is clear from inspection of figure 2.2 that at least in the case of the male rats, it is not reasonable to treat the vehicle control group and the negative control group as equivalent; it appears that the vehicle control group has experienced noticeably inferior survival outcomes compared with the negative control group. To accurately estimate the impact of TAK-536-MII, we therefore exclude the (better performing) negative control group from subsequent analyses, in both sexes.

However, at the request of the Executive Carcinogenesis Assessment Committee, analagous tables and plots using only the negative control have been included in Appendix A. It is inappropriate to combine the vehicle and negative control groups, so no analyses using a combined control have been conducted.

[1]Note that the substrain used for the historical control (F344/Crlj) was different from the substrain used for the experiemnt itself (F344/Jcl).

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Table 2.1: Tumors with an increased incidence rate but which were found not to be treatment related by the sponsor

Organ	Tumor	Group with elevated incidence	Other tumors cited as evidence of no tumorigenic effect
Adrenal gland	Pheochromocytoma	All male treated groups	Focal medullary hyperplasia
Pituitary gland	Anterior adenomas	Low dose female rats	Focal hyperplasia of the anterior lobe
Uterus	Endometrial stromal polyps	Mid dose female rate	
	Adenomas	High dose female rats	
	Adenomas and carcinomas	High dose female rats	
Hemolymphoreticular system	Large granular lymphocytic leukemia	High dose female rats	

Figure 2.1

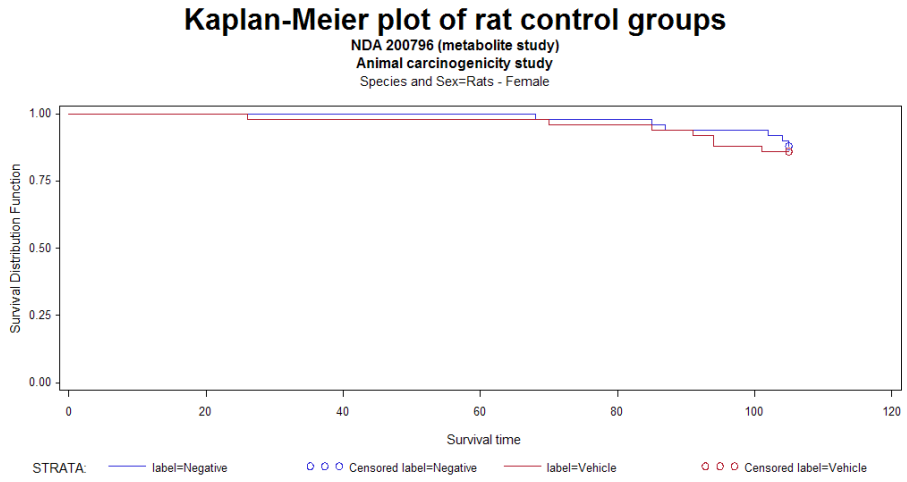

Table 2.2: Results of proportional hazards regression of control rats (metabolite study)

Species and Sex	p-value	Hazard Ratio	Confidence interval for HR
Rats — Female	0.7406	1.202	(0.4043,3.578)
Rats — Male	0.2260	1.443	(0.7969,2.613)

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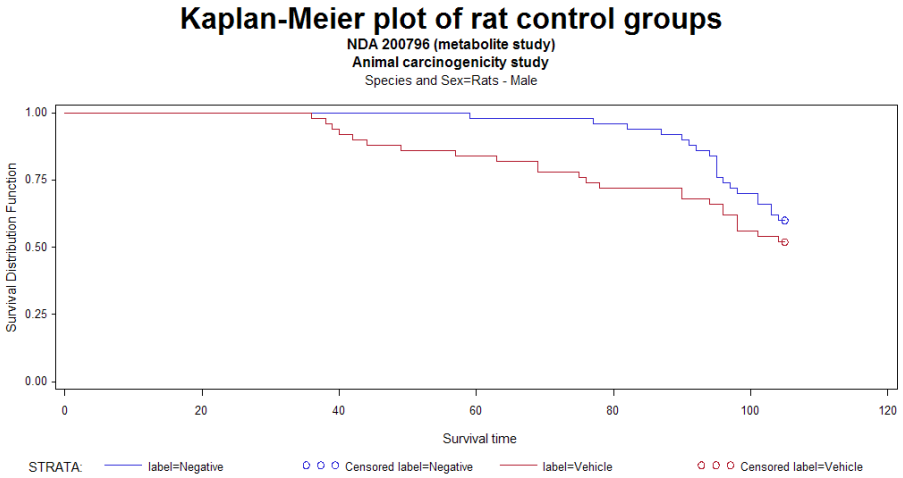
Figure 2.2

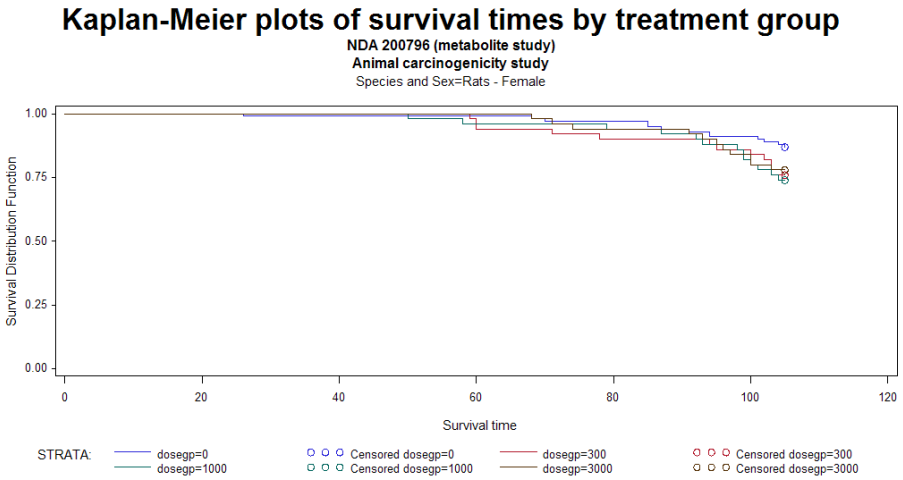


Survival analysis

Kaplan-Meier plots of the survival rates for the two sexes are shown in figures 2.3 and 2.4. The results of log-rank tests for trend and homogeneity are presented in table 2.3 (on page 54). Intercurrent mortality data are presented in table 2.4 (on page 54).

There is no evidence for an decreasing trend in survival times as dosage is increased. There is some evidence of heterogeneity of survival times among the male rats, but from inspection of table 1.3, it is apparent that this is due to the inferior survival rate of the control group. Consequently, this heterogeneity can not be attributed to any toxicity effect of TAK-536-MII.

Figure 2.3



Tumor analysis

The same analysis of tumor types was conducted as for the prodrug study (see section 1.1.3). The results of these analyses, by tumor type, are shown in table 2.6. As before, composite analysis of

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Figure 2.4

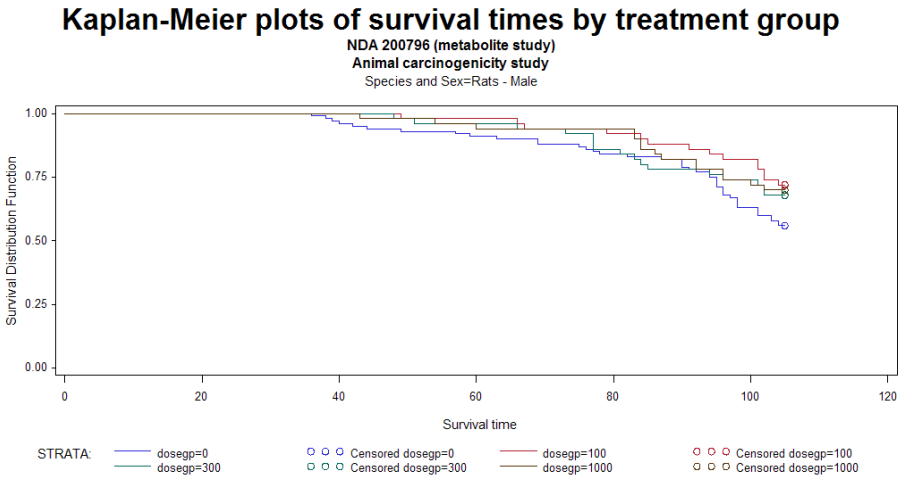

Table 2.3: Results of log-rank tests of survival in metabolite study on rats

Species and Sex	Number of dose groups	p-value (trend)	p-value (homogeneity)
Rats — Female	4	0.3194	0.5363
Rats — Male	4	0.8802	0.0624

Table 2.4: Intercurrent mortality for rats in metabolite study

Sex	Dose group	Dosage	At start	52 weeks		78 weeks		90 weeks		Termination	
		(mg/kg)	Number	Number	%	Number	%	Number	%	Number	%
Female	Negative Control	0	50	50	100.0	49	98.0	47	94.0	46	92.0
	Vehicle Control	0	50	49	98.0	48	96.0	47	94.0	43	86.0
	Low dose	300	50	50	100	46	92.0	45	90.0	38	76.0
	Mid dose	1000	50	49	98.0	48	96.0	46	92.0	38	76.0
	High dose	3000	50	50	100	47	94.0	47	94.0	39	78.0
Male	Negative Control	0	50	50	100.0	48	96.0	46	92.0	31	62.0
	Vehicle Control	0	50	43	86.0	37	74.0	36	72.0	27	54.0
	Low dose	100	50	49	98.0	47	94.0	44	88.0	37	74.0
	Mid dose	300	50	48	96.0	43	86.0	39	78.0	34	68.0
	High dose	1000	50	49	98.0	47	94.0	41	82.0	35	70.0

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all hemangiosarcomas regardless of site, and of uterine adenomas and adenocarcinomas, have been conducted. No hemangiosarcomas were reported in any of the rats in the study (except for one male rat in the negative control group); the results of combining uterine adenomas and adenocarcinomas are presented in table 2.7. An abridged version of table 2.6, comprising all tumor types for which at least one pairwise or trend test yielded a p-value below 0.05 in at least one sex is presented as table 2.8.

- **Adrenal pheochromocytoma**

  Among the male rats, the adjusted incidence rate in the control group is just 2.7%, but it is 18% in the low dose group, 12% in the mid dose group, and 16% in the high dose group. This is not enough to generate a significant trend ($p = 0.1648$), but the comparison tests against control for both the low and high dose group yield p-values below 0.05 ($p = 0.0331$ and $p = 0.0500$ respectively). However, as shown in table 1.6 (on page 21), adrenal pheochromocytomas are a common tumor. Consequently, in order to retain significance after adjusting for multiplicity, the p-value of the pairwise comparison test between the high dose group and the control group would have to be below 0.01. Since it is not, we consider this a negative result.

  Among female rats, there is no suggestion of a dose response relationship, or of a significant result when comparing any of the dose groups with the controls.

- **Large granular lymphocyte leukemia**

  Among the female rats, the incidence rates for large granular lymphocytic leukemia were 13%, 17%, 24% and 27% in the control, low, mid, and high dose groups respectively. The test for trend gave a significant result; a p-value of 0.0433. None of the pairwise comparisons were significant, although the p-value of the test of comparison between the high dose group and the control group was 0.0610.

  The historical control data suggests that such tumors should considered common in F344/Jcl rats[2]. Nonetheless, even if large granular lymphocytic leukemia is considered rare, the result for the test of trend does not remain significant after adjusting for the multiplicity of endpoints. Furthermore, there is no evidence of any corresponding carcinogenic effect among the male rats, so it does not seem appropriate to consider this tumor type further here.

- **Pituitary anterior adenomas**

  The only interesting result concerning anterior adenomas of the pituitary gland is the comparison between the low dose female group and the female controls ($p = 0.0470$). This is driven by adjusted incidence rates of 28% and 46% in the control and low dose groups reapectively. Since these tumors are considered common (see table 1.6 on page 21), the appropriate α-level to use for a pairwise comparison, after adjusting for multiplicity of endpoints, is 0.01. Therefore there seems no reason to to assume that this result is anything other than a false positive, especially when the results are viewed in combination with the results for other dose levels, and with the results for male rats.

- **Uterine adenomas**

  The test for a dose response relationship finds a significant trend ($p = 0.0191$) for uterine adenomas (no cases among control and low dose animals, 2.2% among mid dose animals, and 6.5% among high dose animals). However, given the small number of animals involved, the comparison between the high dose group (3 cases) and the control group has a p-value of 0.1209, which is not significant. Since uterine adenomas are considered a common tumor type (see table 1.6 on page 21), we must conclude that these results are not significant after adjusting for multiplicity.

[2]It is interesting to note that when a determination of rarity is made on the basis of historical data for F344/Jcl rats submitted by the sponsor (see Appendix B), large granular lymphocytic leukemias are classified as rare in female rats; of three hundred animals studied, only one developed such a cancer (so that the 90% confidence interval for the true incidence rate is $(0.02\%, 1.6\%)$). This observation is hard to reconcile with the high incidence rate seen in the control animals for this submission (where the observed incidence rate was 27%. This discrepency provides confirmation that the decision to disregard the historical control data submitted by the sponsor (a decision made on the grounds that the data dated from the mid 1990s, and thus were too old) was correct.

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When the uterine adenomas are combined with uterine adenocarcinomas, the trend becomes less significant; the p-value is 0.0263. After making the suitable adjustment for multiplicity, this result loses its significance, and would even if these were not considered common tumors.

