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January 3, 2001

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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane
Room 1061
Rockville, MD 20852

ATTN: Docket No. 99F-0187 (Neotame)

Dear Sir/Madam:

Enclosed please find comments on Docket No. 99F-0187 (Neotame).

Very truly yours,

HOLLAND & KNIGHT LLP

C. Thomas Swaim

CTS/cc
Enclosure
BOS1 #1099396 v1

99F-0187

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Critical Study Of Food Additive Petition (FAP)
To United States Food And Drug Administration (FDA)
In Respect Of
Neotame (NC-00723)

Introduction

Neotame (NC-00723) is an artificial sweetener which, by weight, is 8,000 times sweeter than sugar. If approved by the United States Food and Drug Administration (FDA), neotame will be used in a wide variety of foods and beverages. Neotame (N-(N-(3,3-dimethylbutyl)-*L*-α-aspartyl)-*L*-phenylalanine 1-methyl ester) is very similar in structure to aspartame but unlike aspartame, neotame does not break down in the body to basic biochemical entities which are then metabolized. However, its metabolic profile and toxicity appears to be very different for reasons which have not been fully elucidated by the petitioner but are probably related to the routes of the compound's systemic elimination. Close inspection of the data show neotame has adverse effects in the dog liver and biliary system and has adverse effects on rabbit reproduction/teratology.

Standard toxicology studies have been carried out to determine the safety of neotame and its demethylated metabolite NC-00751. Both compounds have remarkably similar structures to that of aspartame, but neotame is considerably sweeter. These studies have been submitted in a Food Additive Petition (FAP) to the FDA in order to receive government approval to market the product as a low calorie intense sweetening agent. The two areas of the data which have been analysed in detail show some significant safety issues which remain unaddressed and must be resolved before approval.

The Effects Noted In the Two Long Term Dog Studies Are Due to Neotame Toxicity to the Liver; These Effects Are Not Reversible as Implied by the Petitioner.

The long term studies conducted in the dog species show definite signs of toxicity which, through close inspection of the pharmacokinetic data generated in the study and specific PK metabolism studies, is shown to be related to systemic exposure of the parent compound. These effects are particularly worrisome since they occur in the dog, which, of the animal species tested with this compound, is the species most similar, anatomically and physically, to man. Any effects occurring in the dog can therefore be considered to be highly relevant for human risk assessment.

Increase in Serum Alkaline Phophatase (ALP)

The 13-week dog study (PCR 0990) showed a number of toxic effects which were all related to the liver. The most obvious effect was the increase in serum alkaline phophatase (ALP), as reported by the petitioners. This activity was subsequently found to be of hepatic origin from ALP isoenzyme analyses. The petitioner reported that there was no evidence of test article related cholestasis; however the fact that the dogs were reported to produce discolored feces (white and grey) suggests clearly that there is an effect on bile salt metabolism and excretion. All but two of the dogs at the top two neotame dosages produced white and grey feces and this was severe enough for the results to show it still to be apparent during the 4 week reversibility phase in the top dose group. The petitioners also claim that fecal discoloration is related to fecal excretion of NC-00723 and its metabolite N-00751, both which are white. These data do not, however, close off alternative hypotheses especially since there has been no mention of this same phenomenon occurring in any other species that excretes neotame via the same route and that these compounds, when incorporated into animal diets, were not found to color the dog's or any other animal's feces.

Increase in Plasma Concentrations of Cholesterol and Triglycerides

Also apparent from the clinical chemistry data is the increased plasma concentrations of cholesterol and triglycerides in the neotame treatment groups throughout the study period in both sexes (tables 1-4 taken from report). In fact the triglyceride levels were significantly increased in the males at the top dose group during weeks 2 and 6 of treatment. The accumulation and/or secretion of lipids namely triglycerides is a well known mechanism involved in liver injury (1). Likewise, increased blood cholesterol levels are obviously clinically significant especially over long periods of time. It is known that the liver serves both as the chief source and the chief agent for disposal of plasma cholesterol, a portion of that removed from the blood appearing in the bile. Alterations in the concentration of cholesterol in human bile is also known to result in clinical consequences (2). Both of these effects indicate neotame is having an adverse effect on the liver.

Effects on Organ Weights/Bodyweight

The clearest indication, however, that neotame affects the liver can be evaluated from the organ weight and the microscopic examination. The results show that there is a dose dependent increase in absolute and relative liver weights with a statistically significant increase in the male relative liver weight at the top dose and at the top two doses in the females (figures 1-2). These increased liver weights are still apparent after the 4 week reversal period with the top dose male liver still 11% higher than that of the control and the top dose female liver being 32.8% greater than control (table 5). The increased liver weights are also associated with an increase in hepatocellular glycogen which was reported in both sexes at the top two dose levels. The fact that neotame appears to adversely affect the liver is also indicated in the cumulative body weight gain data where again a clear dose dependent decrease in bodyweight gain is apparent during the treatment period in both sexes (table 6). The petitioners claim that the decreases in bodyweight gain are due to poor diet palatability; however it is also well known that decreased bodyweight gain is also caused by toxicity resulting in suppressed food intake. The decreases in body weight gain in this study are therefore considered to be the result of a toxic effect because the minimal decreases or increases in food intake do not support the large decreases in body weight gain. For example, in females, there is a 24% reduction in bodyweight gain at 600mg/kg/day, whereas the decrease in food intake is only 3.1%. Similarly, at 60mg/kg/day there is a 12% reduction in bodyweight gain and only a 2.2% reduction in food intake. In males, a 15.5% reduction in bodyweight gain at 600mg/kg/day is associated with an increase in food consumption (101%); likewise, at 60mg/kg/day a 4.2% reduction in bodyweight gain is associated with food consumption some 6% higher than that of the control animals. The differences between the level of food intake and bodyweight gain clearly indicates that the effect on bodyweight is most probably a toxic phenomenon and not as a result of a decrease in food consumption.

The toxic effects on the liver leading to decreases in bodyweight gain can be directly related to the systemic circulating levels of the parent compound. The petitioners show clearly in the pharmacokinetic section of the 13-week dog study report that there is a non-linear increase in systemic exposure of the parent compound and its metabolite over the dose range studied, *i.e.* there is a higher dose adjusted AUC_{0-24} and C_{max} at the higher dosages (figures 3-4 taken from the report). In addition, the petitioners claim that there was no evidence of accumulation over the 12-week period. However, this claim is not fully supported by the data which shows quite clearly that the AUC's and C_{max}'s are higher after 12 weeks than the proceeding day 1 and week 6 analysis (tables 7-8 taken from the report). The petitioners fail to provide an explanation for the non-linear increases in exposure with increasing dosage but it is most likely due to a saturation of elimination either by the biliary or renal route and is not due to saturation of metabolism since similar AUC and C_{max} profiles are produced for the parent compound and its metabolite. This non-linearity was similarly identified in the specific dog PK and metabolism studies (PCR1029). Both oral and IV studies showed that less than half of the absorbed dose of neotame was excreted in the urine, indicating biliary excretion is a major component in the elimination process. The fact that neotame is cleared from the dog's systemic system by biliary excretion and that we have saturation of elimination occurring at higher dosages, which is associated with considerable liver toxicity and increased circulating cholesterol and ALP levels producing

significant bodyweight changes, leads us to conclude that these phenomenon are inextricably linked. It is well known clinically that blood cholesterol and alkaline phosphatase levels rise due to either bile duct obstruction or hepatocellular disease/toxicity (3).

