CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-037

PHARMACOLOGY REVIEW(S)

Barry N. Rosloff, Ph.D.
7/8/2009

NDA 22-037 (GUANFACINE) - SUPERVISORY MEMO

I am in agreement with Dr. Elayan's conclusion (review of 7/7/2009) that the potential of guanfacine to induce cardiac valvulopathy in humans should be addressed by the Sponsor. The following are some comments on this issue.

Since the time when fenfluramine (as a component of Fen-Phen) was shown to cause cardiac valvulopathy in humans, there has been research suggesting that this effect (and that of other human valvulopathogens) is mediated by stimulation of cardiac 5-HT2b receptors. In support of this hypothesis, Dr. Bryan Roth of UNC Chapel Hill, whose studies were discussed in Dr. Elayan's review, stated that he screened about 2200 "FDA-approved or investigational medications", and 27 of these were 5-HT2b receptor agonists; of these 27, he states that 7 are "*bona fide*" valvulopathogens. These 7 are norfenfluramine (the metabolite of fenfluramine thought to be responsible for its valvulopathic effect), pergolide, cabergoline, ergonovine, methylergonovine (the metabolite of methysergide thought to be responsible for its purported valvulopathic effect), ergotamine, and dihydroergotamine. This would seem to indicate an association between 5-HT2b agonism and valvulopathy in view of the relatively high proportion of agonists which were valvulopathic, although for comparison one would want to know what proportion of the approximately 2170 compounds which were not agonists were also valvulopathic. The first 3 drugs listed above are considered to be valvulopathogens by FDA (fenfluramine and pergolide removed from market; cabergoline added warning and consideration of echocardiogram testing to labeling). References to the valvulopathic effects of the other drugs listed can be found in the literature; however for at least ergotamine and methysergide (the parent compound of methylergonovine) a recent article referred to case reports but stated that "there are no reports of case-control series...and no dose-dependent relation (to valvulopathy) has ever been described." (Droogmans, et. al., European Journal of Echocardiography Advance Access, published 4/23/2009). Also note that 6 of the 7 valvulopathogens belong to the same chemical/pharmacological class (ergots), which weakens the conclusion that the observed correlation shows a role of 5-HT2b agonism since these drugs likely have other pharmacological effects in common aside from such agonism. (In contrast, Dr. Roth notes the ergot derivative bromocriptine is not a 5-HT2b agonist and has not been associated with valvulopathy. However, although I have not performed a thorough search and evaluation of the clinical literature, articles claiming a valvulopathic effect of bromocriptine can be found, e.g. Tan, et. al., Movement Disorders 24:3, 344-349, 2009). (Dr. Roth also states that the drug methylenedioxymethamphetamine [MDMA] is also both a 5-HT2b agonist and a valvulopathogen, although in my view it is unlikely that adequately controlled clinical studies exist for this drug of abuse). Another consideration is the possibility that for some of the above drugs examination for valvulopathy may have been more likely or intensive based on their pharmacological properties.

There is also some basic animal research suggesting a link between 5-HT and/or the 5-HT2b receptor and cardiac valvulopathy (Elangbam et. al., Experimental and Toxicologic Pathology 60, 253-262, 2008). Overall I consider the preclinical and clinical evidence in support of a link suggestive but far from conclusive and, at best, support an association rather than a causal link.

In view of the evidence that 5-HT2b agonists may be associated with valvulopathy, the Division of Metabolic and Endocrine Drugs has required human echocardiogram monitoring for such agonists under INDs. (Our Division has also intended to require this, although to my knowledge we have not had any 5-HT2b agonists aside from guanfacine. [Note that we do not routinely require screening for binding to this receptor. The Sponsor of the current guanfacine NDA did not screen for this receptor; we became aware of Dr. Roth's results relatively recently]).

As noted in Dr. Elayan's review, according to Dr. Roth's data guanfacine is intermediate in agonism potency between compounds that do and do not cause valvulopathy. Also note that all of the data for the agonist effect of guanfacine are unpublished* and come from a single lab; i.e. they have not been peer reviewed nor independently verified. Finally, there have not been any reports of valvulopathy for guanfacine in humans submitted to FDA (or found in a literature search by Dr. Levin) despite a long history of use (although possibly without a great extent of use).

In conclusion, I believe that this issue should be further explored. One avenue could be attempts to replicate the results of Dr. Roth for guanfacine; another could be to see if guanfacine produces valvulopathy in animals at therapeutically relevant doses. In view of the non-invasiveness of echocardiogram monitoring, the preferred approach might be to perform echocardiograms in subjects receiving or who have received guanfacine. In view of the facts that, as noted above, guanfacine does not appear to have a particularly high potency as an agonist and no human cases of valvulopathy have been reported, such monitoring could be performed as a post-marketing commitment. I have discussed the above issues with Dr. Elayan and her team leader Dr. Fossom, and we are all in agreement with the above.**

*Note added in proof: This work was recently published online—Molecular Pharmacology Fast Forward, July 1, 2009.

**Note added in proof: At an internal team meeting on 7/10/09, also attended by Drs. Stockbridge (Director of Cardio-Renal Division) and Unger (Deputy Director of ODE I), it was decided that postmarketing commitments should consist of (1) examination of heart valves in the ongoing juvenile rat study and (2) performance of a new rat study to see if guanfacine produces valvulopathy, using fenfluramine as a positive control. If an effect of guanfacine is seen, echocardiogram testing in humans will be considered.

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7/14/2009 03:13:08 PM
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22037

SERIAL NUMBER:

DATE RECEIVED BY CENTER:

PRODUCT: Guanfacine Hydrochloride

INTENDED CLINICAL POPULATION: Attention Hyperactivity Disorder (ADHD)

SPONSOR: Shire Pharmaceuticals

DOCUMENTS REVIEWED: no documents were submitted (see background)

REVIEW DIVISION: Division of Psychiatry Products

PHARM/TOX REVIEWER: Ikram Elayan

PHARM/TOX SUPERVISOR: Linda Fossom

DIVISION DIRECTOR: Thomas Laughren

PROJECT MANAGER: ShinYe Chang

Date of review submission to Division File System (DFS): 07/07/2009

Drug:

Trade name: Intuniv

Generic name: Guanfacine hydrochloride

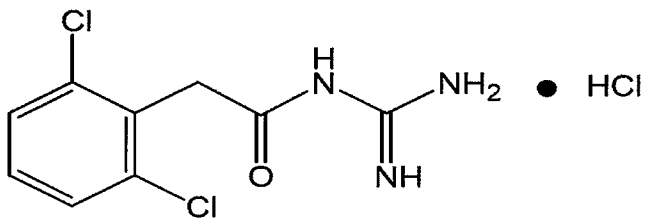
Code name: SPD-503

Chemical name: Benzeneacetamide-N-(aminoiminomethyl)-2,6-dichloro-monohydrochloride

CAS registry number: 29110-48-3

Molecular formula/molecular weight: $C_9H_9C_{l2}N_3O.HCL/282.56$ (246.08 as free base)

Structure:



Background

It was brought to the attention of the review team during the review cycle that some data were presented at the FDA by Dr. Bryan Roth from the University of North Carolina, Chapel Hill, regarding guanfacine binding to the serotonin receptor subtype 5-HT2B. In his presentation, Dr. Roth reported that guanfacine binds as an agonist at this receptor. Later on, Dr. Roth provided more data from his laboratory (in the form of a manuscript that is in preparation for submission to the journal Molecular Pharmacology) with similar findings using different functional assays for this receptor and testing a variety of drugs including guanfacine. (The sponsor of this NDA had provided some in vitro data for guanfacine binding to different receptors, transporters, ion channels or for its effect on the activity of different enzymes using the NOVA Screen test. In this test, guanfacine was tested up to a concentration of 10 μM at several subtypes of the serotonin receptors but no data were presented for its action at the 5-HT2B receptor).

The use of some drugs, such as fenfluramine, pergolide, and cabergoline that have agonistic activity at the 5-HT2B receptor, has been associated with cardiac valvular pathology. Even though the mechanism by which these drugs result in this pathologic finding has not been fully determined, it has been proposed that their activity at this serotonergic receptor subtype is mediating this effect. It is of concern that guanfacine has been suggested to have agonistic activity at this receptor. It is not clear whether guanfacine has a potential to result in such valvular pathologic findings. The following review will summarize the available data that is mainly collected from Dr. Roth's presentation and from the preliminary data he forwarded in the form of an unpublished

manuscript for work conducted in his laboratory. Following the data presentation, an evaluation for the level of concern in view of the available data will be discussed.

Data presentation:

In his presentation at the FDA, Dr. Roth provided data in which guanfacine was shown to have agonistic activity at the 5-HT2B receptor as shown below (directly extracted from Dr. Roth's file of his slide presentation at the FDA):

Figure (1):

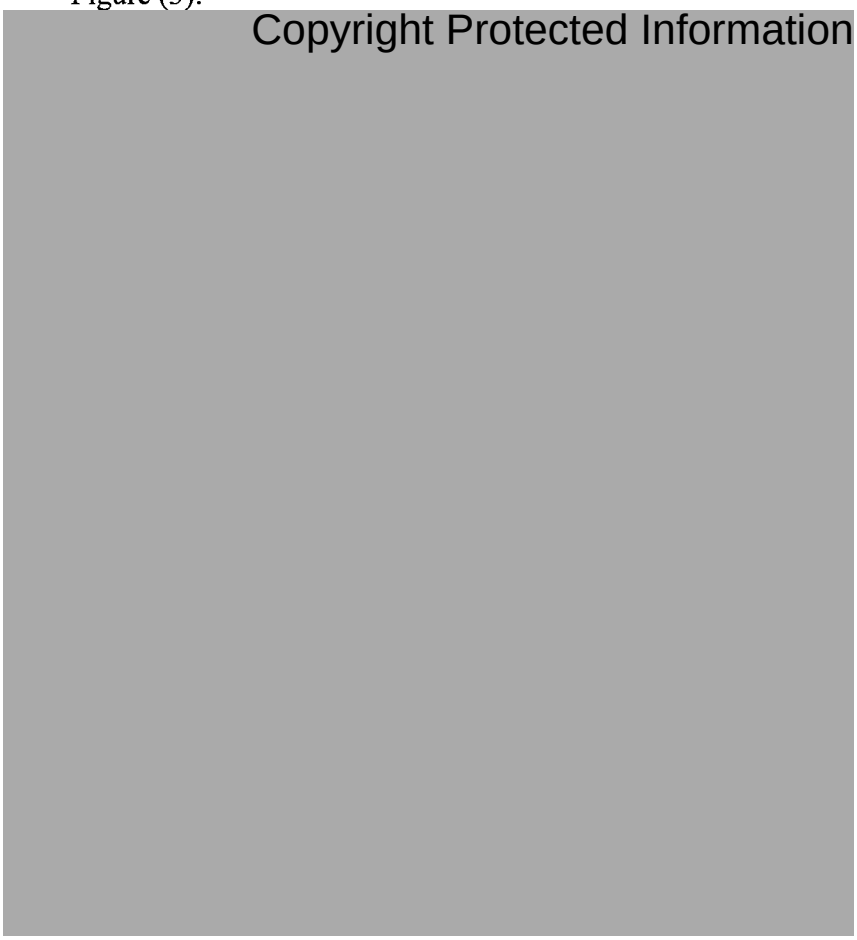
Figure (2):

The following table was extracted from the preliminary manuscript forwarded by Dr. Roth that is planned to be submitted to Molecular Pharmacology for publication as indicated by Dr. Roth (e-mail communication):

(The yellow high-lighting was added to the table by the Reviewer for enhancement of the bolded font used by the authors)

The authors of the manuscript described some previous work conducted in Dr. Roth's laboratory and they stated that: "we have showed that valvulopathogens elicit 5-H2B receptor dependent proliferative responses in primary cultures of human heart valve interstitial cells, consistent with the putative actions of valve heart disease (VHD)-associated drugs in vivo" (Setola et al., 2003). The authors also cited work by other groups in which valves from patients treated with drugs that caused VHD displayed proliferative interstitial foci, which is considered the hallmark feature of valvulopathy (Connolley et al., 1997; Steffee et al., 1999). In this manuscript, the authors presented data to show the effect of the different tested compounds on proliferation of HEK293 cells stably expressing recombinant human 5-HT2B receptors using the tetrazolium salt (XTT)-based proliferation assay. The following figure summarizes the data as provided in the manuscript:

Figure (3):



Discussion:

The data presented by Dr. Roth and summarized here indicate that guanfacine acts as an agonist at the 5-HT2B receptor using in vitro model of HEK293 Flpln cells stably expressing the human recombinant 5-HT2B receptor exposed to different concentrations of guanfacine and other agonistic compounds using a variety of functional activity parameters as end points (see table extracted from Dr. Roth's manuscript for the different assays used). It has to be noted and as stated by Dr. Roth in the manuscript, these agonist-induced responses appear to be specific, since they were blocked by the 5-HT2B receptor antagonist SB 206553 or were not seen in parental cell lines that are not expressing recombinant human 5-HT2B receptors.

The table presented in Dr. Roth's manuscript summarizes the estimated potency (pEC50) and efficacy (Emax, expressed as % of that for serotonin), for the different tested compounds. According to Dr. Roth's data, the pEC50 for guanfacine at the 5-HT2B receptor using the different functional assays was ~6.1 (the average pEC50 value for the different assays, see table 1). In comparison, the pEC50 values for compounds that are

believed to induce valvular heart disease such as pergolide, cabergoline, norfenfluramine, dihydroergotamine and others (see Roth's table 1, above, for tested compounds and values, as designated by bolded font and yellow high-lighting) were estimated to be more than 7.5 on average. On the other hand, one drug that acts as an agonist at the 5-HT2B receptor but is not associated with valvular heart disease, namely ropinirole, had a pEC50 value of ~5.1. These values included here were the average (calculated by the Reviewer) for all the tests used and the ranges for these values were different using the different tests (see the table for these values).

The following are data for human plasma concentrations in clinical studies submitted under this NDA and summarized by the clinical pharmacology review by Dr. Elena V. Mishina (DFS, 6/4/2007).

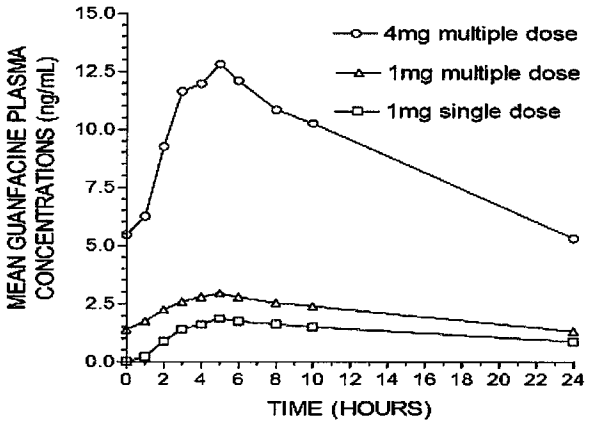


Figure 5. Mean guanfacine plasma concentration vs. time values

Table 3. Summary of Pharmacokinetic Parameters for Guanfacine After Repeated Oral Administration of 2 and 4mg SPD503 Doses Once Daily to Children (6-12 years) and Adolescents (13-17 years) with ADHD

Parameter*	Multiple Dose 2mg		Multiple Dose 4mg	
	Children (6-12 years)	Adolescents (13-17 years)	Children (6-12 years)	Adolescents (13-17 years)
C_{max} (ng/mL)	4.4 ± 1.66	2.9 ± 0.77	10.1 ± 7.09	7.0 ± 1.53
t_{max} (h)	4.98 (3.95-7.97)	4.53 (2.93-7.98)	5.02 (3.97-10.3)	4.97 (1.00-7.97)
AUC_{0-24} (h•ng/mL)	70.0 ± 28.33	48.2 ± 16.06	162.1 ± 115.56	116.7 ± 28.37
CL/F				
(mL/min)	552 ± 215	826 ± 486	522 ± 212	607 ± 166
(mL/min/kg)	15.3 ± 4.11	14.4 ± 8.34	14.3 ± 3.70	10.7 ± 3.11

The log of the average steady-state plasma concentration for children ages 6-12 treated with multiple dosing of 4 mg based on the table above (AUC value of 162.1 h.ng/ml) is calculated as follows:

162 ng.h/ml/24h = 6.75 ng/ml (plasma concentration at steady state)
MW for guanfacine is 247
6.75 ng/ml/246 = 0.027 μM = 27 nM
Log 27 nM = -7.57
For the Cmax of 10 ng/ml: = 0.0407 μM = 41 nM; the log of 41 nM = -7.39

According to the binding graph in figure (2) above for guanfacine binding to the 5-HT2B receptor as provided by Dr. Roth, this concentration of guanfacine will probably result in <20% occupation of the 5-HT2B receptor. Whether the valvulopathy is dependent on the average plasma concentration or the Cmax is not clear and one might take into consideration that Cmax for guanfacine was ~10 ng/ml in children ages 6-12 years of age (see table from the Clinical Pharmacology review for this NDA). This concentration of guanfacine will probably result in <30% occupation of the 5-HT2B receptor.

The data presented in figure (3) demonstrated that at a concentration of 30 nM, guanfacine and compounds believed to result in valvular heart disease, resulted in an increase in cell proliferation. However, at the same concentration (i.e. 30 nM) ropinirole, a 5-HT2B receptor agonist that does not cause valvulopathy, did not induce cell proliferation. The authors noted that the effect on cell proliferation was not observed in cells not expressing the 5-HT2B receptor thus ruling out a non-specific effect, e.g., on metabolism of the XTT colorometric substrate. However, at a higher concentration (1μM) all the tested compounds, including ropinirole, appeared to stimulate cell proliferation (see figure 3), an effect that was blocked by the selective 5-HT2B receptor antagonist SB206553.

Dr. Roth and his group presented data here for ropinirole for its characteristics as an agonist, its effect on the different functional assays used, and its potency, to speculate why it is not causing valvular pathology even though it binds to the 5-HT2B receptor. Even though this compound seems to be as efficacious in binding and activating the receptor as other compounds believed to cause valvulopathy, it seems to be much less potent (pEC50 being ~5.1 while for the other drugs believed to cause valvulopathy being ~7.5). On the other hand, guanfacine appears to be somewhat in the middle of this spectrum, i.e. with a pEC50 of ~6.1 which lies in between ropinirole and the other proposed valvulopathogenic compounds. Therefore the valvulopathogenic effect of guanfacine cannot be predicted from this data.

Moreover, comparing the binding affinity for guanfacine to its proposed pharmacological target (the adrenergic α2A receptor) (as summarized by the sponsor of this NDA in the following table) to that at the 5-HT2B receptor might indicate that at therapeutic plasma concentration guanfacine might bind to the pharmacological receptor with a higher affinity but still might significantly bind to the 5-HT2B receptor.

Table 1 - 1

IC_{50} Determination: Summary Results

Assay Cerep Compound I.D.	Client Compound I.D.		IC_{50} (M)	K_i (M)	n_H
α_{2A} *(h)*					
9242-1	Guanfacine HCl		2.9E-08	1.3E-08	*0.8*
9242-1	Guanfacine HCl	1st experiment	3.1E-08	1.4E-08	*0.7*
9242-1	Guanfacine HCl	2nd experiment	3.5E-08	1.5E-08	*0.8*
9242-1	Guanfacine HCl	3rd experiment	2.5E-08	1.1E-08	*0.7*
	Guanfacine HCl	mean	3.00E-08	1.33E-08	*0.75*
		sem	2.08E-09	8.54E-10	*0.03*

In the table above the Ki value for the adrenergic α2A receptor was 13 nM, based on the sponsor's experiments as submitted in the NDA. The log of this value is -7.89. The log of the concentration for guanfacine that results in 50% binding at the 5-HT2B receptor (as measured by the activation of the receptor and as reported by Dr. Roth, see Figure 1) was approximately -6.87 (135 nM). This means that the Ki value for the binding to the pharmacological receptor was ~10 fold lower than that for the 5-HT2B receptor (i.e. 10 times higher affinity for the pharmacological receptor). In addition, the log of the plasma levels at the therapeutic dose was approximately -7.57 (27 nM, as calculated from the table extracted from the Clinical Pharmacology review for this NDA, see above). By comparing these plasma levels to the Ki for the pharmacological receptor and to the pEC50 for the 5-HT2B receptor, it is evident that at these plasma levels more than 50% of the pharmacological receptor will be predicted to be occupied while less than 20% of the 5-HT2B receptor will be occupied. Therefore, at those plasma concentrations some binding at the 5-HT2B receptor is still possible even though binding at the pharmacological receptor will be predominant. It should however be stressed that the methodologies for the binding studies conducted by Dr. Roth's lab are not the same methodologies used by the sponsor for binding at the pharmacological receptor (activation assays vs. radiological binding assays).