- **Endometrial stromal polyps**

  The interesting result for endometrial stromal polyps is the comparison between the mid dose group and the controls ($p = 0.0170$), driven by an incidence rate of 50% in the mid dose group and a rate of 27% in the control group. Since neither the comparison between the control group and the high dose group nor the test of trend yield significant results, we consider this to be a negative result. Furthermore, since these tumors are considered common (see table 1.6) this result does not remain significant after adjusting for multiplicity. Finally, there is no evidence of increaced incidence in the other treatment groups. Thus, it follows that this result is unlikely to warrant further concern.

Analysis of unexamined and autolytic organs

Autolytic organs The only organ which has been widely reported as being autolytic is the planta, which is autolytic in one hundred and twenty nine animals. One hundred and seven of these animals are male (twenty five controls, twenty eight from the low dose group, twenty nine from the mid dose group, and twenty five from the high dose group). Especially since most of the remaining animals are reported as having their planta unexamined, we cannot make any inference about a possible tumorigenic effect of TAK-536-MII in the planta.

Unexamined organs As in the prodrug studies, several organ/tissue types have been listed as unexamined in many animals. We again assume that these organ/tissue types only underwent histopathological examination when gross exams or other incidental observations directed the pathologist's attention to the organ. Table 2.9 lists the numbers of animals for which these organs have been reported as unexamined, and table 2.10 displays the results for those animals whose abdominal cavity etc. *was* examined.

Regardless of the reason that these organs were unexamined, we must conclude that no inference, either positive or negative, should be made regarding tumors in these organ/tissue types.

The following observations are noteworthy:

- **Planta**

  Two hundred and seventy animals were reported as having had their planta unexamined. Of the remaining one hundred and thirty animals, one hundred and twenty nine were found to have autolysis in the planta, so that a usable tissue sample was not obtainable. The remaining animal (a mid dose male animal) was found to have a fibrosarcoma of the planta. This animal also had a Leydig cell tumor of the testis.

- **Tail and Vertebra**

  Three hundred and ninety seven animals were reported as having had their tail unexamined. In the remaining three cases, two (a control group male animal and a low dose male animal) were found to have had autolysis in the tail, so that a usable sample could not be obtained. The remaining animal (a mid dose female) was found to have a keratoacanthoma of the tail. This animal (number 4116) was also found to have a chordoma of the vertebra and an adrenal pheochromocytoma. It is noteable that this animal was the only animal for whom the vertebra was not reported as unexamined.

Suitability of dose levels

As in the case of the prodrug study, there is no evidence of increased mortality in the high dose groups. The second statistically measurable criterion described in [3] is a detectable reduction of up to 10% in the high dose animals' weight gain over the course of the study, measured relative to the control group. As mentioned in section 2.1.3, there are visably discrenable differences in the survival outcomes of the vehicle control and negative control groups, and so the above tumor analyses have

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been conducted using only the vehicle control group. For the same reason, the following weight change calculations have been made using only the vehicle control group. This decision is further reinforced by inspection of the mean weights of the two control groups over time, as displayed in figures 2.5 and 2.6. It is clear for these plots that the vehicle itself (a daily dose of 10mL/kg of corn oil) has caused increased weight gain compared to the control, and so it is inappropriate to assess the impact of TAK-536-MII on weight gain by comparing treated animals with the negative control animals.

Figure 2.5

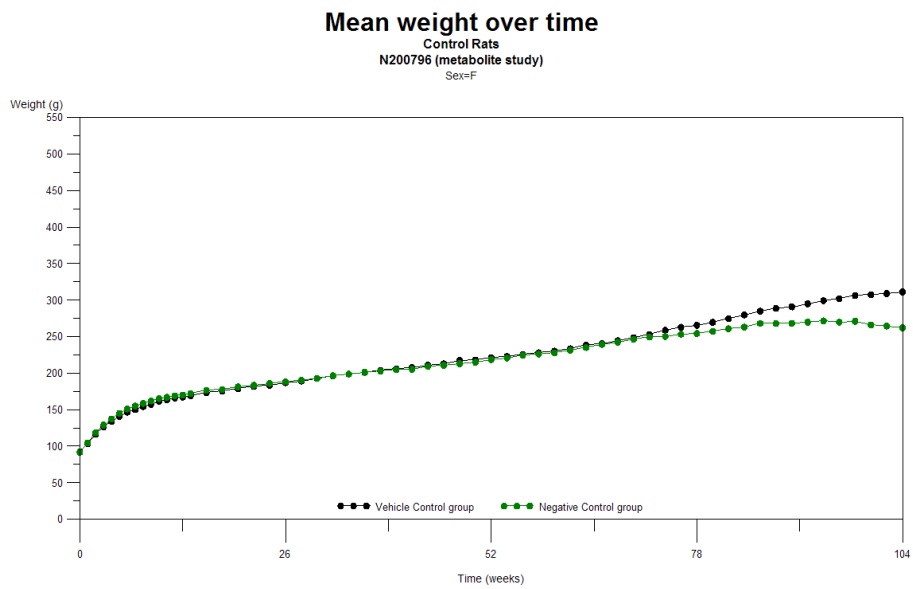

Table 2.5 shows the diminished weight gain in the treatment groups. There does appear to be some sign that the treated animals experienced less weight gain than the control animals. The effect is modest, well under the 10% threshold mentioned in [3], but doses appear to be a genuine effect. It seems therefore reasonable to conclude that the mid and high level dose levels were suitable in both the male and female rat studies. Plots of mean weight for male and female rats over time are presented in figures 2.7 and 2.8.

As with the prodrug, it appears that there is a noticeable diminution in weight gain in the high dose groups, especially for the female rats. Consequently, it seems reasonable to conclude that the selection of the high dose did indeed provide an adequate tumor challenge to the animals in this study.

Table 2.5: Weight change by group — rat metabolite study

Species and Sex	Δ_C	Δ_L	$\frac{\Delta_L}{\Delta_C} - 1$	Δ_M	$\frac{\Delta_M}{\Delta_C} - 1$	Δ_H	$\frac{\Delta_H}{\Delta_C} - 1$
Rats - Female	218.8	221.8	+1.4%	209.6	−4.2%	193.4	−11.6%
Rats - Male	394.6	394.9	+0.1%	373.6	−5.3%	363.4	−7.9%

2.2 Supplementary rat study

The supplementary study of female rats was an investigation of the effect of higher doses of the metabolite TAK-536-MII. Treatment animals received two doses of 3000 mg/kg per day, suspended

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Figure 2.6

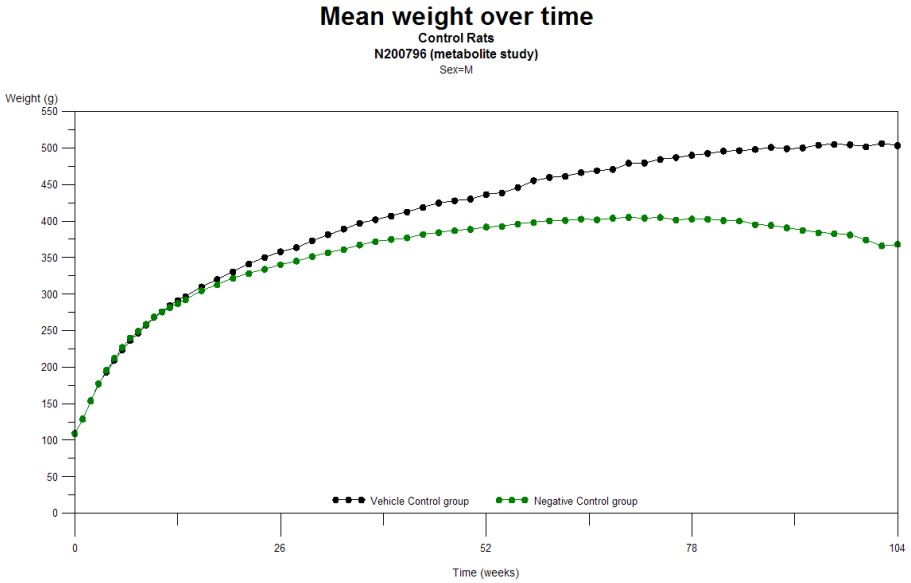

Figure 2.7

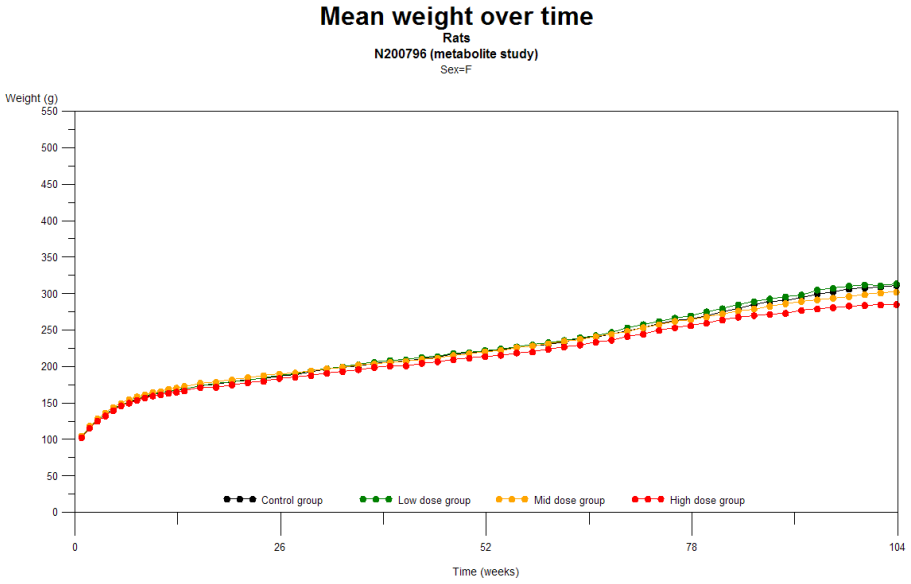

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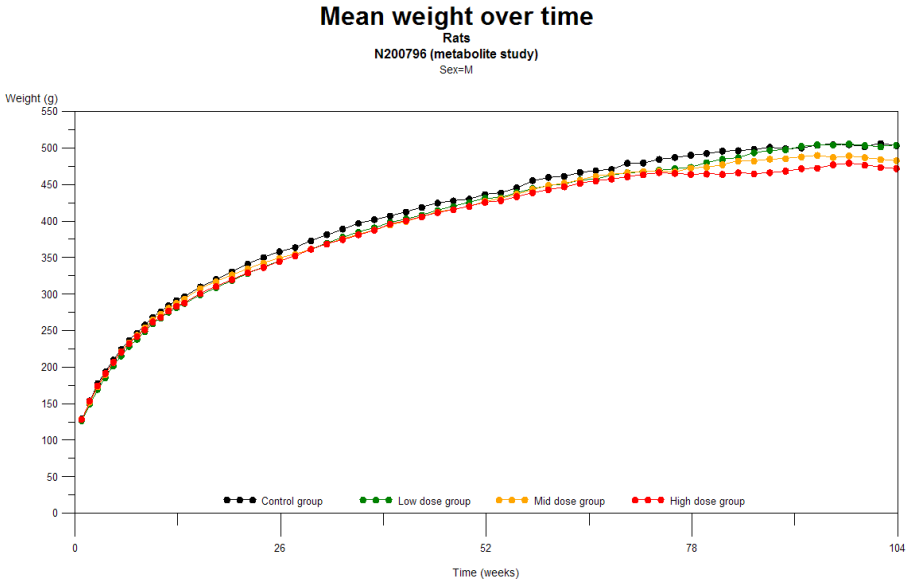
Figure 2.8



in corn oil (so that each animal would consume 20 mL/kg of corn oil per day). However, mortality was high in both the controls and the treatment group. The sponsor presumes that this is due to an overdose of corn oil. After forty three weeks, survival in the control group was below 50%, at which point those animals stopped receiving the vehicle. After seventy three weeks, the number of dose animals was just sixteen (from an original population of fifty), and the study was abandoned. All surviving animals were euthanized, and no histopathological analyses were conducted. Accordingly, there is no data to analyze for the purposes of this review.

