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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-928

Pharmacology Review(s)

MEMORANDUM

May 9, 2006

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-928

I have read the pharmacology/toxicology review for Chantix[®] (varenicline tartrate) and concur with the conclusion by Dr. Mamata De and Dr. Daniel Mellon that the marketing application is approvable. The nonclinical data are reported accurately in the product label.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.

Associate Director

Office of New Drugs

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Kenneth Hastings
5/9/2006 04:33:18 PM
PHARMACOLOGIST



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: 21-928
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 11/09/06
PRODUCT: Varenicline tartrate
INTENDED CLINICAL POPULATION: Treatment of smoking cessation
SPONSOR: Pfizer, Inc.
DOCUMENTS REVIEWED: eCTD Module 2 and 4
REVIEW DIVISION: Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)
PHARM/TOX REVIEWER: Mamata De, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob A. Rappaport, M.D.
PROJECT MANAGER: Dominic Chiapperino, Ph.D.

Date of review submission to Division File System (DFS): May 8, 2006

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: From the nonclinical pharmacology and toxicology perspective, NDA 21-928 may be approved.
- B. Recommendation for nonclinical studies: None
- C. Recommendations on labeling: The labeling recommendations below were sent to the Sponsor on May 8, 2006. Final labeling can be found in the action letter.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Carcinogenesis. Lifetime carcinogenicity studies were performed in CD-1 mice and Sprague-Dawley rats. There was no evidence of a carcinogenic effect in mice administered varenicline tartrate by oral gavage for 2 years at doses up to 20 mg/kg/day (47 times the maximum recommended human daily exposure in terms of AUC). Rats were administered varenicline (1, 5, and 15 mg/kg/day) by oral gavage for 2 years. In male rats (n = 65 per sex per dose group), incidences of hibernoma (tumor of the brown fat) were increased at the mid dose (1 tumor, 5 mg/kg/day, the maximum recommended human daily exposure in terms of AUC) and maximum dose (2 tumors, 15 mg/kg/day, the maximum recommended human daily exposure in terms of AUC). The clinical relevance of this finding to humans has not been established. There was no evidence of carcinogenicity in female rats.

Mutagenesis. Varenicline was not genotoxic, with or without metabolic activation, in the following assays: Ames bacterial mutation assay; mammalian CHO/HGPRT assay; and tests for cytogenetic aberrations in vivo in rat bone marrow and in vitro in human lymphocytes.

Impairment of fertility. There was no evidence of impairment of fertility in either male or female Sprague-Dawley rats administered varenicline succinate up to 15 mg/kg/day ([SPONSOR TO PROVIDE AUC RATIO]).

However, a decrease in fertility was noted in the offspring of pregnant rats who were administered varenicline succinate at an oral dose of 15 mg/kg/day (36 times the human AUC at 1 mg BID). This decrease in fertility in the offspring of treated female rats was not evident at an oral dose of 3 mg/kg/day (SPONSOR TO PROVIDE AUC RATIO).

PREGNANCY

Pregnancy Category C.

Varenicline was not teratogenic in rats and rabbits at oral doses up to 15 and 30 mg/kg/day, respectively (36 and 100% of the maximum recommended human daily exposure based on AUC at 1 mg BID, respectively). (SPONSOR Confirm AUC)

Nonteratogenic effects. Varenicline has been shown to have an adverse effect on the fetus in animal reproduction studies. Administration of varenicline to pregnant rabbits resulted in reduced fetal weights at an oral dose of 30 mg/kg/day (100% of the human AUC at 1 mg BID); this reduction was not evident following treatment with 10 mg/kg/day (33% of the maximum daily human exposure based on AUC). In addition, in the offspring of pregnant rats treated with varenicline there were decreases in fertility and increases in auditory startle response at an oral dose of 15 mg/kg/day (36 times the maximum recommended human daily exposure based on AUC at 1 mg BID).

There are no adequate and well-controlled studies in pregnant women. Varenicline should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

In support of NDA 21-928, the sponsor has completed the appropriate preclinical studies including toxicology studies in rats, dogs, mice and monkeys with duration of single dose to 12 months, 2-year carcinogenicity studies in mice and rats, standard battery of genotoxicity studies, reproductive toxicity studies in rats and rabbits, and special toxicology studies.

The major target organs of toxicity were brain/central nervous system (CNS), gastrointestinal tract (GIT), and lymphoid system. The CNS toxicity was manifested by decreased food consumption and body weight gain or weight loss was noted in all species. The severity of the effects ranged from minimal to profound decreases in food consumption and marked body weight loss, associated with clinical signs suggestive of CNS effects and overall health deterioration at high dose levels.

CP-526,555 was tested in two pivotal repeat dose toxicity studies in Cynomolgus monkey (9-month). In these studies, the compound was administered either by oral gavage or via nasogastral intubation. The doses tested ranged from 0.01-1.2 mg/kg/day. The high dose (1.2 mg/kg/day) was not tolerated as observed by >15% decrease in the body weight within 3-weeks and the treatment with this dose was discontinued. One mortality was seen (1/4 females) at 0.4 mg/kg, the second highest dose tested. Sporadic incidences of the treatment related clinical signs were observed in animals with all doses. The major clinical signs include loose stool and emesis. However, an increase in the lymphocyte infiltration in different tissues (trachea, thyroid etc) along with the chronic inflammation observed histopathologically in some tissues (heart) at the same dose range might implicate a treatment related effect of the compound on the lymphoid system. The changes in the monocytes/lymphocyte counts might be stress related. Increased fibrinogen level was observed in dose above ≥ 0.2 mg/kg/day suggesting underlying

increased inflammatory processes, biological significance of the finding is not known. Megacolon, a rare pathological finding in monkey, was observed at 0.4 mg/kg/day (in 1/4 females), this finding might be related to the increased incidence of emesis and/or stress related effect in GIT and therefore might be considered as treatment related. A NOAEL of 0.2 mg/kg/day (HED=3.8 mg based on body surface area), exposure at this dose (by the end of the 9-month) as described by C_{max} and AUC were found to be 48 ng/mL and 869 ng•h/mL (MRHD=7.6x) respectively.

CP-526,555 tested in 6-month toxicity study in rat showed sporadic clinical signs of reduced and/or soft feces, chromodacryorrhea, and urine staining at all doses (3, 10, 30 mg/kg). A decrease in the body weight gain > 10% was observed at high dose which was correlated with a decrease in food consumption and considered to be treatment-related. Changes in WBC counts were noted with all doses at Week-13 which was partially recovered at Week-26. Clinical pathological changes including increases in ALT and ALKP were noted at high dose; > 10% gain in liver weight in females was noted at the same dose suggesting treatment related functional changes in the liver. Histopathological findings were limited to jejunal epithelial vacuolation (5/15 males and 1/15 females), retinal dysplasia (1/15 males), and infiltration of foamy macrophages in the lung at high dose (6/15, 5/15 male, and females respectively compared to 2/15 in control animals). A NOAEL of 10 mg/kg/day (HED= 96 mg) by oral gavage is established for this 6-month rat study based on the decrease in body weight gain and hepatobiliary parameters. At NOAEL dose exposure as described by C_{max} and AUC were found to be 906 ng/mL and 14,000 ng•h/mL (MRHD=126x) respectively by the end of the 6-month toxicity study.

Single dose toxicity studies as well as subchronic toxicity studies in different nonclinical species showed similar clinical signs as described above in the chronic toxicity studies in monkey and rat. These observations were not always dose related but consistent throughout the different doses that were tested and were found in all nonclinical species studied. The test article showed low affinity for 5HT₃ receptor in the binding study and the emetic effect of the compound was found to be blocked in ferrets by ondansatrone (an antagonist of 5-HT₃ receptor) in the safety pharmacology studies. These results suggest that CP-526,555 might be acting as an agonist of the 5-HT₃ receptor. The emetic effect observed is considered a treatment-related pharmacological effect of the compound and the effect is predicted to be manifested clinically. In addition, to the histopathological observations mentioned above in the chronic toxicity studies dilatation of colon, jejunum, cecum, and ileum were noted at doses higher than the NOAEL dose in the single and repeat dose studies. The study results identify GI tract as the major target organ of toxicity for the compound. The safety pharmacology studies also confirmed the reduction in the geometric center of the GIT for the compound indicating inherent changes in the gastric mobility related to the pharmacology of the compound. In the distribution studies in rat the C¹⁴ labeled compound were noted in the GI tract within one hour after the administration of the test article. The compound was found to reside in the gastric content at least up to 18 hr. The exposure (AUC_{0-last} ng•h/mL) of the compound in GIT mucosa after single dose administration ranged between 633-1563, indicating a moderate exposure of the test article in this tissue. The results indicate that the chronic application of the test article in clinic may have compounded the effects at the GIT and might have

pronounced pathological consequences in GIT, a dire treatment related effect on the digestive function might also be observed clinically.

In the 6-month toxicity study in rat, phospholipidosis was observed in different tissues as indicated by vacuolation and foamy macrophage infiltration. The toxicological implication of the findings is not yet known.