In addition, there is not enough evidence here or in the literature to support the notion that guanfacine has some pharmacodynamic characteristic (e.g., distribution) that might be protective and thus the clinical use of guanfacine will not be associated with these findings.

Therefore, it is reasonable to say that the data presented here do not provide a high confidence level that guanfacine use might not be associated with valvulopathy; however, at the same time the available data are not extremely alarming.

It is interesting that in the manuscript provided by Dr. Roth, the authors proposed a question about whether there are any "additional factor(s)" to distinguish those compounds that bind to 5-HT2B receptors and cause these valvulopthogenic findings from those compounds that bind to this receptor but do not result in these findings.

It is therefore suggested that the sponsor address this issue, as presented by Dr. Roth and his group, of the potential of guanfacine to produce such valvular pathologies. It should be emphasized that the guanfacine data presented by Dr. Roth at the FDA were publicly presented at the IXth World Conference on Clinical Pharmacology and Therapeutics (2008) and at the NIH and NIMH (as confirmed by Dr. Roth). However, no other data for guanfacine binding to the 5-HHT2B receptor could be found in the literature. The sponsor of this NDA is to be informed of the available data and they should respond to our concern about the potential for guanfacine to result in these valvular pathologies based on Dr. Roth's data.

Therefore, the Reviewer suggests that unless data are provided to prove that guanfacine binding to this receptor is not associated with valvulopathy, i.e. as in the case of ropinirole, then guanfacine is to be considered a suspect. It is possible that the sponsor can follow up on this issue clinically by conducting echocardiograms, but this has to be decided by the clinical team. The fact that this drug will be used in children with ADHD who might also be concomitantly treated with stimulants might predispose those children to further risk. Even though there were no data in the literature to indicate amphetamine or other stimulants used in the treatment of ADHD bind to the 5-HT2B receptor, there was one report in which amphetamine use in aging rats resulted in exacerbation of spontaneous mitral valve valvulopthy (Elangbam C.S. et al, Exp Tox Path, 2006).

Recommendations to be conveyed to the Sponsor:

In view of data presented by Dr. Bryan Roth from the University of North Carolina for the possibility that guanfacine acts as an agonist at the serotonin 5-HT2B receptor subtype, it is recommended that receptor binding and activation studies at this receptor subtype be conducted. If such studies confirm that guanfacine is indeed an agonist then further studies might be warranted including echocardiography, due to the seriousness of valvulopathological changes that are believed to be associated from the use of compounds that show agonistic activity at this receptor.

References

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2- Elangbam, C.S., Wehe J.G., Barton J.C., Krull D.L., Nyska A., Crabbs T., and Kissling G.E.: Evaluation of glycosaminoglycans content and 5-hydroxtryptamine 2B receptor in the heart valves of Sprague-Dawley rats with spontaneous mitral valvulopathy – a possible exacerbation by dl-amphetamine sulphate in Fisher 344 rats? Exp Tox Path 58: 89-99, 2006.
3- Setola V., Hufeisen S.J., Grande-Allen K.J., Vesely I., Glennon R.A., Blough B., Rothman R.B., and Roth B.L.: 3,4-methylenedioxymethamphetamine (MDMA, "Ecstacy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. Mol Pharmcol 63: 1223-1229, 2003.
4- Steffee C.H., Singh H.K. and Chitwood W.R.: Histologic changes in three explanted native cardiac valves following use of fenfluramines. Cardiovasc Pathol 8: 245-253, 1999.

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PUBLIC HEALTH SERVICE
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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 22037

SERIAL NUMBER: N-000

DATE RECEIVED BY CENTER: August 24, 2006

PRODUCT: Guanfacine Hydrochloride

INTENDED CLINICAL POPULATION: ADHD (studies were conducted in children and adolescents 6 to 17 years of age)

SPONSOR: Shire Pharmaceuticals.

DOCUMENTS REVIEWED: see list of studies reviewed below

REVIEW DIVISION: Division of Psychiatry Products

PHARM/TOX REVIEWER: Ikram Elayan, Ph.D.

PHARM/TOX SUPERVISOR: Barry Rosloff, Ph.D.

DIVISION DIRECTOR: Dr. Tom Laughren

PROJECT MANAGER: Felecia Curtis

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: the application is considered approvable from a pharmacology/toxicology stand point. Some changes pertaining to the mechanism of action of the drug and preclinical findings in the labeling proposed by the sponsor is recommended.

B. Recommendation for nonclinical studies: no studies are recommended. However, it should be mentioned that a juvenile animal study to investigate the effect of the drug on reproduction was recommended at the time of the pre NDA meeting and before the submission of the application. This study was specified as a Phase IV commitment.

C. Recommendations on labeling

The following are the changes to the labeling as proposed by the pharm/tox team:

MECHANISM OF ACTION

Guanfacine is an alpha-2A-adrenergic receptor agonist. It has a 15 to 20-fold lower affinity for the alpha-2B and alpha-2C adrenergic receptor subtypes compared to the alpha-2A subtype. The mechanism of action of guanfacine in Attention Deficit Hyperactivity Disorder (ADHD) is not known.

Guanfacine is not a central nervous system (CNS) stimulant.

PREGNANCY

Pregnancy Category B - Rat experiments have shown that guanfacine crosses the placenta. However, administration of guanfacine to rats and rabbits at 6 and 4 times, respectively, the maximum recommended human dose of 4 mg/day on a mg/m^2 basis resulted in no evidence of harm to the fetus. Higher doses (20 times the maximum recommended human dose in both rabbits and rats) were associated with reduced fetal survival and maternal toxicity. There are no adequate and well-controlled studies of guanfacine in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

NONCLINICAL TOXICOLOGY

No carcinogenic effect of guanfacine was observed in studies of 78 weeks in mice or 102 weeks in rats at doses up to6-7 times the maximum recommended human dose of 4 mg/day on a mg/m^2 basis.

Guanfacine was not genotoxic in a variety of test models including the Ames test and an *in vitro* chromosomal aberration test; however, an increase in numerical aberrations (polyploidy) was observed in the latter study.

No adverse effects were observed in fertility studies in male and female rats at doses up to 30 times the maximum recommended human dose on a mg/m^2 basis.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The studies reviewed here included those pertaining to the pharmacological action of the drug, studies conducted to investigate the effect on QTc and the HERG channel current, and studies (a 4-week rat study and two genotoxicity studies) to compare an impure form of the drug (spiked with three impurities) to a pure form for the purpose of qualifying these impurities up to a level that covers their proposed specification in the drug substance (see the review for more details). In addition, an overview of the safety of the excipient used in the drug product (b) (4) was presented.

Pharmacology:

The affinity of guanfacine for human recombinant α_2-adrenoceptor subtypes (α_{2A}-, α_{2B}-, and α_{2C}) expressed in CHO cells was examined. In this in vitro study, CHO cells expressing human recombinant α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors were incubated with [^{3}H]RX 821002 (1, 2.5, and 2nM, respectively) in the absence and presence of guanfacine hydrochloride (10 concentrations ranging from 10^{-5}-10^{-10} M for the α_{2A} receptors; 10^{-4}- 10^{-9} M for the α_{2B} and α_{2c} receptors) or yohimbine (8 concentrations ranging from $3x10^{-7}$-10^{-10}M for α_{2A} and α_{2c} and 10^{-6}-10^{-10} M α_{2B}) at 22 °C for 30 min (α_{2A} and α_{2c}) or 20 min (α_{2B}). Non-specific binding was defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand (epinephrine, 100 µM). Radioactivity was determined by liquid scintillation counting. The results indicated that guanfacine has a higher affinity for the α_{2A} than the α_{2B} and α_{2c} receptors (20- and 16-fold for the α_{2B} and α_{2C}, respectively).

In a NOVASCREEN assay, the affinity of guanfacine to different receptors, ion channels, transporters and enzymes, was tested at concentrations of 1 nM, 100 nM and 10 µM. The data suggested that up to a concentration of 100 nM the compound was mostly specific to the adrenergic α_2 receptors. At a higher concentration (10 uM) binding to the other

adrenergic receptors was observed (see the study review for more information about these bindings). The specificity of the compound at the highest dose seems to diminish.

In a behavioral study, the effect of guanfacine in a proposed rat model of ADHD [the spontaneously hypertensive rat (SHR)] was evaluated. The sponsor stated that the SHR model exhibits several of the core symptoms of ADHD (i.e. hyperactivity, impulsiveness, and attentional deficit). In that study, the effects of different concentrations of guanfacine (0.075, 0.15, 0.3 and 0.6 mg base/kg i.p.) given 30 min before testing every third day on behavior of control Wistar Kyoto rats (WKY) and SHR rats was evaluated. The test basically evaluates the animals' response to a certain cue (light) above a choice of two levers to receive a reinforcement. The total number of lever presses was used as an expression of the general activity level and thus a measure of the degree of overactivity, the percentage of correct lever choices of the total number of lever presses when the inforcers are delivered infrequently was used as a measure of *sustained attention*, and the number of correct responses with short inter-response times (<0.67 s) was used as a measure of degree of *impulsiveness* (because the rat had responded correctly again before it had received the water reward). The data obtained from the study suggested that the drug decreased the levels of hyperactivity and impulsivity in SHR animals especially at the higher two doses used (0.3 and 0.6 mg/kg). The data indicated that the control animals (WKY) experienced sedation with guanfacine treatment. It could be interpreted from this observation that the decrease in hyperactivity and impulsiveness in the SHR animals can be as a result of sedation. The sponsor argues against this proposal since attention in the SHR animals was improved with treatment. The sponsor argued that sedation should hinder attention rather than improve it.

The effect of the dug on blood oxygen level dependent (BOLD) response using a modification of functional magnetic resonance (fMRI) called the pharmacological MRI (phMRI) was measured in different rat brain regions. Male Sprague Dawley rats were anesthetized and treated with guanfacine (0.3 mg/kg i.p.). The BOLD effects were measured at 4 min 40 sec intervals, over a 90 min period using a 2.35T Burker magnet, at all brain levels both for basal effects (i.e. prior to treatment) and saline/drug effects (post treatment effect). Respiration rate, blood pressure, and blood gases were monitored. The data indicated negative BOLD effects in some areas of the brain such as the caudate putamen and nucleus accumbens and positive BOLD effects in frontal cortex brain regions (see the review of the study for more specific regions). There were no effects on the physiological parameters measured. The decreases in BOLD effects seen in caudate putamn and the increases in the frontal cortex were interpreted as a decrease and increase in the activity in these areas, respectively. This resulted in the conclusion by the sponsor that guanfacine treatment resulted in these effects and that this ability of guanfacine to change neuronal activity in specific areas of the rat brain (which according to the sponsor are similar to the areas that are believed to be impaired in ADHD) may explain the therapeutic efficacy for guanfacine in ADHD.

Safety pharmacology:

In vivo telemetered dog study:

Four telemetered beagle dogs were treated with 0.5, 1.5 and 5.0 mg/kg guanfacine orally (capsules). The dose of 5 mg/kg was administered to only 1 telemetered dog (partial dose since the capsule was not totally administered) but due to worsening condition of this animal the other telemetered dogs were not treated with this dose. Two untelemetered dogs were treated with this dose and similar signs were observed (vomiting, incoordination of movement, piloerection, poor peripheral circulation, and bradycardia). Oral administration of 1.5 mg/kg of guanfacine caused vomiting in 2/4 animals.

Oral administration of 0.5 and 1.5 mg/kg of the test substance was not associated with effects on arterial blood pressure, QRS, QT, QTcF or QTcQ intervals. A pronounced and prolonged bradycardia was observed following both 0.5 and 1.5 mg/kg administration. This coincided with an increase in the RR and the PR intervals.

Dose-dependent worsening of an existing bradydysrhythmia, associated with sinus bradycardia and delayed conduction through the AV-node was observed in two animals.

The conclusion from this study is that at the doses used there was no effect of treatment on QTc duration; however, the treatment seems to worsen existing condition of bradydysrhythmias that are associated with sinus bradycardia and conduction through the AV-node in treated animals.

In vitro HERG channel study:

The test article (Lot # GF 08002) was used to perfuse HEK293 cells transfected with the HERG channel at a concentration of 1 µg/ml (~4 µM, as calculated by reviewer, MW 246 free base) for 15 min exposure at a rate of 1-2 ml/min under a whole cell clamping conditions (n=5). The other groups were a vehicle group treated with 100% bath solution (n=4) and a reference substance (100 nM E-4301, an agent known to block the HERG current); n=5 (n=2 test substance and n=3 vehicle treated cells). At the concentration used in this study (1 µg/ml or ~4 µM) there appears to be no effect on the HERG channel current. It is not clear if an effect could be seen at higher concentrations.

Toxicology:

In the toxicology section, studies to qualify three impurities found in the drug substance at a specification of up to (b) (4) also labeled as (b) (4) in the study reports) were conducted by testing a pure form of the compound and an impure form (spiked with (b) (4) % of each of these impurities) and comparing the results. A chromosomal aberration study and an Ames test were conducted to compare the genotoxicity of the impure form compared to the pure form and a 4-week toxicology study in rats with a 2-week recovery period was also

conducted. The impurity levels as spiked in the impure form of the compound (b)(4)% for each impurity) can be considered as adequate to qualify these impurities in the genotoxicity studies and in the 4-week rat study (up to 10 mg/kg/day in rats while the human maximal dose was 4 mg/day).

Genotoxicity studies:

In a chromosomal aberration study human peripheral blood lymphocytes were incubated with both the pure and the impure form of the compound (spiked with (b) (4)% of each of the three impurities). The concentrations ranged from 10-240 ug/ml (see review of study for details) and the incubations were for 3h with and without S9 and for 20h without S9. The highest doses chosen were based on the mitotic index at that dose (at least 50% inhibition) and analysis from highly toxic doses was avoided. The data indicated no differences between the two forms of the compound and that small and sporadic increases in the frequencies of numerical aberrations were seen in both forms and were more than that seen in the current control and the normal ranges.

In an Ames test, the two forms of the compound (the impure form was spiked with the impurities at the level of (b) (4) for each) were tested with the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA102. The concentrations of the test article ranged from 20 to 5000 ug/plate (7 concentrations were used) both with and without S9. The results for the genotoxicity of the two forms were negative.

4-Week toxicity study:

A 4-week study was conducted to compare the effects of an impure form of guanfacine (spiked with (b)(4)% of each of the three impurities) with a pure form in an attempt to qualify these impurities. In this study, Crl:OFA(SD) rats (16/sex/group, with 6/sex/group for a recovery period of 2 weeks) were treated with either the vehicle or with each form of guanfacine at a dose level of 10 mg/kg/day orally in the diet. The following parameters were evaluated: mortality, clinical signs, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, gross pathology, organ weights, and histopathology. The results of the study indicated that the two forms of guanfacine resulted in similar findings with very minor differences that can be considered biologically insignificant (see the study review for specific details). Therefore, it is concluded that these impurities did not result in any significant differences in findings that are seen with the pure form and accordingly these impurities will be considered qualified for their toxicologic effects.

Juvenile animal studies:

Juvenile animal studies were reviewed for the IND of the NDA (IND 63551) and can be found in DFS. The general overview of these studies indicted that at the doses tested

there appeared to be no effect of the compound to require description in the labeling. However, since a reproductive segment was not conducted in that study, the sponsor agreed to perform this part as a Phase IV commitment.

B. Pharmacologic activity:

Guanfacine is an alpha 2A adrenergic receptor agonist. It has a higher affinity to the alpha 2A subtype compared to the alpha 2B and 2C subtypes. It is not exactly known how these pharmacologic characteristics of guanfacine apply to its effect in the treatment of ADHD. However, the sponsor's proposal is that the deficits in learning, working memory and attention present in ADHD are likely to result from dysregulation of noradrenergic neurotransmission in the prefrontal cortex. The sponsor based this conclusion on studies that showed that selective lesions in the noradrenergic neurons in the prefrontal cortex with 6-hydroxydopamine, or monoaminergic depletion by reserpine treatment produced profound deficits in spatial working memory of monkeys and that these findings were reversed by administration of low doses of the non-selective alpha 2 agonist, clonidine. The sponsor suggested that cognitive deficits induced by frontal lobe lesions in humans or monkeys closely resemble those present in ADHD and that activation of the postsynaptic alpha 2 adrenergic receptors is critical in maintaining and improving cognitive performance. The sponsor proposed that these cognitive deficits are predicted to be responsive to the actions of guanfacine which is proposed to increase the noradrenergic output via post-synaptic alpha 2 adrenoceptors in the dorsolateral prefrontal cortex. The sponsor continued to propose that guanfacine through its effect in the prefrontal cortex or independent of this effect might be able to modulate the dopamine rich brain areas that are thought to be involved in ADHD such as the pautamen and the nucleus acumbens by decreasing activity in these areas and therefore improving the hyperactivity observed with ADHD. The sponsor proposed that the effect of guanfacine is mainly mediated through alpha 2A receptors that are located in these areas. The behavioral studies that the sponsor submitted were mainly used for supporting these proposals by the sponsor. In the opinion of the reviewer, these proposals seem overly enthusiastic and the weight of evidence or support for them is not proportional.

C. Nonclinical safety issues relevant to clinical use

The conducted study in telemetered dogs did not indicate an effect of treatment on QTc at the tested doses; however, the treatment appeared to worsen an existing condition of bradydysrhythmia associated with bradycardia and delayed conduction through the AV-node in some of the treated animals. In an in vitro study, only one concentration of the compound was used (~4 uM) for the effect on the HERG channel and there was no effect on HERG channel current with this treatment. It is not clear if an effect could be seen at higher concentrations. Therefore, these findings even thought they don't support the notion that the drug might not have an effect on QTc, the data might not be

comprehensive to totally eliminate a possibility for the drug to have an effect on the heart in humans, especially in vulnerable populations (i.e. preexisting heart conditions).

Three impurities needed to be qualified for this NDA ((b) (4) also labeled as (b) (4) in the study reports). These impurities were specified up to (b) (4)% in the drug substance. The sponsor conducted studies with a "pure" and an "impure" form of the compound (spiked with these impurities at a level of approximately (b) (4)% for each impurity) and compared them in a 4-week rat toxicity study (at a dose of 10 mg/kg/day in rats) and in two genotoxicity studies (Ames test and in vitro chromosomal aberrations). The conducted studies did not indicate major differences between the two forms of the compound and the results were quite similar. In addition, the findings did not indicate any significant toxicological findings. The level of spiking of these impurities in the "impure" form of the compound will cover the human exposure to these impurities up to their specification limit in the drug substance (b) (4)%). Their levels were (b) (4), in the genetoxicity studies and the levels obtained from a high dose of 10 mg/kg/day in the rat 4-week study will exceed the human exposure from a high recommended dose of 4 mg/day (50 times based on a mg/kg and 12 times based on mg/m^2 based on a human body wt of 20 kg body). Therefore, these impurities are considered qualified to the specification of (b) (4)% proposed in the drug substance.