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Table 2.6: All reported neoplastic tumors in rat metabolite study

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Abdominal cavity	PARAGANGLIOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.	100%	.	.
		Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
Adrenal	ADENOMA,CORTICAL CELL	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	2	0	0	0
			Poly-3 adjusted incidence rate	4.3%	.00%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.7%	.00%	.00%	.00%
	PHEOCHROMOCYTOMA	Female	*p*-value of pairwise and trend tests	.9402	.3445	.9416	.9416
			Observed number of cases	3	5	1	1
			Poly-3 adjusted incidence rate	6.4%	11%	2.2%	2.2%
		Male	*p*-value of pairwise and trend tests	.1648	.0331	.1397	.0500
			Observed number of cases	1	8	5	7
			Poly-3 adjusted incidence rate	2.7%	18%	12%	16%
	PHEOCHROMOCYTOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
Cerebrum	ASTROCYTOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	.5120		.5385	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.3%	.00%
	OLIGODENDROGLIOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	.8770	1.000	.8750	1.000
			Observed number of cases	2	0	1	0
			Poly-3 adjusted incidence rate	4.2%	.00%	2.2%	.00%
		Male	*p*-value of pairwise and trend tests	.7831	.5556		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
Clitoral gland	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.5000		1.000	
			Observed number of cases	1	0	0	2
			Poly-3 adjusted incidence rate	100%	.	.00%	100%
	ADENOMA	Female	*p*-value of pairwise and trend tests	.6667		.5000	

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Table 2.6: All reported neoplastic tumors in rat metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.	100%	.00%
Harderian gland	ADENOMA	Male	*p*-value of pairwise and trend tests	.7831	.5556		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
Heart	SCHWANNOMA	Female	*p*-value of pairwise and trend tests	.5027		.5000	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
		Male	*p*-value of pairwise and trend tests	.6629	.5556	.2867	
			Observed number of cases	0	1	2	0
			Poly-3 adjusted incidence rate	.00%	2.2%	4.7%	.00%
Hemolymphoreticular(all	LEUKEMIA,LARGE GRANULAR LYMPHO	Female	*p*-value of pairwise and trend tests	.0433	.3696	.1308	.0610
			Observed number of cases	6	8	11	13
			Poly-3 adjusted incidence rate	13%	17%	24%	27%
		Male	*p*-value of pairwise and trend tests	.4451	.9840	.9807	.8414
			Observed number of cases	4	1	1	3
			Poly-3 adjusted incidence rate	11%	2.2%	2.3%	6.9%
	SARCOMA,HISTIOCYTIC	Female	*p*-value of pairwise and trend tests	.9358	.7418	1.000	1.000
			Observed number of cases	1	1	0	0
			Poly-3 adjusted incidence rate	2.1%	2.2%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.2572	.8056	1.000	.5671
			Observed number of cases	1	1	0	2
			Poly-3 adjusted incidence rate	2.7%	2.2%	.00%	4.6%
Intestine,cecum	LEIOMYOSARCOMA	Female	*p*-value of pairwise and trend tests	.7486	.4945		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
Intestine,duodenum	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	.2590			.5443
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
Intestine,jejunum	ADENOMA	Male	*p*-value of pairwise and trend tests	.2590			.5443
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
Intestine,rectum	ADENOMA	Female	*p*-value of pairwise and trend tests	.2514			.5000
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%

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Table 2.6: All reported neoplastic tumors in rat metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Kidney	ADENOMA,RENAL CELL	Female	*p*-value of pairwise and trend tests	.7541	1.000	.7527	1.000
			Observed number of cases	1	0	1	0
			Poly-3 adjusted incidence rate	2.1%	.00%	2.2%	.00%
		Male	*p*-value of pairwise and trend tests	.2590			.5443
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
	LIPOSARCOMA	Female	*p*-value of pairwise and trend tests	.2514			.5000
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
Liver	ADENOMA,HEPATOCELLULAR	Female	*p*-value of pairwise and trend tests	.1892		.5000	.5000
			Observed number of cases	0	0	1	1
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	2.1%
		Male	*p*-value of pairwise and trend tests	.6450	.5840	.7902	.7955
			Observed number of cases	1	2	1	1
			Poly-3 adjusted incidence rate	2.7%	4.4%	2.4%	2.3%
	CARCINOMA,HEPATOCELLULAR	Male	*p*-value of pairwise and trend tests	.2590			.5443
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
	CHOLANGIOMA	Male	*p*-value of pairwise and trend tests	.2590			.5443
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
Lung(bronchus)	ADENOCARCINOMA,BRONCHIOLO−ALVE	Male	*p*-value of pairwise and trend tests	.6530	.5556	.5385	
			Observed number of cases	0	1	1	0
			Poly-3 adjusted incidence rate	.00%	2.2%	2.4%	.00%
	ADENOMA,BRONCHIOLO−ALVEOLAR	Female	*p*-value of pairwise and trend tests	.1415	.4945	.2473	.2473
			Observed number of cases	0	1	2	2
			Poly-3 adjusted incidence rate	.00%	2.2%	4.3%	4.3%
		Male	*p*-value of pairwise and trend tests	.4224	.8818	.9587	.7433
			Observed number of cases	3	2	1	3
			Poly-3 adjusted incidence rate	8.2%	4.4%	2.4%	6.9%
Mammary gland	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.5126	.7418	.7473	.7473
			Observed number of cases	1	1	1	1
			Poly-3 adjusted incidence rate	2.1%	2.2%	2.1%	2.2%
		Male	*p*-value of pairwise and trend tests	.1214	.5556		.2931
			Observed number of cases	0	1	0	2

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Table 2.6: All reported neoplastic tumors in rat metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	4.6%
	ADENOMA	Female	*p*-value of pairwise and trend tests	.4406	1.000	1.000	.7527
			Observed number of cases	1	0	0	1
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	2.2%
	FIBROADENOMA	Female	*p*-value of pairwise and trend tests	.8869	.6510	.9416	.9416
			Observed number of cases	3	3	1	1
			Poly-3 adjusted incidence rate	6.4%	6.6%	2.2%	2.2%
		Male	*p*-value of pairwise and trend tests	.4666	.3056		.5443
			Observed number of cases	0	2	0	1
			Poly-3 adjusted incidence rate	.00%	4.4%	.00%	2.3%
Mesothelium	MESOTHELIOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	.8233	.5932	.4003	.9054
			Observed number of cases	2	3	4	1
			Poly-3 adjusted incidence rate	5.3%	6.7%	9.3%	2.3%
Oral cavity	PAPILLOMA,SQUAMOUS CELL	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.
Pancreas	ADENOMA,ACINAR CELL	Male	*p*-value of pairwise and trend tests	.8126	.2570	.1397	.7955
			Observed number of cases	1	4	5	1
			Poly-3 adjusted incidence rate	2.7%	8.9%	12%	2.3%
	ADENOMA,ACINAR−ISLET CELL	Female	*p*-value of pairwise and trend tests	.7486	.4945		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.9540	.8056	1.000	1.000
			Observed number of cases	1	1	0	0
			Poly-3 adjusted incidence rate	2.7%	2.2%	.00%	.00%
	ADENOMA,ISLET CELL	Female	*p*-value of pairwise and trend tests	.9959	.7351	.9412	1.000
			Observed number of cases	5	4	2	0
			Poly-3 adjusted incidence rate	11%	8.8%	4.3%	.00%
		Male	*p*-value of pairwise and trend tests	.0881	.2036	.6580	.0929
			Observed number of cases	13	21	14	23
			Poly-3 adjusted incidence rate	35%	46%	33%	52%
	CARCINOMA,ISLET CELL	Female	*p*-value of pairwise and trend tests	.4406	1.000	1.000	.7527
			Observed number of cases	1	0	0	1
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	2.2%
		Male	*p*-value of pairwise and trend tests	.1978		.5385	.5443

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Table 2.6: All reported neoplastic tumors in rat metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	0	0	1	1
			Poly-3 adjusted incidence rate	.00%	.00%	2.4%	2.3%
Parathyroid	ADENOMA	Male	*p*-value of pairwise and trend tests	.7901	.5696		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
Pituitary	ADENOMA,ANTERIOR	Female	*p*-value of pairwise and trend tests	.8832	.0470	.1902	.6794
			Observed number of cases	13	21	18	12
			Poly-3 adjusted incidence rate	28%	46%	38%	26%
		Male	*p*-value of pairwise and trend tests	.4744	.7670	.5624	.6439
			Observed number of cases	14	15	17	16
			Poly-3 adjusted incidence rate	37%	32%	38%	36%
	ADENOMA,INTERMEDIATED	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
	CARCINOMA,ANTERIOR	Female	*p*-value of pairwise and trend tests	.5027		.5000	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
Planta	FIBROSARCOMA	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
Preputial gland	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	.5000			
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.	.	.00%	100%
	ADENOMA	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.00%
Prostate	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	.7831	.5556		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
	ADENOMA	Male	*p*-value of pairwise and trend tests	.5015	.5254	.8166	.5904
			Observed number of cases	10	13	9	12
			Poly-3 adjusted incidence rate	27%	29%	21%	27%
Salivary gland,sublingu	ADENOMA	Male	*p*-value of pairwise and trend tests	.2590			.5443
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%

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Table 2.6: All reported neoplastic tumors in rat metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Seminal vesicle	ADENOMA	Male	*p*-value of pairwise and trend tests	.7840	.5625		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
Skin+Subcutis	FIBROMA	Female	*p*-value of pairwise and trend tests	.8135	.2418		
			Observed number of cases	0	2	0	0
			Poly-3 adjusted incidence rate	.00%	4.4%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.5596	.8056	1.000	.7955
			Observed number of cases	1	1	0	1
			Poly-3 adjusted incidence rate	2.7%	2.2%	.00%	2.3%
	KERATOACANTHOMA	Female	*p*-value of pairwise and trend tests	.7486	.4945		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.4521	1.000	1.000	.7955
			Observed number of cases	1	0	0	1
			Poly-3 adjusted incidence rate	2.7%	.00%	.00%	2.3%
	PAPILLOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	.5027		.5000	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
	SCHWANNOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	.9526	.5840	1.000	1.000
			Observed number of cases	1	2	0	0
			Poly-3 adjusted incidence rate	2.7%	4.4%	.00%	.00%
Spinal cord	SCHWANNOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	.5627	.7995	1.000	.7944
			Observed number of cases	1	1	0	1
			Poly-3 adjusted incidence rate	2.7%	2.2%	.00%	2.3%
Stomach	CARCINOID	Female	*p*-value of pairwise and trend tests	.2514			.5000
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
	CARCINOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
	LEIOMYOSARCOMA	Male	*p*-value of pairwise and trend tests	.2590			.5443
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
Tail	KERATOACANTHOMA	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0

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Table 2.6: All reported neoplastic tumors in rat metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	.	.	100%	.
Testis	LEYDIG CELL TUMOR	Male	*p*-value of pairwise and trend tests	.8821	.3261	.1137	.7689
			Observed number of cases	27	35	38	29
			Poly-3 adjusted incidence rate	70%	77%	83%	65%
Thoracic cavity	FIBROMA	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
Thyroid	ADENOMA,C CELL	Female	*p*-value of pairwise and trend tests	.9070	.8608	.7039	.9653
			Observed number of cases	9	6	8	4
			Poly-3 adjusted incidence rate	19%	13%	17%	8.6%
		Male	*p*-value of pairwise and trend tests	.7054	.0564	.1972	.4263
			Observed number of cases	2	9	6	4
			Poly-3 adjusted incidence rate	5.4%	20%	14%	9.1%
	ADENOMA,FOLLICULAR CELL	Female	*p*-value of pairwise and trend tests	.6270	.4945	.5000	
			Observed number of cases	0	1	1	0
			Poly-3 adjusted incidence rate	.00%	2.2%	2.2%	.00%
		Male	*p*-value of pairwise and trend tests	.2349	.5840	.3778	.3778
			Observed number of cases	1	2	3	3
			Poly-3 adjusted incidence rate	2.7%	4.4%	7.0%	6.9%
	CARCINOMA,C CELL	Female	*p*-value of pairwise and trend tests	.2620	.7473	.7527	.5000
			Observed number of cases	1	1	1	2
			Poly-3 adjusted incidence rate	2.1%	2.2%	2.2%	4.3%
		Male	*p*-value of pairwise and trend tests	.6852	.0896	.2867	.5443
			Observed number of cases	0	4	2	1
			Poly-3 adjusted incidence rate	.00%	8.9%	4.7%	2.3%
	CARCINOMA,FOLLICULAR CELL	Female	*p*-value of pairwise and trend tests	.7486	.4945		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
		Male	*p*-value of pairwise and trend tests	.7043	1.000	.5583	1.000
			Observed number of cases	1	0	2	0
			Poly-3 adjusted incidence rate	2.7%	.00%	4.7%	.00%
Tongue	PAPILLOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	.5027		.5000	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
		Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000

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Table 2.6: All reported neoplastic tumors in rat metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.7%	.00%	.00%	.00%
Urinary bladder	PAPILLOMA,TRANSITIONAL CELL	Female	*p*-value of pairwise and trend tests	.5979	1.000	1.000	.8791
			Observed number of cases	2	0	0	1
			Poly-3 adjusted incidence rate	4.3%	.00%	.00%	2.1%
		Male	*p*-value of pairwise and trend tests	.7831	.5556		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
Uterus	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.3953	1.000	.7527	.7527
			Observed number of cases	1	0	1	1
			Poly-3 adjusted incidence rate	2.1%	.00%	2.2%	2.2%
	ADENOMA	Female	*p*-value of pairwise and trend tests	.0191		.5000	.1209
			Observed number of cases	0	0	1	3
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	6.5%
	GRANULAR CELL TUMOR	Female	*p*-value of pairwise and trend tests	.2514			.5000
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.1%
	POLYP,ENDOMETRIAL STROMAL	Female	*p*-value of pairwise and trend tests	.6146	.5000	.0170	.6152
			Observed number of cases	13	14	24	13
			Poly-3 adjusted incidence rate	27%	29%	50%	27%
	SARCOMA,ENDOMETRIAL STROMAL	Female	*p*-value of pairwise and trend tests	.7500	.5000		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
	SCHWANNOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	.8919	.1209		
			Observed number of cases	0	3	0	0
			Poly-3 adjusted incidence rate	.00%	6.5%	.00%	.00%
Vertebra	CHORDOMA	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
Zymbal gland	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.
		Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	3	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.