The petitioners were obviously clearly aware of these toxic effects in the 13-week study and as a result they reduced the dosages for the 52-week dog study (PCR 1017) presumably in an attempt to dilute the above noted effects. However the same adverse liver and clinical chemistry effects are clearly evident even at these reduced dosages. The results show clearly again that there are significant dose dependent increases in the serum alkaline phosphatase levels throughout the study. These reach highly significant levels at the top dose in males and the top two doses in females, but increases are still apparent at 60 mg/kg/day. The petitioners claim that these effects are reversible and not associated with toxicity. However, the results show that even after the reversibility phase, the ALP levels were still significantly higher than in the controls. After 56 weeks the ALP levels were still 1.5 and 2.2 times higher in the females at 200 and 800mg/kg/day and 1.2 and 2.2 times higher in the males respectively (table 9). The increases in ALP are again associated with increases in blood cholesterol levels. For males, there is a clear dose dependent increase in this parameter throughout the treatment period (tables 10-12). The dose dependent increase in females (tables 13-14) however, is not so pronounced, but this is due in part to some suspect control results which are shown to be very variable during the treatment period unlike those animals which were administered test material.

The petitioners also claim that that there were no organ weight changes. However, just as in the 13-week study, there is a clear dose dependent increase in absolute and relative liver weights (to bodyweight and brain weight) with some of these increases again reaching statistical significance. As in the 13-week study, these effects are not seen to be reversible, as the relative liver to bodyweight at the top dose is still some 13.8% and 17.8% higher in males and females respectively after the reversibility period in comparison to control (table 15). Likewise, the petitioners claim that there was no microscopic findings in the liver. However, all of the animals in both sexes except one showed signs of hepatocellular vacuolation at the top dose. The only other group where such effects were noted was the control group. The fact that no such effects were seen in the intermediary doses but were clearly apparent in the controls make these results extremely suspect, especially as all the other clinical data highlighted points to the fact that adverse liver effects are suspected in the treatment groups. The increases in hepatocellular vacuolation may therefore be related to the increases and accumulation of blood ALP, triglycerides and cholesterol. As in the previous study, these effects are associated with white/gray feces which are apparent at doses of 60mg/kg/day and higher.

Overall, the combination of the effects seen in dogs administered neotame are of great concern. A major route of neotame excretion has been shown to occur via the biliary route of elimination. This, coupled with the increases in ALP, cholesterol, triglycerides, discolored feces and increases in liver weight with associated pathology, indicates that a major adverse effect is occurring in the functioning of the liver and biliary system. The toxic nature of this compound and its metabolite is also indicated in the bodyweight gain data generated in the 13-week study. The study showed large decreases in bodyweight gain were associated with small decreases and even increases in food intake. The effects are also apparent in the 52-week study.

These adverse effects are of particular concern because the problems have been produced in the dog, which is the species most similar to man (physiologically and anatomically), and which handles compounds most like that of man (pharmacokinetics). As the dog is the closest species to man in which long term toxicity studies are normally undertaken (except primates which were not tested in this instance), the adverse effects noted can been deemed to be highly significant for man. The effects noted in the dog are consistent with those of either hepatocellular toxicity or obstruction of the bile duct. Due to the likelihood of such an effect occurring in man following consumption of this product, it is therefore essential that such

effects be studied further, especially as both of the dog studies have shown effects at dosages as low as 60mg/kg /day.

In order to establish an ADI, it is necessary to take the No Effect Level (NEL) in mg/kg/day from the pivotal animal study, which, in this case is the dog, because of its degree of similarity to man. The effective NEL established in both of the dog studies can therefore be established as only 20mg/kg/day due to adverse effects occurring at higher levels. Since the effects occurring in the dog are considered to be highly relevant for human risk assessment, this should warrant at least a safety factor of 100, which when applied to the NEL, gives a maximum ADI of only 0.2mg/kg/day. However, sometimes when a toxicity of this nature is uncovered it is deemed prudent based on the category of food additive and its high potential usage, to consider a higher safety factor. Such a response would obviously reduce the ADI still further.

A Neotame-Induced Effect on Implantation Loss, Fetal Size and Limb Development In the Rabbit Teratology Studies May Be Masked by the Quality of the Studies and The High Background Incidences of Effects.

Both the maternal toxicity range-finding study administered by gavage to the rabbit and the teratology study in the rabbit by gavage indicates a direct toxic effect on the rabbit's reproduction cycle, embryogenesis and the fetus. The petitioners point out that these effects are due to the fact that neotame effects bodyweight gain. However, there are only limited effects on food intake. Also, these responses were not due to secondary effects such as gastro-intestinal disturbances or diarrhea and therefore, must be considered to be a direct toxic effect due to the systemic concentrations of neotame.

In the maternal toxicity range-finding study (PCR 1038), the petitioners claim that there were no test article-related effects on fetal resorptions or post implantation loss at dosages up to 1000mg/kg/day. This was due to the fact that the control animals were reported to have a high post-implantation loss (19.1%), which was even higher than the highest value recorded for any animal in the historical control data from the last eleven studies (mean 11.8%, high 17.9%). However, on close inspection of the data, it is apparent that the control data are artificially high because the petitioners have included the data from an animal that aborted on day 29 that had a post-implantation loss of 40%. It is not customary practice to include data from aborted animals. If this animal's data are removed from the calculations as is warranted with this type of study, the post implantation loss in the controls is reduced to 13.3%, which more closely matches the historical mean value of 11.8%. When the post implantation data from the treated groups are re-assessed in light of the above change, it becomes apparent that there is a dose dependent increase in this parameter (i.e. 10.1 to 15.1 to 6.4 to 17.5 and 25.0), indicating neotame affects embryogenesis which probably accounts for the abortion rate in the high dose group. This study also showed that these effects were not due to maternal toxicity, since only minimal effects were noted on the food intake and bodyweight gain of most of the animals even in the 1000mg/kg/day dose group.

The definitive rabbit teratology study (PCR 1023) was then conducted at dosages up to only 500mg/kg/day. No explanation was provided by the petitioner as to why the top dose had been reduced by 50%, especially as there was only limited maternal toxicity in the dose range finding study at 1000mg/kg/day. The international guidelines (4) stipulate that some form of maternal toxicity is a necessity when undertaking this type of study. The low top dose level of 500mg/kg/day is also puzzling given that neotame is supposedly an inert, non-toxic food additive. This dosage is much lower than has been tested for other food additives in its class. If this were to be the case, surely the petitioner would be looking to express the compound's lack of toxicity by dosing at the limit dose. This is clearly not the case.

Overall, the results and interpretation of this study are scientifically flawed given that a number of the results reported for the control group are greatly different from the historical control data. The fact that the control data are so much different from the historical control data makes interpretation of the data from the treated animals very difficult. For example, the control animals show a post implantation loss of 22.1%, which is some 4.2% higher than the maximum level recorded for any animal in the last eleven studies. (It would be worthwhile knowing how close with respect to time the historical control studies had been conducted; only those studies conducted near to the time of the study are relevant due to the strain change over time.) Likewise, the number of small fetuses were greater in the controls than in the historical control group, as were the bilateral and unilateral increased renal pelvic cavitation and the incidence of incomplete and unossified metacarpals and/or phalanges and the unossified heads of limb long bones and the incidence of a folded retina. Such high background incidences precludes the usefulness of the study and indicates that the study should be repeated.