(b) (4)

(b) (4)

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

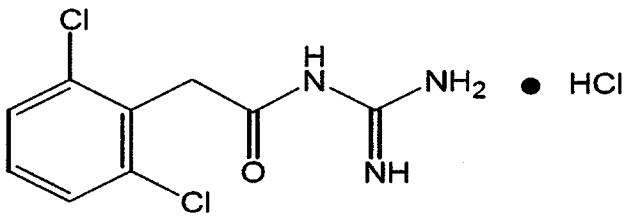
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-037
Review number: 1
Sequence number/date/type of submission: N-000, August 24, 2006
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Shire Pharmaceuticals
Manufacturer for drug substance: (b) (4)

Reviewer name: Ikram Elayan
Division name: Division of Psychiatry Products
HFD #: 130
Review completion date:

Drug:

Trade name: (b) (4)
Generic name: Guanfacine hydrochloride
Code name: SPD-503
Chemical name: Benzeneacetamide-N-(aminoiminomethyl)-2,6-dichloro-monohydrochloride
CAS registry number: 29110-48-3
Molecular formula/molecular weight: $C_9H_9C_{l2}N_3O.HCL/282.56$ (246.08 as free base)
Structure:



Relevant INDs/NDAs/DMFs: IND 63551 and IND (b) (4) DMF (b) (4)

Drug class: α_2 adrenergic agonist

Intended clinical population: ADHD (studies were conducted in children and adolescents 6 to 17 years of age)

Clinical formulation: extended release tablets

Route of administration: oral

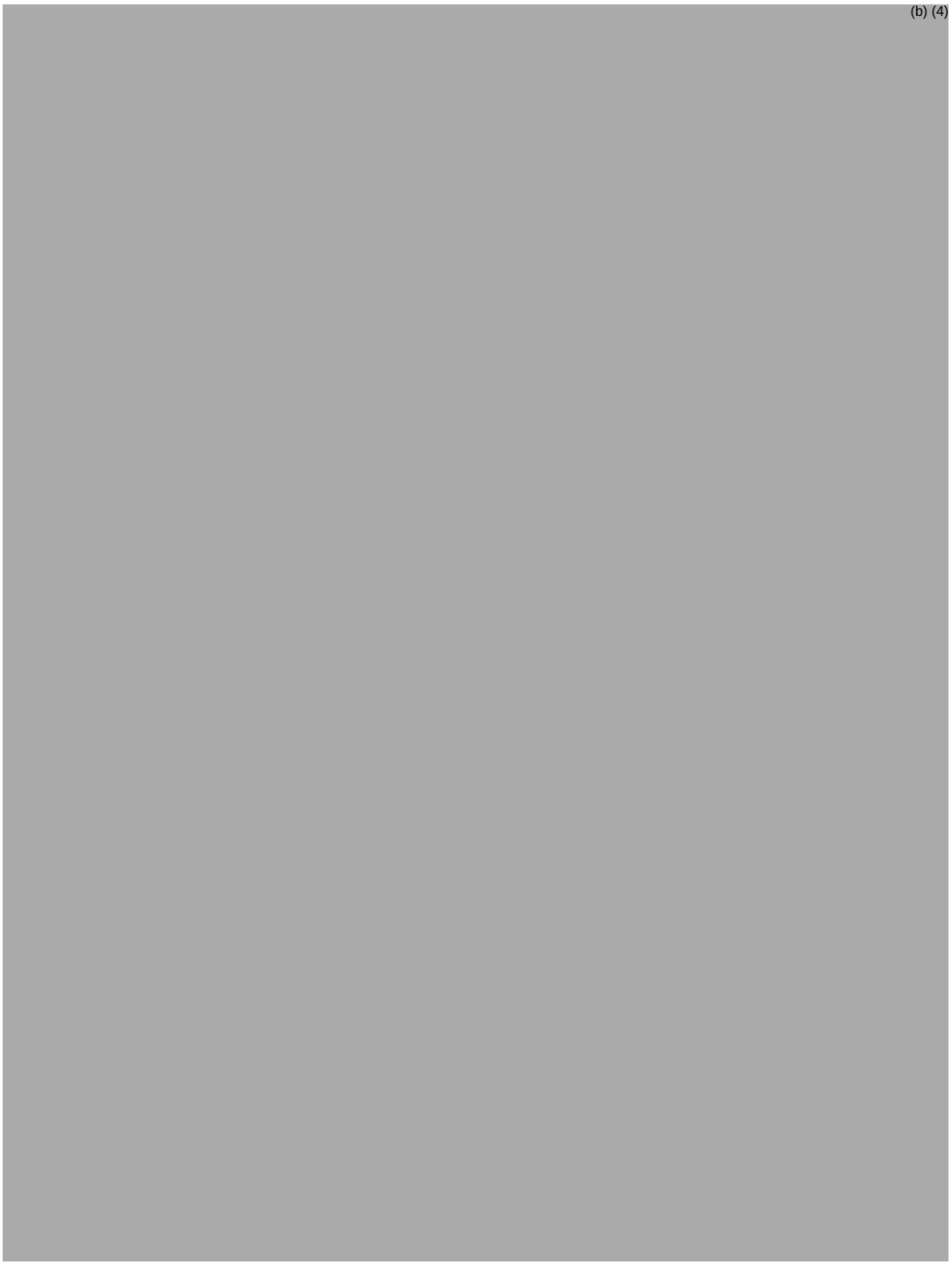
Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

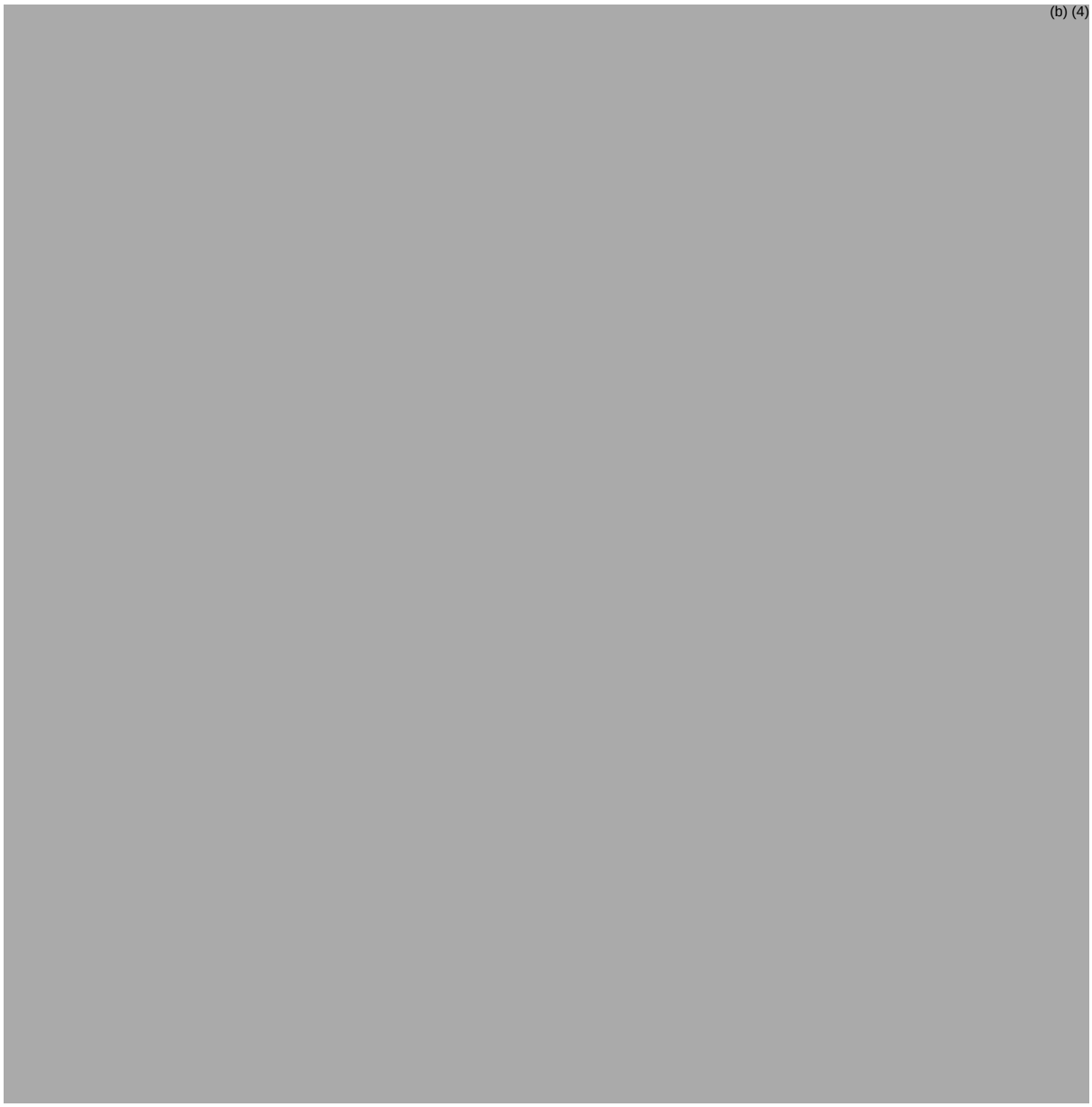
This submission is considered **a (b) (2) application**:

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA number 22037are owned by Shire Inc. or are data for which Shire Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 22037 that Shire Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the **drug's approved labeling. Any** data or information described or referenced below from a previously approved application that Shire Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22037.

Studies reviewed within this submission: All studies submitted for the qualification of impurities: SPD (pure) and SPD (impure): reverse mutation in five histidine-requiring strains of Salmonella typhimurium (Study V01018-SPD503), SPD503 (pure) and SPD503 (impure): induction of chromosome aberrations in cultured human peripheral blood lymphocytes (Study V01019-SPD503), SPD503 (Guanfacine hydrochloride) a comparative toxicity study of pure and impure material by dietary administration to OFA rats for 4 weeks followed by a 2 week recovery period (Report R01020-SPD503). In addition, studies related to QT prolongation: Effect of Guanfacine Hydrochloride on HERG currents recorded from stably transfected HEK293 cells (Study V00168-SPD503), and Guanfacine Hydrochloride: cardiovascular effects in conscious telemetered dogs (study D00023-SLI503). Studies related to the pharmacology of Guanfacine: Behavioral study of effects of Guanfacine in a rat model of attention-deficit/hyperactivity disorder (ADHD) (Study # R00837-SPD503), Guanfacine produces differential effects in frontal cortex compared to striatum: assessed by phMRI BOLD contrast (Study # R0098), NOVASCREEN report (Report # V00662-(b) (4))482-IIIF), and Determination of the affinity of Guanfacine hydrochloride for human recombinant α_2-adrenoceptor subtypes (Study # V00965-SPD503).

Studies not reviewed within this submission: The following studies were not reviewed in here:


(b) (4)



2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

Mechanism of action: guanfacine is a selective α_2-adrenergic receptor agonist. The sponsor proposed that the effect of guanfacine on the post synaptic α_{2A} adrenergic receptors in frontal cortex increases the noradrenergic output and improves cognitive and behavioral performance. The sponsor stated that "there is evidence that α_2-adrenergic

receptor agonists act directly in the prefrontal cortex to enhance executive function". The sponsor continued to conclude that "guanfacine has been demonstrated to enhance prefrontal cortical functions including working memory, behavioral inhibition and attention in rats, monkeys, and humans".

The following studies reviewed here were submitted as part of the pharmacology section of the submission and appear to be submitted to support the sponsor's claim regarding the mechanism of action of the drug in ADHD.

Study title: Determination of the affinity of guanfacine hydrochloride for human recombinant α_2-adrenoceptor subtypes (Study # V00965-SPD503).

The study was conducted by (b) (4) between March and April 2005. The study was not labeled as GLP but seemed to be audited (a letter only indicated that it was audited but no QA report could be found).

CHO cells expressing human recombinant α_{2A}-, α_{2B}-, and α_{2c}-adrenoceptors were incubated with [^{3}H]RX 821002 (1, 2.5, and 2nM, respectively) in the absence and presence of guanfacine hydrochloride (10 concentrations ranging from 10^{-5}-10^{-10} M for the α_{2A} receptors; 10^{-4}-10^{-9} M for the α_{2B} and α_{2c} receptors) or yohimbine (8 concentrations ranging from $3x10^{-7}$-10^{-10}M for α_{2A} and α_{2c} and 10^{-6}-10^{-10} M α_{2B}) at 22 °C for 30 min (α_{2A} and α_{2c}) or 20 min (α_{2B}). Non-specific binding was defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand (epinephrine, 100 µM). Radioactivity was determined by liquid scintillation counting.

The results of the study with comparison to previously reported data using human cloned receptors and tissues are summarized in the following table as provided by the sponsor:

Table 1: Affinity of guanfacine for human α_{2A}- α_{2B}- and α_{2C}-adrenoceptor subtypes

	K_i (nM)			Selectivity ratio	
	α_{2A}	α_{2B}	α_{2C}	α_{2A}/α_{2B}	α_{2A}/α_{2C}
Cerep study	13	255	201	20	15
Renouard et al (1994)[6]	115[a]				
Newman-Tancredi et al (1998)[8]	69[b]				
Uhlén et al (1994)[7]	50[b]	1020[b]	1120[b]	20	22
Devedjian et al (1994)[5]	146[b]	2251[b]	1480[b]	15	10

Receptor sources: [a] Platelets; [b] Cloned/transfected

It is clear from the results that guanfacine has a higher affinity for the α_{2A} than the α_{2B} and α_{2c} receptors and thus a higher selectivity (20- and 16-fold for the α_{2B} and α_{2C}, respectively). The sponsor stated that these values (the fold differences) are in agreement with the literature even though the absolute values for the Ki are different than that obtained in this study (see the previous table). The sponsor compared these results to data from the rat obtained from the literature where guanfacine Ki values for the α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors were 13-56 nM, 971-2020 nM and 353-834 nM, respectively, with selectivity rations of 17-60 and 15-21 fold for α_{2A} vs. the α_{2B} and α_{2c}, respectively.

Conclusion: guanfacine had higher affinity for the α_{2A} than the α_{2B} and α_{2c} as judged by the 15-20 fold different in its affinity to the α_{2A} receptor compared to those receptors using recombinant human versions of those receptors expressed in CHO cells.

NOVASCREEN assay: using this test the sponsor submitted the data with which the compound was tested for its affinity to different receptors at concentrations of 1 nM, 100 nM and 10 µM. The compound at a concentration of up to 100 nM did not have significant binding (>50% inhibition) at any receptor except of the adrenergic alpha 2 (non-specific, ~89%) and the alpha 2A (~67.9%). The binding to the other alpha 2 receptors at this concentration was <50% (~36% for the alpha 2B and ~14% for the alpha 2C receptor). At a concentration of 10 µM, binding of >50% were seen in other receptors in addition to those mentioned earlier (alpha 2 non selective was 100% and alpha 2A was 106%) such as the alpha 1 non-selective (~53%), alpha 2B (102%), alpha 2C (65.8%), human recombinant dopamine transporter (63%), rat recombinant dopamine D3 (77%), dopamine non-selective (55%), Imidazoline (72%), norepinephrine transporter (55%),

human recombinant serotonin 5HT1A (85.9%), human 5HT2A (67.7%), serotonin 5HT2C (70%), serotonin 5HT3 (70%), serotonin non selective (51%), and monoamine oxidase A peripheral (74%).

Conclusion: the data suggested that up to a concentration of 100 nM the compound was mostly specific to the adrenergic α2 receptors. At higher concentration (10 uM) binding to the other adrenergic receptors was seen including the α1, the dopamine transporter, D3 dopamine receptor, the NE transporter, the 5HT receptors and the enzyme monoamine oxidase A (peripheral). However, it should be noted that at this high concentration, which is probably much higher that the in vivo levels, the binding of this compound will be less specific.

Study title: behavioral study of effects of guanfacine in a rat model of attention deficit/hyperactivity disorder (ADHD):

The study was conducted at the University of Oslo, Norway by Professor Terje Sagvolden. The signature date on the study is September 20, 2004.

For the overview of the "validation" of the spontaneously hypertensive rat (SHR) as an animal model of ADHD the following paper was referenced by the sponsor: "Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD)" Terje Sagvolden. Neuroscience and Neurobehavioral Reviews 24:31-39, 2000.

Methods:

The effects of guanfacine at doses of 0.075, 0.15, 0.3 and 0.6 mg base/kg i.p. given 30 min before testing every third day on behavior of Wistar Kyoto (WKY, as the control group) and Spontaneous Hypertensive Rat (SHR), a proposed animal model of ADHD, was evaluated in this study. A total of 32 rats (16 SHR and 16 WKY) were used in the study. The animals were 4 weeks old at the beginning of the study. The animals were housed individually and had free access to food and had access to water at all times before the habituation session. However, after completing the habituation session, the rats were deprived of water for 21h a day. The rats received water as reinforcers during the experimental session and had free access to water for 90 min after the experimental session.

The behavioral test was conducted after training sessions in which the rats were trained to open a lid and press a lever from a 2 choice lever pressing paradigm in order to obtain the water based on cues (light above the correct lever).

Each behavioral session was divided into 18-min ("segment") to monitor intra-session changes in the behavior. For each segment, each lever press was recorded as a function

of time since last response (inter-response time, IRT). In addition, the number of reinforcers delivered; and the number of correct (reinforcer present) and incorrect (no reinforcer present) lid openings were recorded for each segment. A computer and an online system recorded the behavior and scheduled reinforces (i.e. drops of water delivered on average every 180 sec).

The total number of lever presses is an expression of the general activity level and therefore a measure of the degree of *overactivity*, the percentage of correct lever choices of the total number of lever presses when the inforcers are delivered infrequently is a measure of *sustained attention*, and the number of correct responses with short inter-response times (<0.67 s) is a measure of degree of *impulsiveness* (because the rat had responded correctly again before it had received the water reward).

Results:

Overactivity:

The data indicated a pronounced overactivity in the SHR compared to the WKY controls. This pronounced overactivity was reduced by guanfacine in a dose dependent matter with the most effect seen at the highest two doses (0.3 and 0.6 mg/kg). At the highest dose used (0.6 mg/kg) there was no statistical difference between the control and the treated group (t-test) to which the sponsor referred to as **"apparent normalization of the SHR behavior". The following two figures from the sponsor's submission summarize these findings:**

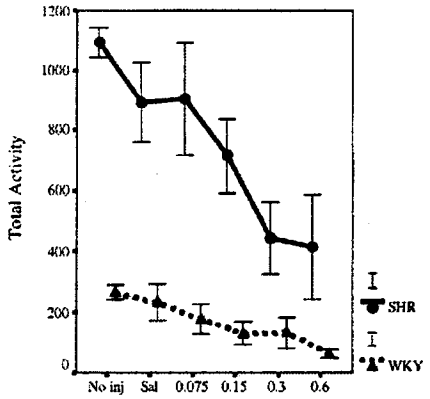


Figure 2. Effect of guanfacine on activity in medicated SHR and WKY controls ± SEM.

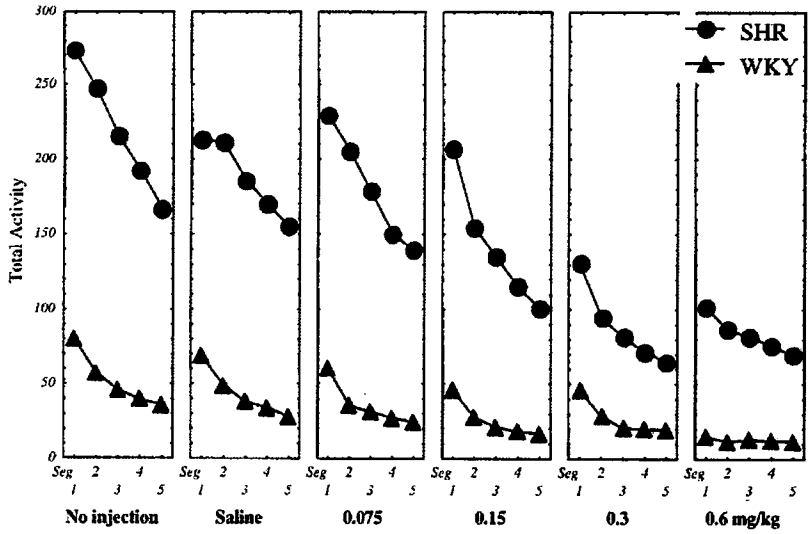


Figure 3. Effect of guanfacine on activity level across the five 18-min within-session segments in SHR and WKY controls.

Impulsiveness:

The SHR showed pronounced impulsiveness compared to the WKY controls. The drug seemed to reduce impulsiveness in both groups. Follow up t-test showed no difference between control and the SHR treated with 0.3 and 0.6 mg/kg doses. The sponsor had concluded that the "0.3 and 0.6 mg/kg doses apparently normalized SHR behavior".

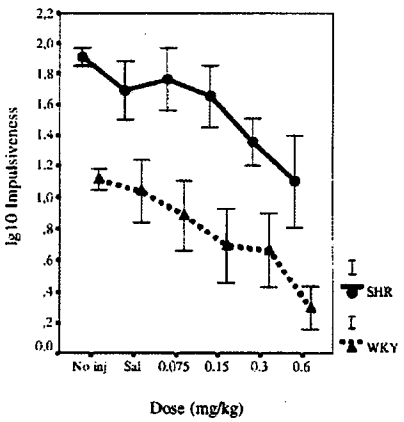


Figure 6. Effect of guanfacine on impulsiveness (lg10 transformed), responding within 0.67 sec following the previous lever press, in the SHR model of ADHD and WKY controls ± SEM.