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Table 2.7: Composite endpoint analyses for rat metabolite study

Endpoint	Sex	Quantity	Control	Low dose	Mid dose	High dose
Uterine adenoma & adenocarcinoma	Female	*p*-value of pairwise and trend tests	0.0263	1	0.5000	0.1805
		Poly-3 adjusted incidence rate	2.1%	0%	4.3%	8.6 %
		Observed number of cases	1	0	2	4
		Adjusted number at risk	46.93	45.55	46.05	46.45

Table 2.8: Neoplastic tumors in rat metabolite study with at least one significant result

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Adrenal	PHEOCHROMOCYTOMA	Female	*p*-value of pairwise and trend tests	.9402	.3445	.9416	.9416
			Observed number of cases	3	5	1	1
			Poly-3 adjusted incidence rate	6.4%	11%	2.2%	2.2%
			Adjusted number at risk	46.93	45.55	46.16	46.45
		Male	*p*-value of pairwise and trend tests	.1648	.0331	.1397	.0500
			Observed number of cases	1	8	5	7
			Poly-3 adjusted incidence rate	2.7%	18%	12%	16%
			Adjusted number at risk	36.64	45.14	42.94	43.91
Hemolymphoreticular(all	LEUKEMIA,LARGE GRANULAR LYMPHO	Female	*p*-value of pairwise and trend tests	.0433	.3696	.1308	.0610
			Observed number of cases	6	8	11	13
			Poly-3 adjusted incidence rate	13%	17%	24%	27%
			Adjusted number at risk	47.97	46.71	46.68	47.30
		Male	*p*-value of pairwise and trend tests	.4451	.9840	.9807	.8414
			Observed number of cases	4	1	1	3
			Poly-3 adjusted incidence rate	11%	2.2%	2.3%	6.9%
			Adjusted number at risk	37.15	45.05	42.89	43.41
Pituitary	ADENOMA,ANTERIOR	Female	*p*-value of pairwise and trend tests	.8832	.0470	.1902	.6794
			Observed number of cases	13	21	18	12
			Poly-3 adjusted incidence rate	28%	46%	38%	26%
			Adjusted number at risk	47.01	45.79	47.04	47.02
		Male	*p*-value of pairwise and trend tests	.4744	.7670	.5624	.6439
			Observed number of cases	14	15	17	16

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Table 2.8: Significant results from rat metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	37%	32%	38%	36%
			Adjusted number at risk	37.79	46.37	44.56	44.57
Uterus	ADENOMA	Female	*p*-value of pairwise and trend tests	.0191		.5000	.1209
			Observed number of cases	0	0	1	3
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	6.5%
			Adjusted number at risk	46.93	45.55	46.05	46.45
	POLYP,ENDOMETRIAL STROMAL	Female	*p*-value of pairwise and trend tests	.6146	.5000	.0170	.6152
			Observed number of cases	13	14	24	13
			Poly-3 adjusted incidence rate	27%	29%	50%	27%
			Adjusted number at risk	47.62	47.98	47.99	48.28

Table 2.9: Organs reported as unexamined in rats (metabolite study)

Organ name	Female rats										Male rats										Total	
	Control		Low dose		Mid dose		High dose		Total		Control		Low dose		Mid dose		High dose		Total			
Abdominal cavity	50	100%	48	96%	49	98%	49	98%	196	98%	50	100%	48	96%	46	92%	50	100%	194	97%	390	97.5%
Clitoral gland	49	98%	50	100%	49	98%	48	96%	196	98%	.	.	.	.	.	.	.	.	.	.	196	98.0%
Oral cavity	50	100%	50	100%	50	100%	50	100%	200	100%	49	98%	50	100%	50	100%	50	100%	199	100%	399	99.8%
Planta	43	86%	44	88%	46	92%	45	90%	178	89%	25	50%	22	44%	20	40%	25	50%	92	46%	270	67.5%
Preputial gland	.	.	.	.	.	.	.	.	.	.	50	100%	49	98%	49	98%	49	98%	197	99%	197	98.5%
Tail	50	100%	50	100%	49	98%	50	100%	199	100%	49	98%	49	98%	50	100%	50	100%	198	99%	397	99.3%
Thoracic cavity	50	100%	50	100%	49	98%	50	100%	199	100%	50	100%	49	98%	49	98%	50	100%	198	99%	397	99.3%
Vertebra	50	100%	50	100%	49	98%	50	100%	199	100%	50	100%	50	100%	50	100%	50	100%	200	100%	399	99.8%
Zymbal gland	49	98%	50	100%	50	100%	50	100%	199	100%	47	94%	50	100%	50	100%	50	100%	197	99%	396	99.0%

Table 2.10: Animals for which commonly unexamined organs were examined (rat metabolite study)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
Abdominal cavity	Female	Low	3115	Abdominal cavity	PARAGANGLIOMA,MALIGNANT
				Uterus	POLYP,ENDOMETRIAL STROMAL
					SCHWANNOMA,MALIGNANT

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Table 2.10: Animals for which commonly unexamined organs were examined (rat metabolite study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
			3142	Abdominal cavity	Autolytic
				Skin+Subcutis	KERATOACANTHOMA
		Mid	4129	Abdominal cavity	Autolytic
		High	5144	Abdominal cavity	Autolytic
	Male	Low	2019	Abdominal cavity	Autolytic
				Planta	Autolytic
				Adrenal	PHEOCHROMOCYTOMA
				Heart	SCHWANNOMA
				Pancreas	ADENOMA,ISLET CELL
				Thyroid	CARCINOMA,C CELL
			2043	Abdominal cavity	Autolytic
		Mid	3016	Abdominal cavity	Autolytic
				Coagulating gland	Autolytic
				Planta	Autolytic
				Scrotum	Autolytic
				Mesothelium	MESOTHELIOMA,MALIGNANT
				Pancreas	ADENOMA,ISLET CELL
				Testis	LEYDIG CELL TUMOR
				Thyroid	CARCINOMA,FOLLICULAR CELL
			3018	Abdominal cavity	PARAGANGLIOMA,MALIGNANT
				Pancreas	ADENOMA,ISLET CELL
				Pituitary	ADENOMA,ANTERIOR
				Testis	LEYDIG CELL TUMOR
			3023	Abdominal cavity	Autolytic
				Mesothelium	MESOTHELIOMA,MALIGNANT
			3028	Abdominal cavity	Autolytic
				Coagulating gland	Autolytic
				Intestine,cecum	Autolytic
				Intestine,colon	Autolytic

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Table 2.10: Animals for which commonly unexamined organs were examined (rat metabolite study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
				Intestine,rectum	Autolytic
				Lymph node,nos	Autolytic
				Planta	Autolytic
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Pituitary	ADENOMA,ANTERIOR
Clitoral gland	Female	Control	1133	Clitoral gland	ADENOCARCINOMA
		Mid	4136	Clitoral gland	ADENOMA
		High	5108	Clitoral gland	ADENOCARCINOMA
			5144	Clitoral gland	ADENOCARCINOMA
				Pituitary	ADENOMA,ANTERIOR
				Uterus	POLYP,ENDOMETRIAL STROMAL
Oral cavity	Male	Control	1040	Planta	Autolytic
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Oral cavity	PAPILLOMA,SQUAMOUS CELL
				Pituitary	ADENOMA,ANTERIOR
				Testis	LEYDIG CELL TUMOR
Planta	Female	Control	1103	Planta	Autolytic
				Mammary gland	FIBROADENOMA
				Pituitary	ADENOMA,ANTERIOR
				Thyroid	ADENOMA,C CELL
			1109	Planta	Autolytic
				Uterus	POLYP,ENDOMETRIAL STROMAL
			1129	Planta	Autolytic
				Pituitary	ADENOMA,ANTERIOR
			1133	Planta	Autolytic
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Pituitary	ADENOMA,ANTERIOR
				Thyroid	ADENOMA,C CELL

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Table 2.10: Animals for which commonly unexamined organs were examined (rat metabolite study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
			1143	Planta	Autolytic
				Mammary gland	FIBROADENOMA
				Pituitary	ADENOMA,ANTERIOR
			1149	Planta	Autolytic
				Pancreas	ADENOMA,ISLET CELL
			1150	Planta	Autolytic
		Low	3104	Planta	Autolytic
				Pituitary	ADENOMA,ANTERIOR
			3107	Planta	Autolytic
				Pituitary	ADENOMA,ANTERIOR
			3118	Planta	Autolytic
				Adrenal	PHEOCHROMOCYTOMA
				Pancreas	ADENOMA,ISLET CELL
			3120	Planta	Autolytic
				Pancreas	ADENOMA,ISLET CELL
				Pituitary	ADENOMA,ANTERIOR
			3130	Planta	Autolytic
				Mammary gland	FIBROADENOMA
				Pituitary	ADENOMA,ANTERIOR
				Thyroid	ADENOMA,C CELL
			3135	Planta	Autolytic
				Mammary gland	ADENOCARCINOMA
				Pituitary	ADENOMA,ANTERIOR
				Thyroid	ADENOMA,C CELL
		Mid	4109	Planta	Autolytic
				Pituitary	ADENOMA,ANTERIOR

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Table 2.10: Animals for which commonly unexamined organs were examined (rat metabolite study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
			4123	Planta	Autolytic
				Uterus	POLYP,ENDOMETRIAL STROMAL
			4131	Planta	Autolytic
				Pituitary	ADENOMA,ANTERIOR
				Thyroid	ADENOMA,C CELL
			4139	Parathyroid	Autolytic
				Planta	Autolytic
				Liver	ADENOMA,HEPATOCELLULAR
				Pituitary	ADENOMA,ANTERIOR
				Uterus	POLYP,ENDOMETRIAL STROMAL
		High	5123	Planta	Autolytic
				Pituitary	ADENOMA,ANTERIOR
			5131	Parathyroid	Autolytic
				Planta	Autolytic
			5143	Planta	Autolytic
				Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Stomach	CARCINOID
			5146	Planta	Autolytic
				Adrenal	PHEOCHROMOCYTOMA
			5149	Planta	Autolytic
				Uterus	ADENOCARCINOMA
Preputial gland	Male	Low	2015	Coagulating gland	Autolytic
				Preputial gland	Autolytic
				Adrenal	PHEOCHROMOCYTOMA
				Liver	ADENOMA,HEPATOCELLULAR
				Pancreas	ADENOMA,ISLET CELL
				Prostate	ADENOMA
				Testis	LEYDIG CELL TUMOR
				Thyroid	ADENOMA,C CELL

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Table 2.10: Animals for which commonly unexamined organs were examined (rat metabolite study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
		Mid	3039	Planta	Autolytic
				Heart	SCHWANNOMA
				Pancreas	ADENOMA,ISLET CELL
				Pituitary	ADENOMA,ANTERIOR
				Preputial gland	ADENOMA
				Testis	LEYDIG CELL TUMOR
		High	4034	Planta	Autolytic
				Preputial gland	ADENOCARCINOMA
				Prostate	ADENOMA
				Thyroid	ADENOMA,FOLLICULAR CELL
Tail	Female	Mid	4116	Adrenal	PHEOCHROMOCYTOMA
	Male	Control	1050	Tail	Autolytic
				Pancreas	ADENOMA,ISLET CELL
				Testis	LEYDIG CELL TUMOR
		Low	2026	Planta	Autolytic
				Tail	Autolytic
				Mammary gland	ADENOCARCINOMA
					FIBROADENOMA
				Pancreas	ADENOMA,ISLET CELL
				Testis	LEYDIG CELL TUMOR
Thoracic cavity	Female	Mid	4129	Thoracic cavity	Autolytic
				Uterus	POLYP,ENDOMETRIAL STROMAL
	Male	Low	2043	Thoracic cavity	Autolytic
		Mid	3008	Coagulating gland	Autolytic
				Planta	Autolytic
				Pancreas	ADENOMA,ACINAR CELL
					ADENOMA,ISLET CELL
				Testis	LEYDIG CELL TUMOR
				Thoracic cavity	FIBROMA

Reference ID: 2868673

Table 2.10: Animals for which commonly unexamined organs were examined (rat metabolite study) (continued)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
				Thyroid	ADENOMA,C CELL
Vertebra	Female	Mid	4116	Tail	KERATOACANTHOMA
				Vertebra	CHORDOMA
Zymbal gland	Female	Control	1123	Hemolymphoreticular(all sites)	LEUKEMIA,LARGE GRANULAR LYMPHOCYTIC
				Uterus	POLYP,ENDOMETRIAL STROMAL
				Zymbal gland	ADENOCARCINOMA
	Male	Control	1009	Prostate	ADENOMA
				Testis	LEYDIG CELL TUMOR
				Zymbal gland	ADENOCARCINOMA
			1046	Trigeminal nerve	Autolytic
				Zymbal gland	ADENOCARCINOMA
			1049	Lymph node,nos	Autolytic
				Planta	Autolytic
				Lung(bronchus)	ADENOMA,BRONCHIOLO−ALVEOLAR
				Prostate	ADENOMA
				Skin+Subcutis	FIBROMA
				Testis	LEYDIG CELL TUMOR
				Zymbal gland	ADENOCARCINOMA

Reference ID: 2868673

2.3 Mouse study

2.3.1 Overview

Two separate experiments were conducted, one in male and one in female mice. In each of these two experiments there were three treated groups (called the low dose group, the mid dose group, and the high dose group respectively), one diet control group, and one active control group. One hundred and twenty five hemizygous Tg.rasH2 mice of each sex were initially randomly allocated to each of these five treatment groups (twenty five animals of each sex per group). The randomization used a block design to ensure homogeneity of group means of bodyweight.

This was a dosed-feed oral administration trial. The low dose animals received the test article in their food, in a concentration of 1.25%. For the mid dose group, the concentration was 3.5%, and for the high dose group, the concentration was 5%. The sponsor has reported the average consumption of the test article, by dose group. This information is presented in table 2.11. The sponsor reports that the "consumption in the females was slightly higher than in the males", but the differences are fairly substantial: 36% for the low dose animals, 38% for the mid dose animals, and 37% for the high dose animals. Notably, the female mid dose group have received a dose closer to the male high dose group than to the male mid dose group.

Table 2.11: Average consumption of TAK-536-MII (metabolite) in mouse study, by dose group

Dose group	Concentration (%)	Average daily dosage (mg/kg)	
		Female	Male
Low dose	1.25	2407	1762
Mid dose	3.5	6951	5026
High dose	5.0	11182	8134

The diet control group were given the basal diet *ad libitum*. The positive control group received three doses of urethane, for a total dose of 1000 mg/kg. The urethane was administered in a saline solution by intraperitoneal injections on days 1, 3, and 5 of the study.

During the administration period, animals were checked twice daily for moribundity and mortality. Each animal received a weekly hands-on exam. After death, all animals were necropsied as soon as possible.