The fact that there was limited, if any, maternal toxicity at the top dose group of 500mg/kg/day also provides further evidence that the study should be repeated. In fact, the report states food intakes and bodyweight gains were similar to control for the first week of dosing and then this changed slightly during the second week for the top dose group only. A possible reason for the change in food intakes and bodyweight gain during the second week could be related to the fact that during the first week, the top-dose animals only received 450 mg/kg/day, which is some 50mg/kg/day or 10% less than the intended dose. During the second week this was increased to the intended dosage of 500mg/kg/day; *i.e.* there was a problem in the preparation of the dosages (see table 16). The increased dosage would therefore affect blood levels during this period. A further reason for the effects on the dam during the second week could also be related to the systemic accumulation; *i.e.* higher C_{max} and AUC of the parent compound. This is shown most graphically in the report (figure 5). In fact, the plasma AUC of neotame at day 14 of dosing is four times higher than on day 1 at 50mg/kg/day and two times higher at 500mg/kg/day. The accumulation of the parent compound over time in the rabbit is also very difficult to understand given the very high clearances reported for other species in the database. The results also suggest that the animals that had a marked decrease in food consumption during the second week of administration also had the highest blood levels of neotame, indicating further a direct mechanism of embryo/fetal toxicity.

The skeletal examinations also identified a greater number of very worrisome vertebrae and limb defects in the top dose group of 500mg/kg/day, some occurring at incidences way above those seen in the historical control data. These include the incidence of incomplete or asymmetric costal elements of sacral vertebrae, 26 pre-sacral vertebrae, the incidence of incomplete and unossified metacarpals and/or phalanges, the incidence of asymmetric and double association pelvis, and the incidence of a small anterior fontanelle.

Overall, the two rabbit studies indicate that neotame has a direct systemic toxic effect on the embryo and fetus. Blood plasma and PK data indicate that these toxic effects occur at doses around 500mg/kg/day and result from the fact that the parent compound accumulates systemically during the two-week period. This leads to death and abortion at doses of 500mg/kg and greater. The abortion may be related to the higher incidence of post implantation loss indicated in the first study but which could not be verified in the repeat study due to the high incidence seen in the control animals. The petitioners concluded that the effects on pregnancy are related to a decrease in food intake. While it is agreed that in some animals there was a reduction in food intake, this was not related to any G-I disturbance, diarrhea or hormone imbalance, and it must therefore be concluded that the effects are due to the systemic accumulation and toxicity of neotame. This is further supported in the fetal observation reports where it can be seen clearly that 500mg/kg/day of neotame increases the incidence of a number of skeletal malformations. It must be pointed out also that these effects may have been more numerous and severe if the correct dosage of 500mg/kg/day had been administered throughout the study.

Conclusion

Scientific studies have been carried out to establish the safety of neotame for human consumption. These studies have highlighted potential concerns of neotame toxicity which, given the similarity in structure with that of aspartame, is difficult to reconcile. In the long-term dog studies the petitioners have failed to establish the relationship between the kinetics of the compounds and the adverse toxic effects on the animal and in particular, the liver and biliary system. Likewise, the effects noted during rabbit reproduction studies indicate an adverse effect on embryogenesis and fetal development. Because of the potential widespread use of neotame, concerns regarding the potential toxic effects of neotame indicate that neotame should not be approved at this time unless more reliable studies are conducted to address these specific concerns.

1) Toxic Responses of the Liver. G.L. Plaa, Casarett & Doull's Toxicology 1986.

2) Sterol Metabolism and its Control. A. White, P. Handler, E. Smith, R. Hill, & I. R. Lehman, Principles of Biochemistry 1978.

3) Liver & Biliary System. W.F. Ganong, Review of Medical Physiology 1979.

4) ICH Guidelines, 1993.

Table 1.

SUMMARY OF CLINICAL CHEMISTRY DATA

MALES WEEK 2

DOSE MG/KG		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI† MG/DL	CHOL MG/DL	TRIG MG/DL
0	MEAN	100	9	.7	5.8	3.4	2.4	1.5	.1	156	30
	S.D.	5.1	1.6	.08	.26	.05	.27	.20	.00	13.9	3.7
	N	6	6	6	6	6	6	6	6	6	6
60	MEAN	96	10	.7	5.5	3.2	2.3	1.4	.1	168	28
	S.D.	11.6	2.2	.05	.45	.29	.24	.16	.05	39.0	6.2
	N	4	4	4	4	4	4	4	4	4	4
200	MEAN	96	10	.8	5.6	3.4	2.2	1.5	.1	176	36
	S.D.	7.2	1.0	.06	.13	.13	.15	.15	.00	29.3	5.2
	N	4	4	4	4	4	4	4	4	4	4
600	MEAN	95	10	.7	5.9	3.4	2.5	1.4	.1	194	39
	S.D.	5.8	1.9	.04	.24	.22	.08	.10	.04	34.5	8.8
	N	6	6	6	6	6	6	6	6	6	6
1,200	MEAN	89	11	.8	5.8	3.4	2.3	1.5	.1	189	45**
	S.D.	6.6	2.0	.05	.19	.15	.15	.13	.00	39.7	10.2
	N	6	6	6	6	6	6	6	6	6	6

* $P \leq 0.05$, Dunnett's t-test.
** $P \leq 0.01$, Dunnett's t-test.
*** $P \leq 0.001$, Dunnett's t-test.
† $P \leq 0.01$, Levene's test for homogeneity of variance; ANOVA done on ranked data.
§ Not analyzed statistically.

Table 2.

SUMMARY OF CLINICAL CHEMISTRY DATA

MALES WEEK 6

DOSE MG/KG		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI† MG/DL	CHOL MG/DL	TRIG MG/DL
0											
	MEAN	101	15	.8	5.6	3.4	2.2	1.6	.0	154	56
	S.D.	3.0	3.7	.08	.28	.10	.33	.23	.05	15.5	8.7
	N	6	6	6	6	6	6	6	6	6	6
60											
	MEAN	102	16	.8	5.4	3.2	2.2	1.5	.0	157	52
	S.D.	5.3	2.5	.10	.39	.36	.13	.22	.06	31.4	10.1
	N	4	4	4	4	4	4	4	4	4	4
200											
	MEAN	97	14	.8	5.6	3.6	2.1	1.7	.0	179	58
	S.D.	4.3	.8	.05	.24	.24	.14	.22	.05	33.6	15.2
	N	4	4	4	4	4	4	4	4	4	4
600											
	MEAN	99	13	.8	5.8	3.4	2.4	1.4	.0	175	68
	S.D.	4.8	3.1	.05	.33	.15	.21	.08	.05	32.0	15.7
	N	6	6	6	6	6	6	6	6	6	6
1,200											
	MEAN	94*	14	.8	5.8	3.4	2.4	1.4	.1	171	88*
	S.D.	3.4	3.5	.06	.19	.09	.19	.12	.00	40.9	30.1
	N	6	6	6	6	6	6	6	6	6	6

* $P \leq 0.05$, Dunnett's t-test.
** $P \leq 0.01$, Dunnett's t-test.
*** $P \leq 0.001$, Dunnett's t-test.
† $P \leq 0.01$, Levene's test for homogeneity of variance; ANOVA done on ranked data.
§ Not analyzed statistically.