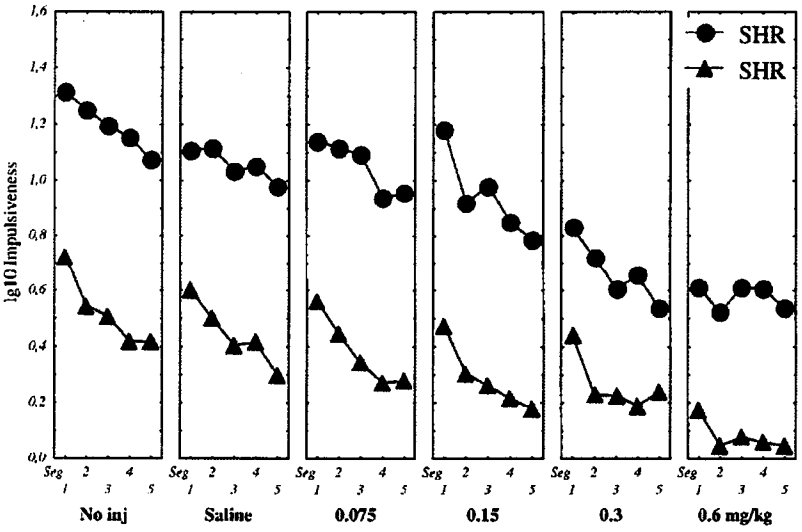


Figure 7. Effect of guanfacine on impulsiveness (lg10 transformed) across the five 18-min within-session segments in medicated SHR and WKY controls.

Sustained attention:

Without active drug, the SHR had a lower percent correct lever choice (i.e. poorer sustained attention) than the WKY controls. Guanfacine improved the poor performance of the SHR. The effect was more pronounced towards the end of the session. The following figures were obtained from the sponsor:

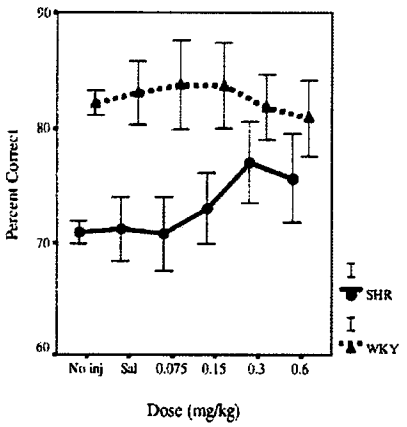


Figure 10. Effect of guanfacine on sustained attention, percent correct choice of the correct lever switch in the animal model of ADHD and WKY controls ± SEM.

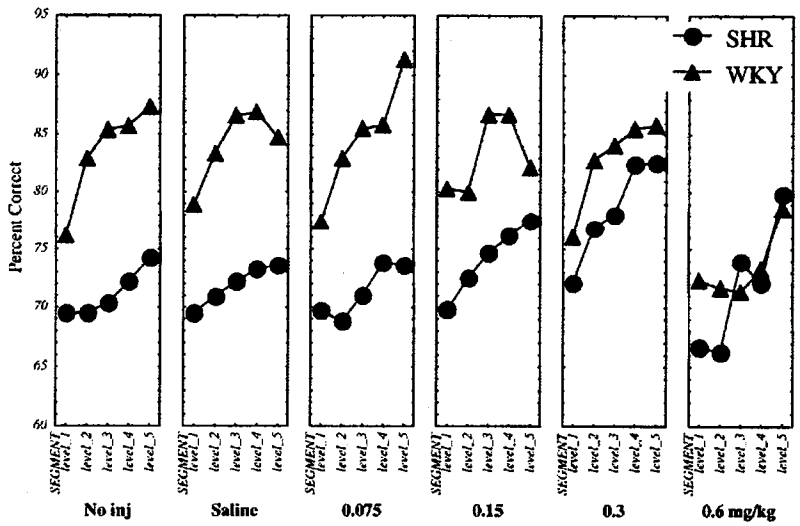


Figure 12. Effect of guanfacine on sustained attention, percent correct choice of the correct lever switch across the five 18-min within-session segments in medicated SHR and WKY controls.

The data demonstrated that the SHR were consistently poorer than the WKY control and that both groups' behavior improved towards the end of the 90-min sessions when under the influence of guanfacine.

The sponsor indicated that the control group seemed to be sedated during treatment with the guanfacine which could indicate that the effect on impulsiveness and hyperactivity could be due to the sedation; however, the sponsor argues that sedation should make attentiveness worse rather than better.

Conclusion: the data presented here from the study with SHR (proposed as a "validated" model of ADHD) appear to show that the drug decreased the levels of hyperactivity and impulsivity in those animals especially at the higher two doses used (0.3 and 0.6 mg/kg). The data suggest that the control animals (WKY) experienced sedation with guanfacine treatment which could be somewhat responsible for this decrease in hyperactivity and impulsiveness in the SHR animals. However, the sponsor argues that the increase in sustained attention in SHR animals treated with the similar paradigm might argue against this possibility since sedation would hinder attention rather than improve it.

As for the validation of the SHR model for ADHD, the reviewer is skeptical about the use of this model as "validated" since even though the behavioral manifestations of increased activity in these animals might be similar to those in children with ADHD, the etiology or biochemistry leading to these behavioral characteristics might not be similar. In addition, despite the fact the guanfacine appeared to improve these behavioral effects in these animals (SHR), it is not known whether this will result in similar observation in children with ADHD. In addition, it is possible that the compound results in a sedative effect that caused the animals to calm down. The sponsor argued against this proposal since the drug improved their attention rather than deteriorate it. Therefore, the general out come of these proposals is that even though guanfacine might improve the behavioral

manifestations in this animal model, it is not clear how this effect can be translated to its action in ADHD.

One suggestion to test the validity of this model as an animal model of ADHD would be to test the effect of already approved drug for the treatment of ADHD in this model to see whether it will respond to this treatment. This could further support the proposal that this model might be a model of ADHD.

Study title: guanfacine produces differential effects in frontal cortex compared to striatum: assessed by phMRI BOLD contrast.

This study was conducted by Dr. Neil Easton at the Institute of Neuroscience, University of Nottingham, Queens Medical Center, UK. The date of the study conduction was not clear.

The objective of the study was to determine with the phMRI (pharmacological magnetic resonance imaging) blood oxygenation level dependent (BOLD) response in different rat brain regions in response to treatment with guanfacine. Male Sprague Dawley rats (8-9) were anesthetized and an intraperitoneal cannula line was inserted into the abdomen for the administration of the drug or the vehicle. A cannula was introduced in each of the femoral arteries for monitoring blood pressure and blood gases. The anesthetized animals were carried in a cradle designed to fit inside the probe of the Burker 2.35T Biospec Avance MR system. Body temperature was maintained at 37 °C. A repetitive phMRI scanning protocol was used to study the effect of guanfacine (0.3 mg/kg, i.p.) evoked changes in brain signal intensity as measured with the T2-weighted blood oxygenation level dependent (BOLD) contrast method. BOLD effects were measured at 4 min 40 sec intervals, over a 90 min period, at all brain levels both for basal effects (i.e. prior to treatment) and saline/drug effects (post treatment effect). The investigator used the 0.3 dose based on personal communication with Dr. Terje Sagvolden (conducted the effect of the drug on SHR animals, a proposed model for ADHD). At this dose, decreases in hyperactivity and compulsivity was seen in SHR in a study conducted by the investigator (see the previous section).

The authors provided the following regarding the positive changes in BOLD contrast, they stated that "the positive effect reflects decreases in deoxyhaemoglobin relative to oxyhaemoglobin, because of increased regional cerebral blood flow (rCBF) above and beyond that required for an increased oxygen metabolism. Therefore any changes in rCBF may be a consequence of increased excitatory and/or inhibitory neuronal activity". In addition, the authors stated that "interpretation of negative BOLD contrast effects is complicated as there is little evidence that these changes are a consequence of decreased neuronal activity and that a mechanism such as "vascular steal" can occur. This is where an increase in activity in one brain area literally steals oxyhemoglobin from nearby areas causing the concentration of deoxyhemoglobin relative to oxyhemoglobin to rise in the area from where the oxyhemoglobin was removed, thus producing a negative BOLD response". Taking these explanations in mind the results of this study are presented here.

Results:

The authors of the study indicated that saline treatment did not result in changes in the BOLD signal intensity to distinguish it from the baseline signal indicating no significant functional changes in rat brain for either the positive or negative response.

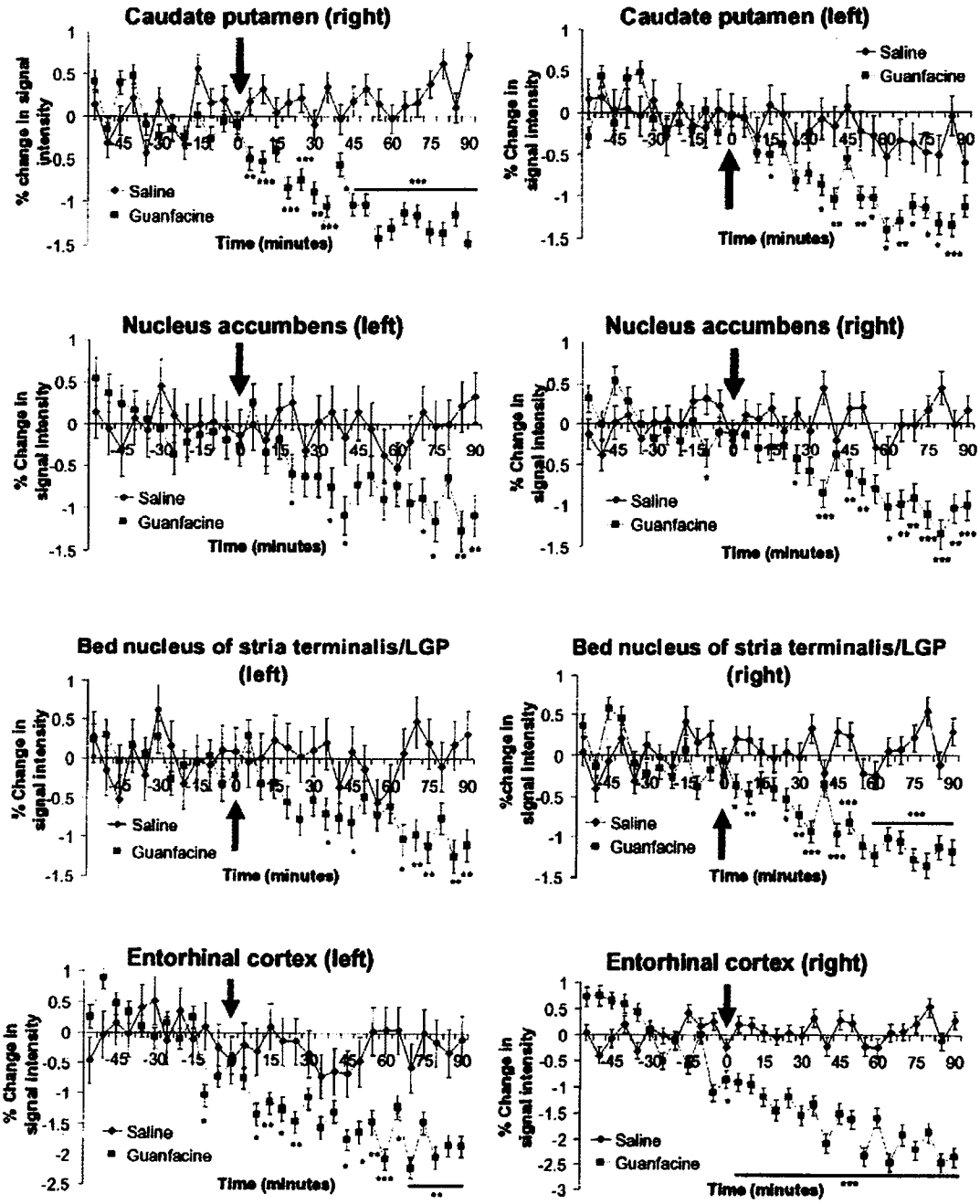
Negative BOLD effect: guanfacine evoked negative BOLD effects compared to the pre-treatment in discrete regions of both sides of the rat brain as listed in the following table provided within the results section of this study:

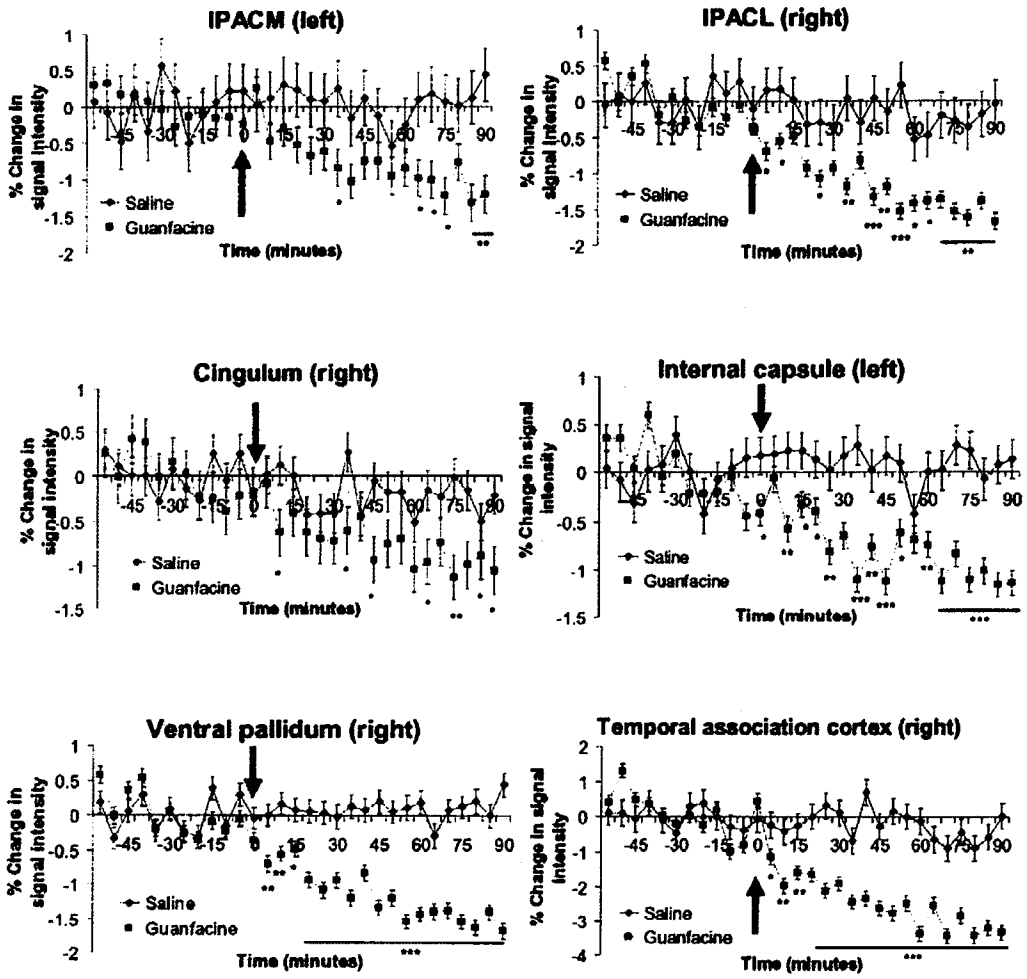
Brain region	Position	T-value	P-value	Maximum Change (% ±sem)	Time to maximal change (mins)
Caudate putamen	Left	8.11	< 0.001	-1.29 ±0.22	60
	Right	12.59	< 0.001	-1.48 ±0.13	90
Nucleus accumbens	Left	11.78	< 0.001	-1.27 ±0.24	85
	Right	7.44	< 0.001	-1.35 ±0.18	80
Bed nuclei of stria terminalis / Lateral globus pallidus	Left	7.67	< 0.001	-1.25 ±0.20	85
	Right	7.42	< 0.001	-1.35 ±0.15	80
Entorhinal cortex	Left	7.33	< 0.001	-2.21 ±0.18	75
	Right	6.38	< 0.001	-2.45 ±0.19	85
Interstitial nucleus of the posterior limb of the anterior commisure, lateral part (IPACL)	Right	7.92	< 0.001	-1.68 ±0.12	90
Interstitial nucleus of the posterior limb of the anterior commisure, medial part (IPACM)	Left	8.05	< 0.001	-1.31 ±0.25	85
Cingulum	Right	8.64	< 0.001	-1.13 ±0.26	75
Internal capsule	Left	4.53	< 0.001	-1.17 ±0.13	85
Ventral pallidum	Right	5.95	< 0.001	-1.68 ±0.12	90
Subiculum / Temporal Association Cortex	Right	6.46	< 0.001	-3.43 ±0.23	75
Ectorhinal Cortex	Left	4.70	< 0.001	-1.44 ±0.21	90

Table 1. CNS areas showing significant ($p < 0.001$) negative BOLD changes by random effect analysis following guanfacine administration (0.3 mg/kg, n = 9). Whole brain BOLD effects obtained using 17 RARE volume data sets over a 90 minute period following drug injection are expressed as a percentage mean difference from corresponding basal signal intensity ± s.e.m. The time at which there was a maximal intensity change in BOLD response is also indicated for each region listed.

The change of BOLD signal at each post-treatment timepoint in guanfacine treated rats was highly significant from the saline treated rats (unpaired t-test analysis). See the following figures provided in the results section from this study:

Figure 3





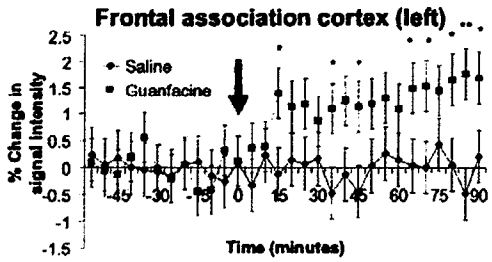
As seen from the results, the time taken to reach peak maximal change in signal ranged from 60-90 min post treatment and it is not clear if it will even get bigger after that time since the collection of the data was up to 90 min only due to the limitation of the technicality of methods (according to the sponsor).

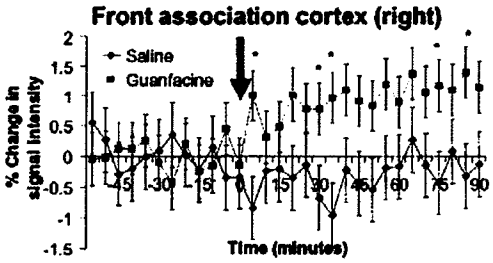
Positive BOLD effect was seen in the following areas of the brain as summarized by the sponsor in the following table:

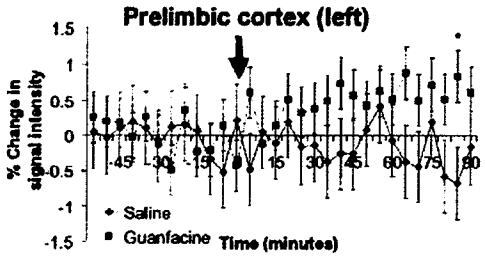
Brain region	Position	P-value	Number of animals showing effect (of 9)	Maximum Change (% ±sem)	Time to maximal change (mins)
Frontal association cortex	Left	-	5	1.76 ±0.49	85
	Right	< 0.05	7	1.40 ±0.44	85
Secondary motor cortex	Right	< 0.05	7	2.64 ±0.55	90
Prelimbic cortex	Left	-	4	0.89 ±0.36	65
	Right	< 0.05	7	0.55 ±0.38	65
Lateral hypothalamic area	Right	< 0.05	7	1.46 ±0.08	80
Field CA1 of hippocampus / Thalamic nuclei	Central	< 0.05	7	2.69 ±0.15	90
Retrosplenial agranular cortex	Central	< 0.10	6	2.14 ±0.06	45
Dentate gyrus	Left	-	4	1.76 ±0.15	75
	Right	-	5	1.03 ±0.08	80
Medial forebrain bundle/ Subincertal nucleus (Sn)	Left	-	5	1.18 ±0.02	40
Mediodorsal thalamic nucleus	Central	< 0.10	6	2.30 ±0.20	90

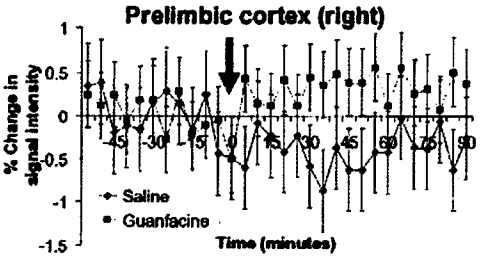
Table 2. CNS areas showing positive BOLD effects following intraperitoneal injection of guanfacine (0.3 mg/kg, n = 9) obtained from probability maps. P-values were calculated using binomial probability. Whole brain BOLD effects obtained using 17 RARE volume data sets over a 90 minute period following drug injection are expressed as a percentage mean difference from corresponding basal signal intensity ± s.e.m. The time at which there was a maximal intensity change in BOLD response is also indicated for each region listed.