Interestingly, although the test article was found to be distributed in the melanin containing tissue in the skin and eye, only a few toxicological manifestations of these pharmacological effects of the test article were noted in any of the chronic toxicity studies. Rough hair coat was reported in rodents in the 2-year carcinogenicity study; however, the toxicological implication of the finding in that study is unclear. Retinal dysplasia was observed in 1/20 rats in the 6-month study at 30 mg/kg dose (HED=288 mg). At this dose a > 200-fold safety margin exist as compared to the clinical dose based on the body surface area.

Mutagenicity and Carcinogenicity: CP-526,555 tested negative in the Ames test, in vitro cytogenetics study human peripheral blood cells, mammalian cell gene mutation assay, and in vivo rat micronucleus test, suggesting it does not have mutagenic potential. Carcinogenicity studies were completed in both the rat (1, 5, 15 mg/kg) and mouse (1, 5, 20 mg/kg/day) models. In the 2-year carcinogenicity studies, drug-related neoplastic alterations were limited to hibernoma in male rats. Specifically, 1/65 male rats showed benign hibernoma at 5mg/kg dose; 2/65 male rats showed malignant hibernoma at 15 mg/kg dose. This finding was not statistically significant. The 'hibernoma' is a rare tumor finding in rodents. Therefore, although the incidence was low, the finding it is considered treatment-related and should be in the label (NOTE: The sponsor included the finding in the proposed drug labeling). No apparent drug-related neoplastic findings were observed in female rats at doses up to 15 mg/kg (HED=145 mg, AUC=674, MRHD=6x) or in mice at doses up to 20 mg/kg (HED= 97.5 mg, AUC=1790 ng•h/mL, MRHD=15x).

Reproductive toxicity: Reproductive toxicology was assessed in both rats and rabbits following oral administration.

In the Segment I reproductive toxicity study in male rats with oral administration of 0, 0.3, 3, and 15 mg/kg a NOAEL of 3 mg/kg (HED=29 mg) was established for F₀ male ; serum levels at the NOAEL dose was approximately 228 ng/mL in males (MRHD=2x). In the Segment I reproductive toxicity study in female rats with oral administration of 0, 0.3, 3, and 15 mg/kg a NOAEL of 3 mg/kg was established for F₀ female; serum levels at the NOAEL dose was approximately 198 ng/mL in females(MRHD=1.8x). A NOAEL of 15 mg/kg/day (HED= 290 mg) was established for the early embryonic toxicity for the test article in this study, serum level at the NOAEL dose was found to be approximately 581 and 500 ng/mL in males and females respectively (MRHD=9x).

In the Segment II reproductive toxicity studies in rat (0, 0.3, 5, 15 mg/kg) and rabbit (0, 1, 10, 30 mg/kg) NOAELs of 1 mg/kg (AUC= 279 ng•h/mL) and 3 mg/kg (AUC= 370

ng/mL) respectively were established for the maternal toxicity. No teratological effect was observed in rats; therefore, 15 mg/kg was established as the NOAEL for the teratology in rat. A significant decrease in the fetal weight was observed in the F₁ fetuses in rabbit at high dose, which might be related to the maternal body weight decrease. A NOAEL of 10 mg/kg (HED=193 mg, serum concentration in fetus = 399 ng/mL, MRHD=3.5x) was determined for rabbit teratology. There was a dose-dependent increase in serum varenicline concentrations in the fetuses following administration of drug to the dam.

Segment III pre-/postnatal development toxicity was assessed in female rat treated with 0, 0.3, 3, and 15 mg/kg/day of varenicline. The F₀ dams showed overall health deterioration with increasing dose. No reproductive toxicity was reported for the F₀ females. F₁ fetuses from the high dose group showed a significant reduction in body weight compared to controls. Auditory startle response significantly decreased in F₁ fetus. Increase in latency time (unknown biological significance) in water maize test (for learning and memory) was noted in F₁ fetus. Fertility in F₁ fetus reduced (60-80%) significantly (P< 0.01) at high dose. A NOAEL of 3 mg/kg (HED=29 mg, MRHD=2x) was established in F₁ fetus based on physical behavior noted and fertility. The test article was quantifiable in the serum in fetuses at Day-6 (approximately 20 ng/mL). The result confirms the transport of the test article via milk.

Local toxicology: In the local toxicity studies, the compound was not found phototoxic in rat up to a oral dose of 100 mg/kg (HED=967 mg); no dermal toxicity was observed in rat up to 2000 mg/kg, and the compound was found negative in the skin sensitization study in the guinea pig up to a challenge dose of 500 mgs. The test article was also considered as a mild irritant in the dermal toxicity study in rabbit at 500 mg (HED=53 mg). These results indicate that the oral formulation of the compound do not cause irritation or phototoxic reaction in rat, predicting low clinical potential for dermal toxicity.

The intraocular administration of the test article showed mild ocular irritation at 45 mg (HED=4.8 mg). Conjunctivae (chemosis, redness, and discharge) and iridial irritation was noted in all treated rabbits. The tissue distribution study in rats indicated drug deposition in skin and eye after single oral administration. Although, the level of the test article was found to be decreasing by Day 7 an acute effect indicated by irritation in skin and eye is considered to be treatment related, since nicotine is also known to be deposited in melanin containing tissue, this effect is considered pharmacologically related and predicted to manifest clinically only if dermal or ocular contact with the drug product occurs.

B. Pharmacologic activity:

Pharmacology:

CP-526,555 (varenicline) is described as a partial agonist at the neuronal nicotinic receptor, specifically neuronal nicotinic receptors containing $\alpha 4$ and $\beta 2$ subunits.

Receptor binding studies demonstrated that the varenicline has high affinity for the $\alpha 4\beta 2$ neuronal nicotinic receptor subtype (rat and human cortex, $K_i \sim 0.2$ nM). The test article >500-fold $\alpha 3\beta 4$, >20,000-fold selective for $\alpha \beta \gamma \delta 1$ (muscle nicotinic receptor), and >3500-fold selective for $\alpha 7$ (nicotinic toxin) receptors. CP-526,555 had low affinity at the $5HT_{3A}$ (K_i 350 nM). The compound do not show any affinity for a variety of other neurotransmitter receptors (approximately 56 different receptors tested), modulatory binding sites, ion channels, and neurotransmitter uptake sites in membranes derived from relevant tissues and cell lines.

The partial agonist efficacy of the compound was further justified by the dopamine release activity of the compound. In the *in vivo* assay in rats an ED_{50} of 0.032 mg/kg PO (maximal response 153%) was obtained which is approximately 63% of that of nicotine (maximal response 184%). The result shows that the test article reduced the peak dopamine release effect of the nicotine to approximately 60%. Rats were responsive to substitution of the nicotine by the test article demonstrating efficacy for nicotine substitution *in vivo*. The test article was also compared to nicotine in the animal model to evaluate the withdrawal effect and physical dependence after self administration. Discontinuation of CP-526,555, did not result in any behavioral disorder or body weight loss which might imply less liability for dependence with this test article.

In safety pharmacology studies varenicline treatment produced mild tremors, decreases in locomotor activity, piloerection and hunched to flattened body posture following a single oral administration of 10 mg/kg in rat [human equivalent dose (HED) = 96 mg based on body surface area]. C_{max} at this dose in rat was found to be 749.0 ng/mL at 1 hr. Plasma concentration at the human efficacious dose of 1 mg is found to be 9.2 ng/mL, indicating that approximately 50-fold safety margin exists for CNS behavioral changes. In addition to the CNS related behavioral changes mentioned above, at 100 mg/kg dose in rat, convulsion, salivation, mild to moderate ptosis, decreases in response to toe pinch, decreases in exploratory behavior, disturbances in gait (splayed hind limbs) and decreases in body/ limb tone were noted. No effects on the incidence of twitch, myoclonus or tonic extension induced by pentylenetetrazol (PTZ) compared to the incidence in vehicle-treated mice were noted with the compound up to 10 mg/kg dose. A significant decrease in the body temperature in rat was noted with the compound at 10 mg/kg ($2^\circ C$); no change in body weight temperature was noted at 1 mg/kg dose.

A significant increase in the sodium (138%) and chlorine (173%) excretion was noted in rat with the test article at 30 mg/kg dose (HED=290 mg), no such changes were noted at 3 mg/kg (HED=29 mg). This is a pharmacological effect of nicotine, the compound being a partial agonist of nicotine might be showing a real manifestation of this pharmacological effect, however, in the repeat dose toxicity studies no toxicological effect related to this findings were observed.

A significant decrease in gastric emptying were noted with CP-526,555 at 3 (55% ↓) and 89% ↓ mg/kg, the reduction in geometric center of the gastrointestinal tract (GIT) indicating reduction in the gastro intestinal motility is a well known pharmacological effect of nicotine and thus might be a valid finding with this compound. This effect is

dose related. In rat one hour after oral administration of CP-526,555 at 0.3 (plasma level=142 ng/mL), 3 (plasma level=656 ng/mL), and 30 mg/kg (plasma level= 1038 ng/mL) 3, 53, and 90% reduction in the geometric center was noted. This safety pharmacology observation was found to be manifested in all the toxicity studies described and reviewed under this submission.