Table 3.

SUMMARY OF CLINICAL CHEMISTRY DATA

FEMALES WEEK 2

DOSE MG/KG		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI† MG/DL	CHOL MG/DL	TRIG MG/DL
0											
	MEAN	101	10	.8	5.6	3.4	2.2	1.5	.1	144	33
	S.D.	4.8	2.3	.08	.27	.13	.19	.10	.00	27.9	5.8
	N	6	6	6	6	6	6	6	6	6	6
60											
	MEAN	104	12	.8	5.8	3.5	2.2	1.6	.1	163	30
	S.D.	3.9	1.7	.06	.13	.13	.13	.14	.05	34.7	5.5
	N	4	4	4	4	4	4	4	4	4	4
200											
	MEAN	93	10	.8	5.8	3.4	2.4	1.5	.1	177	35
	S.D.	19.5	1.7	.05	.19	.08	.24	.19	.00	32.9	8.5
	N	4	4	4	4	4	4	4	4	4	4
600											
	MEAN	98	12	.8	5.9	3.6	2.4	1.5	.1	188	32
	S.D.	4.8	2.7	.09	.37	.25	.24	.17	.06	54.9	8.4
	N	6	6	6	6	6	6	6	6	6	6
1,200											
	MEAN	88	12	.8	5.7	3.6	2.1	1.8	.1	186	43
	S.D.	7.5	3.1	.04	.35	.20	.29	.29	.04	25.1	9.3
	N	6	6	6	6	6	6	6	6	6	6

* $P \leq 0.05$, Dunnett's t-test.
** $P \leq 0.01$, Dunnett's t-test.
*** $P \leq 0.001$, Dunnett's t-test.
† $P \leq 0.01$, Levene's test for homogeneity of variance; ANOVA done on ranked data.
§ Not analyzed statistically

Table 4.

SUMMARY OF CLINICAL CHEMISTRY DATA

FEMALES WEEK 6

DOSE MG/KG		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI† MG/DL	CHOL MG/DL	TRIG MG/DL
0											
	MEAN	96	15	.8	5.6	3.4	2.2	1.6	.0	139	66
	S.D.	7.4	3.0	.08	.23	.16	.19	.17	.05	17.9	19.7
	N	6	6	6	6	6	6	6	6	6	6
60											
	MEAN	103	16	.8	5.7	3.6	2.0	1.8	.0	149	66
	S.D.	6.7	3.6	.10	.17	.21	.10	.17	.05	26.6	9.5
	N	4	4	4	4	4	4	4	4	4	4
200											
	MEAN	96	14	.8	5.7	3.5	2.2	1.6	.0	171	59
	S.D.	12.4	2.5	.05	.17	.08	.15	.13	.06	28.6	13.4
	N	4	4	4	4	4	4	4	4	4	4
600											
	MEAN	103	14	.8	5.9	3.6	2.4	1.5	.1	192	56
	S.D.	10.5	2.8	.10	.35	.19	.28	.18	.05	63.1	16.5
	N	6	6	6	6	6	6	6	6	6	6
1,200											
	MEAN	96	13	.8	5.6	3.5	2.1	1.7	.1	168	64
	S.D.	6.2	3.2	.04	.22	.27	.14	.25	.04	38.0	10.3
	N	6	6	6	6	6	6	6	6	6	6

* $P \leq 0.05$, Dunnett's t-test.
** $P \leq 0.01$, Dunnett's t-test.
*** $P \leq 0.001$, Dunnett's t-test.
† $P \leq 0.01$, Levene's test for homogeneity of variance; ANOVA done on ranked data.
§ Not analyzed statistically.

Table 5.

Summary of Liver-Bodyweight Percentages (Relative Liver Weight) following the Reversibility Phase

Male

	Dose mg/kg/day		
	0	**200**	**600**
Mean	2.7776	3.1816	3.0855
% Increase in Size		14.5%	11%

Female

	Dose mg/kg/day		
	0	**200**	**600**
Mean	2.4396	2.5665	3.2406
% Increase in Size		5.2%	32.8%

Table 6.

Summary of Cumulative-Bodyweight & Food Intake Data (g)
Throughout the Study

Male

	Dose mg/kg/day				
	0	**60**	**200**	**600**	**1,200**
Bodyweight Gain	2,817	2,700	2,650	2,383	1,200
Food Intake	25,979	27,466	25,411	26,290	20,321

Female

	Dose mg/kg/day				
	0	**60**	**200**	**600**	**1,200**
Bodyweight Gain	2,300	2,025	1,850	1,750	567
Food Intake	25,584	25,013	26,373	24,780	19,632

Table 7.

Monsanto Study PCR 1019
Sponsor's Summary

Pharmacokinetic Parameters of NC-00723 in Dogs Given NC-00723 in the Diet for 13 Weeks

AUC

Dose mg/kg		DAY 1 AUC µg.hr/mL	WEEK 6 AUC µg.hr/mL	WEEK 12 AUC µg.hr/mL
60	Mean	1.37	1.67	1.68
	SD	0.67	0.69	0.44
200	Mean	8.29	4.82	7.81
	SD	4.06	2.91	3.19
600	Mean	18.72	24.61	29.48
	SD	9.35	7.22	14.16
2000/1200[a]	Mean	14.04	76.03	107.63
	SD	14.11	28.50	36.25

Cmax

Dose mg/kg		DAY 1 Cmax µg/mL	WEEK 6 Cmax µg/mL	WEEK 12 Cmax µg/mL
60	Mean	0.23	0.25	0.23
	SD	0.13	0.12	0.09
200	Mean	1.20	0.68	0.96
	SD	0.56	0.30	0.36
600	Mean	3.57	3.52	4.6
	SD	1.64	1.02	1.91
2000/1200	Mean	5.28	10.04	14.09
	SD	5.02	1.70	4.43

a - After 2 weeks, the daily dosage of this group was reduced from an offered dosage of 2000 mg/kg to a nominal dose of 1200 mg/kg

Table 8.

Monsanto Study PCR 1019
Sponsor's Summary

Pharmacokinetic Parameters of NC-00751 in Dogs Given NC-00723 in the Diet for 13 Weeks

AUC

Dose Mg/kg		DAY 1 AUC µg.hr/mL	WEEK 6 AUC µg.hr/mL	WEEK 12 AUC µg.hr/mL
60	Mean	8.33	9.19	9.92
	SD	3.59	3.49	3.26
200	Mean	42.79	24.03	36.28
	SD	32.03	9.50	14.65
600	Mean	71.20	96.23	112.55
	SD	27.90	20.87	41.98
2000/1200[a]	Mean	57.59	262.99	384.33
	SD	62.89	94.98	102.02

Cmax

Dose Mg/kg		DAY 1 Cmax µg/mL	WEEK 6 Cmax µg/mL	WEEK 12 Cmax µg/mL
60	Mean	0.77	0.93	0.87
	SD	0.33	0.51	0.26
200	Mean	4.17	2.32	3.06
	SD	2.32	0.77	0.91
600	Mean	9.80	10.58	12.64
	SD	4.37	2.22	6.22
2000/1200	Mean	13.13	26.67	41.27
	SD	12.19	4.26	13.02

a - After 2 weeks, the daily dosage of this group was reduced from an offered dosage of 2000 mg/kg to a nominal dose of 1200 mg/kg

Table 9.