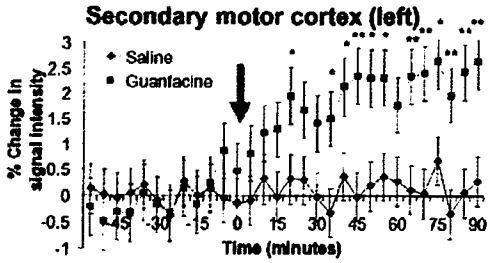
Time course for the same regions showed a clear change between saline controls and guanfacine treated animals (see following figures from the sponsor):

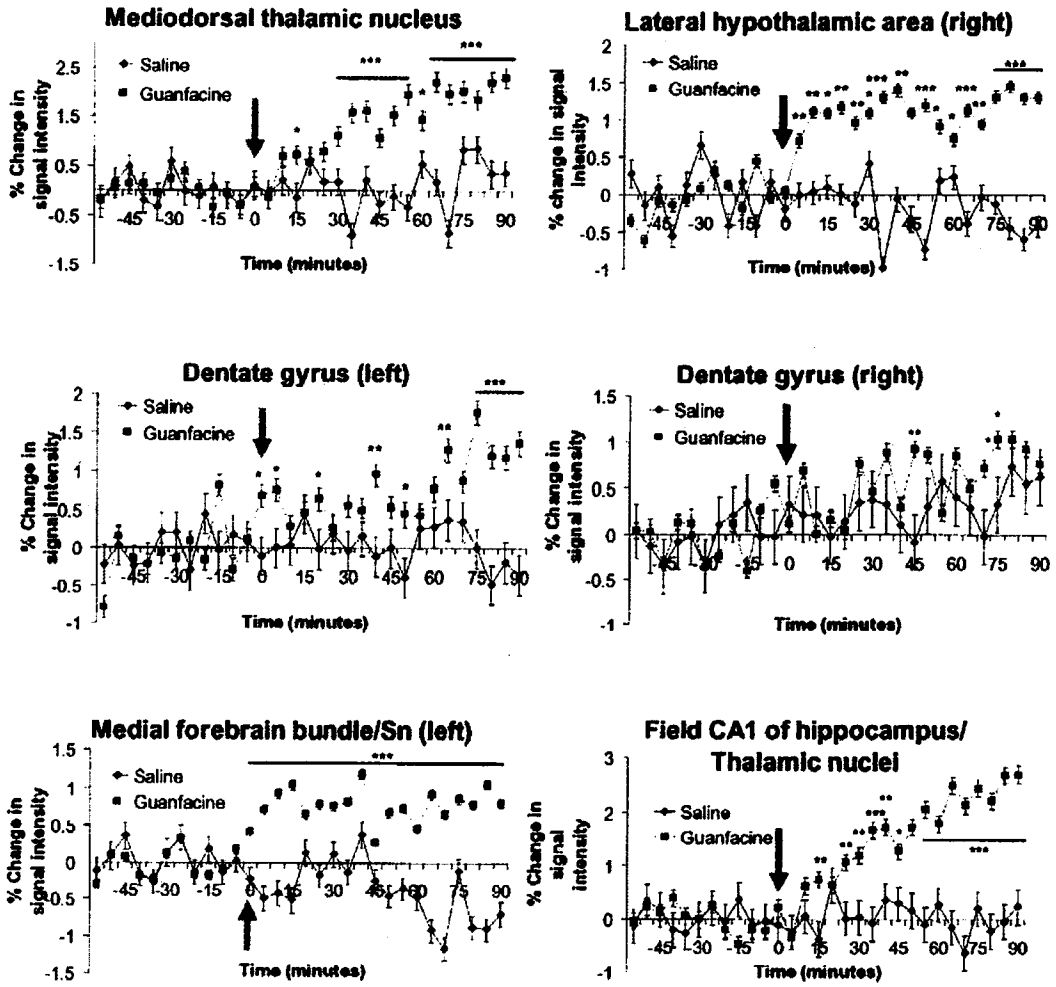

Frontal association cortex (left)
Saline
Guanfacine
% Change in signal intensity
Time (minutes)


Front association cortex (right)
Saline
Guanfacine
% Change in signal intensity
Time (minutes)


Prelimbic cortex (left)
Saline
Guanfacine
% Change in signal intensity
Time (minutes)


Prelimbic cortex (right)
Saline
Guanfacine
% Change in signal intensity
Time (minutes)


Secondary motor cortex (left)
Saline
Guanfacine
% Change in signal intensity
Time (minutes)



Physiological measures:

There was no effect on blood pressure values or respiratory rates throughout the experiment as a result of treatment.

Conclusions: in light of the previously presented data the compound appears to result in decreases in functional BOLD response in specific regions of the brain (such as caudate pautamen, nucleus accumbens and entorhinal cortex). The authors suggested that this results in a deactivation within the dopamine rich regions of the brain; however, this assumption is based on the hypothesis that this decrease in BOLD contrast reflects a decrease in activity of these neuronal pathways. As the authors suggested earlier this **decrease in BOLD contrast could be the result of a "vascular steal" effect, i.e. that the** blood is going to another active area and therefore the rCBF in this area is decreased. The authors argue against the vascular steal effect in this situation because this will result in a more random effect than the local and discrete changes observed here (specific areas

were affected and not a random effect). The authors added that the temporal analysis of the negative BOLD effects produced here showed a time and treatment dependent effect following guanfacine administration in comparison to saline administration. The authors suggested that the data presented here would support the notion that guanfacine produced the inhibitory action in striatal areas which could be linked to activation of alpha 2 adrenergic receptors in prefrontal areas or could be independent of changes in neuronal function in the PFC. The authors sited a paper in which alpha 2 receptors modulated the release of dopamine in rabbit caudate nucleus using fast cyclic voltammetry leading to the conclusion that dopaminergic synapses in the caudate possess inhibitory presynaptic alpha-2 adrenergic receptors (Trendeleburg et al., 1994). The authors also sited studies that showed that most of the adrenergic receptors in this area are of the α_{2A} subtypes and therefore the effect seen is probably mostly through the alpha 2A receptors.

The positive BOLD effect seen in the fronto-cortical areas were viewed by the study authors as an indication of increased activity in this areas and was correlated with other studies where treatment with guanfacine showed an improvement in cognitive performance and increased rCBF values in dorsolateral PFC in young adult rhesus monkeys (Avery et al., 2000). The authors continued to emphasize the role of these areas in different activities such as those needed for purposeful movements (frontal associative area and secondary motor areas) and cognition. Increased activity was also reported in the perlimbic region of the PFC which is thought to be involved in executive functions requiring attentional shift and behavioral flexibility, and in working memory.

The authors indicated that there are certain known and confirmed connections between these brain regions (i.e. the prefrontal cortex and the striatal areas) so it will not be surprising if pharmacological manipulation of one area would have a potential to influence the other through an inhibitory of stimulatory effect.

The authors of the study concluded that the BOLD effect is well known to be closely linked with neuronal activity and that the striatum and the PFC are often associated with locomotor and cognitive aspects of ADHD, respectively. The authors suggested that guanfacine acts on the prefrontal cortex (probably postsynaptically at alpha-2 adrenoreceptors) to increase cognitive and associated function and may also help in the regulation of locomotor activity via inhibitory control of subcortical brain regions such as the caudate pautamen and nucleus accumbens.

2.6.2.3 Secondary pharmacodynamics

N/A

2.6.2.4 Safety pharmacology

Neurological effects: no data submitted.

Cardiovascular effects:

Study title: Guanfacine Hydrochloride: cardiovascular effects in conscious telemetered dogs (Study #D00023 SLI503-IIF).

This study was a GLP study conducted by (b) (4) in July 2000. The drug (batch # GF08002) was administered using gelatin capsules (doses expressed as free base) to a single group of 4 telemeterd male beagle dogs (12-21 months of age) according to the following regiment as described by the sponsor:

Day 1	Placebo (empty gelatin capsules)
Day 5	0.5 mg guanfacine/kg p.o.
Day 11	1.5 mg guanfacine/kg p.o.
Day 18	5.0 mg guanfacine/kg p.o.

According to the sponsor, on day 18 an error in dosing of the first animal (#5941) occurred and as a result of the behavior observed (see results below), the remaining animals were not dosed. Subsequently, two non-telemetered animals (11 months of age) were dosed on Day 34 with 5 mg/kg guanfacine p.o. The animals were allowed to eat approximately 1h post dosing. The following parameters were evaluated as provided by the sponsor:

> Measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and the lead II ECG variables (measuring PR interval, RR interval, QRS duration and QT interval) were taken from each telemetered animal continuously starting 30 min prior to dosing and ending approximately 24 h following dosing. Mean arterial blood pressure (MAP) was calculated as ($DBP+{}^{1}/_{3}(SBP-DBP)$). QTc was calculated using Fridericia's formula ($QTcF= QT/\sqrt[3]{RR}$), and QTcQ interval was calculated as QTc = QT + # (1-RR). # corresponds to a correction factor specific to each dog as it represents the slope of the line from a plot of QT against RR interval generated over a range of heart rates.

According to the sponsor, a visual inspection of all the ECG waveforms (for disturbances in rhythm and waveform morphology) was performed in all dogs on all of the dosing days. All abnormal waveforms were printed off and reviewed (b) (4)

Results:

General observations:

No effects were seen at 0.5 mg/kg. Vomiting was seen at 1.5 mg/kg in two animals (#5941 at ~1h post dosing, #J1671 at ~2h post dosing). Salivation was seen in animal#5491 at ~2h post dosing.

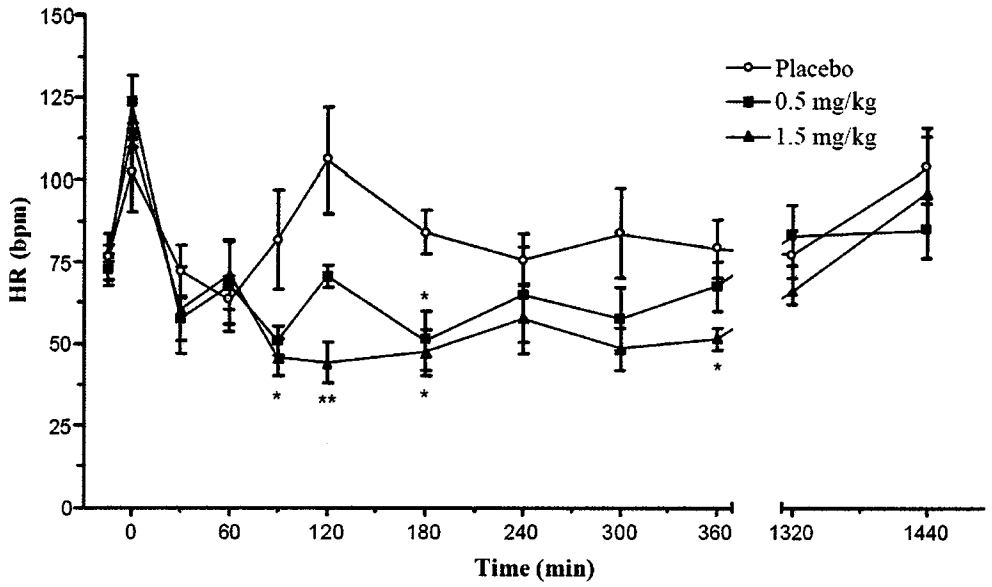
According to the sponsor, when animal # 5941 was dosed with 5 mg/kg of guanfacine, the capsule was damaged during the procedure. This resulted in a small amount of the test substance to be administered in the mouth directly but the remainder (majority) of the dose in the capsule was spilled on the pen floor. Therefore, it was unclear how much test substance was received or the exact route of absorption (sublingual/inhalation). Apparently, the animal vomited ~10 min post this dosing. No clinical signs were observed until ~2h post dose when the animal displayed marked piloerection together with poor limb coordination, notable dragging of forelimbs and weakness of the hindlimbs. Respiration rate was decreased and poor peripheral circulation was noted visually from the pallor of the gums, tongue and skin. The sponsor indicated that bradycardia was also observed as judged from manual palpitation of the pulse. These sings started to improve by 5h post dosing and by 21h the animal appeared to be fine. According to the sponsor, gross behavioral observations of the non-telemetered animals treated with 5 mg/kg revealed a similar profile of clinical signs observed in the partially dosed animal #5941 (vomiting, incoordination of movement, piloerection, poor peripheral circulation, and bradycardia). At ~22h post dose the animals were normal.

Arterial blood pressure and heart rate:

There was no marked effect on arterial blood pressure (mean, systolic, and diastolic). There were some points where systolic blood pressure was significantly lower than the placebo value but these values were not dose related and were similar to the pretreatment value in these animals. Therefore, these are not considered to be drug related.

Hear rate was found to be decreased at around 1.5-6h post treatment at both the 0.5 and 1.5 mg/kg. The maximal effect was seen at 2h. Heart rate was 36 and 62 beats per min lower at 0.5 and 1.5 mg/kg compared to the placebo, respectively. The following figure was extracted from the sponsor's **submission (Figure 4):**

Figure 4
The Effect of Guanfacine on Heart Rate in Conscious, Telemetered Male Beagle Dogs



The effect of partial dosing with the 5 mg/kg/day in one dog (5941) had no effect on blood pressure but caused marked bradycardia.

Lead II ECG:

In correlation with the bradycardia, there was an increase in RR interval seen between 1.5 and 6h post dosing at the 0.5 and 1.5 mg/kg. PR interval was also increased 1-6h after administration of both 0.5 and 1.5 mg/kg test substance (the effect was similar at both doses in both magnitude and duration). QRS duration, QT interval and the QT corrected intervals (QTcF and QTcQ intervals) were relatively unaffected by oral administration of guanfacine at doses of 0.5 or 1.5 mg/kg. The following figures are obtained from the **sponsor's submission (Figures 5-10):**

Figure 5
The Effect of Guanfacine on RR Interval in Conscious, Telemetered Male Beagle Dogs

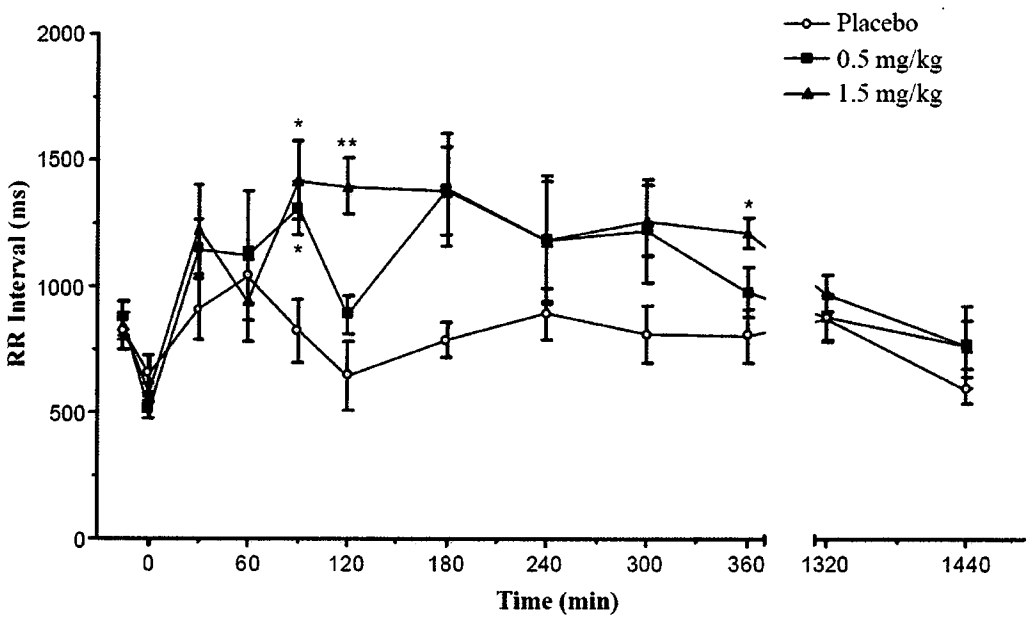


Figure 6
The Effect of Guanfacine on PR Interval in Conscious, Telemetered Male Beagle Dogs

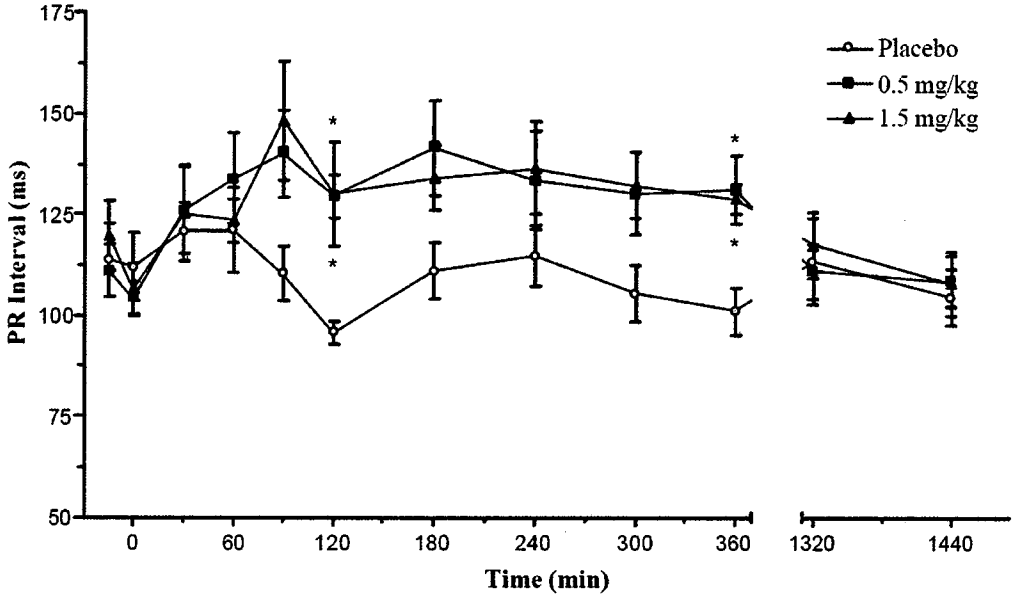


Figure 7
The Effect of Guanfacine on QRS Duration in Conscious, Telemetered Male Beagle Dogs

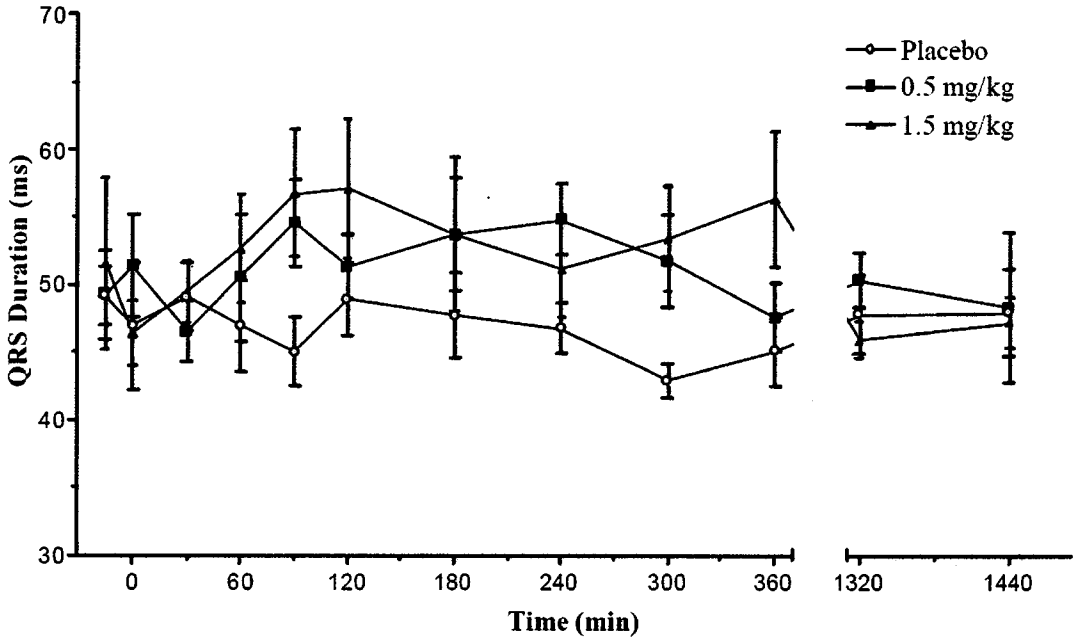


Figure 8
The Effect of Guanfacine on QT Interval in Conscious, Telemetered Male Beagle Dogs

