

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-977

PHARMACOLOGY REVIEW



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

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| NDA NUMBER: | 21-977 |
| SERIAL NUMBER: | 000 |
| DATE RECEIVED BY CENTER: | 12/06/05 |
| PRODUCT: | NRP-104 |
| INTENDED CLINICAL POPULATION: | Children and adolescents ages 6-12 years |
| SPONSOR: | New River Pharmaceuticals 1861 Pratt Drive Suite 1090 Blacksburg, VA 24060 |
| DOCUMENTS REVIEWED: | Module 4, Sequence 1, Vol. 1-33 |
| REVIEW DIVISION: | Division of Psychiatric Products |
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Date of review submission to Division File System (DFS): 9-21-2006

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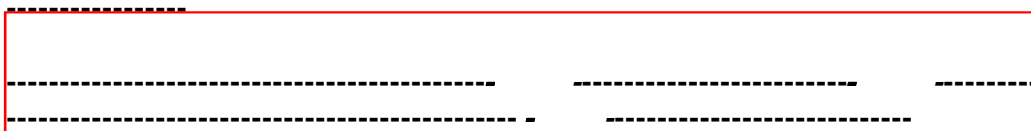
EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: this submission is considered approvable from a pharmacology and toxicology prospective. Some [REDACTED] impurities [REDACTED] are specified in the commercial batch at levels ranging from [REDACTED], while they were either not detected in the non-clinical batches or their levels in the preclinical batch were much less than the specification in the commercial batch (see table under non-clinical issues relevant to clinical use). If the sponsor cannot determine that the levels of these impurities in the non-clinical batches were equal to or above the specification in the commercial batch or if these impurities cannot be removed from the commercial batches then studies to test the toxicity of these impurities are to be conducted. [REDACTED]
- B. Recommendation for nonclinical studies: see the previous section
- C. Recommendations on labeling:

Pediatric use:

[illegible]



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

The submitted studies were generally adequate in evaluating the effects of the compound and in characterizing its effects compared to those of amphetamine. The results indicated that the compound produces its effects almost totally through its metabolite amphetamine. The parent compound was found to be present in minimal amounts in the plasma of rats treated orally and the most prominent metabolite was amphetamine. From conversations with Dr. Andre Jackson, it appears that the parent compound is also present in minimal amounts in humans treated orally as was observed in animals and that the major metabolite was amphetamine (see the clinical pharmacology review). The following sections summarize the different pre clinical studies conducted to characterize the effect of this compound:

Pharmacodynamic:

This compound (NRP104) is considered a prodrug for d-amphetamine since it is composed of lisdexamphetamine dimesylate which is an amphetamine covalently bound to l-lysine by an amide bond that is converted to d-amphetamine *in vivo*. The prodrug itself is not a stimulant; however, since amphetamine is the major product, stimulant effect is seen with treatment. The parent compound does not appear to bind to either the norepinephrine transporter nor to the dopamine transporter when tested *in vitro* using human recombinant transporters. It should also be emphasized that the parent compound has not been detected in the brain of rats treated orally with the compound while amphetamine was detected in the brain in response to this treatment. *In vivo* studies indicated that the compound increases locomotor activity when administered orally to rats similar to d-amphetamine sulfate and produces other clinical signs similar to those seen with d-amphetamine sulfate. However, when administered intravenously or intranasally the increase in activity seen in treated rats was less than that seen with an equivalent dose of d-amphetamine sulfate given through these two routes.

Safety pharmacology:

The effect of the drug on the CVS was assessed in anesthetized beagle dogs treated IV with doses of 0, 0.5, 1, and 5 mg/kg of the test article. In order to compare the effect of the test article to those of amphetamine, the effect of d-amphetamine sulfate was also assessed in a group of animals treated with 0, 0.202, 0.404, and 2.02 mg/kg. The effects of the test article were generally comparable to those of amphetamine, (increases in HR, blood pressure, and cardiac output) with some slight differences (the effect of d-

amphetamine sulfate on blood pressure was slightly higher compared to that with NRP and was seen at an earlier time point, see the review for more details). Sinus tachycardia was observed 30 min post dose in animals treated with the test article at HD in dogs and in one dog treated with amphetamine verntricular extrasystole and sinus tachycardia were observed. In the 28-day study in dogs there were no significant findings observed.

The effect on the CNS was studied within the general toxicity studies and the juvenile animal studies and the general findings were in agreement of the effect of a stimulant on the CNS which included increased activity and stereotypic behavior in treated animals similar to what is seen with amphetamines.

Pulmonary assessment was conducted in anesthetized guinea pigs by IV administration of the test article (0, 1, 5, and 7.5 mg/kg). The results indicated an increase in respiratory rate and minute volume thirty minutes after the treatment.

The effect of the test article on the renal and gastrointestinal systems was not evaluated.

Pharmacokinetics:

The pharmacokinetic characteristics of the test article were studied using different routes of administration (oral, I.V. and I.N.) in rats and in dogs. The parent compound was not detected in the brain of rats following oral administration while d-amphetamine was detected in the brain as a result of this treatment. Following oral administration of NRP-104 in rats, the bioavailability of the parent compound varied with dose. Tmax for the parent compound ranged from 0.25 to 3h at low dose and up to 4-8h at high doses. Cmax for d-amphetamine in plasma following oral administration of NRP-104 (3 mg/kg amphetamine base) was ~one half of Cmax following d-amphetamine sulfate administration in one report and comparable to those of a similar dose in another report (see review for available figures). At higher doses the fraction of amphetamine absorbed as a result of oral administration of NRP-104 decreased compared to lower doses; however, in animals treated with d-amphetamine sulfate the amphetamine absorbed was increased at the highest doses. Following I.V. administration in rats, the plasma concentration of d-amphetamine derived from intact NRP-104 in comparison to d-amphetamine derived from an equimolar dose of d-amphetamine sulfate, were significantly reduced. Similar observations were seen with intranasal administration. The metabolism of the compound following oral administration in rats seems to be fairly simple since the major products were those of amphetamine and amphetamine metabolites. The parent compound was observed only for up to 8 hours after oral administration and the highest levels of the radioactivity produced from the parent compound were less than 2% of the total radioactivity in plasma of F. The levels of radioactivity for the parent compound after I.V. administration were ~20% of the total radioactivity in plasma. The only metabolite that was directly related to the parent compound (M2 or hydroxylated NRP-104) was observed only after I.V. administration. This suggests that after an oral administration, NRP-104 is quickly converted to amphetamine before reaching the plasma circulation. The site of metabolism was not thoroughly tested; however, in vitro testing showed that the liver is not the site of

metabolism for the compound. However, in several places the sponsor stated that the site of metabolism is in the gastrointestinal tract. The major route of elimination of total radioactivity after oral administration in rats is through urine (~77% in M and ~87% in F). The compound did not seem to inhibit a variety of CYP-450 enzymes (see table within review for specific enzymes).

In dogs the pharmacokinetic parameters were evaluated following oral and I.V. administration and that data indicated that the compound has a moderate oral bioavailability (33%) and that plasma levels of d-amphetamine after oral administration of NRP-104 are comparable to those after I.V. administration.

Toxicology:

For detailed description of the studies and findings from these studies please see the overall toxicology summary or the individual study review within this document.

The sponsor conducted the following studies in rats: a single oral dose study, a 7-day oral dose range-finding study, and a 28-day oral toxicity study. The following studies were conducted in dogs: an escalating single oral dose study, a 7-day oral dose range finding study, and a 28-day oral toxicity study.

The single dose studies in rats (doses 0.1, 1, 10, 60, 100, and 1000 mg/kg orally by gavage) and dogs (doses of 3, 10, 18, and 24 mg/kg orally by gavage) were used to evaluate the maximum recommended dose for the long term studies and to evaluate the toxicity of the compound. In rats, the LD50 for NRP-104 was considered to be >1000 mg/kg (equivalent to 399 mg/kg of d-amphetamine), based on the death in 1/3 F and 1/3 M at the 1000 mg/kg, compared to the LD50 for d-amphetamine sulfate of 96.8 mg/kg (equivalent to 70.5 mg/kg of d-amphetamine base). Increased motor activity such as biting and licking of the cage, chromodacryorrhea/chromorhinorrhea, and skin lesions were observed at doses of 60 mg/kg and above. All rats appeared to be normal 4 days after treatment. In dogs, no deaths were observed, increased activity, abnormal gait, restlessness, repetitive behavior, head bobbing and excessive liking were observed at 10, 18, and 24 mg/kg. Circling and emesis were observed at 18 and 24 mg/kg. The MTD for the dogs was considered to be less than 24 mg/kg since emesis was observed in all animals at this dose. The effects of the test article on the observed clinical observations (increased activity and stereotypic behavior) seem to be consistent between the two species.

In the 7-day study in rats (doses 0, 30, 100, and 300 mg/kg orally by gavage) death and self mutilation were observed at 100 and 300 mg/kg and increased activity at all doses. In the 7-day study in dogs (0, 3, 6, or 12 mg/kg/day orally by gavage), no death was observed, increased activity was observed at all doses (seen only on few days at LD) and repetitive behavior, restlessness, vessels over sclera dilated at MD and HD and severe ocular discharge at HD (all seen only on Day 1). Decreases in body wt were observed in both rats and dogs in response to treatment mostly at MD and HD in each species. No histopathology was conducted in these studies.

In the 28-day study rats (10-15/sex/group) were treated with 0, 20, 40, or 80 mg/kg of NRP-104 orally by gavage. Another group of animals (15/sex) were treated with a d-amphetamine sulfate (16 mg/kg). Five animals from the control, HD NRP-104 treated group and d-amphetamine sulfate group were used as a recovery group. There was no death reported but 1/9 F treated with 80 mg/kg in the toxicokinetic group was moribund sacrificed on Day 7 due to self-mutilation. Clinical signs noted in all NRP-104 treated groups and in the d-amphetamine sulfate treated group included increased activity and post dose jumping. Self mutilation and thin body condition were observed in some animals treated with the HD of NRP-104. One F in the d-amphetamine sulfate group had thin condition towards the end of the study. Body wt decreases were observed at MD and HD in the NRP-104 group and in the d-amphetamine sulfate treated group. All animals were normal during the recovery period except for 1M and 1F from HD NRP-104 group with thin body condition for the first few days of the recovery period. Some statistically significant increases in clinical chemistry parameters (glucose, BUN, and ALT) were observed at MD and HD NRP-104 groups. Histopathological changes such as fiber necrosis and degeneration of biceps of thigh muscle in 1/15 F in HD group and degeneration of muscular tone in the esophagus in 2/15 M and 2/15 F were seen at HD. These findings were not considered drug related by the sponsor; however, in the opinion of the reviewer a drug effect cannot be ruled out. Toxicokinetic data indicated that C_{max} and AUC values of NRP-104 were lower than d-amphetamine values in all groups in both M and F. AUC values of both d-amphetamine and NRP-104 were greater at Day 28 than at Day 1 in F and M, particularly in the MD and HD groups. Both AUC and C_{max} were higher in F than in M for all treatment groups.

In the 28-day study, dogs (3-5/sex/group) were treated with 0, 3, 6, and 12 mg/kg/day orally by gavage with an additional group of animals (5/sex) treated with 2.4 mg/kg/day of d-amphetamine sulfate. Two animals per sex from the control, HD NRP-104 treated group and the d-amphetamine sulfate treated group were used for the recovery group (14-days). No deaths were observed. Restlessness and increased activity were observed in few animals at LD (several days), most animals at MD (almost throughout study) and all animals at HD and those treated with d-amphetamine sulfate (throughout the study). Repetitive behavior, head shaking, and pacing in cage were observed in animals treated at MD and HD but they were seen in more animals at HD than at MD. Decreased activity predose was observed in some animals at MD and HD and those treated with d-amphetamine sulfate. Panting, circling and abnormal gait were also observed in some animals treated with HD of NRP-104 and animals treated with d-amphetamine sulfate. Decreases in body wt were observed at MD and HD and in those animals treated with d-amphetamine sulfate and body thinness was observed in some animals at HD and in the d-amphetamine sulfate treated group. There were some decreases in reticulocytes at MD and HD. During the recovery period, a decrease in body wt and body thinness was seen in some animals treated with NRP-104 and d-amphetamine sulfate and decreased activity was seen in 1M treated with HD NRP-104. There were no ophthalmology findings and no ECG findings at the tested times. There were no significant histopathological findings.

The conducted 28-day toxicology studies are considered adequate and the results indicated that an MTD had been reached in those studies in both rats (sacrifice of one animal due self sustained injuries, self mutilation, and the effects on body wt at HD) and in dogs (behavioral abnormalities including restlessness, head shaking, pacing in cage, panting, circling and the effect on body wt at HD). The addition of the group treated with the d-amphetamine sulfate in these studies was valuable since it was appropriate to compare the effect of this compound to the effects of d-amphetamine (the proposed metabolite). According the sponsor's calculations, the doses used for NRP-104 in these studies were comparable to those doses used for the d-amphetamine sulfate group based on the d-amphetamine base value. By comparing the results obtained from treatment with NRP-104 with those with d-amphetamine sulfate, it was evident that the effects of the compound are very similar to those of d-amphetamine sulfate and thus indicating that this compound is acting almost totally through its metabolite d-amphetamine.

Genetic toxicology: the compound was tested in the Ames test, in vitro mouse lymphoma assay and the in vivo micronucleus assay. The overall outcome of the studies indicated that the compound is not genotoxic in any of the tests used. For more details about the studies and the outcomes please see the review for these individual studies.

Carcinogenicity: no studies were conducted. Since the compound is metabolized to amphetamine with minimal amounts of the parent compound present, carcinogenicity studies were not requested. Carcinogenicity studies for amphetamine have been performed by the National Toxicology Program (NTP) and are described in the Adderall labeling.

Reproductive toxicology: no studies were conducted. Studies were not requested for the same rationale why carcinogenicity studies were not requested. Animal reproduction studies of amphetamine are described in the Adderall labeling.

Special studies (juvenile animal studies in rats and dogs):

Rat study:

Rat pups were treated with 0, 4, 10, and 40 mg/kg/day orally by gavage from PND 7 to 63 inclusive. Four subgroups were used in the study:

- Subgroup A (toxicity study): animals were treated from PND 7 to 63. Animals were evaluated for parameters usually done in a general toxicology study and for physical development (crown-to-rump length), functional observational battery (FOB), motor activity and auditory startle habituation. These evaluations were conducted during or at the end of the treatment period. Animals were sacrificed on PND 64
- Subgroup B (regression study): in addition to evaluations done during the treatment period (PND 7-63) such as physical development, preputial separation, auditory startle habituation and vaginal opening, animals were evaluated for the following at the end of a 28 day regression period: FOB, motor activity, auditory

- startle habituation, and Cincinnati water maze. Animals were sacrificed on PND 92.
- Subgroup C (reproductive study): animals were treated from PND 7-63 and then were mated at approximately 85 days of age. The animals were evaluated for the Cincinnati water maze between PND 52-61, for estrous cycle 10 days prior to mating, mating (PND 85) and then they were sacrificed after Day 26-28 post coitum. Paternal performance (mating index, fertility index and conception rate) and maternal performance (gestation index, duration of parturition, # of pups at birth, and #of implantation scars) were also evaluated. The F2 generation pups and litters were observed for death, external malformations, weighed, sexed, and were observed through the lactation period.
 - Subgroup D (toxicokinetic study): blood was collected on PND 64

A toxicokinetic group was also used to measure plasma levels after one day of treatment (PND 7).

Deaths were observed in all groups (1M from control group due to gavage error, 1F from LD, 1F from MD and 1M & 1F from HD group), all of which the sponsor considered as non-drug related. The reviewer considers the death of the 1M from the HD group as possibly drug related since clinical signs seen prior to death included thinness, decreased activity, moderate dehydration, and cold to touch which might indicate that the death was due to deteriorating condition caused by drug treatment. The immediate effects of the drug observed in the toxicity study (increased activity and stereotypic behavior) are similar to those of an amphetamine. In addition, the effect on body weight (decreases seen at MD and HD in M and at HD in F) is also similar to what is usually observed with amphetamine. The decrease observed in M continued to be seen at the end of the regression period. It was clear from the results also that the test article had an effect on the growth of pups as judged by the decrease in length of the crown-to-rump at HD in both M & F. A decrease in the other M treated groups (LD & MD) was also seen towards the end of the study. The decrease seen in M at HD was still seen at the end of the regression period. Therefore, it appears that the drug might have an effect on the growth of pups treated for that length of time. However, it seems from the data that the decrease in body wt and the decrease in the length of the crown-to-rump measurements are correlated in their occurrence in the different groups. Therefore it is not clear if the effects on the length of the crown-to-rump measurement and therefore growth development in the pups is a direct drug effect or it is a consequence of the effect on body wt (see later section on preclinical findings in the relevance to clinical use).

In addition, there was a delay in the onset of vaginal opening in F treated with HD while there was no effect on preputial separation in M. This observation can be interpreted that this compound might have an effect on sexual maturation in F. The slight effect seen on the fertility index and the conception rate at MD and HD might be associated with the effect on sexual maturation in F but it was not clear from the data whether this effect was a male factor or a female factor. With no evaluation of the male sperm count and viability the evaluation of the effect of the drug on the male reproductive system and thus on the fertility index will not be possible. The number of implantation scars was counted

and were found not to be affected by treatment. In addition, there was no drug effect on the number of pups at birth. The exact mechanism by which the drug might have an effect on fertility could not be determined from the findings of this study.

The effect of the compound on the startle response at start and the average startle and the effect on motor activity count in the treated animals (all were decreased compared to the control group) seem to indicate that the compound results in decreased activity in animals treated for the length of time that was used in this study. Note that these measurements were conducted prior to daily dosing when the animals were likely to be hypoactive following a period of drug-induced hyperactivity. In addition, the numbers of treated animals, especially at HD, that appeared lying on the side or curled up were more than those seen in the control group. The data from the Cincinnati water maze test were highly variable and even though the sponsor considered that there is no drug related effect, in the opinion of the reviewer a drug effect cannot be ruled out. In the opinion of the reviewer it looks that the treated animals seemed to take longer time in crossing the path compared to the control group especially on the first path they were tested on (see later for more details). The data as they were examined by the reviewer reflected that the treated animals on several occasions might have been less able to successfully complete the maze path in a short time especially during their first exposure to the test (path A) than the control animals. However, it should be mentioned that during the testing on a second path (path B), which the animals were exposed to after path A, they seemed to be less different from the control animals compared to when they were tested on path A. The Cincinnati water maze test measures the time it takes the animal to complete a certain task and the number of errors made by the animals in finishing this task. It should be mentioned that there was no difference between the control and the treated animals in the number of errors encountered during the test. In addition, an effect on motor activity could be ruled out since there was no difference between control and treated animals in swimming a straight line. However, the data from the maze test, as mentioned earlier, suggested that there might be a difference in the number of animals in the treated groups compared to the control being able to finish the task in a shorter time. However, it should be emphasized that the data were variable among the different groups and there was no statistically significant difference between the groups. It is possible that the sample size was not enough to detect the drug effect and that a larger sample size might be needed to observe the drug effect.

A slight increase in % neutrophils (40-50% compared to control in M&F treated with HD) was seen on day 64 but was not seen on Day 92.

Some increases were observed (ALP, urea, and phosphorus) mainly at HD in both M&F but according to the sponsor were within the historical control data (HC data were not provided).

Some histopathological changes were observed in the liver (necrosis, inflammation and fibrosis), the kidney and/or bladder (pyelonephritis and transitional cell hyperplasia), and lymph nodes (hyperplasia) at HD only or at a higher incidence at the HD. These were not

considered treatment related by the sponsor. The occurrence of these findings in the HD only or at a higher incidence at HD might argue against this suggestion.

Dog study:

In the dog study, pups 10 weeks age were treated with 0, 2, 5, and 12 mg/kg/day orally by gavage for 6 months. The following parameters were evaluated: clinical observations, body weight, growth measurements (height and length), ophthalmology, electrocardiography, observational battery, neurological examinations, hematology and clinical chemistry, urinalysis, hormonal levels, male reproductive assessment, gross pathology, organ weights, histopathology and toxicokinetics.

There were no mortalities in the study. The following clinical signs were observed with treatment and mainly at the HD: stereotypic behavior such as head searching/bobbing/shaking, pacing in cage and repetitive pawing, circling, vocalization and yelping, walking or stumbling on objects, increased activity in F, thin condition, decreased activity prior to dosing and tremors. The condition of some individual animals was deteriorating at certain times such that treatment had to be suspended for a day and then treatment resumed without observing the same complications. These findings indicated that the high dose used is approaching a maximum tolerated dose and therefore with effects seen on body wt the doses used in this study are considered adequate.

The drug resulted in a decrease in body wt of treated animals compared to the control group especially at MD and HD. This effect appears to still be evident, although to a much lesser extent, till the end of the recovery period. There appeared to be no effect on other growth measurements such as height and length.

There was no effect on ophthalmological outcomes as tested here nor on the ECG outcomes.

The functional observational battery indicated that muscle tremors were observed in more animals treated with MD and HD compared to the control group especially towards the end of the study and this was also a finding seen in the animals during the clinical observations. In addition, treated dogs tended to be sleeping more than the control animals during observations which could be due to the hyper activity seen after dosing. It is possible that these animals get tired from the increased activity seen after treatment and that due to this they tend to get tired and to sleep more.

The neurological examinations performed did not indicate a drug effect.

A decrease in urine volume was seen in treated animals and as a result a higher specific gravity at MD and HD was observed in both M and F.

There appears to be no effect on hormonal levels (see methods for the evaluated hormones).

The results of the study might not be adequate to predict the effect of the drug on the male reproductive system. There were issues with the outcome of the studies since it seems that individual variations between the animals could be due to sexual immaturity in some of these animals. To come to a definitive conclusion about the effect of the drug on the male reproductive system would require a better quality of the data from control and treated group and sexual maturity of the animals should be guaranteed for the assessment of the effect of the drug. Whether the drug has an effect or not will not be known unless there was adequate number of animals in the study that reached sexual maturity in order to be able to assess the effect of the drug on these parameters. However, even though it would have been an asset to the study that conclusions about the effect of the drug on male sexual system can be drawn, this will not invalidate the study since an evaluation of the drug on reproduction is usually conducted in the rat study. However, it should be mentioned that in the rat study, the male reproductive system was not thoroughly evaluated either (no sperm evaluation data was presented).

No histopathological findings that are considered drug related were observed in dogs.

B. Pharmacologic activity: the pharmacological activity of the drug appears to be similar to that of amphetamine which is the proposed metabolite of the test article.

C. Nonclinical safety issues relevant to clinical use:

It appears from the chemistry review that there are some impurities that were exceeding the qualification level when their proposed commercial release specifications were compared to their levels in the batches used in the conducted non-clinical studies. The following table was prepared by the reviewer and summarizes the levels of these impurities as supplied by the chemist Dr. Lyudmila Soldatova. For comparison purposes, **NRP-104 is L-lys-D-amphetamine**:

| <u>Impurity</u> (----- ----- -----) | Commercial proposed specification | Levels in non- clinical batch (Batch # 1001D) | Levels in non- clinical batch # N039EH (#1003E) | Levels in non- clinical batch # N040EH (#1004E) |
|---|---|--|--|--|
| ----- ----- ----- | NMT ----- (area %) | Not detected | Not detected | Not detected |
| ----- ----- ----- | NMT ----- | Not detected | ----- (area | ----- (area |

| | | | | |
|--|--|-------|-------|-------|
| ----- | (area %) | | %) | %) |
| ----- | ----- | ----- | ----- | ----- |
| Organic volatile impurities (detected by GC) | | | | |
| ----- | ----- | ----- | ----- | ----- |
| ----- | ----- | ----- | ----- | ----- |
| ----- | ----- | ----- | ----- | ----- |
| □ □----- | ----- | ----- | ----- | ----- |
| Single unknowns | ----- | ----- | ----- | ----- |
| Other impurities | | | | |
| ----- | ----- however, a higher specification for stability testing was assigned ----- | | | |

The levels of -----) that humans are going to be exposed to based on their specification in the commercial batch (NMT -----) and as calculated from the maximum recommended human dose (70 mg/day) will be ≤----- μg/day which is far less than the allowable levels for these compounds (1.5 μg/day). Therefore, there are no concerns in regards of human exposure to this impurity at the proposed human doses and at the specification set for the commercial batch.

As for the ----- impurities, these impurities are -----

----- Of these products only----- is the compound that would be of potential unknown effect since the other compounds have either been used in humans or they are found in the human body. The sponsor has to either demonstrate that these impurities do not impose a potential toxic effect in humans or these impurities have to be eliminated from the commercial batch. If they could not be eliminated then these impurities have to be tested for their genotoxicity as other

impurities (the levels of these impurities were not known in the batches used for the genotoxicity studies, see later) and they might need to be tested in a juvenile rat study. It should be mentioned that the [REDACTED] impurities were excluded from discussion in ICH 3QA guidance.

It is not known whether the sponsor was unable to detect these impurities in the non-clinical batches at the time the studies were conducted because of the unavailability of the method for detecting these impurities. If at all possible the sponsor might need to reevaluate some of the old batches used in the preclinical studies to see if they can determine the levels of these impurities in these batches and compare them to the commercial batch. The sponsor is probably aware that degradation of the parent compound into these compounds could have happened and should take that into consideration while conducting these studies. The chemistry team has requested information about these impurities from the sponsor as seen in the following question sent by the CMC team (DFS dated 8-24-06):

9. Provide qualification data to show that the acceptance limits for [REDACTED] impurities [REDACTED] [NMT [REDACTED] (area %) each], and for [REDACTED] [NMT [REDACTED] (area %)] in the drug substance specification are qualified.

Further correspondence between the CMC team and the sponsor regarding chemistry issues pertaining to the [REDACTED] impurities and other issues are still ongoing. The following comments/questions will be sent by the chemistry team to the sponsor (as indicated by the chemistry reviewer on September 21, 2006):

1. Tighten the drug substance specification limit for impurities [REDACTED] [REDACTED] [currently, NMT [REDACTED] (area %) each], and for [REDACTED] (currently, NMT [REDACTED] (area %)), or provide the data to demonstrate that the acceptance limits are qualified [include the release testing results (e.g., CoAs) of the batches used for qualification].
2. Certificate of Analysis of the Lot #3037652 ([REDACTED] Lot 1001D) you provided in the Amendment dated September 5, 2006 demonstrates different results of testing for several organic volatile impurities and residual solvents from that provided in the original NDA submission. Clarify which results are correct, and why these two CoAs show different results.

As for the organic solvents ([REDACTED]), their levels specified in the commercial batch were slightly different from their levels in some of the non-clinical batches (batch #1001D used for all non-clinical studies except juvenile animals studies); however, they were much lower in the batches used in the juvenile animal studies (N039EH and N040EH) (see previous table). These solvents were not included in the table of ICH Q3C, a guidance that makes recommendations as to what amounts of residual solvents are considered safe in pharmaceuticals. In addition,

literature search indicated that one of these solvents () is widely used in the chemical industry and that the compound itself was found not to be mutagenic in an Ames and in an in vitro micronucleus assay using human lymphocytes; however, an () was found to be mutagenic in these tests. However, it was indicated in these studies that very high concentrations of this () are necessary to induce a mutagenic effect and that this () is efficiently detoxified by different liver enzymes. The other solvent () was found to be of no toxic potential from the literature search (LD in oral dosing in rats was 2gm/kg). Since these solvents seem to be found in levels close to those specified for commercial use in the batches used for the non-clinical studies (except the juvenile animal studies) and these solvents did not appear to warrant a human hazard based on the fact that they were not listed in the ICHQ3C table and the literature search, then they probably will not need further testing. Some single unknown organic volatile impurities were specified as () in the commercial batch and the levels of these in the non-clinical batches were much lower. The CMC team has asked the sponsor to specify these unknowns.

In their repeated genotox studies the sponsor utilized a batch of the test article (Lot #3037652) for which there was no certificate of analysis available. The sponsor was asked by the CMC team to provide the certificate of analysis for this new batch. It is important to know what impurities are present in this batch and how they compare to their levels in the clinical batch.

From findings of the juvenile animal studies, the drug appears to result in a decrease in body weight. In addition, the drug appears to affect the growth in rat pups as judged by the decrease in the length of the crown-to-rump. The effect on both the body weight and the length of the crown-to-rump in pups appears to still be seen at the end of a one month recovery period (in M only for the length of the crown-to-rump measurement). It should be pointed out that the observation of the effect on the body wt was parallel to the observation of the effect on the length of the crown-to-rump in these animals. It is not clear if the effect on growth in the treated pups was as a result of its effect on body weight or a direct effect. Regardless of whether the effect on the length of the crown-to-rump was as a result of the effect of the drug on body wt or it was a direct effect, it is apparent that these effects were not transient and were still seen after the removal of the drug. The effect of the drug on the weight and growth of children treated with this compound should be monitored during clinical use to see whether the treated children will have a similar effect and if they do whether they will catch up with their peers in these parameters.

In addition, there was a delay in the onset of vaginal opening in pup rats treated with the drug during development which might indicate that the drug could result in a delay of sexual maturation in females. There seemed to be a minimal effect on fertility in treated animals at the MD and HD compared to control (was not statistically significant and the sponsor did not consider it drug related). However, a drug effect could not be ruled out. In addition, it was not clear if the effect was due to a male factor since no evaluation of

sperm samples were done and a female factor was not clear. Therefore, it is recommended that children treated with this compound be followed to see the effect of treatment with this compound on their sexual maturation.

From the startle response test results it appears that the response of treated animals to a stimulus as measured in the first msec of a 100 msec window (startle at start) was lower than that seen in control animals and that this effect was seen also at the end of the recovery period. This effect was not statistically significant and the sponsor did not acknowledge it (see review for data and more details). In addition, the average startle response (the average of all responses during the 100 msec interval after the start of the stimulus) appeared to be lower in M treated at HD (statistically significant) and an even larger decrease was seen in F of the same group (not statistically significant). This effect was not reproduced in another group treated in a similar manner nor it was seen at the end of the recovery period. The sponsor considered these effects as drug unrelated. The reviewer believes that there might be a drug effect even though no statistical significance was observed especially in the first startle response at the start of the recording interval. The relevance of this finding to humans is not totally understood; however, it seems to indicate that the animals were not responding to a stimulus in a similar fashion to the control group (either a motor function effect or an alertness effect). Whether the lack of response is related to the decrease in motor activity that was observed in treated animals compared to the control group or not is not clear. However, it should be pointed out that the decrease in motor activity in treated animals compared to the control group was not seen at the end of the recovery period, while the effect on the startle response was still seen at the end of the recovery period. Therefore the correlation between these two findings is not clear. This effect on motor activity and startle response in animals might suggest general observation of the treated children to see whether comparable findings (either on motor activity or levels of alertness) might be associated with treatment. It is important to mention that startle habituation in the treated animals was not different from the control animals which might be an indication that learning in those animals was not different from the control animals.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-977

Review number: 1

Sequence number/date/type of submission: 000/12-06-05

Information to sponsor: Yes () No (X)

Sponsor and/or agent: New River Pharmaceuticals

1861 Pratt Drive

Suite 1090

Blacksburg, VA 24060

Manufacturer for drug substance:

and

Reviewer name: Ikram Elayan, Ph.D.

Division name: Division of Psychiatric Products

Review completion date: August 18, 2006

Drug:

Trade name: none

Generic name: *l*-lysine *d*-amphetamine dimesylate

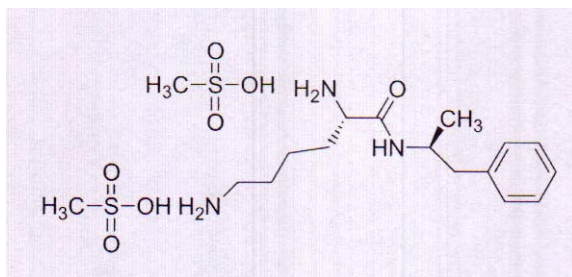
Code name: NRP-104

Chemical name: (2S, 2'S)-2, 6-diamino-N-(1-phenylpropan-2-yl) hexanamide di-methanesulfonic acid (mesylate)

CAS registry number:

Molecular formula/molecular weight: C₁₅H₂₅N₃O.C₂H₈O₆S₂ /mol. Wt. 455.59

Structure:



Relevant INDs/NDAs/DMFs: IND 67482

Drug class: psychostimulant (a prodrug of dextroamphetamine)

Intended clinical population: children and adolescents ages 6-12

Clinical formulation: capsules containing 30, 50, and 70 mg of the active ingredient

Route of administration: oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: all submitted studies

Studies not reviewed within this submission: none.

Note: For NDA reviews, all section headings should be included.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary:

This compound (NRP-104) is considered a prodrug for d-amphetamine since it is composed of lisdexamphetamine dimesylate which is an amphetamine covalently bound to *l*-lysine by an amide bond that is converted to *d*-amphetamine *in vivo*. The prodrug itself is not a stimulant; however, since amphetamine is the major product, stimulant effect is seen with treatment. The parent compound does not appear to bind to either the norepinephrine transporter nor to the dopamine transporter when tested *in vitro* using human recombinant transporters. It should also be emphasized that the parent compound has not been detected in the brain of rats treated orally with the compound while amphetamine levels were found to be present in the brain in response to this treatment. *In vivo* studies indicated that the compound increases locomotor activity when administered orally to rats similar to d-amphetamine sulfate and produces other clinical

signs similar to those seen with d-amphetamine sulfate. However, when administered intravenously or intranasally the increase in activity seen in treated rats was less than that seen with an equivalent dose of d-amphetamine sulfate given through these two routes.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: the proposed mechanism of action for the compound is similar to that of d-amphetamine since it is proposed that the parent compound get metabolized in the gastrointestinal tract to produce *d*-amphetamine and lysine after oral administration.

The following studies were submitted to support the hypothesis that the observed effects of NRP-104 are similar to those produced with d-amphetamine treatment.

Note:

Conversion factors: for the purpose of conversion between the different compounds (i.e. NRP-104, d-amphetamine sulfate, and d-amphetamine base) the conversion factors used in the following studies and others in the submission are as following (as provided by the sponsor in table 8.1 in vol. 2 page 8-98 of the original IND submission N-000):

d-amphetamine base content of amphetamine sulfate = $\frac{\text{MW of d-amphetamine base}}{\text{MW of d-amphetamine sulfate}}$

d-amphetamine base content of lysine-amphetamine mesylate = $\frac{\text{MW of d-amphetamine base}}{\text{MW of lysine-amphetamine mesylate}}$

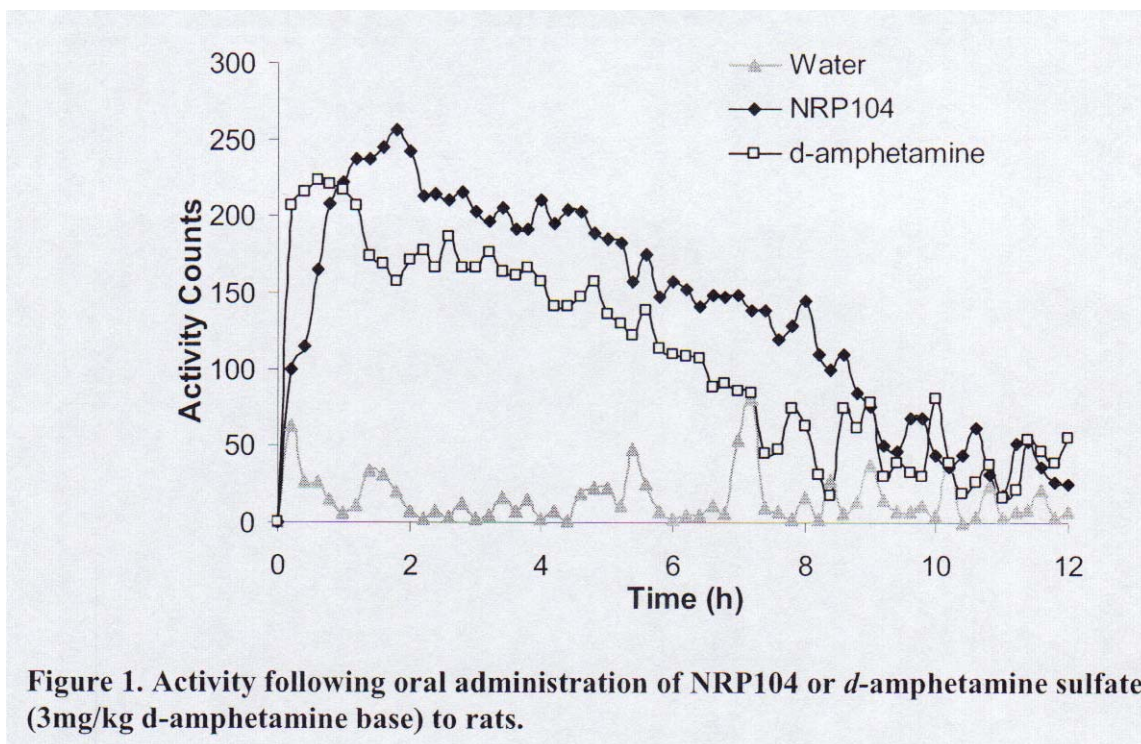
d-amphetamine base content of lysine-amphetamine HCl = $\frac{\text{MW of d-amphetamine base}}{\text{MW of lysine-amphetamine HCl}}$

d-amphetamine sulfate equivalent of lysine-amphetamine mesylate = $\frac{\text{MW of d-amphetamine sulfate}}{\text{MW of lysine-amphetamine mesylate}}$

d-amphetamine base equivalents of NRP104 base = $\frac{\text{MW of d-amphetamine base}}{\text{MW of NRP104 base}}$

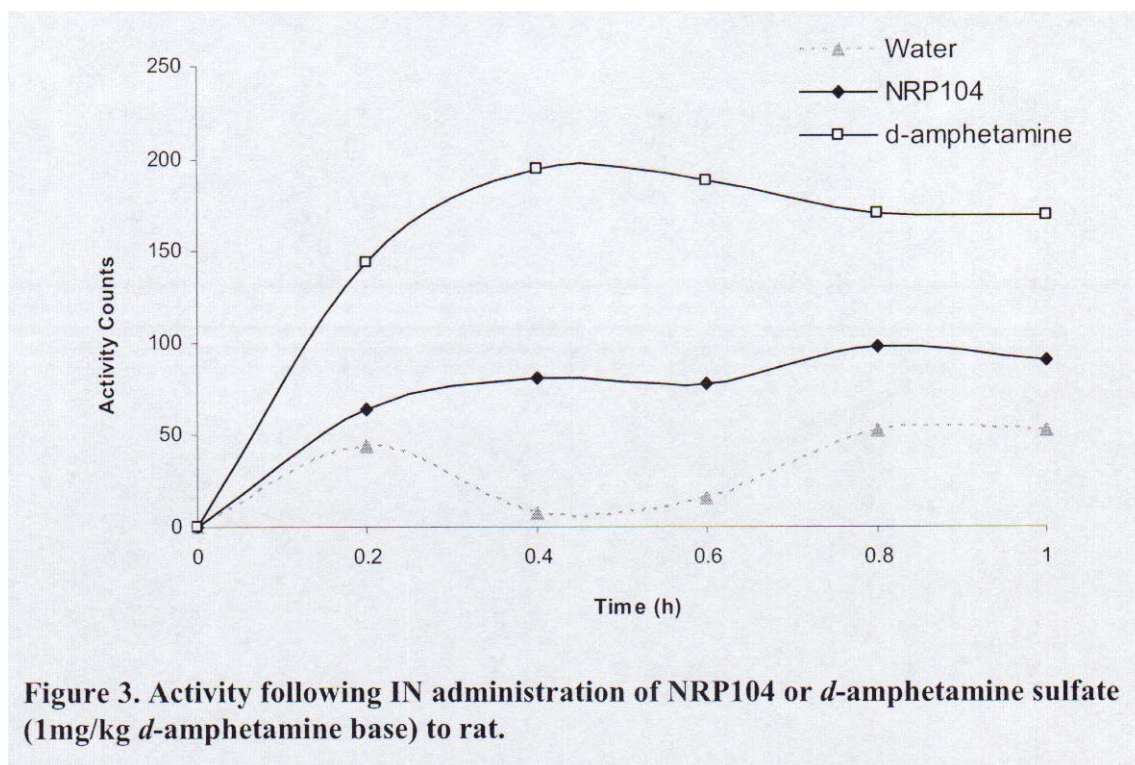
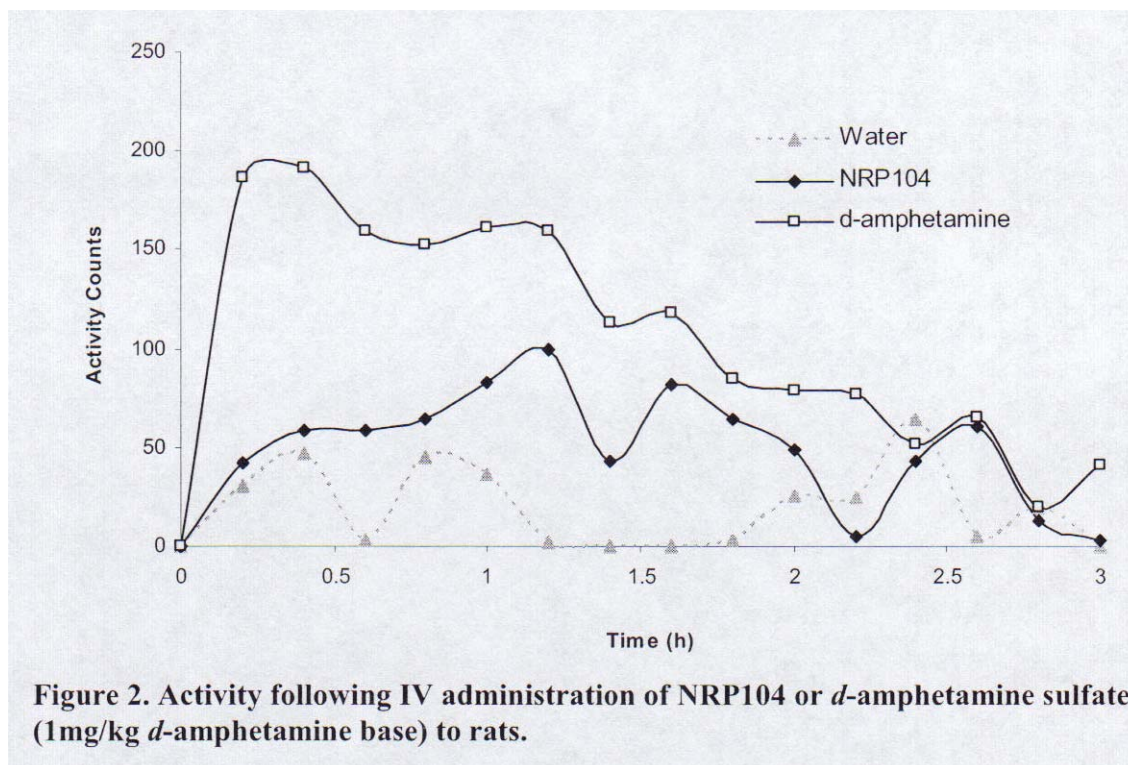
In studies number R01-NRP104-OPD-03, R02-NRP104-IVPD-04, and R03-NRP-104-INPD-05 the pharmacodynamics of NRP-104 following single dose oral, intravenous and intranasal administration in rats were tested (Module 4, Sequence 1, vol. 1 page 1, submission N-000):

Rats were treated with either NRP-104 or d-amphetamine sulfate by the oral route (gavage, single doses of NRP-104 or d-amphetamine sulfate at a dose of 3 mg of d-amphetamine base/kg), the treatment with NRP-104 resulted in increased activity in rats slightly higher than that observed with d-amphetamine sulfate as seen in the following sponsor's figure (figure 1, Module 4, sequence 1, vol. 1 page 14, submission N-000).



From the figure, it is seen that the onset of activity with NRP104 treatment is slightly delayed compared to *d*-amphetamine sulfate; however, the total activity and the peak activity was slightly higher compared to that of *d*-amphetamine.


In contrast to the oral administration, both intravenous (single doses of NRP-104 or *d*-amphetamine sulfate at a dose of 1mg *d*-amphetamine base/kg delivered by the tail vein) and intranasal (I.N., single doses of NRP-104 or *d*-amphetamine sulfate equivalent to a dose of 1mg *d*-amphetamine base/kg by pipetting 0.02 ml of solution into the nasal flares) administration in rats showed an increase in activity; however, the increase in activity observed with NRP-104 treatment was less than the increase in activity observed in response to treatment with the equivalent dose of *d*-amphetamine sulfate (see following figures; 2 and 3, from Module 4, Sequence 1, vol. 1 pages 14-15, submission N-000).



So these data indicate that oral administration of this compound in rats results in increased activity in treated animals comparable to or even slightly higher than that observed with an equivalent dose of *d*-amphetamine sulfate; however, while I.V. and I.N. administration resulted in increased activity in treated animals, the levels of activity observed are decreased and the onset of peak activity was delayed compared to those observed with treatment with a comparable dose of *d*-amphetamine sulfate administered through the same route.

The parent compound (NRP104) does not appear to have affinity for either the norepinephrine transporter (NET) or the dopamine transporter (DAT) when tested *in vitro*. Using human recombinant NE and DA transporters, the compound resulted in the following inhibitions (defined as the competitive displacement of 60-85 Ci/mmol of [³H] Nisoxetine at the NET and 60-87 Ci/mmol of [³H] WIN35,428 at the DAT) by the indicated concentrations as provided by the sponsor (page 8-4, vol. 2, submission N000 for the IND 67482, comparable to data presented on page 5 vol. 1 Module 4, Sequence 1 of the NDA):

| Binding activity of NRP104 for human recombinant NET or DAT sites | | |
|---|------------------------|------------------------|
| NRP104 concentration (M) | Percent inhibition NET | Percent inhibition DAT |
| 10 ⁻⁹ | 8.15 | -10.46 |
| 10 ⁻⁷ | -11.75 | 11.52 |
| 10 ⁻⁵ | 13.89 | -0.71 |
| | | |

When tested at a concentration of 10⁻⁵ M for its binding to a variety of receptors and enzymes , the compound showed ~36% inhibition at the non-selective α₂ adrenergic receptor and ~25% inhibition at the non-selective opioid receptor. See the following summary of binding activity as supplied by the sponsor (page 38-39, vol. 1 Module 2, Sequence 1):

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| Table 2.4.6-5 NRP104: Binding Activity of NRP104 for Various Receptors and Enzymes (Continued) | | | |
|--|-------|--|---------------------------|
| Name of Company: New River Pharmaceuticals | | Report No: V01-NRP104-REB-02 | CRO No: 03-0160 |
| Growth Factors | | Enzymes | |
| Corticotropin releasing factor, Non-selective | 1.72 | Choline Acetyltransferase | 9.15 |
| Oxytocin | -4.82 | Esterase, Acetylcholine | 8.08 |
| Platelet Activating Factor, PAF | 8.36 | Glutamic Acid Decarboxylase | 1.76 |
| Thyrotropin releasing Hormone, TRH | 1.81 | Oxidase, MOA-A, Peripheral | -7.51 |
| | | Oxidase, MOA-A, Peripheral | 4.41 |
| Brain/Gut Peptides | | Brain/Gut Peptides | |
| Angiotensin II, AT1 (h) | -0.46 | Neurokinin, NK1 | 5.13 |
| Angiotensin II, AT2 | 8.56 | Neurokinin, NK2 (NKA) (hr) | 6.07 |
| Bradykinin, BK2 | 10.04 | Neurokinin, NK3 (NKB) | 13.37 |
| Cholecystokinin, CCK1 (CCKA) | -2.47 | Vasoactive Intestinal Peptide, Non-selective | 13.45 |
| Cholecystokinin, CCK2 (CCKB) | -1.60 | Vasopressin | 0.76 |
| Endothelin, ET-A (h) | 6.05 | | |
| Endothelin, ET-B (h) | -2.19 | | |
| Galanin, Non-selective | 2.24 | | |
| Noteworthy Findings: NRP104 did not show over 50% inhibition for any receptor or enzyme tested. It is therefore unlikely to produce side effects mediated by these sites. | | | |

Pharmacokinetic studies indicated that the parent compound was not detected in the brain following oral administration in rats; however, d-amphetamine levels were increased after such treatment (see PK section later).

Drug activity related to proposed indication: the drug is proposed to act as d-amphetamine; _____

_____ (see the Controlled Substance Staff's review for this compound). Accordingly, the drug activity in relation to the proposed indication will be similar to that of d-amphetamine.

2.6.2.3 Secondary pharmacodynamics: no secondary pharmacodynamic mechanisms were proposed. The sponsor pointed to the fact that the compound was tested for its affinity to a variety of receptors and enzymes (see [REDACTED] data summary) and that no significant binding affinity was observed and thus concluded that “there appears to be no direct interaction by NRP104 with active sites considered to be critical for the pharmacological effects of d-amphetamine”. From the results obtained it is safe to conclude that the compound is mainly acting like amphetamine and that there are no secondary pharmacodynamic actions besides its primary pharmacodynamic action as an amphetamine prodrug.

2.6.2.4 Safety pharmacology

Neurological effects: no specific studies were submitted for this purpose but some of the effect of the drug on the CNS in rats can be gathered from the review of the multiple dose studies found later in this review. In general the effect of this compound on the CNS were similar to those produced with d-amphetamine and included hyperactivity, post dose jumping and self mutilation. In addition, the juvenile animal studies reviewed later explored the effect of the drug on the neurodevelopment of juvenile rats and dogs (see the studies for more details).

Cardiovascular effects:

Cardiovascular (hemodynamic) evaluation of intravenously administered NRP-104 in dogs ([REDACTED] Study # 0247DN29.001 or Study D01-NRP104-SPC-06) found in vol. 1, Module 4, sequence 1 (GLP but not with relation to the characterization of test article or the stability of the test article):

Four open-chest anesthetized beagle dogs (2 M and 2 F, 8-10 months) were treated (1 M & 1F) with either vehicle or test article at doses 0, 0.5, 1.0 and 5.0 mg/kg or with vehicle and d-amphetamine sulfate at 0, 0.202, 0.404, and 2.02 mg/kg via intravenous administration (abdominal vena cava via femoral vein). Effects on the cardiovascular system (CVS) were determined by changes in arterial blood pressure (systolic-SAP, diastolic-DAP and mean-MAP), heart rate (HR), left ventricular pressure (LVP), left ventricular end diastolic pressure (LVEDP), +dP/dt, cardiac output (CO), and Lead II ECG (gross analysis). Following stabilization, baseline values for each parameter were established over a minimum of 10 minutes. Additional, electrocardiogram recordings were obtained prior to the first dose administration, at each dose completion and at 30 minutes following the completion of each dose of vehicle and test article.

Results:

At 0.5 mg/kg, NRP-104 increased blood pressure, cardiac output, LVP and +dP/dt and reduced HR. At 1.0 mg/kg, NRP-104 increased blood pressure, heart rate, cardiac output, LVP, +dP/dt and LVEDP. At 5.0 mg/kg, NRP-104 increased blood pressure, heart rate, LVP and +dP/dt, while decreasing cardiac output and LVEDP. See the following table

provided by the sponsor for the changes in these parameters in response to treatment with NRP-104 (Table 1, page 22, vol. 1, Module 4, Sequence 1).

A. Table 1 - The Effects of NRP 104 and d-Amphetamine on Cardiovascular Parameters in the Anesthetized Dog – Summary

| TREATMENT | TIME | SAP | % Change | DAP | % Change | MAP | % Change | HR | % Change | CO | % Change | LVP | % Change | +dP/dt | % Change | LVEDP | % Change |
|-------------|------|-----|-------------|-----|-------------|-----|-------------|-----|-------------|------|-------------|-----|-------------|--------|-------------|-------|-------------|
| 0.9% Saline | 0 | 81 | 0 | 48 | 0 | 61 | 0 | 105 | 0 | 0.64 | 0 | 87 | 0 | 1284 | 0 | 6.90 | 0 |
| 1 ml/kg | 30 | 87 | 7 | 54 | 11 | 67 | 10 | 112 | 6 | 0.76 | 19 | 87 | 0 | 1304 | 2 | 6.75 | -2 |
| NRP 104 | 0 | 84 | 0 | 51 | 0 | 64 | 0 | 108 | 0 | 0.68 | 0 | 86 | 0 | 1322 | 0 | 6.63 | 0 |
| 0.5 mg/kg | 5 | 87 | 4 | 52 | 3 | 66 | 3 | 103 | -5 | 0.69 | 1 | 87 | 2 | 1361 | 3 | 7.04 | 6 |
| | 15 | 93 | 11 | 51 | 1 | 67 | 5 | 87 | -20 | 0.67 | -1 | 95 | 11 | 1732 | 31 | 7.12 | 7 |
| | 25 | 104 | 25 | 55 | 8 | 73 | 15 | 87 | -19 | 0.94 | 38 | 105 | 22 | 1907 | 44 | 7.09 | 7 |
| | 30 | 107 | 28 | 58 | 14 | 77 | 21 | 100 | -7 | 1.27 | 87 | 108 | 26 | 1952 | 48 | 6.81 | 3 |
| NRP 104 | 0 | 105 | 0 | 55 | 0 | 74 | 0 | 97 | 0 | 1.27 | 0 | 108 | 0 | 1995 | 0 | 6.35 | 0 |
| 1.0 mg/kg | 5 | 121 | 15 | 63 | 15 | 85 | 15 | 93 | -4 | 1.59 | 25 | 120 | 11 | 2421 | 21 | 7.39 | 16 |
| | 15 | 142 | 35 | 73 | 33 | 100 | 35 | 98 | 2 | 3.30 | 159 | 140 | 29 | 2808 | 41 | 7.86 | 24 |
| | 25 | 163 | 55 | 97 | 75 | 124 | 68 | 165 | 70 | 3.58 | 182 | 162 | 50 | 3601 | 81 | 5.75 | -9 |
| | 30 | 134 | 28 | 73 | 32 | 98 | 32 | 145 | 50 | 4.43 | 249 | 144 | 33 | 3203 | 61 | 6.02 | -5 |
| NRP 104 | 0 | 132 | 0 | 71 | 0 | 95 | 0 | 141 | 0 | 4.33 | 0 | 144 | 0 | 3175 | 0 | 6.04 | 0 |
| 5.0 mg/kg | 5 | 142 | 7 | 71 | 0 | 99 | 4 | 134 | -5 | 2.43 | -44 | 151 | 5 | 3040 | -4 | 6.16 | 2 |
| | 15 | 176 | 33 | 98 | 39 | 130 | 37 | 196 | 39 | 3.42 | -21 | 184 | 28 | 3723 | 17 | 6.08 | 1 |
| | 25 | 126 | -5 | 69 | -3 | 98 | 1 | 204 | 44 | 3.00 | -31 | 160 | 11 | 3678 | 16 | 4.91 | -19 |
| | 30 | 132 | 0 | 70 | -1 | 99 | 4 | 197 | 39 | 3.13 | -28 | 163 | 13 | 3783 | 19 | 4.55 | -25 |

n=2
SAP - systolic arterial pressure (mmHg)
DAP - diastolic arterial pressure (mmHg)
MAP - mean arterial pressure (mmHg)
HR - heart rate (beats/min)
CO - cardiac output (L/min)
LVP - left ventricular pressure (mmHg)
LVEDP - left ventricular end diastolic pressure (mmHg)
+dP/dt - (mmHg/sec)
%Change- percent change from respective Time 0.

d-Amphetamine sulfate at all doses increased blood pressure, heart rate, cardiac output, and LVP. +dP/dt was increased by d-amphetamine sulfate (at 0.202 mg/kg), while the effects of d-amphetamine sulfate on LVEDP were variable. See the following table provided by the sponsor for the changes in these parameters in response to treatment with d-amphetamine sulfate (table 1, page 23, vol. 1 Module 4, Sequence 1).