The compound induced excessive retching and emesis in ferret at all dose studied (0.025-0.3 mg/kg) with different routes of administration (SC, oral). Mecamylalanine a nicotine receptor blocker only partially blocked this emetic effect. Ondasteron, a receptor antagonist effectively blocked the retching and emesis in ferrets. This suggest an inherent structural effect (the compound showed binding affinity to 5-HT₃, approximately 530 nM) of the compound for induction of emesis.

ADME:

The ADME profile of CP-526, 555 was adequately studied. Extensive metabolism of the compound and interspecies variation of the metabolites were noted; however, none of the metabolites were considered major metabolite since <10 % of the metabolites in total were found in the systemic circulation. All metabolites found in human were found to be present in at least one animal species, and qualified in the toxicology studies. The potential for varenicline to induce CYP isozymes have been investigated. No oxidative metabolism of the test article is reported in the rat, monkey, and human liver microsomes suggesting that it is not a good substrate for cytochrome P450 enzymes. The plasma protein binding was found to be low in all species studied (18, 45, 19, 41, and 20 in mouse, rat, dog, monkey, and human respectively). The test article was found to be absorbed fast (1 hr) after single dose oral administration in rat and mouse. In the primates, however, the T_{max} of the compound was found to be between 3-5 hrs. The t_{1/2} in monkeys (24 h) was higher after single dose than that in human (16 h) under similar conditions. The tissue distribution studies in rodents showed that the test article is extensively deposited in melanin containing tissues like skin and eye. This indicates that the test article might have different effect on skin and eye demographically. The T_{max} for the test article after oral administration was found to reach within 3-4 hrs in the primates, the compound has a long t_{1/2} (approximately 16-20 hrs in primates). The compound is excreted mainly via urine (approximately 80-85 %), feces accounted for approximately 5-6 % of the excretion. The fate of the rest of the compound administered; however, is not carefully studied.

Non clinical safety issues relevant to clinical use:

The major toxicity findings and their clinical relevance are listed below:

In the tissue distribution study (where 1.30 µCi of ¹⁴C was tagged with the compound to analyze the tissue distribution) in rat single administration of 3.4 mg/kg (HED = 33 mg considering 60 kg man) showed drug distribution in the different ocular tissues for a long period of time at least 7 days. A decreasing trend of the exposure of test article in all different tissues was noted after a peak exposure at 18 hrs. No major toxicity related to

the skin and eye was observed in non clinical species, however, the melanin deposition is a pharmacological characteristic of nicotine. The compound being a partial agonist of nicotine might show related toxicity in the melanin containing tissues and the effect might vary demographically.

The CNS and GIT related clinical signs like emesis, loose stool, and salivation was noted sporadically in all dose groups in all non clinical species studied, therefore, similar treatment related clinical signs are predicted to be observed in human. Decrease in body weight and food consumption >10% is observed in the dose > NOAEL doses. The safety margins (comparison of NOAEL dose with the maximal proposed human dose) and the noteworthy findings are tabulated below.

In the reproductive toxicity studies a decrease in the pregnancy rate was observed in F₁. Decrease in behavioral pattern in the F₂ (like rearing, auditory startle) indicating a decrease in motor coordination and exploratory behavior is noted.

In a 2-year carcinogenicity study in rats, hibernoma, a rare tumor finding was noted in males at 10 and 15 mg/kg (HED= \geq 96 mg). The tumor findings are not statistically significant. This finding, however, is considered treatment related due to the rarity of the findings in rodents.

Dose and Toxicity Findings (mg/kg/d)	HED (mg)	AUC _{0-24hr} (ng.hr/mL) @ NOAEL	Multiples *of HED	Multiples of human **Exposure
Rat NOAEL: 10 mg/kg/ day for 6 month toxicity study; Noteworthy finding>10 mg Lymphocyte infiltration in different tissue, ↑ in liver weight in females, ↑ in ALT&ALKP, ↑ monocyte /lymphocytes counts, ↑vacuolation in jejunal wall and foamy macrophages in lung.	96	14,400	96 x	126 x
Monkey NOAEL: 0.2 mg/kg/day for 9 month toxicity study. Noteworthy finding>10 mg; megacolon in ¼ females, ↑ monocyte /lymphocytes counts	3.8	869	4 x	7.5 x
Rat male carcinogenicity NOAEL: 1 mg/kg; >1 mg for showed tumor (hibernoma)	9.6	280	9.6 x	2.4 x
Rat fertility F ₀ NOAEL: ≤0.3mg/kg; Noteworthy findings >0.3 mg/kg ↓ in pregnancy rate up to 15%; ↓ in total occurrence of estrus; ↓ in	2.9	20.8	2.9 x	

number of estrus cycles.				
Rat fertility F₁ females NOAEL: 3 mg/kg Noteworthy findings >3 mg/kg ↓ in pregnancy rate up to 40%	29	20.9	29 x	
Rat post natal development findings: NOAEL 3 mg/kg Noteworthy findings >3 mg/kg ↓ Decrease in rearing, auditory startle.	29	20.9	29 x	
Rat tissue distribution study 3.8 mg; Noteworthy findings: tissue deposition of the compound in skin, eye, pituitary, meninges up to 7 days	31.9	NA	32 x	NA

* per 60 kg person based on body surface area

** 1 mg dose in human with a steady state AUC of 114 ng•h/mL

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21,928
Review number: 1
Sequence/date/type of submission: 000, 11/09/06, eCTD
Information to sponsor: No (x)

Sponsor and/or agent: Pfizer Inc.
Manufacturer for drug substance: Pfizer Inc.

Reviewer name: Mamata De, Ph.D.
Division name: Division of Anesthesia, Analgesia, and Rheumatology Products
HFD: 170
Review completion date: 04/09/06

Drug:

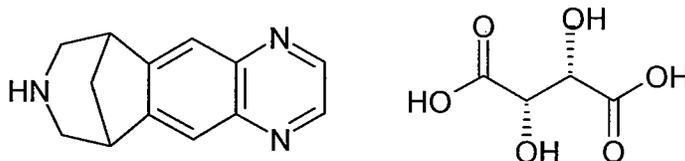
Trade name: To be determined (Chantix®)
Generic name: Varenicline tartrate
Code name: CP-526,555¹
Chemical name: 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino[2,3-*h*][3]benzazepine, (2*R*,3*R*)-2,3-dihydroxybutanedioate (1:1)

CAS registry number: 375815-87-5
Molecular formula: C₁₃H₁₃N₃ • C₄H₆O₆
Molecular weight: 361.35

Structure:

¹ During development of varenicline, several different salt forms were tested. The nomenclature for these forms is reflected in the code name of the drug as follows:

<i>Code Name</i>	<i>Salt form</i>
CP-526,555	Base
CP-526,555-01	[
CP-526,555-18	Tartrate
CP-526,555-24	Succinate



Varenicline tartrate

Relevant INDs/NDAs/DMFs: IND 58,994 Verenicline IR
IND 2 J

Drug class: Selective partial agonist of the $\alpha 4\beta 2$ neuronal nicotinic acetylcholine receptor subtype

Intended clinical population: Smoking cessation aid for those who wish to quit smoking tobacco cigarettes

Clinical formulation: Clinical formulation for 1.0 mg varenicline is provided below:

Clinical formulation for 1.0 mg varenicline

Name of Ingredients	Reference to Standards	Function	Unit formula (mg/Tablet)
Core Tablet			
Varenicline Tartrate ^a	Pfizer	Active	1.71
Microcrystalline Cellulose ^b	NF		
Dibasic Calcium Phosphate, anhydrous	USP		
Croscarmellose Sodium	NF		
Colloidal Silicon Dioxide	NF		
Magnesium Stearate ^c	NF		
Core Tablet Weight			
Film Coat Solution			
Opadry [®] Blue ^d	Pharm	Tablet Coating	
Purified Water ^e	USP	Solvent	
Opadry [®] Clear ^f	Pharm	Tablet Coating	
Total Tablet Weight			
			209.00

There are no novel excipients in the proposed drug product formulation.

Route of administration: Oral tablets.

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

Data reliance: This is a NDA application is filed under Section 505(b)(1). Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-928 are owned by Pfizer, Inc. or are data for which Pfizer, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 21-928 that Pfizer, 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 [name of sponsor] 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 21-928.