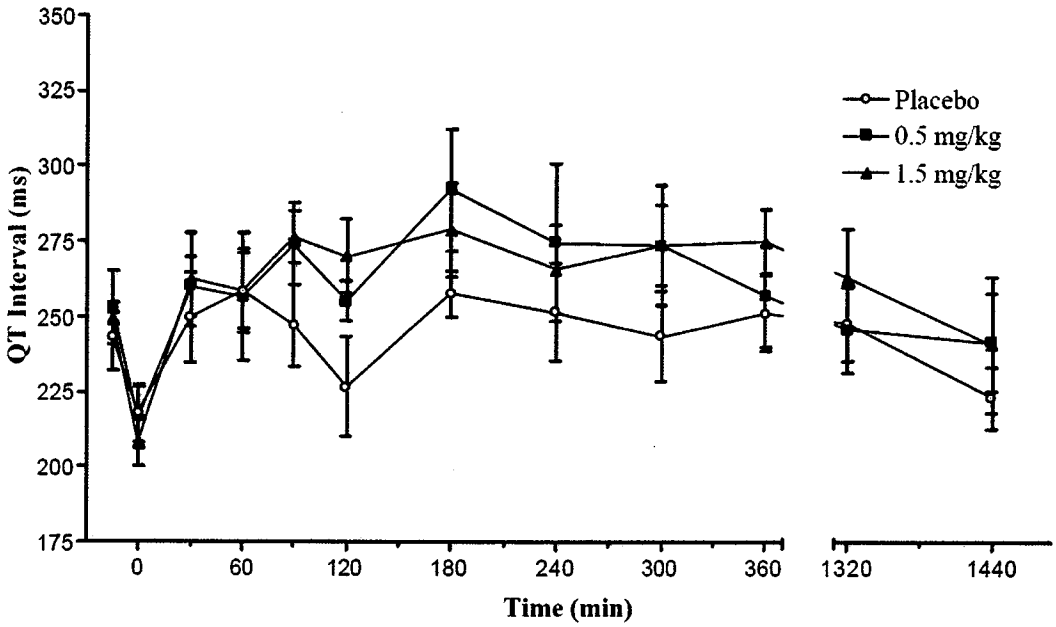


Figure 9
The Effect of Guanfacine on QTcF Interval in Conscious, Telemetered Male Beagle Dogs

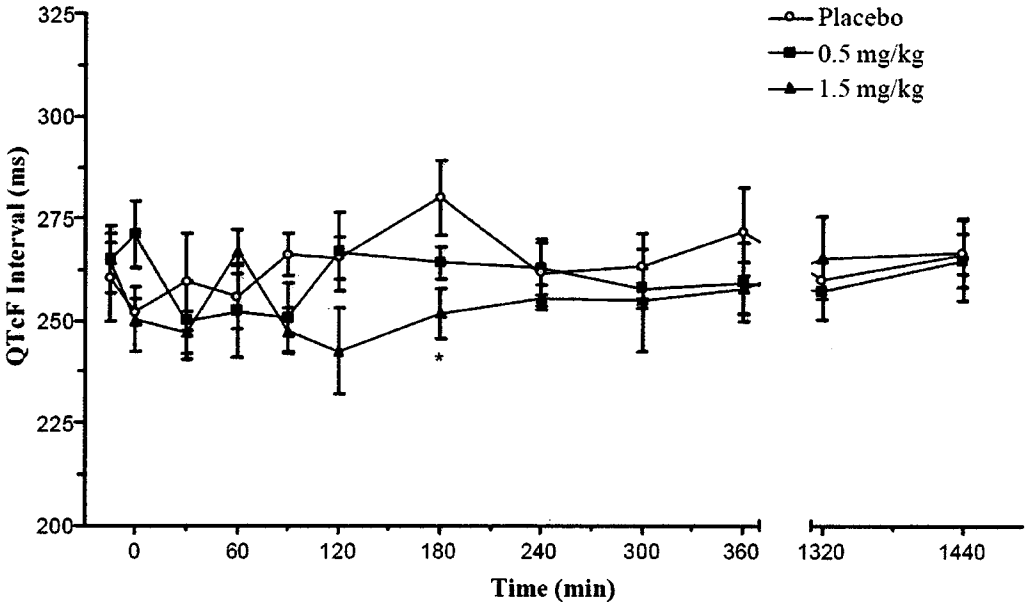
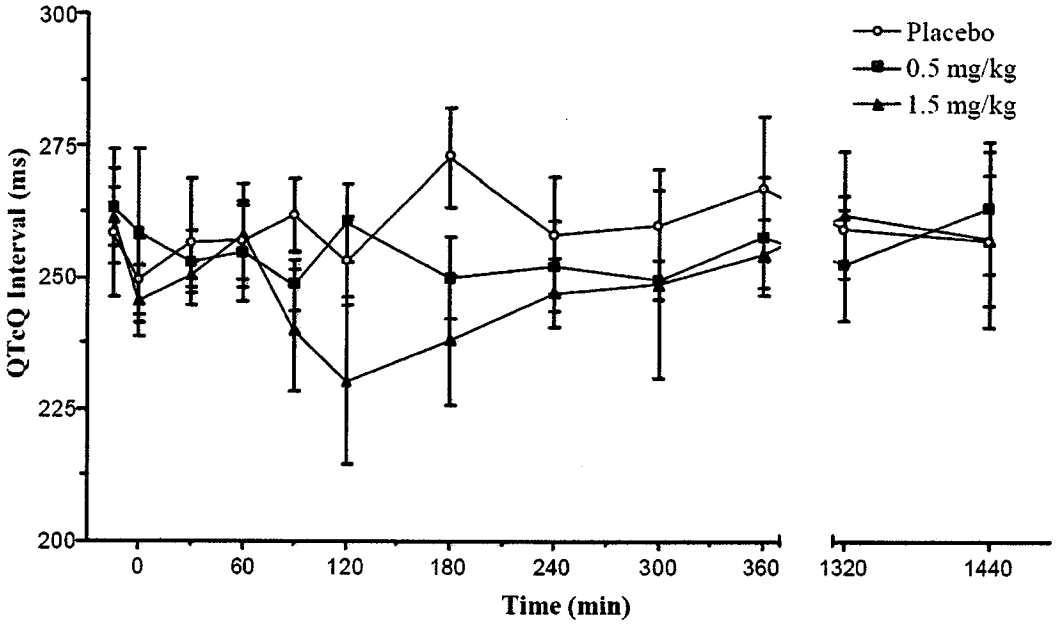


Figure 10
The Effect of Guanfacine on QTcQ Interval in Conscious, Telemetered Male Beagle Dogs



Examination of the lead II ECG waveform indicated a dose-dependent worsening of an existing bradydysrhythmia, associated with sinus bradycardia and delayed conduction through the AV-node, in two animals. One of those two animals was animal # 5941, the administration of the drug caused dose dependent exacerbation on an underlying inherent bradydysrhythmia characterized by sinus pauses and first- and second-degree hear block and associated supraventricular premature complexes (this happened in the first 7h after drug treatment at both treatment doses). The other dog (#4216) had similar bradydysrhythmia, characterized by sinus pauses and first- and second-degree heart block and associated supraventricular premature complexes, was noted following the 0.5 mg/kg dose (1-2h post dose). The incidence of this dysrhythmia increased following 1.5 mg/kg dose (1-5h post dose). No significant drug-related changes were observed in the other two animals.

Summary and Conclusion:

Four telemetered beagle dogs were treated with 0.5, 1.5 and 5.0 mg/kg guanfacine orally (capsules). The dose of 5 mg/kg was administered to only 1 telemetered dog (partial dose since the capsule was not totally administered) but due to worsening condition of this

animal the other telemetered dogs were not treated with this dose. Two untelemetered dogs were treated with this dose and similar signs were observed (see review for details).

Oral administration of 1.5 mg/kg of guanfacine caused vomiting in 2/4 animals and with 5 mg/kg there was increased incidence of these episodes and resulted in incoordination of movement, piloerection and poor peripheral circulation in the only two animals examined.

Oral administration of 0.5 and 1.5 mg/kg of the test substance was not associated with effects on arterial blood pressure, QRS, QT, QTcF or QTcQ intervals. A pronounced and prolonged bradycardia was observed following both 0.5 and 1.5 mg/kg administration. This coincided with an increase in the RR and the PR intervals.

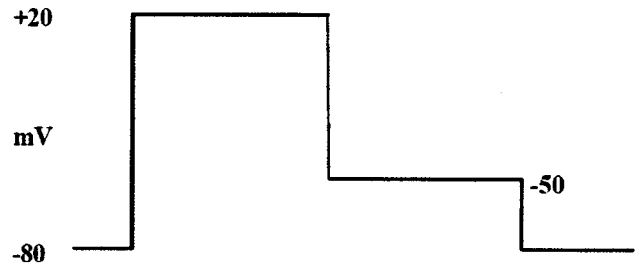
Dose-dependent worsening of an existing bradydysrhythmia, associated with sinus bradycardia and delayed conduction through the AV-node was observed in two animals.

The range of the administered doses was limited since the high dose (5.0 mg/kg) was not used in the telemetered dogs due to concern that the dose might not be tolerated, as was seen in one dog that received only partial amount of that dose. The fact that similar observations were seen in two other dogs (non-telemetered) indicated that the effect was reproducible and was not due to overly sensitive animal. However, it is reasonable to suggest that another dose that is between 1.5 and 5 mg/kg could have been used (i.e. 3 mg/kg) and therefore that dose could have been tolerated and could have indicated a better understanding of the effect of the drug on the CVS. However, one could argue that the 1.3 mg/kg dose could have been adequate due to the observation of vomiting in 2/4 animals. The conclusion is that the study did not indicate an effect for treatment on QTc duration and it seems to worsen existing condition of bradydysrhythmias that are associated with sinus bradycardia and conduction through the AV-node in treated animals.

Study title: effect of guanfacine hydrochloride on HERG currents recorded from stably transfected HEK293 Cells (Study #DIVO1045)

This study was a GLP study conducted by (b) (4) in February 2001. The test article (Lot # GF 08002) was used to perfuse HEK293 cells (transfected with the HERG channel) at a concentration 1 µg/ml (~4 µM, as calculated by reviewer, MW 246 free base) for 15 min exposure at a rate of 1-2 ml/min under a whole cell clamping conditions (n=5). The other groups were a vehicle group treated with 100% bath solution (n=4) and a reference substance (100 nM E-4301, an agent known to block the HERG current); n=5 (n=2 test substance and n=3 vehicle treated cells). The voltage protocol was described by the sponsor in the following diagram extracted from **the sponsor's submission:**

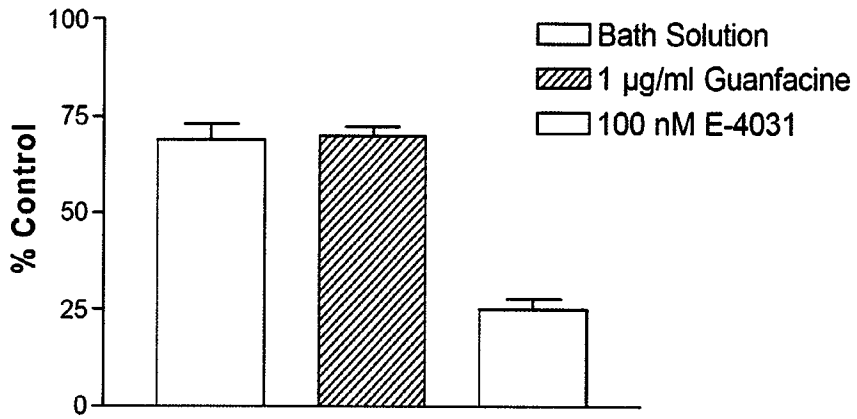
Voltage Protocol



Results:

The results are summarized in the following figure as provided by the sponsor:

Figure 1 Effect of Guanfacine (1 µg/ml), Bath Solution (100%) and E-4031 (100 nM) on HERG Tail Current



Conclusions:

At the concentration that was used in this study (1 µg/ml) there appears to be no effect on the HERG channel current. However, it should be pointed out that only one

concentration was used (which is equivalent to ~4 μM). It is possible that this concentration is too low to test the effect of the drug on the HERG channel current; therefore, additional higher concentrations should have been used to cover a spectrum of concentrations to confirm that the negative results are not due to the inadequacy of the dose use.

Pulmonary effects: no studies submitted

Renal effects: no studies submitted

Gastrointestinal effects: no studies submitted

Abuse liability: no studies submitted

Other:

2.6.2.5 Pharmacodynamic drug interactions:

No studies

2.6.3 PHARMACOLOGY TABULATED SUMMARY

No tables were available.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

No studies were reviewed for this section. The P/K characteristics of the compound in humans are well known in view of the long experience with this drug.

2.6.4.2 Methods of Analysis

N/A

2.6.4.3 Absorption

2.6.4.4 Distribution

2.6.4.5 Metabolism

2.6.4.6 Excretion

2.6.4.7 Pharmacokinetic drug interactions

2.6.4.8 Other Pharmacokinetic Studies

2.6.4.9 Discussion and Conclusions

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

N/A

2.6.6 TOXICOLOGY

This section will be dedicated to the studies conducted to compare a "pure" form of SPD503 to an "impure" form:

2.6.6.1 Overall toxicology summary

Studies were conducted to compare the effects of an impure form of guanfacine (spiked with approximately (b)(4)% for each of three impurities: (b) (4) and (b) (4); also referred to as impurities (b) (4) in the study reports) with a pure form in an attempt to qualify these impurities. These impurities were specified up to (b)(4)% in the drug substance (see CMC review #1 in DFS dated August 24, 2006, page 13, Table 1). These studies were a 4-week toxicity study in rats, and two genotoxicity studies (Ames test and chromosomal aberrations).

General toxicology:

In the 4-week toxicity study, Crl:OFA(SD) rats (16/sex/group, with 6/sex/group for a recovery period of 2 weeks) were treated with either the vehicle or with each form of guanfacine at a dose level of 10 mg/kg/day orally in the diet. The following parameters were evaluated: mortality, clinical signs, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, gross pathology, organ weights, and histopathology. The results of the study indicated that the two forms of guanfacine resulted in similar findings with very minor differences that can be considered biologically insignificant (see the study review for specific details). Therefore, it is concluded that these impurities that are found in the impure form did not result in any significant differences in findings that are seen with the pure form and accordingly these impurities will be considered qualified for their toxic effects.

Genetic toxicology:

In a chromosomal aberration study human peripheral blood lymphocytes were incubated with both the pure and the impure form of the compound (spiked with the impurities but the level was not defined). The concentrations ranged from 10-240 ug/ml (see review of study for details) and the incubations were for 3h with and without S9 and for 20h without S9. The highest doses chosen were based on the mitotic index at that dose (at least 50% inhibition) and analysis from highly toxic doses was avoided. The data

indicated no differences between the two forms of the compound and that small and sporadic increases in the frequencies of numerical aberrations were seen in both forms and were more that seen in the current control and the normal ranges.

In an Ames test, the two forms of the compound (the impure spiked with the impurities at the level of (b) (4) %) were tested with the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA102. The concentrations the test article ranged from 20 to 5000 ug/plate (7 concentrations were used) both with and without S9. The results were negative in this test.

Based on the level to which these impurities were spiked in the "impure" form of the drug ((b) (4) % for each) and the levels of their specification in the drug substance (up to (b) (4) % for each) then, these impurities will considered qualified from a genotoxicity perspective.

Carcinogenicity: no studies conducted. The studies submitted with original guanfacine NDA are described in the labeling.

Reproductive toxicology: no studies conducted. The studies submitted with original guanfacine NDA are described in the labeling.

Special toxicology: no studies submitted here. Juvenile rat studies were submitted prior to the NDA submission and a review of these studies can be found in DFS under the IND for this submission (IND 63551).

2.6.6.2 Single-dose toxicity

No studies conducted.

2.6.6.3 Repeat-dose toxicity

Study title: SPD503 (Guanfacine hydrochloride) a comparative toxicity study of pure and impure material by dietary administration to OFA rats for 4 weeks followed by a 2-week recovery period

Key study findings:

Study no.: R01020-SPD503
Volume #, and page #: electronic submission under toxicology (r01020-pdf)
Conducting laboratory and location:

(b) (4)

Date of study initiation: July 2006
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: Batch # for the impure form is 05MF291/B, the impure form was spiked with (b) (4) % of each of the impurities (b) (4) and the batch # of the pure form was labeled as GF0 5004/A

Methods

Doses: 10 mg/kg/day of either the pure or the impure form (expressed as guanfacine hydrochloride)

Species/strain: Crl:OFA(SD) rats

Number/sex/group or time point (main study): 16/sex/group with 6 in each group for the recovery period (2 weeks of recovery)

Route, formulation, volume, and infusion rate: orally in the diet

Satellite groups used for toxicokinetics or recovery: blood samples were obtained from the recovery group animals at the end of Week 4

Age: 49 days

Weight: 241-297 g for M, 138-185 g for F

Mortality: daily.

Clinical signs: at least twice daily for ill-health or reaction to treatment. A more detailed weekly physical exam was also done.

Body weights: one week before treatment and weekly thereafter to the end of the recovery period.

Food consumption: weekly and the weekly consumption per (g/rat/week) was calculated for each cage.

Ophthalmoscopy: prior to treatment and during Week 4. The pupils were dilated, the adnexae, conjunctiva, cornea, sclera, anterior chamber, iris, lens, vitreous and fundus were examined.

EKG: not performed.

Hematology: during week 4 of treatment (males) or Week 5 immediately prior to termination (F) and Week 3 of recovery (prior to termination). Blood was obtained after an overnight withdrawal of food. With animals under light general anesthesia induced by isoflurane blood samples were withdrawn from sublingual vein. The following parameters were evaluated:

Haematocrit (Hct)
Haemoglobin (Hb)
Erythrocyte count (RBC)
Mean cell haemoglobin (MCH)
Mean cell haemoglobin concentration (MCHC)
Mean cell volume (MCV)
Total white cell count (WBC)
Differential WBC count
- Neutrophils (N)
- Lymphocytes (L)
- Eosinophils (E)
- Basophils (B)
- Monocytes (M)
- Large unstained cells (LUC)

Platelet count (Plt)

Clinical chemistry: blood was collected at the same time from the animals described under the hematology section. The following parameters were evaluated:

Alkaline phosphatase (ALP)
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Total bilirubin (Bili)
Urea
Creatinine (Creat)
Glucose (Gluc)
Total cholesterol (Chol)
Triglycerides (Trig)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Calcium (Ca)
Inorganic phosphorus (Phos)
Total protein (Total Prot)

Urinalysis: during week 4 of treatment and week 2 of recovery. Overnight urine samples were collected for all animals. The animals were placed in metabolic cages without food or water. The following parameters were evaluated: volume, pH, specific gravity, protein, sodium, potassium, chloride, glucose, ketones, bilirubin, and heme pigments. Microscopic examination included:

Crystals (Cryst)
Epithelial cells(Epi)
Leucocytes (Leuc)
Erythrocytes (RBC)
Casts
Spermatozoa and precursors (Sperm)
Other abnormal components (A)

Gross pathology: all animals were subject to a detailed necropsy.

Organ weights : the following organs and tissues were weighed:

Adrenals	Prostate
Brain	Submandibular salivary glands (Submand
Epididymides (Epididymid)	Salivary G)
Heart	Seminal vesicles (Seminal Ve)
Kidneys	Spleen
Liver	Testes
Lungs with mainstem bronchi (Lungs & Br)	Thymus
Ovaries	Thyroid with parathyroids (Thyroids+P)*
Pituitary	Uterus with cervix (Uterus&C)

* Weighed after partial fixation.

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

The following tissues were preserved and examined histologically from all animals:

Adrenals
Aorta - thoracic
Brain
Caecum
Colon
Duodenum
Epididymides
Eyes
Femurs+
Harderian glands
Head#
Heart
Ileum (including Peyer's patches)
Jejunum
Kidneys
Lachrymal glands
Larynx
Liver
Lungs
Lymph nodes - mandibular
- mesenteric
Mammary area - caudal
Oesophagus
Optic nerves
Ovaries
Pancreas
Pituitary
Prostate
Rectum
Salivary glands - submandibular+
- parotid+
- sublingual+
Sciatic nerves+
Seminal vesicles
Skeletal muscle - thighs+
Skin
Spinal cord
Spleen
Sternum (including bone marrow)
Stomach
Testes
Thymus
Thyroid with parathyroids
Tongue
Trachea
Ureters
Urinary bladder
Uterus and cervix
Vagina

+ Only one processed for examination
# Not processed for examination

Tissues reported at macroscopic examination as being grossly abnormal were examined for all main and recovery animals.

Results:

Guanfacine formulation: the mean concentration of guanfacine in test diet formulations analysed for the study were within -6% of the nominal concentrations (105 ppm). The stability was confirmed during ambient temperature storage for 7 days and following frozen storage for 14 days. The homogeneity was confirmed for Guanfacine at a nominal concentration of 200 ppm.