2.3.2 Sponsor's analysis

Survival analysis

The sponsor analyzed survival data using Cox-Tarone binary regression on life tables (see [9]), using a twenty six week window. Continuity-corrected two-sided tail probabilities for trend, and group comparisons were measured against a 5% significance level. Analyses were also conducted using the Gehan-Breslow method. The sponsor argues that the Cox-Tarone method is more sensitive to late deaths and that the Gehan-Breslow method is more sensitive to early deaths, and therefore both methods are appropriate for use in this analysis. However, given the very low mortality rate in the mouse studies, it is hard to see the applicability of the Gehan-Breslow method, except in the case of the urethane study.

In the analysis of the female mice, the Gehan-Breslow analysis found a significant ($p = 0.0340$) improvement in survival as dosage was increased, although no pairwise comparisons with control were significant. No significant results were reported form the study of male mice at all.

Tumor analysis

Neoplasm incidence data were analyzed only in those cases when the incidence count in at least one treatment group differed from the count on the corresponding control group by at least two. In these cases, either Peto's method [7], or an exact method (when the incidence table was sparse)

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Table 2.12: p-values from sponsor's survival analyses: urethane treated mice against control (prodrug study)

	Cox-Tarone test	Gehan-Breslow test
Female	0.0001	0.0000
Male	0.0000	0.0000

were used. Trend and pairwise comparisons were conducted at the 5% significance level; all tests were one tailed.

In the female mouse study, the only significant result reported is that the rate of hemangiosarcomas of the nasal cavity. For these tumors, the p-value of the test of trend was 0.0120, although the comparison between the control group and the high dose group did not lead to a significant result ($p = 0.1024$).

Although the tests conducted were one-tailed, the sponsor claims that the incidence of lung tumors (alveolar-bronchiolar adenomas, and the combination of alveolar-bronchial adenomas and carcinomas) have a reduced incidence in the high dose group compared to the control, and that there is a significant, negative dose response in these cases.

Urethane study

In both male and female mice, the sponsor found that the mice treated with urethane had significantly worse survival times than the control group. The p-values for these tests are shown in table 2.12.

Note that the p-values of 0.0000 have been quoted directly from the sponsor's submission, and are presumably interpretable as < 0.00005.

The sponsor also found strong evidence of increased rates of alveolar bronchiolar adenomas ($p = 0.0003$ for females, $p < 0.0001$ for males), carcinomas ($p = 0.00325$ for females, the results was not significant for males), and adenomas and carcinomas when combined ($p = 0.0003$ for females, $p = 0.0055$ for males, although note that no such carcinomas were reported in the study of male mice), and of hemangiosarcomas, when reported as "multiple organs" ($p = 0.0010$ for females and $p = 0.0003$ for males) and splenetic ($p = 0.0022$ for females, $p = 0.0003$ for males) or in the lungs ($p = 0.0325$ for females, the results were not significant for males) in the urethane treated group, compared with the control group.

2.3.3 Reviewer's analysis

Survival analysis

Kaplan-Meier plots of the survival rates for the two sexes are shown in figures 2.9 and 2.10. The results of log-rank tests for trend and homogeneity are presented in table 2.13.

There is no evidence for heterogeneity of survival times across doses in either females ($p = 0.2883$) or males ($p = 0.9331$). The test of trend dose detect a significant dose response in mortality among the female mice ($p = 0.0312$), but in the absence of a significant indication of heterogeneity, and given the very small number of events reported, it is not reasonable to conclude that this study provides any evidence that TAK-536-MII has an impact on mouse survival times.

Table 2.13: Results of log-rank tests of survival in metabolite study on mice

Species and Sex	Number of dose groups	p-value (trend)	p-value (homogeneity)
Mice — Female	4	0.0312	0.2882
Mice — Male	4	0.3072	0.9331

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Figure 2.9

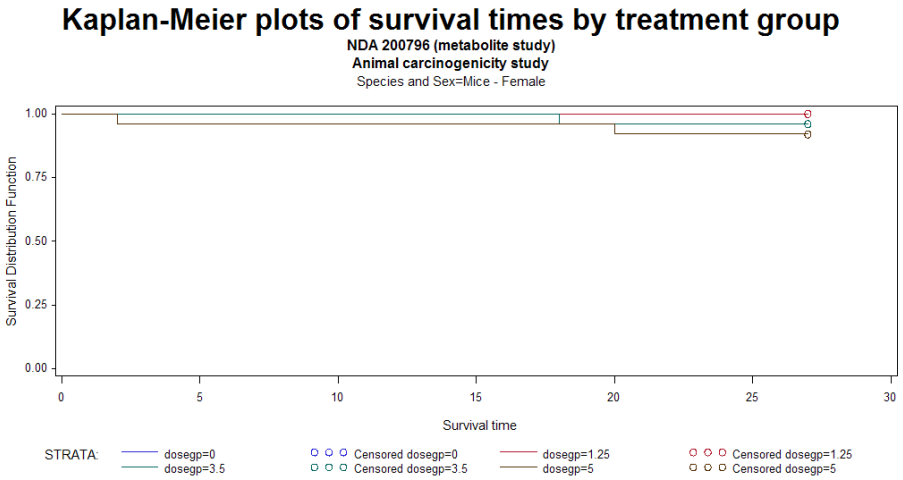
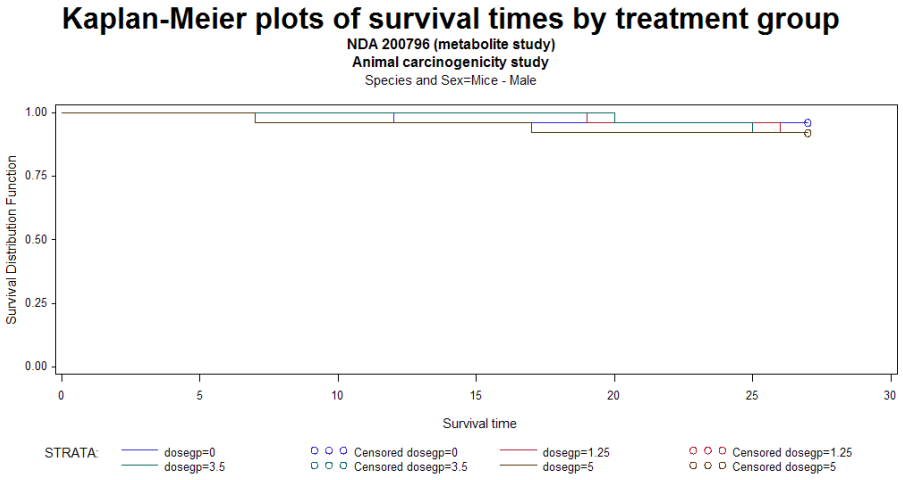


Figure 2.10



Reference ID: 2868673

Tumor analysis

As with the study of the prodrug TAK-491, a poly-k analysis has been conducted for each reported tumor type. For the same reasons as discussed in section 1.2.3, it has been decided to use a value of $k = 1$.

The results of the poly-1 analyses are presented in table 2.14. A composite analysis of all hemangiosarcomas is presented in table 2.15. There were no uterine adenomas or adenocarcinomas reported in this study, so for that composite endpoint, there is nothing to report. An abridged version of table 2.14, consisting of those tumor types for which at least one test, in at least one sex yielded a p-value below 0.05 is presented as table 2.16.

The only result of interest is the incidence of hemangiosarcomas of the nasal cavity in female mice. No such tumors were reported in the control group, the low dose group, or the mid dose group, but three animals in the high dose group were found to have such tumors. The p-value for the test of trend is 0.0120. Since nasal hemangiosarcomas are a rare tumor, this trend retains its significance after adjusting for multiplicity. The test of comparison between the high dose group and the controls is not significant ($p = 0.1024$), and the results from the male mice are weaker (no control animals developed nasal hemangiosarcomas, but two low dose, two mid dose, and one high dose animal did — the p-value for the test of trend is 0.3238). Nonetheless, this result is worthy of some concern.

At the request of the pharmacological reviewer, an additional analysis was done, where all hemangiosarcomas, regardless of site, are pooled. This test did not yield any significant results. The main reason that this analysis dilutes the result for hemangiosarcomas of the nasal cavity is that no high dose mice of either sex developed any hemangiosarcomas except in the nasal cavity.

The extent to which this result should be considered a cause for concern depends on the mechanism by which hemangiosarcomas develop; if they can be developed by direct contact with a carcinogen, then the fact that these tumors were located nasally, taken together with the fact that this was a dietary study, is grounds for further consideration. On the other hand, if it is felt that hemangiosarcomas are more in the nature of systemic tumors, so that the actual site at which one manifests is largely independent of the carcinogenesis process, then consideration of hemangiosarcomas of the nasal cavity independently of other hamangiosarcomas is inappropriate. The determination of how to proceed is beyond the scope of this statistical review.

Autolytic and unexamined organs

Autolytic organs No animals in this study were reported as having any organs autolyzed to the extent that no usuable tissue sample could be obtained.

Unexamined organs Six organs (ears, mediastimum, prepuce, preputial glands, subcutis, tail) were reported as being unexamined in all except a very few animals. As in the previous studies, it is assumed that these organs were not routinely examined in any animals, but that when a tumor was detected in one organ, either by gross exam or incidentally in the necroscopy process, all other animals were then reported as having had that organ unexamined. Regardless, it must be remembered that it is not possible to make any meaningful statistical inference, either positive or negative, in these cases.

It is worth noting, however, that in this study, unlike the other three studies, it is *not* the case that for the above organs, every animal was reported as having had the organ either unexamined, autolytic or tumorous. For example, male mouse number 1369 is not reported as having had its prepuce left unexamined, but neither is its prepuce listed as being tumorous or autolytic (although this animal did have its preputial gland listed as being unexamined). It may therefore be that the mechanism by which animals did or did not have these organs examined is different from the other three studies.

The results of the histopathological exams of those animals who *did* have these organs examined are presented in table 2.17.

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Urethane study

The purpose of the urethane study was to verify that the study could indeed detect the impact of a known toxin. It is worth noting that the histopathological exams conducted on these animals were incomplete; the same organs as in the prodrug urethane study (see table 1.13 on page 43) were left unexamined in all animals.

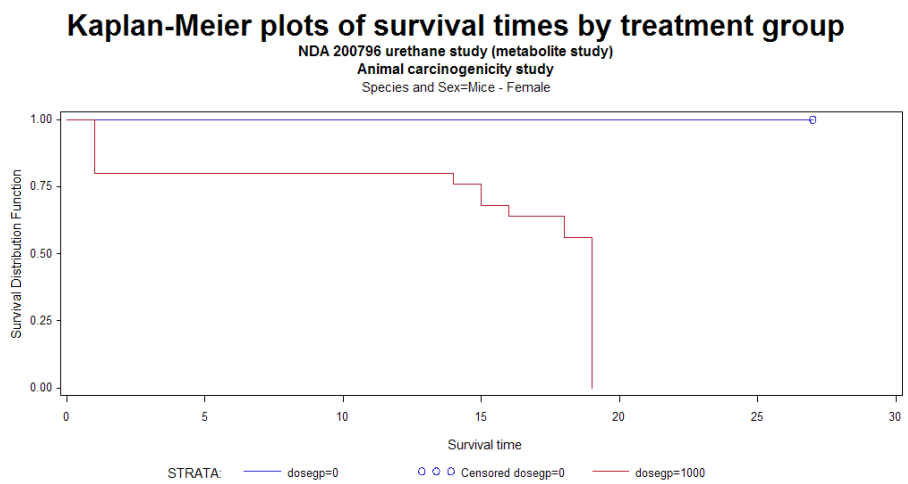
Thus a full comparison of tumor incidence rates between the urethane group and the control is not possible. The only organs in which tumors were reported to have been found in the urethane group are the spleen (hemangiosarcomas) and the lungs (hemangiosarcomas, alveolar bronchiolar adenomas and carcinomas).

The results of these tumor analyses are presented in table 2.18. Note that tumors found in the control group but in organs not examined in any of the urethane-treated animals are not included. There is clearly very strong evidence of urethane's carcinogenic effect; the p-value for the test of comparison between the urethane group and the control group was less than 0.0001 for alveolar bronchiolar adenomas in both sexes, for alveolar-bronchiolar carcinomas in female mice, and for splenetic hemangiosarcomas, in both sexes. The results for alveolar-bronchiolar carcinomas in male mice are almost as strong $p = 0.0002$.

The study has also produced evidence that urethane is connected with hemangiosarcomas of the lungs; the evidence for the female mice is a strong $p = 0.0209$, but the evidence for the same effect in male mice is weaker ($p = 0.3514$).

Survival plots for the urethane treated animals, compared with the controls, are shown as figures 2.11 and 2.12. As can be seen from these plots, the survival profiles of the urethane-treated animals is clearly worse than the control group, in both sexes. It is no surprise that the log-rank test finds strongly significant differences for both males and females ($p < 0.0001$ in both cases). Taken together, we can conclude that the study has successfully verified the toxic and carcinogenic effects of urethane.