A. Table 1 (continued) – The Effects of NRP 104 and d-Amphetamine on Cardiovascular Parameters in the Anesthetized Dog – Summary

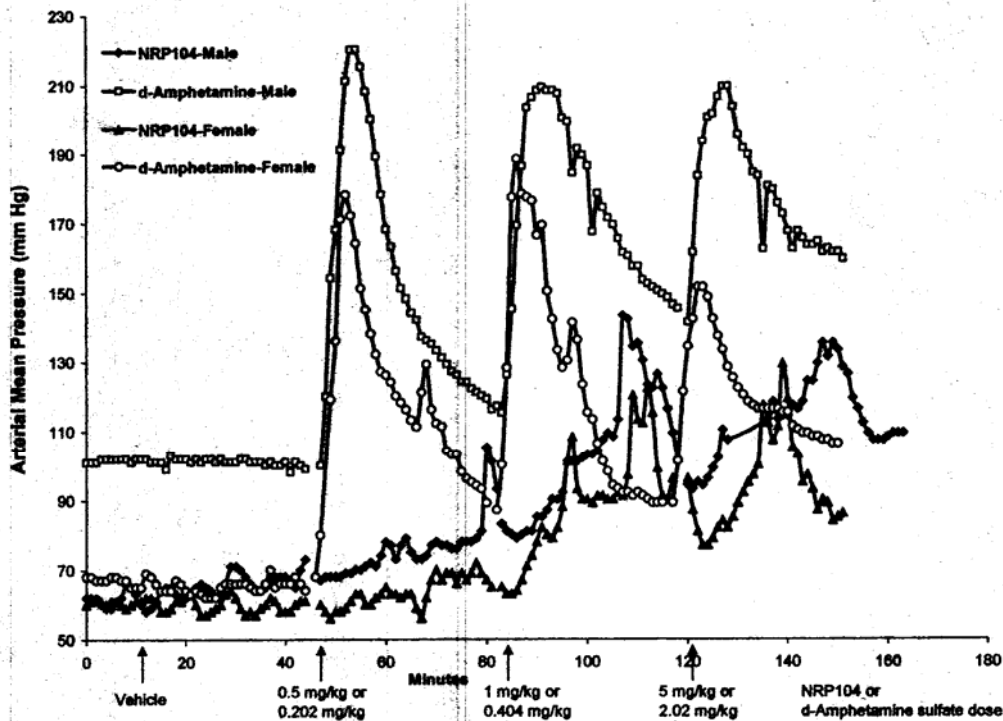
| TREATMENT | TIME | SAP | % Change | DAP | % Change | MAP | % Change | HR | % Change | CO | % Change | LVP | % Change | +dP/dt | % Change | LVEDP | % Change |
|---------------|------|-----|-------------|-----|-------------|-----|-------------|-----|-------------|------|-------------|-----|-------------|--------|-------------|-------|-------------|
| 0.9% Saline | 0 | 110 | 0 | 67 | 0 | 84 | 0 | 108 | 0 | 0.73 | 0 | 105 | 0 | 1380 | 0 | 7.73 | 0 |
| 1 ml/kg | 30 | 108 | -2 | 65 | -3 | 82 | -2 | 104 | -3 | 0.89 | 22 | 101 | -3 | 1374 | 0 | 7.74 | 0 |
| d-Amphetamine | 0 | 111 | 0 | 67 | 0 | 84 | 0 | 105 | 0 | 0.88 | 0 | 104 | 0 | 1394 | 0 | 8.44 | 0 |
| 0.202 mg/kg | 5 | 218 | 97 | 145 | 117 | 176 | 109 | 130 | 24 | 1.33 | 51 | 214 | 107 | 1835 | 32 | 11.21 | 33 |
| | 15 | 168 | 52 | 97 | 45 | 125 | 49 | 113 | 8 | 1.24 | 42 | 157 | 52 | 3245 | 133 | 5.27 | -38 |
| | 25 | 148 | 34 | 87 | 30 | 110 | 31 | 134 | 28 | 1.28 | 46 | 142 | 37 | 3170 | 127 | 4.60 | -45 |
| | 30 | 140 | 26 | 80 | 20 | 103 | 23 | 125 | 19 | 1.00 | 14 | 135 | 30 | 3125 | 124 | 3.96 | -53 |
| d-Amphetamine | 0 | 139 | 0 | 78 | 0 | 101 | 0 | 121 | 0 | 1.02 | 0 | 133 | 0 | 3065 | 0 | 3.90 | 0 |
| 0.404 mg/kg | 5 | 240 | 73 | 147 | 88 | 187 | 85 | 118 | -2 | 1.16 | 13 | 238 | 79 | 2386 | -22 | 9.67 | 148 |
| | 15 | 193 | 39 | 112 | 44 | 145 | 43 | 145 | 20 | 1.26 | 24 | 191 | 43 | 3977 | 30 | 3.26 | -17 |
| | 25 | 166 | 19 | 92 | 17 | 122 | 20 | 148 | 23 | 1.31 | 28 | 168 | 26 | 3990 | 30 | 3.13 | -20 |
| | 30 | 160 | 16 | 87 | 11 | 117 | 16 | 149 | 24 | 1.30 | 27 | 163 | 22 | 3945 | 29 | 1.37 | -65 |
| d-Amphetamine | 0 | 158 | 0 | 87 | 0 | 115 | 0 | 149 | 0 | 1.34 | 0 | 162 | 0 | 4091 | 0 | 0.94 | 0 |
| 2.02 mg/kg | 5 | 228 | 44 | 128 | 48 | 169 | 47 | 151 | 1 | 1.60 | 19 | 227 | 40 | 3678 | -10 | 2.91 | 209 |
| | 15 | 196 | 24 | 107 | 23 | 142 | 23 | 167 | 12 | 1.50 | 12 | 200 | 24 | 4131 | 1 | 0.75 | -20 |
| | 25 | 189 | 20 | 102 | 17 | 135 | 17 | 171 | 15 | 1.32 | -2 | 192 | 19 | 4295 | 5 | -0.81 | -186 |
| | 30 | 183 | 16 | 98 | 13 | 129 | 12 | 175 | 17 | 1.45 | 8 | 187 | 16 | 4310 | 5 | -0.67 | -171 |

n=2
SAP - systolic arterial pressure (mmHg)
DAP - diastolic arterial pressure (mmHg)
MAP - mean arterial pressure (mmHg)
HR - heart rate (beats/min)
CO - cardiac output (L/min)
LVP - left ventricular pressure (mmHg)
LVEDP - left ventricular end diastolic pressure (mmHg)
+dP/dt - (mmHg/sec)
%Change - percent change from respective Time 0.

The data indicate that the effects of NRP-104 and d-amphetamine sulfate were comparable even though the effect of d-amphetamine sulfate on blood pressure was slightly higher compared to that with NRP-104 and was seen at an earlier time point than

that seen with NRP-104 (see the figure 8.4 provided by the sponsor in vol. 1 page 5-10 of Clinical Investigator's Brochure, Edition #1 dated March 20, 2004).

Figure 8.4 Mean blood pressure following intravenous bolus injection of increasing amounts of NRP104 or d-amphetamine in male and female dogs.



The effect on HR was seen mostly as an increase with d-amphetamine treatment while with NRP-104 treatment there was a decrease observed at the lower dose while an increase was observed at MD and HD which was even slightly higher than that observed with the comparable doses of d-amphetamine. The effect on cardiac output was slightly different with NRP-104 since a moderate increase was seen with d-amphetamine at all doses while a moderate increase was seen at LD of NRP-104 and then a larger increase was seen at MD while a decrease was seen at HD.

According to the sponsor, the IV administration of NRP-104 caused sinus tachycardia 30 min post-dose at HD in both dogs (M & F). In one of the dogs treated with d-amphetamine sulfate 3 ventricular extrasystoles followed dose completion of the lowest dose and sinus tachycardia was present in the 30 min post MD record and in the dose completion and post HD record.

Note: it should be noted that the sponsor stated that the actual concentration of the prepared solution was less than the nominal especially at the HD (see table provided by the sponsor on page 62, vol. 1, Module 4 Sequence 1):

Sample batch assay results of NRP104 dosing formulations (presented as salt concentrations) are summarized below:

| Date of Sample Preparation | Date of Sample Assay | Nominal Dosing Formulation Conc'n (mg/ml) | Assayed Dosing Formulation Conc'n (mg/ml) | %Diff from Nominal Conc'n |
|----------------------------|----------------------|---|---|---------------------------|
| 10-Mar-04 | 14-Apr-04 | 0.5 | 0.437 ^b | -12.6 ^b |
| 10-Mar-04 | 14-Apr-04 | 1.0 | 1.04 ^a | 4.6 ^a |
| 10-Mar-04 | 14-Apr-04 | 5.0 | 2.95 ^b | -40.9 ^b |
| 11-Mar-04 | 14-Apr-04 | 0.5 | 0.514 ^a | 2.9 ^a |
| 11-Mar-04 | 14-Apr-04 | 1.0 | 0.722 ^b | -27.8 ^b |
| 11-Mar-04 | 14-Apr-04 | 5.0 | 3.59 ^b | -28.1 ^b |

^aAverage values from duplicate injections

^bReassayed sample; mean data from both analyses (four injections)

In conclusion, the assayed concentrations of the HD of NRP-104 dosing formulations were significantly lower (by ~28 and 41%) of the nominal concentration and the concentration of one of the two formulations at the MD was also significantly lower than the nominal concentration (by ~28%). It is not clear how the effects on the cardiovascular system will differ if these doses were up to the nominal concentration. This finding (i.e. the discrepancy between the actual and the nominal concentration) was not noticed at the time of the IND since at that time only executive summary of the study was provided and no mention of this finding was provided. At the time of the NDA filing this finding was discussed with the team leader (Dr. Barry Rosloff) and the motion was towards the insignificance of the animal data at this point especially in view of the collected data from the human subjects up to that point.

Some electrocardiographic recordings were performed in the dog juvenile study; however, these data might not be optimal since they were collected **only before dosing** and they did not coincide with C_{max}. In general the data did not indicate any adverse drug related finding.

In the 28-day study, dogs (3/sex, strain unspecified) were treated with NPR104 at doses of 0, 3, 6, and 12 mg/kg orally by gavage. No significant findings were observed; however, **no indication of when measurements were done relative to C_{max}.**

Pulmonary effects:

Pulmonary assessment of NRP-104 in the anesthetized guinea pig (----- Study # 1082GN29.001, or G03-NRP104-SPR-07, found in vol. 1 Module 4, Sequence 1):

Sixteen male Hartley guinea pigs were assigned to 4 groups and were treated with one of the following doses of NRP-104: 0, 1, 5, and 7.5 mg/kg by IV administration. Animals were fasted for ~17-20 h before treatment and were anesthetized (1.4-1.5 g/kg of urethane initially and supplemented with 80-160 mg as needed) throughout the experiment. The following parameters were evaluated: changes in airway resistance (cm H₂O/ml/sec), dynamic lung compliance (ml/cm H₂O), respiratory rate (breaths/min), tidal volume (ml) and minute volume (ml/min). These values were recorded and summarized every minute for the first 5 min and every 5 min thereafter for a minimum of 30 min.

Results:

There was an increase in respiratory rate at 1 mg/kg (~35%), at 5 mg/kg (~35%) and at 7.5 mg/kg (~45%), at 20-30 min after treatment (all were compared to baseline). A slight increase in minute volume was seen at 1 mg/kg (26%), and a larger effect was seen at 5 mg/kg (~40%) and at 7.5 mg/kg (~50%) 20-30 min after treatment all compared to baseline values. An increase of ~32% was seen at 7.5 mg/kg compared to the control group at the same time points (25-30 min post dose). It should be noted that the changes seen at 1 mg/kg were mainly due to changes seen in 1 animal (#3328). No significant effect was observed at the other parameters evaluated. The following table was provided by the sponsor summarizing the data (table 1, pages 22-23, vol. 1, Module 4 Sequence 1):

Table 1
Pulmonary Assessment of NRP 104 in the Anesthetized Guinea Pig
Study Number: 1082GN29.001
Mean Summary Data

| Intravenous Treatment | Time (min) | Resistance (cmH ₂ O/ml/sec) | | Compliance (ml/cmH ₂ O) | | Respiratory Rate (breaths/min) | | Tidal Volume (ml) | | Minute Volume (ml/min) | |
|--|------------|--|------|------------------------------------|------|--------------------------------|------|-------------------|------|------------------------|-------|
| | | mean | sem | mean | sem | mean | sem | mean | sem | mean | sem |
| Vehicle 0.9% Saline 1 ml/kg (n=4) | 0 | 0.06 | 0.00 | 1.35 | 0.06 | 62.78 | 3.32 | 1.79 | 0.12 | 111.78 | 4.18 |
| | 1 | 0.06 | 0.00 | 1.37 | 0.06 | 60.85 | 4.94 | 1.81 | 0.11 | 108.91 | 5.77 |
| | 2 | 0.06 | 0.00 | 1.31 | 0.06 | 63.29 | 4.15 | 1.83 | 0.12 | 115.30 | 1.32 |
| | 3 | 0.06 | 0.00 | 1.33 | 0.08 | 62.27 | 3.73 | 1.82 | 0.13 | 111.13 | 3.05 |
| | 4 | 0.06 | 0.00 | 1.33 | 0.06 | 69.38 | 4.56 | 1.84 | 0.13 | 126.90 | 8.06 |
| | 5 | 0.06 | 0.00 | 1.34 | 0.08 | 65.42 | 4.76 | 1.83 | 0.12 | 118.83 | 6.05 |
| | 10 | 0.06 | 0.00 | 1.40 | 0.11 | 65.04 | 4.55 | 1.87 | 0.12 | 119.90 | 6.65 |
| | 15 | 0.06 | 0.00 | 1.46 | 0.23 | 69.99 | 3.89 | 1.82 | 0.12 | 129.28 | 7.76 |
| | 20 | 0.05 | 0.00 | 1.33 | 0.11 | 64.76 | 6.53 | 1.77 | 0.13 | 114.41 | 9.86 |
| | 25 | 0.06 | 0.00 | 1.22 | 0.02 | 66.31 | 4.94 | 1.82 | 0.12 | 118.80 | 9.01 |
| | 30 | 0.05 | 0.01 | 1.15 | 0.03 | 65.23 | 4.75 | 1.95 | 0.22 | 126.46 | 11.14 |

| Intravenous Treatment | Time (min) | Resistance (cmH ₂ O/ml/sec) | | Compliance (ml/cmH ₂ O) | | Respiratory Rate (breaths/min) | | Tidal Volume (ml) | | Minute Volume (ml/min) | |
|-------------------------------|------------|--|------|------------------------------------|------|--------------------------------|-------|-------------------|------|------------------------|-------|
| | | mean | sem | mean | sem | mean | sem | mean | sem | mean | sem |
| NRP 104 1.0 mg/kg (n=4) | 0 | 0.05 | 0.00 | 1.09 | 0.14 | 56.07 | 4.44 | 1.91 | 0.09 | 106.35 | 6.06 |
| | 1 | 0.05 | 0.00 | 1.12 | 0.13 | 56.30 | 4.26 | 1.95 | 0.10 | 108.28 | 4.58 |
| | 2 | 0.05 | 0.00 | 1.09 | 0.12 | 56.68 | 4.77 | 1.99 | 0.07 | 111.59 | 6.60 |
| | 3 | 0.05 | 0.00 | 1.10 | 0.12 | 57.70 | 4.94 | 1.97 | 0.09 | 112.93 | 8.58 |
| | 4 | 0.05 | 0.01 | 1.11 | 0.13 | 55.85 | 4.63 | 1.93 | 0.09 | 107.32 | 6.77 |
| | 5 | 0.05 | 0.01 | 1.13 | 0.15 | 56.00 | 3.04 | 1.93 | 0.10 | 107.43 | 3.46 |
| | 10 | 0.05 | 0.00 | 1.11 | 0.14 | 59.47 | 3.45 | 1.95 | 0.12 | 115.00 | 4.05 |
| | 15 | 0.05 | 0.00 | 1.09 | 0.15 | 60.79 | 6.51 | 1.92 | 0.15 | 114.42 | 6.67 |
| | 20 | 0.05 | 0.00 | 1.10 | 0.16 | 66.65 | 7.75 | 1.88 | 0.12 | 121.90 | 9.11 |
| | 25 | 0.05 | 0.00 | 1.07 | 0.18 | 73.87* | 13.04 | 1.88 | 0.16 | 133.86* | 16.93 |
| | 30 | 0.05 | 0.00 | 1.05 | 0.21 | 77.01* | 11.94 | 1.84 | 0.18 | 136.10* | 12.46 |

Mean and standard error of the mean calculated using non-truncated values.

* = Statistically significant ($p \leq 0.05$) change from time 0

Table 1 (Continued)
Pulmonary Assessment of NRP 104 in the Anesthetized Guinea Pig
Study Number: 1082GN29.001
Mean Summary Data

| Intravenous Treatment | Time (min) | Resistance (cmH ₂ O/ml/sec) | | Compliance (ml/cmH ₂ O) | | Respiratory Rate (breaths/min) | | Tidal Volume (ml) | | Minute Volume (ml/min) | |
|-------------------------------|------------|--|------|------------------------------------|------|--------------------------------|-------|-------------------|------|------------------------|-------|
| | | mean | sem | mean | sem | mean | sem | mean | sem | mean | sem |
| NRP 104 5.0 mg/kg (n=4) | 0 | 0.05 | 0.00 | 1.62 | 0.21 | 53.94 | 5.41 | 1.84 | 0.19 | 97.29 | 4.99 |
| | 1 | 0.05 | 0.00 | 1.65 | 0.23 | 56.79 | 8.38 | 1.91 | 0.21 | 103.45 | 10.11 |
| | 2 | 0.05 | 0.01 | 1.57 | 0.21 | 58.65 | 7.34 | 2.21* | 0.12 | 127.60* | 11.07 |
| | 3 | 0.05 | 0.01 | 1.54 | 0.25 | 62.05 | 9.84 | 2.01 | 0.19 | 124.04* | 13.91 |
| | 4 | 0.05 | 0.01 | 1.60 | 0.22 | 59.27 | 6.84 | 2.01 | 0.16 | 117.64 | 13.68 |
| | 5 | 0.05 | 0.01 | 1.61 | 0.22 | 59.89 | 6.99 | 1.99 | 0.16 | 116.26 | 11.50 |
| | 10 | 0.05 | 0.01 | 1.54 | 0.25 | 65.44 | 7.71 | 2.14* | 0.14 | 135.15* | 10.70 |
| | 15 | 0.05 | 0.01 | 1.46 | 0.23 | 70.61 | 11.87 | 2.02 | 0.23 | 137.21* | 15.66 |
| | 20 | 0.05 | 0.01 | 1.36 | 0.20 | 72.90* | 12.89 | 2.02 | 0.26 | 136.34* | 15.00 |
| | 25 | 0.05 | 0.01 | 1.38 | 0.19 | 68.97 | 11.01 | 2.10 | 0.26 | 134.39* | 17.64 |
| | 30 | 0.06 | 0.01 | 1.49 | 0.35 | 71.95* | 11.42 | 2.00 | 0.28 | 134.83* | 12.98 |
| Intravenous Treatment | Time (min) | Resistance (cmH ₂ O/ml/sec) | | Compliance (ml/cmH ₂ O) | | Respiratory Rate (breaths/min) | | Tidal Volume (ml) | | Minute Volume (ml/min) | |
| | | mean | sem | mean | sem | Mean | sem | mean | sem | Mean | sem |
| NRP 104 7.5 mg/kg (n=4) | 0 | 0.05 | 0.00 | 1.58 | 0.20 | 62.17 | 7.90 | 1.76 | 0.14 | 107.65 | 7.88 |
| | 1 | 0.05 | 0.00 | 1.63 | 0.12 | 70.31 | 10.54 | 1.76 | 0.21 | 122.20 | 9.58 |
| | 2 | 0.06 | 0.00 | 1.49 | 0.13 | 68.27 | 5.79 | 1.98 | 0.17 | 134.98* | 2.54 |
| | 3 | 0.05 | 0.00 | 1.52 | 0.14 | 66.72 | 4.18 | 1.93 | 0.13 | 128.88 | 2.30 |
| | 4 | 0.05 | 0.00 | 1.63 | 0.17 | 71.39 | 7.83 | 1.83 | 0.12 | 131.83 | 8.11 |
| | 5 | 0.05 | 0.00 | 1.83 | 0.36 | 62.62 | 4.94 | 1.94 | 0.11 | 120.50 | 3.62 |
| | 10 | 0.05 | 0.00 | 1.71 | 0.35 | 80.06* | 8.21 | 1.82 | 0.08 | 144.65* | 8.13 |
| | 15 | 0.06 | 0.00 | 1.42 | 0.14 | 75.39 | 5.90 | 1.98 | 0.13 | 147.75* | 4.13 |
| | 20 | 0.06 | 0.00 | 1.40 | 0.13 | 80.10* | 6.23 | 1.94 | 0.14 | 151.90*,** | 3.21 |
| | 25 | 0.06 | 0.01 | 1.23 | 0.17 | 90.22* | 11.74 | 1.80 | 0.19 | 156.28*,** | 4.77 |
| | 30 | 0.06 | 0.01 | 1.27 | 0.19 | 84.10* | 7.03 | 1.97 | 0.11 | 162.70* | 6.70 |

Mean and standard error of the mean calculated using non-truncated values.

* = Statistically significant ($p \leq 0.05$) change from time 0

** = Statistically significant ($p \leq 0.05$) change from 0.9% saline.

Of note, the actual concentrations of the dosing solutions used in this study were within 5% of the nominal concentration.

Renal effects: no studies were submitted.

Gastrointestinal effects: no studies were submitted

Abuse liability: see CSS review

Other: none.

2.6.2.5 Pharmacodynamic drug interactions:

No studies were conducted in this regard. However, as mentioned earlier, the binding of this compound to a variety of receptors and enzymes indicated that there was no significant interaction with a site or enzyme to warrant a potential pharmacodynamic interaction (at least of those sites and enzymes tested).

2.6.3 PHARMACOLOGY TABULATED SUMMARY

The followings are tabulated data provided by the sponsor summarizing the pharmacodynamic results as presented in vol. 1 Module 2 Sequence 1 under non-clinical overview on pages 29, 31, 33, and 35. See also summary of binding study in the pharmacodynamic section of the review:

| Table 2.4.6-1 <i>In vitro</i> Binding Human Recombinant Norepinephrine (NET) and Dopamine (DAT) Transporter Sites Binding Study | | |
|--|------------------------------------|---|
| Name of Company: New River Pharmaceuticals, Inc. | Report No: V01-NRP104-RB-01 | |
| CTD Location: Mod 4, Vol 1, Section 4.2.1.1 | CRO Report No: 03-9575 | Study in Compliance with GLP: No |
| Main Testing Facility: [REDACTED] | | |
| General Pharmacology (<i>in vitro</i>) | | |
| Study Objective: To determine whether or not NRP104 binds human norepinephrine or dopamine transporter sites. | | |
| Binding Assay: The numbers under the heading percent inhibition NET refer to the competitive displacement of [³ H]Nisoxetine (60-85 Ci/mmol) at the human recombinant norepinephrine transporter (NET) binding site. The numbers under percent inhibition DAT refer to the competitive displacement of [³ H]WIN,35,428 (60-87 Ci/mmol) at the human recombinant dopamine transporter (DAT) binding site. Positive controls such as desipramine (NET) and GBR-12909 (DAT) show increasing percent inhibition with increasing concentrations. Stimulants like amphetamine show a concentration-dependent inhibition of radioligand binding to both NET and DAT binding sites (see references in Drouin, <i>et. al.</i> , 2002). | | |
| Binding Activity of NRP104 for Human Recombinant NET or DAT Transporter Sites | | |
| NRP104 Concentration (M) | Percent Inhibition NET* | Percent Inhibition DAT* |
| 10 ⁻⁹ | 8.15 | -10.46 |
| 10 ⁻⁷ | -11.75 | 11.52 |
| 10 ⁻⁵ | 13.89 | -0.71 |
| *No binding activity is defined as producing between -20 and 20 percent inhibition of radioligand binding | | |
| Noteworthy Findings: NRP104 has no affinity for human recombinant norepinephrine or dopamine transporter sites. | | |

Table 2.4.6-2 NRP104: Pharmacodynamics Following Oral Administration of NRP104 or *d*-amphetamine in Rats

| | | | | | |
|---|------------------------------|---|--|---|---|
| Name of Company: New River Pharmaceuticals, Inc. | | New River Pharmaceuticals Study No: R01-NRP104-OPD-03 | | | |
| CTD Location: Module 4, Vol 1 , pg 1 , Section 4.2.1.1 | | | | | |
| General Pharmacokinetics (<i>in vivo</i>) | | | | | |
| Study Objective: To compare the motor activity of rats following oral administration of NRP104 or <i>d</i> -amphetamine or vehicle alone. | | | | | |
| Species/Strain: Rat/Sprague Dawley | | Weight Range on Day 1: Male: 250 - 300 | | Duration of Treatment: 1 day Frequency of Dosing: Once | |
| Test Materials: NRP104 (diHCl), <i>d</i> -amphetamine sulfate | | Route: Oral Dose Volume: ~1.67mL/kg Vehicle: Distilled de-ionized water | | Study Dates: March 26-April 16, 2003 | |
| Batch No: R-II-49 | | Dose: NRP104 (diHCl) – 7.52 mg/kg; <i>d</i> -amphetamine sulfate – 4.12 mg/kg which is equivalent to 3 mg/kg <i>d</i> -amphetamine base. | | Study in Compliance with GLP: No | |
| Main Testing Facilities: New River Pharmaceuticals, Inc. 1861 Pratt Drive, and [REDACTED] [REDACTED] | | | | | |
| Frequency of dosing and study design: Single doses of NRP104 (n=4) or <i>d</i> -amphetamine (n=3) or vehicle (n=1) were administered to rats. Rats were placed in a motor activity chamber and counts were recorded over a period of 5 (study 1), 8 (study 2), and 12 (study 3) hours. Data is presented as the mean of the three studies. | | | | | |
| Pharmacodynamic parameters of rats following oral administration of NRP104 or <i>d</i>-amphetamine sulfate or vehicle. | | | | | |
| Test Material | Total Activity Counts | Total Activity Counts Above Baseline | Peak of activity (Counts per 0.2 h) | Time of Peak (Counts per 0.2 h) | Time of Last Count Above 100 per 0.2 h |
| Vehicle | 936 | 0 | 81 | 7.2 | - |
| NRP104 | 8,423 | 7,487 | 256 | 1.8 | 8.6 h |
| <i>d</i> -amphetamine sulfate | 6,622 | 5,686 | 223 | 0.6 | 6.4 h |
| Noteworthy Findings: NRP104 produced an increase in motor activity in rats similar to that observed with an equimolar dose of <i>d</i> -amphetamine sulfate following oral administration. The onset of motor activity was delayed as compared to that of <i>d</i> -amphetamine sulfate. The total activity above baseline for NRP104 was greater than that of <i>d</i> -amphetamine sulfate. Activity remained above 100 counts per 0.2 h for a longer period in rats administered NRP104 than for rats administered <i>d</i> -amphetamine sulfate. | | | | | |

| Table 2.4.6-3 Pharmacodynamics Following Intravenous Administration of NRP104 or <i>d</i> -Amphetamine Sulfate in Rats | | | | | |
|---|-----------------------|--|---|---|--|
| Name of Company: New River Pharmaceuticals, Inc. | | | New River Pharmaceuticals Report No: R01-NRP104-IVPD-054 | | |
| General Pharmacokinetics (<i>in vivo</i>) | | | | | |
| Study Objective: To compare the motor activity of rats following IV administration of NRP104 or <i>d</i> -amphetamine or vehicle alone. | | | | | |
| Species/Strain: Rat/Sprague Dawley | | Weight Range on Day 1: Male: 250 - 300 | | Duration of Treatment: 1 day Frequency of Dosing: Once | |
| Test Materials: NRP104(diHCl), <i>d</i> -amphetamine sulfate | | Route: Intravenous Dose Volume: ~0.33mL/kg Vehicle: Distilled de-ionized water | | CTD Location: Mod 4, Vol 1 , Section 4.2.1.1 | |
| Batch No: R-II-49 | | Dose: NRP104(diHCl) - 2.51 mg/kg; <i>d</i> -amphetamine Sulfate - 1.37 mg/kg which is equivalent to 1mg/kg <i>d</i> -amphetamine base. | | Study in Compliance with GLP: No | |
| Main Testing Facilities: New River Pharmaceuticals, Inc. 1861 Pratt Drive, and [REDACTED] | | | | | |
| Frequency of dosing and study design: Single doses of NRP104 (n=4) or <i>d</i> -amphetamine (n=3) or vehicle (n=1) were administered to rats by bolus intravenous injection. Rats were placed in a motor activity chamber and counts were recorded over a period of 3 hours. | | | | | |
| Pharmacodynamic parameters of rats following oral administration of NRP104 or <i>d</i> -amphetamine sulfate or vehicle. | | | | | |
| Test Material | Total Activity Counts | Total Activity Counts Above Baseline | Peak of activity (Counts per 0.2 h) | Time of Peak (Counts per 0.2 h) | Time of Last Count Above 100 per 0.2 h |
| Vehicle | 304 | 0 | 64 | 2.4 | - |
| NRP104 | 767 | 463 | 100 | 1.2 | - |
| <i>d</i> -amphetamine sulfate | 1,559 | 1,355 | 191 | 0.4 | 1.6 h |
| Noteworthy Findings: Total activity produced in rats by IV injection of NRP104 was substantially less than that of rats injected with amphetamine sulfate. The peak of activity for NRP104 injected rats was decreased and delayed as compared to activity of rats injected with <i>d</i> -amphetamine sulfate. | | | | | |

| Table 2.4.6-4 Pharmacodynamics Following Intranasal Administration of NRP104 or <i>d</i> -amphetamine sulfate in Rats | | | | | |
|--|-----------------------|--|-------------------------------------|---|--|
| Name of Company: New River Pharmaceuticals, Inc. | | | Report No: R03-NRP104-INPD-05 | | |
| CTD Location: Mod 4, Vol 1 , Section 4.2.1.1 | | | | | |
| General Pharmacodynamics (<i>in vivo</i>) | | | | | |
| Study Objective: To compare the motor activity of rats following IN administration of NRP104 or <i>d</i> -amphetamine sulfate or vehicle alone. | | | | | |
| Species/Strain: Rat/Sprague Dawley | | Weight Range on Day 1: Male: 250 - 300 | | Duration of Treatment: 1 day Frequency of Dosing: Once | |
| Test Materials: NRP104(diHCl), <i>d</i> -amphetamine sulfate | | Route: Intranasal Dose Volume: ~0.067mL/kg Vehicle: Distilled de-ionized water | | Study Dates: March 26-April 16, 2003 | |
| Batch No: R-II-49 | | Dose: NRP104(diHCl) - 2.51 mg/kg; <i>d</i> -amphetamine sulfate - 1.37 mg/kg which is equivalent to 1mg/kg <i>d</i> -amphetamine base. | | Study in Compliance with GLP: No | |
| Main Testing Facilities: New River Pharmaceuticals, Inc. 1861 Pratt Drive, and [REDACTED] [REDACTED] [REDACTED] MA 01960 | | | | | |
| Frequency of dosing and study design: Single doses of NRP104 (n=4) or <i>d</i> -amphetamine sulfate (n=3) or vehicle (n=1) were administered to rats by placing the dose into the nasal flares. Rats were placed in a motor activity chamber and counts were recorded over a period of 1h. | | | | | |
| Pharmacodynamic parameters of rats following oral administration of NRP104 or <i>d</i> -amphetamine or vehicle. | | | | | |
| Test Material | Total Activity Counts | Total Activity Counts Above Baseline | Peak of activity (Counts per 0.2 h) | Time of Peak (Counts per 0.2 h) | Time of Last Count Above 100 per 0.2 h |
| Vehicle | 172 | 0 | 52 | 0.8 | - |
| NRP104 | 408 | 236 | 98 | 0.8 | - |
| <i>d</i> -amphetamine sulfate | 857 | 685 | 194 | 0.4 | 1 h |
| Noteworthy Findings: Total activity produced in rats by IN administration of NRP104 was substantially less than that of rats administered amphetamine sulfate. The peak of activity for NRP104 dosed rats was decreased and delayed as compared to activity of <i>d</i> -amphetamine sulfate dosed rats. | | | | | |

The reviewer's summary of the data does not differ from what has been provided by the sponsor and as attached here in this section. The general overall summary can be summed as that the compound after it is metabolized, is basically acting like amphetamine in its *in vivo* action as judged by its effect on locomotor activity in rats treated by the oral route and that the *in vitro* binding of the parent

compound indicates that the parent does not bind to the DA or NE transporters. Rather it is the effect of the product (i.e. amphetamine) on these transporters that is responsible for the in vivo effect of the parent which resembled the effect of d-amphetamine sulfate when they were compared in different studies. What was also observed is that when given by the I.V. or I.N. routes the compound was much less effective than amphetamine sulfate in its actions as judged by the reduction in the observed effects of treatment using these routes.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary:

A validated study for the detection of test article in the plasma of rats and a “revised draft report” for the detection of test article in dogs were provided. The pharmacokinetic characteristics of the test article were studied using different routes of administration (oral, I.V. and I.N.) in rats and in dogs. The parent compound was not detected in the brain of rats following oral administration while d-amphetamine was present in the brain as a result of this treatment. Following oral administration of NRP-104 in rats, the bioavailability of the parent compound varied with dose. T_{max} for the parent compound ranged from 0.25 to 3h at low dose and up to 4-8h at high doses. C_{max} for d-amphetamine in plasma following oral administration of NRP-104 (3 mg/kg amphetamine base) was ~one half of C_{max} following d-amphetamine sulfate administration in one report and comparable to those of a similar dose in another report (see review for available figures). At higher doses the fraction of amphetamine absorbed as a result of oral administration of NRP-104 decreased compared to lower doses, however, in animals treated with d-amphetamine sulfate the amphetamine absorbed was increased at the highest doses. Following I.V. administration in rats, the plasma concentration of d-amphetamine derived from intact NRP-104 in comparison to d-amphetamine derived from an equimolar dose of d-amphetamine sulfate, were significantly reduced. Similar observations were seen with intranasal administration. The metabolism of the compound following oral administration in rats seems to be fairly simple since the major products were those of amphetamine and amphetamine metabolites. The parent compound was observed only for up to 8 hours after oral administration and the highest levels of the radioactivity produced from the parent compound were less than 2% of the total radioactivity in plasma of F. The levels of radioactivity for the parent compound after I.V. administration were ~20% of the total radioactivity in plasma. The only metabolite that was directly related to the parent compound (M2 or hydroxylated NRP-104) was observed only after I.V. administration. This suggests that after an oral administration, NRP-104 is quickly converted to amphetamine before reaching the plasma circulation. The site of metabolism was not thoroughly tested; however, in vitro testing showed that the liver is not the site of metabolism for the compound. However, in several places the sponsor stated that the site of metabolism is in the “gastrointestinal tract”. The major route of elimination of total radioactivity after oral administration in rats is through urine (~77% in M and ~87% in

F). The compound did not seem to inhibit a variety of CYP-450 enzymes (see table within review for specific enzymes).

In dogs the pharmacokinetic parameters were evaluated following oral and I.V. administration and that data indicated that the compound has a moderate oral bioavailability (33%) and that plasma levels of d-amphetamine after oral administration of NRP-104 are comparable to those after it I.V. administration.

2.6.4.2 Methods of Analysis

The sponsor has provided in a GLP study a validation for ~~XXXXXX~~ analytical procedure AP.100448.PL06 for the determination of NRP-104 (lysine-amphetamine) and d-amphetamine in rat plasma (heparin), by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (~~XXXXXX~~ project # 100448). The validation included selectivity, linearity, lower limit of quantitation (LLOQ), carry-over, intra- and inter-assay precision and accuracy, stock solution stability, injection medium integrity, short-term matrix stability, freeze-thaw stability, long-term matrix stability, and dilution integrity.

In general, the data indicate that the assay has met the validation criteria set by the sponsor. The only finding that indicated some deviation from the set criteria was the stability of the stock solution for the parent compound which was found to be stable up to 9 days at the lowest concentration and up to 28 days at the highest concentration. Similarly, d-amphetamine stability at the lowest concentration was seen up to 11 days and up to 28 days at the highest concentration. Long-term matrix stability (stability of reference standards in rat plasma) was confirmed for 89 and 125 days even though the study was faced with challenges and negative results at earlier days. In the conclusion, the sponsor stated that the analytical procedure demonstrated to be suitable for the determination of NRP-104 and d-amphetamine in rat plasma.

A “revised draft report” for the validation of the method in dog plasma was provided by the sponsor (~~XXXXXX~~ project # 100449). The validation criteria were similar to those in the rat and the results were within the set acceptance criteria set by the sponsor. The sponsor indicated in the conclusion that “partial validation of the analytical procedure demonstrated that the method was suitable for the determination of NRP-1-4 and d-amphetamine in dog plasma”.

A GLP study was performed for the validation of an analytical assay for the detection of NRP-104 concentration in formulations prepared in deionized water using an HPLC method with UV detection (Study #0876VN29.001). The method was acceptable with regard to linearity, precision, accuracy and selectivity aspects of NRP-104.

The sponsor provided summaries, tables and figures for the evaluation of the pharmacokinetic characteristics of the compound using different routes of administration

(oral, I.V., and I.N.) in studies conducted in male Sprague Dawley rats (studies # R05-NRP-104-PKIV-9, R06-NRP104-PKO-10, and R07-NRP104-PKIN-11) and dog (Study #0832DN29.001). The overall summary of these studies is as follows:

2.6.4.3 Absorption:

Rat:

Following oral administration of NRP104 (1.5, 3, 6, 12, and 60 mg/kg as d-amphetamine base), the rate of absorption was rapid at lower doses but more prolonged at higher doses. The fraction of intact NRP-104 absorbed after oral administration in rats was variable with escalating doses from 1.5 to 12 mg/kg (d-amphetamine base). The fraction absorbed (F) at 1.5 mg/kg was only 2.6% whereas it increased to 24.6% at 12 mg/kg. The fraction absorbed fell to 9.3% at the high dose of 60 mg/kg. The fraction absorbed here refers to the oral bioavailability of the parent compound as judged from the AUC value of the parent after oral administration compared to the AUC value after I.V. administration (5.08 mg/kg of NRP-104, which was the only dose used for the IV route). The following table was provided by the sponsor summarizing the PK parameters of the NRP-104 following oral administration in rats (page 6, vol. 3, Module 4, Sequence 1):

| Pooled plasma pharmacokinetic parameters of NRP104 following oral administration of NRP104 | | | | | | | |
|--|--------|--------------|--------------|--------------|--------------------|--------------------|-------|
| Dose Route | Drug | Dose (mg/kg) | Cmax (ng/ml) | Tmax (ng/ml) | AUC(0-8) (ng.ml/h) | AUC(inf) (ng.ml/h) | F (%) |
| Oral | NRP104 | 5.08 | 36.5 | 0.25 | 59.4 | 60 | 2.6 |
| Oral | NRP104 | 10.16 | 135.4 | 1.5 | 329.7 | 332.1 | 7.2 |
| Oral | NRP104 | 20.32 | 676.8 | 0.25 | 1156.8 | 1170.8 | 12.8 |
| Oral | NRP104 | 40.64 | 855.9 | 1 | 4238.6 | 4510.4 | 24.6 |
| Oral | NRP104 | 203.2 | 1870.3 | 3 | 8234.3 | 8499.9 | 9.3 |

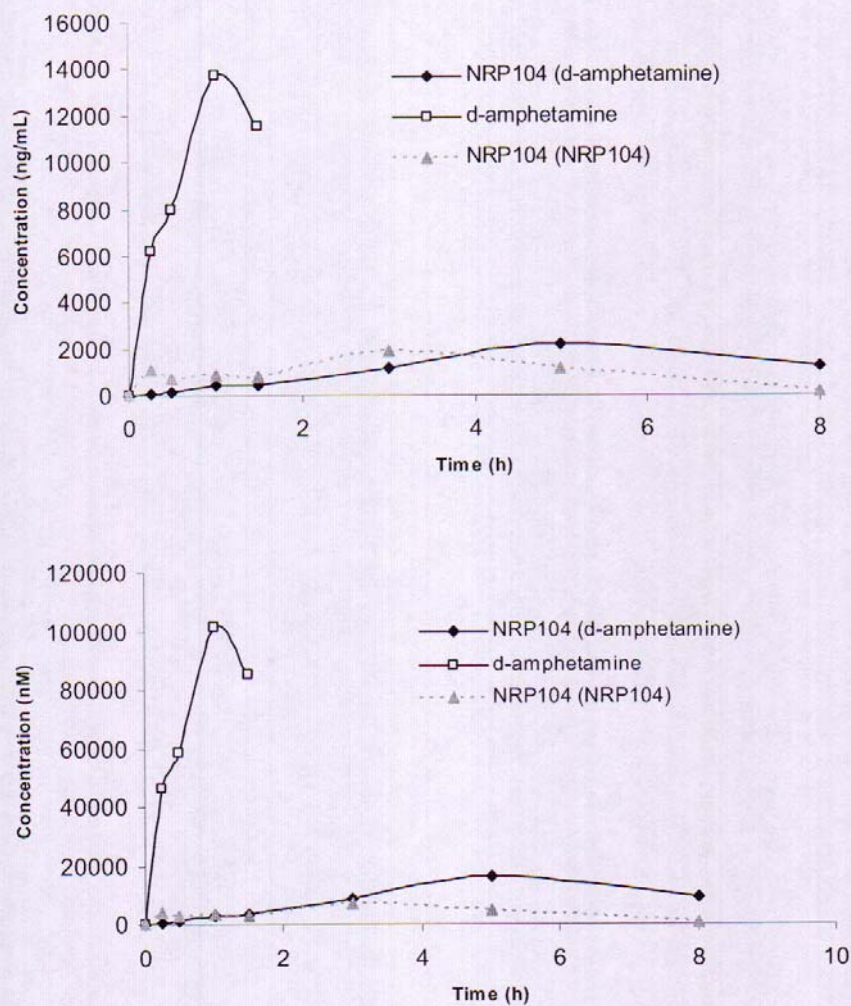
It should be noted that the doses in the table are for lysine-amphetamine mesylate (NRP-104) and the conversion factor for d-amphetamine base content of NRP104 is 0.2948 (so a dose of 5.08 mg/kg of NRP-104 is equal to 1.5 mg/kg of d-amphetamine base and so on for the rest of doses in the table). See conversion factors described earlier in the review.

Plasma d-amphetamine base concentration expressed as Cmax following oral administration of NRP-104 in rats was reduced by approximately half as compared to Cmax following d-amphetamine sulfate administration at doses of 1.5 to 6 mg/kg amphetamine base (which are human equivalent doses of 19.9 to 79.72 mg of d-amphetamine sulfate, using conversion factors provided by sponsor). At higher doses (12 to 60 mg/kg of d-amphetamine base which is the HED of 159.4 to 797.15 mg of d-amphetamine sulfate) Cmax was reduced even further (by 84% at the highest dose, see

the following figure and table provided by the sponsor (pages 24 and 6, respectively of vol. 3, Module 4, Sequence 1):

Figure 7.

Plasma *d*-amphetamine and NRP104 levels following oral administration of NRP104 or *d*-amphetamine sulfate (60mg/kg *d*-amphetamine base) to rats. Note: 8 of the total 12 rats in 60mg/kg *d*-amphetamine dosed groups died, therefore, plasma concentrations could not be determined beyond 1.5 hours.



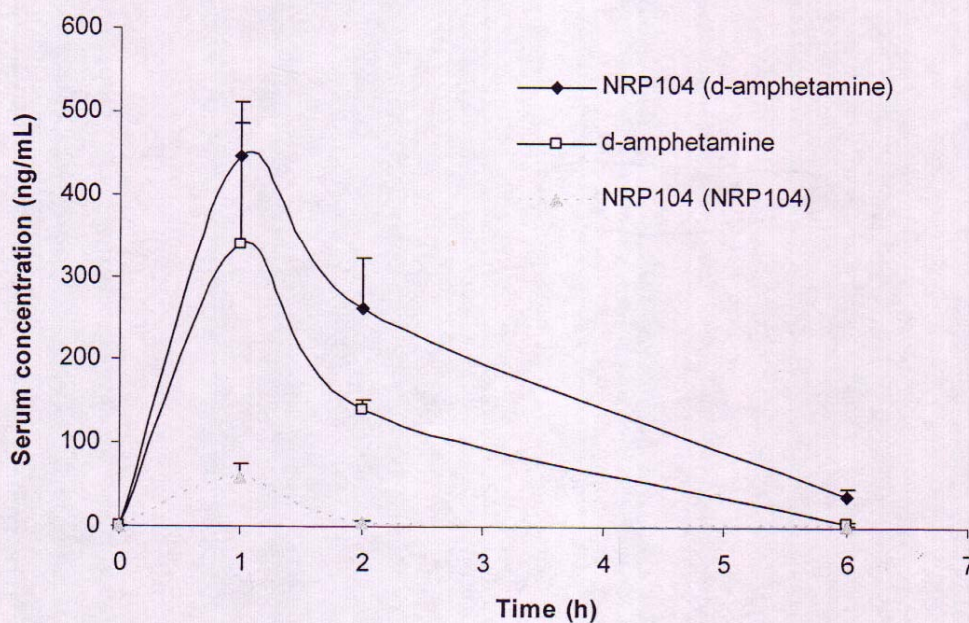
Pooled plasma pharmacokinetic parameters of *d*-amphetamine following administration of NRP104 or *d*-amphetamine at increasing doses

| Dose Route | Drug | Dose (mg/kg) | C _{max} (ng/mL) | T _{max} (h) | AUC(0-8) (ng.mL/h) | AUC(inf) (ng.mL/h) | F (%) |
|------------|-----------------------|--------------|--------------------------|----------------------|--------------------|--------------------|-------|
| Oral | NRP104 | 1.5 | 59.6 | 3 | 308 | 331 | 61 |
| Oral | <i>d</i> -amphetamine | 1.5 | 142.2 | 0.5 | 446 | 461 | 84 |
| Oral | NRP104 | 3 | 126.9 | 1.5 | 721 | 784 | 72 |
| Oral | <i>d</i> -amphetamine | 3 | 217.2 | 1.5 | 885 | 921 | 84 |
| Oral | NRP104 | 6 | 310.8 | 3 | 1,680 | 1,797 | 82 |
| Oral | <i>d</i> -amphetamine | 6 | 815.3 | 0.25 | 1,319 | 1,362 | 62 |
| Oral | NRP104 | 12 | 412.6 | 5 | 2,426 | 2,701 | 62 |
| Oral | <i>d</i> -amphetamine | 12 | 1,533.1 | 0.25 | 4,252 | 4,428 | 101 |
| Oral | NRP104 | 60 | 2,164.3 | 5 | 9995.1 | 11,478 | 52 |
| Oral | <i>d</i> -amphetamine | 60 | 13,735 | 1 | NC | 48,707 | 223 |

NC – not calculated

However; it should be noted that in another report (Report #R04-NRP104-DBT-08) C_{max} for *d*-amphetamine in *serum* of rats following oral administration of NRP-104 (3.64 mg/kg amphetamine base) was similar to that seen with equimolar dose of *d*-amphetamine sulfate as seen in the following figure (figure 8.7 on page 8-17 of volume 2 submission N-000 of IND 67482):

Figure 8.7 Serum levels of *d*-amphetamine and NRP104 following oral administration of equimolar doses NRP104 or *d*-amphetamine sulfate (3.64 mg/kg *d*-amphetamine base) to rats



As for AUC values for d-amphetamine base following oral administration of NRP-104, they were comparable to those observed after oral administration of d-amphetamine sulfate at lower doses; however, at higher doses the values obtained from NRP-104 treatment were much lower than those obtained with d-amphetamine sulfate (reduced by 77% at the highest dose of 60 mg/kg, as was observed with C_{max}, see previous table).

In general what can be gathered from the previous data is that the pharmacokinetics of d-amphetamine obtained from NRP-104 are nearly linear at doses from 1.5 to 60 mg/kg (HED of 19.9 to 797.2 mg) with the fraction absorbed ranging from 52% to 82% (see previous table). For d-amphetamine obtained from d-amphetamine sulfate the pharmacokinetic parameters were also nearly linear at lower doses of 1.5 to 6 mg/kg with the fraction absorbed ranging from 62% to 84% but unlike NRP-104 at higher doses (12 and 60 mg/kg) the fraction absorbed was disproportionately increased at higher doses with the fraction absorbed calculated as 101 to 223%, respectively (according to the sponsor those values were extrapolated from the 1.5 mg/kg dose). The sponsor noted that the fraction absorbed for NRP-104 and d-amphetamine sulfate was extrapolated from the AUC_{inf} of the 1.5 mg/kg NRP-104 and d-amphetamine intravenous doses and the fraction absorbed for the high doses of d-amphetamine may be above 100% due to a difference in clearance rate. The sponsor stated that these results suggest that the capacity for clearance of d-amphetamine when delivered as the sulfate salt becomes saturated at the higher doses whereas the gradual hydrolysis of NRP-104 precludes saturation of d-amphetamine elimination at higher doses.

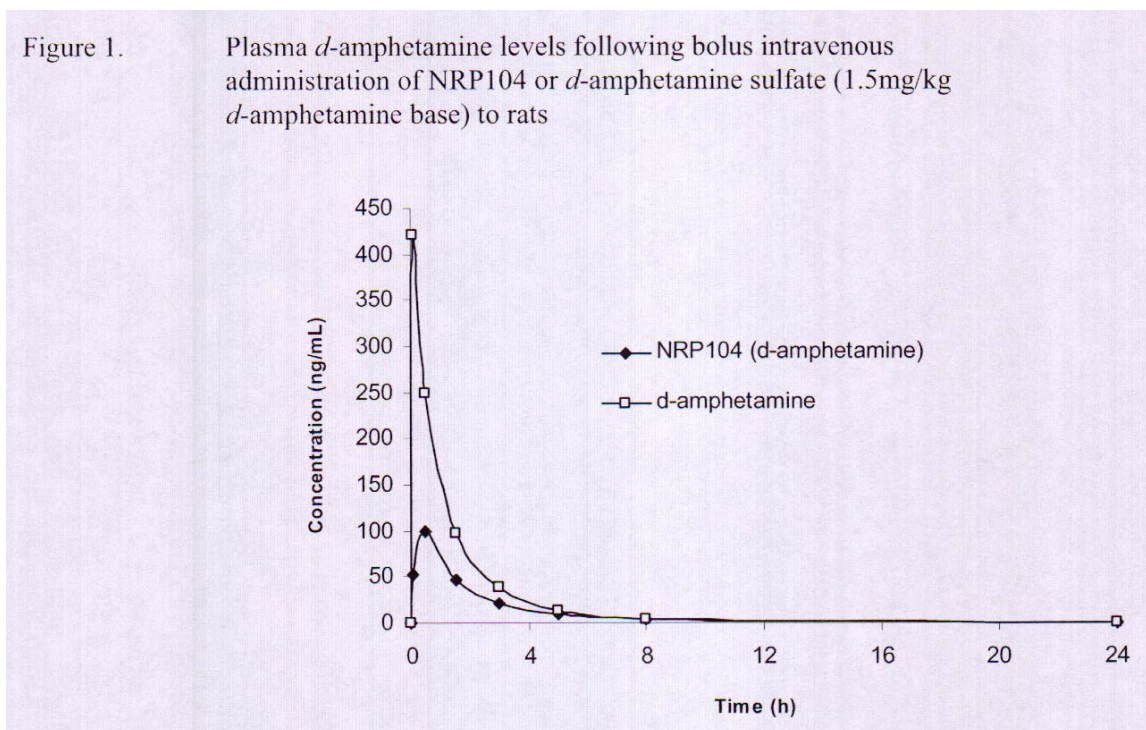
T_{max} for d-amphetamine following oral administration of NRP104 ranged between 1.5-5h as compared to 0.5 to 1.5h following oral administration of d-amphetamine sulfate.

Bioavailability of d-amphetamine following bolus intravenous administration of NRP-104 in rats (1.5 mg d-amphetamine base/kg, plasma sample collected at 5, 30 minutes, 1.5, 3, 5, 8, and 24h post dose) was ~1/2 that of the equimolar d-amphetamine sulfate dose while C_{max} was only about 1/4. T_{max} was delayed with NRP-104 treatment compared to d-amphetamine sulfate which could be due to gradual hydrolysis of NRP-104. . The following table summarizes the pharmacokinetic parameters of d-amphetamine from NRP-104 and d-amphetamine sulfate following I.V. administration (page 4, vol. 3, Module 4, Sequence 1):

Pooled plasma pharmacokinetic parameters of *d*-amphetamine following bolus IV administration of NRP104 versus *d*-amphetamine

| Dose Route | Drug | Dose (mg/kg) | C _{max} (ng/mL) | T _{max} (h) | AUC(0-24) (ng.mL/h) | AUC(inf) (ng.mL/h) |
|------------|-----------------------|--------------|--------------------------|----------------------|---------------------|--------------------|
| IV | NRP104 | 1.5 | 99.5 | 0.5 | 237.8 | 237.9 |
| IV | <i>d</i> -amphetamine | 1.5 | 420.2 | 0.083 | 546.7 | 546.9 |

The following figure was also provided by the sponsor (figure 1, vol. 3, module 4, sequence 1):



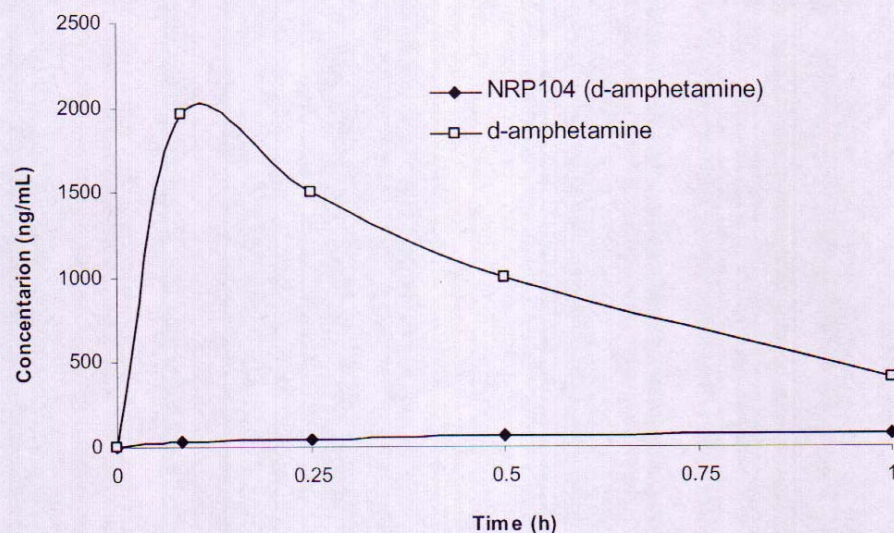
Plasma levels of the parent compound after I.V. administration as provided by the sponsor are seen in the following table (page 4, vol. 3, Module 4, Sequence 1):

| Pooled plasma pharmacokinetic parameters of NRP104 following IV administration of NRP104 | | | | | | |
|--|--------|--------------|--------------------------|----------------------|---------------------|--------------------|
| Dose Route | Drug | Dose (mg/kg) | C _{max} (ng/mL) | T _{max} (h) | AUC(0-24) (ng.mL/h) | AUC(inf) (ng.mL/h) |
| IV | NRP104 | 5.08 | 4513.1 | 0.083 | 2,282 | 2,293 |

Following intranasal administration (I.N., single dose of NRP-104 or *d*-amphetamine sulfate as 3 mg *d*-amphetamine base/kg by pipetting 0.02 ml of solution into the nose flares) in rats, AUC and C_{max} values for *d*-amphetamine following NRP104 were only 5% of those with *d*-amphetamine sulfate (see Figure 8 as provided by the sponsor on page 25, vol. 3 Module 4, Sequence 1).

Figure 8.

Plasma *d*-amphetamine levels following intranasal administration of NRP104 or *d*-amphetamine sulfate (3mg/kg *d*-amphetamine base) to rats



T_{max} of *d*-amphetamine concentration after I.N. administration of NRP-104 was delayed (60 min) as compared to T_{max} of *d*-amphetamine sulfate (5 min), which could reflect the gradual hydrolysis of NRP-104 (see following table provided by sponsor for pharmacokinetic parameters after I.N. administration, page 7, vol. 3, Module 4, Sequence 1):

Pooled plasma pharmacokinetic parameters of *d*-amphetamine following bolus ~~iv~~ ^{IN} administration of NRP104 versus *d*-amphetamine

| Dose Route | Drug | Dose (mg/kg) | C _{max} (ng/mL) | T _{max} (h) | AUC(0-1) (ng.mL/h) | AUC(inf) (ng.mL/h) |
|------------|-----------------------|--------------|--------------------------|----------------------|--------------------|--------------------|
| IN | NRP104 | 10.16 | 78.6 | 1 | 56 | 91 |
| IN | <i>d</i> -amphetamine | 4.12 | 1962.9 | 0.083 | 1032 | 7291 |

The table was labeled as “iv” but it should be I.N. since the values reflect levels after I.N. administration, this will be clarified with the sponsor.

The parent compound was well absorbed which indicates that minimal hydrolysis by nasal mucosa occurred as judged by plasma levels after I.N. administration (table provided by sponsor on page 7, vol. 3, Module 4, Sequence 1):

Pooled plasma pharmacokinetic parameters of NRP104 following IN administration of NRP104

| Dose Route | Drug | Dose (mg/kg) | C _{max} (ng/mL) | T _{max} (h) | AUC(0-1) (ng.mL/h) | AUC(inf) (ng.mL/h) |
|------------|--------|-----------------|-----------------------------|-------------------------|-----------------------|-----------------------|
| IN | NRP104 | 10.16 | 3345.1 | 0.25 | 2,580 | 9,139 |

(It is difficult to understand the high AUC following I.N. administration. With an I.N. dose only 2-fold the dose administered I.V., there is a \approx 4-fold greater AUC with I.N. administration compared to I.V.)

Dogs:

In a non-GLP, non-randomized, two-treatment cross-over study (Study #0832DN29.001), male dogs (n = 3) were fasted overnight prior to each dose they will receive. On Day 1 animals received oral dose by gavage at 2 mg/kg (free base NRP-104) at dose level of 10 ml/kg. On Day 8, animals received 2 mg/kg (free base NRP-104) at a dose volume of 2 ml/kg as a single 30-min I.V. administration into the cephalic vein. Serial blood samples were obtained from each animal via the jugular vein at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, and 71h post oral dose and at 0, 0.167, 0.33, 0.49 (prior to stop of infusion), 0.583, 0.667, 0.75, 1, 2, 3, 4, 8, 12 and 23 h post I.V. infusion start.

The following figures and tables were provided by the sponsor to summarize the pharmacokinetic parameters for NRP-104 and d-amphetamine levels after treatment with NRP-104 (pages 4-5 and pages 20-21, vol. 3, Module 4, Sequence 1):

Pharmacokinetic results are summarized in the tables that follow.

| Mean (SD) plasma pharmacokinetic parameters of NRP104 in male beagle dogs | | | | | | | | | |
|---|-----------------|-----------------------------|--------------------------------------|-----------------------|-------------------------|------------|-------------------|----------------------------|----------|
| Route | Dose (mg/kg) | C _{max} (ng/mL) | T _{max} ^a (h) | AUC(inf) (ng•h/mL) | t _{1/2} (h) | MRT (h) | CL/F (mL/h•kg) | V _{ss} (mL/kg) | F (%) |
| IV | 2 | 1650 | 0.49 | 964 | 0.88 | 0.33 | 2087 | 689 | NA |
| | (0.00) | (178) | (0.49-0.49) | (97.1) | (0.2) | (0.03) | (199) | (105.9) | |
| Oral | 2 | 328.2 | 0.5 | 319 | 0.39 | 0.81 | 6351 | NA | 33 |
| | (0.00) | (91.9) | (0.5-0.5) | (46.3) | (0.1) | (0.19) | (898.3) | | (1.9) |

^a: median (range)

| Mean (SD) plasma pharmacokinetic parameters of d-amphetamine following administration of NRP104 in male beagle dogs | | | | | |
|---|--------------|--------------------------|-----------------------------------|--------------------|----------------------|
| Route | Dose (mg/kg) | C _{max} (ng/mL) | T _{max} ^a (h) | AUC(inf) (ng•h/mL) | t _{1/2} (h) |
| IV | 2 | 113.2 | 1.0 | 672.5 | 3.14 |
| | (0.00) | (3.2) | (0.67 – 2.0) | (85.7) | (0.4) |
| Oral | 2 | 104.3 | 2.0 | 728.0 | 3.48 |
| | (0.00) | (21.8) | (2 – 2) | (204.9) | (0.4) |

^a: median (range)

Figure 3. Mean Plasma Concentration Time Profile of NRP104 and d-amphetamine Following 30-min Intravenous Infusion (2 mg/kg) in Conscious Male Beagle Dogs (n=3).