The achieved dosages were confirmed to be ~10 mg/kg/day. The following table was provided by the sponsor:

Achieved dosage - group mean values (mg/kg/day)

GROUP	:	1	2	3
COMPOUND	:	CONTROL	SPD503:IMPURE	SPD503:PURE
DOSAGE (MG/KG/DAY)	:	0	10	10

SEX:	MALE		FEMALE	
GROUP:	2	3	2	3
WEEK				
1	8.4	8.4	10.3	9.0
2	11.0	11.1	11.7	11.7
3	10.2	10.0	10.0	10.2
4	10.8	10.8	10.6	10.7
1-4	10.1	10.1	10.7	10.4

Results:

The following tables and summary were extracted from the sponsor's submission summarizing the results of the study. The reviewer concurs with these summaries after reviewing the data presented in the submission. The data generally indicated no major difference in the effect of the pure material vs. the impure material (see below).

The toxic potentials of pure and impure SPD503 were considered to be essentially similar, with only a few minor differences considered to be of no toxicological importance.

Common responses to pure and impure SPD503 in males and females, or one sex, were as follows:

- Black staining on the muzzle, reduced bodyweight gains, food consumption and food conversion efficiency in both sexes.
- Higher total and differential white blood cell counts and platelet counts in both sexes and longer prothrombin times in females.
- Higher blood levels of aspartate aminotransferse in both sexes and urea and potassium in males. Lower levels of creatinine, glucose and protein in both sexes and phosphorous in females and triglycerides in males.
- Lower urinary volume, pH, and protein in males. Higher urinary specific gravity, sodium, potassium and chloride in both sexes and protein in females. A shift in the appearance of the urine of both sexes from pale to medium yellow.
- A higher incidence of animals with thin appearance at macroscopic examination.
- Lower weights for the heart, liver, prostate and seminal vesicles in both sexes and adrenals, ovaries, pituitary and thyroids of females which were considered to reflect the lower terminal bodyweights of the animals.
- Higher weights for the uterus and cervix which were considered to be of no toxicological importance as they were not supported by macroscopic or microscopic pathology findings.

- Higher adjusted weights for the submandibular salivary glands of males which were considered to reflect the acinar cell hypertrophy detected at microscopic examination of this tissue. Lower adjusted weights for the submandibular salivary glands of females which were considered to reflect the acinar cell atrophy detected at microscopic examination of this tissue.

- Multifocal or focal myocardial inflammatory cells in the heart, decreased extramedullary haemopoiesis and increased haemosiderosis in the spleen, increased sinus histiocytosis in the mesenteric lymph node, acinar cell hypertrophy in the submandibular salivary gland of males, acinar cell atrophy in the submandibular and parotid salivary glands of females, with increased acinar cells with mitotic figures in the submandibular glands.

There were considered to be no essential differences between animals receiving the pure and impure forms of SPD503 for the heart findings, for extramedullary haemopoiesis in the spleen, or for the mesenteric lymph node or submandibular salivary gland findings, in both sexes. Animals receiving the impure form showed a slightly greater effect of treatment than those receiving the pure form for haemosiderosis in the spleen (both sexes) and for acinar cell atrophy in the parotid salivary gland in females, but this was considered unlikely to be of toxicological importance.

Following the 2-week recovery period, it was considered that complete recovery had occurred from the changes seen in main study animals in the heart and spleen, with partial recovery in the mesenteric lymph node, and submandibular and parotid salivary glands.

The following minor isolated differences in response were recorded but were concluded to be of no toxicological importance:

- A statistically significantly higher mean reticulocyte count was confined to males receiving impure SPD503. A statistically significant higher concentration of alanine aminotransferase was confined to males receiving pure SPD503. A statistically significantly lower sodium concentration was confined to males receiving pure SPD503.

2.6.7.7A Repeat-Dose Toxicity: 4-week comparative toxicity study in rats: SPD503 (Guanfacine hydrochloride: pure and impure)

Species/Strain: Rat/OFA(SD) **Duration of Dosing**: 4 weeks **Study No.**: R01020-SPD503
Initial Age: 7 weeks **Duration of Post-dose**: 15 days **Location in CTD**:
Date of First Dose: 1 February 2006 **Method of Administration**: Oral (dietary) **GLP Compliance**: Yes
Vehicle/Formulation: Basal diet - Rat and Mouse No. 1 Maintenance Diet **Special Features**: The study was performed to compare SPD503 with impurities with pure SPD503.
No Observed Adverse Effect Level: Not applicable for this study

	Male			Female		
Test Substance	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material
Daily Dose (mg/kg/day)	0 (Control)	10	10	0 (Control)	10	10
Number of Animals	16	16	16	16	16	16
Toxicokinetics: Concentration (ng/mL) Week 4 (Midnight)	-	9.13	12.6	-	16.0	9.78
Noteworthy Findings						
Died or Sacrificed Moribund	0	0	0	0	0	0
Body Weight (%) Week 0-4	123.9	55++	57++	52.4	59++	56++
Week R0-R2	13.2	180	254++	0.6	1567+	667+
Food Consumption (%) Week 1-4	193	87	89	143	85	83
Week R1-R2	186	107	112	129	112	108
Clinical Observations: Black staining on the muzzle	0/16	15/16	12/16	0/16	5/16	7/16

(Continued)

2.6.7.7A Repeat-Dose Toxicity: 4-week comparative toxicity study in rats: SPD503 (Guanfacine hydrochloride: pure and impure)

	Male			Female		
Test Substance	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material
Daily Dose (mg/kg/day)	0 (Control)	10	10	0 (Control)	10	10
Number of Animals	16	16	16	16	16	16
Ophthalmoscopy	There were no treatment-related findings					
Haematology						
Reticulocytes (%) Week 4/5	2.38	2.82++†	2.47	2.20	1.87	1.94
Week R3	2.37	2.90+	3.24++	NR	NR	NR
Total white blood cell counts ($x10^9$/L)						
Week 4/5	9.42	12.66++	12.90++	6.50	7.15	8.54+
Week R3	6.11	9.52+	7.76	5.16	6.66††	4.08
Neutrophils ($x10^9$/L) Week 4/5	1.30	2.16++†	1.57	0.61	1.00	0.62
Week R3	0.89	2.02++	1.27	0.54	0.60	0.43
Eosinophils ($x10^9$/L) Week 4/5	0.11	0.16	0.14	0.09	0.15+	0.13+
Monocytes ($x10^9$/L) Week 4/5	0.32	0.45	0.38	0.14	0.28	0.21+
Week R3	0.20	0.41+	0.25	0.14	0.14	0.11+
Lymphocytes ($x10^9$/L) Week 4/5	7.66	9.73+	10.62++	5.61	5.66†	7.49+
Week R3	4.84	6.80	6.04	4.36	5.79††	3.44
Basophils ($x10^9$/L) Week 4/5	0.05	0.11	0.12+	0.03	0.03†	0.04+
Week R3	0.05	0.11	0.07	0.03	0.03†	0.02
Large unstained cells ($x10^9$/L)						
Week 4/5	0.04	0.06+	0.07+	0.04	0.03†	0.05+
Week R3	0.02	0.03	0.03	0.01	0.04++††	0.01

(Continued)

2.6.7.7A Repeat-Dose Toxicity: 4-week comparative toxicity study in rats: SPD503 (Guanfacine hydrochloride: pure and impure)

		Male			Female		
		N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material
		0 (Control)	10	10	0 (Control)	10	10
		16	16	16	16	16	16
Haematology (continued)							
Platelet counts (x10^9/L)	Week 4/5	829	1045++†	929	894	1027	1013
Prothrombin time (sec)	Week 4/5	13.7	13.8	13.8	14.1	14.9+	14.9+
Activated partial thromboplastin time (sec)	Week 4/5	19.4	18.1	17.3+	16.6	14.4++†	16.8
Serum Chemistry							
Alanine aminotransferase (U/L)							
	Week 4/5	28	31	35++	30	34	33
	Week R3	31	25†	32	NR	NR	NR
Aspartate aminotransferase(U/L)							
	Week 4/5	71	90++	97++	83	93++	91+
Creatinine (µmol/L)	Week 4/5	34	29++	27++	34	28++†	31
	Week R3	38	34	34+	45	38+	39+
Urea (mmol/L)	Week 4/5	5.06	6.98++	6.77++	4.85	5.00	4.96
Sodium (mmol/L)	Week 4/5	139	138	137++	142	142†	143
	Week R3	142	143+	142	NR	NR	NR

(Continued)

2.6.7.7A Repeat-Dose Toxicity: 4-week comparative toxicity study in rats: SPD503 (Guanfacine hydrochloride: pure and impure)

		Male			Female		
Test Substance		N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material
Daily Dose (mg/kg/day)		0 (Control)	10	10	0 (Control)	10	10
Number of Animals		16	16	16	16	16	16
Serum Chemistry (continued)							
Potassium (mmol/L)	Week 4/5	5.3	6.3+	6.4+	4.2	4.5+†	4.1
	Week R3	4.3	4.5	4.6	3.9	4.9+†	3.9
Glucose (mmol/L)	Week 4/5	5.56	4.35++	4.06++	6.49	4.94++	5.18+
Total protein (g/L)	Week 4/5	64	59++	59++	60	57++	56++
	Week R3	66	64	64	65	62+	63
Triglyceride (mmol/L)	Week 4/5	0.77	0.51++	0.59+	0.40	0.46	0.42
Phosphate (mmol/L)	Week 4/5	2.38	2.43	2.36	2.61	2.43	2.39+
	Week R3	NR	NR	NR	2.02	2.61++†	2.25
Urinalysis							
Volume (ml)	Week 4	5.3	1.3++	1.4++	4.4	1.2++	1.3++
	Week R2	5.5	6.8	6.7	3.0	2.9††	5.6+
pH	Week 4	7.2	6.7+	6.3++	6.4	6.2+	5.9++
	Week R2	7.3	7.6†	7.0	6.4	6.4†	6.7+
Specific gravity (g/L)	Week 4	1038	1060++††	1078++	1037	1065++	1059++
	Week R2	1039	1028++	1034	1048	1047†	1031+
Urinary potassium (mmol/L)	Week 4	210.2	314.2++	326.2++	176.1	308.2++†	229.4
	Week R2	201.4	107.4++†	162.3	223.4	173.6	122.4

(Continued)

2.6.7.7A Repeat-Dose Toxicity: 4-week comparative toxicity study in rats: SPD503 (Guanfacine hydrochloride: pure and impure)

		Male			Female		
Test Substance		N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material
Daily Dose (mg/kg/day)		0 (Control)	10	10	0 (Control)	10	10
Number of Animals		16	16	16	16	16	16
Urinalysis (continued)							
Urinary chloride (mmol/L)	Week 4	97.4	221.3++	194.8++	107.4	255.9++†	207.0++
Urinary sodium (mmol/L)	Week 4	90.7	160.2++	146.7++	94.0	152.4+	124.7
Protein (g/L)	Week 4	0.80	0.54+	0.49++	0.02	0.22++	0.33++
Organ Weights (%[‡])							
Heart	Main	1.395	89+	91	0.802	96	96
	Recovery	1.325	104††	111++	0.823	97	96
Liver	Main	18.34	93†	102	7.36	96	94
Salivary gland	Main	0.592	123+	116	0.426	92	93
	Recovery	0.644	105	113+	0.439	107	103
Pituitary	Main	0.010	90†	110	0.012	75+	75+
Adrenal	Main	0.060	105	110	0.076	83++	80++
Lung and bronchi	Main	1.549	109†	99	1.064	94	96
	Recovery	1.730	98†	116+	1.080	101	104

(Continued)

2.6.7.7A Repeat-Dose Toxicity: 4-week comparative toxicity study in rats: SPD503 (Guanfacine hydrochloride: pure and impure)

		Male			Female		
Test Substance		N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material
Daily Dose (mg/kg/day)		0 (Control)	10	10	0 (Control)	10	10
Number of Animals		16	16	16	16	16	16
Organ weight (continued)							
Thyroid with parathyroid	Main	0.015	93	147	0.014	79+	79
Prostate	Main	0.788	86	79++	-	-	-
Seminal vesicle	Main	1.182	75++	87	-	-	-
Gross Pathology		There was no difference between the two treated groups					
Histopathology							
Heart:							
Myocardial inflammatory cells	Main	2	5	5	1	2	0
	Recovery	3	4	2	3	1	0
Myocarditis	Main	0	0	1	0	0	0
	Recovery	1	0	0	0	0	0

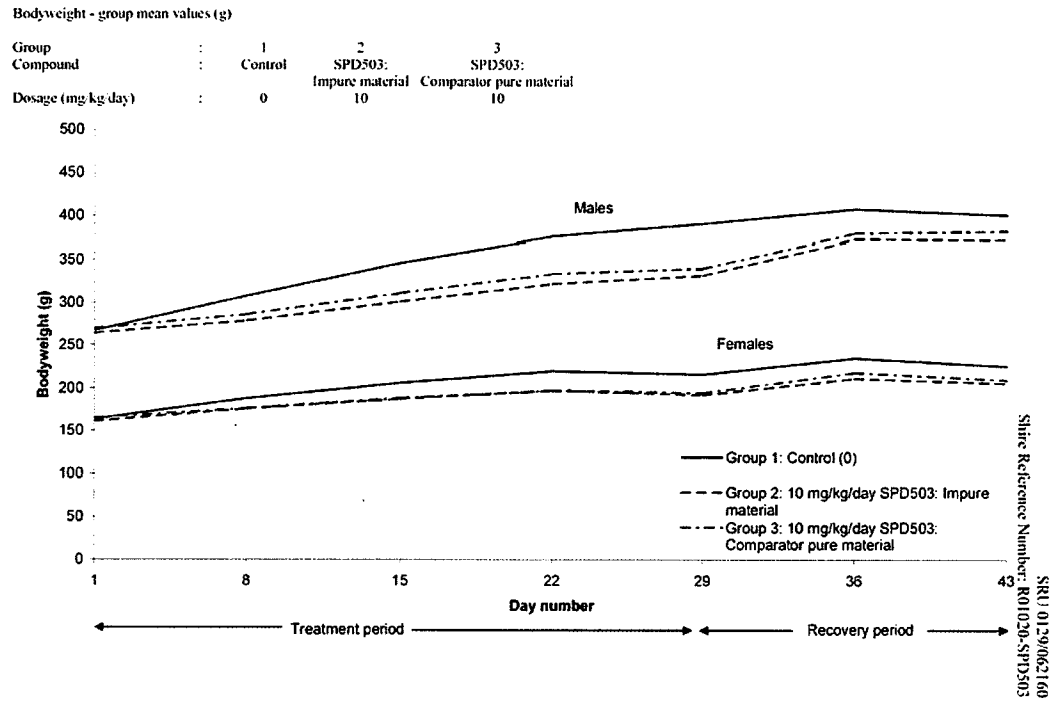
(Continued)

2.6.7.7A Repeat-Dose Toxicity: 4-week comparative toxicity study in rats: SPD503 (Guanfacine hydrochloride: pure and impure)

		Male			Female		
Test Substance		N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material	N/A (Control)	SPD503: Impure material	SPD503: Comparator pure material
Daily Dose (mg/kg/day)		0 (Control)	10	10	0 (Control)	10	10
Number of Animals		16	16	16	16	16	16
Histopathology (continued)							
Spleen:							
Extramedullary haemopoiesis	Main	6	1	2	2	0	0
	Recovery	4	6	6	1	3	3
Haemosiderosis	Main	7	9	10	10	10	10
	Recovery	6	6	6	6	6	6
Mesenteric lymph node:							
Sinus histiocytosis	Main	9	10	10	9	9	10
	Recovery	6	6	6	6	6	5
Submandibular salivary gland:							
Acinar cell hypertrophy	Main	0	10	10	0	0	0
	Recovery	0	2	3	0	0	0
Acinar cell atrophy	Main	0	0	0	0	7	7
	Recovery	0	0	0	0	0	0
Increased acinar cells with mitotic figures	Main	0	0	0	0	3	5
	Recovery	0	0	0	0	0	0
Parotid salivary gland:							
Acinar cell atrophy	Main	0	0	0	0	9	7
	Recovery	-	-	-	0	1	0

\+ p<0.05, ++ p<0.01, Group 2 and 3 versus Group 1
† p<0.05, †† p<0.01, Group 2 versus Group 3
* For controls, group means are shown. For treated groups, percent of control is shown. Statistical significance is based on actual data.
‡ Absolute and relative weights differ from controls in the same direction. For controls, group means are shown. For treated groups, percent of control is shown.
NR This parameter was not measured in the Recovery phase.

Body wt: A decrease in body wt (10-15% was seen with both the pure and the impure compound by the end of the study). The following figure provided by the sponsor summarizes the effect on body wt:



Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

No differences between the pure and the impure form of the compound. See results above as summarized by the sponsor in the tables.

Toxicokinetics: blood samples were obtained from the recovery group animals at the end of Week 4 at ~midnight (Tmax) through the tail vein

No significant differences in plasma levels for the pure and the impure forms of the compound. See table from sponsor for results above.

Conclusion: the results obtained from the treatment with pure and impure SPD503 (10/mg/kg/day) for 4 weeks in OFA rats were very similar with a few minor differences that are of no toxicological importance.

It is not clear to the reviewer the basis for the dose selected in this study. However, there was a decrease in body wt and body wt gain with this dose in both the pure and the impure-treated groups indicating that an effect of treatment is see with this dose. Since the aim of the study was to compare the effects of the two forms of the compound, then the dose used in this study can be considered adequate for the purpose of comparison. Based on calculations from this study in which the impurities were spiked to (b)(4)% for each one, the levels of these impurities obtained from a 10 mg/kg/day dose in the rats will

be more than what humans will be exposed to base on a maximum human dose of 4 mg/day both based on a mg/kg and a mg/m^2 basis (see the comparisons based on a 20 kg human body wt as seen under the toxicology psection on page 9 of this review).

2.6.6.4 Genetic toxicology

Study title: SPD503 (pure) and SPD503 (impure): induction of chromosome aberrations in cultured human peripheral blood samples

A GLP chromosomal aberrations study was conducted using cultured human peripheral blood lymphocytes (Study 2082/25-D6172) at (b) (4) starting in January and completed March 2006 (QA report provided).

Methods:

Duplicate samples of human lymphocytes (male donors) were incubated with SPD503 (pure, lot GFO 5004A with a stated purity of 100.2%) and SPD503 (impure; lot number GFO 5001 was spiked with impurities (b) (4), the spike level for each of these impurities was (b) (4)%, and the resultant batch was given the number 05MF291/B) at the following concentrations as summarized in the following tables:

SPD503 (pure)

S-9	Treatment + recovery (hours)	Vehicle control	Concentration (µg/mL)	Positive control
-	20+0	0[a]	10.00, 20.00, 30.00, 40.00	NQO, 2.50 µg/mL
+	3+17	0[a]	150.0, 175.0, 220.0, 240.0	CPA, 12.5 µg/mL

[a] Vehicle control was purified water only

SPD503 (impure)

S-9	Treatment + recovery (hours)	Vehicle control	Concentration (µg/mL)	Positive control
-	20+0	0[a]	10.00, 20.00, 30.00	NQO, 2.50 µg/mL
+	3+17	0[a]	100.0, 175.0, 210.0, 220.0	CPA, 6.25 µg/mL

[a] Vehicle control was purified water only

The S-9 used was prepared from the liver of rats treated with Aroclor 1254.

The doses for the current study were based on findings of a preliminary study in which precipitation at the end of treatment period was observed at a concentration of 1696 ug/ml and precipitation at the time of harvest was observed at a concentration of 2826 ug/ml (equivalent to 10 mM, dissolved in water). In addition, the highest concentrations chosen was based on the mitotic index at that dose (at least 50% inhibition), but analysis from highly cytotoxic concentrations was avoided.

Results

Study validity: the test article was tested in duplicate in the presence and absence of the S-9 mix. The sponsor avoided analysis of slides from highly cytotoxic concentrations. Almost 200 cells were evaluated for each concentration (on some occasions fewer numbers were used. i.e. 160). Appropriate positive concentrations were used (adequate responses). The appropriate incubations times were used (both 3h in the presence and absence of the S-9 and 20 h in the absence of S-9).