Figure 2.11

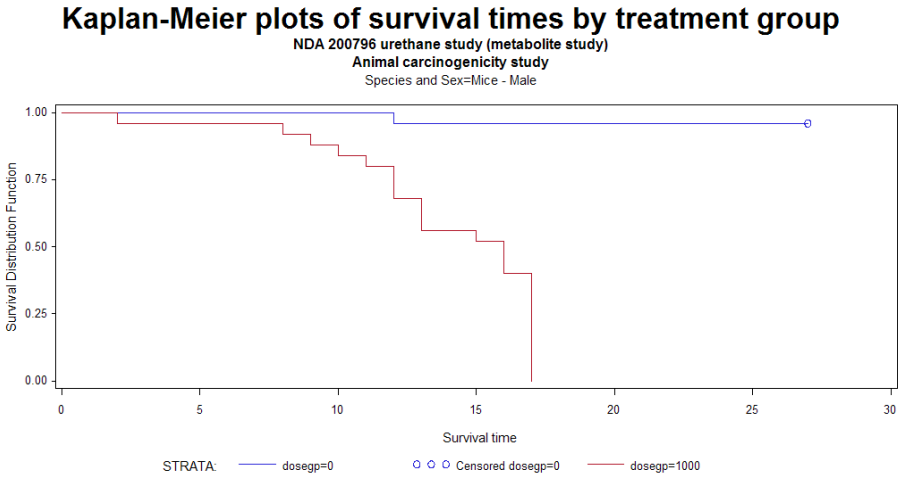


2.3.4 Suitability of dose levels

As with the prodrug study, there are currently no accepted statistical guidelines to determine whether a dose level has been found to be appropriate in a transgenic mouse study. The retrospective determination of suitability of the dose level therefore depends on the interpretation of clinical signs, and so falls outside the scope of this review.

Reference ID: 2868673

Figure 2.12



Reference ID: 2868673

Table 2.14: All reported neoplastic tumors in mouse metabolite study

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
brain	hemangiosarcoma	Male	*p*-value of pairwise and trend tests	.4947		.5000	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
cavity, nasal	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	.0120			.1024
			Observed number of cases	0	0	0	3
			Poly-1 adjusted incidence rate	.00%	.00%	.00%	13%
		Male	*p*-value of pairwise and trend tests	.3238	.2447	.2447	.4894
			Observed number of cases	0	2	2	1
			Poly-1 adjusted incidence rate	.00%	8.1%	8.1%	4.2%
epididymides	hemangiosarcoma	Male	*p*-value of pairwise and trend tests	.4947		.5000	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
harderian glands	adenoma	Female	*p*-value of pairwise and trend tests	.8759	.3046	.7551	1.000
			Observed number of cases	1	3	1	0
			Poly-1 adjusted incidence rate	4.0%	12%	4.0%	.00%
		Male	*p*-value of pairwise and trend tests	.7221	.5000	1.000	.7447
			Observed number of cases	1	2	0	1
			Poly-1 adjusted incidence rate	4.1%	8.1%	.00%	4.2%
intestine, rectum	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	.4845		.4898	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
kidneys	hemangiosarcoma	Male	*p*-value of pairwise and trend tests	.4947		.5000	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
liver	hepatocellular adenoma	Male	*p*-value of pairwise and trend tests	.3657	.5000		.4894
			Observed number of cases	0	1	0	1
			Poly-1 adjusted incidence rate	.00%	4.0%	.00%	4.2%
	hepatocellular carcinoma	Male	*p*-value of pairwise and trend tests	.4947		.5000	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
lungs with bronchi	alveloar−bronchiolar carcinoma	Female	*p*-value of pairwise and trend tests	.1458	.5000		.2347
			Observed number of cases	0	1	0	2
			Poly-1 adjusted incidence rate	.00%	4.0%	.00%	8.3%
		Male	*p*-value of pairwise and trend tests	.7474	.5000		

Reference ID: 2868673

Table 2.14: All reported neoplastic tumors in mouse metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	0	1	0	0
			Poly-1 adjusted incidence rate	.00%	4.0%	.00%	.00%
	alveolar−bronchiolar adenoma	Female	*p*-value of pairwise and trend tests	.6213	.9308	.7519	.8389
			Observed number of cases	6	3	5	4
			Poly-1 adjusted incidence rate	24%	12%	20%	17%
		Male	*p*-value of pairwise and trend tests	.9836	.9317	.8568	1.000
			Observed number of cases	6	3	4	0
			Poly-1 adjusted incidence rate	25%	12%	16%	.00%
	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	4.0%	.00%	.00%	.00%
mediastinum	adenoma	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	0	1
			Poly-1 adjusted incidence rate	.	.	.	100%
multicentric	sarcoma	Male	*p*-value of pairwise and trend tests	.2500			.5000
			Observed number of cases	0	0	0	1
			Poly-1 adjusted incidence rate	.00%	.00%	.00%	4.1%
ovaries	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	.7369	1.000	.7449	1.000
			Observed number of cases	1	0	1	0
			Poly-1 adjusted incidence rate	4.0%	.00%	4.0%	.00%
prepuce	papilloma	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	0
			Poly-1 adjusted incidence rate	.	50%	.	.
skeletal muscle (thigh)	hemangiosarcoma	Male	*p*-value of pairwise and trend tests	.4947		.5000	
			Observed number of cases	0	0	1	0
			Poly-1 adjusted incidence rate	.00%	.00%	4.0%	.00%
spleen	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	.4208	.2449	.1099	
			Observed number of cases	0	2	3	0
			Poly-1 adjusted incidence rate	.00%	8.0%	12%	.00%
		Male	*p*-value of pairwise and trend tests	.8227	.8752	.6798	1.000
			Observed number of cases	2	1	2	0
			Poly-1 adjusted incidence rate	8.0%	4.0%	8.1%	.00%
subcutis	hemangiosarcoma	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	100%	.	.	.

Reference ID: 2868673

Table 2.14: All reported neoplastic tumors in mouse metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
		Male	p-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	100%	.	.	.
thymus	thymoma	Female	p-value of pairwise and trend tests	.6697	.2449	.4898	
			Observed number of cases	0	2	1	0
			Poly-1 adjusted incidence rate	.00%	8.0%	4.0%	.00%
uterus	hemangiosarcoma	Female	p-value of pairwise and trend tests	.8182	.7551	.7449	1.000
			Observed number of cases	1	1	1	0
			Poly-1 adjusted incidence rate	4.0%	4.0%	4.0%	.00%
vagina	hemangiosarcoma	Female	p-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-1 adjusted incidence rate	4.0%	.00%	.00%	.00%

Table 2.15: Composite endpoint analyses for mouse metabolite study

Endpoint	Sex	Quantity	Control	Low dose	Mid dose	High dose
All hemangiosarcomas	Female	p-value of pairwise and trend tests	.4078	.7913	.3351	.7556
		Poly−1 adjusted incidence rate	16%	12%	24%	13%
		Observed number of cases	4	3	6	3
		Adjusted number at risk	25.00	25.00	24.69	23.85
	Male	p-value of pairwise and trend tests	.5088	.6465	.1279	.9350
		Poly−1 adjusted incidence rate	12%	12%	28%	4.2%
		Observed number of cases	3	3	7	1
		Adjusted number at risk	25.00	24.73	24.77	23.92

Table 2.16: Neoplastic tumors in mouse prodrug study with at least one significant result

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
cavity, nasal	hemangiosarcoma	Female	p-value of pairwise and trend tests	.0120			.1024
			Observed number of cases	0	0	0	3
			Poly-1 adjusted incidence rate	.00%	.00%	.00%	13%
			Adjusted number at risk	25.00	25.00	24.69	23.85

Reference ID: 2868673

Table 1.7: Significant results from mouse metabolite study (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
		Male	p-value of pairwise and trend tests	.3238	.2447	.2447	.4894
			Observed number of cases	0	2	2	1
			Poly-1 adjusted incidence rate	.00%	8.1%	8.1%	4.2%
			Adjusted number at risk	24.46	24.73	24.73	23.92

Table 2.17: Animals for which commonly unexamined organs were examined (mouse metabolite study)

Typically unexamined organ	Sex	Dose group	Animal number	Organ/tissue name	Tumor name
ears	Male	Control	1303	No tumors	
		Low dose	1369	No tumors	
mediastinum	Male	High	1406	mediastinum	adenoma
prepuce	Male	Low	1365	prepuce	papilloma
			1369	No tumors	
preputial glands	Male	Low	1358	lungs with bronchi	alveolar−bronchiolar adenoma
subcutis	Female	Control	1450	lungs with bronchi	alveolar−bronchiolar adenoma
					hemangiosarcoma
				subcutis	hemangiosarcoma
	Male	Control	1312	subcutis	hemangiosarcoma

Table 2.18: All tumors reported in urethane study (metabolite study)

Organ	Tumor type	Sex	Quantity	Control	Urethane
lungs with bronchi	alveloar-bronchiolar carcinoma	Female	p-value of pairwise test		< 0.0001
			Observed number of cases	0	15
			Poly-1 adjusted incidence rate	.00%	81%
		Male	p-value of pairwise test		.0002
			Observed number of cases	0	8
			Poly-1 adjusted incidence rate	.00%	50%

Reference ID: 2868673

Table 2.18: All reported neoplastic tumors in urethane study (metabolite study) (continued)

Organ	Tumor type	Sex	Quantity	Control	Urethane
	alveolar−bronchiolar adenoma	Female	p-value of pairwise test		< 0.0001
			Observed number of cases	6	20
			Poly-1 adjusted incidence rate	24%	99%
		Male	p-value of pairwise test		< 0.0001
			Observed number of cases	6	24
			Poly-1 adjusted incidence rate	25%	100%
	hemangiosarcoma	Female	p-value of pairwise test		.0209
			Observed number of cases	1	5
			Poly-1 adjusted incidence rate	4.0%	32%
		Male	p-value of pairwise test		.3514
			Observed number of cases	0	1
			Poly-1 adjusted incidence rate	.00%	7.3%
spleen	hemangiosarcoma	Female	p-value of pairwise test		< 0.0001
			Observed number of cases	0	19
			Poly-1 adjusted incidence rate	.00%	95%
		Male	p-value of pairwise test		< 0.0001
			Observed number of cases	2	21
			Poly-1 adjusted incidence rate	8.0%	95%

Reference ID: 2868673

Chapter 3

Conclusions

Two separate groups of studies have been conducted; an investigation into the prodrug TAK-491 and an investigation into the metabolite TAK-536-MII. Each group of studies included a twenty four month study on both male and female rats, and a twenty six week study using male and female transgenic mice. Additionally, a study was attempted with the goal of assessing the effect of very high doses of TAK-536-MII on female rats, but this study was abandoned after experiencing high mortality in both the control and the treatment arms. Of the completed studies, both rat studies and the mouse study into TAK-491 were gavage studies; the mouse study of TAK-536-MII was a diet study.

In addition to these carcinogenicity studies, two verification studies were conducted with the transgenic mice. In each case, a group of mice were treated with urethane, a chemical known to be carcinogenic. The goal of these studies was to verify that the studies were sufficiently well administered to detect the known carcinogenicity effect associated with urethane.

3.1 Urethane studies

The urethane studies were both successful, in that the performance of the treatment group was strikingly worse than that of the control group, both in terms of overall survival, and in terms of those few carcinogenicity effects which were investigated, primarily tumors of the lungs and spleen. The success of this experiment provides some assurance that the studies have been conducted well, although some caveats must be added: the urethane studies were not blinded to the pathologists, the carcinogenic effect of urethane is much larger (and therefore easier to detect) than is likely in most proposed drugs, and the limited pathological exams given to the urethane treated mice can only provide limited assurance of adequate conduct of the more extensive pathological exams given to the mice in the main study.

3.2 Indications of carcinogenicity

In the case of the prodrug, the strongest signal was hemolymphoreticular histiocytic sarcomas in female rats, where the adjusted incidence rate demonstrated a significant trend (1.1% in the control group, 2.2% in the low diose group, 2.1% in the mid dose group, and 8.2% in the high dose group. The p-value for the test of trend is 0.0168, is considered significant, even after adjusting for multiplicity, only when such tumors are considered rare. For the purposes of this review, histiocytic sarcomas are considered rare, so that this signal is considered statistically significant, but a strong case can nonetheless be made to consider them common.

The incidence rate for adrenal cortical cell adenomas in female rats in the prodrug study is also somewhat worrying. The significant result is in the low dose group, but all three treatment groups displayed elevated incidence rates compared to the control group. Since the historical data suggests (just) that this be considered a rare tumor type, the observed rates in the treatment groups must be viewed as indicative of a possible tumorigenic effect. Mitigating against this concern, however, is

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the fact that as in the case of histiocytic sarcomas, the decision to consider such tumors rare is not clear cut — if they are considered common, then the results are less striking, and could be largely attributed to an unusually low incidence rate in the control group — and the fact that there is no evidence of a corresponding tumorigenic effect in the male rats.

There were no other noteworthy signals in either the rat or the mouse prodrug study.

In the case of the metabolite study, the only results which retains its significance after adjusting for the multiplicity of endpoints are for the analysis of hemangiosarcomas of the nasal cavity in female mice.

In the case of the hemangiosarcomas of the nasal cavity, only three such tumors were detected; all in the high dose group. In such a small study (with twenty five animals per treatment group) this is enough for the trend to be strongly significant, with a p-value of 0.0120, which remains significant after adjusting for multiplicity as long as these tumors are considered rare. Arguing against the carcinogenicity effect are the fact that there is no detectable pattern of nasal cavity hemangiosarcomas in the male mice (although none of the five animals with such tumors were in the control group) and the fact that when pooled across all sites, incidence of hemangiosarcomas seems to bear little relationship to dosage.

3.3 Autolytic and missing organs

Several organs have been reported as being so widely unexamined or (in one case) autolytic that, if the data are taken at face value, no inference should be made about such tumors. It is probable that these organs underwent a gross physical exam, the results of which were noted, but were not subjected to a complete histiopathological analysis. Regardless, the studies cannot tell us anything about possible carcinogenic effects at these sites. The list of all such sites is presented in table 3.1.

Of particular concern is the fact that nasal cavities of the prodrug rats have not been examined, given the strong result for hemangiosarcomas of the nasal cavity in mice in the metabolite study.