Studies reviewed within this submission:

Study Title	Study Number
Primary Pharmacodynamics	
Effects of CP-526,555-01 (Varenicline) on Basal and Nicotine Induced Dopamine Release in Rat N. Accumbens	1997-37293.60
<i>In Vitro</i> Functional Effects of CP-526,555	1997-38226
<i>In Vivo</i> Effects of CP-526,555-01 (Varenicline) as a Nicotinic Partial Agonist: Intrinsic Effects Alone (Oral and S.C.) and on Nicotine-Induced Increases in Dopamine Turnover (Rat Nucleus Accumbens)	1997-38271
Summary Report for CP-526,555: I. <i>In Vitro</i> Binding Affinity at Nicotinic Receptors II Effects on <i>In Vitro</i> Nicotine Stimulated [³ H]Dopamine Release in the Rat Corpus Striatum	1997-38428
Effects of CP-526,555-01-01 (Varenicline) on Nicotine Drug Discrimination in Rats	1997-43203
Effects of CP-526,555 (Varenicline) Pretreatment (or Substitution) on Rats Trained to Self-Administer Nicotine	1998-41516.020
Toleration and Withdrawal Assessment of CP-526,555-18 (Varenicline) in Rats	2004-63943
<i>In Vitro</i> Functional Effects of Varenicline	2005-104026
Safety Pharmacology	
General Pharmacology of CP-526,555	CP 5265550705/GP
Effects of CP-526,555 on HERG-Encoded Potassium Current in Stably Transfected HEK-293 Cells	CP 526555-Herg
Effects of CP 526,555 in the Ferret Emesis Model	CP 526,555/GP/0705/FE
Effects of CP-526,555-18 on Action Potentials Recorded from Dog Isolated Purkinje Fibers <i>In Vitro</i>	CP 526555-18/IC/001/02
Pharmacokinetics	
Assay Characterization for CP-526,555 Using HPLC with MS/MS Detection in Mouse Serum	DM2004-526555-054
Serum Concentrations of CP-526,555 and Total Drug Related Material after Oral Administration of [¹⁴ C]CP-526,555-24 to Male and Female Sprague-Dawley Rats. and Identification of Circulating Metabolites	DM2000-526555-026
Metabolism and Excretion of CP-526,555 in Cynomolgus Monkeys after Oral Administration of 0.08 mg/kg [¹⁴ C]CP-526,555-24 and Identification of Circulating and Excretory Metabolites	DM2000-526555-030
Metabolism and Excretion of CP-526,555 in Healthy Human Subjects after Oral Administration of 1.0 Mg [¹⁴ C]CP-526,555 and Identification of Circulating and	DM2000-526555-031

Excretory Metabolites	
Metabolism And Excretion of Cp-526,555 in CD-1 Mice after Oral Administration of 3.0 mg/kg [¹⁴ C]CP-526,555-24 and identification of Circulating and Excretory Metabolites	DM2001-526555-042
<i>In Vitro</i> Assessment of Human Intestinal Permeation of CP-526,555 Using Caco-2 Cell Monolayers	DM2003-526555-053
Oral Pharmacokinetics of CP-526,555 in Organic Cation Transporter 2 (OCT2) Knockout and RT1490 Wild Type Mice	DM2004-526555-055
Assessment of Blood Cell Partitioning and Plasma Protein Binding of CP-526,555 in Rat, Dog and Human Blood	DM1998-526555-006
Plasma Protein Binding and Blood Cell Partitioning of CP-526,555 in Plasma and Whole Blood from Cynomolgus Monkeys	DM1998-526555-009
Tissue Distribution in Long-Evans Rats of a Neuronal Nicotinic Partial Agonist (CP-526,555) for Treatment of Nicotine Addiction	DM2000-526555-012
Serum Protein Binding For CP-526,555 in the Mouse	DM2002-526555-050
Skin Concentrations of Varenicline in Male Long-Evans Rats after Daily Administration for One or Three Days	DM2004-526555-066
Radiolabeled Mass Balance, Excretion, and Metabolite Identification of CP-526,555 in Urine, Feces, and Bile of Sprague-Dawley Rats after Oral Administration of [¹⁴ C]CP-526,555	DM2000-526555-011
Urinary Excretion of CP-708,075 in Rats Following a Single Oral Dose of CP-526,555 at 30 mg/kg	DM2001-526555-041
Identification and Quantization of Circulating Metabolites of CP-526,555 in Female New Zealand Rabbits after Oral Administration of [¹⁴ C]CP-526,555	DM2001-526555-044
Induction Potential of Varenicline (CP-526555-18) on Cytochrome P450 1A2 and 3A4 in Human Hepatocytes	RR 764-04912
Substrate Disappearance Studies of CP-526,555 in Rat, Dog, Monkey and Human Hepatic Microsomes	DM1998-526555-008
The Effect of Probenecid, Cimetidine, and NH ₄ Cl on the Pharmacokinetics of CP-526,555 in Rats Following Intravenous Administration	DM2001-526555-043
Effect of CP-526,555 on Human Drug Metabolizing Enzymes <i>In Vitro</i>	DM2001-526555-045
<i>In Vitro</i> Transport of CP-526,555	DM2003-526555-052
Determination of the Enzyme Kinetics and UGTs Involved in the Metabolism of Varenicline to the N-Carbamoylglucuronide Conjugate of Varenicline	DM2005-526555-076
Induction Potential of Varenicline (CP-526555-18) on Cytochrome P450 1A2 and 3A4 in Human Hepatocytes	RR 764-04912
Monomethyl Tartrate (CE-157,254) Incubations at 37°C in Human Blood, Simulated Gastric Fluid and Buffer	DM2002-526555-051
Single Dose Toxicity	
Rat	
Exploratory Single Dose Oral Pharmacokinetic Study of CP-526,555-18 in Rats	00-1545-26
CP-526,555-01 Single-Dose Oral Toxicity Study in Rats	97-1545-06
Cynomolgus Monkey	
Exploratory Single Dose Oral Pharmacokinetic Study of CP-526,555-18 in Monkeys	1545-27
5 Day Exploratory Bridging Study in Cynomolgus Monkeys	98-1545-09
CP-526,555-24 Acute Oral Toxicity Study in Cynomolgus Monkeys	98-1545-14
Escalating Dose Toxicity Study of CP-526,555-18 in Monkeys	RR 745-03502
Acute Intravenous Toxicity Study of CP-526,555-18 in Monkeys	RR 745-03516
Chronic Toxicity	
Mouse	
CP-526,555-18 2-Week Oral Range-Finding Study in CD-1 Mice	01-1545-29
CP-526,555-18 3-Month Oral Toxicity Study in CD-1 Mice	01-1545-31

Rat	
CP-526,555-24 Exploratory 10-Day Oral Toleration Study in Sprague Dawley Rats	97-1545-08
CP-526,555-24 6-Week Oral Toxicity Study in Sprague-Dawley Rats	98-1545-11
CP-526,555-24 3-Month Oral Toxicity Study in Sprague-Dawley Rats	00054
6-Month Oral Toxicity Study of CP-526,555-18 in Rats	RR 745-03536
Dog	
Exploratory Oral Escalation/Toleration Study o Cp-526,555-01 in Beagle Dogs	97-1545-05
Cynomolgus Monkey	
CP-526,555-01 Exploratory Escalation/Toleration Study in Monkeys	97-1545-07
10-Day Oral Dose Range-Finding Study of CP-526,555-24 in Monkeys	00-1545-23
1-Month Oral Dose Range-Finding Study with 14-Day Recovery Phase of CP-526,555-18 in Monkeys	04-1545-34
CP-526,555-24 Six-Week Oral Toxicity Study in Cynomolgus Monkeys	98-1545-10
3-Month Oral Toxicity Study in Cynomolgus Monkeys	00-1545-22
Nine-Month Oral Toxicity Study of CP-526,555-18 in Cynomolgus Monkeys	00-1545-28
9-Month Oral Toxicity Study with 5-Week Recovery Phase of CP-526,555-18 in Monkeys	04-1545-35
Genetic Toxicology	
CP-526,555-24 <i>In Vitro</i> Cytogenetic Studies	97-1545-03
Microbial Reverse Mutation Assays	97-1545-04
Mammalian Cell Gene Mutation Assays; CP-526,555-01	98-1545-12
Rat <i>In Vivo</i> Micronucleus: Oral Route; CP-526,555-24	98-1545-13
Carcinogenicity	
Two-Year Oral Gavage Carcinogenicity and Toxicokinetic Study with CP-526,555 in Mice	02-1545-32
Two-Year Oral Gavage Carcinogenicity and Toxicokinetic Study with CP-526,555 in Rats	02-1545-33
Reproductive Toxicity	
CP-526,555-24 Reproductive Study I: Fertility and Early Embryonic Development Oral Gavage Study in Female Sprague-Dawley Rats	99-1545-20
CP-526,555: An Oral Teratology Study of CP-526,555-24 in the Rabbit	99-1545-18
An Oral Teratology Study of CP-526,555-24 In The Rat	99-1545-18
Oral Pre- and Postnatal Development Study in Sprague-Dawley Rats with CP-526,555-24	01-1545-30
Local Toxicity	
Three Day Dosage Tolerance and Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of CP-526,555 in Pigmented Rats	04-1545-36
CP-5269555-18: Acute Dermal Toxicity (Limit Test) in the Rat	1131/550
CP-5269555-18: Acute Eye Irritation in the Rabbit	1131/552
CP-5269555-18: Skin Sensitization in the Guinea Pig - Magnusson and Kingman Maximization Method	1131/553

Studies not reviewed within this submission:

All submitted studies were reviewed.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary:

Scientific Background:

The molecular mechanisms that contribute to nicotine addiction are poorly understood. Neuronal adaptations, both in the nicotinic cholinergic receptor (nAChR) system and in other mesolimbic neurochemical systems, are critical for understanding nicotine dependence. The neuronal nAChR is a ligand-gated ion channel composed of five subunits that differ in composition from skeletal muscle cholinergic receptors. Molecular cloning techniques identified 16 genes encoding nAChR subunits: $\alpha 1$, β , δ , and γ subunits in skeletal muscle, and $\alpha 2$, $\alpha 4$, $\beta 2$, $\beta 4$ subunits in brain, sensory systems, and autonomic ganglia. Most neuronal nAChRs are formed by a heteropentameric assembly of α and β subunits, with the functional properties depending on the subunit composition. The distribution of the various subunits in the rat brain has distinct expression patterns. The regional distribution of α and β subunits coincides with high-affinity binding sites for [^3H] nicotine. Several findings suggest that the $\alpha 4\beta 2$ nAChR subtype plays a major role in the reinforcing effects of nicotine. Humans and rodent studies demonstrated that chronic exposure to nicotine increases the density of nicotine binding, mainly to the $\alpha 4\beta 2$ nAChR. Nicotine self-administration is reduced in rats pretreated with the selective $\alpha 4\beta 2$ nAChR antagonist dihydro- β -erythroidian DH β E and in genetically modified mice with functional deletion of the $\beta 2$ subunit. The $\beta 2$ subunit is crucial to mediating the dopamine-releasing effects of nicotine as demonstrated by the absence of striatal dopamine release in $\beta 2$ subunit knockout mice treated with nicotine. This cholinergic release of mesolimbic dopamine release, mimicked by nicotine and shared by other addictive drugs, is considered central in the acquisition and maintenance of nicotine addiction.

Regulatory Background:

The United States Food and Drug Administration has previously approved several nicotine replacement therapy products and bupropion as treatment aids to smoking cessation. Bupropion (Zyban), a dopamine and norepinephrine reuptake inhibitor, is thought to act by increasing the concentration of dopamine in the nucleus accumbens, similar to the effect of nicotine. Interestingly; bupropion also acts as a noncompetitive antagonist at rat $\alpha 4\beta 2$, $\alpha 3\beta 4$, and $\alpha 7$ nAChR subtypes.

Varenicline tartrate, is a new molecular entity with partial agonist properties at neuronal nicotinic receptors. The sponsor hypothesizes that the partial agonist activity of varenicline allows the drug to stimulate the $\alpha 4\beta 2$ nAChR and simultaneously prevent nicotine from binding to the same receptor.

Functional Activity:

The Sponsor provides evidence that CP-526,555 (varenicline) is a partial agonist of nicotinic receptors. Receptor binding studies demonstrated that varenicline has high affinity for the $\alpha 4\beta 2$ neuronal nicotinic receptor subtype (rat and human cortex, $K_i \sim 0.2$

nM). The affinity for $\alpha 4\beta 2$ was substantially greater than its affinity for $\alpha 3\beta 4$ (>500-fold), the muscle nicotinic receptor, $\alpha\beta\gamma\delta 1$ (>20,000-fold), and the nicotinic toxin receptor, $\alpha 7$ (>3500-fold). CP-526,555 had low affinity at the 5HT_{3A} (K_i 350 nM). CP-526,555 also lacked significant affinity to a variety of other neurotransmitter receptors (56 different receptors tested), modulatory binding sites, ion channels, and neurotransmitter uptake sites. Therefore, **CP-526,555's mechanism of action** involves the selective binding to the $\alpha 4\beta 2$ neuronal nicotinic receptor subtype. The therapeutic benefit related to smoking cessation is thought to occur from CP-526,555's disruption of nicotine's interaction with this $\alpha 4\beta 2$ neuronal nicotinic receptor subtype.

The Sponsor demonstrated functional activity of CP-526,555 in both *in vitro* and *in vivo* studies. In electrophysiologic studies using oocytes and HEK cells expressing human $\alpha 4\beta 2$ neuronal nicotinic receptors, the application of CP-526,555 induced currents with an EC₅₀ of 2.6 μ M and 3.5 μ M, respectively. In another *ex vivo* functional assay (rat striatal slices), CP-526,555 stimulated [³H]dopamine release at 1 μ M concentration with a maximal efficacy of 51% (relative to release evoked by 10 μ M nicotine). Greater concentrations of CP-526,555 resulted in no additional dopamine release, suggesting only partial agonistic activity of the compound. This partial agonist activity of CP-526,555 was verified by *in vivo* microdialysis studies in rats. Administration of the CP-526,555 resulted in dopamine release with an ED₅₀ of 0.032 mg/kg PO (maximal response 153%) which is approximately 63% of that of nicotine (maximal response 184%). Thus, CP-526,555 reduced the peak dopamine-releasing effect in conscious rats similar to its effects demonstrated *in vitro*. The **primary pharmacodynamics** of CP-526,555 results from this partial agonist activity.

Both nicotine and CP-526,555 activate the $\alpha 4\beta 2$ neuronal nicotinic receptor subtype resulting in mesolimbic dopamine that is believed to play a key role in the 'reward system' in brain mediating addiction. In animal models designed to evaluate withdrawal and physical dependence after repeated administration, discontinuation of CP-526,555 did not result in any behavioral disorder or body weight loss, implying that continuous CP-526,555 administration did not result in physical dependence to this compound. This observation is in contrast to similar findings following repeated nicotine treatments, and may be due to the partial agonistic property of CP-526,555 on the $\alpha 4\beta 2$ neuronal nicotinic receptor subtype, suggesting reduced addictive potential of CP-526,555 compared to nicotine.

CP-526,555 was tested in numerous *ex vivo* tissue experiments to identify **secondary pharmacodynamics** effects. No effect was demonstrated in the epinephrine-stimulated contraction of guinea pig aorta at concentrations ≤ 10 μ M, on histamine-(H₁)-stimulated contraction of isolated segments of guinea pig ileum, and on either the rate of basal beating (histamine-independent) or histamine-(H₂)-stimulated positive chronotropic activity at concentrations ≤ 10 μ M. CP-526,555 had no effect on oxytocin-induced contraction of the rat uterus at concentrations ≤ 10 μ M. CP-526,555 produced concentration dependent relaxation of oxotremorine-contracted longitudinal muscle of the rat colon with an EC₅₀ of 2.68 μ M. Nicotine produced a similar effect but was 2.7 times more potent. The biological significance of this finding is not known.

Safety Pharmacology:

Neurological: Mild tremors, decreased locomotor activity, piloerection and hunched to flattened body posture was noted with a single oral administration of 10 mg/kg CP-526,555 in rats [human equivalent dose (HED) = 96 mg/60 kg person based on body surface area] corresponding to a mean plasma C_{max} of 749.0 ng/mL at 1 hr. Mean plasma concentrations at the human efficacious dose of 1 mg was 9.2 ng/mL, corresponding to an approximately 50-fold safety margin for CNS behavioral changes. In rats, 100 mg/kg CP-526,555 produced convulsion, salivation, mild to moderate ptosis, decreased response to toe pinch, decreased exploratory behavior, gait disturbances (splayed hind limbs) and decreased muscular tone. In mice, doses up to 10 mg/kg CP-526,555, resulted in no significant effect on the incidence of pentylenetetrazole (PTZ)-induced twitch, myoclonus or tonic extension. At 10 mg/kg, but not at 1 mg/kg CP-526,555, rat body temperature was significantly lower (2°C). These effects are often observed with toxic doses of other cholinergic agonists such as nicotine.

Renal: In rats, CP-526,555 at 30 mg/kg (HED=290 mg/60 kg person based on body surface area), but not at 3 mg/kg (HED=29 mg/60 kg person based on body surface area), significantly increased sodium (138%) and chlorine (173%) excretion. This is a known pharmacological effect of nicotine.

Gastrointestinal Oral administration of CP-526,555 resulted in a dose-related decrease in gastric emptying and gastrointestinal motility in rat. Reduced gastric emptying of 55% and 89% occurred at doses of 3 and 30 mg/kg, respectively. One hour after oral administration of CP-526,555, reductions in the geometric center were 3% at 0.3 mg/kg (plasma level=142 ng/mL), 53% at 3.0 mg/kg (plasma level=656 ng/mL), and 90% at 30 mg/kg (plasma level= 1038 ng/mL). The reduction in geometric center of the gastrointestinal tract is a well known pharmacological effect of nicotine and thus might be expected with this partial agonist.

Cardiovascular and Respiratory: In conscious monkeys, CP-526,555 was administered orally at 0.1, 0.3, and 3.0 mg/kg (HED=58 mg/60 kg person based on body surface area). There were no changes in pH, arterial blood gases and heart rate. One of the six monkeys administered 0.1 mg/kg (plasma level= 21 ng/mL) exhibited decreased heart rate (31%, 90 mins post dose) and slightly increased PR at 45, 60, and 90 min post dose. The other 5 monkeys with similar plasma level of CP-526,555 did not have these effects. Since these effects were absent at the C_{max} (32 ng/mL) for the 3 mg/kg dose, the response of this one monkey may be unrelated to CP-526,555.