Summary of Clinical Chemistry Data
Alkaline Phosphatase IU/L – Week 56

Male

	Dose mg/kg/day		
	0	**200**	**800**
Mean	44	50	94

Female

	Dose mg/kg/day		
	0	**200**	**800**
Mean	57	84	128

Table 10.

Covance 6211-304

SUMMARY OF CLINICAL CHEMISTRY DATA

MALES WEEK 13

DOSE MG/KG/DAY		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI MG/DL	CHOL MG/DL	TRIG MG/DL
0											
	MEAN	102	14	1.0	6.0	3.4	2.5	1.4	.1	160	33
	S.D.	5.1	1.9	.10	.30	.23	.29	.18	.04	10.5	5.6
	N	6	6	6	6	6	6	6	6	6	6
20											
	MEAN	104	16	1.0	6.0	3.5	2.6	1.4	.1	179	43
	S.D.	5.7	1.9	.05	.10	.05	.05	.00	.00	31.4	10.2
	N	4	4	4	4	4	4	4	4	4	4
60											
	MEAN	98	13	1.0	6.2	3.4	2.8	1.2	.1	166	40
	S.D.	2.8	1.7	.06	.25	.16	.19	.13	.00	15.4	5.6
	N	4	4	4	4	4	4	4	4	4	4
200											
	MEAN	101	13	.9	6.0	3.4	2.6	1.4	.1	176	41
	S.D.	4.4	2.8	.04	.29	.22	.37	.27	.00	35.2	7.9
	N	6	6	6	6	6	6	6	6	6	6
800											
	MEAN	97	14		6.1	3.4	2.7	1.3	.1	195	45
	S.D.	6.9	2.0		.24	.16	.15	.08	.04	26.3	5.9
	N	6	6		6	6	6	6	6	6	6

Table 11.

Covance 6211-304

SUMMARY OF CLINICAL CHEMISTRY DATA

MALES WEEK 26

DOSE MG/KG/DAY		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI MG/DL	CHOL MG/DL	TRIG MG/DL
0											
	MEAN	97	15	1.1	6.5	3.6	2.8	1.3	.0	159	27
	S.D.	5.0	1.8	.13	.44	.31	.33	.18	.05	13.5	4.6
	N	6	6	6	6	6	6	6	6	6	6
20											
	MEAN	100	16	1.1	6.6	3.5	3.1	1.1	.1	167	38
	S.D.	1.0	2.2	.10	.23	.18	.08	.05	.05	21.3	10.7
	N	4	4	4	4	4	4	4	4	4	4
60											
	MEAN	97	12	1.0	6.7	3.4	3.2	1.0	.0	154	30
	S.D.	3.9	3.1	.13	.37	.13	.26	.06	.05	30.4	8.6
	N	4	4	4	4	4	4	4	4	4	4
200											
	MEAN	95	12	.9*	6.3	3.4	2.9	1.2	.1	172	35
	S.D.	9.7	1.0	.10	.41	.35	.35	.21	.04	35.7	5.5
	N	6	6	6	6	6	6	6	6	6	6
800											
	MEAN	91	12*	.9	6.7	3.5	3.2	1.1	.1	202	32
	S.D.	6.5	1.8	.10	.30	.23	.27	.13	.06	36.1	5.0
	N	6	6	6	6	6	6	6	6	6	6

* $P \leq 0.05$, Dunnett's t-test

APP-12927

Table 12.

Covance 6211-304

SUMMARY OF CLINICAL CHEMISTRY DATA

MALES WEEK 39

DOSE MG/KG/DAY		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI MG/DL	CHOL MG/DL	TRIG MG/DL
0											
	MEAN	92	15	1.1	6.5	3.6	2.9	1.2	.1	145	29
	S.D.	5.1	1.7	.13	.37	.28	.35	.20	.08	15.3	4.3
	N	6	6	6	6	6	6	6	6	6	6
20											
	MEAN	103	17	1.2	6.8	3.6	3.3	1.1	.1	160	48
	S.D.	2.6	.8	.10	.24	.17	.26	.14	.10	31.2	16.5
	N	4	4	4	4	4	4	4	4	4	4
60											
	MEAN	97	13	1.0	6.9	3.4	3.5*	1.0	.0	145	34
	S.D.	4.3	2.8	.17	.31	.18	.24	.10	.05	21.1	9.0
	N	4	4	4	4	4	4	4	4	4	4
200											
	MEAN	100	13	1.0	6.7	3.5	3.2	1.1	.1	167	38
	S.D.	7.2	2.3	.10	.32	.23	.32	.17	.04	42.8	5.4
	N	6	6	6	6	6	6	6	6	6	6
800											
	MEAN	96	12	1.0	6.8	3.4	3.4*	1.0	.0	178	35
	S.D.	7.4	2.0	.08	.32	.15	.28	.10	.05	34.6	9.4
	N	6	6	6	6	6	6	6	6	6	6

* $P \leq 0.05$, Dunnett's t-test

APP-12931

Table 13.

Covance 6211-304

SUMMARY OF CLINICAL CHEMISTRY DATA

FEMALES WEEK 13

DOSE MG/KG/DAY		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI MG/DL	CHOL MG/DL	TRIG MG/DL
0											
	MEAN	107	14	1.0	6.1	3.6	2.5	1.5	.1	168	36
	S.D.	6.0	2.0	.10	.40	.22	.41	.30	.08	19.3	5.2
	N	6	6	6	6	6	6	6	6	6	6
20											
	MEAN	100	14	1.0	6.1	3.7	2.4	1.5	.1	164	40
	S.D.	10.1	1.0	.05	.16	.08	.14	.13	.00	25.4	10.3
	N	4	4	4	4	4	4	4	4	4	4
60											
	MEAN	102	14	1.0	6.2	3.8	2.4	1.6	.1	183	48
	S.D.	7.6	2.4	.10		.34	.14	.19	.00	36.9	5.8
	N	4	4	4	4	4	4	4	4	4	4
200											
	MEAN	104	14	1.0		3.7	2.4	1.6	.1	186	49
	S.D.	8.0	1.7	.04		.14	.27	.21	.05	30.4	13.2
	N	6	6	6		6	6	6	6	6	6
800											
	MEAN	101	15	.9		3.4	2.5	1.4	.1	186	42
	S.D.	6.4	1.2	0		.14	.29	.14	.04	36.3	2.3
	N	6	6	6		6	6	6	6	6	6

Table 14.

Covance 6211-304

SUMMARY OF CLINICAL CHEMISTRY DATA

FEMALES WEEK 39

DOSE MG/KG/DAY		GLU MG/DL	UN MG/DL	CREAT MG/DL	T PRO G/DL	ALB G/DL	GLOB G/DL	A/G RATIO	T BILI MG/DL	CHOL MG/DL	TRIG MG/DL
0											
	MEAN	101	14	1.1	6.5	3.8	2.8	1.4	.1	166	36
	S.D.	7.7	2.5	.12	.33	.21	.37	.23	.08	16.3	3.7
	N	6	6	6	6	6	6	6	6	6	6
20											
	MEAN	98	14	1.1	6.8	3.7	3.1	1.2	.2	193	34
	S.D.	15.1	3.6	.05	.37	.31	.38	.21	.06	54.4	5.6
	N	4	4	4	4	4	4	4	4	4	4
60											
	MEAN	96	13	1.0	6.6	3.8	2.8	1.4	.0	179	40
	S.D.	12.1	1.0	.13	.16	.29	.17	.17	.05	37.8	11.1
	N	4	4	4	4	4	4	4	4	4	4
200											
	MEAN	103	14	1.0	6.4	3.6	2.8	1.3	.1	201	44
	S.D.	9.6	1.9	.08	.38	.10	.40	.19	.08	48.7	9.2
	N	6	6	6	6	6	6	6	6	6	6
800											
	MEAN	100	15	1.0	6.6	3.5	3.0	1.2	.1	217	43
	S.D.	5.5	1.9	.10	.37	.10	.34	.12	.06	44.7	5.3
	N	6	6	6	6	6	6	6	6	6	6

APP-12933

Table 15.