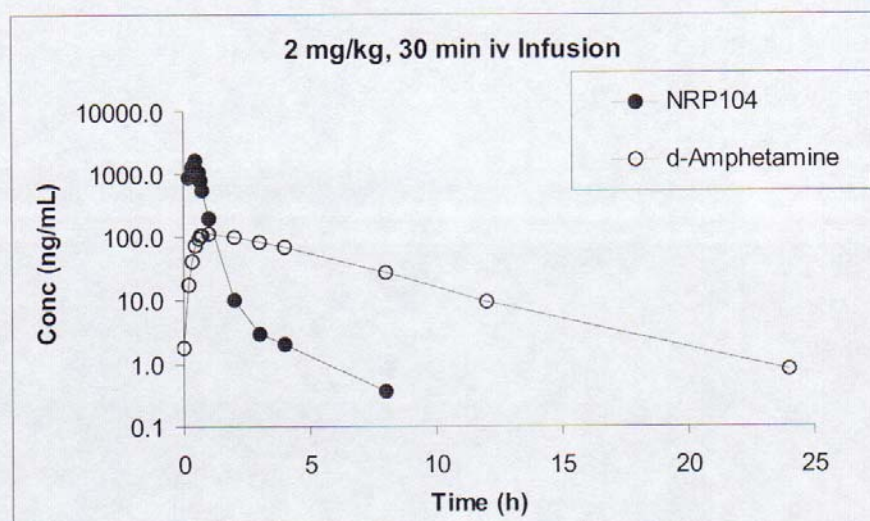
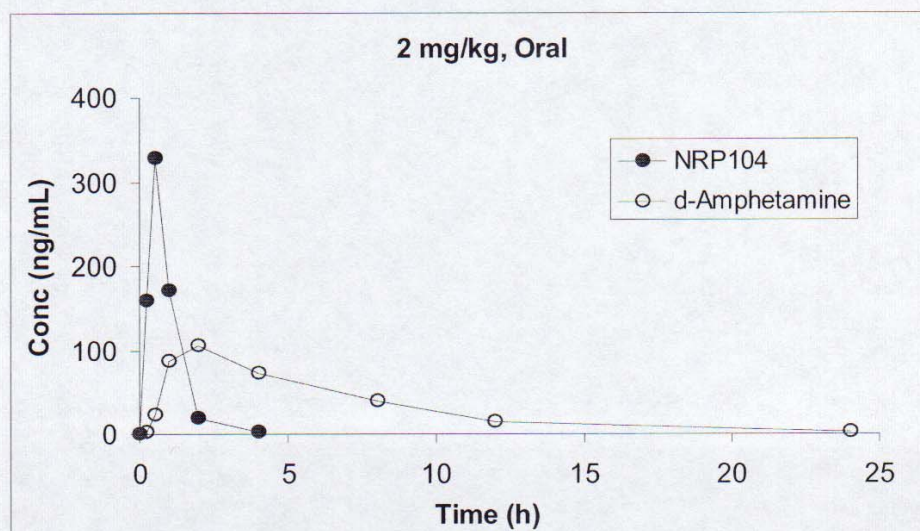


Figure 4. Mean Plasma Concentration Time Profile of NRP104 and d-amphetamine Following Oral Administration of NRP104 (2 mg/kg) in Conscious Male Beagle Dogs (n=3).



From the data it is apparent that the parent compound has a moderate oral bioavailability (33%) and that plasma levels of d-amphetamine after oral administration of NRP-104 are comparable to those obtained after its I.V. administration. It should be noted that the sponsor did not compare the levels of d-amphetamine obtained after administration of d-amphetamine sulfate to those obtained after NRP-104 administration as was done in rats.

2.6.4.4 Distribution

In a non-GLP study (Study #R04-NRP104-DBT-08), four groups of rats (n = 9/group) were treated with a single oral dose of NRP-104 (2 dose levels) or amphetamine (2 dose levels) in the fasted state as outlined in the table below obtained from sponsor (page 7, vol. 3, Module 4, Sequence 1). Serum samples and brain tissues were collected for all groups from 3 animals at each of 3 terminal time points (1, 2, and 6 hours) after animals were euthanized (see table below from sponsor).

| Group No. | Test Article | N | Route of Administration | Dose (mg/kg)* | Dose Volume (mL/kg) | Dose Conc. (mg/mL) |
|-----------|--------------------|---|-------------------------|---------------|---------------------|--------------------|
| 1 | Lysine-amphetamine | 9 | Oral | 3.09 | 5 | 0.618 |
| 2 | Lysine-amphetamine | 9 | Oral | 10.29 | 5 | 2.058 |
| 3 | d-Amphetamine | 9 | Oral | 1.5 | 5 | 0.3 |
| 4 | d-Amphetamine | 9 | Oral | 5 | 5 | 1 |

* equivalent $\mu\text{mol/kg}$ doses of lysine-amphetamine and amphetamine

| Group | Test Article | N | Serum sample times* | Brain sample times * |
|-------|--------------------|---|---------------------|----------------------|
| 1 | Lysine-amphetamine | 9 | 1, 2, 6 h | 1, 2, 6 h |
| 2 | Lysine-amphetamine | 9 | 1, 2, 6 h | 1, 2, 6 h |
| 3 | d-Amphetamine | 9 | 1, 2, 6 h | 1, 2, 6 h |
| 4 | d-Amphetamine | 9 | 1, 2, 6 h | 1, 2, 6 h |

* 3 rats per sample time, terminal samples

Following oral administration of NRP104 to rats, the parent compound was not detected in the brain tissue samples at either dose administered (all samples were below quantifiable limits, < 12.5 ng/g). Tmax was 1h; however, it should be noted that this was the first collection time and that an earlier Tmax could have been missed. The following tables were provided by the sponsor for the levels of parent compound in the brain and serum of rats treated with the specified doses (tables 1 & 3, pages 12-13, vol. 3, Module 4, Sequence 1):

Table 1. Serum and Brain Lysine-Amphetamine Concentrations in Rats Following a Single Oral Dose of 3.09 mg/kg Lysine-Amphetamine

| Time (h) | Serum Concentration (ng/mL) | | | | |
|------------------------|---------------------------------|-------------|--------------|-------|-------|
| | Rats 1, 4, 7 | Rat 2, 5, 8 | Rats 3, 6, 9 | Mean | SD |
| 1 | 8.919 | 5.821 | 10.147 | 8.296 | 2.229 |
| 2 | bql | bql | bql | bql | NC |
| 6 | bql | bql | bql | bql | NC |
| C_{max} (ng/mL) | | | | 8.296 | |
| T_{max} (h) | | | | 1.0 | |
| AUC_{0-6h} (h*ng/mL) | | | | NC | |
| bql = <2.5 ng/mL | | | | | |
| Time (h) | Brain Concentration (ng/g) | | | | |
| | Rats 1, 4, 7 | Rat 2, 5, 8 | Rats 3, 6, 9 | Mean | SD |
| 1 | bql | bql | bql | bql | NC |
| 2 | bql | bql | bql | bql | NC |
| 6 | bql | bql | bql | bql | NC |
| C_{max} (ng/g) | | | | NC | |
| T_{max} (h) | | | | NC | |
| AUC_{0-6h} (h*ng/g) | | | | NC | |
| bql = <12.5 ng/g | | | | | |
| Time (h) | Brain/Serum Concentration Ratio | | | | |
| | Rats 1, 4, 7 | Rat 2, 5, 8 | Rats 3, 6, 9 | Mean | SD |
| 1 | NC | NC | NC | NC | NC |
| 2 | NC | NC | NC | NC | NC |
| 6 | NC | NC | NC | NC | NC |

Table 3. Serum and Brain Lysine-Amphetamine Concentrations in Rats Following a Single Oral Dose of 10.29 mg/kg Lysine-Amphetamine

| Time (h) | Serum Concentration (ng/mL) | | | | |
|--------------------------------|-----------------------------|-----------------|-----------------|--------|--------|
| | Rats 10, 13, 16 | Rats 11, 14, 17 | Rats 12, 15, 18 | Mean | SD |
| 1 | 42.018 | 68.321 | 67.703 | 59.347 | 15.011 |
| 2 | 3.753 | 4.985 | 6.953 | 5.230 | 1.614 |
| 6 | 3.202 | bql | bql | 1.067 | NC |
| C _{max} (ng/mL) | | | | | 59.347 |
| T _{max} (h) | | | | | 1.0 |
| AUC _{0-6 h} (h*ng/mL) | | | | | 74.55 |
| bql = <2.5 ng/mL | | | | | |

| Time (h) | Brain Concentration (ng/g) | | | | |
|-------------------------------|----------------------------|-----------------|-----------------|------|----|
| | Rats 10, 13, 16 | Rats 11, 14, 17 | Rats 12, 15, 18 | Mean | SD |
| 1 | bql | bql | bql | bql | NC |
| 2 | bql | bql | bql | bql | NC |
| 6 | bql | bql | bql | bql | NC |
| C _{max} (ng/g) | | | | | NC |
| T _{max} (h) | | | | | NC |
| AUC _{0-6 h} (h*ng/g) | | | | | NC |
| bql = <12.5 ng/g | | | | | |

| Time (h) | Brain/Serum Concentration Ratio | | | | |
|----------|---------------------------------|-----------------|-----------------|------|----|
| | Rats 10, 13, 16 | Rats 11, 14, 17 | Rats 12, 15, 18 | Mean | SD |
| 1 | NC | NC | NC | NC | NC |
| 2 | NC | NC | NC | NC | NC |
| 6 | NC | NC | NC | NC | NC |

As for amphetamine levels in both the brain and serum, the maximum concentration was also observed at the first collection time point (1h) after dosing with both the parent and d-amphetamine sulfate (it should be noted that in observation of the effect on locomotor activity, the T_{max} for peak effect was about 2 hrs for NRP104 and ≤ 1 hr for d-amphetamine sulfate). Average brain to serum levels of amphetamine ranged from 4.5 to 9.6 for both NRP-104 and d-amphetamine sulfate-dosed animals. Serum and brain $AUC_{(0-6h)}$ and C_{max} were similar after 3.09 mg/kg NRP-104 and 1.5 mg/kg amphetamine doses. However, both C_{max} and AUC were slightly higher after the 10.29 mg/kg NRP-104 dose than after the 5 mg/kg amphetamine dose. See the following Summary Tables provided by the sponsor (pages 38 & 39, vol. 2, Module 2, Sequence 1):

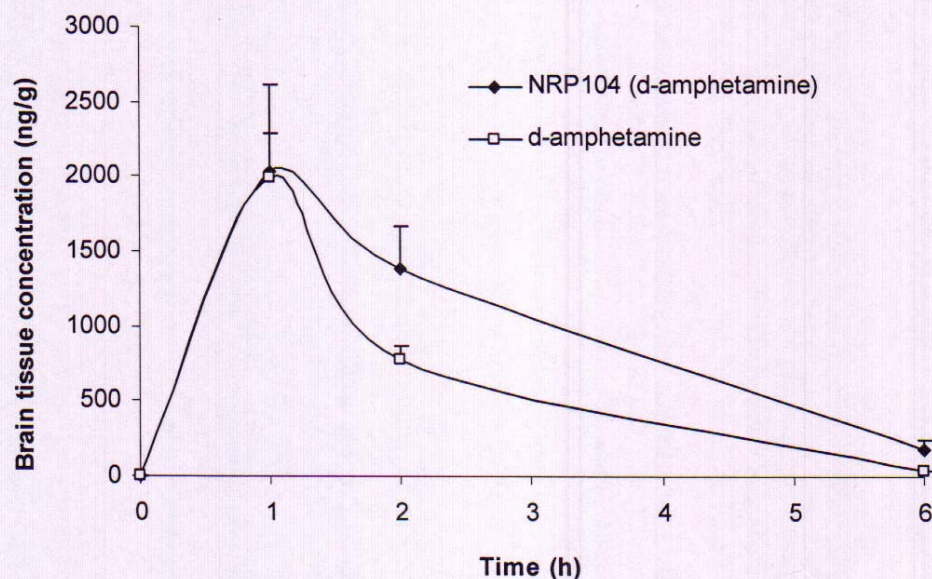
Table 2.6.4-9 Brain Uptake of Lysine-Amphetamine (NRP104) and Amphetamine in Rats Following Oral Administration of Lysine-Amphetamine or Amphetamine

| | | | | |
|---|-------|---|---|-------|
| Name of Company: New River Pharmaceuticals, Inc. | | Report No: R04-NRP104-DBT-08 | CRO Report No: 98D0303 | |
| CTD Location: Mod 4, Vol 3 , Section 4.2.2.3 | | | | |
| General Pharmacokinetics (<i>in vivo</i>) and Distribution | | | | |
| Study Objective: The objectives of this study were to evaluate the brain uptake of lysine-amphetamine and amphetamine after oral administration of lysine-amphetamine or amphetamine in rats. Four groups of rats were administered a single oral dose of lysine-amphetamine (2 dose levels) or amphetamine (2 dose levels) in the fasted state (N=9 per group). Serum and brain specimens were collected at each of three terminal time points and assayed for lysine-amphetamine and amphetamine. | | | | |
| Species/Strain: Rat/Sprague- Dawley | | Weight Range on Day 1: Male: 200-250 g | Duration of Treatment: 1 day Frequency of Dosing: Once | |
| Test Materials: NRP104, <i>d</i> -amphetamine | | Route: Oral (gavage) | Dose Volume: 5 mL/kg | |
| Batch No: R-II-49 (diHCl) | | Vehicle: 0.5% aqueous methylcellulose | Study in Compliance with GLP: No | |
| Main Testing Facilities: [REDACTED] | | | | |
| Frequency of dosing and study design: There were four groups of nine animals each. All animals were administered a single dose of lysine-amphetamine or amphetamine by oral gavage (PO), as outlined in the table below. Food was returned at 4 h post-dose if animals were not terminated by then. Serum samples and brain tissue were collected for all groups from three animals at each of 3 terminal time points (1, 2, and 6 hours) after animals were euthanized via CO ₂ inhalation. | | | | |
| Pharmacokinetic parameters of <u>NRP104</u> in serum and brain tissue following oral administration of <u>NRP104</u> | | | | |
| Sample Analyzed | Serum | Brain | Serum | Brain |
| Drug | | | | |
| Dose (mg/kg) : NRP104 | 3.09 | 3.09 | 10.29 | 10.29 |
| <i>d</i> -amphetamine base | 1.5 | 1.5 | 5 | 5 |
| HED (mg <i>d</i> -amphetamine sulfate equivalent, 60 kg adult) | 20 | 20 | 60 | 60 |
| C _{max} (ng/mL) | 8.296 | bql | 59.347 | bql |
| AUC _{0-6h} (ng.h/mL) | NC | | 74.55 | - |
| T _{max} (hours) | 1.0 | - | 1.0 | - |

| Table 2.6.4-9 Brain Uptake of Lysine-Amphetamine (NRP104) and Amphetamine in Rats Following Oral Administration of Lysine-Amphetamine or Amphetamine (Continued) | | | | |
|--|---------|--|----------|----------------------------|
| Name of Company: New River Pharmaceuticals, Inc. | | New River Pharmaceuticals No: R04-NRP104-DBT-08 | | CRO Report No: 98D-0303 |
| General Pharmacokinetics (<i>in vivo</i>) and Distribution | | | | |
| Pharmacokinetic parameters of <u>d-amphetamine</u> in serum and brain tissue following oral administration of <u>NRP104</u> | | | | |
| Sample Analyzed | Serum | Brain | Serum | Brain |
| Drug | | | | |
| Dose (mg/kg) : NRP104 | 3.09 | 3.09 | 10.29 | 10.29 |
| d-amphetamine base | 1.5 | 1.5 | 5 | 5 |
| HED (mg d-amphetamine sulfate equivalent, 60 kg adult) | 20 | 20 | 60 | 60 |
| C _{max} (ng/mL) | 119.361 | 531.155 | 446.087 | 2,024.935 |
| AUC _{0-6h} (ng.h/mL) | 231.96 | 1,206.57 | 1,286.68 | 6,371.97 |
| T _{max} (hours) | 1.0 | 1.0 | 1.0 | 1.0 |
| Pharmacokinetic parameters of <u>NRP104</u> in serum and brain tissue following oral administration of <u>d-amphetamine</u> | | | | |
| Sample Analyzed | Serum | Brain | Serum | Brain |
| Drug | | | | |
| Dose (mg/kg) : NRP104 | NA | NA | NA | NA |
| d-amphetamine base | 1.5 | 1.5 | 5 | 5 |
| C _{max} (ng/mL) | - | - | - | - |
| AUC _{0-6h} (ng.h/mL) | - | - | - | - |
| T _{max} (hours) | - | - | - | - |
| Pharmacokinetic parameters of <u>d-amphetamine</u> in serum and brain tissue following oral administration of <u>d-amphetamine</u> | | | | |
| Sample Analyzed | Serum | Brain | Serum | Brain |
| Drug | | | | |
| Dose (mg/kg) : NRP104 | 3.09 | 3.09 | 10.29 | 10.29 |
| d-amphetamine base | 1.5 | 1.5 | 5 | 5 |
| C _{max} (ng/mL) | 134.214 | 692.873 | 339.563 | 1,994.819 |
| AUC _{0-6h} (ng.h/mL) | 240.49 | 1,320.17 | 699.03 | 3,989.37 |
| T _{max} (hours) | 1.0 | 1.0 | 1.0 | 1.0 |
| Brain/Serum Concentration Ratios | | | | |
| Drug | NRP104 | d-amphetamine | NRP104 | d-amphetamine |
| Dose (mg/kg) : NRP104 | 3.09 | 3.09 | 10.29 | 10.29 |
| d-amphetamine base | 1.5 | 1.5 | 5 | 5 |
| 1 hour | 4.525 | 5.139 | 4.528 | 6.322 |
| 2 hours | 6.451 | 5.968 | 5.353 | 5.509 |
| 6 hours | 9.609 | 5.701 | 4.740 | 6.878 |
| Noteworthy Findings: No measurable amounts of NRP104 was detected in the brain. | | | | |

The following figure was provided by the sponsor in the original submission of IND 67482 (submission N-000, Figure 6, page 5-17 of Investigator's Brochure, Edition 1, dated March 20, 2004). It should be noted that the dose level indicated in the figure (3.64 mg/kg amphetamine base) is comparable to a dose of 10.29 mg/kg lysine amphetamine HCl salt (pure) as calculated by the sponsor (see last page of study report submitted in the original IND 67482, N000 vol. C2.2 for Study R-04-NRP104-DBT-08) and as calculated by the reviewer using the conversion factors provided by the sponsor and considering the purity of this salt (see conversion factors presented previously in the review).

Figure 6 Brain tissue levels of *d*-amphetamine and NRP104 following oral administration of to rats of equimolar doses of NRP104 or *d*-amphetamine sulfate (3.64 mg/kg *d*-amphetamine base)
Note: all brain samples were blq for intact NRP104



No other studies were conducted to investigate the distribution of this compound (whole body autoradiography).

2.6.4.5 Metabolism

The sponsor has proposed a metabolic profile for NRP-104 and suggested "tentative" metabolites in plasma, urine, bile, and feces based on two studies conducted in Sprague Dawley rats following either a single oral or intravenous dose of a mixture of NRP-104 and [^{14}C]NRP-104 (Study# R10-NRP104-ADME-25). The study designs are described

in the following table provided by the sponsor (page 15, vol. 3, module 4, Sequence 1) for the study entitled “Pharmacokinetics of radioactivity in bile duct-cannulated and intact Sprague-Dawley rats following a single oral or IV administration of [^{14}C]NRP104” (groups 1-6 and 9-10) and the second study entitled “Pharmacokinetics and excretion mass balance of radioactivity in bile duct-cannulated and intact Sprague-Dawley rats following oral administration of a mixture of NRP 104 and [^{14}C]NRP104” (groups 7 & 8):

Table S-1. Dosing and Blood Collection Summary

| Group Number | M/F | Dose Route | Dose Level (mg/kg) | Dose Conc. (mg/mL) | Target Radioactivity Level ($\mu\text{Ci/kg}$) | Plasma (hr) | Urine/Feces (hr) | Bile (hr) | Frozen Carcass (hr) |
|--|-----|------------|--------------------|--------------------|--|---------------------------------------|--|--------------------------------------|---------------------|
| 1 (Intact) | 2/2 | Oral | 10 | 2 | 80 | 0, 0.5, 1 | N/A | N/A | 1 |
| 2 (Intact) | 2/2 | Oral | 10 | 2 | 80 | 0.5, 1, 3 | N/A | N/A | 3 |
| 3 (Intact) | 2/2 | Oral | 10 | 2 | 80 | 1, 3, 5 | N/A | N/A | 5 |
| 4 (Intact) | 2/2 | Oral | 10 | 2 | 80 | 5, 8, 12 | N/A | N/A | 12 |
| 5 (Intact) | 2/2 | Oral | 10 | 2 | 80 | 8, 12, 24 | N/A | N/A | 24 |
| 6 (Intact) | 2/2 | Oral | 10 | 2 | 80 | 12, 24, 48 | N/A | N/A | 48 |
| 7 (Intact) | 3/3 | Oral | 10 | 2 | 80 | N/A | Pre-dose, 0-5, 5-12, 12-24 hr, and every 24 hr until 168 hr. | N/A | 168 |
| 8 (BDC, JVC) | 3/3 | Oral | 10 | 2 | 80 | Pre-dose, 0.5, 1, 3, 5, 8, 12, 24, 48 | N/A | Pre-dose, 0-3, 3-5, 5-8, 8-24, 24-48 | N/A |
| 9 (JVC) | 3/3 | IV | 3 | 3 | 80 | 0, 0.083, 1, 3, 5, 8, 12, 24, 48 | N/A | N/A | N/A |
| 10 (BDC, JVC) | 3/3 | IV | 3 | 3 | 80 | 0, 0.083, 1, 3, 5, 8, 12, 24, 48 | N/A | Pre-dose, 0-3, 3-5, 5-8, 8-24, 24-48 | N/A |
| BDC: Bile duct-cannulated JVC: Jugular vein cannulated N/A: Not applicable | | | | | | | | | |

Results:

A total of 14 putative metabolites were detected and/or identified. See the following table provided by the sponsor for those metabolites detected in plasma, urine, bile and feces (page 9, vol. 3, Module 4, Sequence 1):

TABLE A. NRP 104 and its Metabolites Detected in Plasma, Urine, Bile and Feces from Sprague-Dawley Rats following either a Single Oral or Intravenous Dose of a Mixture of NRP 104 and [¹⁴C]NRP 104

| | Parent | Metabolites | | | | | | | | | | | | | |
|----------------------|--------|-------------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Component | NRP104 | M1 | M2 | M3 | M4 | M5 | M6 | M7 | M8 | M9 | M10 | M11 | M12 | M13 | M14 |
| Retention time (min) | 7.75 | 3.0 | 6.33 | 8.58 | 9.33 | 10.25 | 10.75 | 13.17 | 14.50 | 17.58 | 18.50 | 19.33 | 19.65 | 20.99 | 22.67 |
| Molecular Weight | 263 | 329 | 279 | 327 | 151 | 151 | 327 | 135 | 151 | 369 | 179 | 193 | 193 | 193 | 327 |
| MRM | 264>84 | 330>136 | 280>84 | 328>135 | 152>135 | 152>135 | 328>135 | 136>119 | 152>135 | 370>194 | 180>105 | 194>135 | 194>119 | 194>119 | 328>135 |
| Plasma (Male) | D | N | D | M | N | N | d | M | D | d | d | N | d | N | D |
| Plasma (Female) | D | N | D | M | N | N | d | M | D | d | d | N | d | N | D |
| Urine | D | D | D | M | D | D | D | M | D | D | D | D | d | d | D |
| Bile | D | M | N | M | D | M | N | M | D | M | D | D | d | d | d |
| Feces | D | N | N | N | N | M | N | M | N | N | d | d | d | N | N |

M: Predominant metabolite in each sample

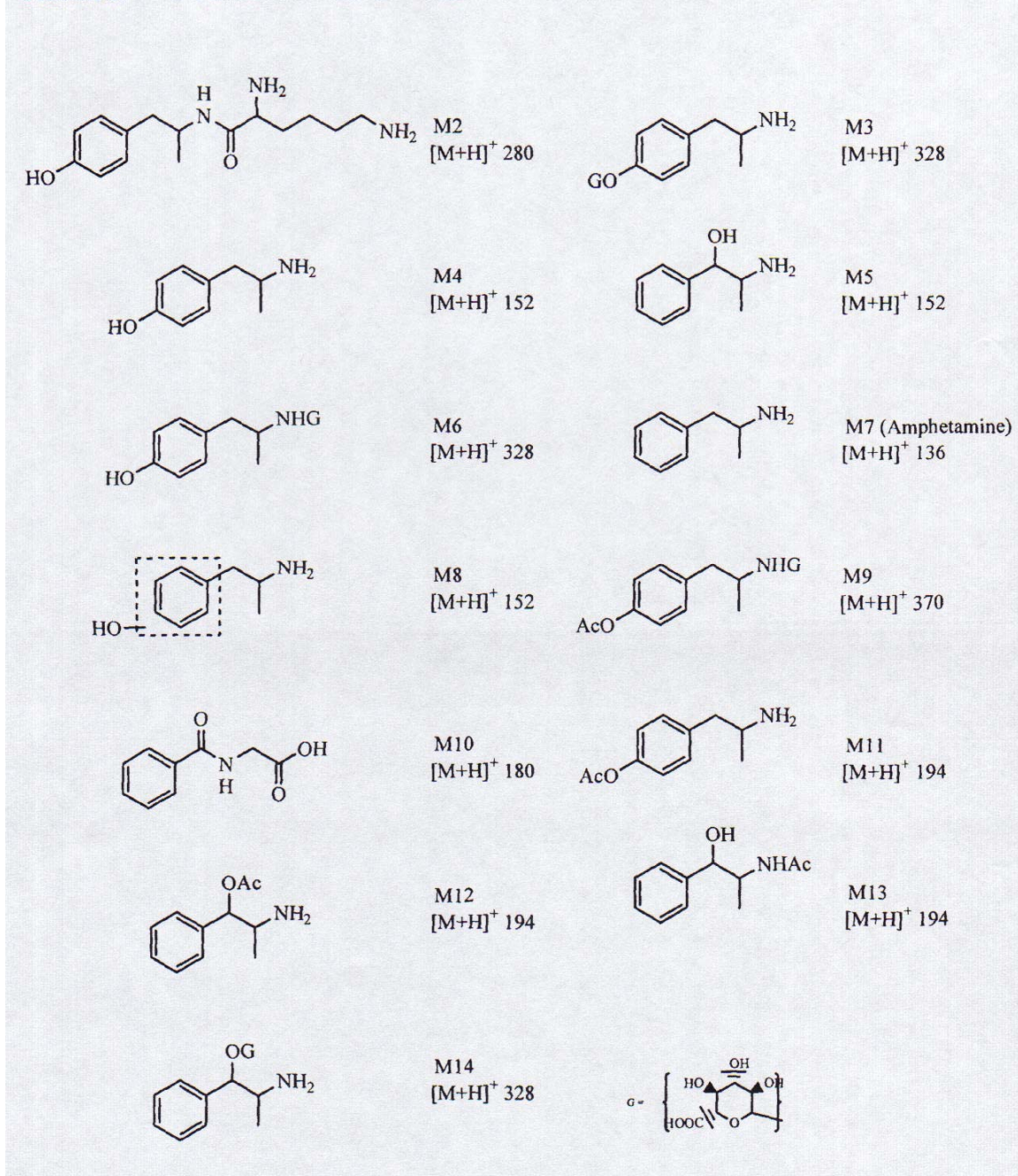
D: Detected by both radiodetector and MS

d: Detected by MS only

N: Not detected

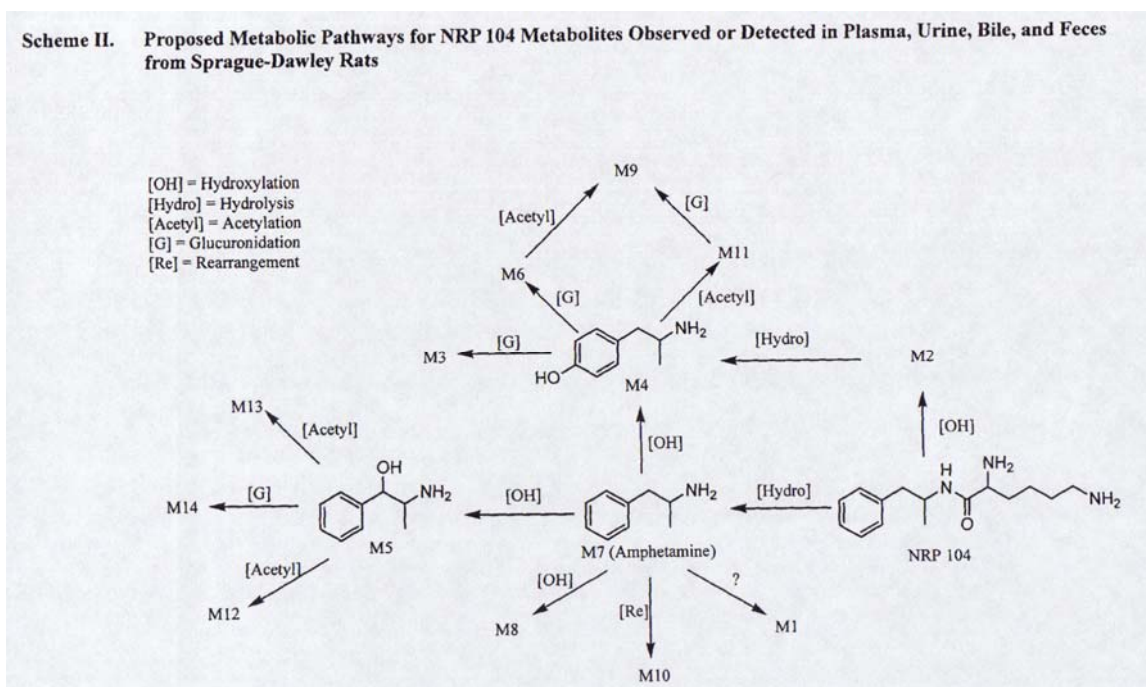
* Amphetamine

The proposed structures for these metabolites (mainly based on MS/MS fragmentation patterns) are shown in the following scheme provided by the sponsor (page 10, vol. 3, Module 4, Sequence 1):

Scheme I. Structures of NRP 104 Tentative Metabolites Identified in Rats

It should be noted that the sponsor stated that “in the absence of authentic compound and confirmatory evidence using techniques such as NMR, the structure assignment for these metabolites are considered preliminary and tentative”.

On the bases of structure assignment for these metabolites, possible transformation pathways for NRP-104 include amide hydrolysis, hydroxylation, rearrangement, acetylation and glucuronidation. The proposed metabolic pathways for NRP-104 metabolites derived from the observed metabolites were provided by the sponsor in the following Scheme II (page 11, vol. 3, Module 4, Sequence 1):



The major findings of the study:

The levels of NRP-104, the parent compound, were higher in rats receiving an IV dose than in those receiving an oral dose. The levels of NRP-104 in plasma after oral administration were less than 2% of the total radioactivity 30 min after dosing as summarized in the following tables provided by the sponsor (tables 1-4 pages 23-27, vol. 3, Module 4, Sequence 1):

Table 1. Concentrations and % of Total Radioactivity of Individual Metabolites and NRP 104 in Pooled Plasma Samples from Male Intact Rats (Groups 1 to 6) following Oral Administration of a Mixture of NRP 104 and [¹⁴C]NRP 104 at a Target Dose of 10 mg/kg

| Time Point (hr) | ng Equivalents/g | | | | | |
|---|------------------|---------|---------|------------------|---------|-------|
| | M2 | NRP 104 | M3 | M7 (Amphetamine) | M8 | M14 |
| Predose | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.5 | 0.000 | 55.067 | 48.676 | 262.644 | 0.000 | 0.000 |
| 1.0 | 0.000 | 0.000 | 198.534 | 388.809 | 35.419 | 0.000 |
| 3.0 | 0.000 | 0.000 | 484.640 | 150.481 | 0.000 | 0.000 |
| 5.0 | 0.000 | 0.000 | 406.435 | 103.304 | 347.285 | 0.000 |
| 8 | 0.000 | 0.000 | 244.142 | 0.000 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 151.965 | 0.000 | 0.000 | 0.000 |
| 24 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 48 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| % of Total Radioactivity in Plasma ^a | 0.0 | 0.4 | 67.2 | 18.3 | 14.2 | 0.0 |

Specific activity of [¹⁴C]NRP 104 in dose formulation was 9.763 µCi / mg.

^a % of total radioactivity in plasma was calculated from:

(AUC(0-48h) of individual metabolite/ Total AUC(0-48h) of all metabolites) x 100 where AUC(0-48h) is the area under the curve, radioactivity concentrations in plasma from 0-48 hr postdose, calculated by the trapezoidal rule.

Table 2. Concentrations and % of Total Radioactivity of Individual Metabolites and NRP 104 in Pooled Plasma Samples from Female Intact Rats (Groups 1 to 6) following Oral Administration of a Mixture of NRP 104 and [¹⁴C]NRP 104 at a Target Dose of 10 mg/kg

| Time Point (hr) | ng Equivalents/g | | | | | |
|---|------------------|---------|---------|------------------|-------|-------|
| | M2 | NRP 104 | M3 | M7 (Amphetamine) | M8 | M14 |
| Predose | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.5 | 0.000 | 293.095 | 173.089 | 499.865 | 0.000 | 0.000 |
| 1.0 | 0.000 | 0.000 | 244.227 | 696.476 | 0.000 | 0.000 |
| 3.0 | 0.000 | 0.000 | 664.626 | 452.919 | 0.000 | 0.000 |
| 5.0 | 0.000 | 0.000 | 495.713 | 239.759 | 0.000 | 0.000 |
| 8 | 0.000 | 0.000 | 312.081 | 51.629 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 73.283 | 0.000 | 0.000 | 0.000 |
| 24 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 48 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| % of Total Radioactivity in Plasma ^a | 0.0 | 1.9 | 61.1 | 37.0 | 0.0 | 0.0 |

Specific activity of [¹⁴C]NRP 104 in dose formulation was 9.763 µCi / mg.

^a % of total radioactivity in plasma was calculated from:

(AUC(0-48h) of individual metabolite/ Total AUC(0-48h) of all metabolites) x 100 where AUC(0-48h) is the area under the curve, radioactivity concentrations in plasma from 0-48 hr postdose, calculated by the trapezoidal rule.

Table 3. Concentrations and % of Total Radioactivity of Individual Metabolites and NRP 104 in Pooled Plasma Samples from Male BDC/JVC Rats (Group 8) following Oral Administration of a Mixture of NRP 104 and [¹⁴C]NRP 104 at a Target Dose of 10 mg/kg

| Time (hr) | ng Equivalents/g | | | | | |
|---|------------------|---------|---------|------------------|-------|-------|
| | M2 | NRP 104 | M3 | M7 (Amphetamine) | M8 | M14 |
| Predose | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 1 | 0.000 | 0.000 | 0.000 | 320.744 | 0.000 | 0.000 |
| 3 | 0.000 | 0.000 | 0.000 | 453.676 | 0.000 | 0.000 |
| 5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 8 | 0.000 | 0.000 | 453.676 | 1397.955 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 24 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 48 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| % of Total Radioactivity in Plasma ^a | 0.0 | 0.0 | 20.4 | 79.6 | 0.0 | 0.0 |

Specific activity of [¹⁴C]NRP104 in dose formulation was 8.380 µCi / mg.

^a % of total radioactivity in plasma was calculated from:

(AUC(0-48h) of individual metabolite/ Total AUC(0-48h) of all metabolites) x 100 where AUC(0-48h) is the area under the curve, radioactivity concentrations in plasma from 0-48 hr postdose, calculated by the trapezoidal rule.

Table 4. Concentrations and % of Total Radioactivity of Individual Metabolites and NRP 104 in Pooled Plasma Samples from Female BDC/JVC Rats (Group 8) following Oral Administration of a Mixture of NRP 104 and [¹⁴C]NRP 104 at a Target Dose of 10 mg/kg

| Time (hr) | ng Equivalents/g | | | | | |
|---|------------------|---------|---------|------------------|-------|-------|
| | M2 | NRP 104 | M3 | M7 (Amphetamine) | M8 | M14 |
| Predose | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.5 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 1 | 0.000 | 0.000 | 224.688 | 737.868 | 0.000 | 0.000 |
| 3 | 0.000 | 0.000 | 811.295 | 352.996 | 0.000 | 0.000 |
| 5 | 0.000 | 0.000 | 352.996 | 0.000 | 0.000 | 0.000 |
| 8 | 0.000 | 0.000 | 485.927 | 160.560 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 24 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 48 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| % of Total Radioactivity in Plasma ^a | 0.0 | 0.0 | 67.2 | 32.8 | 0.0 | 0.0 |

Specific activity of [¹⁴C]NRP104 in dose formulation was 8.380 µCi / mg.

^a % of total radioactivity in plasma was calculated from:

(AUC(0-48h) of individual metabolite/ Total AUC(0-48h) of all metabolites) x 100 where AUC(0-48h) is the area under the curve, radioactivity concentrations in plasma from 0-48 hr postdose, calculated by the trapezoidal rule.

One hour after an IV administration, NRP-104 levels were 18.1 to 22.7% of the radioactivity in plasma (tables 5-8, pages 27-30, vol. 3, module 4, Sequence 1):

Table 5. Concentrations and % of Total Radioactivity of Individual Metabolites and NRP 104 in Pooled Plasma Samples from Male JVC Rats (Group 9) following Intravenous Administration of a Mixture of NRP 104 and [¹⁴C]NRP 104 at a Target Dose of 3 mg/kg

| Time (hr) | ng Equivalents/g | | | | | |
|---|------------------|---------|---------|------------------|--------|--------|
| | M2 | NRP 104 | M3 | M7 (Amphetamine) | M8 | M14 |
| Predose | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.5 | 69.385 | 878.852 | 0.000 | 143.985 | 0.000 | 0.000 |
| 1 | 0.000 | 23.502 | 86.149 | 137.282 | 76.857 | 20.877 |
| 3 | 0.000 | 0.000 | 92.519 | 49.996 | 0.000 | 0.000 |
| 5 | 0.000 | 0.000 | 176.446 | 0.000 | 0.000 | 0.000 |
| 8 | 0.000 | 0.000 | 42.138 | 0.000 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 26.476 | 0.000 | 0.000 | 0.000 |
| 24 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 48 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| % of Total Radioactivity in Plasma ^a | 1.7 | 22.7 | 53.0 | 16.7 | 4.7 | 1.3 |

Specific activity of [¹⁴C]NRP104 in dose formulation was 25.741 µCi / mg.

^a % of total radioactivity in plasma was calculated from:

(AUC(0-48h) of individual metabolite/ Total AUC(0-48h) of all metabolites) x 100 where AUC(0-48h) is the area under the curve, radioactivity concentrations in plasma from 0-48 hr postdose, calculated by the trapezoidal rule.

Table 6. Concentrations and % of Total Radioactivity of Individual Metabolites and NRP 104 in Pooled Plasma Samples from Female JVC Rats (Group 9) following Intravenous Administration of a Mixture of NRP 104 and [¹⁴C]NRP 104 at a Target Dose of 3 mg/kg

| Time (hr) | ng Equivalents/g | | | | | |
|---|------------------|---------|---------|------------------|--------|--------|
| | M2 | NRP 104 | M3 | M7 (Amphetamine) | M8 | M14 |
| Predose | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.5 | 65.658 | 867.670 | 0.000 | 161.904 | 0.000 | 47.371 |
| 1 | 0.000 | 0.000 | 215.242 | 215.627 | 47.021 | 70.487 |
| 3 | 0.000 | 0.000 | 136.145 | 75.370 | 0.000 | 0.000 |
| 5 | 0.000 | 0.000 | 150.319 | 65.658 | 0.000 | 0.000 |
| 8 | 0.000 | 0.000 | 18.287 | 31.709 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 24 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 48 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| % of total radioactivity in plasma ^a | 1.4 | 18.1 | 41.0 | 32.4 | 2.5 | 4.7 |

Specific activity of [¹⁴C]NRP104 in dose formulation was 25.741 µCi / mg.

^a % of Total radioactivity in plasma was calculated from:

(AUC0-48 of individual metabolite/ Total AUC0-48 of all metabolites) x 100 where AUC0-48 is the area under the curve radioactivity concentrations in plasma from 0-48 hr postdose, calculated by the trapezoidal rule.

Table 7. Concentrations and % of Total Radioactivity of Individual Metabolites and NRP 104 in Pooled Plasma Samples from Male BDC/JVC Rats (Group 10) following Intravenous Administration of a Mixture of NRP 104 and [¹⁴C]NRP 104 at a Target Dose of 3 mg/kg

| Time (hr) | ng Equivalents/g | | | | | |
|---|------------------|---------|---------|------------------|--------|--------|
| | M2 | NRP 104 | M3 | M7 (Amphetamine) | M8 | M14 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.5 | 29.836 | 627.439 | 0.000 | 108.548 | 0.000 | 47.738 |
| 1 | 13.072 | 33.949 | 89.159 | 129.058 | 61.545 | 28.734 |
| 3 | 0.000 | 0.000 | 126.468 | 47.371 | 0.000 | 0.000 |
| 5 | 0.000 | 0.000 | 115.636 | 0.000 | 0.000 | 0.000 |
| 8 | 0.000 | 0.000 | 49.996 | 0.000 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 18.287 | 0.000 | 0.000 | 0.000 |
| 24 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 48 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| % of Total Radioactivity in Plasma ^a | 1.7 | 19.7 | 53.9 | 17.2 | 4.3 | 3.3 |

Specific activity of [¹⁴C]NRP104 in dose formulation was 25.741 µCi / mg.

^a % of total radioactivity in plasma was calculated from:

(AUC(0-48h) of individual metabolite/ Total AUC(0-48h) of all metabolites) x 100 where AUC(0-48h) is the area under the curve, radioactivity concentrations in plasma from 0-48 hr postdose, calculated by the trapezoidal rule.

Table 8. Concentrations and % of Total Radioactivity of Individual Metabolites and NRP 104 in Pooled Plasma Samples from Female BDC/JVC Rats (Group 10) following Intravenous Administration of a Mixture of NRP 104 and [¹⁴C]NRP 104 at a Target Dose of 3 mg/kg

| Time (hr) | ng Equivalents/g | | | | | |
|---|------------------|---------|---------|------------------|-------|--------|
| | M2 | NRP 104 | M3 | M7 (Amphetamine) | M8 | M14 |
| Predose | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 0.5 | 0.000 | 788.205 | 0.000 | 125.698 | 0.000 | 45.498 |
| 1 | 0.000 | 0.000 | 163.391 | 220.842 | 0.000 | 73.497 |
| 3 | 0.000 | 0.000 | 152.559 | 110.806 | 0.000 | 0.000 |
| 5 | 0.000 | 0.000 | 140.992 | 0.000 | 0.000 | 0.000 |
| 8 | 0.000 | 0.000 | 52.586 | 0.000 | 0.000 | 0.000 |
| 12 | 0.000 | 0.000 | 34.684 | 0.000 | 0.000 | 0.000 |
| 24 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 48 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| % of Total Radioactivity in Plasma ^a | 0.0 | 16.5 | 55.3 | 23.4 | 0.0 | 4.8 |

Specific activity of [¹⁴C]NRP104 in dose formulation was 25.741 µCi / mg.

^a % of total radioactivity in plasma was calculated from:

(AUC(0-48h) of individual metabolite/ Total AUC(0-48h) of all metabolites) x 100 where AUC(0-48h) is the area under the curve, radioactivity concentrations in plasma from 0-48 hr postdose, calculated by the trapezoidal rule.

Metabolite profiling in plasma:

For both oral and IV dosed rats, the total number of metabolites detected in plasma by LCMS/MS was 9, but only 5 of these metabolites were quantifiable by a radiodetector. The most prominent metabolites in plasma were glucuronide-conjugated amphetamine metabolite (M3), and amphetamine (M7). M2, which is the hydroxylated NRP-104, was observed in IV dosed rats, but not in orally dosed rats. This finding might suggest that with an oral dose administration, NRP-104 was quickly hydrolyzed to amphetamine before reaching the plasma circulation.

In groups 1 through 6 (intact; oral dose), M3 (glucuronide conjugated amphetamine metabolite) accounted for 67.2% of plasma radioactivity from M and 61.1% in F. Amphetamine (M7), accounted for 18.3% and 37% of plasma radioactivity from M and F, respectively. Interestingly a gender difference was observed with M8 (hydroxylated amphetamine) which was observed in plasma from M only and accounted for 14.2% of plasma radioactivity (see previous tables).

For group 8 [bile duct cannulated (BDC)/jugular vein cannulated (JVC); oral dose], M3 and M7 were also the major metabolites in plasma from both M and F rats. However, their relative percent of total radioactivity in plasma is reversed (M7 was dominant in M accounting for 79.6% of plasma radioactivity while M3 was dominant in F accounting for 67.2% of plasma radioactivity).

In groups 9 (JVC; IV dose) and 10 (BDC/JCV; IV dose), one hour after intravenous dose administration, NRP-104 represented 18.1 to 22.7% of the radioactivity in plasma (see previous tables 5 to 8 attached from sponsor). M3 and M7 were the major metabolites in plasma from both M and F. Other metabolites were M2, M8, and M14 (see metabolic pathway for the identification of these metabolites).

Metabolite profiling in urine:

For orally dosed rats (group 7), the total number of NRP-104 metabolites detected in urine by LCMS/MS was 14; however, only 9 of those were quantifiable by a radiodetector. During the first 0-5 h period, NRP-104 was detected in urine, accounting for 4.8% of administered dose. No quantifiable NRP-104 was detected after the 5h postdose time point. Glucuronide-conjugated amphetamine metabolite (M3) and amphetamine (M7), were the major metabolites in urine samples, accounting for 29.8 and 13.6% of orally administered dose. Other quantifiable, although minor, metabolites were M2, M8, M9, M11, and M14 (see the following table 9 from sponsor, page 31, vol. 3, Module 4, Sequence 1):

Table 9. Amounts of Individual Metabolites (μg Equivalents) Recovered in Pooled Urine and Feces Samples from Both Male and Female Intact Rats following 10 mg/kg Oral Dose of a Mixture of NRP 104 and [^{14}C]NRP 104 (Group 7)

| Time Period (hr) | μg Equivalents | | | | | | Accumulated μg Equivalents | | Accumulated % Dose | |
|------------------|---------------------------|-------|-------|-------|------|-------|---------------------------------------|-------|--------------------|-------|
| | Urine | | | Feces | | | Urine | Feces | Urine | Feces |
| | 0-5 | 5-12 | 12-24 | 0-5 | 5-12 | 12-24 | | | | |
| M2 | 19.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 19.3 | 0.0 | 0.7 | 0.0 |
| NRP 104 | 127.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 127.4 | 0.0 | 4.8 | 0.0 |
| M3 | 215.0 | 343.0 | 233.6 | 0.0 | 0.0 | 0.0 | 791.5 | 0.0 | 29.8 | 0.0 |
| M4 | 50.8 | 26.0 | 45.8 | 0.0 | 5.4 | 32.8 | 122.7 | 38.1 | 4.6 | 1.4 |
| M7 | 170.1 | 134.0 | 56.5 | 0.9 | 5.0 | 20.4 | 360.5 | 26.3 | 13.6 | 1.0 |
| M8 | 31.8 | 11.2 | 6.8 | 0.0 | 0.0 | 0.0 | 49.8 | 0.0 | 1.9 | 0.0 |
| M9 | 4.4 | 16.9 | 31.8 | 0.0 | 0.0 | 0.0 | 53.1 | 0.0 | 2.0 | 0.0 |
| M10 | 27.4 | 16.1 | 11.2 | 0.0 | 0.0 | 0.0 | 54.7 | 0.0 | 2.1 | 0.0 |
| M11 | 8.7 | 5.6 | 24.4 | 0.0 | 0.0 | 0.0 | 38.7 | 0.0 | 1.5 | 0.0 |
| M14 | 7.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 7.1 | 0.0 | 0.3 | 0.0 |
| Total | | | | | | | 1624.8 | 64.4 | 61.3 | 2.4 |

Average % of dose recovery in urine: 86.9.

Average % of dose recovery in feces: 4.4.

Average μCi administered: 23.17.

Average μg equivalents administered: 2652.

Specific activity of [^{14}C]NRP 104 in dose formulation: 8.738 μCi / mg.

Metabolite profiling in feces:

For orally dosed rats (group 7), the total number of metabolites detected in feces by LC/MS/MS was 6, but only 2 of those were quantifiable by a radiodetector.

Amphetamine (M7) and hydroxylated amphetamine (M4) were the major metabolite in feces accounting for 1 and 1.4% of the orally administered dose, respectively (see previous table 9).

Metabolite profiling in bile:

The total number of metabolites detected in bile by LCMS/MS was 12, but only 6 of those were quantifiable by radiodetector. For group 8 (BDC/JVC; oral dose), two glucuronide conjugated metabolites, M3 and M9 were the major metabolites in bile samples, accounting for 1.8 and 2% of the orally administered dose. Other quantifiable metabolites, M1, M4, and M7 were also observed as minor metabolites in bile samples (see table 10 from sponsor, page 32, vol. 3, Module 4, Sequence 1):

Table 10. Amount of Individual Metabolites (µg Equivalents) Recovered in Pooled Bile Samples from Both Male and Female BDC Rats following 10 mg/kg Oral (Group 8) or 3 mg/kg Intravenous (Group 10) Dose of a Mixture of NRP 104 and [14C]NRP 104

| Group | µg Equivalents | | | | | | Accumulated µg Equivalents | | Accumulated % Dose | |
|------------------|----------------|--------|--------|-------|-------|--------|----------------------------|--------|--------------------|-----|
| | 8 | | | 10 | | | 8 | 10 | 8 | 10 |
| Time Period (hr) | 0-3 | 3-5 | 5-8 | 0-3 | 3-5 | 5-8 | | | | |
| M1 | 1.887 | 10.459 | 0.000 | 0.000 | 0.000 | 0.000 | 12.346 | 0.000 | 0.4 | 0.0 |
| NRP 104 | 0.769 | 4.061 | 8.573 | 1.022 | 0.000 | 0.000 | 13.403 | 1.022 | 0.5 | 0.1 |
| M3 | 1.112 | 9.194 | 39.805 | 5.507 | 5.966 | 10.312 | 50.111 | 21.785 | 1.8 | 2.6 |
| M4 | 0.313 | 1.969 | 6.744 | 0.388 | 0.000 | 0.000 | 9.026 | 0.388 | 0.3 | 0.0 |
| M7 | 0.996 | 4.914 | 14.500 | 6.690 | 4.274 | 4.329 | 20.410 | 15.293 | 0.7 | 1.8 |
| M9 | 1.401 | 8.845 | 47.648 | 3.875 | 3.653 | 7.229 | 57.894 | 14.757 | 2.0 | 1.8 |
| M11 | 0.000 | 0.000 | 0.000 | 1.003 | 0.620 | 0.734 | 0.000 | 2.357 | 0.0 | 0.3 |
| Total | | | | | | | 163.190 | 55.602 | 5.7 | 6.6 |

Average % of dose recovery in bile: 16.9 for Group 8; 12.9 for Group 10

Average µCi administered: 24.252 for Group 8; 21.670 for Group 10.

Average µg equivalents administered: 2830 for Group 8; 842 for Group 10.

Specific activity of [14C]NRP 104 in dose formulation: 8.099 to 8.738 µCi / mg for Group 8; 25.714 µCi / mg for Group 10.

For group 10 (BDC/JVC; IV dose), amphetamine, M3 and two glucuronide-conjugated metabolites, M7 and M9, were the major metabolites in bile samples, accounting for 2.6, 1.8, and 1.8%, respectively. M11 was also observed as a minor but quantifiable metabolite in bile samples (see the previous table 10).

The following tables summarize PK parameters for the parent and d-amphetamine in the different animals from the different groups described under the study design (see table at the beginning of the metabolism section) as provided by the sponsor (pages 31-32 section 4.2.2.4, vol. 3, Module 4, Sequence 1):

TABLE 14. Comparison of d-Amphetamine and Lysine-Amphetamine Pharmacokinetics after a 10 mg/kg Oral Dose in Intact (Groups 1-6, intact) Rats

| d-Amphetamine | | | | | | |
|---------------------------|--------------------------|----------------------|---------------------------------|--------------------------------|----------------------|---------------------------|
| Male | | | | | | |
| Group# | C _{max} (ng/mL) | t _{max} (h) | AUC _(last) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | Relative Availability (%) |
| 1-6 | 66.4 | 1.0 | 226.0 | 229.3 | 2.0 | 59 |
| Female | | | | | | |
| Group# | C _{max} (ng/mL) | t _{max} (h) | AUC _(last) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | Relative Availability (%) |
| 1-6 | 144.8 | 1.0 | 632.2 | 641.5 | 4.1 | 149 |
| Lysine-Amphetamine | | | | | | |
| Male | | | | | | |
| Group# | C _{max} (ng/mL) | t _{max} (h) | AUC _(last) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | Relative Availability (%) |
| 1-6 | 126.2 | 0.5 | 129.4 | 130.4 | 1.2 | 18 |
| Female | | | | | | |
| Group# | C _{max} (ng/mL) | t _{max} (h) | AUC _(last) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | Relative Availability (%) |
| 1-6 | 242.7 | 0.5 | 208.4 | 211.6 | 0.9 | 23 |

NC: Not Calculated

Relative Availability was calculated by comparing the d-Amphetamine or Lysine-Amphetamine dose-normalized AUCs after oral (groups 1-6) and IV dosing (Groups 9 or 10).

TABLE 15. Comparison of d-Amphetamine and Lysine-Amphetamine Pharmacokinetics after a 3 mg/kg IV Dose in Intact (Group 9, JVC) and Bile Duct Cannulated (Group 10, JVC) Rats

| d-Amphetamine | | | | | |
|---------------------------|---------------------------------|--------------------------------|---------------------------------|--------------------------------|----------------------|
| Male | | | | | |
| Group# | C _{max} (ng/mL) | t _{max} (h) | AUC _(last) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) |
| 9 | 42.8 ± 16.0 | 1.0 ± 0 | 103.7 ± 34.9 | 117.1 ± 46.5 | 1.6 ± 0.4 |
| 10 (BDC) | 25.7 ± 6.7 | 0.4 ± 0.5 | 58.5 ± 4.4 | 61.8 ± 4.7 | 1.5 ± 0.7 |
| Female | | | | | |
| Group# | C _{max} (ng/mL) | t _{max} (h) | AUC _(last) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) |
| 9 | 34.7 ± 4.3 | 1.0 ± 0 | 120.2 ± 38.7 | 128.9 ± 47.0 | 4.5 ± 4.9 |
| 10 (BDC) | 31.0 ± 6.0 | 1.0 ± 1 | 92.4 ± 28.0 | 107.5 ± 36.1 | 5.0 ± 4.8 |
| Lysine-Amphetamine | | | | | |
| Male | | | | | |
| Group# | AUC _(last) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | Vdss (L/kg) | CL (L/hr/kg) |
| 9 | 210.0 ± 13.6 | NC | NC | NC | NC |
| 10 (BDC) | 273.0 ± 29.4 | NC | NC | NC | NC |
| Female | | | | | |
| Group# | AUC _(last) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | Vdss (L/kg) | CL (L/hr/kg) |
| 9 | 272.9 ± 22.0 | NC | NC | NC | NC |
| 10 (BDC) | 293.2 ± 101.1 | NC | NC | NC | NC |

NC: Not Calculated

In animals treated with oral or IV dose (see treatments as described in table S-1 at the beginning of this section), the levels of total radioactivity in plasma (expressed as ng/ml of lysine amphetamine equivalents) were greater than the sum of lysine-amphetamine and d-amphetamine indicating that the radioactivity is also present as metabolites of amphetamine-lysine and/or d-amphetamine. The apparent half life of lysine-amphetamine after oral administration was 1.2h in M and 0.9h in F. AUC and C_{max} of d-amphetamine were greater than AUC and C_{max} of lysine-amphetamine after oral administration which could be due to pre-systemic conversion of lysine-amphetamine to d-amphetamine. In contrast, AUC and C_{max} of lysine-amphetamine were greater than AUC and C_{max} of d-amphetamine after IV dosing. This could be due to less rapid clearance or less extensive hydrolysis of lysine amphetamine after IV dosing.

The effect of biliary excretion on the PK profiles of amphetamine lysine and d-amphetamine after IV dosing of amphetamine lysine can be evaluated by a comparison of Group 9 (JVC) and Group 10 (BDC/JVC). There was no influence for bile collection on

lysine amphetamine systemic exposure in both M&F and there was no influence on d-amphetamine exposure in F but the AUC and C_{max} values for d-amphetamine were lower in bile duct-cannulated M rats (group10) compared to intact rats (group 9).

In vitro metabolism:

In an in vitro study using human liver microsomes and fresh human and rat hepatocytes (Document # 37-304-TP), the stability of NRP-104 was assessed by incubating 12.5 and 125 ng/ml NRP-104 in pooled human liver microsomes (0.1 and 0.5 mg/ml) for up to thirty minutes and in fresh human and rat hepatocytes for up to 4h. The levels of NRP-104 and d-amphetamine were evaluated using two analytical methods (LC-MS/MS and radio-HPLC), preceded by either a liquid-liquid extraction or protein precipitation of the sample. Using a liquid-liquid extraction method and LC-MS/MS, the concentrations of NRP-104 and amphetamine were evaluated in human liver microsomes at 0, 15, and 30 min after the addition of NADPH.

The concentration of NRP-104 did not decrease in the incubated samples and no amphetamine formation was detected. No NRP-104 metabolites were observed in 30 min incubations with human liver microsomes or with incubations up to 4h in fresh human or rat hepatocytes. A positive control (7-hydroxycoumarin) was used in this study to confirm that the hepatocytes used in this study are of acceptable quality. According to the sponsor, based on the activity results and the morphology of the cells, the cells were determined by the sponsor to be of sufficient quality (the following data were provided by sponsor in section 4.2.24, on page 18, vol. 3, Module 4, Sequence 1):

| Table SD1: Hepatocyte Quality Control Incubations to Evaluate 7-Hydroxycoumarin Glucuronidation and Sulfation | | |
|--|---|--|
| | 7-Hydroxycoumarin Sulfation pmol/min/million cells | 7-Hydroxycoumarin Glucuronidation pmol/min/million cells |
| Rat Hepatocytes Lot Rs140 | 118 | 563 |
| Human Hepatocytes Lot Hu147 | 0 | 136 |
| Human Hepatocytes Lot Hu159 | 63 | 629 |

It is not clear why the levels of 7-hydroxysulfation was null in Human Hepatocytes Lot Hu147 and whether this reflects a poor quality of those cells.