Study outcome:

The results indicated that both the pure and the impure form of the compound did not induce structural chromosome aberrations that are different from what is observed in the negative control. There were some small and sporadic increases in the frequencies of numerical aberrations which were more than seen in the current control and the normal ranges with both the pure and the impure form of the compound. There seemed to be no differences between the results obtained from the pure vs. those produced from the impure form. The following tables summarizing the data were provided by the sponsor:

Appendix Table 17 SPD503 (pure): 3+17 hours, -S-9, Experiment 1, Donor sex: n

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
150.0	A	103	0	3	0	3	**2.9**
	B	93	0	0	0	0	0
	Total	196	0	3	0	3	1.5
175.0	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
250.0	A	102	0	2	0	2	2.0
	B	102	0	0	2	2	2.0
	Total	204	0	2	2	4	2.0
275.0	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
NQO, 5.00	A	32	0	0	0	0	0
	B	34	0	0	0	0	0
	Total	66	0	0	0	0	0

** Total cells examined for numerical aberrations
Numbers highlighted exceed historical negative control range (APPENDIX 5)
For abbreviations and classification see APPENDIX 2

Appendix Table 18 SPD503 (pure): 3+17 hours, +S-9, Experiment 1, Donor sex: n

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
100.0	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
175.0	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
225.0	A	103	0	1	2	3	**2.9**
	B	101	0	0	1	1	1.0
	Total	204	0	1	3	4	2.0
CPA, 12.5	A	34	0	0	0	0	0
	B	44	0	0	0	0	0
	Total	78	0	0	0	0	0

** Total cells examined for numerical aberrations
Numbers highlighted exceed historical negative control range (APPENDIX 5)
For abbreviations and classification see APPENDIX 2

Appendix Table 19 SPD503 (impure): 3+17 hours, -S-9, Experiment 1, Donor sex: male

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
175.0	A	100	0	0	0	0	0
	B	102	0	0	2	2	2.0
	Total	202	0	0	2	2	1.0
200.0	A	102	0	0	2	2	2.0
	B	100	0	0	0	0	0
	Total	202	0	0	2	2	1.0
225.0	A	104	0	1	**3**	4	**3.8**
	B	100	0	0	0	0	0
	Total	204	0	1	3	4	2.0
NQO, 2.50	A	100	0	0	0	0	0
	B	93	0	0	0	0	0
	Total	193	0	0	0	0	0

** Total cells examined for numerical aberrations
Numbers highlighted exceed historical negative control range (APPENDIX 5)
For abbreviations and classification see APPENDIX 2

Appendix Table 20 SPD503 (impure): 3+17 hours, +S-9, Experiment 1, Donor sex:

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	64	0	0	1	1	1.6
	B	100	0	0	0	0	0
	Total	164	0	0	1	1	0.6
100.0	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
200.0	A	102	0	0	2	2	2.0
	B	101	0	0	1	1	1.0
	Total	203	0	0	3	3	1.5
225.0	A	105	1	1	**3**	5	**4.8**
	B	104	0	0	**4**	4	**3.8**
	Total	209	1	1	7	9	4.3
CPA, 6.25	A	31	0	0	0	0	0
	B	39	0	0	0	0	0
	Total	70	0	0	0	0	0

** Total cells examined for numerical aberrations
Numbers highlighted exceed historical negative control range (APPENDIX 5)
For abbreviations and classification see APPENDIX 2

Appendix Table 21 SPD503 (pure): 20+0 hours, -S-9, Experiment 2, Donor sex: m

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	101	0	0	1	1	1.0
	B	101	0	0	1	1	1.0
	Total	202	0	0	2	2	1.0
10.00	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
20.00	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
30.00	A	101	0	1	0	1	1.0
	B	101	0	0	1	1	1.0
	Total	202	0	1	1	2	1.0
40.00	A	100	0	0	0	0	0
	B	101	0	1	0	1	1.0
	Total	201	0	1	0	1	0.5
NQO, 2.50	A	24	0	0	0	0	0
	B	42	0	0	0	0	0
	Total	66	0	0	0	0	0

** Total cells examined for numerical aberrations
For abbreviations and classification see APPENDIX 2

Appendix Table 22 SPD503 (pure): 3+17 hours, +S-9, Experiment 2, Donor sex: I

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	101	0	1	0	1	1.0
	Total	201	0	1	0	1	0.5
150.0	A	102	0	0	2	2	2.0
	B	101	0	0	1	1	1.0
	Total	203	0	0	3	3	1.5
175.0	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
220.0	A	101	0	0	1	1	1.0
	B	103	0	1	2	3	**2.9**
	Total	204	0	1	3	4	2.0
240.0	A	102	0	0	2	2	2.0
	B	101	0	0	1	1	1.0
	Total	203	0	0	3	3	1.5
CPA, 12.5	A	60	0	0	0	0	0
	B	42	0	0	0	0	0
	Total	102	0	0	0	0	0

** Total cells examined for numerical aberrations
Numbers highlighted exceed historical negative control range (APPENDIX 5)
For abbreviations and classification see APPENDIX 2

Appendix Table 23 SPD503 (impure): 20+0 hours, -S-9, Experiment 2, Donor sex: male

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
10.00	A	102	0	1	1	2	2.0
	B	100	0	0	0	0	0
	Total	202	0	1	1	2	1.0
20.00	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
30.00	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
NQO, 2.50	A	48	0	0	0	0	0
	B	78	0	0	0	0	0
	Total	126	0	0	0	0	0

** Total cells examined for numerical aberrations
For abbreviations and classification see APPENDIX 2

Appendix Table 24 SPD503 (impure): 3+17 hours, +S-9, Experiment 2, Donor sex: m

Treatment (µg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	Total	200	0	0	0	0	0
100.0	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	Total	201	0	0	1	1	0.5
175.0	A	103	0	0	**3**	3	**2.9**
	B	104	0	0	**4**	4	**3.8**
	Total	207	0	0	7	7	3.4
210.0	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	Total	201	0	0	1	1	0.5
220.0	A	105	0	1	**4**	5	**4.8**
	B	100	0	0	0	0	0
	Total	205	0	1	4	5	2.4
CPA, 6.25	A	80	0	0	0	0	0
	B	66	0	0	0	0	0
	Total	146	0	0	0	0	0

** Total cells examined for numerical aberrations
Numbers highlighted exceed historical negative control range (APPENDIX 5)
For abbreviations and classification see APPENDIX 2

H = Hyperdiploid, E=Endoreduplicated, P = Polyploid

Study title: SPD503 (pure) and SPD503 (impure): reverse mutation in five histidine-requiring strains of Salmonella typhimurium (study #V01018-SPD503)

A GLP study was conducted by (b) (4) in January-February 2006 using SPD503 (pure, batch #GF0 5004/A. purity stated as 100.2%) and SPD503 (impure; batch #GF0 5001 spiked with (b) (4)% of each of impurities (b) (4) and was designated as batch #05MF291/B after spiking. Purity stated as 98.5% by HPLC).

Methods:

Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 were tested with the following concentrations (table as provided by the sponsor):

Experiment	S-9	Concentration of treatment solution (mg/mL)	Final concentration (µg/plate)
Range-finder Experiment and Mutation Experiment 1	– and +	0.008	1.6
		0.04	8
		0.20	40
		1.00	200
		5.00	1000
		25.00	5000
Mutation Experiment 2	– and +	0.1024[a]	20.48[a]
		0.256	51.2
		0.64	128
		1.60	320
		4.00	800
		10.00	2000
		25.00	5000

[a] Concentration used for treatments of strain TA102 only.

The range finding study was carried out in the absence and in the presence of S-9 in strain TA100 only. The mammalian liver post-mitochondrial fraction (S-9) was prepared from male Sprague Dawley rats induced with Aroclor 1254. The negative control was the solvent (water) and the positive controls as specified in the following table by the sponsor:

Chemical	Source	Stock * concentration (µg/mL)	Final concentration (µg/plate)	Use Strain(s)	S-9
2-nitrofluorene (2NF)	(b) (4)	50	5.0	TA98	–
Sodium azide (NaN_3)		20	2.0	TA100, TA1535	–
9-aminoacridine (AAC)		500	50.0	TA1537	–
Mitomycin C (MMC)		2	0.2	TA102	–
Benzo[a]pyrene (B[a]P)		100**	10.0	TA98	+
2-aminoanthracene (AAN)		50**	5.0	TA100, TA1535, TA1537	+
		200**	20.0	TA102	+

* With the exception of NaN_3 and MMC, which were prepared in water, all stock solutions were prepared in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO). All stock solutions were stored in aliquots at 1-10°C in the dark, with the exception of B[a]P and MMC which were stored in aliquots at –80°C in the dark.

** For Experiment 2 pre-incubation treatments, stock solutions of these positive control compounds were twice the concentration stated. This enabled the volume additions to be reduced to 0.05 mL (thus avoiding solvent-induced toxicity) whilst maintaining the final concentrations per plate detailed above.

The samples were done in triplicates. Colonies were counted electronically or manually where confounding factors affected the accuracy of the automated counter (bubbles, split agar, or microcolonies). The background lawn was inspected for signs of toxicity.

Results:

Study validity: the study is considered valid according the protocol presented and the negative and positive control results.

Study outcome:

The results did not indicate a drug related induced mutation in the five strains tested here at a concentration up to 5000 ug/plate and under the conditions employed in the study. See the following tables as provided by the sponsor:

SPD503 (pure): summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water	200 µl	21 ± 4	121 ± 9	13 ± 3	19 ± 5	335 ± 23
SPD503 (pure)	1.6	23 ± 6	111 ± 12	15 ± 5	20 ± 3	323 ± 24
	8	37 ± 8	124 ± 3	12 ± 6	16 ± 1	323 ± 37
	40	22 ± 3	113 ± 8	14 ± 4	19 ± 4	315 ± 5
	200	29 ± 5	125 ± 11	17 ± 4	19 ± 2	326 ± 20
	1000	30 ± 7	124 ± 4	15 ± 7	31 ± 9	236 ± 6
	5000	12 ± 3	142 ± 8	8 ± 1	18 ± 5	0 ± 0 (A+M)
Positive controls	Compound	2NF	NaN_3	NaN_3	AAC	MMC
	Dose Level	5 µg	2 µg	2 µg	50 µg	0.2 µg
	Mean ± SD	971 ± 109	962 ± 42	768 ± 58	177 ± 24	625 ± 41

SD Standard deviation

2NF 2-Nitrofluorene
NaN_3 Sodium azide
AAC 9-Aminoacridine
MMC Mitomycin C

V : Very thin background lawn
A : Absence of background lawn
M : plate counted manually

SPD503 (pure): summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water	200 µl	38 ± 4	145 ± 13	15 ± 6	29 ± 6	287 ± 20
SPD503 (pure)	1.6	36 ± 8	139 ± 6	16 ± 6	22 ± 1	282 ± 30
	8	29 ± 5	140 ± 8	23 ± 3	21 ± 3	278 ± 11
	40	32 ± 6	152 ± 10	19 ± 5	29 ± 7	273 ± 30
	200	37 ± 2	147 ± 10	17 ± 7	22 ± 2	251 ± 19
	1000	39 ± 9	155 ± 5	17 ± 2	30 ± 5	223 ± 10
	5000	27 ± 4	141 ± 5	15 ± 1	14 ± 5	0 ± 0 (M+A)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	431 ± 13	1596 ± 78	281 ± 30	173 ± 12	1346 ± 80

SD Standard deviation

B[a]P Benzo[a]pyrene
AAN 2-Aminoanthracene

M : Plate counted manually
A : Absence of a background lawn

SPD503 (impure): summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water	200 µl	23 ± 7	122 ± 10	13 ± 3	18 ± 4	323 ± 17
SPD503 (impure)	1.6	23 ± 2	124 ± 12	12 ± 6	27 ± 11	353 ± 12
	8	20 ± 6	122 ± 12	14 ± 3	25 ± 5	355 ± 9
	40	26 ± 9	128 ± 11	11 ± 3	25 ± 7	384 ± 76
	200	25 ± 5	137 ± 27	23 ± 9	27 ± 6	297 ± 28
	1000	24 ± 2	131 ± 4	15 ± 2	26 ± 9	218 ± 13
	5000	16 ± 7	97 ± 1	10 ± 7	23 ± 3	0 ± 0 (V)
Positive controls	Compound	2NF	NaN_3	NaN_3	AAC	MMC
	Dose Level	5 µg	2 µg	2 µg	50 µg	0.2 µg
	Mean ± SD	867 ± 18	954 ± 64	730 ± 1	244 ± 42	591 ± 47

SD Standard deviation

2NF 2-Nitrofluorene
NaN_3 Sodium azide
AAC 9-Aminoacridine
MMC Mitomycin C

V : Very thin background lawn

SPD503 (impure): summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water	200 µl	34 ± 4	128 ± 22	15 ± 4	27 ± 2	274 ± 18
SPD503 (impure)	1.6	25 ± 5	148 ± 16	18 ± 4	22 ± 3	278 ± 19
	8	44 ± 5	158 ± 13	12 ± 2	37 ± 8	268 ± 4
	40	39 ± 11	152 ± 14	14 ± 2	34 ± 8	284 ± 10
	200	51 ± 12	137 ± 24	16 ± 9	28 ± 5	268 ± 9
	1000	36 ± 4	143 ± 12	17 ± 2	28 ± 7	247 ± 26
	5000	32 ± 9	109 ± 9	11 ± 6	23 ± 10	0 ± 0 (V)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	440 ± 20	1509 ± 139	284 ± 82	301 ± 118	1654 ± 79

SD Standard deviation

B[a]P Benzo[a]pyrene
AAN 2-Aminoanthracene

V : Very thin background lawn

SPD503 (pure): summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water	200 µl	24 ± 2	104 ± 8	21 ± 6	18 ± 3	352 ± 58
SPD503 (pure)	20.48	NA	NA	NA	NA	377 ± 12
	51.2	21 ± 5	86 ± 9	11 ± 2	14 ± 8	367 ± 27
	128	17 ± 1	78 ± 5	12 ± 4	16 ± 3	350 ± 49
	320	18 ± 5	89 ± 12	14 ± 4	13 ± 4	336 ± 21
	800	22 ± 3	83 ± 4	15 ± 3	21 ± 1	303 ± 5
	2000	21 ± 6	80 ± 6	17 ± 5	14 ± 2	232 ± 16
	5000	11 ± 1 (S)	35 ± 7 (S)	10 ± 4	12 ± 3 (S)	- (T)
Positive controls	Compound	2NF	NaN_3	NaN_3	AAC	MMC
	Dose Level	5 µg	2 µg	2 µg	50 µg	0.2 µg
	Mean ± SD	975 ± 40	1112 ± 101	849 ± 138	156 ± 39	796 ± 43

SD Standard deviation

MMC Mitomycin C
2NF 2-Nitrofluorene
NaN_3 Sodium azide
AAC 9-Aminoacridine

S : Slight thinning of the background lawn
T : Toxic, no revertant colonies

SPD503 (pure): summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water	200 µl	33 ± 4	106 ± 10	19 ± 6	22 ± 4	290 ± 14
SPD503 (pure)	20.48	NA	NA	NA	NA	299 ± 18
	51.2	41 ± 6	106 ± 19	10 ± 4	17 ± 4	280 ± 3
	128	38 ± 5	103 ± 9	14 ± 4	16 ± 3	309 ± 9
	320	42 ± 3	107 ± 13	17 ± 2	18 ± 8	211 ± 8
	800	40 ± 4	100 ± 18	14 ± 8	9 ± 1	164 ± 25 (S)
	2000	25 ± 4 (S)	64 ± 3 (S)	13 ± 4 (S)	7 ± 1 (S)	- (T)
	5000	11 ± 5 (Ppn+M+S)	15 ± 5 (Ppn+M+V)	6 ± 2 (Ppn+M+S)	2 ± 0 (Ppn+M+S)	- (Ppn+T)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	409 ± 54	1957 ± 52	326 ± 14	124 ± 13	1240 ± 77

SD Standard deviation

AAN 2-Aminoanthracene
B[a]P Benzo[a]pyrene

S : Slight thinning of the background lawn
Ppn : Precipitation of test article observed
T : Toxic, no revertant colonies
V : Very thin background lawn
M : Plate counted manually

SPD503 (impure): summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water	200 µl	23 ± 6	104 ± 7	15 ± 3	14 ± 4	347 ± 46
SPD503 (impure)	20.48	NA	NA	NA	NA	395 ± 62
	51.2	21 ± 7	91 ± 4	11 ± 3	19 ± 6	294 ± 36
	128	24 ± 7	106 ± 8	14 ± 3	23 ± 8	351 ± 98
	320	18 ± 2	92 ± 15	13 ± 4	15 ± 6	365 ± 9
	800	29 ± 7	103 ± 7	18 ± 5	19 ± 5	346 ± 22
	2000	23 ± 3	89 ± 9	15 ± 4	17 ± 0	271 ± 36
	5000	18 ± 1	36 ± 4 (S)	13 ± 2	10 ± 3 (S)	- (T)
Positive controls	Compound	2NF	NaN_3	NaN_3	AAC	MMC
	Dose Level	5 µg	2 µg	2 µg	50 µg	0.2 µg
	Mean ± SD	928 ± 36	917 ± 35	716 ± 22	171 ± 40	651 ± 22

SD Standard deviation

MMC Mitomycin C
2NF 2-Nitrofluorene
NaN_3 Sodium azide
AAC 9-Aminoacridine

S : Slight thinning of background lawn
T : Toxic, no revertant colonies

SPD503 (impure): summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Water	200 µl	36 ± 8	99 ± 10	17 ± 3	17 ± 5	320 ± 16
SPD503 (impure)	20.48	NA	NA	NA	NA	274 ± 17
	51.2	31 ± 3	112 ± 9	18 ± 4	19 ± 2	318 ± 11
	128	35 ± 18	106 ± 11	22 ± 2	22 ± 4	264 ± 32
	320	34 ± 7	116 ± 17	21 ± 2	21 ± 9	213 ± 18
	800	36 ± 11	104 ± 12	22 ± 7	13 ± 6	184 ± 8 (S)
	2000	30 ± 1 (S)	60 ± 15 (S)	13 ± 6 (S)	10 ± 6 (S)	- (T)
	5000	16 ± 8 (Ppn+S)	14 ± 7 (Ppn+M+V)	5 ± 1 (Ppn+M+S)	3 ± 0 (Ppn+M+S)	- (T)
Positive controls	Compound	B[a]P	AAN	AAN	AAN	AAN
	Dose Level	10 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	323 ± 12	1596 ± 71	237 ± 55	213 ± 10	936 ± 36

SD Standard deviation

AAN 2-Aminoanthracene
B[a]P Benzo[a]pyrene

S : Slight thinning of the background lawn
Ppn : Precipitation of test article observed
T : Toxic, no revertant colonies
V : Very thin background lawn
M : Plate counted manually

Therefore the compound is considered to have negative gentoxic potential using the Ames test.

2.6.6.5 Carcinogenicity

Carcinogenicty studies were conducted with the original NDA for guanfacine

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA report: yes ( ) no ( )
Drug, lot #, and % purity:
CAC concurrence:

Methods

Doses:
Basis of dose selection (MTD, MFD, AUC etc.): Species/strain:
Number/sex/group (main study):
Route, formulation, volume:
Frequency of dosing:
Satellite groups used for toxicokinetics or special groups:
Age:
Animal housing:
Restriction paradigm for dietary restriction studies:
Drug stability/homogeneity:
Dual controls employed:
Interim sacrifices:
Deviations from original study protocol:

Observation times
Mortality:
Clinical signs:
Body weights:
Food consumption:
Histopathology: Peer review: yes ( ), no ( )
Toxicokinetics:

Results
Mortality:

Clinical signs:

Body weights:

Food consumption:

Gross pathology:

Histopathology:

Non-neoplastic:

Neoplastic:

Toxicokinetics:

2.6.6.6 Reproductive and developmental toxicology

Reproductive studies were conducted with the original NDA for guanfacine.

Fertility and early embryonic development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality:

Clinical signs:

Body weight:

Food consumption:

Toxicokinetics:

Necropsy:

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Embryofetal development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality (dams):

Clinical signs (dams):

Body weight (dams):

Food consumption (dams):

Toxicokinetics:

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Offspring (malformations, variations, etc.):

Prenatal and postnatal development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

F_0 in-life:

F_0 necropsy:

F_1 physical development:

F_1 behavioral evaluation:

F_1 reproduction:

F_2 findings:

2.6.6.7 Local tolerance

2.6.6.8 Special toxicology studies

The review of the juvenile animal studies can be found in DFS under the IND 63551

The general overview of these studies indicted that at the doses tested there appeared to be no effect of the compound in juvenile animal studies to require description in the labeling. However, since a reproductive segment of the study was conducted, the sponsor committed to perform this part as a Phase IV commitment.

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:
Formulation/vehicle:

Methods

Doses:

Study design:

Results:

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

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/

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