Summary of Liver-to-Bodyweight Percentages (Relative Liver Weight) Following the Reversibility Phase

Male

	Dose mg/kg/day		
	0	**200**	**800**
Mean	2.6165	2.8365	2.9791

Female

	Dose mg/kg/day		
	0	**200**	**800**
Mean	2.8201	2.5703	3.3233

Table 16.

Formulation analysis – concentration of NC-00723 in formulations prepared for the first and last weeks of treatment

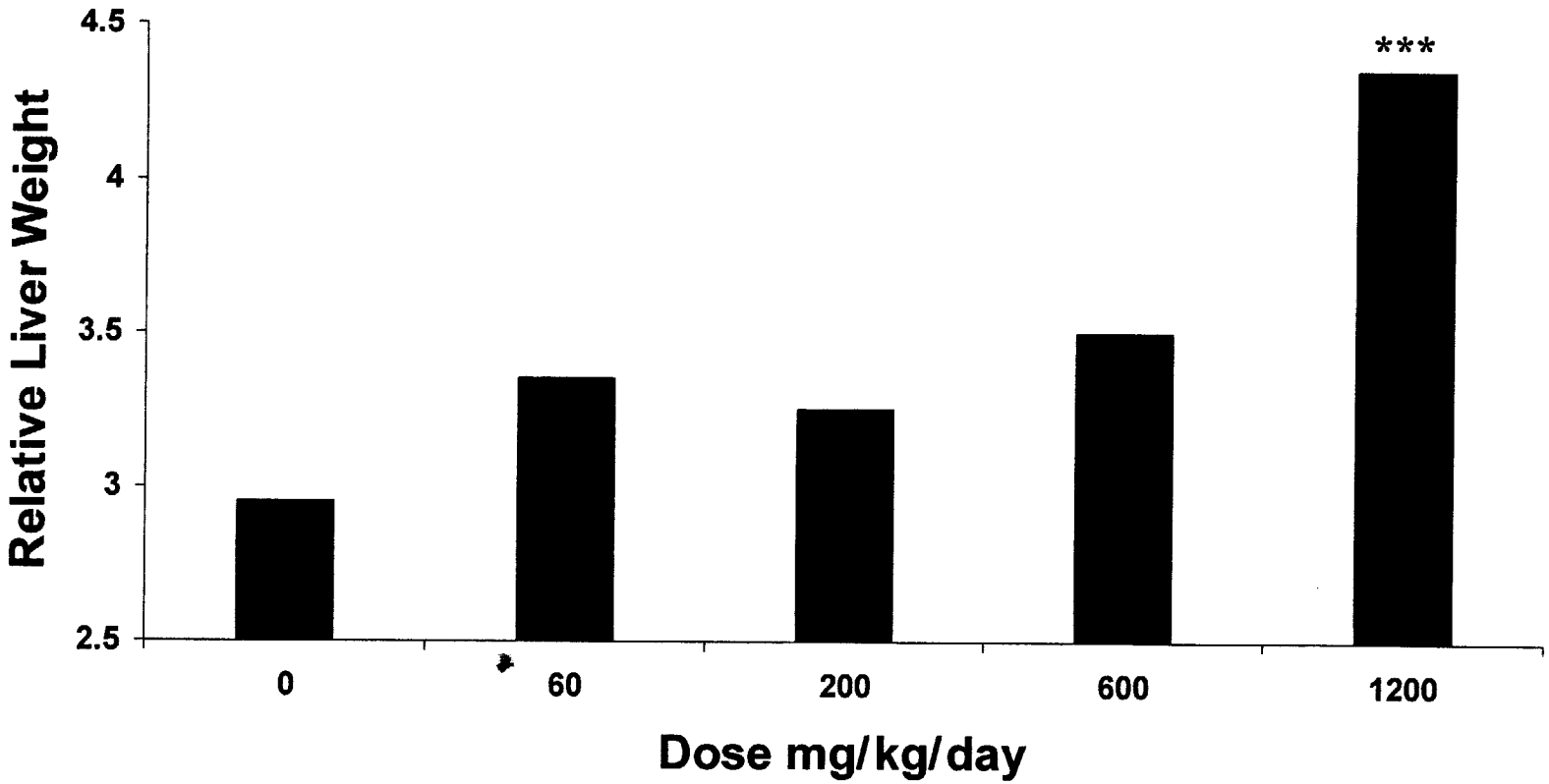
Group	:	1	2	3	4
Compound	:	Control	----------NC00723 ----------		
Dosage (mg/kg/day)	:	0	50	150	500

Group and Sex	Dose level (mg/kg/day)	Intended concentration (mg/ml)	Found concentration (mg/ml)	Mean
First Week				
1F	0	0	ND, ND	ND
2F	50	5	4.96, 5.02	4.99
3F	150	15	13.5, 14.5	14.0
4F	500	50	46.3, 45.3	45.8
Last Week				
1F	0	0	ND, ND	ND
2F	50	5	5.20, 4.84	5.02
3F	150	15	14.3,14.3	14.3
4F	500	50	50.3 49.8	50.1

ND None detected.