The following figures were provided by the sponsor for the levels of NRP-1-4 and d-amphetamine (section 4.2.2.4, page 21-22, vol. 3, module 4, sequence 1):

Figure SD1: Stability of 12.5 ng/mL NRP-104 with 0.1 and 0.5 mg/mL Human Liver Microsomes

Data from folder MJWIO

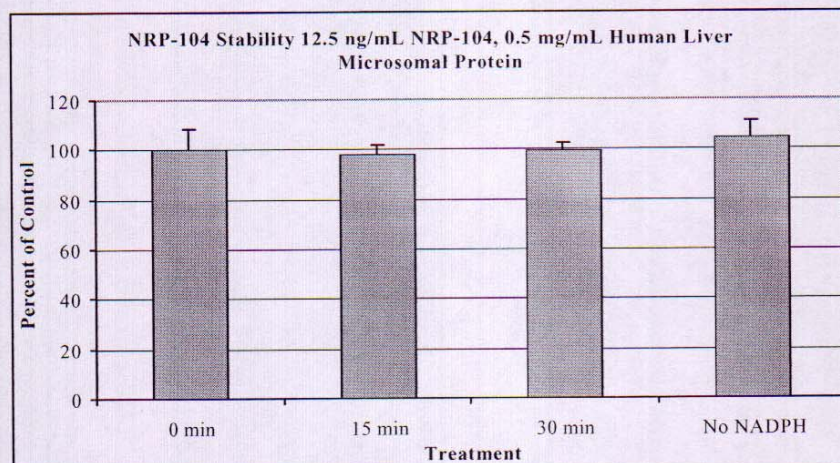
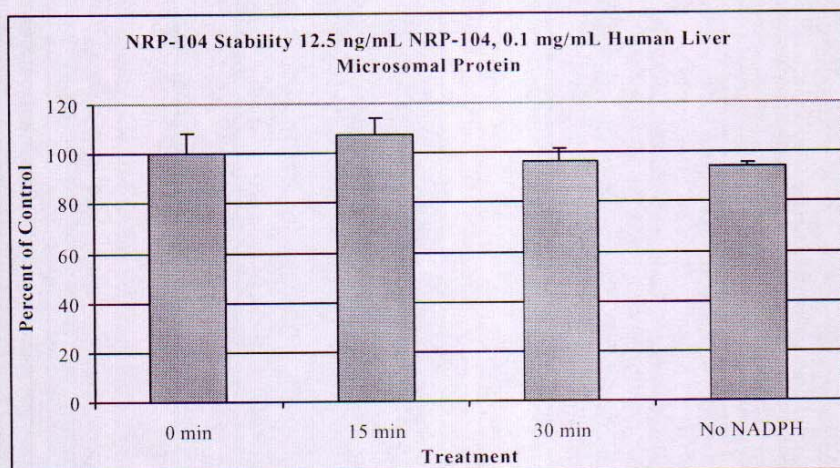


Figure SD2: Stability of 125 ng/mL NPR-104 with 0.1 and 0.5 mg/mL Human Liver Microsomes

Data from folder MJWIO

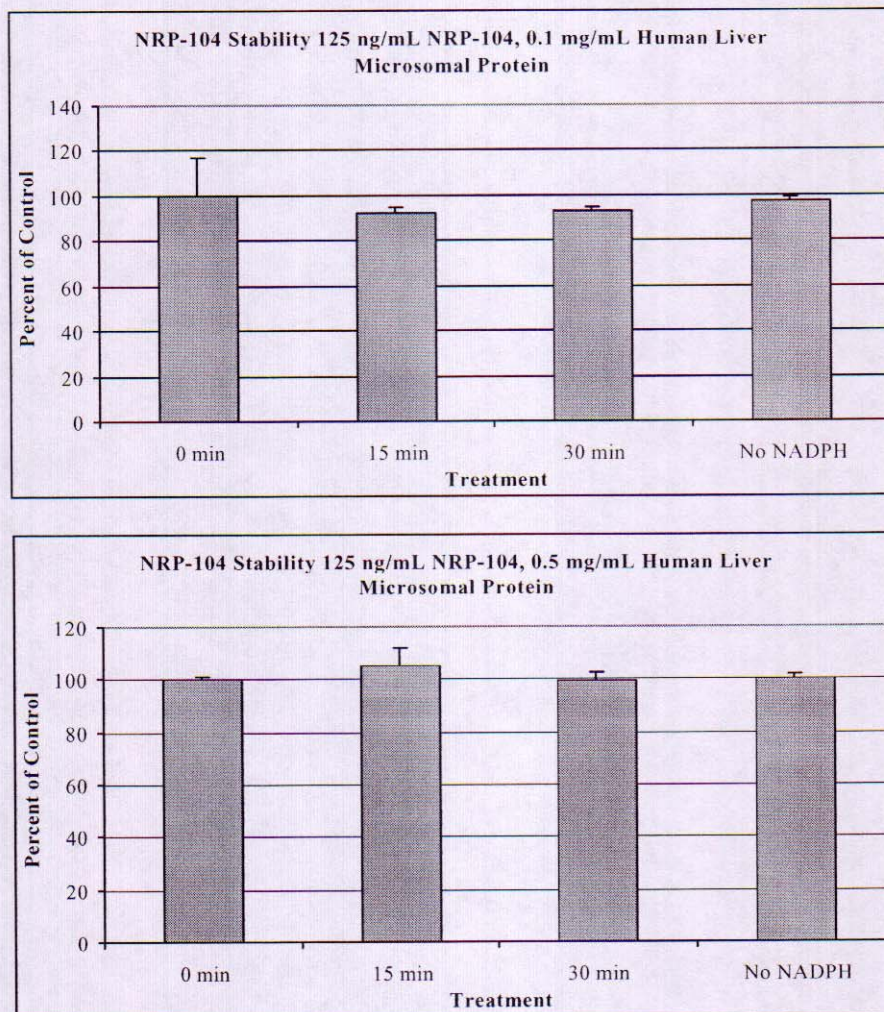


Figure SD3: Amphetamine Formation by Incubation of NPR-104 with Human Liver Microsomes

Data from folder MJWIO

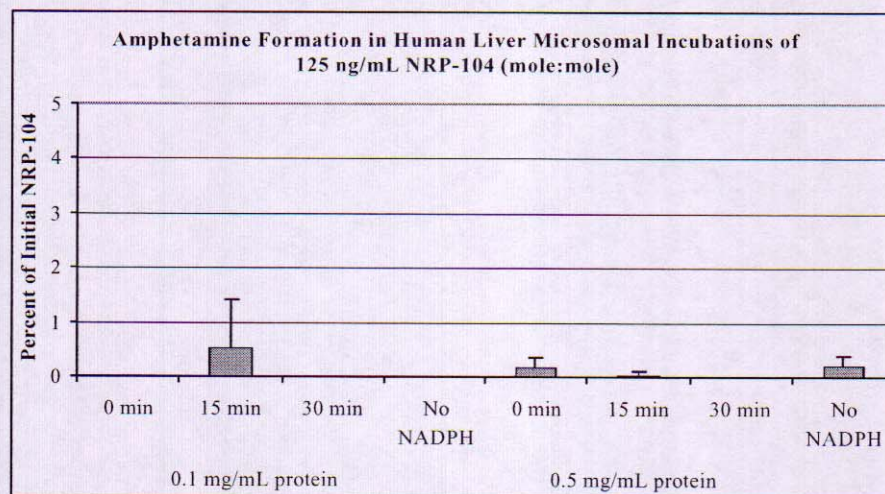
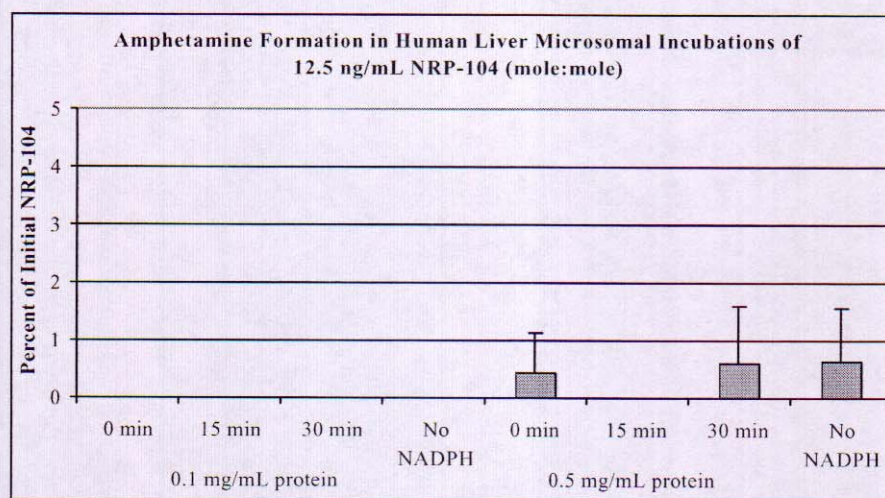
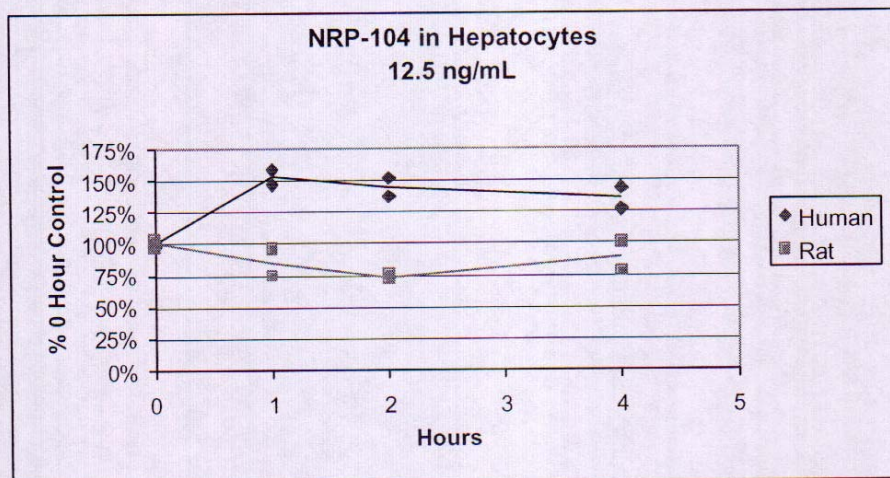


Figure SD4: Metabolic Stability of NRP-104 (12.5 ng/mL) in Fresh Human and Rat Hepatocytes

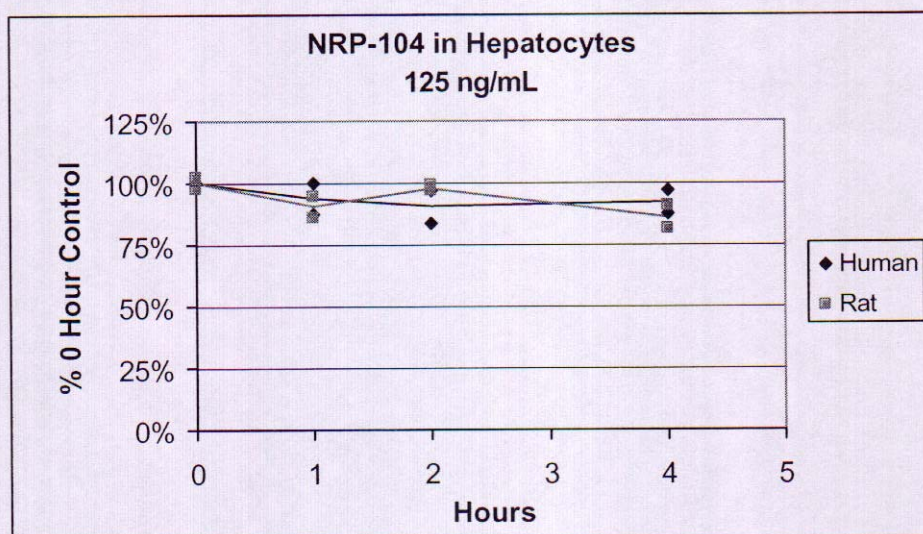
Data from folder MJPHO



* 0-Hour control samples were spiked with organic solvent before addition of NRP-104. All other samples were spiked with NRP-104 and incubated for the appropriate time before addition of organic solvent. This may account for the lower 0 hour control value observed in the human hepatocytes.

Figure SD5: Metabolic Stability of NRP-104 (125 ng/mL) in Fresh Human and Rat Hepatocytes

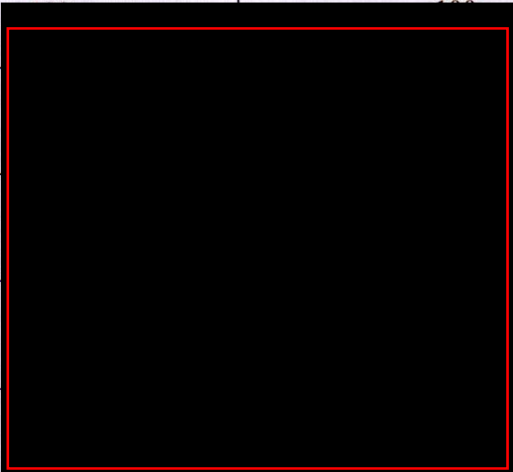
Data from folder MJAIO



A small amount of a non-enzymatically generated degradant was seen in some samples analyzed using the protein precipitation method in which NADPH was added and the samples were dried and reconstituted in mobile phase. The source of this degradant is not known but it appears that the addition of NADPH has a role in this process since all dried down samples that contained NADPH, irrespective if the sample contained microsomes or not contained this degradant. The sponsor concluded that this degradant is not generated through an enzymatic process and indicated that it could be due to the analytical method. One possibility according to the sponsor is that a non-specific free radical based mechanism of NRP-104 degradation caused by the analytical method might be responsible for the observed results (see the following table provided by the sponsor (section 4.2.2.4, page 19, vol. 3, module 4, sequence 1):

Table SD2: Degradant Formation: Effect of Microsomal Protein, NADPH, and Time

Data from MJZGO

| | NRP-104 as % of total signal | |
|--------------------------|--|--------------|
| | 1 Day Old* | 2 Days Old * |
| Buffer Only |  | |
| Buffer + 1 mM NADPH | | |
| Buffer + 10 mM NADPH | | |
| Microsomes + 1 mM NADPH | | |
| Microsomes + 10 mM NADPH | | |

* 1-Day-old samples were prepared on 7/27 and 2-day-old samples were prepared on 7/26. Samples were treated with acetonitrile, dried down and reconstituted with mobile phase on the day they were prepared. All samples were analyzed on 7/27 immediately after the 1-Day-old samples were prepared.

It should be noted that so far the sponsor has not provided evidence for where the metabolism of this compound take place. The sponsor has stated in different parts of the submission that the site of metabolism for the compound in the gastrointestinal tract; however, there were no studies conducted to prove the sponsor's claim.

2.6.4.6 Excretion

In intact male and female rats (Group 7, oral dose, see table S-1 included previously summarizing treatment in this group), urinary excretion of total radioactivity was the predominant route of elimination, accounting for 77.3% of the administered dose in M and 86.9% in F. Excretion in feces accounted for only 10.9% of the dose in M and 4% in F. Elimination in urine and feces occurred within the first 48h. Biliary excretion of total radioactivity in M and F BDC/JVC rats (Group 8, oral dose, see table S-1 in previous section for treatment design) accounted for a mean of 18.3% of the dose in M and 7.24% of the dose in F over a collection period of 48h. Biliary excretion in BDC rats was not less than fecal excretion in intact rats, suggesting that fecal radioactivity in intact rats could be due to biliary excretion rather than elimination of unabsorbed radioactivity.

This indicates that at this dose (oral, 10 mg/kg) NRP-104 seems to be well absorbed. The mean total recovery of radioactivity in all samples collected was 90% for intact M and 95.4% for intact F. The following table summarizes the cumulative recovery of radioactivity following oral administration in rats (intact and BDC/JVC) as provided by the sponsor (section 4.2.2.5, page 20, vol. 3, Module 4, Sequence 1):

Table 1. Mean (\pm SD) Cumulative Recovery of Radioactivity Following Oral Administration of a Mixture of [14 C]NRP104 and NRP104 (10 mg/kg) to Intact (Group 7) Male and Female Rats, and BDC/JVC (Group 8) Males and Females

| Matrix | Percent of Administered Dose | | | |
|-----------|-----------------------------------|-------------------------------------|-----------------------------------|-------------------------------------|
| | Group 7 Intact Males (0-168 h) | Group 7 Intact Females (0-168 h) | Group 8 BDC/JVC Males (0-48 h) | Group 8 BDC/JVC Females (0-48 h) |
| Urine | 77.3 \pm NC ^a | 86.9 \pm 5.0 | NA | NA |
| Feces | 10.9 \pm 2.6 | 3.96 \pm 0.84 | NA | NA |
| Cage Wipe | 0.123 \pm 0.051 | 0.123 \pm 0.036 | 0.108 \pm 0.078 | 0.184 \pm 0.103 |
| Cage Wash | 2.21 \pm 0.62 | 4.36 \pm 3.36 | 3.33 \pm 2.16 | 4.68 \pm 3.16 |
| Bile | NA | NA | 18.3 \pm 4.9 | 7.24 \pm 0.93 |
| Total | 90.0 \pm NC ^a | 95.4 \pm 3.0 | 21.7 \pm 4.5 | 12.1 \pm 4.1 |

Notes: Values are the mean \pm standard deviation (N=3), except when otherwise indicated.

BDC: Bile duct cannulated

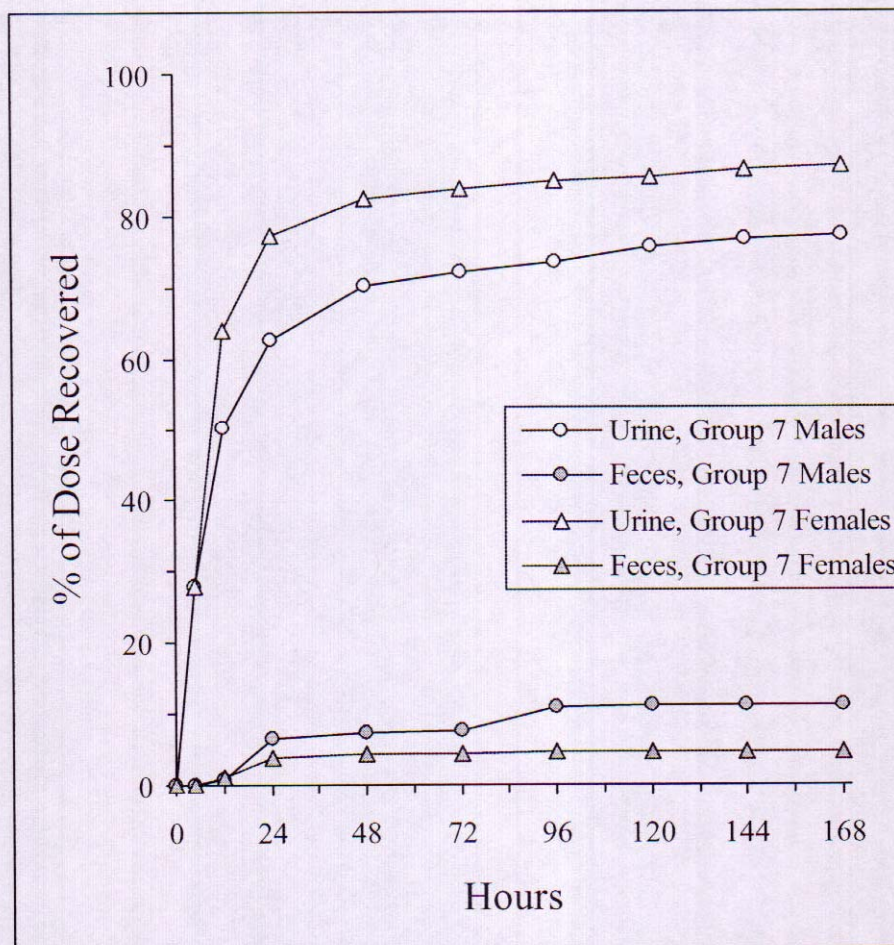
JVC: Jugular vein cannulated

NA: Not Applicable

^a N=2 (Rat #26 was not included due to apparent metabolic cage malfunctions in urine collection.)

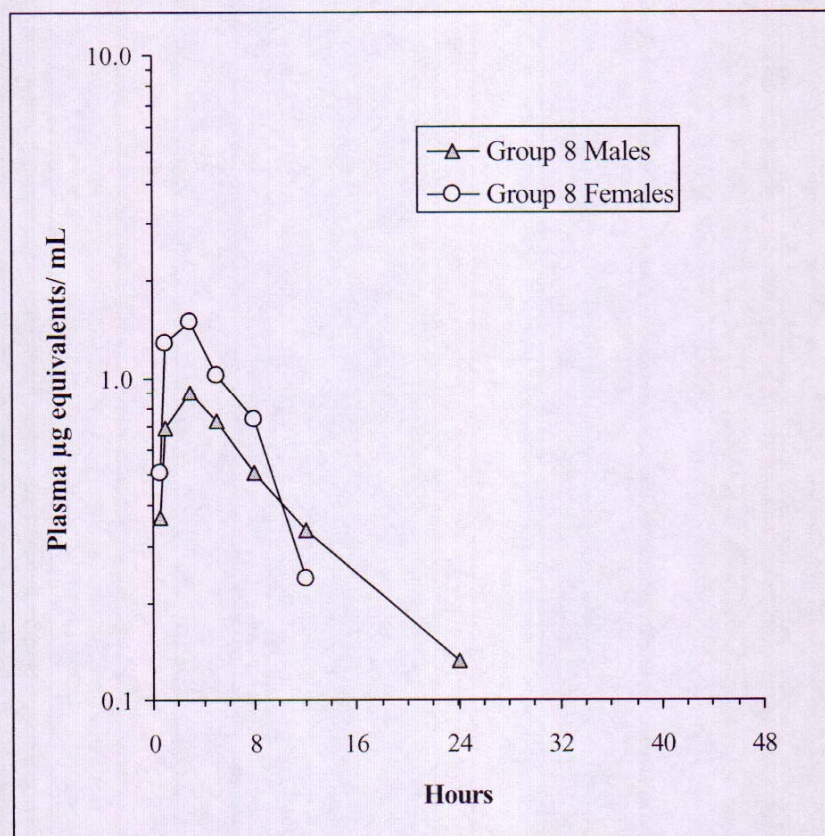
The profiles of urinary and fecal elimination of radioactivity vs. time are shown in the following figure as provided by the sponsor (section 4.2.2.5, page 25, vol. 3, Module 4, Sequence 1):

Figure 2. Cumulative Elimination of Radioactivity in Urine and Feces of Inta (Group 7) versus Time After a Single Oral Dose



The plasma radioactivity vs. time profiles for group 8 (oral dose, BDC/JVC) for both M and F are shown in the following figure as provided by the sponsor (section 4.2.2.5, page 26, vol. 3, Module 4, Sequence 1):

Figure 3. Plasma Radioactivity versus Time in BDC Rats (Group 8) After a Single Oral 10 mg/kg Dose of [14 C]NRP104



The peak plasma levels after oral administration was mainly seen at 3h. No radioactivity was detected in plasma at the termination collection point of 48h and the mean terminal half-life for elimination of radioactivity was 7.9h in M and 3.5h in F.

The following table summarizes biliary excretion for Group 10 (IV dose, JVC/BDC, see previous section for treatment) as provided by sponsor (page 33 section 4.2.2.4, vol. 3, Module 4, Sequence 1):

TABLE 16. Biliary Excretion of Total Radioactivity after an IV Bolus Dose in Group 10 (JVC/BDC) Rats

| Group 10, Males | | | | | |
|------------------------|------------|----|----|------|-----|
| Hours | % Excreted | | | | |
| | Rat # | | | Mean | SD |
| | 43 | 44 | 45 | | |
| 0-3 | | | | 3.9 | 3.3 |
| 3-5 | | | | 4.5 | 3.7 |
| 5-8 | | | | 3.4 | 2.4 |
| 8-24 | | | | 2.0 | 0.6 |
| 24-48 | | | | 0.2 | 0.1 |

| Cumulative % Excreted | | | | | |
|-----------------------|-------|----|----|------|-----|
| Hours | Rat # | | | Mean | SD |
| | 43 | 44 | 45 | | |
| 0 | | | | 0 | 0 |
| 3 | | | | 3.9 | 3.3 |
| 5 | | | | 8.5 | 6.7 |
| 8 | | | | 11.9 | 9.0 |
| 24 | | | | 13.9 | 9.4 |
| 48 | | | | 14.1 | 9.4 |

| Group 10, Females | | | | | |
|--------------------------|------------|----|----|------|-----|
| Hours | % Excreted | | | | |
| | Rat # | | | Mean | SD |
| | 46 | 47 | 48 | | |
| 0-3 | | | | 4.6 | 2.0 |
| 3-5 | | | | 3.1 | 1.7 |
| 5-8 | | | | 2.3 | 0.3 |
| 8-24 | | | | 1.2 | 0.1 |
| 24-48 | | | | 0.3 | 0.1 |

| Cumulative % Excreted | | | | | |
|-----------------------|-------|----|----|------|-----|
| Hours | Rat # | | | Mean | SD |
| | 46 | 47 | 48 | | |
| 0 | | | | 0 | 0 |
| 3 | | | | 4.6 | 2.0 |
| 5 | | | | 7.7 | 3.6 |
| 8 | | | | 10.1 | 3.7 |
| 24 | | | | 11.3 | 3.6 |
| 48 | | | | 11.6 | 3.6 |

Cumulative biliary excretion up to 48h after IV dosing accounted for 14.1% of the dose in M rats and 11.6% of the dose in F rats. The majority of radioactivity was excreted in bile within 8h of dosing.

2.6.4.7 Pharmacokinetic drug interactions

An in vitro study was conducted to evaluate the potential of NRP-104 to inhibit seven specific isoforms of cytochrome P450 in pooled human liver microsomes. The test system was evaluated and validated by using known enzyme substrates and specific inhibitors for these enzymes. The supporting data were provided by the sponsor and are not included here. The IC₅₀ values were determined using probe substrates for those different enzymes in the presence of different concentrations of NRP-014 as summarized by the following table provided by the sponsor (section 4.2.2.6, page 6, vol. 3, Module 4, Sequence 1):

| Isoform | Substrate | Reaction | Concentration of NRP-104(μM) | | | | | IC ₅₀ |
|---------|------------------|------------------|------------------------------|------|------|------|------|------------------|
| | | | 0.01 | 0.1 | 1 | 10 | 100 | |
| CYP1A2 | Phenacetin | O-Deethylation | 99.6 | 99.7 | 97.8 | 95.1 | 94.2 | NI ^a |
| CYP2A6 | Coumarin | 7-Hydroxylation | 102 | 96.7 | 101 | 98.2 | 92.5 | NI |
| CYP2B6 | Bupropion | Hydroxylation | 101 | 99.3 | 101 | 88.9 | 90.8 | NI |
| CYP2C9 | Tolbutamide | Hydroxylation | 96.6 | 93.6 | 91.4 | 90.6 | 89.0 | NI |
| CYP2C19 | (S)-Mephenytoin | 4'-Hydroxylation | 95.2 | 98.4 | 102 | 99.6 | 87.3 | NI |
| CYP2D6 | Dextromethorphan | O-Demethylation | 98.4 | 98.3 | 101 | 97.8 | 90.0 | NI |
| CYP3A4 | Midazolam | 1'-Hydroxylation | 107 | 111 | 95.4 | 98.6 | 92.1 | NI |
| CYP3A4 | Testosterone | 6β-Hydroxylation | 97.0 | 94.5 | 82.4 | 94.6 | 101 | NI |

^a NI: No inhibition exceeding 50% was observed within the 0.01-100 μM concentration range of NRP-104.

The results of the study did not indicate a significant inhibition of those enzymes by the test article.

2.6.4.8 Other Pharmacokinetic Studies: none

2.6.4.9 Discussion and Conclusions:

The fraction absorbed of the parent compound NRP-104 was variable when administered orally to rats. The fraction absorbed at 1.5 mg/kg was only 2.6% whereas it increased to

24.6% at 12 mg/kg and it fell to 9.3% at the highest dose of 60 mg/kg. This observation seems to indicate that the compound might be delivered to the plasma via a plausible delivery system that gets to be saturated at higher doses. The nature of this system, if exists, is yet to be proven. However, plasma levels of d-amphetamine in response to oral administration of NRP-104 were increased and they appeared to be increased in a near linear fashion at lower doses (fraction absorbed ranged from 61 to 82% at doses from 1.5 to 6 mg/kg); however, at highest dose (60 mg/kg) the fraction absorbed was dropped to 52%. In contrast, rats administered equivalent oral doses of d-amphetamine sulfate the fraction of d-amphetamine absorbed increased with increasing dose and at the highest dose the fraction reached a level of 223%. It is not clear why this was observed but the sponsor stated that these results suggest that the capacity of clearance of d-amphetamine when delivered as the sulfate salt becomes saturated at the higher doses whereas the gradual hydrolysis of NRP-104 precludes saturation of d-amphetamine elimination at higher doses which is a possibility. It is also possible that the conversion of the parent compound to d-amphetamine, wherever that might happen, gets saturated at higher doses and thus the levels of d-amphetamine delivered to the plasma is reduced.

The plasma C_{max} after treatment with comparable doses of NRP-104 and d-amphetamine sulfate seems to be lower after NRP-104 treatment compared to d-amphetamine sulfate treatment in rats, at least in one of the reports, even though this was not the case in another (see the body of the review). However, the sponsor seems to be inclined towards presenting the earlier finding than the later and indicated that this might be a positive feature of the compound especially at those doses that are close to the human equivalent doses since the AUC values obtained from NRP-104 treatment and d-amphetamine sulfate at these doses were comparable (see review for details). This is a positive feature since it will, according to the sponsor, decrease the potential for abuse of this compound because there will be no large increase in C_{max} that might lead to a “rush” while the AUC values are comparable. In addition, the fact that the plasma levels of d-amphetamine after I.V. and I.N. administration were significantly lower than those observed with comparable dose of d-amphetamine sulfate in rats adds to the positive features of this compound to be less abused, according to the sponsor’s evaluation.

The pharmacokinetic parameters of the parent and d-amphetamine produced in response to both oral and I.V. treatment were evaluated in dogs and the studies indicated that the oral bioavailability of the compound in dogs was 33% and that both oral and I.V. administration of the drug produce comparable levels of d-amphetamine. However, there were no comparisons between NRP-104 treatment and d-amphetamine sulfate treatment in dogs compared to what was done in rats.

Distribution of the parent compound was investigated only in the rat brain and as expected from the structure of the parent compound the levels of the compound in the brain were below the quantitation levels while amphetamine levels were increased in the brain in response to this treatment. The metabolic profile (the sponsor indicated that these metabolites are tentative) of the parent compound seems to be fairly straight forward since the parent compound was not detected for a long time after oral treatment in the plasma (only up to 8h) and the levels of radioactivity of the parent in the plasma

was up to 2% of the total radioactivity in F. The major metabolites presented after oral administration were amphetamine and its metabolites. The metabolism of the compound does not appear to be in the liver since the in vitro studies conducted did not indicate the liver as a potential site of metabolism. There seems to be no inhibition of a variety of CYP-450 enzymes as judged by the in vitro studies conducted. The elimination of the compound was mainly through urine.

2.6.4.10 Tables and figures to include comparative TK summary:

See review and the following section for tables and figures pertaining to TK parameters.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

The following tables were provided by the sponsor to summarize the pharmacokinetic parameters (pages 12, 13, 20, and 24, in vol. 2, Module 2, Sequence 1):

| Table 2.6.4-1 Pharmacokinetics of <i>d</i> -amphetamine Following Oral Administration of NRP104 or <i>d</i> -amphetamine in Rats | | | | | |
|---|---|---|---|---|---------------------|
| Name of Company: New River Pharmaceuticals, Inc. | | New River Pharmaceuticals Report No: R06-NRP104-PKO-10 | | LC/MS/MS by [REDACTED] [REDACTED], Protocol No: 98D0306 | |
| CTD Location: Mod 4, Vol 3 , 4.2.2.2 | | | | | |
| General Pharmacokinetics (<i>in vivo</i>) | | | | | |
| Study Objective: To determine the pharmacokinetics of <i>d</i> -amphetamine released by hydrolysis of NRP104 as compared to that of <i>d</i> -amphetamine sulfate following oral administration. | | | | | |
| Species/Strain: Rat/Sprague Dawley | Weight Range on Day 1: Male: 250 – 300 g | | Duration of Treatment: 1 day Frequency of Dosing: Once | | |
| Test Materials: NRP104, <i>d</i> -amphetamine sulfate | Route: Oral Dose Volume: 1.67mL/kg – Oral | | Study Dates: 06 August 2003 through 16 October 2003 | | |
| Batch No: 1001D | Vehicle: distilled de-ionized water | | Study in Compliance with GLP: No | | |
| Main Testing Facilities: New River Pharmaceuticals, Inc. 1861 Pratt Drive, and [REDACTED] [REDACTED] | | | | | |
| Frequency of dosing and study design: Single doses of NRP104 or <i>d</i> -amphetamine sulfate were administered orally at doses of 1.5 (base), 3 (base), 6 (base), 12 (base), and 60 (base) mg/kg. Plasma samples were collected at 15 and 30 minutes, 1, 1.5, 3, 5, and 8 hours post dose and analyzed for <i>d</i> -amphetamine concentrations using LC/MS/MS. Pharmacokinetic parameters were determined from <i>d</i> -amphetamine plasma concentration profiles. | | | | | |
| Pharmacokinetic parameters of <i>d</i> -amphetamine following oral administration of NRP104 to rats | | | | | |
| Dose: mg/kg <i>d</i> -amphetamine base | 1.5 | 3 | 6 | 12 | 60 |
| C _{max} (ng/mL) | 59.6 | 126.9 | 310.8 | 412.6 | 2,164.3 |
| AUC _{last} (ng.h/mL) | 308 | 721 | 1,680 | 2,426 | 9,995 |
| AUC _{inf} (ng.h/mL) | 331 | 784 | 1,797 | 2,701 | 11,478 |
| T _{max} (h) | 3 | 1.5 | 3 | 5 | 5 |
| AUC / Dose (ng.h.kg/mL/mg) | 221 | 261 | 300 | 225 | 191 |
| C _{max} / Dose (ng.kg/mL/mg) | 39.7 | 42.3 | 51.8 | 34.4 | 36.1 |
| F (%) | 61 | 72 | 82 | 62 | 52 |
| Pharmacokinetic parameters of <i>d</i> -amphetamine following oral administration of <i>d</i> -amphetamine to rats | | | | | |
| Dose: mg/kg <i>d</i> - amphetamine base | 1.5 | 3 | 6 | 12 | 60 |
| C _{max} (ng/mL) | 142.2 | 217.2 | 815.3 | 1,533.1 | 13,735.2 |
| AUC _{last} (ng.h/mL) | 446 | 885 | 1,319 | 4,252 | 14,281 ^a |
| AUC _{inf} (ng.h/mL) | 461 | 921 | 1,362 | 4,428 | 48,707 |
| T _{max} (h) | 0.5 | 1.5 | 0.25 | 0.25 | 1 |
| AUC / Dose (ng.h.kg/mL/mg) | 307 | 307 | 227 | 369 | 812 |
| C _{max} / Dose (ng.kg/mL/mg) | 94.8 | 72.4 | 135.9 | 127.8 | 228.9 |
| F (%) | 84 | 84 | 62 | 101 | 223 |
| ^a AUC _{last} for 0-1.5h, due to deaths in high dose <i>d</i> -amphetamine group. | | | | | |
| Noteworthy Findings: Bioavailability of <i>d</i> -amphetamine from NRP104 decreased at higher doses. | | | | | |

Table 2.6.4-2 Pharmacokinetics of NRP104 Following Oral Administration of NRP104 in Rats

| | | | | | |
|--|------------|---|----------|---|-----------|
| Name of Company: New River Pharmaceuticals, Inc. | | New River Pharmaceuticals Report No: R06-NRP104-PKO-10 | | LC/MS/MS by [REDACTED] [REDACTED] Protocol No: 98D0306 | |
| CTD Location: Mod 4, Vol 3 , Section 4.2.2.2 | | | | | |
| General Pharmacokinetics (<i>in vivo</i>) | | | | | |
| Study Objective: To determine the pharmacokinetics of NRP104 following oral administration. | | | | | |
| Species/Strain: Rat/Sprague Dawley | | Weight Range on Day 1: Male: 250 – 300 g | | Duration of Treatment: 1 day Frequency of Dosing: Once | |
| Test Materials: NRP104, | | Route: Oral Dose Volume: 1.67mL/kg – Oral | | Study Dates: 06 August 2003 through 16 October 2003 | |
| Batch No: 1001D | | | | Study in Compliance with GLP: No | |
| Main Testing Facilities: New River Pharmaceuticals, Inc. 1861 Pratt Drive, and [REDACTED] [REDACTED] | | | | | |
| Frequency of dosing and study design: Single doses of NRP104 or <i>d</i> -amphetamine sulfate were administered orally at doses of 1.5 (base), 3 (base), 6 (base), 12 (base), and 60 (base) mg/kg. Plasma samples were collected at 15 and 30 minutes, 1, 1.5, 3, 5, and 8 hours post dose and analyzed for <i>d</i> -amphetamine concentrations using LC/MS/MS. Pharmacokinetic parameters were determined from <i>d</i> -amphetamine plasma concentration profiles. | | | | | |
| Pharmacokinetic parameters of <u>NRP104</u> following <u>oral</u> administration of <u>NRP104</u> to rats | | | | | |
| Dose: mg/kg <i>d</i> -amphetamine base | 1.5 | 3 | 6 | 12 | 60 |
| C_{max} (ng/mL) | 36.5 | 135.4 | 676.8 | 855.9 | 1,870.3 |
| AUC(last) (ng.h/mL) | 59.4 | 329.7 | 1,157 | 4,239 | 8,234 |
| AUC(inf) (ng.h/mL) | 60 | 332 | 1,171 | 4,510 | 8,500 |
| T_{max} (h) | 0.25 | 1.5 | .25 | 1 | 3 |
| AUC / Dose (ng.h.kg/mL/mg) | 40 | 110.7 | 195.2 | 375.8 | 141.7 |
| C_{max} / Dose (ng.kg/mL/mg) | 24.3 | 45.1 | 112.8 | 71.3 | 31.2 |
| F (%) | 2.6 | 7.2 | 12.8 | 24.6 | 9.3 |
| Noteworthy Findings: Absorption of NRP104 showed a trend of increase with increasing doses. | | | | | |

| Table 2.6.4-3 Pharmacokinetics Following Intravenous Administration of NRP104 or <i>d</i> -amphetamine in Rats | | |
|---|---|---|
| Name of Company: New River Pharmaceuticals, Inc. | New River Pharmaceuticals Report No: R05-NRP104-PKIV-9 | LC/MS/MS by [REDACTED] [REDACTED], Protocol No: 98D0306 |
| CTD Location: Mod 4, Vol 3 , Section 4.2.2.2 | | |
| General Pharmacokinetics (<i>in vivo</i>) | | |
| Study Objective: To determine the pharmacokinetics of <i>d</i> -amphetamine released by hydrolysis of NRP104 as compared to that of <i>d</i> -amphetamine sulfate following intravenous administration. | | |
| Species/Strain: Rat/Sprague Dawley | Weight Range on Day 1: Male: 250 – 300 g | Duration of Treatment: 1 day Frequency of Dosing: Once |
| Test Materials: NRP104, <i>d</i> -amphetamine sulfate | Route: IV Dose Volume: 0.33mL/kg intravenous | Study Dates: 06 August 2003 through 16 October 2003 |
| Batch No: 1001D | Vehicle: phosphate buffered saline, pH 7.2 | Study in Compliance with GLP: No |
| Main Testing Facilities: New River Pharmaceuticals, Inc. 1861 Pratt Drive, and [REDACTED] [REDACTED] | | |
| Frequency of dosing and study design: Single doses of NRP104 or <i>d</i> -amphetamine sulfate were administered intravenously to rats at a dose of 1.5 mg (<i>d</i> -amphetamine base)/kg. Plasma samples were collected at 5 and 30 minutes, 1.5, 3, 5, 8, and 24 hours post dose after intravenous administration and analyzed for <i>d</i> -amphetamine and NRP104 concentrations using LC/MS/MS. Pharmacokinetic parameters were determined from <i>d</i> -amphetamine and NRP104 plasma concentration profiles. | | |
| Pharmacokinetic parameters of <i>d</i> -amphetamine following IV administration of NRP104 or <i>d</i> -amphetamine to rats | | |
| Dose | NRP104 1.5mg/kg | <i>d</i> -amphetamine 1.5mg/kg |
| C _{max} (ng/mL) | 99.5 | 420.2 |
| AUC _{last} (ng.h/mL) | 237.8 | 546.7 |
| AUC _{inf} (ng.h/mL) | 237.9 | 546.9 |
| T _{max} (Min.) | 30 minutes | 5 minutes |
| AUC / Dose (ng.h.kg/mL/mg) | 158.5 | 364.5 |
| C _{max} / Dose (ng.kg/mL/mg) | 66.3 | 280.1 |
| Pharmacokinetic parameters of NRP104 following IV administration of NRP104 or <i>d</i> -amphetamine to rats | | |
| Dose | NRP104 1.5mg/kg | <i>d</i> -amphetamine 1.5mg/kg |
| C _{max} (ng/mL) | 4,513.1 | NA |
| AUC _{last} (ng.h/mL) | 2,282 | NA |
| AUC _{inf} (ng.h/mL) | 2,293 | NA |
| T _{max} (Min.) | 5 minutes | NA |
| Noteworthy Findings:: Bioavailability of <i>d</i> -amphetamine from NRP104 was substantially reduced compared to that of <i>d</i> -amphetamine sulfate | | |

| Table 2.6.4-4 Pharmacokinetics Following Intranasal Administration of NRP104 or <i>d</i>-Amphetamine in Rats | | |
|---|--|--|
| Name of Company: New River Pharmaceuticals, Inc. | New River Pharmaceuticals Report No: R06-NRP104-PKO-11 | LC/MS/MS by [REDACTED] [REDACTED], Protocol No: 98D0306 |
| CTD Location: Vol 3 , pg 1 , Section 4.2.2.2 | | |
| General Pharmacokinetics (<i>in vivo</i>) | | |
| Study Objective: To determine the pharmacokinetics of <i>d</i> -amphetamine released by hydrolysis of NRP104 as compared to that of <i>d</i> -amphetamine sulfate following intranasal administration. | | |
| Species/Strain: Rat/Sprague Dawley | Weight Range on Day 1: Male: 250 – 300 g | Duration of Treatment: 1 day Frequency of Dosing: Once |
| Test Materials: NRP104 <i>d</i> -amphetamine sulfate | Route: IN Dose Volume: 0.067mL/kg Vehicle: distilled de-ionized water | Study Dates: 06 August 2003 through 16 October 2003 |
| Batch No: 1001D | | Study in Compliance with GLP: No |
| Main Testing Facilities: New River Pharmaceuticals, Inc. 1861 Pratt Drive, and [REDACTED] [REDACTED] | | |
| Frequency of dosing and study design: Single doses of NRP104 or <i>d</i> -amphetamine sulfate were administered intranasally to rats at a dose of 3mg (<i>d</i> -amphetamine base)/kg. Plasma samples were collected at 5, 15, and 30 minutes and 1 hour post dose and analyzed for <i>d</i> -amphetamine and NRP104 concentrations using LC/MS/MS. Pharmacokinetic parameters were determined from <i>d</i> -amphetamine and NRP104 plasma concentration profiles. | | |
| Pharmacokinetic parameters of <i>d</i>-amphetamine following IN administration of NRP104 or <i>d</i>-amphetamine to rats | | |
| Dose | NRP104 3mg/kg | <i>d</i> -amphetamine 3mg/kg |
| C _{max} (ng/mL) | 78.6 | 1,962.9 |
| AUC _{last} (ng.h/mL) | 56 | 1,032 |
| AUC _{inf} (ng.h/mL) | 91 | 7291 |
| T _{max} (Min.) | 60 minutes | 5 minutes |
| AUC _{inf} / Dose (ng.h.kg/mL/mg) | 30.3 | 2,430.3 |
| C _{max} / Dose (ng.kg/mL/mg) | 26.2 | 654.3 |
| Pharmacokinetic parameters of NRP104 following IN administration of NRP104 or <i>d</i>-amphetamine to rats | | |
| Dose | NRP104 3mg/kg | <i>d</i> -amphetamine 3mg/kg |
| C _{max} (ng/mL) | 3,345.1 | NA |
| AUC _(last) (ng.h/mL) | 2,580 | NA |
| AUC _(inf) (ng.h/mL) | 9,139 | NA |
| T _{max} (Min.) | 15 minutes | NA |
| Noteworthy Findings: Bioavailability of <i>d</i> -amphetamine from NRP104 was substantially reduced compared to that of <i>d</i> -amphetamine sulfate. | | |

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The sponsor conducted the following studies in rats: a single oral dose study, a 7-day oral dose range-finding study, and a 28-day oral toxicity study (doses 0, 20, 40, and 80 mg/kg/day). The following studies were conducted in dogs: an escalating single oral dose study, a 7-day oral dose range finding study, and a 28-day oral toxicity study.

The single dose studies in rats were conducted to evaluate the maximum tolerated dose and to determine the LD50 at doses 0.1, 1, 10, 60, 100, and 1000 mg/kg administered orally by gavage. Based on the finding at 1000 mg/kg dose in which 1/3F was found dead and 1/3 M was euthanized on day 3 for humane reasons (skin lesions), the LD50 for NRP-104 was considered to be >1000 mg/kg. This dose of NRP-104 diHCl salt (which is equivalent to 399 mg/kg of d-amphetamine base) was compared to the LD50 reported for d-amphetamine sulfate which equals 96.8 mg/kg (equivalent to 70.5 mg/kg d-amphetamine base). Increased motor activity (excessive biting and licking of cage), chromodacryorrhea/chromorhinorrhea, and skin lesions were seen in groups treated with 60 mg/kg and above with the severity increasing with the doses. All surviving animals appeared to be normal 4 days after treatment.

In the seven day study rats (5/sex/group) were treated with 0, 30, 100, and 300 mg/kg of NRP-104 orally by gavage, death and self mutilation were observed at 300 and 100 mg/kg, while increased activity was observed at all doses. Decreases in body wt were observed at MD and HD. Statistically significant changes in some clinical chemistry parameters were observed. No histopathology was conducted in this study. A toxicokinetic group was also included in this study.

In the 28-day study, rats (10-15/sex/group) were treated with 0, 20, 40, or 80 mg/kg of NRP-104 orally by gavage. Another group of animals (15/sex) were treated with a d-amphetamine sulfate (16 mg/kg). Five animals from the control, HD NRP-104 treated group and d-amphetamine sulfate group were used as a recovery group. A toxicokinetic group was also included in this study. Mortality, clinical signs, body wts, food consumption, ophthalmology, hematology, clinical chemistry, gross pathology, organ wts, and histopathology were all evaluated (see review for more details). There was no death reported but 1/9 F treated with 80 mg/kg in the toxicokinetic group was moribund sacrificed on Day 7 due to self-mutilation. Clinical signs noted in all NRP-104 treated groups and in the d-amphetamine sulfate treated group included increased activity and

post dose jumping. Self mutilation and thin body condition were observed in some animals treated with the HD of NRP-104. One F in the d-amphetamine sulfate group had thin condition towards the end of the study. Body wt decreases were observed at MD and HD in the NRP-104 group and in the d-amphetamine sulfate treated group. All animals were normal during the recovery period except for 1M and 1F from HD NRP-104 group with thin body condition for the first few days of the recovery period. Some statistically significant increases in clinical chemistry parameters (glucose, BUN, and ALT) were observed at MD and HD NRP-104 groups. Histopathological changes such as fiber necrosis and degeneration of biceps of thigh muscle were seen at HD in 1/15 F and degeneration of muscular tone in the esophagus in 2/15 F from HD group also. These histopathological findings were considered to be drug unrelated by the sponsor; however, a drug effect cannot be ruled out. Toxicokinetic data indicated that Cmax and AUC values of NRP-104 were lower than d-amphetamine values in all groups in both M and F. AUC values of both d-amphetamine and NRP-104 were greater at Day 28 than at Day 1 in F and M, particularly in the MD and HD groups. Both AUC and Cmax were higher in F than in M for all treatment groups.

In Pyramid Study in dogs, the purpose of the study was to establish a maximum tolerated dose (MTD) of NRP-104 when administered orally once to beagle dogs. The dogs (2/sex/group) were treated with 3, 10, 18, and 24 mg/kg of NRP-104 separated by at least two days. No deaths were observed. Emesis was observed in 1 animal treated with 3 mg/kg. Increased activity, abnormal gait and stance, restlessness, repetitive behavior, head bobbing, excessive licking were observed at 10, 18, and 24 mg/kg. At 18 and 24 mg/kg, circling and emesis were also observed. The animals lost wt over the course of the study. The MTD was considered to be less than 24 mg/kg since emesis was observed in all animals at this dose.

In the 7-day study, dogs (1/sex/group) were treated with 0, 3, 6, or 12 mg/kg/day orally by gavage. No deaths were observed. Increased activity was observed at all doses (seen only on few days at LD) and repetitive behavior, restlessness, vessels over sclera dilated at MD and HD and severe ocular discharge at HD (all seen only on Day 1). Decreased body wt was observed at HD. Decreases in reticulocytes at MD and HD. No histopathology was conducted.

In the 28-day study, dogs (3-5/sex/group) were treated with 0, 3, 6, and 12 mg/kg/day with an additional group of animals (5/sex) treated with 2.4 mg/kg/day of d-amphetamine sulfate. Two animals from the control, HD NRP-104 treated group and the d-amphetamine sulfate treated group were used for the recovery group (14-days). Mortality, clinical signs, body wts, food consumption, ophthalmology, ECG, hematology, clinical chemistry, gross pathology, organ wts, and histopathology were all evaluated (see the review for more details). No deaths were observed. Restlessness and increased activity were observed in few animals at LD (several days), most animals at MD (almost throughout study) and all animals at HD and those treated with d-amphetamine sulfate (throughout the study). Repetitive behavior, head shaking, and pacing in cage were observed in animals treated at MD and HD but they were seen in more animals at HD than at MD. Decreased activity predose was observed in some animals at MD and HD

and those treated with d-amphetamine sulfate. Panting, circling and abnormal gait were also observed in some animals treated with HD of NRP-104 and animals treated with d-amphetamine sulfate. Decreases in body wt were observed at MD and HD and in those animals treated with d-amphetamine sulfate and body thinness was observed in some animals at HD and in the d-amphetamine sulfate treated group. There were some decreases in reticulocytes at MD and HD. During the recovery period, a decrease in body wt and body thinness was seen in some animals treated with NRP-104 and d-amphetamine sulfate during and decreased activity was seen in 1M treated with HD NRP-104. There were no ophthalmology findings and no ECG findings at the tested times. There were no significant histopathological findings.

The conducted 28-day toxicology studies are considered adequate and the results indicated that an MTD had been reached in those studies in both rats (sacrifice of one animal due self sustained injuries, self mutilation, and the effects on body wt at HD) and in dogs (behavioral abnormalities including restlessness, head shaking, pacing in cage, panting, circling and the effect on body wt at HD). The addition of the group treated with the d-amphetamine sulfate in these studies was valuable since it was appropriate to compare the effect of this compound to the effects of d-amphetamine (the proposed metabolite). According the sponsor's calculations, the doses used for NRP-104 in these studies were comparable to those doses used for the d-amphetamine sulfate group based on the d-amphetamine base value. By comparing the results obtained from treatment with NRP-104 with those with d-amphetamine sulfate, it was evident that the effects of the compound are very similar to those of d-amphetamine sulfate and thus indicating that this compound is acting totally through its metabolite d-amphetamine.

At the time of the IND meeting with the sponsor, the Division had agreed that the 28-day study would probably be considered adequate to prove that this compound is not different from amphetamine and accordingly other long term toxicology studies might be needed. This seems to be the case and it is for this reason that the longest studies conducted in both the rodent and the non-rodent species were the 28 day studies.

Genetic toxicology: the compound was tested in the Ames test, in vitro mouse lymphoma assay and the in vivo micronucleus assay.

Even though there were some technical issues with some parts of the definitive study in the Ames test, these were resolved by repeating these parts and by depending on the preliminary study findings. In the mouse lymphoma assay the sponsor was asked to repeat part of the study due to large differences in the duplicates. In the in vivo micronucleus assay the sponsor also was asked to repeat part of the study due to the invalidity of the high dose used. These issues were found to be resolved and the reviewer considers these studies adequate and valid. The overall outcome of the studies indicated that the compound is not genotoxic in any of the tests used. For more details about the studies and the outcomes please see the review for these individual studies.

Carcinogenicity: no studies were conducted. At the time of the pre-NDA meeting the sponsor was told by the division that if the compound produces effects that are due to the metabolite amphetamine with the levels of the parent present minimal as they claimed at that time, then carcinogenicity studies will not be need. Carcinogenecity studies of amphetamine have been perfomed by NTP and are described in the Adderall labeling.

Reproductive toxicology: no studies were conducted. Similar to the reason given for the carcinogenicity studies. Animal reproductive studies of amphetamine are described in the Adderall labeling.

2.6.6.2 Single-dose toxicity

Rats:

A single oral dose study was conducted in Sprague-Dawley rats (3/sex/group) where animals were treated with 0.1, 1, 10, 60, 100, and 1000 mg/kg (Project # 98D-0301). The animals were observed for up to 7 days.

Results:

The following table was provided by the sponsor summarizing the findings of the study (section 4.2.3.1, page 7, vol. 4, Module 4, Sequence 1):

| 6. TABLES | | |
|--|--|--|
| Table 1. Summary of Cage-Side Observations and Necropsy Findings | | |
| Group, Dosage | Cage-Side Observations | Gross Necropsy Findings |
| Gp. 1, 0.1mg/kg | <ul style="list-style-type: none"> No observable abnormalities throughout study | No Findings |
| Gp. 2, 1.0mg/kg | <ul style="list-style-type: none"> No observable abnormalities throughout study | No Findings |
| Gp. 3, 10mg/kg | <ul style="list-style-type: none"> No observable abnormalities throughout study | No Findings |
| Gp. 4, 60mg/kg | <ul style="list-style-type: none"> Increased motor activity (excessive biting and licking of caging) on Days 1 and 2. Slight Chromodacryorrhea/Chromorhinorrhea (Day 1 only). No observable abnormalities on Days 3-7. | No Findings |
| Gp. 5, 100mg/kg | <ul style="list-style-type: none"> Increased motor activity (excessive biting and licking of caging) at 1-24 h post-dose. Slight Chromodacryorrhea/Chromorhinorrhea (Days 2-3). Decreased fecal output over Days 1 and 2. No observable abnormalities on Days 3-7. | No Findings |
| Gp. 6, 1000mg/kg | <ul style="list-style-type: none"> Increased motor activity (excessive biting and licking of caging) over Days 1 and 2. Chromodacryorrhea/Chromorhinorrhea (Days 2-3). 1 Female found dead on morning of Day 2. 1 Male euthanatized on Day 3 due to poor condition (severe, self-inflicted open wounds). 4 rats had skin lesions of varying degrees of severity on Day 3. Little to no fecal/urine output until Day 4. No observable abnormalities on Days 3-7. | <p><u>Female (found dead)</u> Chromodacryorrhea, Chromorhinorrhea, enlarged adrenal glands, distended stomach (gas), distended & edematous intestines.</p> <p><u>Male (euthanatized)</u> – Chromodacryorrhea, enlarged adrenal glands, skin, neck – 2, large, red lesions.</p> |

The sponsor considered the lethal dose to be above 1000 mg/kg since only one animal out of six died in the study while the second animal was euthanatized for human reason (open

wounds). The sponsor compared this lethal dose (>1000 mg/kg) to the LD50 for amphetamine which was reported as 96.8 mg/kg (Physician Desk Reference, 2005). Clinical signs observed at doses higher than 60 mg/kg (mostly increased motor activity and biting and licking) were similar to those of observed with d-amphetamine. The animals seemed to recover few days after the treatment indicating that the effect of the drug is reversible.

Dogs:

Pyramid oral toxicity study in dogs with NRP-104 (Study #0433DN29.001): an escalating single oral dose study was conducted in beagle dogs (2M and 2F/ dose, same dogs for all doses) at dose levels of 3, 10, 18, and 24 mg/kg separated with at least two days to establish an MTD. Additional dosing with NRP-104 at a dose level of 18 mg/kg was done to the same four dogs for collection of plasma samples for toxicokinetic analysis. After this final dose administration, dogs remained on test, untreated, until sacrifice and were necropsied 5 days following the final dose. Animals were observed for clinical signs of effect or toxicity 1h post dosing and as needed, body wt was recorded each day, food consumption was recorded daily, whole blood samples were collected prior to terminal sacrifice for hematology, serum clinical chemistry, coagulation profiles, and toxicokinetic evaluation (at the following time points: pre-dose, 30 min, 1h, 3h, 6h, 8h, 12h, 16h, and 24h).

Results:


No deaths were observed. The following table was prepared by the reviewer summarizing clinical signs observed:

| | | |
|---|----|--|
| Escalating single dose N=2/sex/ grp | 3 | 1/2 M had emesis |
| | 10 | ↑ activity (all animals), abnormal gait & stance (1/2 M), restlessness (all), repetitive behavior (all), head bobbing (2/2F), excessive licking (2/2F) |
| | 18 | All CNS signs observed at 10 mg/kg and abnormal gait and stance (all), circling (1/2M, 1/2F), emesis (1/2M) |
| | 24 | All clinical signs observed at lower doses and emesis in all animals |

All animals lost wt over the course of the study (ranged between 0.4-0.5 kg). Food consumption was sporadic over the course of the study but was mostly seen after administering the 24 mg/kg dose. No test article related findings in hematological, clinical chemistry, coagulation parameters or necropsy findings. The maximum tolerated dose (MTD) was considered to be less than 24 mg/kg due to the observation of emesis in all animals treated with that dose.

Plasma concentrations for NRP-104 and d-amphetamine are summarized in the following tables provided by the sponsor (section 4.2.3.2, page 163, vol. 7, module 4, sequence1):

TABLE 6A. Concentration (ng/mL) of Lysine-Amphetamine in Individual Dog Plasma Samples

Client Study No.: 0433DN29.001
 Project No.: 35-0306

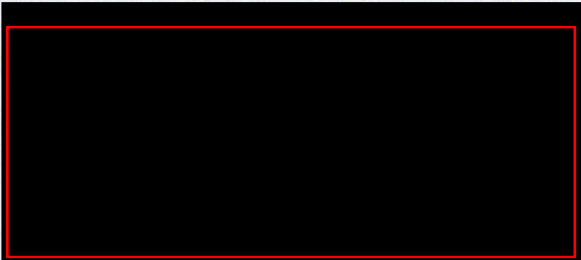

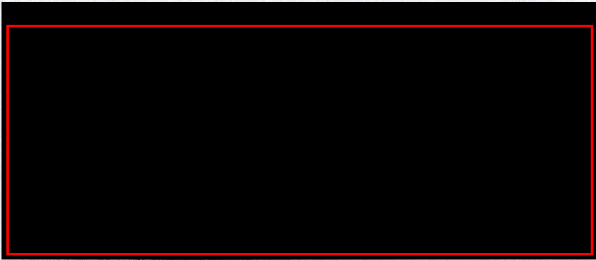
| Timepoint | Subject 0005 | Subject 0006 | Subject 0011 | Subject 0012 |
|------------|--|-----------------|-----------------|-----------------|
| Day 1 0h |  | | | |
| Day 1 0.5h | | | | |
| Day 1 1h | | | | |
| Day 1 3h | | | | |
| Day 1 6h | | | | |
| Day 1 8h | | | | |
| Day 1 12h | | | | |
| Day 1 16h | | | | |
| Day 1 24h | | | | |

TABLE 6B. Concentration (ng/mL) of D-Amphetamine in Individual Dog Plasma Samples

Client Study No.: 0433DN29.001
 Project No.: 35-0306

| Timepoint | Subject 0005 | Subject 0006 | Subject 0011 | Subject 0012 |
|------------|--|-----------------|-----------------|-----------------|
| Day 1 0h |  | | | |
| Day 1 0.5h | | | | |
| Day 1 1h | | | | |
| Day 1 3h | | | | |
| Day 1 6h | | | | |
| Day 1 8h | | | | |
| Day 1 12h | | | | |
| Day 1 16h | | | | |
| Day 1 24h | | | | |

The plasma levels of the parent compound appear to be higher than the d-amphetamine levels after oral administration as judged by the values in the previous tables at the indicated time points.