Other: CP-526,555 induced excessive retching and emesis in ferrets at all dose studied (0.025-0.3 mg/kg) administered either orally or subcutaneously. Nicotine receptor antagonists, mecamylamine, only partially blocked the emetic effect, but ondansetron effectively blocked both the retching and emesis. The retching and emesis in ferrets would appear to be the result of activity of CP-526,555 at serotonergic 5-HT₃ receptors (K_i = 530 nM).

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

CP-526,555 inhibited [³H]nicotine binding to human $\alpha 4\beta 2$ receptors transfected and expressed in HEK293 cells ($K_i = 0.11$ nM), $\alpha 4\beta 2$ receptors in human cortical membranes ($K_i = 0.15$ nM), and $\alpha 4\beta 2$ receptors in rat brain membranes ($K_i = 0.174$ nM).

Summary of Binding Affinities of Varenicline

<u>Tissue/receptor</u>	<u>K_i, nM</u>
Rat Cortex, $\alpha_4\beta_2$	0.17
Human Cortex, $\alpha_4\beta_2$	0.15
Human $\alpha_3\beta_4$	84
IMR32, α_7	620
Electroplax, $\alpha_1 \beta_1 \gamma \delta$	3400

The specificity of CP-526,555 was tested in a screening assay using standard filtration receptor binding techniques. Different neuronal receptors, ion channels, regulatory and second messenger sites in CNS and peripheral tissue were screened. CP-526,555 produced 100% inhibition of specific binding at the rat cortex neuronal nicotinic receptor and had low affinity at the 5HT_{3A} (K_i 530 nM) receptor. No other receptor interactions were detected (IC_{50} values >1 μ M) (see table below).

Screening Assay for CP-526,555 Binding

Appears This Way
On Original

Neurotransmitter Receptors	Ligand	Species	Tissue	IC ₅₀ (nM)	Notebook Number
Adenosine A ₁	NECA	Human	CHO cells	>10000	101332.065 101332.093
Adenosine, non-selective	NECA	Bovine	Striatum	>1000	28034.212
Alpha-1 adrenergic	Prazosin	Human	Cortex	>10000	101332.065 101332.093
Alpha-1 adrenergic, non-selective	Prazosin	Rat	Forebrain	>1000	28034.212
Alpha-2 adrenergic	UK-14,304	Human	Cortex	>10000	101332.065 101332.093
Alpha-2 adrenergic, non-selective	RX 821002	Rat	Cortex	>1000	28034.212
Benzodiazepine, peripheral	PK11195	Rat	Kidney	>1000	28034.212
Beta Adrenergic, non-specific	Dihydroalprenolol	Human	Cortex	>10000	101332.065 101332.093
Beta Adrenergic, non-selective	Dihydroalprenolol	Rat	Cortex	>1000	28034.212
Clozapine	Clozapine	Rat	Striatum	>1000	28034.212
Dopamine, non-selective	Spiperone	Bovine	Striatum	>1000	28034.212
Dopamine, D2	Spiperone	Human	LTK-cells	>10000	101301.160 101301.192
γ-amino-butyric acid GABA-A	Muscimol	Human	Cortex	>10000	101332.065 101332.093
Gaba-A Agonist	Gaba	Bovine	Cerebellum	>1000	28034.212
Gaba-A, Benzodiazepine, central	Flunitrazepam	Bovine	Cortex	>1000	28034.212
Glutamate, AMPA site	AMPA	Rat	Forebrain	>1000	28034.212
Glutamate, NMDA Agonist site	CGP-39653	Rat	Forebrain	>1000	28034.212
Glycine, Strychnine- sensitive	Strychnine	Rat	Spinal Cord	>1000	28034.212
Histamine, H1	Pyrilamine	Human	Cortex	>10000	101332.129 101332.138
Imidazoline 2, central	2-BFI	Rabbit	Brain	>1000	28034.212
Muscarinic, central, non-selective	Quinuclidinyl benzilate	Rat	Cortex	>1000	28034.212
Muscarinic, peripheral, non-selective	Quinuclidinyl benzilate	Guinea Pig	Bladder	>1000	28034.212
Muscarinic, m1	N-methyl scopolamine	Human	CHO	>10000	101301.160 101301.192
Muscarinic, m2	N-methyl scopolamine	Human	CHO	>10000	101301.160 101301.192
Muscarinic, m3	N-methyl scopolamine	Human	CHO	>10000	101301.160 101301.192

Neurotransmitter Receptors	Ligand	Species	Tissue	IC ₅₀ (nM)	Notebook Number
Muscarinic, m4	N-methyl scopolamine	Human	CHO	>10000	101301.160 101301.192
Muscarinic, m5	N-methyl scopolamine	Human	CHO	>10000	101301.160 101301.192
Nicotinic, neuronal	N-methylcarbamyl choline iodide	Rat	Cortex	105% @ 1000	28034.212
Purinergic, P2Y	ADP-beta-S	Rat	PC-12 cells	>1000	28034.212
Serotonin, 5HT1A	8-OH-DPAT	Human	HeLa cells	>10000	101301.160 101301.192
Serotonin, 5HT1A	8-OH-DPAT	Human	CHO cells	>1000	28034.212
Serotonin, 5HT2A	Ketanserin	Human	Cortex	>10000	101332.065 101332.093
Serotonin, 5HT3A	LY-278584	Human	HEK-293	350*	56980.012 101301.008 101301.160
Serotonin, 5HT1D	5-Carboxamido tryptamine	Bovine	Striatum	>1000	28034.212
Serotonin, 5HT7	LSD	Rat	SP9 cells	>1000	28034.212
Serotonin, non-selective	Lysergic acid diethylamide	Rat	Cortex	>1000	28034.212
Sigma, non-selective	DTG	Guinea Pig	Brain	>1000	28034.212
Opioids					
Opiate, nonselective	Naloxone	Rat	Forebrain	>1000	28034.212
Uptake Sites					
Choline uptake	Choline Chloride	Rat	Cortex	>1000	28034.212
Dopamine uptake, cocaine site	WIN-35,428	Guinea Pig	Striatum	>1000	28034.212
GABA uptake	GABA	Rat	Cortex	>1000	28034.212
Norepinephrine uptake	Desmethylimipramine	Rat	Cortex	>1000	28034.212
Serotonin uptake	Citalopram	Rat	Forebrain	>1000	28034.212
Glutamate uptake	glutamate	Rat	Cerebellum	>1000	28034.212
Ion Channels/Regulatory Sites					
Potassium, voltage	Charybdotoxin	Rat	Whole Brain	>1000	28034.212
Potassium, ATP sensitive	Glibenclamide	Rat	Cortex	>1000	28034.212
Calcium Channels (N-Type)	Omega Conotoxin	Rat	Cortex	>1000	28034.212
Calcium Channels (L-type)	D888, desmethoxy verapamil	Rat	Heart	>10000	41303.238 41303.244
Calcium channel (L-type)	Nitrendipine	Rat	Cortex	>1000	28034.212
Gaba-A, chloride, TBOB	TBOB	Rat	Cortex	>1000	28034.212
Glutamate, MK801	MK-801	Rat	Forebrain	>1000	28034.212
Sodium, Site 1	Saxitoxin	Rat	Forebrain	>1000	28034.212
Adenylate cyclase	Forskolin	Rat	Forebrain	>1000	28034.212
Inositol Trisphosphate	IP3	Rat	Cerebellum	>1000	28034.212
NOS neuronal binding	L-N-Ng-Nitro-Arginine	Rat	Brain	>1000	28034.212
Protein Kinase C	Phorbol ester dibutyrate	Mouse	Brain	>1000	28034.212

* denotes Ki value

DRUG ACTIVITY RELATED TO PROPOSED INDICATION:Study Title and: *In vitro* functional effects of varenicline

Study Number: 2005.104026

In vitro nicotinic partial agonist potency and efficacy of varenicline was evaluated using whole-cell patch clamp electrophysiologic techniques to measure receptor mediated currents in HEK293 cells expressing human $\alpha 4\beta 2$ neuronal nicotinic receptors. Currents were evoked using a micro perfusion system.

Key Study Findings:

- Varenicline induced currents in HEK293 cells expressing human $\alpha_4\beta_2$ neuronal nicotinic receptors, with an EC_{50} of 3.5 μM . Maximal responses elicited by varenicline were only 43% of that elicited by nicotine. Concurrent administration of nicotine and varenicline demonstrated that varenicline can partially block nicotine-induced currents. These results suggest that varenicline acts as a partial agonist in this *in vitro* model.

Electrophysiological Currents Induced by Varenicline in HEK-293 Cells Expressing Human $\alpha_4\beta_2$ Nicotinic Receptors.

Treatment	$\alpha_4\beta_2$ Current; % of maximum nicotine response (n)
0.1 μM varenicline	1.75% (1)
0.3 μM varenicline	5.60% (3)
1.0 μM varenicline	13.3% (3)
3.0 μM varenicline	23.5% (3)
10 μM varenicline	29.6% (3)
30 μM varenicline	42.8% (3)
100 μM varenicline	43.3% (3)

Electrophysiological Currents Induced by Nicotine in HEK293 Cells Expressing Human $\alpha_4\beta_2$ Nicotinic Receptors.