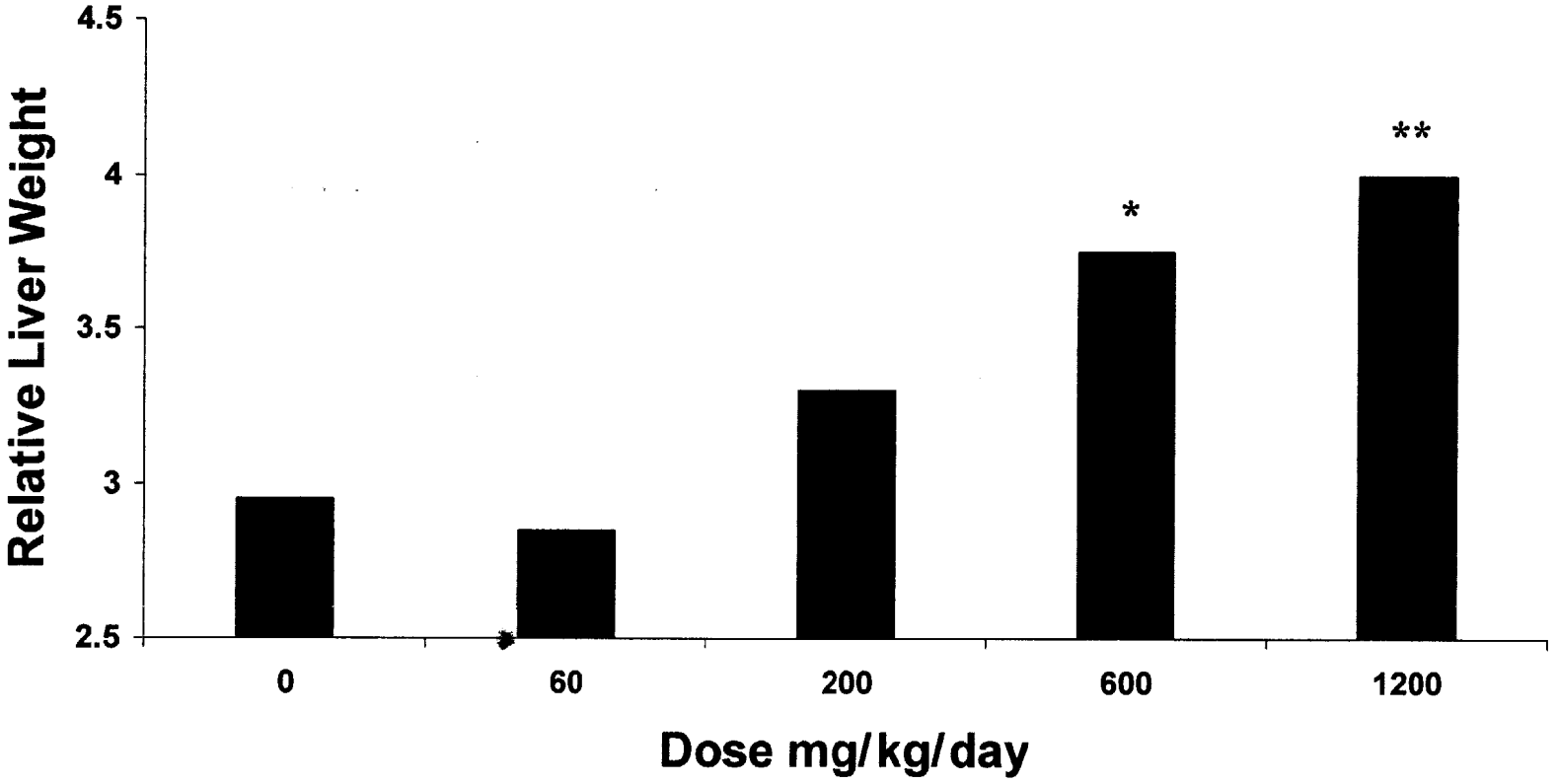
Figure

Male Relative Liver Weights



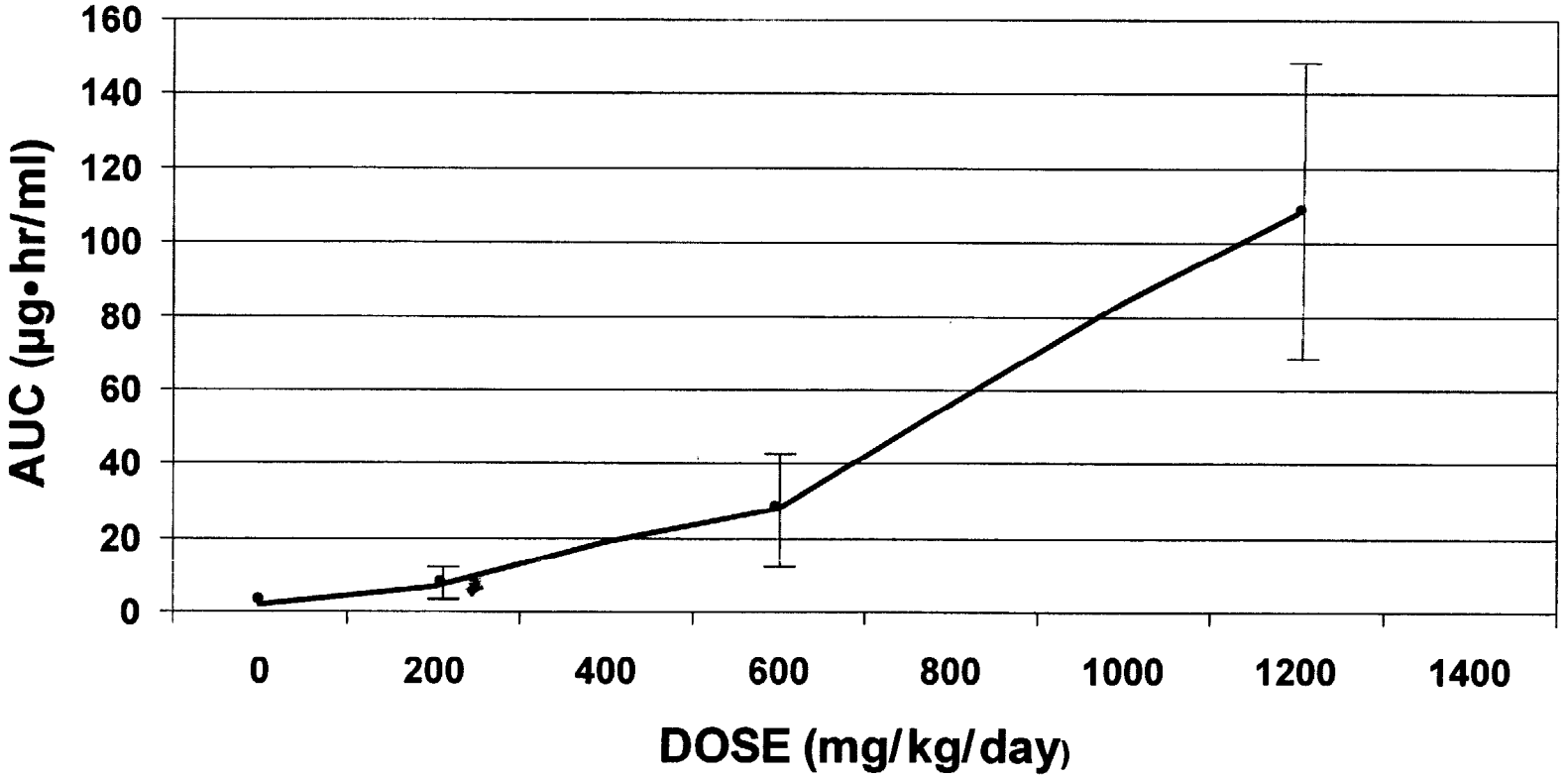
Figure

Female Relative Liver Weights



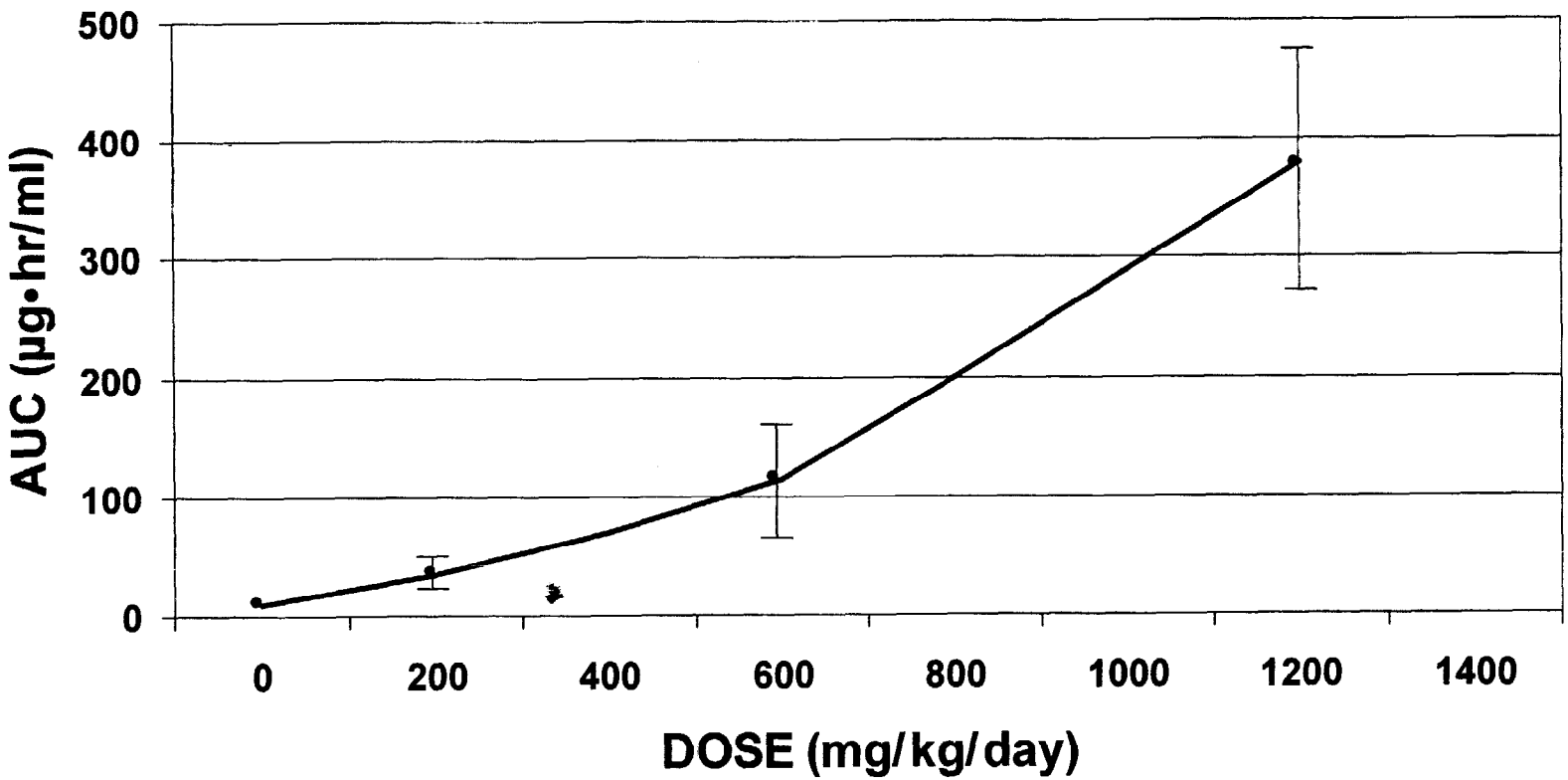
Figure

Mean AUC_{0-24} (±Std Dev) of NC-00723 in Plasma of Dogs given NC-00723 in Diet for 13 Weeks (12 Weeks)



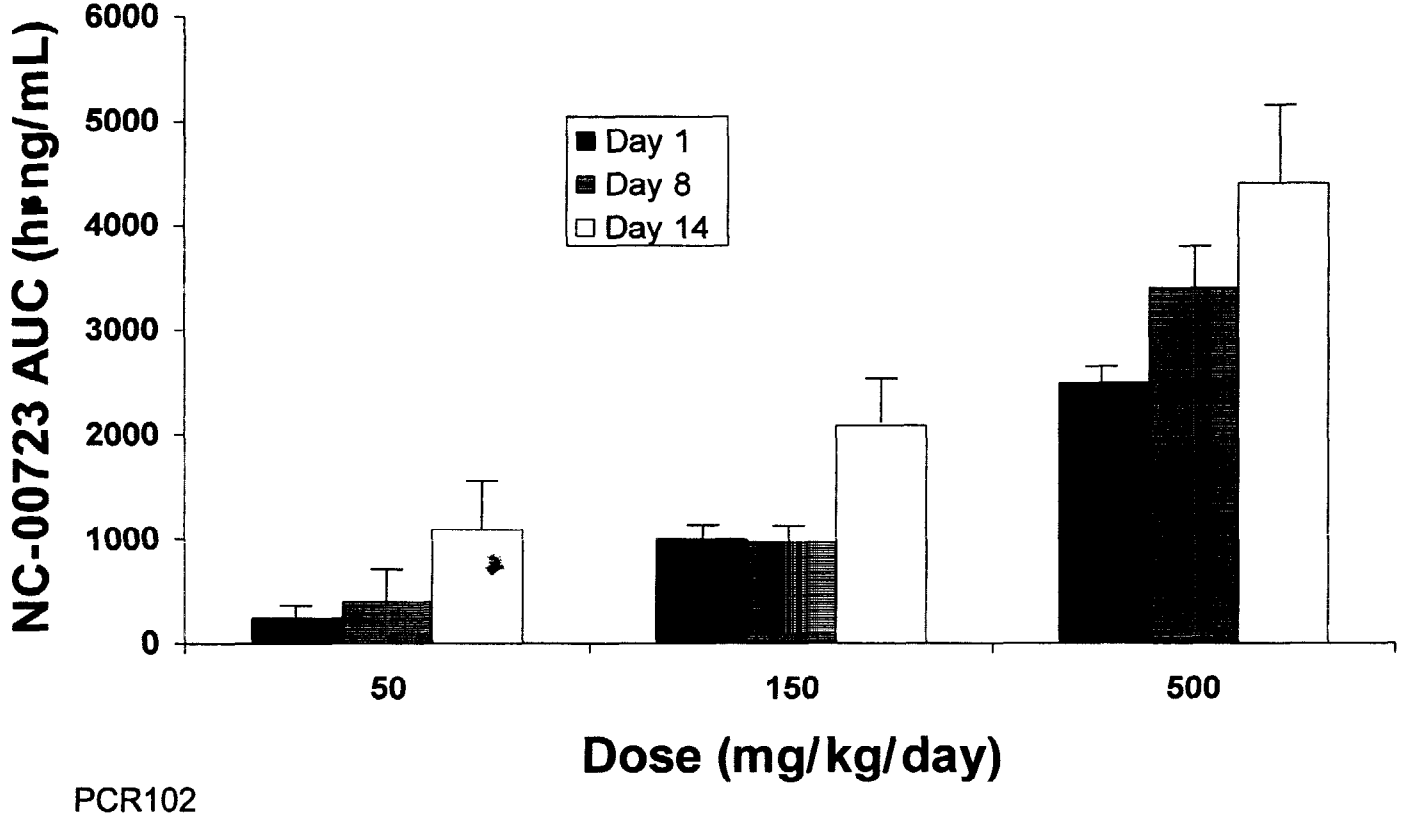
Figure

Mean AUC_{0-24} (±Std Dev) of NC-00751 in Plasma of Dogs given NC-00723 in Diet for 13 Weeks (12 Weeks)



Figure

Plasma AUC's of NC-00723 in Pregnant Rabbits at Different Doses During a Teratology Study (Mean, Standard Error)



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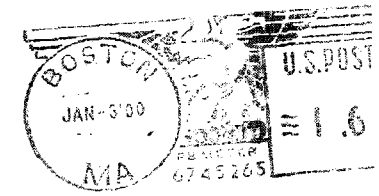

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