2.6.6.3 Repeat-dose toxicity

Rats:

Study title: 7-day dose range-finding oral toxicity study in rats with NRP-104

Key study findings: deaths and stimulant effects (increased activity and self mutilation) were observed in animals treated with 100 and 300 mg/kg. Decreases in body wt were also observed at these doses. Some clinical chemistry changes were also seen at these doses (see review for details). The NOEL was considered to be < 30 mg/kg/day.

Study no.: 0440RN29.002

Volume #, and page #: vol. 4, page 1 (Module 4, Sequence 1)

Conducting laboratory and location: -----

Date of study initiation: November 7, 2003

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: NRP-104 (AIB 17936-4), Lot # BJP-I-152(1), According to certificate of analysis HPLC analysis indicated % purity (peak area was %)

Methods

Doses: 0, 30, 100, and 300 mg/kg/day

Species/strain: Sprague Dawley rats

Number/sex/group or time point (main study): 5/sex/group

Route, formulation, and volume: orally by gavage, solution, 10 ml/kg

Satellite groups used for toxicokinetics or recovery: toxicokinetic group with 9/sex/group. The following table was provided by the sponsor summarizing the animal assignments (pages 14 & 173, vol. 4):

| Toxicology Groups | | | | | | |
|-------------------|-----------|----------------------|----------------------------|--------------------------|-------------------|---------|
| Group | Treatment | Dose* (mg/kg/day) | Dose Volume (ml/kg/day) | Concentration (mg/ml) | Number of Animals | |
| | | | | | Males | Females |
| 1 | Vehicle | 0 | 10 | 0 | 5 | 5 |
| 2 | NRP104 | 30 | 10 | 3.0 | 5 | 5 |
| 3 | NRP104 | 100 | 10 | 10.0 | 5 | 5 |
| 4 | NRP104 | 300 | 10 | 30.0 | 5 | 5 |

| Toxicokinetic Groups | | | | | | |
|----------------------|-----------|---------------------|----------------------------|--------------------------|-------------------|---------|
| Group | Treatment | Dose (mg/kg/day) | Dose Volume (ml/kg/day) | Concentration (mg/ml) | Number of Animals | |
| | | | | | Males | Females |
| 5 | NRP104 | 30 | 10 | 3.0 | 9 | 9 |
| 6 | NRP104 | 100 | 10 | 10.0 | 9 | 9 |
| 7 | NRP104 | 300 | 10 | 30.0 | 9 | 9 |

| Toxicology Groups (Groups 1-4) | | |
|--------------------------------|-----------|------------|
| Group | Males | Females |
| 1 | 4401-4405 | 4406-4410 |
| 2 | 4411-4415 | 4416-4420 |
| 3 | 4421-4425 | 4426-4430 |
| 4 | 4431-4435 | 4436- 4440 |

| Toxicokinetic Groups (Groups 5-7) | | |
|-----------------------------------|-----------|-----------|
| Group | Males | Females |
| 5 | 4441-4449 | 4450-4458 |
| 6 | 4459-4467 | 4468-4476 |
| 7 | 4477-4485 | 4486-4494 |

Age: ~7 weeks

Weight: 204-235 g for M, and 171-197 g for F

Sampling times: for toxicokinetics blood samples were collected on Days 1 and Day 7 at the following time points: pre-dose, 1, 2, 4, 8, and 24h. Animals were sacrificed on Day 8.

Unique study design or methodology (if any): none

Observations and times:

Mortality: animals were observed at least twice daily. According to the sponsor, any animal judged to be in a moribund condition was necropsied.

Clinical signs: animals were observed prior to dosing, at ~1h post dose and additionally as appropriate.

Body weights: at the time of randomization/selection, prior to dose administration on Day 1, and following the final dose on Day 7. A fasted body wt was recorded prior to sacrifice on Day 8.

Food consumption: recorded from days 1-7.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: whole blood samples were collected for hematology, clinical chemistry, and coagulation profile prior to terminal sacrifice on Day 8. Blood samples were collected via cardioventesis. Animals were fasted overnight prior to blood collection. The following parameters were examined: differential white blood cell count (Diff), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet count (PLT), red blood cell count and morphology (RBC), white blood cell count (WBC) and reticulocyte count (Retic). Coagulation parameters including prothrombin time (PT) and activated partial thromboplastin time (APTT).

Clinical chemistry: for blood collection see previous section. The following parameters were evaluated:

| | |
|--|-------------------------------|
| <i>Serum Clinical Chemistry:</i> | |
| • Alanine Aminotransferase (ALT) | • Globulin (calculated)(GLOB) |
| • Albumin (ALB) | • Glucose (GLU) |
| • Albumin/Globulin ratio (calculated)(A/G) | • Phosphorus (PHOS) |
| • Alkaline Phosphatase (ALP) | • Potassium (K) |
| • Aspartate Aminotransferase (AST) | • Sodium (NA) |
| • Calcium (CA) | • Total Bilirubin (T-BIL) |
| • Chloride (CL) | • Total Protein (TP) |
| • Cholesterol (CHOL) | • Triglycerides (TRIG) |
| • Creatinine (CREAT) | • Urea Nitrogen (BUN) |

Urinalysis: not performed.

Gross pathology: animals sacrificed on completion of the study (Day 8), or earlier for humane reasons, were euthanized via overdose of CO₂ asphyxiation and necropsied. The necropsy included examination of the external body surface, all orifices and the cranial, thoracic and abdominal cavities and their contents. A complete necropsy was conducted for any animal euthanized in a moribund condition.

Organ weights: adrenal glands, kidneys, spleen, brain, heart, liver, ovaries, testes.

Histopathology: Adequate Battery: yes (), no (X)—explain: Tissues were collected and preserved in 10% formalin but histopathology evaluation was not conducted and tissues were retained for possible future evaluation (table was provided by the sponsor on page 18, vol. 4, module 4, sequence 1):

The following organs and tissues were preserved in 10% neutral buffered formalin:

- | | |
|---------------------------------------|--|
| • Unique animal identifier | • Pancreas |
| • Abnormalities (gross) | • Pituitary gland |
| • Adrenal glands | • Prostate |
| • Aorta | • Rectum |
| • Brain | • Salivary glands |
| • Cecum | • Sciatic Nerve |
| • Cervix | • Seminal vesicles |
| • Colon | • Skin |
| • Duodenum | • Spinal cord :cervical, midthoracic and lumbar |
| • Epididymides | • Spleen |
| • Esophagus | • Sternum with bone marrow |
| • Exorbital lachrymal gland | • Stomach |
| • Eyes with optic nerve | • Submandibular lymph nodes |
| • Femur – including articular surface | • Testes |
| • Heart | • Thigh muscle (biceps formis) |
| • Ileum | • Thymus |
| • Jejunum | • Thyroid with parathyroids |
| • Kidneys | • Tongue |
| • Liver | • Trachea |
| • Lungs with mainstem bronchus | • Urinary bladder |
| • Mammary gland | • Uterus |
| • Mesenteric lymph nodes | • Vagina |
| • Ovaries | |

Results

The following table was prepared by the reviewer summarizing the findings of the study:

| Species | Study | Dose mg/kg/day | Responses |
|---------|--|-------------------|---|
| Rat | 7-days N=5/sex/ grp for main and 9/sex/grp for TK | 0 | |
| | | 30 | ↑ activity (all F) |
| | | 100 | 1/5 F moribund sacrifice on day 3. From TK group 2/9 F were moribund sacrificed on day 3. ↑ activity (all) until the time of subsequent dosing. <u>Self mutilation</u> (1F). All deaths and sacrifices were due to self mutilation. ↓ body wt (10% in M and 5% in F). ↓ in reticulocytes (67% M, 47% F), ↑ APTT (30%, F only). ↑ in glucose (30% M, 33% F), ↑ BUN (75% M, 95% F), ↑ ALP (23% M, 12% F), ↑ ALT (68% F), ↑ AST (70% in M, 56% in F). Small spleen 1/5 (M & 1/4F) |
| | | 300 | 3/5M & 5/5 F were moribund sacrificed (2M on day 3 and 1 on day 6 and all F on day 3). From TK group 7/9 M moribund sacrificed (5 on day 3 and 2 on day 5) and 1/9M died (on day 5) & 6/9 F were moribund sacrificed (4 on day 3 and 2 on day 5). All deaths and sacrifices were due to self mutilation. ↑ activity (all) until the time of subsequent dosing. <u>Self mutilation</u> (all animals). Licking and jumping. ↓ body wt (19% M, relative to control, no data from F because of death). ↓ in reticulocytes (67% M, no data in F), ↑ glucose (61% M), ↑ BUN (75% M), ALP (53% M), ↑ ALT (163% M), ↑ AST (116% M). F had higher plasma levels compared to M at all doses (both parent and d-amphetamine, 2-5X for parent, 2-10X for metabolite). Small spleen (2/2M). <i>No histopathology was done.</i> |

Toxicokinetics: On Days 1 and 7 blood samples were collected from animals at the following time points: pretreatment, 1, 2, 4, 8 and 24h.

The data indicated an increase in the concentration of the parent in response to treatment and both C_{max} of the parent and d-amphetamine increased approximately proportionally with the increasing dose from 30 to 300 mg/kg/day in both genders. AUC of NRP-104 increased more than proportionally with the increasing dose from 30 to 300 mg/kg/day in both genders. AUC of d-amphetamine increased more than dose proportional from 30 to 300 mg/kg/day in F. Both C_{max} and AUC valued of NRP-104 and d-amphetamine were higher in F rats than in M at all dose levels. In both M and F and on both Day 1 and Day 7, NRP-104 C_{max} and AUC values were lower than d-amphetamine C_{max} and AUC values. At the dose level of 300 mg/kg/day, for both M and F, Day 7 AUC values of NRP-104 were higher than Day 1 AUC values, indicating some accumulation over this dosing period.

The following is a summary of the toxicokinetic data as provided by the sponsor (page 10, of the Toxicology Written Summary section in Module 2, Sequence 1, vol. 2):

| Table 2.6.6-2 7-Day Dose Range-Finding Oral Toxicity Study in Rats | | | | | | | | |
|--|------|--------------------------------------|--------|--------|--------|---|--------|---------|
| Name of Company: New River Pharmaceuticals | | NRP Report No: R08-NRP104-TK7O-14 | | | | CRO Report No: 0440RN29.002 | | |
| CTD Location: Mod 4, Vol 4 , Section 4.2.3.2 | | Route: Oral (gavage) | | | | Duration of Treatment: 7 days | | |
| Species/Strain: Rat: Sprague Dawley | | Test Material: NRP104 | | | | Dosing Frequency: Once daily | | |
| | | Batch No: 1001D | | | | | | |
| | | | | | | Dose Volume: 10mL/kg/day | | |
| Weight Range on Day 1: Male 204-235g; Female 171-197g | | Vehicle: de-ionized water | | | | Dosing period: November 12 -18, 2003 | | |
| Age on Day 1: 7 weeks | | Treatment of controls: Vehicle | | | | Necropsy Dates: November 19, 2003 | | |
| No adverse effect dose level: <30 mg/kg/day | | | | | | Study in Compliance with GLP: Yes | | |
| Main Testing Facility: [REDACTED] | | | | | | | | |
| Study Design | | | | | | | | |
| | Male | | | | Female | | | |
| Daily Dose: mg/kg/day NRP104 | 0 | 30 | 100 | 300 | 0 | 30 | 100 | 300 |
| Number of Animals: Main | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Toxicokinetic | 0 | 6 | 6 | 6 | 0 | 6 | 6 | 6 |
| Toxicokinetics: | | | | | | | | |
| AUC _{0-24h} (ng.mL/h) <i>d</i> -amphetamine Day1 | - | 1,248 | 6,238 | 28,670 | - | 2,095 | 16,184 | 33,176 |
| Day 7 | - | 1,509 | 12,938 | 10,694 | - | 2,422 | 19,190 | 130,661 |
| C _{max} (ng/mL) <i>d</i> -amphetamine Day 1 | | 157 | 660 | 2,230 | - | 388 | 1,593 | 2,363 |
| Day 7 | - | 212 | 1,238 | 3,927 | - | 448 | 1,641 | 6,704 |
| AUC _{0-24h} (ng.mL/h) NRP104 Day1 | - | 124 | 1,001 | 7,172 | - | 227 | 4,123 | 1,0751 |
| Day 7 | - | 240 | 1,831 | 4,771 | - | 293 | 2,294 | 22,445 |
| C _{max} (ng/mL) NRP104 Day 1 | - | 69.9 | 193 | 646 | - | 107 | 983 | 1,122 |
| Day 7 | - | 75.3 | 469 | 1,487 | - | 123 | 394 | 1,978 |

Study title: 28-Day oral toxicity study in rats

Key study findings: one F was moribund sacrificed due to self mutilation. Increased activity was observed at all doses of NRP-104 and in the d-amphetamine sulfate treated group, jumping was observed at MD and HD, and self mutilation at HD. Decreased body wt at MD (M only) and HD and the d-amphetamine sulfate treated group (M only). Body thinness was observed at HD and in the d-amphetamine sulfate treated group (1F). Some clinical chemistry changes were observed.

Study no.: 0436RN29.002

Volume #, and page #: vol. 5, module 4, sequence 1 (section 4.2.3.2, page 1)

Conducting laboratory and location: -----

| |
|-------|
| ----- |
| ----- |
| ----- |
| ----- |

Date of study initiation: December 16, 2003

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: NRP-104, Batch # 1001D, ----- % by HPLC. D-amphetamine from ----- Batch # 043K0803

Methods

Doses: for NRP-104 doses were: 0, 20, 40, and 80 mg/kg/day. For d-amphetamine treated group, a dose of 16 mg/kg d-amphetamine was used

Species/strain: Sprague Dawley rats

Number/sex/group or time point (main study): 10-15/sex/group (see table below for animal assignment)

Route, formulation, volume, and infusion rate: orally by gavage, solution, 10 ml/kg

Satellite groups used for toxicokinetics or recovery: satellite groups for toxicokinetics and recovery (see table below)

Age: ~7 weeks

Weight: 219-274 g for M & 155-221 g for F

Sampling times: for the toxicokinetic group blood was collected on Days 1 and 28 at the following timepoints: predose, 1, 2, 4, 6, 8, 12, 16, and 24h post dose.

Unique study design or methodology (if any): an additional group treated with d-amphetamine (16 mg/kg/day) was included in the study. 5/sex/dose animals from the control, HD NRP-104 group and the d-amphetamine group were kept for a 14-day recovery period. Animals were individually housed. The following tables provided by the sponsor summarize the assignment of animals to the different groups (pages 15 and 16, vol. 5, module 4, sequence 1):

Toxicology Groups (Groups 1-5)

| Group | Treatment | Dose (mg/kg/day) | Dose Volume (ml/kg/day) | Concentration (mg/ml) | Number of Animals | |
|-------|---------------|---------------------|-------------------------------|--------------------------|----------------------|--------|
| | | | | | Male | Female |
| 1 | Vehicle | 0 | 10 | 0 | 15* | 15* |
| 2 | d-amphetamine | 16 | 10 | 1.6 | 15* | 15* |
| 3 | NRP104 | 20 | 10 | 2.0 | 10 | 10 |
| 4 | NRP104 | 40 | 10 | 4.0 | 10 | 10 |
| 5 | NRP104 | 80 | 10 | 8.0 | 15* | 15* |

* 10/sex/group were sacrificed on Day 29. The additional animals (5/sex/group) remained on study, untreated, for a 14-day recovery period.

Toxicokinetic Groups (Groups 5-7)

| Group | Treatment | Dose (mg/kg/day) | Dose Volume (ml/kg/day) | Concentration (mg/ml) | Number of Animals | |
|-------|---------------|---------------------|-------------------------------|--------------------------|----------------------|--------|
| | | | | | Male | Female |
| 6* | d-amphetamine | 16 | 10 | 1.6 | 9 | 9 |
| 7 | NRP104 | 20 | 10 | 2.0 | 9 | 9 |
| 8 | NRP104 | 40 | 10 | 4.0 | 9 | 9 |
| 9 | NRP104 | 80 | 10 | 8.0 | 9 | 9 |

* Dose administration and time of administration were inadvertently not documented for Group 6 male (#4643) on Day 4 and Day 7. This deviation had no impact on the outcome of the study.

Toxicology Groups (Groups 1-5)

| Group | Males | Females |
|-------|-----------|------------|
| 1 | 4301-4315 | 4366-4380 |
| 2 | 4316-4330 | 4381-4395 |
| 3 | 4331-4340 | 4601-4610 |
| 4 | 4341-4350 | 4611-4620 |
| 5 | 4351-4365 | 4621- 4635 |

Toxicokinetic Groups (Groups 6-7)

| Group | Males | Females |
|-------|-----------|-----------|
| 6 | 4636-4644 | 4645-4653 |
| 7 | 4654-4662 | 4663-4671 |
| 8 | 4672-4680 | 4681-4689 |
| 9 | 4690-4698 | 4699-4707 |

It should be noted that the toxicokinetic groups were groups 6-9 and not 5-7 or 6-7 as the sponsor had included in the title of the tables above.

Observations and times:

Mortality: twice daily. Any animal judged to be in a moribund condition was necropsied.

Clinical signs: prior to dosing, 1h post-dose and additionally as appropriate. During the recovery phase, animals were observed once daily. Two animals were not observed pre- and post dose on Day 4. However, this deviation might not have affected the results.

Body weights: at the time of randomization/selection, prior to dosing on Day 1, 8, 15, 22 and following the final dose on Day 28. Recovery animals were weighed on Days 35 and 42. A fasted body weight was recorded prior to sacrifice on Day 29 or Day 43 (recovery animals).

Food consumption: total food was recorded weekly (Days 1-8, 8-15, 15-22, 22-28 for main study animals and Days 28-35 and 35-42 for recovery animals).

Ophthalmoscopy: before study initiation and prior terminal sacrifice on Day 29. A dilating agent was used and examinations were performed by a consulting veterinary pathologist.

EKG: not performed.

Hematology: whole blood samples were collected for hematology, coagulation profile and clinical chemistry analysis prior to terminal sacrifice on Day 29 or Day 43 (recovery animals). The following parameters were evaluated: differential white blood cell count (Diff), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet count (PLT), red blood cell count and morphology (RBC), white blood cell count (WBC) and reticulocyte count (Retic). Coagulation parameters including prothrombin time (PT) and activated partial thromboplastin time (APTT).

Clinical chemistry: for blood collection see previous section. The following parameters were evaluated:

Serum Clinical Chemistry:

- | | |
|--|-------------------------------|
| • Alanine Aminotransferase (ALT) | • Globulin (calculated)(GLOB) |
| • Albumin (ALB) | • Glucose (GLU) |
| • Albumin/Globulin ratio (calculated)(A/G) | • Phosphorus (PHOS) |
| • Alkaline Phosphatase (ALP) | • Potassium (K) |
| • Aspartate Aminotransferase (AST) | • Sodium (NA) |
| • Calcium (CA) | • Total Bilirubin (T-BIL) |
| • Chloride (CL) | • Total Protein (TP) |
| • Cholesterol (CHOL) | • Triglycerides (TRIG) |
| • Creatinine (CREAT) | • Urea Nitrogen (BUN) |

Urinalysis: not performed.

Gross pathology: at terminal sacrifice (Day 29 or Day 43) animals were euthanized via CO₂ asphyxiation and necropsy was performed. The necropsy included an examination of the external body surface, all orifices and the cranial, thoracic and abdominal cavities and their contents.

Organ weights: adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, and testes.

Histopathology: Adequate Battery: yes (X), no () explain

Peer review: yes (), no (X)

According to the sponsor, histopathological evaluations were performed on samples of tissues collected at necropsy on Day 29 for the vehicle, control article (d-amphetamine), mid-dose, and high dose (Groups 1, 2, 4, and 5). In addition, histopathological examinations were performed for all samples of all gross lesions, except those for which the diagnosis was judged unnecessary of the outcome of the study by the veterinary pathologist. The following tissues were collected and slides were stained with hematoxylin and eosin:

The following organs and tissues were preserved in 10% neutral buffered formalin with the exception of the Testes which were preserved in Bouin's:

- Unique animal identifier
- Abnormalities (gross)
- Adrenal glands
- Aorta
- Brain
- Cecum
- Cervix
- Colon
- Duodenum
- Epididymides
- Esophagus
- Exorbital lachrymal gland
- Eyes with optic nerve
- Femur – including articular surface
- Heart
- Ileum
- Jejunum
- Kidneys
- Liver
- Lungs with mainstem bronchus
- Mammary glands
- Mandibular lymph nodes
- Mesenteric lymph nodes
- Ovaries
- Pancreas
- Pituitary gland
- Prostate
- Rectum
- Salivary glands
- Sciatic nerve
- Seminal vesicles
- Skin
- Spinal cord :cervical, midthoracic and lumbar
- Spleen
- Sternum with bone marrow
- Stomach
- Testes
- Thigh muscle (biceps formis)
- Thymus
- Thyroids with parathyroids
- Tongue
- Trachea
- Urinary bladder
- Uterus
- Vagina

Results:

The following table was prepared by the reviewer to capture the significant findings with NRP-104 and d-amphetamine treatment:

| Study | Dose (mg/kg/day) | Responses |
|-------------------|---------------------------------|--|
| 28-days rat study | 0 | |
| | 20 (NRP-104) | <u>Clinical signs</u> : ↑ activity (90% of animals throughout study), post dose jumping (2/10F on day 2) |
| | 40 (NRP-104) | <u>Clinical signs</u> : ↑ activity (all animals throughout), post dose jumping (1-2/10 for both M&F on few days). <u>Body wt</u> : ↓ in body wt in M (11% compared to control). <u>Clinical chemistry</u> : ↑ in ALT (34% M, 45% F). <u>Histopath</u> : fiber necrosis and degeneration of the biceps of thigh muscle (1/15 M, minimal focal) |
| | 80 (NRP-104) | <u>Mortality</u> : 1/9 F from TK group was moribund sacrificed (day 7 due to self mutilation). <u>Clinical signs</u> : ↑ activity (all animals throughout). Post dose jumping (1-3/15 for M & 1-8/15 in F on few days), Self mutilation in 1-3/15 M and 1-7/15 F almost throughout the study . Thin body condition 1-4/15 M starting on day 10 to the end of the study for most with 1-3/5 M continued to be seen for the first few days of the recovery period and 1-4/15F seen starting on day 10 and to the end of the study for most with 1-2/5 F seen through the first few days of the recovery period. <u>Body wt</u> : ↓ body wt in M (20% compared to control) and in F (8%) relative to control. <u>Clinical chemistry</u> : ↑ glucose (F only, 18%), ↑ BUN (19% M, 16% F), ↑ ALT (34% M, 45% F). <u>Histopath</u> : Degeneration of muscular tunic in the esophagus (2/15 M; 1 minimal multifocal & 1 mild focal; 2/15 F; 1 mild focal & 1 minimal focal) according to sponsor due to gavage. The fact that it was seen in this group only was described as “anomalous” . Fiber necrosis and degeneration of the biceps of thigh muscle (1/15 F minimal multifocal). |
| | 16 (amphetamine sulfate) | <u>Clinical signs</u> : increased activity (all animals throughout the study), thin body condition 1/15F starting on day 17 to the end of the study. <u>Body wt</u> : ↓ in bd wt (4-6% in M only, between days 8-22 but an increase in bd wt was seen towards the end of the study). |

Ophthalmoscopy: no test related findings.

EKG: not performed.

Urinalysis: not performed

Gross pathology: no drug related findings.

Organ weights: in M decreases in absolute wt of several organs were observed in treated animals treated with NRP-104 especially at MD and HD (heart, kidney, liver, spleen and testes). However, increased brain, kidney-, and testes-to-body wt ratio were observed. Similar trends were observed with d-amphetamine. In F the HD group had decreased kidney and spleen absolute weights, increased brain-, heart-, kidney-, liver-, and ovary-to-body wt ratios. Similar trends were observed for the MD as well as the d-amphetamine treated group. In F, liver to body wt ratio was increased in all animals treated with NRP-104.

Toxicokinetics: on Days 1 and 28, whole blood samples were collected from each group of animals (9/sex/group) at the following timepoints: predose, 1h, 2h, 4h, 6h, 8h, 12h, 16h, and 24h post dose (3 animals/sex/group/time point alternating at specified timepoints). Blood was collected by retroorbital sinus puncture.

Results:

- Plasma Cmax for d-amphetamine levels after treatment with both NRP-104 and d-amphetamine was reached within 1-2 h except for HD of NRP-104 where it was reached at 2-6 h.
- In both sexes, there was a dose dependent increase in AUC of NRP-104 that was dose proportional from 20 to 40 mg/kg/day and more than dose proportional from 40-80 mg/kg/day. Similarly the AUC of the metabolite (d-amphetamine) increased in a similar pattern.
- There was no gender difference in Cmax and AUC of NRP-104, except at 80 mg/kg/day dose, where the levels (both Cmax and AUC) were higher in F than in M. However, gender differences were observed in Cmax and AUC of the metabolite (d-amphetamine) where the levels (both Cmax and AUC) were greater in F than in M at all doses.
- Accumulation with dosing was observed between Day 1 and Day 28 for both NRP-104 (in all groups) and the metabolite (d-amphetamine) (especially at MD and HD).
- In both M and F $t_{1/2}$ of lysine-amphetamine was generally lower than $t_{1/2}$ of d-amphetamine.
- According to the sponsor, “if converted to the molar equivalent doses (calculation not shown), by comparison, the exposure of d-amphetamine in the test animals after dosing with NRP-104 is very similar to the exposure of d-amphetamine after dosing with lysine-amphetamine”.
- According the sponsor’s calculations, a dose of 16 mg/kg/day of d-amphetamine is equivalent to 11.6×10^4 nmol/kg and NRP-1-4 at a dose level of 40 mg/kg/day is equivalent to 15.2 nmol/kg. The sponsor stated that “therefore, the comparison indicating levels of exposure to d-amphetamine in the test animals, was a rough estimation”.

The following tables summarize the toxicokinetic parameters of d-amphetamine and lysine amphetamine in rats in this study as provided by the sponsor (page 429-430, vol. 6 module 4, Sequence 1):

TABLE 20. Toxicokinetic Parameters of d-Amphetamine and Lysine-Amphetamine in Male Rats for Days 1 and 28 Following Once Daily Oral Administration of Test Articles

Client Study No.: 0436RN29.002

Project No.: 35-0402PK

Day 1, Males

| d-Amphetamine | | | | | | | |
|---------------|-------------------|----------------|--------------------------|----------------------|----------------------|----------------------------------|--------------------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | t _{1/2} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) |
| 6 | d-Amphetamine | 16 | 315.0 | 2.0 | 3.2 | 1341.9 | 1356.9 |
| 7 | NRP104 | 20 | 126.3 | 2.0 | 2.8 | 722.9 | 722.9 |
| 8 | NRP104 | 40 | 164.3 | 1.0 | 4.2 | 1299.8 | 1326.7 |
| 9 | NRP104 | 80 | 460.4 | 4.0 | 8.3 | 3377.7 | 3976.9 |

| Lysine-Amphetamine | | | | | | | |
|--------------------|-------------------|----------------|--------------------------|----------------------|----------------------|----------------------------------|--------------------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | t _{1/2} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) |
| 6 | d-Amphetamine | 16 | NC | NC | NC | NC | NC |
| 7 | NRP104 | 20 | 60.7 | 1.0 | 1.4 | 106.7 | 106.8 |
| 8 | NRP104 | 40 | 140.8 | 1.0 | 2.5 | 255.4 | 268.3 |
| 9 | NRP104 | 80 | 130.0 | 1.0 | 3.0 | 674.1 | 698.4 |

NC: Not calculated due to insufficient data for PK calculation.

Day 28, Males

| d-Amphetamine | | | | | | |
|---------------|-------------------|----------------|--------------------------|----------------------|----------------------|----------------------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | t _{1/2} (h) | AUC _(0-24h) (ng·h/mL) |
| 6 | d-Amphetamine | 16 | 424.1 | 1.0 | 4.9 | 1748.5 |
| 7 | NRP104 | 20 | 170.3 | 1.0 | 3.9 | 882.7 |
| 8 | NRP104 | 40 | 332.4 | 1.0 | 4.0 | 2023.3 |
| 9 | NRP104 | 80 | 669.0 | 2.0 | 7.8 | 6327.5 |

| Lysine-Amphetamine | | | | | | |
|--------------------|-------------------|----------------|--------------------------|----------------------|----------------------|----------------------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | t _{1/2} (h) | AUC _(0-24h) (ng·h/mL) |
| 6 | d-Amphetamine | 16 | NC | NC | NC | NC |
| 7 | NRP104 | 20 | 76.3 | 1.0 | 4.0 | 168.9 |
| 8 | NRP104 | 40 | 166.3 | 1.0 | 4.4 | 403.1 |
| 9 | NRP104 | 80 | 243.6 | 1.0 | 3.2 | 1044.6 |

NC: Not calculated due to insufficient data for PK calculation.

TABLE 21. Toxicokinetic Parameters of d-Amphetamine and Lysine-Amphetamine in Female Rats for Days 1 and 28 Following Once Daily Oral Administration of Test Articles

Client Study No.: 0436RN29.002

Project No.: 35-0402PK

Day 1, Females

| d-Amphetamine | | | | | | | |
|---------------|-------------------|----------------|--------------------------|----------------------|----------------------|----------------------------------|--------------------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | t _{1/2} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) |
| 6 | d-Amphetamine | 16 | 455.1 | 1.0 | 4.1 | 2002.3 | 2039.9 |
| 7 | NRP104 | 20 | 218.0 | 2.0 | 4.2 | 1090.6 | 1120.4 |
| 8 | NRP104 | 40 | 338.5 | 2.0 | 4.5 | 1991.8 | 2046.9 |
| 9 | NRP104 | 80 | 1083.2 | 6.0 | 2.7 | 6609.9 | 7096.5 |

| Lysine-Amphetamine | | | | | | | |
|--------------------|-------------------|----------------|--------------------------|----------------------|----------------------|----------------------------------|--------------------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | t _{1/2} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) |
| 6 | d-Amphetamine | 16 | NC | NC | NC | NC | NC |
| 7 | NRP104 | 20 | 47.5 | 1.0 | 0.5 | 99.6 | 99.5 |
| 8 | NRP104 | 40 | 117.5 | 1.0 | 3.3 | 254.4 | 255.5 |
| 9 | NRP104 | 80 | 269.5 | 2.0 | 1.6 | 1296.0 | 1293.5 |

NC: Not calculated due to insufficient data for PK calculation.

Day 28, Females

| d-Amphetamine | | | | | | |
|---------------|-------------------|----------------|--------------------------|----------------------|----------------------|----------------------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | t _{1/2} (h) | AUC _(0-24h) (ng·h/mL) |
| 6 | d-Amphetamine | 16 | 438.7 | 2.0 | 5.6 | 2619.0 |
| 7 | NRP104 | 20 | 235.0 | 1.0 | 4.1 | 1232.3 |
| 8 | NRP104 | 40 | 479.0 | 1.0 | 6.4 | 2818.2 |
| 9 | NRP104 | 80 | 1282.8 | 4.0 | 6.0 | 9174.5 |

| Lysine-Amphetamine | | | | | | |
|--------------------|-------------------|----------------|--------------------------|----------------------|----------------------|----------------------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | t _{1/2} (h) | AUC _(0-24h) (ng·h/mL) |
| 6 | d-Amphetamine | 16 | NC | NC | NC | NC |
| 7 | NRP104 | 20 | 90.7 | 1.0 | 4.3 | 199.6 |
| 8 | NRP104 | 40 | 201.3 | 1.0 | 6.0 | 400.5 |
| 9 | NRP104 | 80 | 389.8 | 1.0 | 4.9 | 1362.5 |

NC: Not calculated due to insufficient data for PK calculation.

Dogs:

Study title: 7-Day dose-range-finding oral toxicity study

Key study findings: No deaths were observed. Increased activity was observed at all doses (seen only on few days at LD) and repetitive behavior, restlessness, vessels over sclera dilated at MD and HD and severe ocular discharge at HD (all seen only on Day 1). Decreased body wt was observed at HD. Decreases in reticulocytes at MD and HD.

Study no.: 0440DN29.001

Volume #, and page #: vol. 8, page 1 (Module 4, Sequence 1)

Conducting laboratory and location: -----

| |
|-------|
| ----- |
| ----- |
| ----- |
| ----- |

Date of study initiation: November 19, 2003

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: NRP-104 (ALB 17936-4), Lot BJP-I-152(1), according to the certificate of analysis % by HPLC (% peak area).

Methods

Doses: 0, 3, 6, and 12 mg/kg

Species/strain: beagle dogs

Number/sex/group or time point (main study): 1/sex/group

Route, formulation, volume, and infusion rate: oral by gavage, solution, 10 ml/kg

Satellite groups used for toxicokinetics or recovery: none

Age: 5-6 months

Weight: 7.7 to 9.3 mg/kg for both M & F

Sampling times: animals were dosed daily for 7 days. For toxicokinetic portion of the study, whole blood samples were collected on Days 1, 3, 4, 5, 6, and 7.

Unique study design or methodology (if any): no

Mortality: animals were observed twice daily.

Clinical signs: animals were observed prior to dose administration, at ~1h post-dose and additionally as appropriate.

Body weights: at time of randomization, prior to dosing on Day 1 and following the final dose administration on Day 7. A fasted body wt was recorded prior to sacrifice on Day 8.

Food consumption: daily.

Ophthalmoscopy: not performed.

EKG: not performed.

Hematology: blood was collected from the jugular vein prior to treatment initiation and on the day of experimental termination (Day 8) for hematology, serum clinical chemistry, and coagulation profiles. Animals were fasted prior to blood collection. The following parameters were evaluated: differential white blood cell count (Diff), hematocrit (HCT),

hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet count (PLT), red blood cell count and morphology (RBC), white blood cell count (WBC) and reticulocyte count (Retic). Coagulation parameters including prothrombin time (PT) and activated partial thromboplastin time (APTT).

Clinical chemistry: for blood collection see previous section. The following parameters were evaluated:

| | |
|--|-------------------------------|
| <i>Serum Clinical Chemistry:</i> | |
| • Alanine Aminotransferase (ALT) | • Globulin (calculated)(GLOB) |
| • Albumin (ALB) | • Glucose (GLU) |
| • Albumin/Globulin ratio (calculated)(A/G) | • Phosphorus (PHOS) |
| • Alkaline Phosphatase (ALP) | • Potassium (K) |
| • Aspartate Aminotransferase (AST) | • Sodium (NA) |
| • Calcium (CA) | • Total Bilirubin (T-BIL) |
| • Chloride (CL) | • Total Protein (TP) |
| • Cholesterol (CHOL) | • Triglycerides (TRIG) |
| • Creatinine (CREAT) | • Urea Nitrogen (BUN) |

Urinalysis: not performed.

Gross pathology: gross necropsies included examination of the external body surface, all orifices and the cranial, thoracic and abdominal cavities and their contents.

Organ weights: adrenals, brain, heart, kidneys, liver, ovaries, spleen, and testes.

Histopathology: Adequate Battery: yes (), no (X)—explain: tissues were collected and retained for possible evaluation “in the future”.

Peer review: yes (), no ()

The following tissues were preserved in 10% neutral buffered formalin (table provided by the sponsor on page 16, vol. 8, Module 4, Sequence 1):

The tissues from all dogs were preserved in 10% neutral buffered formalin:

- Unique animal identifier
- Abnormalities (gross)
- Adrenal glands
- Aorta
- Brain
- Cecum
- Colon
- Cervix
- Duodenum
- Epididymides
- Esophagus
- Lachrymal gland
- Eyes with optic nerve
- Femur including articular surface
- Gall bladder
- Heart
- Ileum
- Jejunum
- Kidney
- Liver
- Lung with mainstem bronchus
- Mammary glands
- Mandibular lymph nodes
- Mesenteric lymph nodes
- Ovaries
- Pancreas
- Pituitary gland
- Prostate
- Rectum
- Salivary glands
- Sciatic nerve
- Skin
- Spinal cord: cervical, midthoracic, lumbar
- Spleen
- Sternum with bone marrow
- Stomach
- Testes
- Thymus
- Thyroids with parathyroids
- Tongue
- Thigh muscle (biceps femoris)
- Trachea
- Urinary bladder
- Uterus
- Vagina

Results

The following table was prepared by the reviewer to summarize the finding of this study:

| Study | Dose (mg/kg/day) | Responses |
|-------|------------------|--|
| 7-day | 0 | |
| | 3 | <u>Clinical signs: ↑ activity on few days</u> |
| | 6 | <u>Clinical signs: ↑ activity, ↑ salivation, repetitive behavior, restlessness, vessels over sclera dilated, all seen on day 1, but only ↑ activity continued to be seen to the end of the study (↑ activity was seen ~8h after dosing on day 1 but was observed at 1h post dosing after day 3). Hematology: ↓ in reticulocytes (84% in M, 89% in F)</u> |
| | 12 | <u>Clinical signs: ↑ activity, ↑ salivation, repetitive behavior, restlessness, vessels over sclera dilated and sever ocular discharge, all seen on day 1, but only ↑ activity continued to be seen to the end of the study (↑ activity was seen ~8h after dosing on day 1 but was observed at 1h post dosing after day 3). Abrasion on the chest (M). Body wt: ↓ in body wt (6% M, 12% F) relative to control. Hematology: ↓ in reticulocytes (84% M, 89% F). Gross pathology: fluid in the cranial cavity, small spleen and dark red lobes of the lung (M only). NO HISTOPATHOLOGY WAS CONDUCTED.</u> |

Organ weights: a decrease in the wt of the heart of the M at MD and HD (~30% compared to the control) was observed. However, it should be noted that the body wt of the control M was slightly higher than those in the MD and HD groups and thus the heart wt relative the body wt was not significantly decreased from that of the control. Changes in other organs were seen but were inconsistent and did not show a dose response pattern. The sponsor did not consider the changes in organ weights as drug related.

Toxicokinetics: blood was collected from animals according to the following schedule as summarized by the sponsor in the following table (page 213, vol. 8, sequence 1, module 4):

Table 1. Dosing and Blood Collection

| Group | Treatment | No. of Dogs | | Dose mg/kg/day | Dose Volume mL/kg/day | Blood Collection Times ^a |
|-------|-----------|-------------|--------|----------------|-----------------------|---|
| | | Male | Female | | | |
| 2 | NRP104 | 1 | 1 | 3 | 10 | Predose, 1, 2, 4, 8, and 24 hours |
| 3 | NRP104 | 1 | 1 | 6 | 10 | postdose on Day 1; predose, 0.5, 1, 2, |
| 4 | NRP104 | 1 | 1 | 12 | 10 | 4, 6, 8, and 24 hours postdose on Day 7; and predose on Days 3, 4, 5 and 6. |

^a Blood (≈ 1 mL/time point) was collected from dog by jugular vein puncture into tubes containing sodium heparin as anticoagulant.

Results:

The following can be summarized from the data:

- C_{max} of lysine amphetamine was observed at the first sampling time point at all dose levels (1h on Day 1 and 0.5h at Day 7)
- C_{max} and AUC of lysine amphetamine increased approximately proportionally with increasing dose from 3 to 12 mg/kg/day in both genders
- No gender difference was observed.
- Lysine amphetamine AUC values were lower than d-amphetamine AUC values in both M & F in each group and on both days. Elimination half life ($t_{1/2}$) of lysine-amphetamine were generally lower than $t_{1/2}$ of d-amphetamine ($t_{1/2}$ for lysine-amphetamine was 0.4-4h and that of d-amphetamine ranged from 3.3 to 5.1h).

The following tables were provided by the sponsor to summarize the findings (pages 214-215, vol. 8, Module 4, Sequence 1):

Table 2. Individual Toxicokinetic Parameters of d-Amphetamine in Male and Female Dogs on Days 1 and 7 Following Once Daily Oral Administration of Lysine-Amphetamine for 7-Consecutive Days

Day 1

| Male | | | | | | | |
|--------|-------------------|-----------------------------|----------------------|-------------------------------------|-----------------------------------|----------------------|-------------|
| Group# | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | No. of Dogs |
| 2 | | | | | | | 1 |
| 3 | | | | | | | 1 |
| 4 | | | | | | | 1 |

Female

| Group# | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | No. of Dogs |
|--------|-------------------|-----------------------------|----------------------|-------------------------------------|-----------------------------------|----------------------|-------------|
| 2 | | | | | | | 1 |
| 3 | | | | | | | 1 |
| 4 | | | | | | | 1 |

Day 7

| Male | | | | | | |
|--------|-------------------|-----------------------------|----------------------|-------------------------------------|----------------------|-------------|
| Group# | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | t _{1/2} (h) | No. of Dogs |
| 2 | | | | | | 1 |
| 3 | | | | | | 1 |
| 4 | | | | | | 1 |

Female

| Group# | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | t _{1/2} (h) | No. of Dogs |
|--------|-------------------|-----------------------------|----------------------|-------------------------------------|----------------------|-------------|
| 2 | | | | | | 1 |
| 3 | | | | | | 1 |
| 4 | | | | | | 1 |

NC: Not Calculated

Table 3. Individual Toxicokinetic Parameters of Lysine-Amphetamine in Male and Female Dogs on Days 1 and 7 Following Once Daily Oral Administration of Lysine-Amphetamine for 7-Consecutive Days

Day 1

| Male | | | | | | | |
|--------|-------------------|-----------------------------|----------------------|-------------------------------------|-----------------------------------|----------------------|-------------|
| Group# | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | No. of Dogs |
| 2 | | | | | | | 1 |
| 3 | | | | | | | 1 |
| 4 | | | | | | | 1 |
| Female | | | | | | | |
| Group# | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-∞) (ng·h/mL) | t _{1/2} (h) | No. of Dogs |
| 2 | | | | | | | 1 |
| 3 | | | | | | | 1 |
| 4 | | | | | | | 1 |

Day 7

| Male | | | | | | |
|--------|-------------------|-----------------------------|----------------------|-------------------------------------|----------------------|-------------|
| Group# | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | t _{1/2} (h) | No. of Dogs |
| 2 | | | | | | 1 |
| 3 | | | | | | 1 |
| 4 | | | | | | 1 |
| Female | | | | | | |
| Group# | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | t _{1/2} (h) | No. of Dogs |
| 2 | | | | | | 1 |
| 3 | | | | | | 1 |
| 4 | | | | | | 1 |

ND: Not Determined

Study title: 28-day oral toxicity study in dogs with NRP-104

Key study findings: see table within review for summary

Study no.: 0436DN29.001

Volume #, and page #: vol. 9, Module 4, Sequence 1

Conducting laboratory and location: -----

| |
|-------|
| ----- |
| ----- |
| ----- |
| ----- |

Date of study initiation: December 18, 2003

GLP compliance: yes

QA report: yes (X) no ()

Drug, lot #, and % purity: NRP104 (NRP104-lys Dex Amide), purity of -----% by HPLC. For d-amphetamine the batch # was 043K0803

Methods

Doses: 0, 3, 6, and 12 mg/kg/day of NRP-104 and a group was treated with d-amphetamine at a dose of 2.4 mg/kg/day

Species/strain: beagle dogs

Number/sex/group or time point (main study): 3/sex/group except for the control, HD NRP104 group and the d-amphetamine group where 5/sex/group were used so that 2/sex/group from these groups could be used for a 14-day recovery group

Route, formulation, volume, and infusion rate: orally by gavage, solution, vol. 10 mg/kg

Satellite groups used for toxicokinetics or recovery: 2/sex/group were used from control, HD NRP104 and d-amphetamine as a recovery group

Age: ~5 months

Weight: 6-8.4 kg for M and 4.9-7.2 kg for F

Sampling times: blood samples for the toxicokinetic measurements were conducted on Days 1 and 28

Unique study design or methodology (if any): no

Observations and times:

Mortality: at minimum twice daily (some deviation in the observation times were reported but did not seem to be significant to affect the outcome of the study).

Clinical signs: daily prior to dosing and at ~1h after dosing. During recovery phase animals were observed once daily.

Body weights: at the time of randomization/selection, prior to dosing on Days 1, 8, 15, 22 and following the final dose administration on Day 28. Recovery animals were weighed on Days 35 and 42. A fasted body wt was recorded prior to sacrifice on Day 29 or Day 43.

Food consumption: daily.

Ophthalmoscopy: before treatment initiation and during the final week of treatment. A dilating agent was used and the exam was performed by a veterinary pathologist. Examinations to the recovery group were “inadvertently not performed” but since there were no findings were observed in the main study animals this deviation was considered with no impact on the outcome of the study.

EKG: According to the sponsor ECGs were obtained from all animals using lateral recumbency. Recordings were made using limb leads I, II, II, VR, aVL and aVF and two chest leads V10 and RV2. Three leads were monitored simultaneously and a rhythm strip with two chest leads were obtained at the appropriate time intervals. ECGs were obtained from all animals prior to treatment initiation and during the final week of treatment. The sponsor did not indicate when relative to the dosing time the ECGs were obtained.

According to the sponsor ECGs were inadvertently not obtained prior to recovery sacrifice. However, as there were no findings prior to the terminal sacrifice, this deviation had no impact on the outcome of the study.

Hematology: whole blood samples were collected for hematology, coagulation profile and clinical chemistry prior to treatment initiation, on Day 29 and all recovery animals prior to sacrifice on Day 43. All blood samples were collected from the jugular vein with animals being fasted over night prior to blood collection. The following parameters were evaluated: differential white blood cell count (Diff), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet count (PLT), red blood cell count and morphology (RBC), white blood cell count (WBC) and reticulocyte count (Retic). Coagulation parameters including prothrombin time (PT) and activated partial thromboplastin time (APTT).

Clinical chemistry: see previous section for blood collection. The following parameters were evaluated:

Serum Clinical Chemistry:

- | | |
|--|-------------------------------|
| • Alanine Aminotransferase (ALT) | • Globulin (calculated)(GLOB) |
| • Albumin (ALB) | • Glucose (GLU) |
| • Albumin/Globulin ratio (calculated)(A/G) | • Phosphorus (PHOS) |
| • Alkaline Phosphatase (ALP) | • Potassium (K) |
| • Aspartate Aminotransferase (AST) | • Sodium (NA) |
| • Calcium (CA) | • Total Bilirubin (T-BIL) |
| • Chloride (CL) | • Total Protein (TP) |
| • Cholesterol (CHOL) | • Triglycerides (TRIG) |
| • Creatinine (CREAT) | • Urea Nitrogen (BUN) |

Urinalysis: not performed.

Gross pathology: animals were sacrificed on Days 29 or 43 and necropsy was performed. The necropsy included an examination of the external body surface, all orifices and the cranial, thoracic and abdominal cavities and their contents.

Organ weights: organ weights were expressed as absolute and relative to body and brain wt values. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, spleen, and testes.

Histopathology: Adequate Battery: yes (X), no ()—explain

Peer review: yes (), no (X)

Histopathological evaluations were performed on samples of tissues collected at necropsy. In addition, according to the sponsor, histopathological examinations were performed for samples of all gross lesions, except those for which the diagnosis was judged unnecessary for the outcome of the study by the veterinary pathologist. The following tissues were evaluated:

- | | |
|---------------------------------------|--|
| • Unique animal identifier | • Mesenteric lymph nodes |
| • Abnormalities (gross) | • Ovaries |
| • Adrenal glands | • Pancreas |
| • Aorta | • Pituitary gland |
| • Brain | • Prostate |
| • Cecum | • Rectum |
| • Cervix | • Salivary glands |
| • Colon | • Sciatic nerve |
| • Duodenum | • Skin |
| • Epididymides | • Spinal cord :cervical, midthoracic and lumbar |
| • Esophagus | • Spleen |
| • Lachrymal gland | • Sternum with bone marrow |
| • Eyes with optic nerve | • Stomach |
| • Femur – including articular surface | • Testes |
| • Gall bladder | • Thigh muscle (biceps formis) |
| • Heart | • Thymus |
| • Ileum | • Thyroids with parathyroids |
| • Jejunum | • Tongue |
| • Kidney | • Trachea |
| • Liver | • Urinary bladder |
| • Lung with mainstem bronchus | • Uterus |
| • Mammary glands | • Vagina |
| • Mandibular lymph nodes | |

Results

The following table was prepared by the reviewer summarizing data from this study:

| Study | Dose (mg/kg/day) | Responses |
|-------------------------------------|---------------------|--|
| 28-day study with a 2-week recovery | 0 | |
| | 3 (NRP-104) | <u>Clinical signs:</u> <u>restlessness</u> (1-2/3 M&F, on several days), <u>↑ activity</u> (1-2 M&F, more than several days), <u>pacing in cage</u> (1-2 M&F, few occasions), <u>↓ in reticulocytes</u> (70% in F) |
| | 6 (NRP-104) | <u>Clinical signs:</u> <u>restlessness</u> (2-3/3 M&F, almost throughout study), <u>↑ activity</u> (2-3/3M, 3/3F, throughout study), <u>repetitive behavior</u> (1/3 M on day 1 only), <u>head shaking</u> (1/3M & 1-2/3F on few occasions), <u>pacing in cage</u> (1-3/3 M&F several days), <u>decreased activity predose</u> (1-3/3M on few occasions and 1-3/3 F on several occasions). <u>Panting</u> (1-3M & F on several days). <u>Body wts:</u> <u>↓ body wt</u> (19% M, 7% F compared to control). <u>Hematology:</u> <u>↓ in reticulocytes</u> (60% F) |
| | 12 (NRP-104) | <u>Clinical signs:</u> <u>restlessness</u> (all animals, almost throughout study), <u>↑ activity</u> (all animals throughout study), <u>repetitive behavior</u> (4/5 M & 5/5 F on day 1 only) <u>head shaking</u> (1-3/5 M & 1-4/5 F on several occasions), <u>circling</u> (1-3/5 M&F on several occasions), <u>pacing in cage</u> (1-3/5M & 1-4/5 F almost throughout study), <u>Abnormal gait</u> (1-2/5 M&F on several occasions), <u>decreased activity predose</u> (3-5/5 M towards the end of study and 4-5/5 F almost throughout study), <u>panting</u> (1-5/5 M & F almost throughout study). <u>Post dose emesis</u> (1/5 M & F) on one occasion. <u>Predose decreased activity</u> (1-5/5 M & F starting on day 10 and continued almost to the end of the study). <u>Thin body condition</u> (2/5 F started on day 8 to the end and 1/5 started on day 22 to the end of study). <u>Body wt:</u> <u>↓ body wt</u> (16% in M and 20% in F compared to control). <u>Hematology:</u> <u>↓ reticulocytes</u> (50% F). <u>Clinical chemistry:</u> <u>↑ BUN</u> (36% F), <u>↑ in Na</u> (4% in F). According to the sponsor it these changes were within the historical control range (no HC data were provided), no urinalysis was done. <u>Histopathology:</u> No drug related findings. <u>Recovery group:</u> 1HDF had thin body condition through day 35, decreased activity in 1HDM on day 30, and emesis in 1HDM on day 35. The decrease in body wt at the end of recovery period was 15% compared to control in M (statistically significant). |

| | | |
|--|---------------------------------|--|
| | 2.4 mg/kg (amphetamine sulfate) | <p><u>Clinical signs:</u> restlessness (all animals almost throughout the study), ↑ activity (all animals throughout the study), head shaking (2-5/5 M&F on several occasions), repetitive behavior (5/5 M and 4/5 F on day 1 only), circling (1-2/5 M & 1-2/5 F on several days), pacing in cage (2-5/5 M & 2-5/5 F on several days), abnormal gait (1-2/5 M & 1-3/5 F on few days), panting (1-5 M & F almost throughout the study for some and on several occasions for others), post dose emesis (1-2/5 M on a couple of occasions & 1/5 F on one occasion), predose decreased activity (1/5 M on one day & 1/5 F on 3 days), thin body condition (1/5 M reported on day 26 to the end of study & 3/5 F, one started from day 5, one from day 17 and one from day 22 to the end of the study). <u>Body wt:</u> ↓ body wt (21% in M compared to control by the end of study and 26% in F by the end of study. <u>Recovery group:</u> decrease in body wt in M seen at the end of the recovery period was 14% which was statistically significant from control while a similar decrease in F at the time was not statistically significant)</p> |
|--|---------------------------------|--|

Ophthalmoscopy:

EKG:

Urinalysis: not performed

Gross pathology: no drug related findings

Organ weights: a slight increase in ratio of liver wt to body wt in M (~20% compared to control) with a similar finding seen in the d-amphetamine treated M. This increase was not clearly seen in F.

Toxicokinetics:

The following tables summarizing the toxicokinetic findings in this study as provided by the sponsor (page 455 & 456, vol. 10, Module 4, Sequence 4) are attached here:

TABLE 20. Summary of Mean Toxicokinetic Parameters of d-Amphetamine and Lysine-Amphetamine in Male Dogs for Days 1 and 28 Following Once Daily Oral Administration of d-Amphetamine or NRP104 (Lysine-Amphetamine)

Study No.: 0436DN29.001

Project No.: 35-0401PK

Day 1, Males

| | | d-Amphetamine | | | | | |
|--------|-------------------|-------------------|-----------------------------|----------------------|-------------------------------------|-------------------------------------|----------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-inf) (ng·h/mL) | t _{1/2} (h) |
| 2 | d-Amphetamine | 4 | 433 | 1.6 | 3053 | 3089 | 3.4 |
| 3 | NRP104 | 3 | 96.4 | 2.0 | 762 | 775 | 3.8 |
| 4 | NRP104 | 10 | 292 | 2.0 | 2362 | 2383 | 3.1 |
| 5 | NRP104 | 15 | 471 | 2.8 | 4512 | 4574 | 3.5 |

| | | Lysine-Amphetamine | | | | | |
|--------|-------------------|--------------------|-----------------------------|----------------------|-------------------------------------|-------------------------------------|----------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-inf) (ng·h/mL) | t _{1/2} (h) |
| 2 | d-Amphetamine | 4 | NC | NC | NC | NC | NC |
| 3 | NRP104 | 3 | 222 | 1.0 | 370 | 390 | 0.8 |
| 4 | NRP104 | 10 | 715 | 1.0 | 1024 | 1027 | 2.3 |
| 5 | NRP104 | 15 | 976 | 1.0 | 1472 | 1481 | 3.9 |

NC: Not Calculated; insufficient data to perform PK analysis

Day 28, Males

| | | d-Amphetamine | | | | |
|--------|-------------------|-------------------|-----------------------------|----------------------|-------------------------------------|----------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | t _{1/2} (h) |
| 2 | d-Amphetamine | 4 | 232 | 1.2 | 1162 | 2.6 |
| 3 | NRP104 | 3 | 93.4 | 2.0 | 626 | 4.0 |
| 4 | NRP104 | 10 | 198 | 2.0 | 1362 | 3.3 |
| 5 | NRP104 | 15 | 368 | 2.0 | 2175 | 3.3 |

| | | Lysine-Amphetamine | | | | |
|--------|-------------------|--------------------|-----------------------------|----------------------|-------------------------------------|----------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | t _{1/2} (h) |
| 2 | d-Amphetamine | 4 | NC | NC | NC | NC |
| 3 | NRP104 | 3 | 178 | 1.0 | 339 | 0.6 |
| 4 | NRP104 | 10 | 329 | 1.0 | 426 | 6.8 |
| 5 | NRP104 | 15 | 658 | 1.0 | 1220 | 3.5 |

NC: Not Calculated; insufficient data to perform PK analysis

TABLE 21. Summary of Mean Toxicokinetic Parameters of d-Amphetamine and Lysine-Amphetamine in Female Dogs for Days 1 and 28 Following Once Daily Oral Administration of d-Amphetamine or NRP104 (Lysine-Amphetamine)

Study No.: 0436DN29.001

Project No.: 35-0401PK

Day 1, Females

| d-Amphetamine | | | | | | | |
|---------------|-------------------|-------------------|-----------------------------|----------------------|-------------------------------------|-------------------------------------|----------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-inf) (ng·h/mL) | t _{1/2} (h) |
| 2 | d-Amphetamine | 4 | 343 | 2.8 | 2640 | 2667 | 3.3 |
| 3 | NRP104 | 3 | 72.4 | 2.0 | 527 | 532 | 3.4 |
| 4 | NRP104 | 10 | 294 | 2.7 | 2543 | 2569 | 3.3 |
| 5 | NRP104 | 15 | 461 | 2.6 | 4124 | 4174 | 3.3 |

| Lysine-Amphetamine | | | | | | | |
|--------------------|-------------------|-------------------|-----------------------------|----------------------|-------------------------------------|-------------------------------------|----------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | AUC _(0-inf) (ng·h/mL) | t _{1/2} (h) |
| 2 | d-Amphetamine | 4 | NC | NC | NC | NC | NC |
| 3 | NRP104 | 3 | 154 | 1.0 | 273 | 285 | 0.6 |
| 4 | NRP104 | 10 | 738 | 1.0 | 1023 | 1023 | 2.0 |
| 5 | NRP104 | 15 | 765 | 1.0 | 1150 | 1155 | 4.6 |

NC: Not Calculated; insufficient data to perform PK analysis.

Day 28, Females

| d-Amphetamine | | | | | | |
|---------------|-------------------|-------------------|-----------------------------|----------------------|-------------------------------------|----------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | t _{1/2} (h) |
| 2 | d-Amphetamine | 4 | 199 | 1.6 | 1276 | 2.9 |
| 3 | NRP104 | 3 | 113 | 1.7 | 702 | 3.2 |
| 4 | NRP104 | 10 | 174 | 2.0 | 1188 | 3.4 |
| 5 | NRP104 | 15 | 314 | 2.4 | 2158 | 3.2 |

| Lysine-Amphetamine | | | | | | |
|--------------------|-------------------|-------------------|-----------------------------|----------------------|-------------------------------------|----------------------|
| Group# | Treatment article | Dose mg/kg/day | C _{max} (ng/mL) | t _{max} (h) | AUC _(0-24h) (ng·h/mL) | t _{1/2} (h) |
| 2 | d-Amphetamine | 4 | NC | NC | NC | NC |
| 3 | NRP104 | 3 | 94.1 | 1.0 | 140 | 0.5 |
| 4 | NRP104 | 10 | 316 | 1.0 | 438 | 1.0 |
| 5 | NRP104 | 15 | 604 | 1.0 | 925 | 4.4 |

NC: Not Calculated; insufficient data to perform PK analysis.