Treatment	$\alpha_4\beta_2$ Current; % of 10 μM nicotine response (n)
0.1 μM nicotine	3% (3)
0.3 μM nicotine	9% (3)
1.0 μM nicotine	25% (3)
3.0 μM nicotine	43% (6)
10.0 μM nicotine	100% (3)
30.0 μM nicotine	141% (3)
100.0 μM nicotine	148% (3)
300.0 μM nicotine	129% (3)

Concurrent Application of Varenicline Partially Blocks Nicotine Induced $\alpha_4\beta_2$ Nicotinic Receptor Currents in HEK293 Cells Expressing Human Neuronal Nicotinic Receptors.

Treatment	$\alpha_4\beta_2$ Current; % of control
10 μM nicotine	100%
10 μM varenicline + 10 μM nicotine	47%

Study Title: In vitro functional effects of CP-526,555**Study Number: 1997-38226**

This study was done to determine the partial nicotine agonistic potency of CP-526,555 and to determine its functional efficacy. *Xenopus* oocytes expressing human neuronal nicotinic receptors were used as the experimental model. *Xenopus* oocytes were injected with 10-50 ng of each neuronal nicotinic receptor cRNA and stored in Barth's saline for up to 2 weeks. Electrophysiological measurements of the receptor mediated current were recorded using two-electrode voltage clamp techniques.

Key Study Findings:

- CP-526,555 (0.1 – 100 μM) and nicotine (0.3 – 100 μM) induced dose-dependent inward currents in oocytes expressing human $\alpha 4\beta 2$ nicotinic receptors. Inward currents were also dose-dependently evoked in oocytes expressing $\alpha 3\beta 4$ nicotinic receptors.
- Maximal responses from CP-526,555 occurred at or above 30 μM . Maximal responses with nicotine occurred at 100 μM . The dose-response analysis for CP-526,555 resulted in an EC_{50} = 2.6 μM and a Hill slope = 1.0; the results for nicotine were an EC_{50} = 12.5 μM and a Hill slope = 1.2.

Electrophysiological currents induced by CP-526,555 in oocytes expressing human neuronal nicotinic receptors

Dose (μM)	$\alpha 4\beta 2$ Current (% of 10) (n)	$\alpha 3\beta 4$ Current (% of 30) (n)
0.1	6% (2)	N/A
0.3	18% (2)	2% (1)
1.0	37% (1)	N/A
3.0	N/A	18% (3)
10	100% (13)	66% (3)
30	123% (2)	100% (15)
100	122% (2)	134% (2)

Study Title: Effects of CP-526,555-01 (Varenicline) on basal and nicotine induced dopamine release in rat n. accumbens**Study Number: 1997-37293.60**

In vivo microdialysis was performed in awake, freely moving male Sprague-Dawley rats to evaluate the effect of CP-526,555 on DA release within the nucleus. One hour after oral CP-526,555 administration (0.01 to 10 mg/kg), rats were administered SC either vehicle, 0.32 mg/kg (-)-nicotine or 1 mg/kg mecamylamine, a non-selective nicotinic

antagonist. Drug effects were expressed as the percentage of baseline (i.e., average of last 5 pre-drug baseline levels) \pm SEM (n=3-6). The maximal effect of each dose was calculated as the mean \pm SEM of three sequential samples around the T_{max} (i.e. 54-108 min after PO CP-526,555 and 27-81 min after SC nicotine).

Key Study Findings:

- CP-526,555 increased DA release in nucleus accumbens. DA release reached maximal levels 2 hours after drug administration and started to decline to baseline levels at 4-5 hours after dosing.
- Doses of CP-526,555 from 0.01 to 10 mg/kg resulted in an inverted U-shaped dose-response curve. Doses of 0.01, 0.032, 0.1, 0.32 and 1.0 mg/kg PO increased DA levels, but higher doses of 3.2 and 10 mg/kg had a smaller or no effect.
- The maximal response of CP-526,555 was 153% of baseline and the ED_{50} , estimated from the dose-response curve, was 0.032 mg/kg. The partial agonist efficacy of CP-526,555 (maximal response 153%) was about 63% of that of the full agonist nicotine (maximal response 184%).

Effect of CP-526-555 on Dopamine release

Drug Treatment	Maximum Dopamine Release (% of basal levels \pm SEM) (n)
Vehicle p.o. + Vehicle s.c.	100.3 \pm 4.5 % (5)
0.01 mg/kg p.o. CP-526,555 + Vehicle s.c.	119.8 \pm 7.4 % (3)
0.03 mg/kg p.o. CP-526,555 + Vehicle s.c.	126.3 \pm 10.8 % (4)
0.1 mg/kg p.o. CP-526,555 + Vehicle s.c.	144.9 \pm 4.5 % (4)
0.32 mg/kg p.o. CP-526,555 + Vehicle s.c.	130.8 \pm 4.9 % (5)
1.0 mg/kg p.o. CP-526,555 + Vehicle s.c.	152.8 \pm 15.6 % (3)
3.2 mg/kg p.o. CP-526,555 + Vehicle s.c.	111.6 \pm 7.6 % (3)
Vehicle p.o. + 0.32 mg/kg s.c. Nicotine	183.7 \pm 11.7 % (10)
0.01 mg/kg p.o. CP-526,555 + 0.32 mg/kg s.c. Nicotine	155.9 \pm 9.9 % (5)
0.03 mg/kg p.o. CP-526,555 + 0.32 mg/kg s.c. Nicotine	155 % (1)
0.1 mg/kg p.o. CP-526,555 + 0.32 mg/kg s.c. Nicotine	171.4 \pm 13.8 % (5)
1.0 mg/kg p.o. CP-526,555 + 0.32 mg/kg s.c. Nicotine	134.6 \pm 11.5 % (5)
0.32 mg/kg p.o. CP-526,555 + Vehicle s.c.	166.4 \pm 23.8 % (3)
0.32 mg/kg p.o. CP-526,555 + 1 mg/kg s.c. Mecamylamine	126.8 \pm 7.1 % (3)

Study Title: In vivo effects of CP-526,555-01 (varenicline) as a nicotinic partial agonist: intrinsic effects alone (oral and S.C.) and on nicotine-induced increases in dopamine turnover (rat nucleus accumbens)

Study Number: 1997-38271

Sprague Dawley rats (male: 200 and 300 g) were pretreated either with CP-526,555 alone (SC or PO) or in combination with nicotine (SC, concurrent injection). Rats were sacrificed 1 hr after SC dosing or 2 hr after PO dosing. Following decapitation, the nucleus accumbens was rapidly dissected, homogenized, centrifuged, and then DA and its metabolites were quantitated by HPLC. Dopamine turnover (DATO) was calculated as the ratio of tissue concentrations of metabolites to DA, (i.e. ([DOPAC] + [HVA])/ [DA]) and expressed as % control animals. This measure was used as an index of dopamine utilization in rat brain.

Key Study Findings:

- **Effects of CP-526,555 (SC) compared to nicotine (SC) on DATO:** DATO for CP-526, 555 was 129% of control at 3.2 mg/kg SC dose. The peak mean nicotine effect was 177% of control 1 mg/kg SC. Both drugs were dose-responsive across a broad dosing range (0.032 and 5.6 mg/kg). Calculated ED₅₀'s for CP-526,555 and nicotine were 50 and 156 µg/kg, respectively. A comparison of maximal responses indicated that CP-526,555 increased DATO that is 38% of the nicotine-induced DATO increase.
- **Effects of CP-526,555 (oral) on DATO:** The maximal increase DATO by oral CP-526,555 (1.78 mg/kg) was 34%.
- **Ability of CP-526,555 to attenuate nicotine challenge:** CP-526,555 dose-dependently reversed increases in DATO caused by nicotine alone. The ID₅₀ for SC. CP-526,555 in combination with nicotine was estimated to be 750 µg/kg.
- **Ability of mecamylamine to block the effects of nicotine and CP-526,555 on DATO:** Mecamylamine antagonized both CP-526,555 and nicotine mediated increased DATO.

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Summary of CP-526,555- and nicotine-induced DA turnover in the nucleus accumbens

Drug Treatment		DA Turnover		SEM
CP-526,555 (mg/kg, s.c.)	Nicotine (mg/kg, s.c.)	(% Nicotine)	(% control)	(%)
vehicle			100	1.5
0.178	-		178	4.2
0.56	-		121	4.4
1.78	-		122	4.4
3.2	-		129	10.5
5.6	-		123	10.6
	1.0 CP-004994 (Nicotine)		170	4.6
vehicle	-	0		5.4
0.56	1.0	70.1		4.6
1.78	1.0	59.2		4.0
5.6	1.0	43.3		4.1
-	1.0 CP-004994 (Nicotine)	100		4.0

4.0

4.0

Study Title: Summary Report for CP-526,555 in Vitro Binding:

I Affinity at Nicotinic Receptors

II. Effects on In Vitro Nicotine Stimulated [³H] Dopamine Release in the Rat Corpus Striatum

Study Number: 1997-38428

Studies of $\alpha 4\beta 2$ receptor binding were performed with human cortex tissue, rat brain, and HEK293 cells transfected with and expressing the human $\alpha 4\beta 2$ or $\alpha 3\beta 4$ receptors. For $\alpha 1\beta \gamma \delta$ nicotinic receptor binding, *Torpedo* electroplax membranes were used. Human $\alpha 7$ and rat $\alpha 7$ nicotinic receptor binding studies used IMR32 cells or PC12 cells, respectively. CP-526,555-induced dopamine release was assayed *ex vivo* by quantitating [³H]dopamine release from rat corpora striatal slices in alone or in combination with the nicotine.