Table 3.1: Sites for which no conclusion may be drawn

	Prodrug	Metabolite
Rats	Abdominal cavity	Abdominal cavity
	Clitoral gland	Clitoral gland
	Cranial bone	Oral cavity
	Ear	Planta
	Mesothelium	Preputial gland
	Nasal cavity	Tail
	Oral cavity	Thoracic cavity
	Preputial gland	Vertebra
	Thoracic cavity	Zymbal's gland
	Vertebra	
	Zymbal's gland	
Mice	Ears	Ears
	Perinium	Mediastinum
	Scrotal sac	Prepuce
	Skeletal muscle	Preputial glands
	Skin, leg	Subcutis
	Skin, nasal	Tail

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3.4 Leydig cell tumors

The incidence rates of Leydig cell tumors in the rat studies are very high; around 90% in the prodrug study and about 70% in the metabolite study. These rates are in line with those experiences historically in studies involving F344 rats (actually, the rate in the metabolite study is somewhat below that suggested by the historical norms). However, it must be noted that as a result of this high background rate, any dose related increase in the tumor rate, no matter how large, would be undetectable by the standard statistical methods used in studies such as these. It follows that we must consider the study inconclusive regarding this type of tumor.

Note that this is a consideration that applies to all studies where certain tumor types are almost ubiquitous, and therefore to assessment of Leydig cell tumors in most if not all studies of F344 rats.

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Appendix A

Analysis of rat metabolite study using negative control

Figure A.1

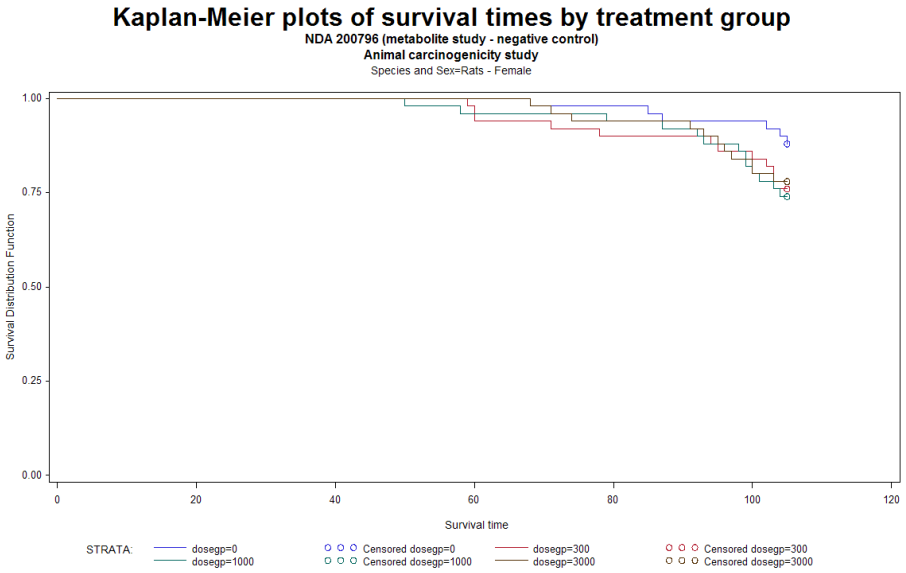

Table A.1: Results of log-rank tests of survival in metabolite study (negative control)

Species and Sex	Number of dose groups	p-value (trend)	p-value (homogeneity)
Rats — Female	4	0.2454	0.3239
Rats — Male	4	0.6561	0.7060

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Figure A.2

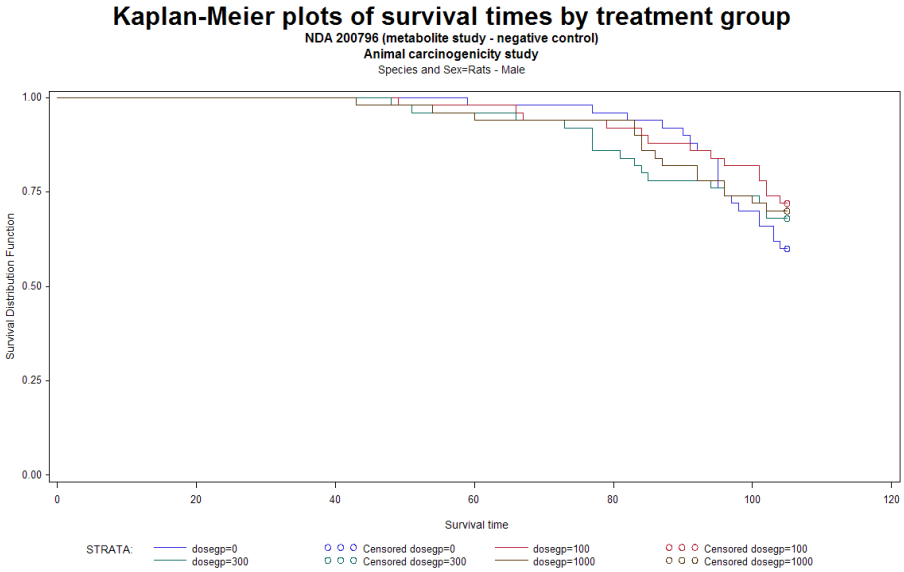

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Table A.2: All reported neoplastic tumors in rat metabolite study (negative control)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Abdominal cavity	PARAGANGLIOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.	100%	.	.
			Adjusted number at risk	0.00	1.00	0.00	0.00
		Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
			Adjusted number at risk	0.00	0.00	1.00	0.00
Adrenal	ADENOMA,CORTICAL CELL	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
	PHEOCHROMOCYTOMA	Female	*p*-value of pairwise and trend tests	.7278	.0235	.4894	.4894
			Observed number of cases	0	5	1	1
			Poly-3 adjusted incidence rate	.00%	11%	2.2%	2.2%
			Adjusted number at risk	48.35	45.55	46.16	46.45
		Male	*p*-value of pairwise and trend tests	.2813	.1765	.4555	.2346
			Observed number of cases	4	8	5	7
			Poly-3 adjusted incidence rate	8.7%	18%	12%	16%
			Adjusted number at risk	45.77	45.14	42.94	43.91
Cerebrum	ASTROCYTOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.4885		.4884	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.3%	.00%
			Adjusted number at risk	44.67	45.05	42.94	43.41
	OLIGODENDROGLIOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	.4973		.4894	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
			Adjusted number at risk	48.35	45.55	46.47	46.45
		Male	*p*-value of pairwise and trend tests	.7471	.5056		
			Observed number of cases	0	1	0	0

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	44.67	45.05	42.42	43.41
Clitoral gland	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.5000		1.000	
			Observed number of cases	1	0	0	2
			Poly-3 adjusted incidence rate	100%	.	.00%	100%
			Adjusted number at risk	1.00	0.00	1.00	2.00
	ADENOMA	Female	*p*-value of pairwise and trend tests	.6667		.5000	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.	100%	.00%
			Adjusted number at risk	1.00	0.00	1.00	1.76
Harderian gland	ADENOMA	Male	*p*-value of pairwise and trend tests	.7471	.5056		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	44.67	45.14	42.42	43.41
Heart	SCHWANNOMA	Female	*p*-value of pairwise and trend tests	.7486	1.000	.7419	1.000
			Observed number of cases	1	0	1	0
			Poly-3 adjusted incidence rate	2.1%	.00%	2.2%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.6339	.5056	.2356	
			Observed number of cases	0	1	2	0
			Poly-3 adjusted incidence rate	.00%	2.2%	4.7%	.00%
			Adjusted number at risk	44.67	45.14	42.42	43.41
Hemolymphoreticular(all	LEUKEMIA,LARGE GRANULAR LYMPHO	Female	*p*-value of pairwise and trend tests	.2758	.9161	.7216	.5661
			Observed number of cases	13	8	11	13
			Poly-3 adjusted incidence rate	27%	17%	24%	27%
			Adjusted number at risk	48.81	46.71	46.68	47.30
		Male	*p*-value of pairwise and trend tests	.9872	1.000	1.000	.9996
			Observed number of cases	15	1	1	3
			Poly-3 adjusted incidence rate	32%	2.2%	2.3%	6.9%
			Adjusted number at risk	47.13	45.05	42.89	43.41
	SARCOMA,HISTIOCYTIC	Female	*p*-value of pairwise and trend tests	.7405	.4839		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.2324	.7584	1.000	.4913

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	1	1	0	2
			Poly-3 adjusted incidence rate	2.2%	2.2%	.00%	4.6%
			Adjusted number at risk	44.75	45.05	42.42	43.63
Intestine,cecum	LEIOMYOSARCOMA	Female	*p*-value of pairwise and trend tests	.7405	.4839		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	48.35	45.81	46.05	46.13
Intestine,duodenum	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	.2471			.4943
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.83
Intestine,jejunum	ADENOMA	Male	*p*-value of pairwise and trend tests	.2471			.4943
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
			Adjusted number at risk	44.02	45.05	42.42	43.41
Intestine,rectum	ADENOMA	Female	*p*-value of pairwise and trend tests	.2486			.4894
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
			Adjusted number at risk	48.35	45.55	46.05	46.45
Kidney	ADENOMA,RENAL CELL	Female	*p*-value of pairwise and trend tests	.4973		.4894	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.2471			.4943
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	CARCINOMA,TRANSITIONAL CELL	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.2%	.00%	.00%	.00%
			Adjusted number at risk	44.91	45.05	42.42	43.41
	LIPOSARCOMA	Female	*p*-value of pairwise and trend tests	.2486			.4894
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
			Adjusted number at risk	48.35	45.55	46.05	46.45

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
	RENAL MESENCHYMAL TUMOR	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.2%	.00%	.00%	.00%
			Adjusted number at risk	44.91	45.05	42.42	43.41
Liver	ADENOMA,HEPATOCELLULAR	Female	*p*-value of pairwise and trend tests	.1851		.4894	.4894
			Observed number of cases	0	0	1	1
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	2.1%
			Adjusted number at risk	48.35	45.55	46.05	46.67
		Male	*p*-value of pairwise and trend tests	.5993	.5085	.7412	.7471
			Observed number of cases	1	2	1	1
			Poly-3 adjusted incidence rate	2.2%	4.4%	2.4%	2.3%
			Adjusted number at risk	44.83	45.05	42.42	43.41
	CARCINOMA,HEPATOCELLULAR	Male	*p*-value of pairwise and trend tests	.2471			.4943
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	CHOLANGIOMA	Male	*p*-value of pairwise and trend tests	.2471			.4943
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.63
Lung(bronchus)	ADENOCARCINOMA,BRONCHIOLO−ALVE	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.9228	.8834	.8706	1.000
			Observed number of cases	2	1	1	0
			Poly-3 adjusted incidence rate	4.5%	2.2%	2.4%	.00%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	ADENOMA,BRONCHIOLO−ALVEOLAR	Female	*p*-value of pairwise and trend tests	.2616	.7363	.4839	.4839
			Observed number of cases	1	1	2	2
			Poly-3 adjusted incidence rate	2.1%	2.2%	4.3%	4.3%
			Adjusted number at risk	48.35	45.55	46.05	46.56
		Male	*p*-value of pairwise and trend tests	.0788	.2528	.4884	.1164
			Observed number of cases	0	2	1	3
			Poly-3 adjusted incidence rate	.00%	4.4%	2.4%	6.9%

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Adjusted number at risk	44.67	45.05	42.42	43.41
Mammary gland	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.2908	.4839	.4894	.4894
			Observed number of cases	0	1	1	1
			Poly-3 adjusted incidence rate	.00%	2.2%	2.1%	2.2%
			Adjusted number at risk	48.35	45.55	46.94	46.48
		Male	*p*-value of pairwise and trend tests	.1053	.5056		.2414
			Observed number of cases	0	1	0	2
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	4.6%
			Adjusted number at risk	44.67	45.31	42.42	43.85
	ADENOMA	Female	*p*-value of pairwise and trend tests	.2486			.4894
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
			Adjusted number at risk	48.35	45.55	46.05	46.45
	FIBROADENOMA	Female	*p*-value of pairwise and trend tests	.8057	.4691	.8710	.8710
			Observed number of cases	2	3	1	1
			Poly-3 adjusted incidence rate	4.1%	6.6%	2.2%	2.2%
			Adjusted number at risk	48.35	45.55	46.05	46.48
		Male	*p*-value of pairwise and trend tests	.4299	.2528		.4943
			Observed number of cases	0	2	0	1
			Poly-3 adjusted incidence rate	.00%	4.4%	.00%	2.3%
			Adjusted number at risk	44.67	45.31	42.42	43.41
	TUMOR,MIXED,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
			Adjusted number at risk	48.77	45.55	46.05	46.45
Mesothelium	MESOTHELIOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	.6024	.1250	.0527	.4943
			Observed number of cases	0	3	4	1
			Poly-3 adjusted incidence rate	.00%	6.7%	9.3%	2.3%
			Adjusted number at risk	44.67	45.05	42.91	43.41
Ovary	GRANULOSA CELL TUMOR,MALIGNANT	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
Pancreas	ADENOMA,ACINAR CELL	Male	*p*-value of pairwise and trend tests	.6789	.0610	.0244	.4943
			Observed number of cases	0	4	5	1