The following summarize the toxicokinetic findings:

- When dosed with lysine-amphetamine the average lysine-amphetamine concentrations had a t_{max} of 1h, where d-amphetamine had a t_{max} of 1.7 to 2.8h. When dosed with d-amphetamine t_{max} was between 1.2 to 2.8h.
- No gender difference in both NRP-014 levels or amphetamine levels except for HD of NRP where AUC values of NRP-104 were slightly higher in M than in F

- There was no accumulation effect observed. To the contrary, levels of both NRP-104 and the metabolite amphetamine were lower on Day 28 compared to Day 1.
- The $t_{1/2}$ of lysine amphetamine was generally lower than the $t_{1/2}$ values of d-amphetamine ($t_{1/2}$ of lysine amphetamine ranged from 0.5 to 4.6h and the $t_{1/2}$ of d-amphetamine ranged from 2.6 to 4h)
- According to the sponsor, if converted to molar equivalent doses, the exposure (C_{max} and AUC) of d-amphetamine in the test animals after dosing with NRP-104 is very similar to the exposure of d-amphetamine after dosing with d-amphetamine.
- According to the sponsor's calculations, d-amphetamine at a dose level of 4 mg/kg/day is equivalent to 28.9×10^3 nmole/kg, and NRP-104 at a dose level of 10 mg/kg/day is equivalent to 37.9×10^3 nmole/kg. So even though not exactly equivalent but roughly equivalent.

2.6.6.4 Genetic toxicology

Study title: Bacterial reverse mutation assay

Key findings: the results indicate negative genotoxic potential for the compound using the Ames assay

Study no.: Sponsor project # 11- -112503.BTL

Volume #, and page #: 11, page 1

Conducting laboratory and location: -----

Date of study initiation: December 17, 2003

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: NRP-104, Batch 1001D, %

Methods

Strains/species/cell line: Salmonella typhimurium strains TA98, TA100, TA1353, and TA1537 and Escherichia coli strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9

Doses used in definitive study: 75, 200, 600, 1800, and 5000 µg/plate

Basis of dose selection: doses were selected for the definitive study based on results obtained from the preliminary study in which the following doses were tested: 2.5, 7.5, 25, 75, 200, 600, 1800, and 5000 µg/plate

Negative controls: water was selected as the solvent for the test article and used as vehicle control

Positive controls: the following table was provided by the sponsor for the positive controls used in the study (page 47, vol. 11, Module 4, Sequence 1):

COMPARISON WITH THE TEST ARTICLE

| Strain | S9 | Positive Control | Concentration (µg/plate) |
|---------------------------|------|-------------------------|--------------------------|
| <i>Salmonella</i> Strains | Rat | 2-aminoanthracene | 1.0 |
| WP2 <i>uvrA</i> | | | 10 |
| TA98 | None | 2-nitrofluorene | 1.0 |
| TA100, TA1535 | | sodium azide | 1.0 |
| TA1537 | | 9-aminoacridine | 75 |
| WP2 <i>uvrA</i> | | methyl methanesulfonate | 1,000 |

Incubation and sampling times:

The plate incorporation method was used in which the tester strain, the test article, negative control, positive controls, and the S9 mix when applicable were added to molten selective top agar that was then overlaid onto the surface of minimal bottom agar. The solidified plates were then inverted and incubated for 48-72h at 37 ± 2 °C. The plates were then evaluated for revertant colonies either by automated colony counter or entirely by hand.

Results

Study validity:

The preliminary study (Experiment #B1) was performed using duplicate samples with adequate standard deviations, using 8 concentrations and using the adequate tester strains. According to the sponsor there were no contamination observed on the sterility plates for the vehicle control, the test article dilutions and the S9 and sham mixes. The sponsor indicated in the conclusion that the “criteria for a valid study were met as described in the protocol”. (The criteria for a valid study were described in the protocol and they included: tester strain integrity, negative control values within historical control values, appropriate numbers of bacteria are plated which must be equal to or greater than 0.3×10^9 cells per milliliter, positive control values must exhibit at least a 3-fold increase over the respective mean negative control value for each tester strain, and a minimum of

at least 3 non-toxic doses be used). The preliminary study is considered to be adequate and the results indicated that the test article up to a concentration of 5000 µg/plate and using 7 additional lower concentrations was not associated with an indication of positive mutagenic potential. The results of the study are summarized in the following table as provided by the sponsor (Table 21, page 37, vol. 11, Sequence 1, Module 4):

| Bacterial Mutation Assay Summary of Results | | | | | | | | | | |
|---|-------|------------------|-------|-----|--------|----|--------------------|----|----------|----|
| Table 21 | | | | | | | | | | |
| Test Article Id | | : NRP104 | | | | | | | | |
| Study Number | | : AA85UC.503.BTL | | | | | Experiment No : B1 | | | |
| Average Revertants Per Plate ± Standard Deviation | | | | | | | | | | |
| Liver Microsomes: None | | | | | | | | | | |
| Dose (µg/plate) | TA98 | | TA100 | | TA1535 | | TA1537 | | WP2 uvrA | |
| Vehicle | 21 ± | 13 | 230 ± | 13 | 21 ± | 4 | 7 ± | 2 | 24 ± | 4 |
| 2.5 | 20 ± | 6 | 231 ± | 6 | 22 ± | 2 | 6 ± | 1 | 13 ± | 1 |
| 7.5 | 15 ± | 4 | 225 ± | 3 | 23 ± | 1 | 6 ± | 0 | 17 ± | 5 |
| 25 | 17 ± | 3 | 228 ± | 3 | 23 ± | 1 | 4 ± | 1 | 18 ± | 4 |
| 75 | 12 ± | 3 | 225 ± | 3 | 25 ± | 1 | 7 ± | 2 | 15 ± | 2 |
| 200 | 14 ± | 0 | 224 ± | 23 | 25 ± | 1 | 6 ± | 0 | 17 ± | 1 |
| 600 | 21 ± | 0 | 232 ± | 8 | 30 ± | 1 | 6 ± | 1 | 15 ± | 1 |
| 1800 | 19 ± | 4 | 213 ± | 11 | 21 ± | 3 | 8 ± | 1 | 19 ± | 4 |
| 5000 | 20 ± | 2 | 207 ± | 11 | 22 ± | 1 | 4 ± | 1 | 13 ± | 2 |
| Positive | 158 ± | 8 | 691 ± | 2 | 395 ± | 70 | 622 ± | 28 | 92 ± | 14 |
| Liver Microsomes: Rat liver S9 | | | | | | | | | | |
| Dose (µg/plate) | TA98 | | TA100 | | TA1535 | | TA1537 | | WP2 uvrA | |
| Vehicle | 28 ± | 5 | 218 ± | 16 | 16 ± | 5 | 6 ± | 1 | 19 ± | 2 |
| 2.5 | 21 ± | 1 | 238 ± | 1 | 13 ± | 2 | 4 ± | 1 | 16 ± | 4 |
| 7.5 | 19 ± | 4 | 238 ± | 1 | 13 ± | 5 | 5 ± | 1 | 13 ± | 1 |
| 25 | 23 ± | 4 | 235 ± | 6 | 16 ± | 2 | 5 ± | 0 | 20 ± | 2 |
| 75 | 21 ± | 0 | 231 ± | 1 | 14 ± | 0 | 6 ± | 1 | 17 ± | 4 |
| 200 | 18 ± | 1 | 238 ± | 3 | 18 ± | 1 | 5 ± | 1 | 24 ± | 5 |
| 600 | 30 ± | 0 | 237 ± | 2 | 16 ± | 1 | 8 ± | 2 | 17 ± | 1 |
| 1800 | 29 ± | 1 | 237 ± | 0 | 14 ± | 4 | 7 ± | 4 | 19 ± | 0 |
| 5000 | 24 ± | 4 | 233 ± | 1 | 17 ± | 1 | 7 ± | 1 | 22 ± | 5 |
| Positive | 440 ± | 129 | 793 ± | 208 | 196 ± | 0 | 169 ± | 31 | 522 ± | 23 |
| Vehicle = Vehicle Control | | | | | | | | | | |
| Positive = Positive Control (50 µL plating aliquot) | | | | | | | | | | |
| Plating aliquot: 100 µL | | | | | | | | | | |

As for the definitive or confirmatory study, even though triplicate samples were used and the appropriate tester strains were used, there were some technical problems associated with the study. In the first conducted part (Experiment #B2), according to the sponsor an unacceptable vehicle control value was seen with tester strain TA100 in the presence of S9 and therefore this was reevaluated in another experiment (Experiment #B4). In

addition, due to contamination, tester strains TA98 in the presence of S9 activation was retested in another experiment (Experiment # B3). Due to an agar preparation error all plates with the tester strains in the absence of S9 activation were reevaluated in Experiment B3. It should be mentioned also that the concentrations of the dosing solutions were between 71 and 122% of the target and that those used for the part of Study # B2 that were considered adequate (TA1535, TA1537, and WP2 uvrA in the presence of S9 conducted on December 31, 2003) the concentrations used were mostly lower than the nominal concentration (ranged between 71-92% of the nominal with the highest concentration being the lowest value from the nominal). The results of the definitive study are summarized in the following table as provided by the sponsor and the results were put together even though they were conducted in different experiments on different days (table 22, page 38, Sequence 1, Module 4):

| Bacterial Mutation Assay Summary of Results | | | | | | | | | | |
|--|-------------------|------------------|--------------------|----|---------------------|---------------------------|---------------------|----|-----------------------|----|
| Table 22 | | | | | | | | | | |
| Test Article Id | | : NRP104 | | | | Experiment Nos : B2/B3/B4 | | | | |
| Study Number | | : AA85UC.503.BTL | | | | | | | | |
| Average Revertants Per Plate \pm Standard Deviation | | | | | | | | | | |
| Liver Microsomes: None | | | | | | | | | | |
| Dose (μ g/plate) | TA98 ^a | | TA100 ^a | | TA1535 ^a | | TA1537 ^a | | WP2 uvrA ^a | |
| Vehicle | 25 \pm | 4 | 196 \pm | 20 | 27 \pm | 9 | 8 \pm | 1 | 24 \pm | 8 |
| 75 | 20 \pm | 4 | 199 \pm | 27 | 30 \pm | 6 | 7 \pm | 2 | 24 \pm | 2 |
| 200 | 23 \pm | 2 | 226 \pm | 23 | 23 \pm | 5 | 4 \pm | 2 | 26 \pm | 2 |
| 600 | 26 \pm | 4 | 214 \pm | 11 | 20 \pm | 3 | 8 \pm | 1 | 21 \pm | 4 |
| 1800 | 23 \pm | 2 | 194 \pm | 17 | 25 \pm | 1 | 8 \pm | 4 | 23 \pm | 4 |
| 5000 | 23 \pm | 4 | 217 \pm | 21 | 26 \pm | 3 | 8 \pm | 4 | 23 \pm | 2 |
| Positive | 128 \pm | 20 | 638 \pm | 22 | 329 \pm | 19 | 467 \pm | 44 | 121 \pm | 35 |
| Liver Microsomes: Rat liver S9 | | | | | | | | | | |
| Dose (μ g/plate) | TA98 ^a | | TA100 ^b | | TA1535 | | TA1537 | | WP2 uvrA | |
| Vehicle | 38 \pm | 19 | 130 \pm | 19 | 14 \pm | 3 | 5 \pm | 2 | 21 \pm | 4 |
| 75 | 28 \pm | 4 | 125 \pm | 2 | 15 \pm | 3 | 5 \pm | 1 | 19 \pm | 3 |
| 200 | 38 \pm | 5 | 109 \pm | 12 | 15 \pm | 3 | 5 \pm | 1 | 20 \pm | 4 |
| 600 | 38 \pm | 10 | 134 \pm | 13 | 14 \pm | 4 | 8 \pm | 1 | 16 \pm | 3 |
| 1800 | 36 \pm | 6 | 134 \pm | 8 | 14 \pm | 3 | 8 \pm | 1 | 23 \pm | 4 |
| 5000 | 34 \pm | 6 | 127 \pm | 5 | 14 \pm | 5 | 8 \pm | 2 | 23 \pm | 7 |
| Positive | 568 \pm | 235 | 977 \pm | 30 | 93 \pm | 16 | 94 \pm | 17 | 506 \pm | 92 |
| Vehicle = Vehicle Control | | | | | | | | | | |
| Positive = Positive Control (50 μ L plating aliquot) | | | | | | | | | | |
| Plating aliquot: 100 μ L | | | | | | | | | | |
| a = Data from Experiment B3 | | | | | | | | | | |
| b = Data from Experiment B4 | | | | | | | | | | |

The following table summarizes the calculated concentration of the dosing formulations as compared to the nominal concentration as provided by the sponsor (page 66, vol. 11, Sequence 1, Module 4):

Sample batch assay results of NRP104 dosing formulations are summarized below:

| Date of Dosing Formulation Preparation | Date of Sample Assay | Nominal Dosing Formulation NRP104 Conc (mg/ml) ^a | Assayed Dosing Formulation Conc NRP104 (mg/ml) ^b | %Diff from Nominal Conc ^b |
|--|----------------------|---|---|--------------------------------------|
| 19 Dec 2003 | 8 Mar 2004 | 0.0 | 0.00 | BLQ |
| 19 Dec 2003 | 8 Mar 2004 | 0.025 | 0.0177 ^c | -29.2 |
| 19 Dec 2003 | 8 Mar 2004 | 0.075 | 0.0706 | -5.9 |
| 19 Dec 2003 | 8 Mar 2004 | 0.25 | 0.227 | -9.3 |
| 19 Dec 2003 | 8 Mar 2004 | 0.75 | 0.690 | -8.0 |
| 19 Dec 2003 | 8 Mar 2004 | 2.0 | 1.88 | -6.2 |
| 19 Dec 2003 | 8 Mar 2004 | 6.0 | 5.96 | -0.6 |
| 19 Dec 2003 | 8 Mar 2004 | 18 | 17.5 | -2.9 |
| 19 Dec 2003 | 8 Mar 2004 | 50 | 51.5 | 3.0 |
| 31 Dec 2003 | 8 Mar 2004 | 0.0 | 0.00 | BLQ |
| 31 Dec 2003 | 8 Mar 2004 | 0.75 | 0.677 | -9.7 |
| 31 Dec 2003 | 8 Mar 2004 | 2.0 | 1.60 | -19.9 |
| 31 Dec 2003 | 8 Mar 2004 | 6.0 | 5.53 | -7.8 |
| 31 Dec 2003 | 8 Mar 2004 | 18 | 13.9 | -22.7 |
| 31 Dec 2003 | 8 Mar 2004 | 50 | 35.6 | -28.7 |
| 16 Jan 2004 | 8 Mar 2004 | 0.0 | 0.00 | BLQ |
| 16 Jan 2004 | 8 Mar 2004 | 0.75 | 0.828 | 10.5 |
| 16 Jan 2004 | 8 Mar 2004 | 2.0 | 2.18 | 8.9 |
| 16 Jan 2004 | 8 Mar 2004 | 6.0 | 7.31 | 21.8 |
| 16 Jan 2004 | 8 Mar 2004 | 18 | 18.3 | 1.7 |
| 16 Jan 2004 | 8 Mar 2004 | 50 | 49.0 | -2.0 |
| 3 Feb 2004 | 8 Mar 2004 | 0.0 | 0.00 | BLQ |
| 3 Feb 2004 | 8 Mar 2004 | 0.75 | 0.849 | 13.2 |
| 3 Feb 2004 | 8 Mar 2004 | 2.0 | 2.16 | 8.1 |
| 3 Feb 2004 | 8 Mar 2004 | 6.0 | 6.17 | 2.9 |
| 3 Feb 2004 | 8 Mar 2004 | 18 | 18.4 | 2.0 |
| 3 Feb 2004 | 8 Mar 2004 | 50 | 50.2 | 0.4 |

^a Salt form dimethane sulfonate salt

^b Average values from duplicate injections.

^c Estimate only; sample assayed below the limit of quantitation.

BLQ Below the limit of quantitation

Study outcome:

The compound is considered to be non-genotoxic as tested in the Ames test. Even though the definitive study encountered some technical problems (such as contamination, unacceptable vehicle control values, errors in agar preparation, and lower concentrations from the nominal) the results of the repeated studies (Experiments B3 and B4) and the results of the preliminary study were reassuring and did not indicate a positive genotoxic potential for the test article. It should be noted that the study results as they are presented here were considered acceptable in the original IND submission mainly because the findings of the preliminary study were considered adequate and the preliminary study is

considered qualified and an adequate study. It is also worth pointing out that the parts that were reevaluated in the definitive study indicated that the compound did not have a genotoxic potential. The only part of the definitive study that might not be optimal is those performed in Experiment B2 on December 31, 2003 where the concentrations were below the nominal value. However, in the preliminary study these concentrations especially the highest doses were within 10% from the nominal value and the results did not indicate any concerns for a genotoxic potential.

The overall outcome of the study is, the compound is considered not genotoxic as tested by the Ames test and the study is considered valid.

Study title: In vitro mammalian cell gene mutation test (L5178Y/TK^{+/+} mouse lymphoma assay)

Key findings: the compound is not considered genotoxic as judged by the results of this assay

Study no.: Sponsor project # 11- -112503-SK5

Volume #, and page #: vol. 11, page 1, section 4.2.3.3.1, Module 4, Sequence 1)

Conducting laboratory and location: -----

Date of study initiation: original study was started in December 2003, supplemental study was started sometime after October 2004 (the test article was received by the conducting laboratory on that date but it is not clear when after that date the study was initiated)

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: NRP-104. The original study used Batch # 1001D (referred to as sample 1 in the report) and the supplemental study used Lot #3037652 (referred to as sample 2 in the report). As for the purity it was indicated only as % (provided by the sponsor) and no reference was made to which of the two lots this purity was designated, however, from earlier studies Batch 1001D had a purity designated by the sponsor as %

Methods

Strains/species/cell line: mouse L5178Y/^{+/+} cell line

Doses used in definitive study: doses used in the definitive study (both the initial study and the supplemental) ranged from 500 to 2500 µg/ml. However, it should be noted that in one of the conducted studies that was considered equivocal in its findings and inadequate due to large differences in the duplicates (referred to by the sponsor as Experiment #B3) doses used in this study ranged from 2000 to 4600 µg/ml.

Basis of dose selection: The doses selected for the definitive study were based on the findings from a preliminary study in which the following doses were used: 0.5, 1.5, 5, 15, 50, 150, 500, 1500, and 4600 µg/ml. Based on the decrease in suspension growth at these concentrations compared to the control doses were chosen for the definitive study.

Negative controls: the test article was dissolve in distilled water which was use as the negative control or vehicle

Positive controls: methyl methanesulfonate (MMS) was used as the positive control for the non-activated test system at a concentration of 15 and 20 µg/ml and 7,12-dimethyl benz(a)anthracene (7,12-DMBA) was used as the positive control for the S9-activated test system at a concentration of 2.5 and 4 µg/ml.

Incubation and sampling times: the cells were incubated with the different concentration for 4h with and without the S9 preparation and for 24h without S9 preparation. Since the 4h incubation with the S9 preparation was negative the 24h incubation was done only without the S9 preparation. At the end of the incubation period cells were washed and resuspended in F₁₀P media for two days and then for the selection of T/K-/- cells (the trifluorothymidine (TFT)-resistant phenotype) cells were plated into three replicate dishes and will be incubated for 10-14 days. The cells were counted with a counter unless the counter was not able to count then they were counted manually. The diameters of the positive controls and solvent controls were determined over a range of mm. There were no positive findings with the test article and therefore no colony sizing was done.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The study was conducted in several trials as summarized by the sponsor in the following table (page 14, section 4.2.3.3.1, Module 4, Sequence 1):

| Trial # | Activation | Exposure Time | Treatment Date | Tables | Figures |
|---------|------------|---------------|----------------|--------------|---------|
| B1 | +/- S9 | 4 hours | 20 Jan 2004 | 2-5 | 1-2 |
| | -S9 | 24 hours | 20 Jan 2004 | 8-9 | 5 |
| B2 | + S9 | 4 hours | 09 Feb 2004 | not reported | |
| B3 | + S9 | 4 hours | 17 Feb 2004 | 6-7 | 3-4 |
| B4 | + S9 | 4 hours | 26 Oct 2004 | 10-11 | 6-7 |

In Experiment B1, the test with S9 activation system did not produce adequate toxicity (% total growth was 47 and 45% at the highest dose tested of 2000 µg/ml). The part of the experiment without the S9 preparation incubated for 4h was adequate and the results of these tow parts (the experiment with and the experiment without S9 incubated for 4h) are presented in tables 2-5 and they are included here as presented by the sponsor (page 19, vol. 11, Module 4, Sequence 1):

TABLE 2

**CLONING DATA FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay (4-hour exposure)**

| Test Article Concentration (µg/mL) | | TFT Colonies | | | | VC Colonies | | | | Mutant Freq. ^a | Induced Mutant Freq. ^b | % Total Growth ^c |
|--|---|--------------|-----|------|---------|-------------|-----|------|---------|------------------------------|---|-----------------------------------|
| | | Counts | | Mean | | Counts | | Mean | | | | |
| Solvent | 1 | 45 | 68 | 55 | 56 ±9 | 145 | 194 | 158 | 166 ±21 | 68 | | |
| Solvent | 2 | 57 | 73 | 74 | 68 ±8 | 205 | 120 | 221 | 182 ±44 | 75 | | |
| Mean Solvent Mutant Frequency= 71 | | | | | | | | | | | | |
| 500 | A | 53 | 39 | 24 | 39 ±12 | 190 | 211 | 225 | 209 ±14 | 37 | -34 | 105 |
| 500 | B | 12 | 21 | 21 | 18 ±4 | 191 | 191 | 170 | 184 ±10 | 20 | -52 | 92 |
| 750 | A | 63 | 72 | 51 | 62 ±9 | 182 | 165 | 156 | 168 ±11 | 74 | 3 | 66 |
| 750 | B | 79 | 57 | 27 | 54 ±21 | 217 | 230 | 211 | 219 ±8 | 50 | -22 | 83 |
| 1000 | A | 73 | 63 | 63 | 66 ±5 | 194 | 199 | 222 | 205 ±12 | 65 | -6 | 61 |
| 1000 | B | 51 | 54 | 72 | 59 ±9 | 145 | 159 | 168 | 157 ±9 | 75 | 4 | 48 |
| 1500 | A | 32 | 13 | 50 | 32 ±15 | 177 | 172 | 185 | 178 ±5 | 36 | -36 | 13 |
| 1500 | B | 41 | 18 | 13 | 24 ±12 | 185 | 171 | 201 | 186 ±12 | 26 | -45 | 15 |
| ----- | | | | | | | | | | | | |
| Positive Control - Methyl Methanesulfonate (µg/mL) | | | | | | | | | | | | |
| 15 | | 179 | 152 | 182 | 171 ±13 | 97 | 96 | 98 | 97 ±1 | 353 | 281 | 29 |
| 20 | | 139 | 104 | 145 | 129 ±18 | 55 | 40 | 35 | 43 ±8 | 597 | 526 | 10 |

Solvent = water

A and B or 1 and 2 are duplicate cultures

$$^a - \text{Mutant frequency (per } 10^6 \text{ surviving cells)} = \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$$

$$^b - \text{Induced mutant frequency (per } 10^6 \text{ surviving cells)} = \frac{\text{average mutant frequency}}{\text{mutant frequency of solvent controls}}$$

$$^c - \% \text{ total growth} = \frac{(\% \text{ suspension growth} \times \% \text{ cloning growth})}{100}$$

TABLE 3

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay (4-hour exposure)

| Test Article Concentration (µg/mL) | | Cell Concentration (X 10 ⁶) ^a | | Susp Growth | | Cloning Growth | | % Total Growth ^e |
|--|---|---|-------|--------------------|--------------------|----------------|--------------------|--------------------------------|
| | | Day 1 | Day 2 | Total ^b | %Cntl ^c | Avg VC | %Cntl ^d | |
| Solvent | 1 | 1.016 | 1.443 | 16.3 | | 166 | | |
| Solvent | 2 | 1.107 | 1.306 | 16.1 | | 182 | | |
| 500 | A | 0.988 | 1.292 | 14.2 | 88 | 209 | 120 | 105 |
| 500 | B | 0.944 | 1.342 | 14.1 | 87 | 184 | 106 | 92 |
| 750 | A | 0.820 | 1.213 | 11.1 | 68 | 168 | 96 | 66 |
| 750 | B | 0.871 | 1.105 | 10.7 | 66 | 219 | 126 | 83 |
| 1000 | A | 0.707 | 1.072 | 8.4 | 52 | 205 | 118 | 61 |
| 1000 | B | 0.676 | 1.153 | 8.7 | 54 | 157 | 91 | 48 |
| 1500 | A | 0.214 | 0.622 | 2.1 | 13 | 178 | 102 | 13 |
| 1500 | B | 0.214 | 0.672 | 2.2 | 14 | 186 | 107 | 15 |
| 2000 | A | 0.025 | 0.088 | 0.0 | 0 | ++ | | |
| 2000 | B | 0.057 | 0.210 | 0.0 | 0 | ++ | | |

Positive Control - Methyl Methanesulfonate (µg/mL)

| | | | | | | | |
|----|-------|-------|-----|----|----|----|----|
| 15 | 0.801 | 0.938 | 8.4 | 52 | 97 | 56 | 29 |
| 20 | 0.688 | 0.852 | 6.5 | 40 | 43 | 25 | 10 |

Solvent = water

A and B or 1 and 2 are duplicate cultures

++ - Too toxic to clone

^a - Cultures containing <0.3x10⁶ cells/mL on day 1 and 2 are considered to have 0% total suspension growth.

^b - Total suspension growth = $\frac{\text{Day 1 cell conc.}}{0.3 \times 10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$

^c - % of control suspension growth = $\frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$

^d - % control cloning growth = $\frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$

^e - % total growth = $\frac{(\% \text{ suspension growth})(\% \text{ cloning growth})}{100}$

TABLE 4

CLONING DATA FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
First Trial of the Initial Assay with S9 (4-hour exposure)

| Test Article Concentration (µg/mL) | | TFT Colonies | | | | VC Colonies | | | | Mutant Freq. ^a | Induced Mutant Freq. ^b | % Total Growth ^c |
|---|---|--------------|-----|------|---------|-------------|-----|------|---------|------------------------------|---|-----------------------------------|
| | | Counts | | Mean | | Counts | | Mean | | | | |
| Solvent | 1 | 40 | 59 | 18 | 39 ±17 | 123 | 91 | 173 | 129 ±34 | 60 | | |
| Solvent | 2 | 20 | 19 | 27 | 22 ±4 | 185 | 70 | 153 | 136 ±48 | 32 | | |
| Mean Solvent Mutant Frequency= 46 | | | | | | | | | | | | |
| 500 | A | 51 | 40 | 15 | 35 ±15 | 189 | 173 | 172 | 178 ±8 | 40 | -7 | 140 |
| 500 | B | 11 | 6 | 7 | 8 ±2 | 81 | 104 | 127 | 104 ±19 | 15 | -31 | 88 |
| 750 | A | 25 | 17 | 18 | 20 ±4 | 176 | 208 | 208 | 197 ±15 | 20 | -26 | 145 |
| 750 | B | 25 | 14 | 12 | 17 ±6 | 160 | 84 | 91 | 112 ±34 | 30 | -16 | 99 |
| 1000 | A | 57 | 58 | 55 | 57 ±1 | 169 | 158 | 159 | 162 ±5 | 70 | 24 | 122 |
| 1000 | B | 37 | 35 | 21 | 31 ±7 | 63 | 45 | 101 | 70 ±23 | 89 | 43 | 51 |
| 1500 | A | 65 | 47 | 50 | 54 ±8 | 158 | 184 | 209 | 184 ±21 | 59 | 12 | 80 |
| 1500 | B | 64 | 48 | 63 | 58 ±7 | 137 | 124 | 160 | 140 ±15 | 83 | 37 | 79 |
| 2000 | A | 72 | 30 | 84 | 62 ±23 | 183 | 184 | 158 | 175 ±12 | 71 | 24 | 47 |
| 2000 | B | 47 | 19 | 18 | 28 ±13 | 143 | 157 | 165 | 155 ±9 | 36 | -10 | 45 |
| Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL) | | | | | | | | | | | | |
| 2.5 | | 37 | 35 | 79 | 50 ±20 | 157 | 111 | 164 | 144 ±24 | 70 | 23 | 83 |
| 4 | | 117 | 104 | 151 | 124 ±20 | 101 | 127 | 114 | 114 ±11 | 218 | 171 | 47 |

Solvent = water

A and B or 1 and 2 are duplicate cultures

$$^a - \text{Mutant frequency (per } 10^6 \text{ surviving cells)} = \frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$$

$$^b - \text{Induced mutant frequency per } 10^6 \text{ surviving cells) = } \frac{\text{average mutant frequency}}{\text{mutant frequency of solvent controls}}$$

$$^c - \% \text{ total growth} = \frac{(\% \text{ suspension growth} \times \% \text{ cloning growth})}{100}$$

TABLE 5

**TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
First Trial of the Initial Assay (4-hour exposure)**

| Test Article Concentration (µg/mL) | | Cell Concentration (X 10 ⁶) ^a | | Susp Growth | | Cloning Growth | | % Total Growth ^e |
|---|---|---|-------|--------------------|--------------------|----------------|--------------------|--------------------------------|
| | | Day 1 | Day 2 | Total ^b | %Cntl ^c | Avg VC | %Cntl ^d | |
| Solvent | 1 | 0.767 | 1.212 | 10.3 | | 129 | | |
| Solvent | 2 | 0.789 | 1.280 | 11.2 | | 136 | | |
| 500 | A | 0.771 | 1.313 | 11.3 | 104 | 178 | 134 | 140 |
| 500 | B | 0.779 | 1.398 | 12.1 | 112 | 104 | 78 | 88 |
| 750 | A | 0.750 | 1.260 | 10.5 | 97 | 197 | 149 | 145 |
| 750 | B | 0.771 | 1.475 | 12.6 | 117 | 112 | 84 | 99 |
| 1000 | A | 0.727 | 1.336 | 10.8 | 100 | 162 | 122 | 122 |
| 1000 | B | 0.672 | 1.407 | 10.5 | 97 | 70 | 53 | 51 |
| 1500 | A | 0.470 | 1.182 | 6.2 | 57 | 184 | 139 | 80 |
| 1500 | B | 0.524 | 1.386 | 8.1 | 75 | 140 | 106 | 79 |
| 2000 | A | 0.310 | 1.125 | 3.9 | 36 | 175 | 132 | 47 |
| 2000 | B | 0.298 | 1.230 | 4.1 | 38 | 155 | 117 | 45 |
| 3000 | A | 0.017 | 0.060 | 0.0 | 0 | ++ | | |
| 3000 | B | 0.009 | 0.016 | 0.0 | 0 | ++ | | |
| ----- | | | | | | | | |
| Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL) | | | | | | | | |
| 2.5 | | 0.597 | 1.235 | 8.2 | 76 | 144 | 109 | 83 |
| 4 | | 0.504 | 1.046 | 5.9 | 54 | 114 | 86 | 47 |
| ----- | | | | | | | | |

Solvent = water

A and B or 1 and 2 are duplicate cultures

++ - Too toxic to clone

^a - Cultures containing <0.3x10⁶ cells/mL on day 1 and 2 are considered to have 0% total suspension growth.

^b - Total suspension growth = $\frac{\text{Day 1 cell conc.}}{0.3 \times 10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$

^c - % of control suspension growth = $\frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$

^d - % control cloning growth = $\frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$

^e - % total growth = $\frac{(\% \text{ suspension growth})(\% \text{ cloning growth})}{100}$

As it is clear from tables 4 and 5, the highest dose used with the S9 preparation was not associated with the adequate toxicity therefore this part of the study (S-9-activated portion) was repeated. In the second trial of the S9 activated portion the study was terminated prior to cloning according to the sponsor due to insufficient toxicity. According to the sponsor, the results of this trial were recorded but not reported. A third trial was performed over a range of 500 to 4600 µg/ml with intermediate concentrations.

The results of this trial are presented in Tables 6 and 7 provided by the sponsor (pages 23 & 24, vol. 11, module 4, sequence 1):

TABLE 6

CLONING DATA FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Third Trial of the Initial Assay with S9 (4-hour exposure)

| Test Article Concentration (µg/mL) | | TFT Colonies | | | | VC Colonies | | | | Mutant Freq. ^a | Induced Mutant Freq. ^b | % Total Growth ^c |
|---|---|--------------|-----|------|--------|-------------|------|-----|---------|---------------------------|-----------------------------------|-----------------------------|
| | | Counts | | Mean | Counts | | Mean | | | | | |
| Solvent | 1 | 46 | 34 | 18 | 33 ±11 | 132 | 113 | 112 | 119 ±9 | 55 | | |
| Solvent | 2 | 19 | 15 | 14 | 16 ±2 | 160 | 92 | 111 | 121 ±29 | 26 | | |
| Mean Solvent Mutant Frequency= 41 | | | | | | | | | | | | |
| 2000 | A | 59 | 47 | 58 | 55 ±5 | 133 | 110 | 152 | 132 ±17 | 83 | 42 | 104 |
| 2000 | B | 39 | 17 | 8 | 21 ±13 | 129 | 79 | 113 | 107 ±21 | 40 | -1 | 84 |
| 2500 | A | 53 | 38 | 24 | 38 ±12 | 117 | 172 | 150 | 146 ±23 | 52 | 12 | 107 |
| 2500 | B | 17 | 15 | 9 | 14 ±3 | 139 | 152 | 144 | 145 ±5 | 19 | -22 | 105 |
| 3000 | A | 59 | 21 | 46 | 42 ±16 | 107 | 110 | 85 | 101 ±11 | 83 | 43 | 65 |
| 3000 | B | 11 | 17 | 48 | 25 ±16 | 92 | 131 | 109 | 111 ±16 | 46 | 5 | 74 |
| 4000 | A | 54 | 61 | 71 | 62 ±7 | 159 | 110 | 119 | 129 ±21 | 96 | 55 | 63 |
| 4000 | B | 15 | 19 | 15 | 16 ±2 | 97 | 100 | 111 | 103 ±6 | 32 | -9 | 50 |
| 4600 | A | 58 | 38 | 71 | 56 ±14 | 116 | 138 | 125 | 126 ±9 | 88 | 47 | 49 |
| 4600 | B | 58 | 65 | 54 | 59 ±5 | 106 | 126 | 118 | 117 ±8 | 101 | 60 | 39 |
| ----- | | | | | | | | | | | | |
| Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL) | | | | | | | | | | | | |
| 2.5 | | 146 | 47 | 78 | 90 ±41 | 68 | 83 | 74 | 75 ±6 | 241 | 200 | 43 |
| 4 | | 183 | 202 | 198 | 194 ±8 | 57 | 91 | 60 | 69 ±15 | 561 | 520 | 28 |
| ----- | | | | | | | | | | | | |
| Solvent = water | | | | | | | | | | | | |
| A and B or 1 and 2 are duplicate cultures | | | | | | | | | | | | |
| ^a - Mutant frequency (per 10 ⁶ surviving cells = $\frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$ | | | | | | | | | | | | |
| ^b - Induced mutant frequency (per 10 ⁶ surviving cells) = $\frac{\text{mutant frequency} - \text{average mutant frequency of solvent controls}}{\text{average mutant frequency of solvent controls}}$ | | | | | | | | | | | | |
| ^c - % total growth = $\frac{(\% \text{ suspension growth} \times \% \text{ cloning growth})}{100}$ | | | | | | | | | | | | |

Table 7

**TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Third Trial of the Initial Assay (4-hour exposure)**

| Test Article Concentration (µg/mL) | | Cell Concentration (X 10 ⁶) ^a | | Susp Growth | | Cloning Growth | | % Total Growth ^e |
|---|---|---|-------|--------------------|--------------------|----------------|--------------------|--------------------------------|
| | | Day 1 | Day 2 | Total ^b | %Cntl ^c | Avg VC | %Cntl ^d | |
| Solvent | 1 | 0.806 | 1.574 | 14.1 | | 119 | | |
| Solvent | 2 | 0.815 | 1.562 | 14.1 | | 121 | | |
| 2000 | A | 0.729 | 1.656 | 13.4 | 95 | 132 | 110 | 104 |
| 2000 | B | 0.733 | 1.635 | 13.3 | 94 | 107 | 89 | 84 |
| 2500 | A | 0.693 | 1.614 | 12.4 | 88 | 146 | 122 | 107 |
| 2500 | B | 0.724 | 1.528 | 12.3 | 87 | 145 | 121 | 105 |
| 3000 | A | 0.663 | 1.477 | 10.9 | 77 | 101 | 84 | 65 |
| 3000 | B | 0.669 | 1.516 | 11.3 | 80 | 111 | 92 | 74 |
| 4000 | A | 0.501 | 1.474 | 8.2 | 58 | 129 | 108 | 63 |
| 4000 | B | 0.523 | 1.413 | 8.2 | 58 | 103 | 86 | 50 |
| 4600 | A | 0.420 | 1.404 | 6.6 | 46 | 126 | 105 | 49 |
| 4600 | B | 0.389 | 1.326 | 5.7 | 41 | 117 | 97 | 39 |
| ----- | | | | | | | | |
| Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL) | | | | | | | | |
| 2.5 | | 0.583 | 1.496 | 9.7 | 69 | 75 | 63 | 43 |
| 4 | | 0.486 | 1.271 | 6.9 | 49 | 69 | 58 | 28 |
| ----- | | | | | | | | |
| Solvent = water | | | | | | | | |
| A and B or 1 and 2 are duplicate cultures | | | | | | | | |
| ^a - Cultures containing <0.3x10 ⁶ cells/mL on day 1 and 2 are considered to have 0% total suspension growth. | | | | | | | | |
| ^b - Total suspension growth = $\frac{\text{Day 1 cell conc.}}{0.3 \times 10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$ | | | | | | | | |
| ^c - % of control suspension growth = $\frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$ | | | | | | | | |
| ^d - % control cloning growth = $\frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$ | | | | | | | | |
| ^e - % total growth = $\frac{(\% \text{ suspension growth})(\% \text{ cloning growth})}{100}$ | | | | | | | | |

Even though the toxicity obtained in that study was not adequate (see tables 6 & 7), the highest dose used in the study (4600 µg/ml) was equal to 10 mM which is the maximum dose recommended by the ICH guidance. The results of this experiment were considered equivocal by the reviewer at the time of the IND submission based on the finding that the # of mutant cells above the control value (induced mutant frequency) were more than 55 in some of the replicates even though the average value was below 55 (see table 6). (The results are considered equivocal if the cultures exhibited mutant frequency between 55

and 99 mutants per 10^6 clonable cells over that of the solvent control). The sponsor at that time did not consider the results equivocal since the average value of the duplicates was below the criteria of the equivocal finding. In addition, it should be pointed out that the replicate values were largely variable. The sponsor was told that this study was not acceptable and that it should be repeated. The Sponsor repeated the study with the S9 activation for 4h and the results of the repeated study (also referred to as the supplemental assay) are summarized in tables 10 and 11 (pages 27 & 28, vol. 11, Module 4, Sequence 1):

Table 10

**CLONING DATA FOR L5178Y/TK^{-/-} MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Supplemental Assay with S9 (4-hour exposure)**

| Test Article Concentration (µg/mL) | TFT Colonies | | | | VC Colonies | | | | Mutant Freq. ^a | Induced Mutant Freq. ^b | % Total Growth ^c |
|--|--------------|---------|-------------|---------|-------------|------|--------|------|------------------------------|---|-----------------------------------|
| | Counts | Mean | Counts | Mean | Counts | Mean | Counts | Mean | | | |
| Solvent 1 | 93 85 76 | 85 ±7 | 173 197 176 | 182 ±11 | 93 | | | | | | |
| Solvent 2 | 58 58 58 | 58 ±0 | 173 177 152 | 167 ±11 | 69 | | | | | | |
| Mean Solvent Mutant Frequency= 81 | | | | | | | | | | | |
| 1000 A | 50 51 45 | 49 ±3 | 168 144 117 | 143 ±21 | 68 | -13 | 79 | | | | |
| 1000 B | 59 64 66 | 63 ±3 | 160 178 192 | 177 ±13 | 71 | -10 | 94 | | | | |
| 1500 A | 60 65 61 | 62 ±2 | 205 204 195 | 201 ±4 | 62 | -20 | 84 | | | | |
| 1500 B | 55 52 34 | 47 ±9 | 205 171 162 | 179 ±19 | 52 | -29 | 71 | | | | |
| 2000 A | 70 79 86 | 78 ±7 | 181 168 179 | 176 ±6 | 89 | 8 | 39 | | | | |
| 2000 B | 68 72 71 | 70 ±2 | 208 164 212 | 195 ±22 | 72 | -9 | 62 | | | | |
| 2250 A | 42 58 53 | 51 ±7 | 178 172 122 | 157 ±25 | 65 | -16 | 38 | | | | |
| 2250 B | 61 59 54 | 58 ±3 | 215 212 224 | 217 ±5 | 53 | -28 | 61 | | | | |
| 2500 A | 54 63 63 | 60 ±4 | 182 136 165 | 161 ±19 | 75 | -7 | 10 | | | | |
| 2500 B | 67 63 55 | 62 ±5 | 136 144 106 | 129 ±16 | 96 | 15 | 15 | | | | |
| Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL) | | | | | | | | | | | |
| 2.5 | 104 216 86 | 135 ±58 | 151 110 142 | 134 ±18 | 201 | 120 | 54 | | | | |
| 4 | 205 238 106 | 183 ±56 | 113 114 83 | 103 ±14 | 354 | 273 | 24 | | | | |
| Solvent = water | | | | | | | | | | | |
| A and B or 1 and 2 are duplicate cultures | | | | | | | | | | | |
| ^a - Mutant frequency (per 10^6 surviving cells) = $\frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$ | | | | | | | | | | | |
| ^b - Induced mutant frequency (per 10^6 surviving cells) = $\frac{\text{mutant frequency} - \text{average mutant frequency of solvent controls}}{\text{average mutant frequency of solvent controls}}$ | | | | | | | | | | | |
| ^c - % total growth = $\frac{(\% \text{ suspension growth} \times \% \text{ cloning growth})}{100}$ | | | | | | | | | | | |

TABLE 11

**TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Supplemental Assay with S9 (4-hour exposure)**

| Test Article Concentration (µg/mL) | | Cell Concentration (X 10 ⁶) ^a | | Susp Growth | | Cloning Growth | | % Total Growth ^e |
|--|---|---|-------|--------------------|--------------------|----------------|--------------------|--------------------------------|
| | | Day 1 | Day 2 | Total ^b | %Cntl ^c | Avg VC | %Cntl ^d | |
| Solvent | 1 | 1.077 | 1.287 | 15.4 | | 182 | | |
| Solvent | 2 | 1.120 | 1.411 | 17.6 | | 167 | | |
| 1000 | A | 1.008 | 1.414 | 15.8 | 96 | 143 | 82 | 79 |
| 1000 | B | 0.963 | 1.438 | 15.4 | 93 | 177 | 101 | 94 |
| 1500 | A | 0.857 | 1.268 | 12.1 | 73 | 201 | 115 | 84 |
| 1500 | B | 0.748 | 1.380 | 11.5 | 70 | 179 | 103 | 71 |
| 2000 | A | 0.453 | 1.266 | 6.4 | 39 | 176 | 101 | 39 |
| 2000 | B | 0.627 | 1.312 | 9.1 | 55 | 195 | 111 | 62 |
| 2250 | A | 0.486 | 1.289 | 7.0 | 42 | 157 | 90 | 38 |
| 2250 | B | 0.603 | 1.214 | 8.1 | 49 | 217 | 124 | 61 |
| 2500 | A | 0.165 | 0.510 | 1.7 | 10 | 161 | 92 | 10 |
| 2500 | B | 0.269 | 0.994 | 3.3 | 20 | 129 | 74 | 15 |
| 2750 | A | 0.134 | 0.397 | 1.3 | 8 | ++ | | |
| 2750 | B | 0.112 | 0.340 | 1.1 | 7 | ++ | | |

Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)

| | | | | | | | |
|-----|-------|-------|------|----|-----|----|----|
| 2.5 | 0.848 | 1.237 | 11.7 | 71 | 134 | 77 | 54 |
| 4 | 0.534 | 1.122 | 6.7 | 40 | 103 | 59 | 24 |

Solvent = water

A and B or 1 and 2 are duplicate cultures

++ - Too toxic to clone

^a - Cultures containing <0.3x10⁶ cells/mL on day 1 and 2 are considered to have 0% total suspension growth.

^b - Total suspension growth = $\frac{\text{Day 1 cell conc.}}{0.3 \times 10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$

^c - % of control suspension growth = $\frac{\text{total treatment suspension growth}}{\frac{\text{average solvent control}}{\text{total suspension growth}}} \times 100$

^d - % control cloning growth = $\frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$

^e - % total growth = $\frac{(\% \text{ suspension growth})(\% \text{ cloning growth})}{100}$

The toxicity levels in this study were adequate at the doses used (even though they were lower than the doses used in the original study) and the data did not indicate an increase in induced mutant frequency (see table 10). **It should be noted that the sponsor used a**

different batch for the drug substance in this experiment compared to the earlier parts of the experiment and it is not know how these batches differ (Batch # 1001D was used in the earlier studies and Lot #3037652 was used in the supplemental assay).

The part of the study with the long term treatment (24h) was done earlier by the sponsor in Experiment B1 and the results are summarized in tables 8 & 9 provided by the sponsor (pages 25 & 26, vol. 1, module 4, sequence 1):

| TABLE 8 | | | | | | | | | | | | | |
|--|---|--------------|-----|------|---------|-------------|------|-----|---------|------------------------------|---|-----------------------------------|--|
| CLONING DATA FOR L5178Y/TK ⁺ MOUSE LYMPHOMA CELLS TREATED WITH NRP104 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION Extended Treatment Assay (24-hour exposure) | | | | | | | | | | | | | |
| Test Article Concentration (µg/mL) | | TFT Colonies | | | | VC Colonies | | | | Mutant Freq. ^a | Induced Mutant Freq. ^b | % Total Growth ^c | |
| | | Counts | | Mean | Counts | | Mean | | | | | | |
| Solvent | 1 | 30 | 54 | 25 | 36 ±13 | 190 | 124 | 177 | 164 ±29 | 44 | | | |
| Solvent | 2 | 21 | 12 | 11 | 15 ±4 | 90 | 111 | 155 | 119 ±27 | 25 | | | |
| Mean Solvent Mutant Frequency= 35 | | | | | | | | | | | | | |
| 500 | A | 18 | 31 | 11 | 20 ±8 | 162 | 175 | 101 | 146 ±32 | 27 | -7 | 81 | |
| 500 | B | 27 | 12 | 19 | 19 ±6 | 144 | 136 | 107 | 129 ±16 | 30 | -5 | 84 | |
| 600 | A | 26 | 40 | 25 | 30 ±7 | 179 | 176 | 178 | 178 ±1 | 34 | 0 | 93 | |
| 600 | B | 44 | 20 | 18 | 27 ±12 | 203 | 137 | 181 | 174 ±27 | 31 | -3 | 53 | |
| 750 | A | 42 | 38 | 19 | 33 ±10 | 160 | 171 | 125 | 152 ±20 | 43 | 9 | 50 | |
| 750 | B | 32 | 25 | 32 | 30 ±3 | 132 | 138 | 137 | 136 ±3 | 44 | 9 | 44 | |
| 1000 | A | 51 | 45 | 53 | 50 ±3 | 159 | 170 | 164 | 164 ±4 | 60 | 26 | 38 | |
| 1000 | B | 27 | 58 | 37 | 41 ±13 | 156 | 144 | 165 | 155 ±9 | 52 | 18 | 13 | |
| 1250 | A | 27 | 46 | 58 | 44 ±13 | 144 | 181 | 140 | 155 ±18 | 56 | 22 | 23 | |
| 1250 | B | 44 | 66 | 22 | 44 ±18 | 140 | 112 | 103 | 118 ±16 | 74 | 40 | 15 | |
| ----- | | | | | | | | | | | | | |
| Positive Control - Methyl Methanesulfonate (µg/mL) | | | | | | | | | | | | | |
| 5 | | 111 | 139 | 70 | 107 ±28 | 92 | 104 | 107 | 101 ±6 | 211 | 177 | 46 | |
| 7.5 | | 85 | 68 | 83 | 79 ±8 | 58 | 63 | 59 | 60 ±2 | 262 | 228 | 22 | |
| ----- | | | | | | | | | | | | | |
| Solvent = water | | | | | | | | | | | | | |
| A and B or 1 and 2 are duplicate cultures | | | | | | | | | | | | | |
| ^a - Mutant frequency (per 10 ⁶ surviving cells) = $\frac{\text{Average \# TFT colonies}}{\text{average \# VC colonies}} \times 200$ | | | | | | | | | | | | | |
| ^b - Induced mutant frequency (per 10 ⁶ surviving cells) = $\frac{\text{average mutant frequency}}{\text{mutant frequency of solvent controls}}$ | | | | | | | | | | | | | |
| ^c - % total growth = $\frac{(\% \text{ suspension growth} \times \% \text{ cloning growth})}{100}$ | | | | | | | | | | | | | |

TABLE 9

**TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK⁺ MOUSE LYMPHOMA CELLS
TREATED WITH NRP104
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Extended Treatment Assay (24-hour exposure)**

| Test Article Concentration (µg/mL) | | Cell Concentration (X 10 ⁶) ^a | | Susp Growth Total ^b | %Cntl ^c | Cloning Growth Avg VC | %Cntl ^d | % Total Growth ^e |
|--|---|---|-------|-----------------------------------|--------------------|--------------------------|--------------------|--------------------------------|
| | | Day 1 | Day 2 | | | | | |
| Solvent | 1 | 1.299 | 1.296 | 18.7 | | 164 | | |
| Solvent | 2 | 1.162 | 1.248 | 16.1 | | 119 | | |
| 500 | A | 0.947 | 1.294 | 13.6 | 78 | 146 | 103 | 81 |
| 500 | B | 1.125 | 1.274 | 15.9 | 92 | 129 | 91 | 84 |
| 600 | A | 0.878 | 1.314 | 12.8 | 74 | 178 | 126 | 93 |
| 600 | B | 0.574 | 1.170 | 7.5 | 43 | 174 | 123 | 53 |
| 750 | A | 0.567 | 1.271 | 8.0 | 46 | 152 | 108 | 50 |
| 750 | B | 0.597 | 1.200 | 8.0 | 46 | 136 | 96 | 44 |
| 1000 | A | 0.398 | 1.285 | 5.7 | 33 | 164 | 116 | 38 |
| 1000 | B | 0.112 | 0.607 | 2.0 | 12 | 155 | 110 | 13 |
| 1250 | A | 0.182 | 1.079 | 3.6 | 21 | 155 | 110 | 23 |
| 1250 | B | 0.161 | 0.921 | 3.1 | 18 | 118 | 84 | 15 |
| 1500 | A | 0.026 | 0.116 | 0.0 | 0 | ++ | | |
| 1500 | B | 0.005 | 0.048 | 0.0 | 0 | ++ | | |

Positive Control - Methyl Methanesulfonate (µg/mL)

| | | | | | | | |
|-----|-------|-------|------|----|-----|----|----|
| 5 | 0.921 | 1.106 | 11.3 | 65 | 101 | 72 | 46 |
| 7.5 | 0.818 | 0.975 | 8.9 | 51 | 60 | 43 | 22 |

Solvent = water

A and B or 1 and 2 are duplicate cultures

++ - Too toxic to clone

^a - Cultures containing <0.3x10⁶ cells/mL on day 1 and 2 are considered to have 0% total suspension growth.

^b - Total suspension growth = $\frac{\text{Day 1 cell conc.}}{0.3 \times 10^6 \text{ cells/mL}} \times \frac{\text{Day 2 cell conc.}}{\text{Day 1 adjusted cell conc.}}$

^c - % of control suspension growth = $\frac{\text{total treatment suspension growth}}{\text{average solvent control total suspension growth}} \times 100$

^d - % control cloning growth = $\frac{\text{average VC of treated culture}}{\text{average VC of solvent control}} \times 100$

^e - % total growth = $\frac{(\% \text{ suspension growth})(\% \text{ cloning growth})}{100}$

Earlier at the time of the IND submission, the sponsor indicated that the 24h treatment was done only in the absence of the S9-activation system because the 4h treatment with S9 activation system did not produce positive findings. However, it should be noted that the earlier 4h study in the presence of the S9 activation was considered inadequate and was repeated. The findings from the newly conducted 4h treatment with S9 activation system also indicated negative findings in the presence of the S9 activation system for 4h treatment and thus the fact that the 24h treatment was not done in the presence of an activation system is still acceptable.

Study outcome: even though the study was done on different trials and some parts of the study were considered inadequate and the sponsor was asked to repeat those parts, the overall studies as they are presented now are considered adequate, valid and the findings did not indicate a genotoxic potential for the compound using this study. Therefore, the compound is considered non-genotoxic using the mouse lymphoma assay.

Study title: mammalian erythrocyte micronucleus test

Key findings: the compound is considered non-genotoxic as judged by the results of this assay

Study no.: sponsor project # 11-~~-----~~-112503-SK6

Volume #, and page #: vol. 11, section 4.2.3.3.2, page 1, Module 4, Sequences 1,
Conducting laboratory and location:-----

| |
|-------|
| ----- |
| ----- |

Date of study initiation: the initial study was started December 2003 while the supplemental study was started January 2005

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: NRP-104, the initial study used batch # 1001D/N011DP (referred to as sample 0001 by the sponsor) and the purity of this batch was ~~-----~~ % w/w (as per certificate of analysis). The supplemental study used batch 3037652 (referred to as sample 0002 by the sponsor) and the purity was ~~-----~~ % (as per certificate of analysis according to the report, however, the certificate of analysis was not attached to the submission).

Methods

Strains/species/cell line: ICR mice (both M and F)

Doses used in definitive study: for M: 18.7, 37.5 or 75 mg/kg. For F: 50, 100, or 200 mg/kg in the initial study and in the supplemental study two doses were used (400 and

600 mg/kg), however, results were obtained only from animals treated with 400 mg/kg due to death at the 600 mg/kg dose.

Basis of dose selection: the doses selected for the initial study were based on findings from a dose range finding study in which M and F mice were exposed to NRP-104 at the following concentrations: 200, 800, 1000, 1500, or 2000 mg/kg. Based on the findings from this study which are included in the following table as summarized by the sponsor (table 1, page 18, vol. 11, Module 4, Sequence 1) doses for the main study were decided:

| Treatment (20 mL/kg) | Observation | Number of Animals With Observed Signs/Total Number of Animals Dosed | | Number of Animals Died/Total Number of Animals Dosed | |
|----------------------|------------------|---|---------|--|---------|
| | | Males | Females | Males | Females |
| NRP104 200 mg/kg | Hyperactivity | 5/5 | 5/5 | | |
| | Piloerection | 5/5 | 0/5 | 2/5 | 0/5 |
| | Lethargy | 1/5 | 0/5 | | |
| | Hunched position | 1/5 | 0/5 | | |
| 800 mg/kg | Hyperactivity | 5/5 | 5/5 | 5/5 | 4/5 |
| | Piloerection | 0/5 | 1/5 | | |
| 1000 mg/kg | Hyperactivity | 5/5 | 5/5 | 5/5 | 2/5 |
| | Piloerection | 0/5 | 3/5 | | |
| 1500 mg/kg | Hyperactivity | 5/5 | 5/5 | 5/5 | 3/5 |
| | Piloerection | 0/5 | 2/5 | | |
| 2000 mg/kg | Hyperactivity | 5/5 | 5/5 | 5/5 | 5/5 |

At a dose of 200 mg/kg in M death was observed in 2/5 animals while no deaths were observed in F at the same dose. Hyperactivity (all animals), lethargy (1/5 M) and hunched position (1/5 M) were observed at 200 mg/kg. The higher doses were all associated with deaths in both M and F. The sponsor indicated that due to mortality and adverse clinical signs seen in M, but not in F at 200 mg/kg, different doses were selected for M and F. The highest dose used in the initial definitive study in M was 75 mg/kg while the highest dose used in F was 200 mg/kg. At the time of the IND review the high dose used in F in the initial study was considered inadequate since the dose was not associated with dose limiting toxicities (hyperactivity might not be considered dose limiting in this study unless it was associated with self mutilation which was not described here). The fact that in M the next highest dose used (200 mg/kg) was associated with death and that other clinical signs that might be limiting were seen in 1/5 animals (lethargy and hunched position), then the 75 mg/kg high dose used in M could be acceptable.

In the supplement study, only F were used and the doses used (400 and 600 mg/kg) were chosen by the sponsor based on the fact that the 200 mg/kg dose used in the initial study was not associated with dose limiting toxicities and the next higher dose tested in F (800 mg/kg in the dose range finding study) was associated with death. Therefore, the sponsor

chose these doses (400 and 600 mg/kg) in the supplemental study which were between the 200 mg/kg and the 800 mg/kg. Mortality was observed at the 600 mg/kg (4/10 F) and hyperactivity was observed in animals treated with both the 400 and 600 mg/kg. Due to the mortality at 600 mg/kg the sponsor did not analyze the bone marrow obtained from the surviving animals at this dose and only analyzed those samples from animals treated with 400 mg/kg. According to the OECD Guideline “the highest dose is defined as the dose producing signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce lethality”. According to the findings of the supplemental study in F the highest dose used could be considered acceptable.

Negative controls: control animals were treated with deionized water the vehicle in which the test article was dissolved.

Positive controls: animals were treated with cyclophosphamide monohydrate (CP) at a dose concentration of 50 mg/kg

Incubation and sampling times: in the dose finding study animals were treated with a single dose of the test article orally by gavage and were observed for up to 3 days for clinical signs of toxicity. For the definitive study animals were treated with vehicle, test article, or positive control (single dose, orally by gavage) and were sacrificed either at 24h or 48h after treatment.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): there were a total of 5 animals per treatment group per time point for all studies conducted. For the bone marrow preparation, two slides were prepared from each mouse. Two thousand polychromatic erythrocytes per animal were scored for the presence of micronuclei. The proportion of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes (PCEs/ECs ratio) for each animal. These are acceptable for the criteria of validation of the assay. In addition, the sponsor indicated that for the assay to be valid, the incidence of micronucleated polychromatic erythrocytes must not exceed 5/1000 polychromatic erythrocytes (0.5%) in the vehicle control. The incidence of micronucleated polychromatic erythrocytes in the positive control must be significantly increased relative to the vehicle control group. These two criteria were met as judged by the results of the study.

In the definitive study in M the results did not indicate a genotoxic potential for the test article at the doses used (see the following table provided by the sponsor on page 21, vol. 11):

**Table 4: Summary of Bone Marrow Micronucleus Analysis
Following a Single Dose of NRP104 in ICR Mice**

-Initial Micronucleus Study-

| Treatment (20 mL/kg) | Sex | Time (hr) | Number of Mice | PCE/Total Erythrocytes (Mean +/- SD) | Change from Control (%) | Micronucleated Polychromatic Erythrocytes | |
|------------------------------|-----|--------------|-------------------|--|----------------------------------|---|--|
| | | | | | | Number per 1000 PCEs (Mean +/- SD) | Number per PCEs Scored ¹ |
| Deionized water | M | 24 | 5 | 0.511 ± 0.07 | --- | 0.4 ± 0.22 | 4 / 10000 |
| | F | 24 | 5 | 0.482 ± 0.05 | --- | 0.5 ± 0.35 | 5 / 10000 |
| NRP104 18.7 mg/kg | M | 24 | 5 | 0.450 ± 0.04 | -12 | 0.5 ± 0.35 | 5 / 10000 |
| | F | 24 | 5 | 0.487 ± 0.07 | 1 | 0.3 ± 0.27 | 3 / 10000 |
| 37.5 mg/kg | M | 24 | 5 | 0.486 ± 0.04 | -5 | 0.6 ± 0.42 | 6 / 10000 |
| | F | 24 | 5 | 0.433 ± 0.03 | -10 | 0.5 ± 0.50 | 5 / 10000 |
| 75 mg/kg | M | 24 | 5 | 0.431 ± 0.05 | -16 | 0.4 ± 0.22 | 4 / 10000 |
| | F | 24 | 5 | 0.436 ± 0.03 | -10 | 0.3 ± 0.27 | 3 / 10000 |
| Cyclophosphamide 50 mg/kg | M | 24 | 5 | 0.347 ± 0.04 | -32 | 18.4 ± 4.20 | *184 / 10000 |
| | F | 24 | 5 | 0.326 ± 0.02 | -32 | 21.8 ± 4.67 | *218 / 10000 |
| Deionized water | M | 48 | 5 | 0.478 ± 0.06 | --- | 0.4 ± 0.22 | 4 / 10000 |
| | F | 48 | 5 | 0.466 ± 0.07 | --- | 0.1 ± 0.22 | 1 / 10000 |
| NRP104 75 mg/kg | M | 48 | 5 | 0.470 ± 0.05 | -2 | 0.6 ± 0.42 | 6 / 10000 |
| | F | 48 | 5 | 0.474 ± 0.04 | 2 | 0.6 ± 0.22 | 6 / 10000 |

¹*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

Since the study was considered inadequate in F, the supplement study was conducted to investigate the effect of the test article at higher doses in F. The following table was provided by the sponsor for the results of the supplement study in which the test article dose not seem to have a genotoxic potential (table 8, page 25, vol. 11):

**Table 8: Summary of Bone Marrow Micronucleus Analysis
Following a Single Dose of NRP104 in Female ICR Mice**

- Supplemental Micronucleus Study-

| Treatment (20 mL/kg) | Sex | Time (hr) | Number of Mice | PCE/Total Erythrocytes (Mean +/- SD) | Change from Control (%) | Micronucleated Polychromatic Erythrocytes | |
|------------------------------|-----|--------------|-------------------|--|----------------------------------|---|--|
| | | | | | | Number per 1000 PCEs (Mean +/- SD) | Number per PCEs Scored ¹ |
| Deionized water | F | 24 | 5 | 0.504 ± 0.08 | --- | 0.3 ± 0.45 | 3 / 10000 |
| NRP104 400 mg/kg | F | 24 | 5 | 0.435 ± 0.12 | -14 | 0.3 ± 0.45 | 3 / 10000 |
| Cyclophosphamide 50 mg/kg | F | 24 | 5 | 0.470 ± 0.05 | -7 | 9.6 ± 2.22 | *96 / 10000 |
| Deionized water | F | 48 | 5 | 0.577 ± 0.05 | --- | 0.8 ± 0.67 | 8 / 10000 |
| NRP104 400 mg/kg | F | 48 | 5 | 0.554 ± 0.07 | -4 | 0.4 ± 0.42 | 4 / 10000 |

¹*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

Study outcome: the studies conducted to test the effect of the test article in the micronucleus assay are considered adequate and the results indicate that the test article is has negative genotoxic potential as judged by the findings of this study.

2.6.6.4 Carcinogenicity :

No studies were conducted based on the fact that this compound is a prodrug for amphetamine and that the main product is amphetamine with insignificant levels of the parent compound circulating or accumulating in the body. Carcinogenicity studies for amphetamine were conducted by NTP and are found in Adderall labeling.

2.6.6.5 Reproductive and developmental toxicology:

No studies were conducted for the same reason given in the previous section. Animal reproductive toxicity studies of amphetamine are described in the Adderall labeling.

2.6.6.7 Local tolerance: no studies were conducted

2.6.6.8 Special toxicology studies: juvenile animal studies

1. Study title: NRP104: an 8-week subchronic oral neonatal toxicity study in the Sprague Dawley rat

Key study findings:

Study no.: ----- project #900572

Volume #, and page #: vol. 12, page 1

Conducting laboratory and location: -----

| |
|-------|
| ----- |
| ----- |
| ----- |
| ----- |

Date of study initiation: January 2005

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: NRP104, lot # N040EH, purity -----%

Formulation/vehicle: solution/ deionized water

Methods

Doses: 0, 4, 10, and 40 mg/kg/day

Rational for dose selection: doses were selected based on the findings of a dose finding study in which neonatal rats (12/sex/group) were dosed orally by gavage with 0, 4, 15, and 40 mg/kg/day of NRP-104 (10ml/kg) from post natal day (PND) 7 to PND 30 inclusive. There were no mortalities reported. Increased activity (according to the sponsor was manifested as increased cage exploration, excessive grooming, stereotypic sniffing, biting of the cage floor, rapid head turning from side to side, increased rearing, sniffing and/or spatial disorientation) was seen in animals treated with 15 or 40 mg/kg/day starting on PND 21 to the end of the study. Mean body weights were ~ 8% lower in M and 12% lower in F at 15 mg/kg/day and ~18% lower in M and 22% lower in F at 40 mg/kg/day compared to control group from PND 22 to 31. Based on these findings the sponsor considered these doses appropriate for the definitive study. The reviewer agrees with the sponsor that these doses are appropriate for the definitive study.

Study design: Sprague Dawley pups (60/sex/group) were dosed with the appropriate dose orally by gavage (10 ml/kg/day) from PND 7 to 63 inclusive. The study was subdivided by the sponsor into Phase I (the main study with 4 subgroups, see later) and Phase II (toxicokinetic study). In Phase I study each dose group was subdivided into 4 subsets (15/sex/group) according to the following assignments: subgroup A (main toxicity study, animals sacrificed on PND 64), subgroup B (regression study, animals sacrificed on PND 92), subgroup C (reproductive study, animals sacrificed after Day 26-28 post coitum), and subgroup D (toxicokinetic bleed on PND 64). Phase II is a toxicokinetic study in which blood was collected from animal on PND 7. The following table was prepared by the reviewer and summarizes the different treatments conducted and their timing:

Phase I Study subgroups:

| Subgroup | Dosing duration | Subgroup investigations | Time/paradigm of investigation |
|-------------------------------|-----------------|---|---|
| Subgroup A (main toxicity) | PND 7-63 | Behavior and clinical observations | Once or twice daily |
| | | Detailed examinations | Once weekly |
| | | Body wt | Days 4, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, and 42 post partum and weekly thereafter |
| | | Food consumption | Per cage (PND 21-28), individually weekly (PND 28-63) |
| | | Physical development (crown-to-rump measurements) | weekly |
| | | Vaginal opening | PND 26 until |

| | | | |
|--|--|--|---|
| | | | development |
| | | Preputial separation | PND 34 until development |
| | | Ophthalmology (funduscopy and biomicroscopic) | PND 21-22 & PND 62-63 |
| | | Functional observation battery (FOB) | 10/sex/group on PND 22 or 23 and PND 59-60, prior to daily dosing |
| | | Motor activity (Figure 8 enclosures) | 10/sex/group on PND 22 or 23 & on PND 59 or 60, prior to daily dosing |
| | | Auditory startle habituation | PND 62 or 63, prior to daily dosing |
| | | Laboratory tests (hematology, biochemistry, and urinalysis), | 10/sex/group, overnight fasted animals (blood from abdominal aorta), at time of termination (PND 64). Urine samples were collected between days 63 and 64 from fasted animals |
| | | Gross pathology | PND 64 (5/sex/group were subject to whole body perfusion fixation at necropsy, 10/sex/group were euthanized and a complete gross pathology was conducted). |
| | | Organ wt | PND 64 (10/sex/group) |
| | | histopathology | PND 64 tissues from all groups were embedded and stained (H&E). Neurophthaology was done on HD and control (several sections of the brain and spinal cord H&E stain, brain wt, brain length, and brain width) |

| Subgroup | Dosing duration | Subgroup investigations | Time/paradigm of investigation |
|-------------------------|-----------------|------------------------------------|--------------------------------|
| Subgroup B (regression) | PND 7-63 | Behavior and clinical observations | Once or twice daily |

| | | | |
|--|--|--|---|
| | | | |
| | | Detailed examinations | Once weekly |
| | | Body wt | Days 4, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, and 42 post partum and weekly thereafter |
| | | Physical development (crown-to-rump measurements) | weekly |
| | | Vaginal opening | PND 26 until development |
| | | Preputial separation | PND 34 until development |
| | | Ophthalmology (funduscopy and biomicroscopy) | PND 21-22 & PND 90-91 |
| | | Laboratory tests (hematology, biochemistry, and urinalysis), | 10/sex/group, overnight fasted animals (blood from abdominal aorta), at time of termination (PND 92). Urine samples were collected at termination from fasted animals |
| | | FOB | 10/sex/group on PND 88-89 (regression) |
| | | Motor activity (Figure 8 enclosures) | 10/sex/group on PND 88-89 (regression) |
| | | Auditory startle habituation | Prior to daily dosing on PND 62 or 63 and between PND 90 & 91 (regression) |
| | | Cincinnati Water maze | 10/sex/group between PND 76 to 84 (regression) |
| | | Laboratory tests (hematology, biochemistry, and urinalysis), | 10/sex/group, overnight fasted animals (blood from abdominal aorta), at |

| | | | |
|--|--|------------------------------|---|
| | | | time of termination (PND 92) |
| | | Gross pathology and organ wt | At termination, 5/sex/group were subject to whole body perfusion fixation. The rest of animals (10/sex/group) were euthanized and a complete gross pathology was conducted. |
| | | histopathology | Not performed |


| Subgroup | Dosing duration | Subgroup investigations | Time/paradigm of investigation |
|---------------------------|-----------------|--|--|
| Subgroup C (reproduction) | PND 7-63 | Cincinnati Water Maze | 10/sex/group between PND 52 and 61, prior to daily dosing |
| | | Estrous cycle | The estrous cycles were determined for 10 days prior to mating, during mating and until the day of positive identification of mating by vaginal lavage (# of days in estrous, # of cycles, and average cycle length) |
| | | Mating: confirmed by presence of spermatozoa in vaginal lavage | At PND 85 for up to 14 days |
| | | Maternal/paternal performance: Gestation index (# of rats with live litters/# of pregnant ratsx100), mating index (# of mating M/# of M placed for | |

| | | | |
|--|--|--|---|
| | | matingx100), fertility index (# of M producing pregnancy/# of M placed for mating x 100), conception rate (#of pregnant F/# of F mated x 100) | |
| | | Parturition | Animals were observed each day from Day 20 of gestation |
| | | Pups and litter observation (F2 generation): on Day 0 post partum, the pups were examined for external malformation, sexed, and the number of alive and dead was recorded. Pups were weighed individually on Day 0 and Day 4 post partum. Pups found dead or dying on or before Day 5 post partum and pups born malformed or externally abnormal were euthanized and placed in Bouin's fluid for subsequent visceral examination. The surviving animals were evaluated during the lactation period | Day 0 to 6 post partum |

| Subgroup | Dosing duration | Subgroup investigations | Time/paradigm of investigation |
|----------|-----------------|-------------------------|--------------------------------|
|----------|-----------------|-------------------------|--------------------------------|

| | | | |
|-------------------------------|----------|------------------------------------|--------|
| Subgroup D (toxicokinetic) | PND 7-63 | Blood samples for toxicokinetic | PND 63 |
|-------------------------------|----------|------------------------------------|--------|

Tests performed:

- 1. Functional observational battery:** the test was performed with equipment built for this purpose (the equipment was not described). The test consisted of observations in home cage, removal from home cage, observations in arena (the arena was described as a 2' square of plexiglass placed on a raised platform), handling observations, on surface (auricular startle and air righting reflex) and on top of box (positional passivity).
- 2. Motor activity:** activity levels were measured individually in figure 8 enclosures. The sessions were 1h long and activity counts were recorded by computer in 6 successive 10 min intervals. The sponsor stated that "in addition to the "diagnostic" function in the system, a check of each beam was made by manually "breaking" each beam a predetermined number of times and verify that the breaks were properly recorded. These checks were made at least prior to the start of testing and at the completion of testing each day".
- 3. Auditory startle habituation:** a  apparatus was used for this test. Animals were given a 4-min acclimation period with a background sound of ~67dBA, and then the startle response was measured in 50 identical trials at a sound level of 120 dBA, for 20 msec with a 100 msec record window and with an 8-second intertrial interval. According to the sponsor, the sound levels are checked at the beginning and end of each test session with a sound meter and the recording platforms' motion sensors are also checked and/or calibrated. The following parameters were measured: startle at start (voltage, which seems to be the startle at the beginning), time of maximum startle (msec), maximum startle (voltage, which appears to be the maximum startle value), and average startle (voltage, which is the average startle value for the measurements over the 100 msec interval).
- 4. Cincinnati Water Maze:** the animals' ability to swim was assessed by measuring the time to swim a straight channel. Learning and memory tests were conducted using the Cincinnati maze. The maze consisted of two paths. On the first day of testing, each animal was tested twice (at least 10 min apart) by measuring the time to complete the first path (Path A). This was repeated on two additional consecutive days (second trial on Day 1 and first trial on Day 2 were at least 25 hours apart) using the same path. The same paradigm (i.e., 3 days of testing) was repeated using a second path (Path B). At least one day separated testing of the two paths.

Laboratory investigations: including hematology, clinical chemistry and urinalysis.

For hematology the following parameters were evaluated:

* activated partial thromboplastin time
blood cell morphology
erythrocyte indices (MCV, MCH, MCHC and RDW)
hematocrit
hemoglobin
mean platelet volume
platelet count
* prothrombin time
red blood cell count
reticulocytes (absolute and percent)
white blood cell count (total, absolute and percent differential)

For clinical chemistry, the following parameters were evaluated:

A/G ratio (calculated)
alanine aminotransferase
albumin
alkaline phosphatase
aspartate aminotransferase
blood urea nitrogen
calcium
chloride
cholesterol
creatinine
globulin (calculated)
glucose
inorganic phosphorus
potassium
sodium
total direct and indirect bilirubin
total protein
triglycerides

For Urinalysis the following parameters were evaluated:

bilirubin
 blood
 color and appearance
 glucose
 ketones
 microscopy of centrifuged deposit
 nitrite
 pH
 protein
 specific gravity
 urobilinogen
 volume

The following organs were weighed:

adrenals
 brain
 heart
 kidneys
 liver
 ovaries
 pituitary
 prostate
 spleen
 testes
 thymus
 thyroid and parathyroids

For histopathology, the following tissues were retained from 10 rats/sex/group from Subgroup A and B (not examined because no findings were seen in Subgroup A). For subgroup C animals only specified tissue (#) were retained:

abnormalities
 # animal identification (retained but not processed)
 adrenals
 aorta (thoracic)
 ** bone and marrow (sternum)
 brain (3 levels: forebrain [through septum], midbrain and midcerebellum and medulla oblongata as selected for neuropathology.)
 cecum

colon
duodenum
*, # epididymides
esophagus
* eyes
harderian glands
heart (including section of aorta)
ileum
jejunum
kidneys
lacrimal glands
liver (sample of 2 lobes)
+ lungs (sample of 2 lobes)
lymph nodes (mandibular and mesenteric)
#, ++ mammary gland (inguinal)
*, ++ optic nerves
ovaries
pancreas
pituitary
prostate
rectum (retained but not processed)
salivary gland (mandibular)
sciatic nerve
seminal vesicles
skeletal muscle
skin (inguinal)
spinal cord (cervical, thoracic, lumbar)
spleen
stomach
*, # testes
thymus
++ thyroid lobes (and parathyroids)
tongue
trachea

| | |
|----|--|
| | urinary bladder |
| # | uterus (cervix, uterine horns and body) |
| # | vagina |
| * | Fixed with Zenker's fluid for euthanized rats only |
| ** | Bone decalcified prior to sectioning |
| + | Infused with neutral buffered 10% formalin |
| ++ | Examined histopathologically only if present in routine sections of eyes (optic nerves), thyroid lobes (parathyroid glands) or skin (mammary gland). |

Neuropathology test: Tissues from the main study from control and HD group (Subgroup A) were processed for neuropathological evaluation. The following tissues were prepared for examination by embedding in paraffin wax, sectioned at 6 microns and stained with hematoxylin and eosin. The sections were examined by light microscopy:

Brain (7 levels) including olfactory bulbs, forebrain (through the septum), center of the cerebrum (through the hypothalamus), midbrain, cerebellum and pons, midcerebellum and medulla oblongata, and medulla oblongata

Spinal cord: cervical, thoracic, lumbar (longitudinal and cross-sections)

It should be noted that these evaluations did not include special neurohistopathology staining (i.e. silver staining).

Results:

Analysis of dose formulation: data analysis of prepared formulations indicated that the prepared solutions were within 10% of the intended dose. There were no results presented to support the stability of the solution within the time frame of the experiment. However, the sponsor indicated that “the analytical method was validated with respect to selectivity, linearity, carry-over, precision and accuracy, injection medium stability, stock solution stability and matrix stability.

The following table prepared by the reviewer summarizes other findings:

| | |
|------------------|--|
| Mortality | 1M from control group was found dead on PND 38 the cause of death was due to gavage error. 1F from LD was found dead on PND 54 no clinical signs or histopathology was found. 1 F treated with MD and 1F treated with HD were found dead on PND 8 and 9, respectively. No adverse effects were noted and no pathological |
|------------------|--|

| | |
|--|---|
| | findings indicated the cause of death. 1M from HD was found dead on PND 19 clinical signs seen prior to death included thinness, decreased activity, moderate dehydration and cold to touch. All the previous deaths were not considered drug related by the sponsor. In the reviewer's view, it is possible that the 1M from HD died due to deteriorating condition caused by poor condition that might be related to drug treatment. |
| Clinical observations | ↑ <u>activity</u> (in both sexes at HD from PND 21 to 63, at MD from PND 22-63 and at LD from PND 44-63), stereotypic behavior such as licking and digging (in both sexes at HD only from PND 22-63), salivation (9/15M at HD, intermittently from PND 49-63). Other individual observations in some animals attributed to drug treatment by the sponsor (1M #451/2 from HD had severe uncoordination on PND 19, 1M from HD group #4111 was thin and had erected fur, 1F #3591 from MD was dehydrated on PND 44). |
| Body wt | ↓ in body wt was observed in M at HD compared to control group from PND 14 to the end of the study (11% on PND 14 and 20% on PND 63) and in F at HD from PND 10 to the end of the study (9% on PND 10 and 13% on PND 63). This decrease in body wt was continuously seen throughout the study (always ≥ 10% compared to control). A statistically significant decrease was seen in M at MD by the end of the study (6%) and a similar decrease was also seen in F (not statistically significant). The decreases at HD in M continued to be seen during the regression or recovery period (from 20% on PND 70 to 13% on PND 91) and to a much lesser extent in F (from 9% on PND 70 to 4% on PND 91). In M treated with MD a slight decrease in body wt was observed during the regression period (9% on PND 70 & 4% on PND 90) but not in F (only 3-4%). |
| Food consumption | Significant reductions at HD between PND 21 and 28 in both M&F and to a lesser extent at MD also. Reductions were also noted in M&F at HD to the end of the study. Reductions at MD were observed but less dramatic than those at HD. |
| Physical development (crown-to-rump measurements) | Crown-to-rump length was statistically significantly reduced in M&F treated with HD from PND 14 to the end of dosing (4-7%). In M the decrease was also seen during the regression period (up to PND 90, from 4-6% compared to control). The decrease in F during the regression period was seen only up to PND 70 (4%). Reductions were seen in M treated with MD on PND 56 and 63 (3-4%). Some reductions were also seen in M treated with LD and MD on PND 70 and 90 (3-4% compared to control). |
| Vaginal opening | There was a delay in the onset of vaginal opening in HD compared to control group (delayed by 1.9-2.2 days) |
| Preputial separation | There appeared to be no drug effect |

| | |
|-------------------------------------|--|
| Ophthalmology | No treatment related findings |
| FOB | There appear to be no drug effect. However, the number of treated animals, especially at HD, that appeared to be lying on the side or curled up were more than seen in control group. This was not seen in the regression group. |
| Motor activity | Total activity counts were statistically significantly decreased in M treated with HD compared to control on PND 22/23 (63%) and in M&F of this group on PND 59/60 (~50%) and in F treated with MD and LD on PND 59/60 (~40%). A decrease in activity was also seen in M at LD and MD on PND 22/23 and 59/60 (~25% & 40%, respectively), but it was not statistically significant. <i>Since activity counts were measured prior to daily dosing, this decrease in total activity in treated animals might be due to exhaustion of the animals caused by the increase of activity seen after treatment.</i> The difference between control and treated group during the recovery period was minimal (~10% decrease from control) and was not statistically significant. |
| Auditory startle habituation | The data indicate a decrease in “startle response at start” for treated animals compared to control and this decrease was still seen in those animals in the regression group (the decrease ranged from ~10-40% compared to control). This decrease was not statistically significant at any time point and was not acknowledged by the sponsor. The effect on “maximum startle” response was not consistent (increases and decreases were observed in the different groups) and no consistent drug effect was seen. There was a statistically significant decrease in “average startle” in M treated with HD (38% compared to control) while a larger decrease (46%) was seen in F but not statistically significant. At the end of the regression period the decrease in average startle seen in M at MD (44%) and HD (32%) compared to control was not dose related nor it was statistically significant. There was no difference between control and treated animals for the effect on time to maximum startle response. See data from sponsor attached immediately after this table. |
| Cincinnati Water maze | Swimming ability as measured by the time to finish the task was comparable between control and treated animals. There was no apparent drug effect upon the number of errors crossing the two paths (path A and path B). Some statistically significant increases were observed in treated animals compared to control in time it took to cross the maze paths and mainly path A. This effect was not as obvious with path B. However, it should be emphasized that the data was highly variable (see the discussion for more details). |
| Hematology | A slight increase in % neutrophils (40-50% compared to control in M&F treated with HD on day 64). Not seen on Day 92. |

| | |
|---|---|
| Clinical chemistry | Some increases were observed (ALP, urea, and phosphorus) mainly at HD in both M&F but according to the sponsor were within the historical control data (HC data were not provided). |
| Organ wt | Decreases in absolute wt of several organs (heart, kidney, liver, pituitary-M only, prostate, spleen, thyroid-F only) at end of dosing at HD but not evident when expressed as relative wt. There were increases relative to body wt in the wts of brain, testes, ovaries and liver (F only) and adrenals (F only). No clear histopath was associated with these findings (see histopath findings). These changes were not seen at the end of the regression period. |
| Histopathology | In two M (#4101 & # 4111) from HD group changes in the kidneys were reported (pyelonephritis and transitional cell hyperplasia, 1 minimal & 1 moderate) with changes seen in the bladder (transitional cell hyperplasia, 1 mild & 1 moderate) and one of those two animals (#4111) had dilatation & inflammation in the ureter (mild). Both of those two animals had inflammation in the prostate (both slight). In F pyelonephritis and hyperplasia of transitional cell (minimal) in the kidney and urinary bladder (slight) was seen in 1 F treated with HD (#4511) and 1F from MD (#3571) with similar severity to that seen in F#4511. In the liver, necrosis and inflammation (minimal) was seen in 1 M (#4011) & 1F (#4511) from HD. In 1 F from HD (#4521) fibrosis with multinucleated cells and supcasular area (moderate) was seen in the liver. Lymph node hyperplasia was seen in treated M at a rate higher than control (in mandibular node 1/10 in control, 3/10 at LD, 3/10 at MD, and 6/10 at HD and in mesenteric 4/10 at HD), in F lymphoid hyperplasia was seen in spleen only (2/10 at HD). These were not considered treatment related by the sponsor; however, a drug effect cannot be ruled out. |
| Neuropathology | No drug effect on the measured parameters (brain wt, width and length and no histopathology findings in the sectioned layers) |
| Estrous cycle | No difference between control and drug treated groups |
| Mating index | No difference between control and drug treated groups except for a lower rate at LD (85.7%) compared to 100% in the control due to the failure of 2 pairs to mate |
| Fertility index | This was 85.7% at LD, 93.3% at MD, and 85.7% at HD compared to 100% in the control group (was not considered biologically significant by the sponsor); however, a drug effect cannot be ruled out. |
| Conception rate | 93.3% at MD and 85.7% at HD and 100% in the control group |
| Maternal performance (gestation index, length of gestation, duration of parturition) | No drug related findings |

Pups and litter observation (F2 generation)

No significant findings

Data for the startle test (startle at start and average startle) as provided by the sponsor in table 14, page 185, volume 12, module 4, sequence 1):

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Table 14 Group Mean Startle Habituation Data
Subgroup A - Main Toxicity - Males
Day 63 Post Partum
Startle at Start (mVolts)

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|-------|-------|-------|-------|-------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 4.95 | 6.23 | 7.36 | 6.54 | 8.56 | 6.73 | 7.53 |
| | SD | 2.69 | 4.45 | 4.45 | 2.68 | 5.93 | 2.01 | 11.72 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 6.89 | 6.01 | 5.41 | 8.79 | 7.42 | 6.90 | 3.84 |
| | SD | 4.47 | 4.58 | 3.78 | 9.07 | 5.06 | 3.64 | 14.97 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 5.90 | 5.53 | 6.04 | 4.60 | 5.90 | 5.59 | -0.93 |
| | SD | 2.79 | 2.34 | 2.84 | 1.81 | 3.37 | 1.91 | 8.55 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 3.93 | 4.48 | 7.33 | 5.92 | 5.91 | 5.51 | 5.40 |
| | SD | 2.55 | 2.78 | 4.14 | 2.31 | 1.67 | 1.69 | 8.20 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

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Table 14 Group Mean Startle Habituation Data
Subgroup A - Main Toxicity - Females
Day 63 Post Partum
Startle at Start

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|-------|-------|-------|-------|-------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 5.86 | 4.76 | 8.75 | 7.95 | 7.08 | 6.88 | 5.63 |
| | SD | 4.76 | 3.38 | 8.82 | 5.92 | 2.91 | 3.77 | 7.49 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 3.83 | 5.38 | 5.42 | 4.14 | 6.48 | 5.05 | 4.06 |
| | SD | 1.95 | 4.05 | 2.77 | 2.48 | 5.35 | 2.80 | 8.91 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 5.49 | 4.37 | 4.98 | 3.85 | 4.28 | 4.59 | -2.94 |
| | SD | 5.78 | 1.58 | 2.59 | 1.68 | 2.82 | 1.81 | 11.87 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 5.76 | 4.77 | 4.96 | 4.41 | 5.18 | 5.02 | -1.52 |
| | SD | 3.63 | 1.33 | 2.40 | 2.07 | 2.90 | 1.39 | 5.56 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

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Table 14

Group Mean Startle Habituation Data
Subgroup B - Regression - Males
Day 63 Post Partum
Startle at Start

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|-------|-------|-------|-------|-------|------------------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 6.22 | 5.72 | 6.50 | 5.37 | 6.22 | 6.01 | -0.36 |
| | SD | 3.28 | 4.13 | 3.77 | 3.94 | 2.15 | 2.30 | 9.55 |
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 2 - NRP104 4 mg/kg/day | Mean | 5.95 | 3.45 | 5.01 | 6.21 | 7.25 | 5.57 $\downarrow 8\%$ | 5.36 |
| | SD | 2.27 | 1.46 | 1.96 | 3.95 | 4.36 | 1.66 | 10.33 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 5.26 | 3.30 | 5.89 | 4.50 | 5.17 | 4.82 $\downarrow 20\%$ | 1.02 |
| | SD | 2.29 | 1.46 | 3.13 | 1.67 | 2.45 | 1.07 | 7.88 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 4.03 | 3.38 | 5.84 | 5.61 | 5.90 | 4.95 $\downarrow 18\%$ | 5.97 |
| | SD | 1.96 | 2.29 | 3.93 | 4.76 | 3.46 | 2.34 | 8.69 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

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Table 14

Group Mean Startle Habituation Data
Subgroup B - Regression - Females
Day 63 Post Partum
Startle at Start

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|-------|-------|-------|-------|-------|------------------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 6.75 | 5.13 | 6.77 | 7.00 | 6.56 | 6.44 | 1.49 |
| | SD | 5.15 | 2.33 | 5.81 | 5.69 | 3.63 | 3.28 | 10.31 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 5.21 | 3.59 | 5.39 | 6.10 | 3.80 | 4.82 $\downarrow 25\%$ | -0.31 |
| | SD | 3.42 | 2.65 | 3.74 | 3.21 | 1.48 | 1.94 | 8.56 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 4.81 | 3.25 | 3.25 | 5.19 | 5.42 | 4.38 $\downarrow 32\%$ | 3.16 |
| | SD | 4.16 | 2.12 | 1.46 | 2.69 | 3.21 | 2.37 | 7.22 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 4.16 | 3.60 | 4.24 | 4.38 | 3.92 | 4.06 $\downarrow 37\%$ | 0.30 |
| | SD | 2.59 | 1.39 | 2.18 | 1.73 | 4.37 | 1.54 | 9.29 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Table 14

Group Mean Startle-Habituation Data
Subgroup B - Regression - Males
Days 90 and 91 Post Partum
Startle at Start

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|-------|-------|-------|-------|-------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 7.41 | 7.75 | 7.18 | 7.61 | 8.35 | 7.66 | 1.74 |
| | SD | 4.01 | 7.45 | 4.36 | 3.10 | 10.22 | 3.91 | 15.74 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 6.76 | 5.19 | 5.41 | 7.68 | 6.11 | 6.23 ↓ 19% | 1.19 |
| | SD | 5.05 | 2.26 | 2.85 | 3.46 | 3.03 | 2.20 ↓ 28% | 7.88 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 5.11 | 6.53 | 6.21 | 5.45 | 4.38 | 5.54 ↓ 28% | -2.54 |
| | SD | 2.94 | 3.23 | 3.70 | 3.07 | 1.75 | 1.64 ↓ 34% | 9.57 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 4.14 | 3.94 | 6.26 | 5.38 | 5.81 | 5.11 ↓ 34% | 4.78 |
| | SD | 2.32 | 2.10 | 3.33 | 2.46 | 6.54 | 1.69 ↓ 34% | 16.46 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Table 14

Group Mean Startle-Habituation Data
Subgroup B - Regression - Females
Days 90 and 91 Post Partum
Startle at Start

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|-------|-------|-------|-------|-------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 7.65 | 7.45 | 7.55 | 7.14 | 5.13 | 6.98 | -5.35 |
| | SD | 5.39 | 5.00 | 4.41 | 5.03 | 3.08 | 3.18 | 15.29 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 5.43 | 5.26 | 6.05 | 5.45 | 5.66 | 5.57 ↓ 20% | 0.65 |
| | SD | 3.61 | 4.69 | 2.97 | 3.11 | 2.83 | 2.17 | 15.09 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 4.71 | 4.28 | 4.86 | 5.66 | 5.11 | 4.92 ↓ 30% | 2.18 |
| | SD | 2.12 | 1.67 | 3.10 | 3.02 | 1.78 | 1.58 ↓ 30% | 4.82 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 4.87 | 6.34 | 6.80 | 5.75 | 6.39 | 6.03 ↓ 14% | -2.45 |
| | SD | 2.26 | 4.09 | 3.65 | 2.93 | 3.54 | 1.78 ↓ 14% | 19.27 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Table 14

Group Mean Startle Habituation Data
Subgroup A - Main Toxicity - Males
Day 63 Post Partum
Average Startle

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|--------|--------|--------|--------|--------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 188.12 | 134.50 | 119.55 | 110.12 | 122.71 | 135.00 | -155.20 |
| | SD | 57.63 | 60.21 | 49.89 | 36.80 | 44.97 | 38.32 | 149.34 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 128.14 | 104.51 | 72.17 | 68.01 | 77.62 | 90.09 | 137.54 |
| | SD | 47.19 | 76.38 | 57.82 | 46.81 | 35.89 | 49.64 | 86.73 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 144.53 | 106.62 | 95.74 | 88.02 | 75.32 | 102.05 | 157.02 |
| | SD | 79.20 | 74.92 | 76.53 | 99.56 | 70.22 | 75.97 | 142.55 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 125.65 | 99.80 | 72.98 | 66.15 | 57.01 | 84.32 | -170.93 |
| | SD | 40.26 | 39.15 | 38.54 | 36.03 | 30.68 | 26.20 | 111.77 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Significantly different from control group (group 1) value: A - P <= 0.05 B - P <= 0.01 C - P <= 0.001 (Dunnett)

Table 14

Group Mean Startle Habituation Data
Subgroup A - Main Toxicity - Females
Day 63 Post Partum
Average Startle

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|--------|--------|--------|-------|-------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 153.67 | 121.13 | 94.39 | 93.18 | 87.76 | 110.03 | -159.77 |
| | SD | 67.66 | 50.37 | 35.45 | 26.88 | 37.39 | 40.13 | 92.78 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 123.85 | 116.92 | 101.32 | 93.91 | 72.47 | 101.69 | -125.77 |
| | SD | 39.09 | 48.96 | 42.11 | 48.58 | 35.09 | 38.53 | 66.62 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 103.50 | 87.80 | 85.37 | 77.77 | 82.38 | 87.36 | -52.27B |
| | SD | 61.90 | 49.78 | 64.79 | 66.29 | 63.66 | 56.56 | 88.64 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 94.73 | 50.57 | 54.19 | 50.22 | 51.81 | 60.30 | -86.19 |
| | SD | 45.47 | 34.06 | 26.79 | 32.35 | 44.70 | 34.85 | 47.02 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Table 14

Group Mean Startle Habituation Data
Subgroup B - Regression - Males
Day 63 Post Partum
Average Startle

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|--------|--------|--------|-------|--------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 193.88 | 109.37 | 103.76 | 97.17 | 83.08 | 117.45 | -233.80 |
| | SD | 139.92 | 72.02 | 56.53 | 71.37 | 55.08 | 66.69 | 227.98 |
| | N | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 2 - NRP104 4 mg/kg/day | Mean | 139.38 | 108.73 | 91.81 | 73.44 | 80.85 | 98.84 | -152.35 |
| | SD | 79.50 | 66.15 | 50.28 | 51.03 | 54.17 | 56.93 | 106.85 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 107.31 | 63.37 | 63.87 | 48.38 | 50.45 | 66.68 | -128.71 |
| | SD | 55.38 | 49.86 | 51.04 | 38.00 | 27.61 | 40.10 | 104.56 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 174.82 | 116.32 | 130.33 | 95.09 | 102.62 | 123.84 | -165.63 |
| | SD | 89.79 | 81.11 | 71.69 | 70.45 | 69.30 | 68.95 | 129.02 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Table 14

Group Mean Startle Habituation Data
Subgroup B - Regression - Females
Day 63 Post Partum
Average Startle

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|--------|-------|-------|-------|-------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 111.62 | 71.81 | 60.20 | 76.37 | 67.17 | 77.43 | -84.34 |
| | SD | 66.21 | 42.74 | 44.80 | 74.21 | 33.74 | 44.18 | 89.99 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 93.84 | 72.01 | 55.05 | 56.48 | 41.83 | 63.84 | -119.55 |
| | SD | 23.58 | 37.66 | 33.82 | 30.26 | 21.65 | 24.74 | 63.53 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 122.56 | 97.26 | 83.04 | 66.14 | 59.25 | 85.65 | -157.74 |
| | SD | 46.92 | 44.83 | 42.70 | 41.43 | 37.05 | 38.94 | 73.35 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 105.17 | 65.48 | 73.47 | 62.98 | 49.23 | 71.27 | -114.38 |
| | SD | 53.12 | 36.17 | 42.04 | 35.22 | 21.62 | 34.23 | 93.70 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Table 14

Group Mean Startle Habituation Data
Subgroup B - Regression - Males
Days 90 and 91 Post Partum
Average Startle

Project No. 900572

| Group | | Trial | | | | | Mean Level | Linear Time Contrast |
|-------------------------|------|--------|--------|--------|--------|--------|------------|----------------------|
| | | 1-10 | 11-20 | 21-30 | 31-40 | 41-50 | | |
| 1 - Vehicle Control | Mean | 216.87 | 178.40 | 167.98 | 130.00 | 130.68 | 164.79 | -220.78 |
| | SD | 79.86 | 78.67 | 71.25 | 56.99 | 59.26 | 60.86 | 161.29 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 - NRP104 4 mg/kg/day | Mean | 228.27 | 174.72 | 145.61 | 123.89 | 120.45 | 158.59 | -266.47 |
| | SD | 310.01 | 209.01 | 157.05 | 119.14 | 102.18 | 167.03 | 663.67 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 - NRP104 10 mg/kg/day | Mean | 145.11 | 80.99 | 82.44 | 70.66 | 83.96 | 92.63 | 132.63 |
| | SD | 58.31 | 49.85 | 77.32 | 61.51 | 61.68 | 56.85 | 111.79 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 - NRP104 40 mg/kg/day | Mean | 173.59 | 95.69 | 97.22 | 99.44 | 90.39 | 111.27 | 162.65 |
| | SD | 95.10 | 66.66 | 73.86 | 79.89 | 67.83 | 72.87 | 92.38 |
| | N | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

Toxicokinetics:

The following table summarizes the plasma levels of both NRP-104 and d-amphetamine in response to treatment with NRP-104 (groups 5, 6, and 7 treated with 4, 10, and 40 mg/kg/day, respectively for 1 day, and groups 2, 3, and 4 treated with 4, 10, and 40 mg/kg/day, respectively for 54 days). AUC values increased more than dose proportional after a single dose and somewhat dose proportionally after multiple dosing of NRP-104 treatment while Cmax appeared to increase dose proportionally. There appeared to be no gender effect. Exposure to d-amphetamine was greater than that of NRP-104. The amphetamine to NRP-104 AUC ratio ranged from between 25.5 (low dose M on Day 63) and 5.93 (high dose M on Day 7). The metabolite to parent ratio generally decreased with increasing dose. Table 3 page 50, vol. 12, Module 4 Sequence 1.

Text Table 3: Toxicokinetic Parameters

| Analyte | Occasion | Group | T _{max} h | C _{max} ng/mL | T _{last} h | t _{1/2el} h | AUC _{0-inf} h*ng/mL |
|---------------|----------|-------|-----------------------|---------------------------|------------------------|-------------------------|---------------------------------|
| NRP104 | Day 7 | 5M | 1 | 24.2 | 6 | 1.30 | 55.2 |
| | | 6M | 1 | 100 | 12 | 3.32 | 221 |
| | | 7M | 1 | 348 | 12 | 2.52 | 772 |
| | | 5F | 0.57 | 23.0 | 3 | 0.798 | 42.4 |
| | | 6F | 1 | 72.6 | 6 | 1.18 | 147 |
| | | 7F | 1 | 292 | 12 | 2.45 | 823 |
| | Day 63 | 2M | 0.5 | 12.7 | 1 | - | - |
| | | 3M | 0.5 | 43.5 | 3 | 0.636 | 70.8 |
| | | 4M | 1 | 144 | 6 | 0.848 | 333 |
| | | 2F | 0.5 | 27.8 | 1 | - | - |
| | | 3F | 0.5 | 78.5 | 3 | 0.478 | 82.8 |
| | | 4F | 0.5 | 245 | 6 | 1.48 | 534 |
| d-Amphetamine | Day 7 | 5M | 3 | 83.9 | 12 | 3.81 | 603 |
| | | 6M | 3 | 199 | 12 | 3.41 | 1500 |
| | | 7M | 3 | 737 | 12 | - | - |
| | | 5F | 3 | 68.2 | 12 | - | - |
| | | 6F | 3 | 211 | 12 | 3.81 | 1417 |
| | | 7F | 3 | 838 | 12 | 3.47 | 6252 |
| | Day 63 | 2M | 1 | 37.8 | 12 | 2.60 | 217 |
| | | 3M | 1 | 124 | 12 | 2.04 | 705 |
| | | 4M | 3 | 610 | 12 | 2.57 | 3425 |
| | | 2F | 1 | 62.3 | 12 | 1.88 | 345 |
| | | 3F | 1 | 165 | 12 | 2.10 | 878 |
| | | 4F | 3 | 911 | 12 | 3.59 | 6157 |

2. Study title: NRP-104: a 2-week dose range-finding and 26-week oral (gavage) toxicity study in the juvenile beagle dog

Key study findings:

Study no.: D07-NRP104-JT-24

Volume #, and page #: vol. #19 module 4 sequence 1

Conducting laboratory and location: _____

Date of study initiation: December 2004

GLP compliance: yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: N040EH, N039EH, purity is ----- % for both

Formulation/vehicle: solution/deionized water

Methods

Doses: 0, 2, 5, and 12 mg/kg/day

Basis for dose selection: the dose was selected for this study based on the findings from a dose finding study (Phase I study) and findings from a 28-day study in adult animals.

In the dose finding study (Phase I study), 10-week old dogs (2/sex/group) were treated with 0, 3, or 10 mg/kg/day of NRP-104 orally by gavage (10 ml/kg) for 14 days. There were no mortalities reported. Clinical findings seen between 2 and 6 hours post dosing at 10 mg/kg/day included increased activity (all animals), pacing (2/2 M, 1/3F), circling (2/2 M, 1/3F), head shaking (1/2 M, 2/2F) and vocalization (all animals). These observations continued for up to 6h post dosing and were generally repeated on each day of the dosing period. A decrease in mean body wt compared to control was seen at 10 mg/kg/day by day 15 in M (13%) and in F (12%). Gross pathology findings seen as single dark, firm raised areas on the atrioventricular valve were seen for both M and 1/2 F dosed with 10 mg/kg/day (these findings were considered to be spontaneous in origin by the sponsor because they were not seen in the definitive study at 12 mg/kg/day).

In the 28-day study in adult dogs doses used were 0, 3, 6, and 12 mg/kg/day. At 12 mg/kg/day restlessness was seen in all animals almost throughout study, increased activity in all animals throughout the study, repetitive behavior (most of the animals on first day only), head shaking (1-4/5 animals on several occasions), pacing and racing (1-4/5 animals throughout the study), and abnormal gait (1-2/5 animals on several occasions). A decrease in body wt of 16% in M and 20% in F compared to control group was observed. Based on the findings from the dose finding study and from the observations in the 28-day study in adult animals the sponsor chose the following dose levels for the definitive study 0, 2, 5, and 12 mg/kg/day.

Study design for the definitive study (Phase II study):

10-Week old beagle dogs were used for the definitive study. The following table provided by the sponsor summarizes dose levels, animals used and subgroups (text table 4, page 23, vol. 19, Module 4, Sequence 1):

Text Table 4 Study Design - Phase II, Main Study and Recovery

| Group No. / <u>Identification</u> | Dose Level (mg/kg/day) | Concentration (mg/mL) | Animal Number | | | |
|--------------------------------------|---------------------------|--------------------------|-------------------|----------------|-------------------------------|----------------|
| | | | Toxicity Subgroup | | 4-Week Regression Subgroup | |
| | | | <u>Males</u> | <u>Females</u> | <u>Males</u> | <u>Females</u> |
| 1 Control | 0 | 0.0 | 101-104 | 151-154 | 105-108 | 155-158 |
| 2 Low Dose | 2 | 0.2 | 201-204 | 251-254 | 205-208 | 255-258 |
| 3 Mid Dose | 5 | 0.5 | 301-304 | 351-354 | 305-308 | 355-358 |
| 4 High Dose | 12 | 1.2 | 401-404 | 451-454 | 405-408 | 455-458 |

The following table was prepared by the reviewer to summarize the different measurements/parameters evaluated and the time and frequency of these measurements:

| parameter | time/paradigm of parameter investigation |
|---|---|
| Clinical observations | Twice daily for mortality and ill health signs or reaction to treatment. Detailed examination once weekly. |
| Body wt | Twice weekly |
| Growth measurements | Nose to tail and height measurements |
| Ophthalmology | Pretreatment, weeks 4, 13, and 26 and during week 4 for the recovery animals. Funduscopy and biomicroscopic examinations |
| Electrocardiography | Pretreatment, weeks 4, 13, and 26 and during week 4 for recovery animals (<u>all before dosing</u>) leads I, II, III, aVR, aVL, and aVF. |
| Observational battery | Qualitative measures (see list later) during weeks 1, 4, 8, 13, 18, 22, and 26 prior to daily dosing and during week 4 of the recovery period |
| Neurological examination | Once pretreatment, during Weeks 1, 4, 8, 13, 18, 22, and 26 prior to daily dosing and during Week 4 of the recovery period (see list of tests later) |
| Laboratory test (hematology, clinical chemistry, and urinalysis) | Pretreatment, during Weeks 4, 8, 13, and 26 of treatment and during Week 4 of recovery. Animals were fasted for 3-5h for the pretreatment and the Week 4 measurements and overnight for the other times. See list of measured parameters later. Blood samples were collected for hormone assay during Week 13 (from 8:30 to 9:00) and 26 (from 10:30 to |

| | |
|-------------------------------------|---|
| | 11:30) and during Week 4 of recovery (from 9:30 to 10:30). Urine was collected either for 7-8h (pretreatment) or over night (Week 4, 8, 13, and 26). Animals were fasted for the 8, 13, and 26 and during the 4-week recovery period collection times only. |
| Male reproductive assessment | Towards the end of the study (PND 154-180), semen samples were collected approximately 3 days apart to assess for volume, color, appearance, sperm concentration (millions/ml and millions/ejaculate), motility (percent motile sperm based on a 200 sperm count) and morphology (two slides, the % of abnormal sperm/100 sperm observed/slide) |
| Gross pathology | Animals were fasted overnight before necropsy which consisted of external examination, including identification of clinically recorded lesions, as well as a detailed internal examination |
| Organ weights | See list of organs listed later |
| Histopathology | See list of tissues prepared for evaluation later from all animals. Tissues were embedded in paraffin wax, sectioned and stained with hematoxylin and eosin. Testes were stained with PAS hematoxylin and examined for spermatogenic cycle (staging) on the right testicle |
| Toxicokinetics | Blood collected on first day of treatment, during Week 16 and on the last day at the following time points: 0.25, 0.5, 1, 3, 6, and 12h post dose. |
| | |

The following parameters were evaluated for the following tests:

Observation Battery:

The qualitative observational battery consisted of the following tests:

| | | |
|---------------------------------|---------------------------|------------------------|
| Abnormal body position/ posture | Piloerection | Pupil size |
| Activity level | Respiration rate/ pattern | Palpebral closure |
| Bizarre / stereotypic behavior | Urination/ defecation | Nystagmus/exophthalmos |
| Convulsions | Lacrimation | Diarrhea |
| Muscle tremors/ twitches | Salivation | Vocalization |

Neurological examination:

The neurological examination consisted of the following tests:

| General Attitude and Behavior | Postural Reactions | Cranial Nerves |
|--------------------------------------|----------------------------|---------------------------|
| Gait | Proprioceptive Positioning | Head – movements/symmetry |
| | Hemihopping/Hemistanding | Head Muscle Tone |
| Spinal Nerves | Wheelbarrowing | Eye Reactions |
| Muscle Tone | Hopping | Eye Symmetry |
| Patellar Reflexes | Placing Reactions-Visual | Vestibular Nystagmus |
| Flexor Reflexes | Placing Reactions-Tactile | Eye Position |
| Panniculus Reflex | Righting Reaction | Corneal Reflex |
| Perineal Reflex | | Pupillary Light Reflex |
| | | Nasal Septum Test |
| | | Mouth Test |
| | | Tongue Test |
| | | Pharynx Test |

Hematology:

Parameters examined:

activated partial thromboplastin time*
blood cell morphology
erythrocyte indices (MCV, MCH, MCHC and RDW)
hematocrit
hemoglobin
mean platelet volume
platelet count
prothrombin time*
red blood cell count
reticulocyte count (absolute and percent)
white blood cell count (total, absolute and percent differential)

For all euthanized animals, 3 femoral bone marrow smears were prepared, stained and evaluated (500 cell count).

Clinical Chemistry:

A/G ratio (calculated)
alanine aminotransferase
albumin
alkaline phosphatase
aspartate aminotransferase
blood urea nitrogen
calcium
chloride
cholesterol
creatinine
globulin (calculated)
glucose
inorganic phosphorus
potassium
sodium
total, direct and indirect bilirubin
total protein
triglycerides

Hormone assays:

Parameters examined:

LH

FSH

testosterone[@]

progesterone[@]

estradiol[@]

prolactin

[@] Validation of the analysis of these parameters is ongoing. Note to file has been issued.

Urinalysis:

Parameters examined:

bilirubin

blood

color and appearance

glucose

ketones

microscopy of centrifuged deposit

nitrite

pH

protein

specific gravity

urobilinogen

volume

Organ Weights:

The following organs were weighed

adrenal glands
brain
heart
kidneys
liver
lungs
ovaries/testes
pituitary
prostate
spleen

thymus
thyroid lobes (with parathyroids)

Paired organs were weighed separately. Organ weight ratios relative to brain weights were calculated.

Tissue preservation:

The following tissues and organs were retained in neutral buffered 10% formaline (unless otherwise indicated):

abnormalities
animal identification^a
adrenals
aorta (thoracic)
bone and marrow (sternum)^b
brain [unilateral sections to include: caudate putamen, cerebral cortex, piriform cortex,
thalamus/hypothalamus, hippocampus, midbrain, cerebellum and medulla oblongata]
cecum
colon
cranial nerve (trigeminal nerve)^{*}
duodenum
epididymides
esophagus
eyes^d
gallbladder
heart (including section of aorta)
ileum
jejunum
kidneys
liver (sample of 2 lobes)
lungs^c
lymph nodes (mandibular unilateral and mesenteric)
mammary gland (inguinal)⁺
meninges⁺⁺
optic nerves^d
ovaries

pancreas
 peripheral nerves [bilateral: longitudinal and cross-sections of sciatic, sural and tibial (at knee)]
 pituitary
 prostate
 salivary gland (mandibular unilateral)
 skeletal muscle [longitudinal and cross-section of gastrocnemius]
 skin (inguinal)
 spinal cord [longitudinal and cross-sections of cervical, thoracic and lumbar]
 spleen
 stomach
 testes[#]
 thymus
 thyroid lobes (and parathyroids)⁺
 tongue
 trachea
 urinary bladder
 uterus (cervix, horns and body)
 vagina

^a Retained but not processed.

^b Bone decalcified prior to sectioning.

^c Infused with neutral buffered 10% formalin (all animals).

^d Fixed in Zenker's solution

⁺ Examined histopathologically only if present in routine sections of thyroid lobes (parathyroid glands), or skin (mammary gland).

⁺⁺ Meninges included the dura mater (submitted on a piece of plastic), arachnoid and pia mater (submitted with brain).

^{*} Due to the small size of the left and right cranial nerve (trigeminal nerve), only the longitudinal section was examined histologically.

[#] Fixed in Bouin's fluid (all animals).

For all euthanized animals, 3 femoral bone marrow smears, were prepared, stained and evaluated (500 cell count).