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	.00%	8.9%	12%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	ADENOMA,ACINAR−ISLET CELL	Female	*p*-value of pairwise and trend tests	.7405	.4839		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.7471	.5056		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	ADENOMA,ISLET CELL	Female	*p*-value of pairwise and trend tests	.9290	.1611	.4839	1.000
			Observed number of cases	1	4	2	0
			Poly-3 adjusted incidence rate	2.1%	8.8%	4.3%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.0032	.0007	.0277	.0001
			Observed number of cases	6	21	14	23
			Poly-3 adjusted incidence rate	13%	46%	33%	52%
			Adjusted number at risk	44.88	45.40	42.47	44.10
	CARCINOMA,ISLET CELL	Female	*p*-value of pairwise and trend tests	.2486			.4894
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.1800		.4884	.4943
			Observed number of cases	0	0	1	1
			Poly-3 adjusted incidence rate	.00%	.00%	2.4%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.72
Parathyroid	ADENOMA	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.2%	.00%	.00%	.00%
			Adjusted number at risk	46.35	43.66	44.34	43.48
		Male	*p*-value of pairwise and trend tests	.7485	.5114		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	43.67	45.05	41.63	42.41
Pituitary	ADENOMA,ANTERIOR	Female	*p*-value of pairwise and trend tests	.9856	.4350	.7460	.9778

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	21	21	18	12
			Poly-3 adjusted incidence rate	43%	46%	38%	26%
			Adjusted number at risk	49.13	45.79	47.04	47.02
		Male	*p*-value of pairwise and trend tests	.7612	.9720	.9028	.9357
			Observed number of cases	23	15	17	16
			Poly-3 adjusted incidence rate	49%	32%	38%	36%
			Adjusted number at risk	46.93	46.37	44.56	44.57
	CARCINOMA,ANTERIOR	Female	*p*-value of pairwise and trend tests	.4973		.4894	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
			Adjusted number at risk	48.35	45.55	46.08	46.45
Planta	FIBROSARCOMA	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
			Adjusted number at risk	0.00	0.00	1.00	0.00
Preputial gland	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	.7500		1.000	
			Observed number of cases	2	0	0	1
			Poly-3 adjusted incidence rate	100%	.	.00%	100%
			Adjusted number at risk	2.00	0.00	1.00	1.00
	ADENOMA	Male	*p*-value of pairwise and trend tests	.5000		.5000	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.	100%	.00%
			Adjusted number at risk	1.41	0.00	1.00	0.69
Prostate	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	.7471	.5056		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	ADENOMA	Male	*p*-value of pairwise and trend tests	.3188	.2312	.5614	.2886
			Observed number of cases	9	13	9	12
			Poly-3 adjusted incidence rate	20%	29%	21%	27%
			Adjusted number at risk	45.06	45.05	43.16	44.84
Salivary gland,sublingu	ADENOMA	Male	*p*-value of pairwise and trend tests	.2471			.4943
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.41

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Seminal vesicle	ADENOMA	Male	*p*-value of pairwise and trend tests	.7471	.5114		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	43.35	45.27	39.45	43.41
Skin+Subcutis	FIBROMA	Female	*p*-value of pairwise and trend tests	.8068	.2314		
			Observed number of cases	0	2	0	0
			Poly-3 adjusted incidence rate	.00%	4.4%	.00%	.00%
			Adjusted number at risk	48.35	45.61	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.3086	.5056		.4943
			Observed number of cases	0	1	0	1
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	KERATOACANTHOMA	Female	*p*-value of pairwise and trend tests	.7405	.4839		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.4343	1.000	1.000	.7471
			Observed number of cases	1	0	0	1
			Poly-3 adjusted incidence rate	2.2%	.00%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	PAPILLOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	.4973		.4894	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
			Adjusted number at risk	48.35	45.55	46.47	46.45
	SCHWANNOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	.8056	.2528		
			Observed number of cases	0	2	0	0
			Poly-3 adjusted incidence rate	.00%	4.4%	.00%	.00%
			Adjusted number at risk	44.67	45.11	42.42	43.41
Spinal cord	SCHWANNOMA,MALIGNANT	Male	*p*-value of pairwise and trend tests	.5356	.7584	1.000	.7529
			Observed number of cases	1	1	0	1
			Poly-3 adjusted incidence rate	2.2%	2.2%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	44.22
Spleen	HEMANGIOSARCOMA	Male	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.2%	.00%	.00%	.00%

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Adjusted number at risk	44.70	45.05	42.42	43.41
Stomach	CARCINOID	Female	*p*-value of pairwise and trend tests	.2486			.4894
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.2%
			Adjusted number at risk	48.35	45.55	46.05	46.45
	LEIOMYOSARCOMA	Male	*p*-value of pairwise and trend tests	.2471			.4943
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.41
Tail	KERATOACANTHOMA	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
			Adjusted number at risk	0.00	0.00	1.00	0.00
Testis	LEYDIG CELL TUMOR	Male	*p*-value of pairwise and trend tests	.9946	.9672	.8526	.9989
			Observed number of cases	43	35	38	29
			Poly-3 adjusted incidence rate	89%	77%	83%	65%
			Adjusted number at risk	48.10	45.67	45.58	44.80
Thoracic cavity	FIBROMA	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
			Adjusted number at risk	0.00	0.00	1.00	0.00
Thyroid	ADENOMA,C CELL	Female	*p*-value of pairwise and trend tests	.6990	.4538	.2486	.7353
			Observed number of cases	5	6	8	4
			Poly-3 adjusted incidence rate	10%	13%	17%	8.6%
			Adjusted number at risk	48.35	45.64	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.7645	.1234	.3545	.6303
			Observed number of cases	4	9	6	4
			Poly-3 adjusted incidence rate	8.9%	20%	14%	9.1%
			Adjusted number at risk	44.78	45.11	43.07	43.91
	ADENOMA,FOLLICULAR CELL	Female	*p*-value of pairwise and trend tests	.6189	.4839	.4894	
			Observed number of cases	0	1	1	0
			Poly-3 adjusted incidence rate	.00%	2.2%	2.2%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.1981	.5085	.2994	.2994
			Observed number of cases	1	2	3	3

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Poly-3 adjusted incidence rate	2.2%	4.4%	7.0%	6.9%
			Adjusted number at risk	44.67	45.05	43.08	43.72
	CARCINOMA,C CELL	Female	*p*-value of pairwise and trend tests	.2543	.7363	.7419	.4839
			Observed number of cases	1	1	1	2
			Poly-3 adjusted incidence rate	2.1%	2.2%	2.2%	4.3%
			Adjusted number at risk	48.35	45.55	46.22	46.45
		Male	*p*-value of pairwise and trend tests	.6427	.0610	.2356	.4943
			Observed number of cases	0	4	2	1
			Poly-3 adjusted incidence rate	.00%	8.9%	4.7%	2.3%
			Adjusted number at risk	44.67	45.14	42.63	43.41
	CARCINOMA,FOLLICULAR CELL	Female	*p*-value of pairwise and trend tests	.7405	.4839		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.4915		.2356	
			Observed number of cases	0	0	2	0
			Poly-3 adjusted incidence rate	.00%	.00%	4.7%	.00%
			Adjusted number at risk	44.67	45.05	42.42	43.41
Tongue	PAPILLOMA,SQUAMOUS CELL	Female	*p*-value of pairwise and trend tests	.4973		.4894	
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
Urinary bladder	PAPILLOMA,TRANSITIONAL CELL	Female	*p*-value of pairwise and trend tests	.2486			.4894
			Observed number of cases	0	0	0	1
			Poly-3 adjusted incidence rate	.00%	.00%	.00%	2.1%
			Adjusted number at risk	48.35	45.55	46.05	46.56
		Male	*p*-value of pairwise and trend tests	.9838	.8792	1.000	1.000
			Observed number of cases	2	1	0	0
			Poly-3 adjusted incidence rate	4.4%	2.2%	.00%	.00%
			Adjusted number at risk	45.26	45.05	42.42	43.41
Uterus	ADENOCARCINOMA	Female	*p*-value of pairwise and trend tests	.3886	1.000	.7419	.7419
			Observed number of cases	1	0	1	1
			Poly-3 adjusted incidence rate	2.1%	.00%	2.2%	2.2%
			Adjusted number at risk	48.35	45.55	46.05	46.45
	ADENOMA	Female	*p*-value of pairwise and trend tests	.0182		.4894	.1132

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Observed number of cases	0	0	1	3
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	6.5%
			Adjusted number at risk	48.35	45.55	46.05	46.45
	GRANULAR CELL TUMOR	Female	*p*-value of pairwise and trend tests	.4365	1.000	1.000	.7419
			Observed number of cases	1	0	0	1
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	2.1%
			Adjusted number at risk	48.35	45.55	46.05	46.56
	LEIOMYOMA	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
	POLYP,ENDOMETRIAL STROMAL	Female	*p*-value of pairwise and trend tests	.7450	.7230	.0613	.8129
			Observed number of cases	16	14	24	13
			Poly-3 adjusted incidence rate	33%	29%	50%	27%
			Adjusted number at risk	48.81	47.98	47.99	48.28
	SARCOMA,ENDOMETRIAL STROMAL	Female	*p*-value of pairwise and trend tests	.7419	.4894		
			Observed number of cases	0	1	0	0
			Poly-3 adjusted incidence rate	.00%	2.2%	.00%	.00%
			Adjusted number at risk	48.35	46.36	46.05	46.45
	SARCOMA,NOS	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
	SCHWANNOMA,MALIGNANT	Female	*p*-value of pairwise and trend tests	.8874	.1132		
			Observed number of cases	0	3	0	0
			Poly-3 adjusted incidence rate	.00%	6.5%	.00%	.00%
			Adjusted number at risk	48.35	46.51	46.05	46.45
Vagina	GRANULAR CELL TUMOR	Female	*p*-value of pairwise and trend tests	1.000	1.000	1.000	1.000
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	2.1%	.00%	.00%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
Vertebra	CHORDOMA	Female	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	0	0	1	0
			Poly-3 adjusted incidence rate	.	.	100%	.
			Adjusted number at risk	0.00	0.00	1.00	0.00

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Table 2.6: All reported neoplastic tumors in rat metabolite study (negative control) (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Zymbal gland	ADENOCARCINOMA	Male	*p*-value of pairwise and trend tests	1.000			
			Observed number of cases	1	0	0	0
			Poly-3 adjusted incidence rate	100%	.	.	.
			Adjusted number at risk	1.00	0.00	0.00	0.00

Table A.3: Neoplastic tumors in rat metabolite study with at least one significant result using negative control

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
Adrenal	PHEOCHROMOCYTOMA	Female	*p*-value of pairwise and trend tests	.7278	.0235	.4894	.4894
			Observed number of cases	0	5	1	1
			Poly-3 adjusted incidence rate	.00%	11%	2.2%	2.2%
			Adjusted number at risk	48.35	45.55	46.16	46.45
		Male	*p*-value of pairwise and trend tests	.2813	.1765	.4555	.2346
			Observed number of cases	4	8	5	7
			Poly-3 adjusted incidence rate	8.7%	18%	12%	16%
			Adjusted number at risk	45.77	45.14	42.94	43.91
Pancreas	ADENOMA,ACINAR CELL	Male	*p*-value of pairwise and trend tests	.6789	.0610	.0244	.4943
			Observed number of cases	0	4	5	1
			Poly-3 adjusted incidence rate	.00%	8.9%	12%	2.3%
			Adjusted number at risk	44.67	45.05	42.42	43.41
	ADENOMA,ISLET CELL	Female	*p*-value of pairwise and trend tests	.9290	.1611	.4839	1.000
			Observed number of cases	1	4	2	0
			Poly-3 adjusted incidence rate	2.1%	8.8%	4.3%	.00%
			Adjusted number at risk	48.35	45.55	46.05	46.45
		Male	*p*-value of pairwise and trend tests	.0032	.0007	.0277	.0001
			Observed number of cases	6	21	14	23
			Poly-3 adjusted incidence rate	13%	46%	33%	52%
			Adjusted number at risk	44.88	45.40	42.47	44.10
Uterus	ADENOMA	Female	*p*-value of pairwise and trend tests	.0182		.4894	.1132
			Observed number of cases	0	0	1	3
			Poly-3 adjusted incidence rate	.00%	.00%	2.2%	6.5%

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Table 2.8: Significant results from rat metabolite study using negative control (continued)

Organ	Tumor type	Sex	Quantity	Control	Low dose	Mid dose	High dose
			Adjusted number at risk	48.35	45.55	46.05	46.45

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Appendix B

Historical control data

The sponsor provided tumor incidence data for control animals from three historical studies involving F344/Jcl rats. Due to concerns over the age of the data (the studies were conducted in the mid 1990s) and the fact that the studies were not conducted at the (b) (4), these results have not been used in this review.

Table B.1: Incidence rates of relevant tumor types in F344/Jcl rats

Organ	Tumor	Prevalence				Category	
		Female		Male		Female	Male
		Rate (%)	90% CI (%)	Rate (%)	90% CI (%)		
Adrenal gland	Cortical cell adenoma	1.3%	(0.4, 3.4)	0.3%	(0.02, 1.6)	Rare	Rare
	Pheochromocytoma	2.0%	(0.9, 3.9)	11.7%	(8.7, 15.2)	Rare	Common
Hemolymphoreticular	Histiocytic sarcoma	0.0%	(0.0, 1.0)	0.0%	(0.0, 1.0)	Rare	Rare
	Large granular lymphocyte leukeima	0.3%	(0.02, 1.6)	0.3%	(0.02, 1.6)	Rare	Rare
Pancreas	Islet cell carcinoma	1.0%	(0.3, 2.6)	0.3%	(0.02, 1.6)	Rare	Rare
Pituitary gland	Anterior adenomas	37.3%	(32.7, 42.2)	32.3%	(27.9, 37.1)	Common	Common
Uterus	Adenomas	7.6%	(5.0, 11.0)	—	—	Common	—
	Endometrial stromal polyps	38.0%	(32.9, 43.3)	—	—	Common	—
	Leiomyosarcoma	0.0%	(0.0, 1.2)	—	—	Rare	—

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