Results:

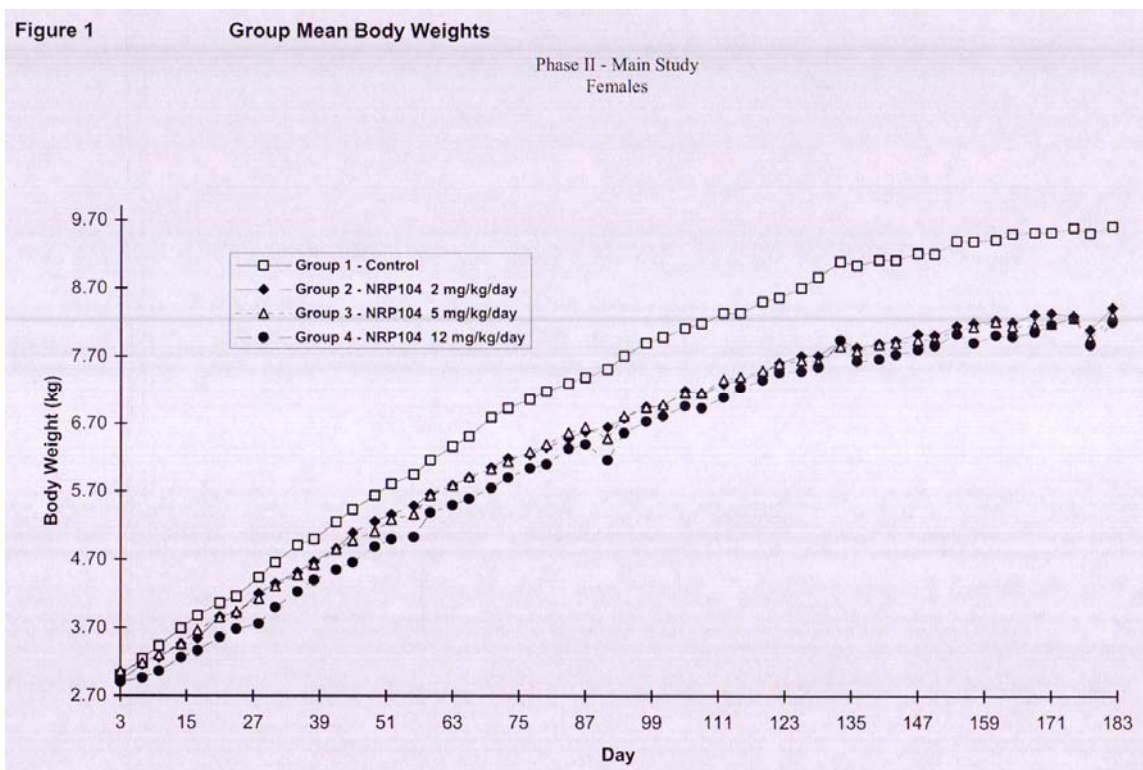
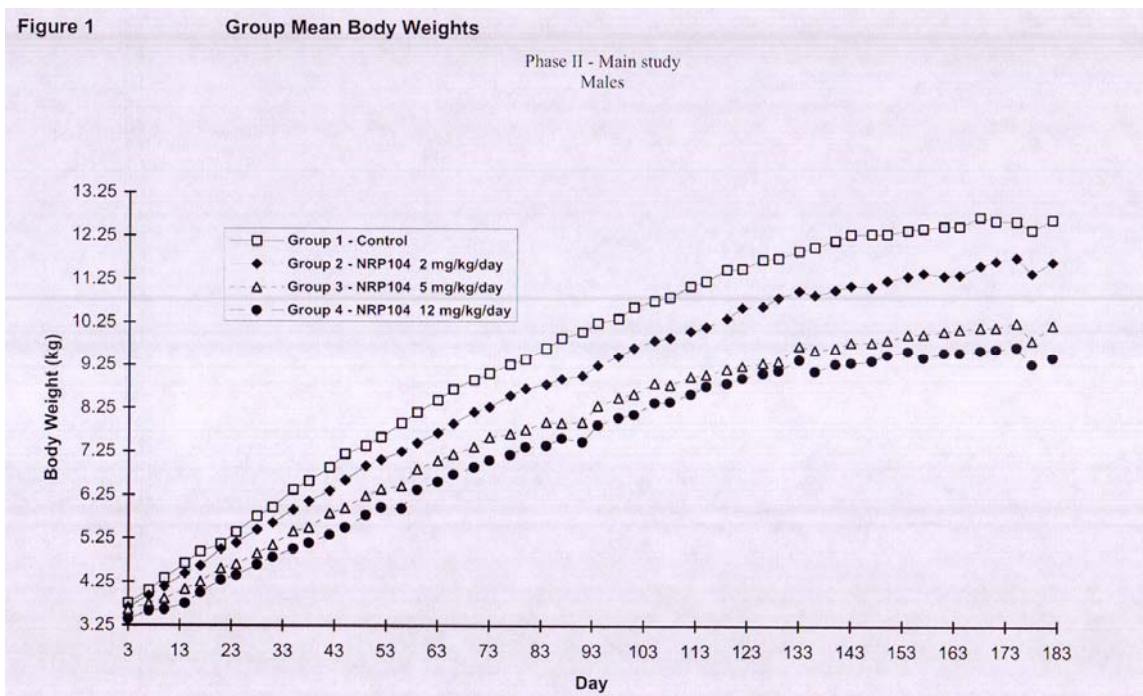
| Definitive study (Phase II study) | |
|-----------------------------------|---|
| Mortality | No mortalities were observed (one F #457 was entangled in water supply apparatus and was euthanized as moribund on Day 141) |
| Clinical observations | Stereotypic behavior such as head searching/bobbing/shaking , pacing in cage with repetitive pawing was seen in all M & F, circling (2/8 M at LD, 7/8 M & 4/8 F at MD, 6/8 M and 7/8 F at |

| | |
|---|---|
| | <p>HD), not responsive to humans and not socializing in cage (all treated M & F), vocalization and yelping (6/8 M & F at LD, 8/8 M & 7/8 F at MD, and 8/8 M & F at HD, walking or stumbling into objects (all treated M & F), increased activity was seen in all M including control but in F it was seen in all treated animals only. <u>These observations were seen between 2-6 h post dosing during the entire study.</u> Increased incidence of decreased activity and tremors was observed prior to dosing in the MD and HD animals. The majority of M in the MD and HD and all treated F were described as being thin. Dosing was suspended on Days 7 and 8 for F # 452 and on Day for F #454 in HD group because of clinical findings of increased or decreased activity, moderate/sever tremors, head shaking and/or weakness however these observations did not appear with the resumption of dosing. During regression decreased activity was seen in animals treated with MD (4/4 M & F) and HD (3/4 M & F), tremors were also seen in some animals treated with MD (1/4 M) and HD (2/4 M & 1/4 F).</p> |
| Body wt | <p>A decrease in body wt was seen in M and F treated with MD and HD from Day 10 or 14 to the end of the study (by the end of the study the decrease in M was 19% at MD and 25% at HD and in F the decrease was 14% at MD and 15% at HD compared to the control group). See figure of the effect on body wt during study attached in this review. Full recovery of the effect on body wt in treated animals compared to the control group was not seen at the end of the regression period.</p> |
| Growth measurements | <p>There were no significant differences in length or height for the treated M or F compared to control.</p> |
| Ophthalmology | <p>No treatment related findings</p> |
| Electrocardiography | <p>No treatment related findings. It should be noted that measurements did not coincide with Cmax since all measurements were done before dosing.</p> |
| Functional observational battery | <p>Muscle tremors (slight to moderate) seen in M & F of all groups but seen in more animals at MD & HD starting around week 4. Towards the end of the study these observations were seen mostly in animals treated with MD & HD (2-7/8 animals). These were not seen during the regression period. Treated dogs tended to be sleeping more than the control animals during observations which could be due to the hyper activity seen after dosing.</p> |

| | |
|--|--|
| Neurological observations | No significant findings that indicate a drug effect. |
| Hematology | ↑ platelet levels at week 4 (both M & F ~41% at HD compared to control), this increase continued to be seen to a somewhat less extent to the end of treatment (between 20-49%) but according to the sponsor was within the historical control (historical control data were not provided). The ↑ continued to be seen during regression but to a much less extent (14% in M and 27% in F). No effects on PT or APTT were observed (except for a 13% decrease in M treated with HD compared to control at week 26 only). |
| Clinical Chemistry | A slight increase in triglycerides was seen in M at MD (40% compared to control) and HD (53%) and in F at HD (31%) during week 4 only. An increase was seen in ALP in M at MD (34%) and HD (38%) and in F at HD (39%) during week 26. An increase in ALP (45%, not statistically significant) was seen in M at HD only during regression period. Other statistically significant findings were observed (mostly decreases in some parameters) that did not indicate a drug effect were observed. |
| Hormonal levels | No drug related findings. The levels of some of these hormones (leutinizing hormone and progesterone for both sexes and follicle stimulating hormone in M) were occasionally below the level of detection. A wide variability was observed between individual animals. |
| Urinalysis | Lower urine volume and a higher specific gravity at MD and HD in both M and F almost throughout the study |
| Male reproductive system assessment | The data were very variable and the samples collected were not from all animals. It seems that because of the sexual immaturity of the animals at this stage in the study, samples could not be collected from all the animals on many occasions. According to the sponsor, sample collection was unsuccessful on many occasions for many animals regardless of their group, the ejaculated volume was too small to be analyzed or interpreted and/or the samples had too low a concentration of spermatozoa to perform the sperm motility assessment. The sponsor stated that "In animals/sample occasions where sufficient ejaculated volume/spermatozoa counts were produced, the administration of the NRP-104 did not appear to induce changes on the sperm motility, spermatozoa |

| | |
|---------------------------------|---|
| | counts or spermatozoa morphology". The reviewer generally agrees with this statement; however, it will be difficult to conclude that the drug does not have an effect on the male reproductive system in view of quality of the data on some occasions (n=1 sometimes). However, if we considered that in those animals with the appropriate sample the sperm count/ml was a good reflection of the group then we can come to a similar conclusion that the sponsor has come to. However, it will be more appropriate if the data were more consistent and the sample size was larger. |
| Organ weights | Decreases in absolute wt of several organs (brain, liver, lung, spleen, and thymus) were seen during treatment, which could have been related to the decrease in body wt. ↓ in relative wt/brain wt for spleen in M treated with MD (47% compared to control) and HD (43%) and a relative wt/brain wt for thymus at HD (41%). ↑ in relative wt/brain wt of thymus in F at HD (38%). After regression period relative wt/brain wt of thymus was increased in M at MD (78%) and HD (155%) and in F at MD (48%) and HD (100%). ↑ in relative wt/brain wt of adrenal gland in all treated F at the end of regression period (33-35% compared to control). |
| Gross and histopathology | In F treated with MD and HD mottled lymph nodes were described for 2/4 animals and 3/4 F from those groups were described to have erythrocytosis/hemorrhage in lymph nodes. It is not clear if these were drug related or stress related. No other drug related findings were observed. |

The following graphs for the effect on body wt during the study in both M and F were obtained from the sponsor (pages 45-46, vol. 19, Module 4, Sequence 1):



Toxicokinetics:

The increase in C_{max} and AUC seen for both the parent compound (NRP-104) and the metabolite (d-amphetamine) was mostly dose proportional. There seems to be a slight accumulative effect with repeated dosing for both parent and metabolite. The terminal half life of the parent appeared to increase at HD. This was not obvious for the metabolite. There were no gender differences for both the parent and the metabolite. The following summary table was provided by the sponsor (page 1922, vol. 24, module 4 sequence 1):

Text Table 1

Summary Toxicokinetic Parameters

| <u>Analyte</u> | <u>Occasion</u> | <u>Group</u> | <u>T_{max}</u> <u>h</u> | <u>C_{max}</u> <u>ng/mL</u> | <u>T_{last}</u> <u>h</u> | <u>t_{1/2el}</u> <u>h</u> | <u>AUC_{0-inf}</u> <u>h*ng/mL</u> |
|----------------|-----------------|--------------|------------------------------------|--|-------------------------------------|--------------------------------------|--|
| d-Amphetamine | D1 | 2M | 1.50 | 39.9 | 12 | 2.27 | 210 |
| | | 3M | 2.00 | 131.5 | 12 | 2.89 | 784 |
| | | 4M | 3.00 | 392.0 | 12 | 2.15 | 2340 |
| | | 2F | 1.00 | 46.4 | 11 | 2.38 | 214 |
| | | 3F | 1.00 | 101.4 | 12 | 2.39 | 472 |
| | | 4F | 3.00 | 275.0 | 12 | 2.11 | 1695 |
| | D106-107 | 2M | 2.50 | 62.3 | 12 | 2.56 | 399 |
| | | 3M | 2.50 | 153.8 | 12 | 2.59 | 1026 |
| | | 4M | 3.00 | 372.0 | 12 | 3.17 | 2780 |
| | | 2F | 1.50 | 65.8 | 12 | 2.67 | 411 |
| | | 3F | 2.00 | 146.5 | 12 | 2.44 | 903 |
| | | 4F | 3.00 | 383.8 | 12 | 2.58 | 2638 |
| | D182-183 | 2M | 3.00 | 68.6 | 12 | 2.77 | 454 |
| | | 3M | 3.00 | 174.5 | 12 | 2.98 | 1230 |
| | | 4M | 3.00 | 441.3 | 12 | 2.92 | 2861 |
| | | 2F | 2.50 | 68.6 | 12 | 2.62 | 436 |
| | | 3F | 3.00 | 165.5 | 12 | 2.64 | 1071 |
| | | 4F | 3.00 | 532.8 | 12 | 2.51 | 3542 |
| NRP104 | D1 | 2M | 0.63 | 164.9 | 3 | 0.514 | 256 |
| | | 3M | 0.50 | 376.0 | 9 | - | - |
| | | 4M | 0.75 | 1292.0 | 6 | 0.584 | 1898 |
| | | 2F | 0.50 | 155.3 | 4 | 0.485 | 213 |
| | | 3F | 0.63 | 437.3 | 8 | 0.443 | 654 |
| | | 4F | 0.63 | 863.0 | 6 | 0.669 | 1402 |
| | D106-107 | 2M | 0.50 | 297.0 | 3 | 0.339 | 350 |
| | | 3M | 0.50 | 515.0 | 4 | 0.508 | 719 |
| | | 4M | 0.63 | 1509.0 | 8 | 0.598 | 2070 |
| | | 2F | 0.50 | 293.0 | 3 | 0.324 | 319 |
| | | 3F | 0.50 | 774.0 | 4 | 0.325 | 909 |
| | | 4F | 0.63 | 1468.8 | 8 | - | - |
| | D182-183 | 2M | 0.58 | 240.5 | 3 | 0.477 | 323 |
| | | 3M | 0.63 | 666.5 | 4 | 0.382 | 982 |
| | | 4M | 0.50 | 1623.3 | 6 | 0.563 | 2469 |
| | | 2F | 0.50 | 270.8 | 3 | 0.380 | 324 |
| | | 3F | 0.50 | 820.3 | 5 | 0.326 | 1102 |
| | | 4F | 0.63 | 1997.8 | 6 | 0.533 | 2544 |

2.6.6.9 Discussion and Conclusions:

A preliminary dose finding study in pup rats was conducted to establish the dose for the definitive study. In this study the pups (12/sex/group) were treated orally by gavage with NRP-014 (0, 4, 15, and 40 mg/kg/day) from PND 7 to PND 30 inclusive. There were no mortalities reported. Increased activity (described as increased cage exploration, excessive grooming, stereotypic sniffing, biting of the cage floor, rapid head turning, increased rearing, sniffing and/or spatial disorientation) was observed in animals treated with 15 or 40 mg/kg/day from PND 21 to the end of the study. Mean body wts were decreased compared to the control group at MD in M (8%) and F (12%) and at HD in M (18%) and in F (22%). These doses were considered appropriate for the definitive study by the sponsor and the reviewer concurs.

In the definitive study, Sprague Dawley pups (60/sex/group) were dosed with 0, 4, 10, and 40 mg/kg/day of NRP-104 orally by gavage (10 ml/kg/day) from PND 7 to 63 inclusive. In the main study (Phase I), each dose group was subdivided into 4 subsets (15/sex/group) according to the following assignments:

- subgroup A (toxicity study) animals were treated from PND 7 to 63. Animals were evaluated for: clinical observation, detailed examinations, body wt, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, gross pathology, organ wt, histopathology, physical development (crown-to-rump), functional observation battery (FOB), motor activity, and auditory startle habituation. These evaluations were conducted during or at the end of the treatment period (see the review for exact timing). Animals were sacrificed on PND 64.
- subgroup B (regression study): in addition to evaluations done during the treatment period (PND 7-63) such as physical development, preputial separation and vaginal opening, animals were evaluated for the following at the end of a 28 day regression period: FOB, motor activity, auditory startle habituation, and Cincinnati water maze (see the tables within the review for exact timing of these evaluations and other conducted tests). Animals were sacrificed on PND 92
- Subgroup C (reproductive study): animals were treated from PND 7-63 and then were mated at approximately 85 days of age. The animals were evaluated for the Cincinnati water maze between PND 52-61, for estrous cycle 10 days prior to mating, mating (PND 85) and then they were sacrificed after Day 26-28 post coitum. Paternal performance (mating index, fertility index and conception rate) and maternal performance (gestation index, duration of parturition, # of pups at birth, and #of implantation scars) were also evaluated. The F2 generation pups and litters were observed for death, external malformations, weighed, sexed, and were observed through the lactation period.
- subgroup D (toxicokinetic study): blood was collected on PND 64

Phase II study was a toxicokinetic study in which pups (15/sex/group) were treated for one day only (PND7) and blood samples were collected before they were sacrificed.

There were deaths observed in all groups (1M from control group due to gavage error, 1F from LD, 1F from MD and 1M & 1F from HD group), all of which the sponsor considered as non-drug related. The reviewer considers the death of the 1M from the HD group as possibly drug related since clinical signs seen prior to death included thinness, decreased activity, moderate dehydration, and cold to touch which might indicate that the death was due to deteriorating condition caused by drug treatment. Increased activity was observed in both M and F from HD group from PND 21 to the end of the treatment period and at MD from PND 22 to the end of treatment and at LD from PND 44 to the end of treatment. Stereotypic behavior was observed in both M and F at HD only from PND 22-63 and salivation was observed in some animals intermittently from PND 49-63. Some individual observations in some animals such as severe uncoordination, thin condition, and dehydration were observed on single days.

A decrease in body wt compared to the control was observed in M at HD (11-20%) and in F (9-13%) an effect that was seen continuously in both sexes from either PND10 or 14 to the end of the treatment period. A decrease in body wt compared to control was seen in M at MD by the end of the study (6%). A decrease in body wt compared to control was still seen in M treated with HD at the end of the regression period (13%) and to a much less extent in F treated with HD (4%). A slight decrease was seen in M treated with MD at the end of the regression period (4%).

Based on these observations the doses used in the definitive study are considered adequate and an MTD was reached.

The length of the crown-to-rump was reduced in M&F treated with HD (4-7%) from PND 14 to the end of dosing and in M the effect was still seen during the regression period (about the same amount up to day 90). Some reductions were also seen in M treated with LD and MD on PND 70 and 90 (3-4% compared to control).

There was a delay in the onset of vaginal opening in F treated with HD compared to control (delayed by 1.9-2.2 days). There appears to be no drug effect on preputial separation in M.

There appears to be no drug effect on FOB; however, the number of treated animals, especially at HD, that were observed to be lying on the side or curled up were more than seen in the control group. This was not seen at the end of the recovery period.

Total activity counts were statistically significantly decreased in M treated with HD compared to control on PND 22/23 (63%) and in M&F of this group on PND 59/60 (~50%) and in F treated with MD and LD on PND 59/60 (~40%). A decrease in activity was also seen in M at LD (~25%) and MD (~40%) for both PND 22/23 and 59/60, but these decreases were not statistically significant. *Since activity counts were measured prior to daily dosing, this decrease in total activity in treated animals might be due to exhaustion of the animals caused by the increase of activity seen after treatment.* The difference between control and treated group during the recovery period was minimal (~10% decrease from control) and was not statistically significant.

The data suggest a decrease in “startle response at start” for treated animals compared to control and this decrease was still seen in those animals in the regression group at the end of the recovery period (the decrease ranged from ~10-40% compared to control in the different groups. This decrease was not statistically significant at any time point and the sponsor did not acknowledge it. The reviewer’s interpretation of the data is that treated animals had a decreased startle response at the start as seen with the lower startle response values at start in treated animals compared to the control even though this decrease was not statistically significant. This could be due to the lower activity in those animals (see motor activity earlier) since these measures were also conducted prior to dosing. However, this proposal does not fully explain why there was still a difference in the start startle response in the treatment group at the end of the regression period compared to control even though there was no difference in the motor activity of the treated and the control groups at this time of the study. The effect on “maximum startle” response was not consistent (increases and decreases were observed in the different groups) and no clear drug effect was seen. There was a statistically significant decrease in “average startle” in M treated with HD compared to control (38%) in Subgroup A while a larger decrease (46%) was seen in F treated with HD of the same group but this decrease was not statistically significant. In the same subgroup (A) a decrease in M treated with LD (33%) and MD (24%) was not dose related nor was statistically significant. At the end of the regression period the decrease in average startle seen in M at MD (44%) and HD (32%) compared to control was not dose related nor it was statistically significant. The sponsor stated that the decrease in average startle observed in M at PND 63 in Subgroup A was not seen in the regression group (Subgroup B) at the same time of the study, suggesting that this finding in M might not be consistent and that this could be due to the “small size of the sample”. The sponsor pointed out that the habituation pattern was unaffected by treatment (the different groups seemed to habituate to the situation in a similar pattern as judged by the linear time contrast). There was no difference between control and treated animals for the effect on time to maximum startle response. Therefore, the general interpretation of the data is that the drug seems to have an inhibiting effect on the response to an auditory stimulus as judged by the decrease in the startle response at start in all groups and in the average startle (at HD) compared to the control group even though the exact mechanism by which this is done is not clear. This effect was not seen in the average startle at the end of the regression period but the trend of decrease was still seen in the startle at start response at the end of the regression period. The meaning of this finding to humans is not clear.

The data from the Cincinnati water maze test were highly variable and even though the sponsor considered that there is no drug related effect, in the opinion of the reviewer a drug effect cannot be ruled out. In the opinion of the reviewer it looks that the treated animals seemed to take longer time in crossing the path compared to the control group especially on the first path they were tested on (see later for more details). The data as they were examined by the reviewer reflected that the treated animals on several occasions might have been less able to successfully complete the maze path in a short time especially during their first exposure to the test (path A) than the control animals. However, it should be mentioned that during the testing on a second path (path B), which

the animals were exposed to after path A, they seemed to be less different from the control animals compared to when they were tested on path A. The Cincinnati water maze test measures the time it takes the animal to complete a certain task and the number of errors made by the animals in finishing this task. It should be mentioned that there was no difference between the control and the treated animals in the number of errors encountered during the test. In addition, an effect on motor activity could be ruled out since there was no difference between control and treated animals in swimming a straight line. However, the data from the maze test, as mentioned earlier, suggested that there might be a difference in the number of animals in the treated groups compared to the control being able to finish the task in a shorter time. However, it should be emphasized that the data were variable among the different groups and there was no statistically significant difference between the groups. It is possible that the sample size was not enough to detect the drug effect and that a larger sample size might be needed to observe the drug effect.

A slight increase in % neutrophils (40-50% compared to control in M&F treated with HD on day 64). Not seen on Day 92.

Some increases were observed (ALP, urea, and phosphorus) mainly at HD in both M&F but according to the sponsor were within the historical control data (HC data were not provided).

Histopathological findings in the kidneys, the bladder, ureter, the liver, and the Lymph nodes were seen only at HD or at a higher rate in the HD group (see review for details). These were not considered treatment related by the sponsor. The reviewer listed these findings here since they indicated that they might be drug related since they are seen in both M and F and at the HD only or at higher incidence at the HD especially those in the liver.

There was no effect of the drug on estrous cycle length. The effect on mating index, fertility index, and conception rate is summarized in the following table as prepared by the reviewer.

| Group | # placed for mating | | # mating | # of F pregnant | Mating index | Fertility index | Conception rate |
|-------|---------------------|----|----------|-----------------|--------------|-----------------|-----------------|
| | M | F | | | | | |
| 1 | 15 | 15 | 15 | 15 | 100 | 100 | 100 |
| 2 | 14 | 14 | 12 | 12 | 85.7 | 85.7 | 100 |
| 3 | 15 | 15 | 15 | 14 | 100 | 93.3 | 93.3 |
| 4 | 14 | 14 | 14 | 12 | 100 | 85.7 | 85.7 |

The effect on mating index and the fertility index at LD was due to the failure of two of pairs from mating. The effect on the fertility index and the conception rate at MD and HD, even though small, might indicate a drug effect. It is not clear; however, if the effect observed is due to an effect on male fertility or female fertility. It should be pointed out that there was no evaluation for the male sperm count, viability, and motility in this study. However, it is also important to point out that there was no effect on maternal

performance (gestation index, length of gestation, number of implantation scars, duration of parturition, and live birth index). There was no effect on the pups of the F2 generation (malformations, viability, clinical condition, and pup wt).

The study is considered adequate as for the different parts that were conducted (toxicity, reproduction, and neurobehavioral) and for the doses used (0, 4, 10, and 40 mg/kg/day). The length of treatment (from PND 7 to 63 inclusive) was appropriate for the tests evaluated and for the age of the intended population (children of 6-12 years of age). The doses used are considered adequate and the HD is considered the MTD based on the possibility of a drug related death, the clinical signs observed at HD and the effect on body wt. The immediate effects of the drug observed in the toxicity study (increased activity and stereotypic behavior) are similar to those of an amphetamine. In addition, the effect on body wt is also similar to what is usually observed with amphetamine.

It was clear from the results that the test article had an effect on the growth of pups as judged by the decrease in length of the crown-to-rump at HD in both M & F. A decrease in the other M treated groups (LD & MD) was also seen towards the end of the study. The decrease seen in M at HD was still seen at the end of the regression period. Therefore, it appears that the drug might have an effect on the growth of pups treated for that length of time. However, it seems from the data that the decrease in body wt and the decrease in the length of the crown-to-rump measurements are correlated in their occurrence in the different groups. Therefore it is not clear if the effects on the length of the crown-to-rump measurement and therefore growth development in the pups is a direct drug effect or it is a consequence of the effect on body wt.

In addition, there was a delay in the onset of vaginal opening in F treated with HD while there was no effect on preputial separation in M. This observation can be interpreted that this compound might have an effect on sexual maturation in F. The slight effect seen on the fertility index and the conception rate at MD and HD might be associated with the effect on sexual maturation in F but it was not clear from the data whether this effect was a male factor or a female factor. With no evaluation of the male sperm count and viability the evaluation of the effect of the drug on the male reproductive system and thus on the fertility index will not be possible. The number of implantation scars was counted and were found not to be affected by treatment. In addition, there was no drug effect on the number of pups at birth. The exact mechanism by which the drug might have an effect on fertility could not be predicted from the findings of this study.

The effect of the compound on the startle response at start and the average startle and the effect on motor activity count in the treated animals seem to indicate that the compound results in decreased activity in animals treated for the length of time that was used in this study. It should be noted; however, that the habituation of animals to the stimulus as judged by the changes in average startle response with the successive treatments was not different between the control and treated animals. There was no effect on the Cincinnati water maze test indicating the test article does not have an effect on learning and memory in these animals.

In the dog juvenile animal study, the doses used for the definitive study were based on the findings of the preliminary study and the findings of the 28-day study in adult dogs. Based on the findings from these two studies the sponsor decided to use doses of 0, 2, 5, and 12 mg/kg/day that were more comparable to those used in adult animals. The sponsor's decision to use higher doses than those used in the preliminary study was reasonable and the reviewer considers those doses used in the definitive study more appropriate than those used in the preliminary study based on the fact that they were associated with more pronounced toxicities to indicate that an MTD was closer to be reached (see review for more details).

Since the intended population for the drug is children and adolescents of 6-12 years of age, the sponsor stated that dogs will be treated starting on week 10 of age for 6 months. This is acceptable, however, it should be noted that at this age (6 months) the dogs are possibly not fully sexually mature since the starting age of maturity in dogs is 6 months and dogs tend to be sexually mature after 8 months of age. Therefore, the findings of this study might suffice for this intended population (6-12 years) but if the drug is to be used in older adolescents (>12 years) then the length of this study might not be enough to cover for the extension of age in this population. In addition, if the animals are not sexually mature by the end of the treatment period (6 months) then it will be difficult to interpret the long term effect of the drug on the male reproductive system even though the length of the study was assumed to parallel the intended population age (6-12 years of age). This is due to the fact that if the animals are not fully sexually mature at this stage then it will be difficult to interpret the data due to difficulty distinguishing whether the effect is due to sexual immaturity or it is a drug effect.

There were no mortalities in the definitive study. The following clinical signs were observed with treatment and mainly at the HD: stereotypic behavior such as head searching/bobbing/shaking, pacing in cage and repetitive pawing, circling, vocalization and yelping, walking or stumbling on objects, increased activity in F, thin condition, decreased activity prior to dosing and tremors. The condition of some individual animals was deteriorating at certain times that treatment has to be suspended for a day; however, when treatment resumed similar complications were not observed. These findings indicated that the high dose used is approaching a maximum tolerated dose and therefore with effects seen on body wt the doses used in this study are considered adequate.

The drug had an effect on body wt of treated animals especially at MD and HD where decreases were observed at these doses compared to control group (by the end of the study the decrease in M was 19% at MD and 25% at HD and in F the decrease was 14% at MD and 15% at HD compared to the control group). This effect appears to still be evident, although to a much lesser extent, till the end of the recovery period (see the review for more details). There appeared to be no effect on other growth measurements such as height and length. The sensitivity of these parameters to drug treatment in the dogs is not clear.

There was no effect on ophthalmological outcomes as tested here nor on the ECG outcomes.

The functional observational battery indicated that muscle tremors were observed in more animals treated with MD and HD compared to the control group especially towards the end of the study. This was also a finding seen in the animals during the clinical observations. In addition, treated dogs tended to be sleeping more than the control animals during observations which could be due to the hyper activity seen after dosing. It is possible that these animals got tired from the increased activity seen after treatment and due to this they tended to sleep more especially prior to dosing the next day.

The neurological examinations performed did not indicate a drug effect.

A decrease in urine volume was seen in treated animals and as a result a higher specific gravity at MD and HD was observed in both M and F.

There appears to be no effect on hormonal levels (see methods for the evaluated hormones). There was some individual variability in the detection of some of these hormones and some technical difficulties (below the levels of quantitation), however, generally the data did not indicate a drug effect.

As indicated in the review the data that was presented for the effect of the drug on the male reproductive system were very variable and the sample size was inadequate on different occasions (n=1). As indicated earlier the sponsor proposed to dose the animals for 26 weeks starting from week 10 of age. According to the sponsor, sample collection was unsuccessful on many occasions for many animals regardless of their group, the ejaculated volume was too small to be analyzed or interpreted and/or the samples had too low a concentration of spermatozoa to perform the sperm motility assessment. The sponsor stated that "In animals/sample occasions where sufficient ejaculated volume/spermatozoa counts were produced, the administration of the NRP-104 did not appear to induce changes on the sperm motility, spermatozoa counts or spermatozoa morphology". The reviewer generally agrees with this statement; however, it will be difficult to conclude that the drug does not have an effect on the male reproductive system in view of quality of the data on some occasions (n=1 sometimes). However, if we considered that in those animals with the appropriate sample the sperm count/ml was a good reflection of the group then we can come to a similar conclusion that the sponsor has come to. However, it will be more appropriate if the data were more consistent and the sample size was larger.

The division's recommendations were that the dog study be conducted up to 8 months of age (see meeting minutes in DFS 9-21-04). It appears that when the sponsor submitted the protocol for input from the division the sponsor proposed that the dog study will be up to 6 months of age. This was based on the proposal that this age in dogs will match the age of the intended population (6-12 years of age). As mentioned earlier the results of the study might not be adequate to predict the effect of the drug on the male

reproductive system since there were issues with the outcome of the studies since it seems that individual variations between the animals could be due to sexual immaturity in some of these animals. To come to a definitive conclusion about the effect of the drug on the male reproductive system would require a better quality of the data from control and treated group and sexual maturity of the animals should be guaranteed for the assessment of the effect of the drug. Whether the drug has an effect or not will not be known unless there was adequate number of animals in the study that reached sexual maturity to be able to come to conclusions about the effect of the drug on male sexual parameters.

No histopathological findings that are considered drug related were observed.

Conclusions:

Toxicological effects of the compound: the findings from the dog and the rat juvenile studies in regards to clinical signs and the decreases in body wt are generally consistent with those obtained in response to amphetamine treatment. In addition, the decrease in the length of the crown- to- rump in treated pup rats compared to the control group indicates that this compound might have an effect on physical development.

The effect of the compound on neurobehavioral parameters: the compound seems to decrease motor activity as judged by the decrease in total activity counts in rat pups treated with the compound especially at HD. In addition, in both dogs and rats the animals appeared to be sleeping or lying on the side with continued treatment. It is possible that with continuous treatment and as a result of increased activity, those treated animals got tired and had a more tendency to be sleeping or lying on the side compared to untreated animals. In addition, in the rat study the compound decreased the startle response in animals which could be due to a variety of reasons including a decrease in motor activity and thus a decrease in response to an auditory stimulus. It should be mentioned that inconsistency between the effect on motor activity and the effect on startle response were observed in the regression group at the end of the recovery period arguing against the possibility of a correlation between the two. There appears to be no effect on the neurological functions in the dogs as tested in juvenile dog study, but an increase in muscle tremors was seen in juvenile dogs at MD and HD especially towards the end of the study as seen during the functional observational battery. The data from the Cincinnati water maze test were variable; however, a deficiency in treated animals to complete the water maze test compared to the control group could not be ruled out. However, it should be pointed out that in the startle habituation test there was no effect on startle habituation in treated rats compared to control group which is an indication that there was no treatment effect on learning in treated animals.

The effect of the compound on the reproductive system in the rats indicates that the compound might have some effect on sexual maturation in F since vaginal opening in treated F rat pups tended to be delayed compared to the control group. In addition, a slight decrease in the fertility index and conception rate in rats was observed even though it was not clear if the effect was due to a male factor or female. In the dog study it was

difficult to interpret the data due to the small sample size in some cases or due to the larger variability between the individual animals which could be due to the fact that these animals might not have reached sexual maturity at this stage (6 months). Therefore, the dog study might be considered inadequate in evaluating the male reproductive system due to deficiencies in the data obtained from the study.

2.6.6.10 Tables and Figures: see the body of the review for table and figures obtained from the sponsor or created by the reviewer.

2.6.7 TOXICOLOGY TABULATED SUMMARY

For the sake of reducing the size of this document summary tables provided by the sponsor are not included here. However, the reviewer summarized the results in a tabulated format in the body of the review. The summary and interpretation of the data by the reviewer was in general agreement with those of the sponsor except in few situations in which the reviewer pointed those out (mainly in the rat juvenile study on the effects on motor activity, startle response and the effect on reproduction).

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Pharmacodynamic:

This compound (NRP104) is considered a prodrug for d-amphetamine since it is composed of lisdexamphetamine dimesylate which is an amphetamine covalently bound to *L*-lysine by an amide bond that is converted to *d*-amphetamine *in vivo*. The prodrug itself is not a stimulant; however, since amphetamine is the major product, stimulant effect is seen with treatment. The parent compound does not appear to bind to either the norepinephrine transporter nor to the dopamine transporter when tested *in vitro* using human recombinant transporters. It should also be emphasized that the parent compound has not been detected in the brain of rats treated orally with the compound while amphetamine was detected in the brain in response to this treatment. *In vivo* studies indicated that the compound increases locomotor activity when administered orally to rats similar to d-amphetamine sulfate and produces other clinical signs similar to those seen with d-amphetamine sulfate. However, when administered intravenously or intranasally the increase in activity seen in treated rats was less than that seen with an equivalent dose of d-amphetamine sulfate given through these two routes.

Safety pharmacology:

The effect of the drug on the CVS was assessed in anesthetized beagle dogs treated IV with the test article. In order to compare the effect of the test article to those of amphetamine, the effect of d-amphetamine sulfate was also assessed in another group of

animals. The effects of the test article were generally comparable to those of amphetamine, (increases in HR, blood pressure, and cardiac output) with some slight differences (the effect of d-amphetamine sulfate on blood pressure was slightly higher compared to that with NRP and was seen at an earlier time point, see the review for more details). Sinus tachycardia was observed 30 min post dose in animals treated with the test article at HD in dogs and in one dog treated with amphetamine verntricular extrasystole and sinus tachycardia were observed. In the 28-day study in dogs there were no significant findings observed.

The effect on the CNS was studied within the general toxicity studies and the juvenile animal studies and the general findings were in agreement of the effect of a stimulant on the CNS which included increased activity and stereotypic behavior in treated animals similar to what is seen with amphetamines.

Pulmonary assessment was conducted in anesthetized guinea pigs by IV administration of the test article. The results indicated an increase in respiratory rate and minute volume thirty minutes after the treatment.

The effect of the test article on the renal and gastrointestinal systems was not evaluated.

Pharmacokinetics:

The pharmacokinetic characteristics of the test article were studied using different routes of administration (oral, I.V. and I.N.) in rats and in dogs. The parent compound was not detected in the brain of rats following oral administration while d-amphetamine was present in the brain as a result of this treatment. Following oral administration of NRP-104 in rats, the bioavailability of the parent compound varied with dose. Tmax for the parent compound ranged from 0.25 to 3h at low dose and up to 4-8h at high doses. Cmax for d-amphetamine in plasma following oral administration of NRP-104 (3 mg/kg amphetamine base) was ~one half of Cmax following d-amphetamine sulfate administration in one report and comparable to those of a similar dose in another report (see review for available figures). At higher doses the fraction of amphetamine absorbed as a result of oral administration of NRP-104 decreased compared to lower doses; however, in animals treated with d-amphetamine sulfate the amphetamine absorbed was increased at the highest doses. Following I.V. administration in rats, the plasma concentraion of d-amphetamine derived from intact NRP-104 in comparison to d-amphetamine derived from an equimolar dose of d-amphetamine sulfate, were significantly reduced. Similar observations were seen with intranasal administration. The metabolism of the compound following oral administration in rats seems to be fairly simple since the major products were those of amphetamine and amphetamine metabolites. The parent compound was observed only for up to 8 hours after oral administration and the highest levels of the radioactivity produced from the parent compound were less than 2% of the total radioactivity in plasma of F. The levels of radioactivity for the parent compound after I.V. administration were ~20% of the total radioactivity in plasma. The only metabolite that was directly related to the parent

compound (M2 or hydroxylated NRP-104) was observed only after I.V. administration. This suggests that after an oral administration, NRP-104 is quickly converted to amphetamine before reaching the plasma circulation. The site of metabolism was not thoroughly tested; however, in vitro testing showed that the liver is not the site of metabolism for the compound. However, in several places the sponsor stated that the site of metabolism is in the gastrointestinal tract. The major route of elimination of total radioactivity after oral administration in rats is through urine (~77% in M and ~87% in F). The compound did not seem to inhibit a variety of CYP-450 enzymes (see table within review for specific enzymes).

In dogs the pharmacokinetic parameters were evaluated following oral and I.V. administration and that data indicated that the compound has a moderate oral bioavailability (33%) and that plasma levels of d-amphetamine after oral administration of NRP-104 are comparable to those after it I.V. administration.

Toxicology:

For detailed description of the studies and findings from these studies please see the overall toxicology summary or the individual study review within this document.

The sponsor conducted the following studies in rats: a single oral dose study, a 7-day oral dose range-finding study, and a 28-day oral toxicity study. The following studies were conducted in dogs: an escalating single oral dose study, a 7-day oral dose range finding study, and a 28-day oral toxicity study.

The single dose studies in rats (doses 0.1, 1, 10, 60, 100, and 1000 mg/kg orally by gavage) and dogs (doses of 3, 10, 18, and 24 mg/kg) were used to evaluate the maximum recommended dose for the long term studies and to evaluate the toxicity of the compound. In rats, the LD50 for NRP-104 was considered to be >1000 mg/kg (equivalent to 399 mg/kg of d-amphetamine), based on the death in 1/3 F and 1/M at the 1000 mg/kg, compared to the LD50 for d-amphetamine sulfate of 96.8 mg/kg (equivalent to 70.5 mg/kg of d-amphetamine base). Increased motor activity such as biting and licking of the cage, chromodacryorrhea/chromorhinorrhea, and skin lesions were observed at doses of 60 mg/kg and above. All surviving rats appeared to be normal 4 days after treatment. In dogs, no deaths were observed, increased activity, abnormal gait, restlessness, repetitive behavior, head bobbing and excessive liking were observed at 10, 18, and 24 mg/kg. Circling and emesis were observed at 18 and 24 mg/kg. The MTD for the dogs was considered to be less than 24 mg/kg since emesis was observed in all animals at this dose. The effects of the test article on the observed clinical observations (increased activity and stereotypic behavior) seem to be consistent between the two species.

In the 7-day study in rats (doses 0, 30, 100, and 300 mg/kg orally by gavage) death and self mutilation were observed at 100 and 300 mg/kg and increased activity at all doses. In the 7-day study in dogs (0, 3, 6, or 12 mg/kg/day orally by gavage), no death was

observed, increased activity was observed at all doses (seen only on few days at LD) and repetitive behavior, restlessness, vessels over sclera dilated at MD and HD and severe ocular discharge at HD (all seen only on Day 1). Decreases in body wt were observed in both the rats and the dogs in response to treatment mostly at MD and HD in each species. No histopathology was conducted in these studies.

In the 28-day study rats (10-15/sex/group) were treated with 0, 20, 40, or 80 mg/kg of NRP-104 orally by gavage. Another group of animals (15/sex) were treated with a d-amphetamine sulfate (16 mg/kg). Five animals from the control, HD NRP-104 treated group and d-amphetamine sulfate group were used as a recovery group. There was no death reported but 1/9 F treated with 80 mg/kg in the toxicokinetic group was moribund sacrificed on Day 7 due to self-mutilation. Clinical signs noted in all NRP-104 treated groups and in the d-amphetamine sulfate treated group included increased activity and post dose jumping. Self mutilation and thin body condition were observed in some animals treated with the HD of NRP-1-4. One F in the d-amphetamine sulfate group had thin condition towards the end of the study. Body wt decreases were observed at MD and HD in the NRP-104 group and in the d-amphetamine sulfate treated group. All animals were normal during the recovery period except for 1M and 1F from HD NRP-104 group with thin body condition for the first few days of the recovery period. Some statistically significant increases in clinical chemistry parameters (glucose, BUN, and ALT) were observed at MD and HD NRP-104 groups. Histopathological changes such as fiber necrosis and degeneration of biceps of thigh muscle in 1/15 F and degeneration of muscular tone in the esophagus in 2/15 F were seen; however, these were not considered drug related by the sponsor. Toxicokinetic data indicated that C_{max} and AUC values of NRP-104 were lower than d-amphetamine values in all groups in both M and F. AUC values of both d-amphetamine and NRP-104 were greater at Day 28 than at Day 1 in F and M, particularly in the MD and HD groups. Both AUC and C_{max} were higher in F than in M for all treatment groups.

In the 28-day study, dogs (3-5/sex/group) were treated with 0, 3, 6, and 12 mg/kg/day with an additional group of animals (5/sex) treated with 2.4 mg/kg/day of d-amphetamine sulfate. Two animals from the control, HD NRP-104 treated group and the d-amphetamine sulfate treated group were used for the recovery group (14-days). No deaths were observed. Restlessness and increased activity were observed in few animals at LD (several days), most animals at MD (almost throughout study) and all animals at HD and those treated with d-amphetamine sulfate (throughout the study). Repetitive behavior, head shaking, and pacing in cage were observed in animals treated at MD and HD but they were seen in more animals at HD than at MD. Decreased activity predose was observed in some animals at MD and HD and those treated with d-amphetamine sulfate. Panting, circling and abnormal gait were also observed in some animals treated with HD of NRP-104 and animals treated with d-amphetamine sulfate. Decreases in body wt were observed at MD and HD and in those animals treated with d-amphetamine sulfate and body thinness was observed in some animals at HD and in the d-amphetamine sulfate treated group. There were some decreases in reticulocytes at MD and HD. During the recovery period, a decrease in body wt and body thinness was seen in some animals treated with NRP-104 and d-amphetamine sulfate and decreased activity was seen in 1M

treated with HD NRP-104. There were no ophthalmology findings and no ECG findings at the tested times. There were no significant histopathological findings.

The conducted 28-day toxicology studies are considered adequate and the results indicated that an MTD had been reached in those studies in both rats (sacrifice of one animal due self sustained injuries, self mutilation, and the effects on body wt at HD) and in dogs (behavioral abnormalities including restlessness, head shaking, pacing in cage, panting, circling and the effect on body wt at HD). The addition of the group treated with the d-amphetamine sulfate in these studies was valuable since it was appropriate to compare the effect of this compound to the effects of d-amphetamine (the proposed metabolite). According the sponsor's calculations, the doses used for NRP-104 in these studies were comparable to those doses used for the d-amphetamine sulfate group based on the d-amphetamine base value. By comparing the results obtained from treatment with NRP-104 with those with d-amphetamine sulfate, it was evident that the effects of the compound are very similar to those of d-amphetamine sulfate and thus indicating that this compound is acting totally through its metabolite d-amphetamine.

At the time of the pre IND meeting with the sponsor, the Division had agreed that the 28-day study would probably be considered adequate to prove that this compound is not different from amphetamine and accordingly other long term toxicology studies might not be needed. This seems to be the case and it is for this reason that the longest studies conducted in both the rodent and the non-rodent species were the 28 day studies.

Genetic toxicology: the compound was tested in the Ames test, in vitro mouse lymphoma assay and the in vivo micronucleus assay.

Even though there were some technical issues with some parts of the definitive study in the Ames test, these were resolved by repeating these parts and by depending on the preliminary study findings. In the mouse lymphoma assay the sponsor was asked to repeat part of the study due to large differences in the duplicates. In the in vivo micronucleus assay the sponsor also was asked to repeat part of the study due to the invalidity of the high dose used. These issues were found to be resolved and the reviewer considers these studies adequate and valid. The overall outcome of the studies indicated that the compound is not genotoxic in any of the tests used. For more details about the studies and the outcomes see the review for these individual studies.

Carcinogenicity: no studies were conducted. At the time of the pre-NDA meeting the sponsor was told by the division that if the compound produces effects that are due to the metabolite amphetamine with the levels of the parent present minimal as they claim at that time, then carcinogenicity studies will not be need. Carcinogenicity studies for amphetamine have been performed by the National Toxicology Program (NTP) and are described in the Adderall labeling.

Reproductive toxicology: no studies were conducted. Similar to the reason given for the carcinogenicity studies. Animal reproduction studies of amphetamine are described in the Adderall labeling.

Special studies (Juvenile animal studies in rats and dogs):

A preliminary or a dose finding study in pup rats was conducted to establish the dose for the definitive study. In this study the pups (12/sex/group) were treated orally by gavage with NRP-014 (0, 4, 15, and 40 mg/kg/day) from PND 7 to PND 30 inclusive. There were no mortalities reported. Increased activity (described as increased cage exploration, excessive grooming, stereotypic sniffing, biting of the cage floor, rapid head turning, increased rearing, sniffing and/or spatial disorientation) was observed in animals treated with 15 or 40 mg/kg/day from PND 21 to the end of the study. Mean body wts were decreased compared to the control group at MD in M (8%) and F (12%) and at HD in M (18%) and in F (22%). These doses were considered appropriate for the definitive study by the sponsor and the reviewer concurs.

In the definitive study, Sprague Dawley pups (60/sex/group) were dosed with 0, 4, 10, and 40 mg/kg/day of NRP-104 orally by gavage (10 ml/kg/day) from PND 7 to 63 inclusive. In the main study (Phase I), each dose group was subdivided into 4 subsets (15/sex/group) according to the following assignments:

- subgroup A (toxicity study) animals were treated from PND 7 to 63. Animals were evaluated for: clinical observation, detailed examinations, body wt, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, gross pathology, organ wt, histopathology, physical development (crown-to-rump), functional observation battery (FOB), motor activity, and auditory startle habituation. These evaluations were conducted during or at the end of the treatment period (see the review for exact timing). Animals were sacrificed on PND 64.
- subgroup B (regression study): in addition to evaluations done during the treatment period (PND 7-63) such as physical development, preputial separation and vaginal opening, animals were evaluated for the following at the end of a 28 day regression period: FOB, motor activity, auditory startle habituation, and Cincinnati water maze (see the tables within the review for exact timing of these evaluations and other conducted tests). Animals were sacrificed on PND 92
- subgroup C (reproductive study): animals were treated from PND 7-63 and then were mated at approximately 85 days of age. The animals were evaluated for the Cincinnati water maze between PND 52-61, for estrous cycle 10 days prior to mating, mating (PND 85), and then they were sacrificed after Day 26-28 post coitum. Paternal performance (mating index, fertility index and conception rate) and maternal performance (gestation index, duration of parturition, # of pups at birth, and #of implantation scars) were also evaluated. The F2 generation pups and litters were observed for death, external malformations, weighed, sexed, and were observed through the lactation period.
- subgroup D (toxicokinetic study): blood was collected on PND 64

Phase II study was a toxicokinetic study in which pups (15/sex/group) were treated for one day only (PND7) and blood samples were collected before they were sacrificed.

Deaths were observed in all groups (1M from control group due to gavage error, 1F from LD, 1F from MD and 1M & 1F from HD group), all of which the sponsor considered as non-drug related. The reviewer considers the death of the 1M from the HD group as possibly drug related since clinical signs seen prior to death included thinness, decreased activity, moderate dehydration, and cold to touch which might indicate that the death was due to deteriorating condition caused by drug treatment. Increased activity was observed in both M and F from HD group from PND 21 to the end of the treatment period and at MD from PND 22 to the end of treatment and at LD from PND 44 to the end of treatment. Stereotypic behavior was observed in both M and F at HD only from PND 22-63 and salivation was observed in some animals intermittently from PND 49-63. Some individual observations in some animals such as severe uncoordination, thin condition, and dehydration were observed on single days.

A decrease in body wt compared to the control was observed in M at HD (11-20%) and in F (9-13%) an effect that was seen continuously in both sexes from either PND10 or 14 to the end of the treatment period. A decrease in body wt compared to control was seen in M at MD by the end of the study (6%). A decrease in body wt compared to control was still seen in M treated with HD at the end of the regression period (13%) and to a much less extent in F treated with HD (4%). A slight decrease was seen in M treated with MD at the end of the regression period (4%).

Based on these observations the doses used in the definitive study are considered adequate and an MTD was reached.

The length of the crown-to rump was reduced in M&F treated with HD (4-7%) from PND 14 to the end of dosing and in M the effect was still seen during the regression period (about the same amount up to day 90). Some reductions were also seen in M treated with LD and MD on PND 70 and 90 (3-4% compared to control).

There was a delay in the onset of vaginal opening in F treated with HD compared to control (delayed by 1.9-2.2 days). There appears to be no drug effect on preputial separation in M.

There appears to be no drug effect on FOB; however, the number of treated animals, especially at HD, that were observed to be lying on the side or curled up were more than seen in the control group. This was not seen at the end of the recovery period.

Total activity counts were statistically significantly decreased in M treated with HD compared to control on PND 22/23 (63%) and in M&F of this group on PND 59/60 (~50%) and in F treated with MD and LD on PND 59/60 (~40%). A decrease in activity was also seen in M at LD (~25%) and MD (~40%) for both PND 22/23 and 59/60, but these decreases were not statistically significant. *Since activity counts were measured prior to daily dosing, this decrease in total activity in treated animals might be due to exhaustion of the animals caused by the increase of activity seen after treatment.* The difference between control and treated group during the recovery period was minimal (~10% decrease from control) and was not statistically significant.

The data suggest a decrease in “startle response at start” for treated animals compared to control and this decrease was still seen in those animals in the regression group at the end of the recovery period (the decrease ranged from ~10-40% compared to control in the different group). This decrease was not statistically significant at any time point and the sponsor did not acknowledge it. The reviewer’s interpretation of the data is that treated animals had a decreased startle response at the start as seen with the lower startle response values at start in treated animals compared to the control even though this decrease was not statistically significant. This could be due to the lower activity in those animals (see motor activity earlier) since these measures were also conducted prior to dosing. However, this proposal does not fully explain why there was still a difference in the start startle response in the treatment group at the end of the regression period compared to control even though there was no difference in the motor activity of the treated and the control groups at this time of the study. The effect on “maximum startle” response was not consistent (increases and decreases were observed in the different groups) and no clear drug effect was seen. There was a statistically significant decrease in “average startle” in M treated with HD compared to control (38%) in Subgroup A while a larger decrease (46%) was seen in F treated with HD of the same group but this decrease was not statistically significant. In the same subgroup (A) a decrease in M treated with LD (33%) and MD (24%) was not dose related nor was statistically significant. At the end of the regression period the decrease in average startle seen in M at MD (44%) and HD (32%) compared to control was not dose related nor it was statistically significant. The sponsor stated that the decrease in average startle observed in M at PND 63 in Subgroup A was not seen in the regression group (Subgroup B) at the same time of the study, suggesting that this finding in M might not be consistent and that this could be due to the “small size of the sample”. The sponsor pointed out that the habituation pattern was unaffected by treatment (the different groups seemed to habituate to the situation in a similar pattern as judged by the linear time contrast). There was no difference between control and treated animals for the effect on time to maximum startle response. Therefore, the general interpretation of the data is that the drug seems to have an inhibiting effect on the response to an auditory stimulus as judged by the decrease in the startle response at start in all groups and in the average startle (at HD) compared to the control group even though the exact mechanism by which this is done is not clear. This effect was not seen in the average startle at the end of the regression period but the trend of decrease was still seen in the startle at start response at the end of the regression period. The significance of this finding to humans is not clear.

The data from the Cincinnati water maze test were highly variable and even though the sponsor considered that there is no drug related effect, in the opinion of the reviewer a drug effect cannot be ruled out. In the opinion of the reviewer it looks that the treated animals seemed to take longer time in crossing the path compared to the control group especially on the first path they were tested on (see later for more details). The data as they were examined by the reviewer reflected that the treated animals on several occasions might have been less able to successfully complete the maze path in a short time especially during their first exposure to the test (path A) than the control animals. However, it should be mentioned that during the testing on a second path (path B), which

the animals were exposed to after path A, they seemed to be less different from the control animals compared to when they were tested on path A. The Cincinnati water maze test measures the time it takes the animal to complete a certain task and the number of errors made by the animals in finishing this task. It should be mentioned that there was no difference between the control and the treated animals in the number of errors encountered during the test. In addition, an effect on motor activity could be ruled out since there was no difference between control and treated animals in swimming a straight line. However, the data from the maze test, as mentioned earlier, suggested that there might be a difference in the number of animals in the treated groups compared to the control being able to finish the task in a shorter time. However, it should be emphasized that the data were variable among the different groups and there was no statistically significant difference between the groups. It is possible that the sample size was not enough to detect the drug effect and that a larger sample size might be needed to observe the drug effect.

A slight increase in % neutrophils (40-50% compared to control in M&F treated with HD on day 64). Not seen on Day 92.

Some increases were observed (ALP, urea, and phosphorus) mainly at HD in both M&F but according to the sponsor were within the historical control data (HC data were not provided).

Some histopathological changes were observed in the liver (necrosis, inflammation and fibrosis), the kidney and/or bladder (pyelonephritis and transitional cell hyperplasia), and lymph nodes (hyperplasia) at HD only or at a higher incidence at the HD. These were not considered treatment related by the sponsor. The occurrence of these findings in the HD only or at a higher incidence at HD might argue against this suggestion (see the review for incidence and severity for these findings).

There was no effect of the drug on estrous cycle length. The effect on mating index, fertility index, and conception rate is summarized in the following table as prepared by the reviewer.

| Group | # placed for mating | | # mating | # of F pregnant | Mating index | Fertility index | Conception rate |
|-------|---------------------|----|----------|-----------------|--------------|-----------------|-----------------|
| | M | F | | | | | |
| 1 | 15 | 15 | 15 | 15 | 100 | 100 | 100 |
| 2 | 14 | 14 | 12 | 12 | 85.7 | 85.7 | 100 |
| 3 | 15 | 15 | 15 | 14 | 100 | 93.3 | 93.3 |
| 4 | 14 | 14 | 14 | 12 | 100 | 85.7 | 85.7 |

The effect on mating index and the fertility index at LD was due to the failure of two of pairs from mating. The effect on the fertility index and the conception rate at MD and HD, even though small, might indicate a drug effect. It is not clear; however, if the effect observed is due to an effect on male fertility or female fertility since the parameters evaluated suggest that there was a lower rate of conception and fertility in those two groups compared to the control but it could not point whether it was a male effect or a

female effect. However, it should be pointed out that there was no effect on maternal performance (gestation index, length of gestation, number of implantation scars, duration of parturition, and live birth index). On the other hand, it is possible that there was an effect on the sperm (either motility or numbers); however, there was no evaluation of these parameters in this study. Therefore, it will be difficult to predict the cause of this effect on fertility index seen in this study from the obtained results. There was no effect on the pups of the F2 generation (malformations, viability, clinical condition, and pup wt).

The study is considered adequate as for the different parts that were conducted (toxicity, reproduction, and neurobehavioral) and for the doses used (0, 4, 10, and 40 mg/kg/day). The length of treatment (from PND 7 to 63 inclusive) was appropriate for the tests evaluated and for the age of the intended population (children of 6-12 years of age). The doses used are considered adequate and the HD is considered the MTD based on the possibility of a drug related death, the clinical signs observed at HD and the effect on body wt. The immediate effects of the drug observed in the toxicity study (increased activity and stereotypic behavior) are similar to those of an amphetamine. In addition, the effect on body wt is also similar to what is usually observed with amphetamine.

It was clear from the results that the test article had an effect on the growth of pups as judged by the decrease in length of the crown-to-rump at HD in both M & F. A decrease in the other M treated groups (LD & MD) was also seen towards the end of the study. The decrease seen in M at HD was still seen at the end of the regression period. Therefore, the drug seems to have an effect on growth of pups treated for that length of period. However, it should be pointed out that this decrease in crown-to-rump was accompanied by a decrease in body wt in the affected groups.

In addition, there was a delay in the onset of vaginal opening in F treated with HD while there was no effect on preputial separation in M. This observation can be interpreted that this compound might have an effect on sexual maturation in F. The effect seen on the fertility index and the conception rate at MD and HD might be associated with the effect on sexual maturation in F but it was not clear from the data whether this effect was a male factor or a female factor. In addition, there was no evaluation for the male sperm count and viability. However, it should be pointed out that the number of implantation scars were counted and were found not to be affected by treatment. In addition, there was no drug effect on the number of pups at birth. The exact mechanism by which the drug might have an effect on fertility could not be predicted from the findings of this study. The effect of the compound on the startle response at start and the average startle and the effect on motor activity count in the treated animals (all were decreased compared to the control group) seem to indicate that the compound results in decreased activity in animals treated for the length of time that was used in this study. In addition, the numbers of treated animals, especially at HD, that appeared lying on the side or curled up were more than those seen in the control group. There was no effect on the Cincinnati water maze test indicating the test article does not have an effect on learning and memory in these animals.

In the dog juvenile animal study, the doses used for the definitive study were based on the findings of the preliminary study and the findings of the 28-day study in adult dogs. In the preliminary study beagle dogs (2/sex/group) were treated with doses of 0, 3, and 10 mg/kg/day orally by gavage at 10 weeks of age for 14 days. In the 28-day study the doses used were 0, 3, 6, and 12 mg/kg/day. In the preliminary study there was no death reported and clinical observations seen 2-6h post dosing at HD included increased activity, pacing, circling, head shaking, and vocalization (see the body of the review for more details). A decrease in mean body wt compared to control was seen at 10 mg/kg/day by day 15 in M (13%) and in F (12%). The findings from the 28-day study at the HD (12 mg/kg/day) were more pronounced than those seen at the HD in the preliminary study (restlessness, increased activity, head shaking, pacing in cage, abnormal gait, decreased activity predose, panting, post dose emesis). A decrease in body wt of 16% in M and 20% in F compared to the control was also observed in that study. Based on these findings from these two studies the sponsor decided to use doses of 0, 2, 5, and 12 mg/kg/day that were more comparable to those used in adult animals. The sponsor's decision to use higher doses than those used in the preliminary study was reasonable and the reviewer considers those dose used in the definitive study more appropriate than those used in the preliminary study based on the fact that they were associated with more pronounced toxicities to indicate that an MTD was closer to be reached.

Since the intended population for the drug is children and adolescents of 6-12 years of age, the sponsor stated that dogs will be treated starting on week 10 of age for 6 months. This is acceptable, however, it should be noted that at this age (6 months) the dogs are possibly not fully sexually mature since the starting age of maturity in dogs is 6 months and dogs tend to be sexually mature after 8 months of age. Therefore, the findings of this study might suffice for this intended population (6-12 years) but if the drug is to be used in older adolescents (>12 years) then the length of this study might not be enough to cover for the extension of age in this population. In addition, if the animals are not sexually mature by the end of the treatment period (6 months) then it will be difficult to interpret the long term effect of the drug on the male reproductive system even though the length of the study was assumed to parallel the intended population age (6-12 years of age). This is due to the fact that if the animals are not fully sexually mature at this stage then it will be difficult to interpret the data due to difficulty distinguishing whether the effect is due to sexually immaturity or it is a drug effect.

There were no mortalities in the definitive study. The following clinical signs were observed with treatment and mainly at the HD: stereotypic behavior such as head searching/bobbing/shaking, pacing in cage and repetitive pawing, circling, vocalization and yelping, walking or stumbling on objects, increased activity in F, thin condition, decreased activity prior to dosing and tremors. The condition of some individual animals was deteriorating at certain times that treatment has to be suspended for a day; however, when treatment resumed similar complications were not observed. These findings indicated that the high dose used is approaching a maximum tolerated dose and therefore with effects seen on body wt the doses used in this study are considered adequate.

The drug had an effect on body wt of treated animals especially at MD and HD where decreases were observed at these doses compared to control group (by the end of the study the decrease in M was 19% at MD and 25% at HD and in F the decrease was 14% at MD and 15% at HD compared to the control group). This effect appears to still be evident, although to a much lesser extent, till the end of the recovery period (see the review for more details). There appeared to be no effect on other growth measurements such as height and length. The sensitivity of these parameters to drug treatment in the dogs is not clear.

There was no effect on ophthalmological outcomes as tested here nor on the ECG outcomes.

The functional observational battery indicated that muscle tremors were observed in more animals treated with MD and HD compared to the control group especially towards the end of the study. This was also a finding seen in the animals during the clinical observations. In addition, treated dogs tended to be sleeping more than the control animals during observations which could be due to the hyper activity seen after dosing. It is possible that these animals got tired from the increased activity seen after treatment and due to this they tended to sleep more especially prior to dosing the next day.

The neurological examinations performed did not indicate a drug effect.

A decrease in urine volume was seen in treated animals and as a result a higher specific gravity at MD and HD was observed in both M and F.

There appears to be no effect on hormonal levels (see methods for the evaluated hormones). There were some individual variability in the detection of some of these hormones and some technical difficulties (below the levels of quantitation); however, generally the data did not indicate a drug effect.

As indicated in the review the data that was presented for the effect of the drug on the male reproductive system were very variable and the sample size was inadequate on different occasions (n=1). As indicated earlier the sponsor proposed to dose the animals for 26 weeks starting from week 10 of age. According to the sponsor, sample collection was unsuccessful on many occasions for many animals regardless of their group, the ejaculated volume was too small to be analyzed or interpreted and/or the samples had too low a concentration of spermatozoa to perform the sperm motility assessment. The sponsor stated that "In animals/sample occasions where sufficient ejaculated volume/spermatozoa counts were produced, the administration of the NRP-104 did not appear to induce changes on the sperm motility, spermatozoa counts or spermatozoa morphology". The reviewer generally agrees with this statement; however, it will be difficult to conclude that the drug does not have an effect on the male reproductive system in view of quality of the data on some occasions (n=1 sometimes). However, if we considered that in those animals with the appropriate sample the sperm count/ml was a good reflection of the group then we can come to a similar conclusion that the sponsor

has come to. However, it will be more appropriate if the data were more consistent and the sample size was larger.

The division's recommendations were that the dog study be conducted up to 8 months of age (see meeting minutes in DFS 9-21-04). It appears that when the sponsor submitted the protocol for input from the division the sponsor proposed that the dog study will be up to 6 months of age. This was based on the proposal that this age in dogs will match the age of the intended population (6-12 years of age). As mentioned earlier the results of the study might not be adequate to predict the effect of the drug on the male reproductive system since there were issues with the outcome of the studies since it seems that individual variations between the animals could be due to sexual immaturity in some of these animals. To come to a definitive conclusion about the effect of the drug on the male reproductive system would require a better quality of the data from control and treated group and sexual maturity of the animals should be guaranteed for the assessment of the effect of the drug. Whether the drug has an effect or not will not be known unless there was adequate number of animals in the study that reached sexual maturity to be able to come to conclusions about the effect of the drug on male sexual parameters.

No histopathological findings that are considered drug related were observed.

Unresolved toxicology issues (if any):

Recommendations:

Suggested labeling:

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ____ No ____

APPENDIX/ATTACHMENTS

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

/s/

Ikram Elayan
9/21/2006 02:00:30 PM
PHARMACOLOGIST

Barry Rosloff
9/27/2006 03:44:54 PM
PHARMACOLOGIST
A few minor disagreements--see my memo of 9/26/06