Key Study Findings:

- CP-526,555 stimulated [³H]dopamine release in a dose-dependent manner. The maximal efficacy relative to 10 μM nicotine was 51% at 1 μM CP-526,555. Higher concentrations resulted in slightly less release, indicating CP-526,555 is a partial agonist. Dopamine release evoked by nicotine at 10 μM was reduced by 53% in the presence of 10 μM CP-526,555, demonstrating its antagonist effect.

CP-526,555 Dose Response for [³H]Dopamine Release:

DOSE RESPONSE	nicotine 10 μM	CP- 526,555 0.032 μM	CP- 526,555 0.1 μM	CP- 526,555 1.0 μM	CP- 526,555 10 μM	CP- 526,555 100 μM
% nicotine evoked release	100	22	42	53	42	38

Study Title: Effects of CP-526,555 (varenicline) pretreatment (or substitution) on rats trained to self-administer nicotine

Study Number: 1998-41516.020

Rats were initially trained to lever-press under a fixed ratio 1 (FR1) schedule of food reinforcement associated with the right lever. Behavior was then maintained under a 30 second variable interval (VI 30) schedule until steady response rates were observed (about 7 sessions).

Two variations of the self-administration procedure were employed. In the first procedure, rats were permitted to self-administer nicotine under the FR5 schedule during daily (Monday-Friday) sessions of 90 min duration. After the subjects' rate of self-administration became stable, studies involving CP-526,555 pretreatment were initiated. In the second procedure, after behavior was maintained under a progressive ratio (PR) schedule of nicotine reinforcement, substitution of CP-526,555 for nicotine was continued for 4 days.

Key Study Findings:

- **Effect of CP-526,555 substitution on rats trained to self-administer nicotine on an FR5 schedule:** Saline substitution was used for the 0 dose. Nicotine was statistically different from the saline substitution condition at the 10 μg/kg dose and trended in that direction at the 30 μg/kg dose (p=0.07) and the 56 μg/kg dose (p=0.10). Only one dose of CP-526,555 substituted for nicotine under the FR5 schedule; the 56 μg/kg dose was statistically different from the saline substitution condition. Therefore, nicotine was self-administered over a wider dose range than was CP-526,555.
- **Effect of CP-526,555 substitution on rats trained to self-administer nicotine on a PR schedule:** Only the 56 μg/kg dose of CP-526,555 might have maintained self administration greater than saline treatment. There was a significant difference in the number of infusions earned, but not a significant difference in the change from baseline (delta). In addition, direct comparisons

between CP-526,555 and nicotine treatments revealed that CP-526,555 infusions were significantly fewer, but with larger changes from baseline than nicotine infusions. These data suggest that CP-526,555 cannot maintain behavior to the same extent as nicotine.

Study Title: Toleration and withdrawal assessment of CP-526,555-18 (varenicline) in rats;

Study Number: 2004-63943

Rats were trained in a lever pressing paradigm at a fixed ratio of 10 (FR10) using successive approximation training, such that at the completion of training 10 lever presses resulted in a reward food pellet being dispensed. Each daily session was composed of up to 50 trials, and each trial successfully completed (10 lever presses) resulted in a reward pellet with a maximum of 50 reward food pellets per rat, per session. An acute dose-response curve was determined for CP-526,555-18. The dose of CP-526,555-18 that decreased the response rate by an average of > 50%, 1.7 mg/kg, was then administered daily for 14 days in an effort to assess tolerance (i.e., a return to day 0 or basal response rate). Animals were observed daily prior to dosing, for a minimum of 5 minutes post dose and once again several hours later.

Key Study Findings:

- Subcutaneous administration of CP-526,555-18 produced a dose-dependent disruption of performance. The maximal effect was observed at 1.7 mg/kg with no effect on the number of trials completed. Varenicline disrupted behavioral performance approximately 50% when dosed acutely at 1.7 mg/kg.
- Tolerance, defined by a return to day 0 or basal response rates, developed to 1.7 mg/kg varenicline treatment after 10 days of dosing. Discontinuation of varenicline after 14 days and substitution with sterile water on days 15 through 21 resulted in no change in response rate, and no observable behavioral effects. Withdrawal from varenicline did not result in any nicotinic withdrawal behaviors such as teeth chattering, chewing, gasping, writhing, head shakes, body shakes, tremors and ptosis.

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2.6.2.3 Secondary pharmacodynamics

Ex vivo tissue bath experiments were performed to identify secondary pharmacodynamic effects of the compound.

Isolated guinea pig aorta: CP-526,555 was examined for effects on norepinephrine stimulated contraction of guinea pig vascular smooth muscle. CP-526,555 had no effect on either basal tension or on norepinephrine-stimulated contraction of guinea pig aorta at concentrations $\leq 10 \mu\text{M}$

Isolated guinea pig right atria: CP-526,555 was tested for effects on spontaneously beating guinea pig right atria. CP-526,555 had no effect on either the rate of basal beating (histamine-independent), or on histamine-(H₂)-stimulated positive chronotropic activity at concentrations $\leq 10 \mu\text{M}$.

Isolated guinea pig ileum: CP-526,555 was tested for effects on histamine-(H₁)-stimulated contraction of isolated segments of guinea pig ileum. CP- 526,555 had no effect at concentrations $\leq 10 \mu\text{M}$.

Isolated rat colon: CP-526,555 produced a concentration-related relaxation of the oxotremorine-contracted longitudinal muscle of the rat colon with an EC₅₀ of 2680 nM. Nicotine produced a similar effect but was 2.7 times more potent than CP-526,555. The selective nicotinic receptor antagonist, mecamylamine, completely inhibited relaxation caused by CP-526,555 ($< 100 \mu\text{M}$).

Isolated rat uterus: CP-526,555 was tested for effects on oxytocin-induced contraction in the rat uterus. CP-526,555 had no effect at concentrations $\leq 10 \mu\text{M}$.

2.6.2.4 Safety pharmacology

Study Title: General Pharmacology of CP-526,555

Study Number: CP 5265550705/GP

Neurological effects:

The acute behavioral effects of CP-526,555 were evaluated in mice given oral doses from 0.32 to 100 mg/kg. Within 0.5 hours after 10 mg/kg of CP-526,555, mice exhibited mild, transient tremors approximately 4 min post dose (n= 1 of 3), decreased locomotor activity, piloerection (n= 2 of 3) and hunched to flattened body posture (all mice). Mice appeared normal at the 1 and 2 hours post dose time points. Mice given 32 mg/kg of CP-526,555 had decreased locomotor activity, mild to moderate tremors, twitches (n=1 of 3), flattened body posture and convulsions (n=2 of 3). Behavioral changes observed in the open field were decreased exploratory behavior, gait disturbance (wobble) and splayed

hind limbs (slight to mild). An increase in pupil size was observed at all time points post dose. By 1 and 2 hours post dose, the effects were a slightly impaired gait (n=1 of 3) and decreased body and limb tone (n=1 of 3). By 2 hours post dose, all CP-526,555 treated-animals appeared normal except for increased pupil size and slightly decreased body tone. At 100 mg/kg of CP-526,555, mice had decreased locomotor activity, twitches, tremors and hunched or flattened body postures at 0.5, 1 and 2 hours post dose. One mouse exhibited tremors, convulsions, loss of righting reflex and death within 0.5 hour post dose. Other behaviors included salivation, mild to moderate ptosis (n=1 of 2 at 0.5 and 1 hour post dose, and n=2 at 2 hours), decreased body and limb tone and splayed hind limbs (n=2 at 0.5 hour and n=1 at 1 and 2 hours post dose), decreased response to toe pinch (n=2 at 1 and 2 hours post dose), impaired inverted screen performance (n=1 of 2 at 0.5, 1 and 2 hours post dose), and increased pupil size (n=2 at 0.5, 1 and 2 hours post dose). In the open field, mice had reduced exploratory behavior and mild gait disturbances at all time points post dose.

For comparison, the acute behavioral effects were evaluated in mice given doses of 0.032 to 32 mg/kg; SC nicotine. Nicotine had no significant effects on behavior in mice at doses up to 0.32 mg/kg. However, mice given 1 to 10 mg/kg of nicotine had dose-related decreased locomotor activity and increased pupil size. Doses of 10 mg/kg nicotine resulted in tremors, convulsions, decreased body and limb tone, decreased transfer arousal, decreased response to toe pinch and disturbances in gait, body position and coordination at 0.5 and 1 hour post dose. Mice appeared normal at 2 hours post dose. All mice (n=3) given 32 mg/kg nicotine had convulsions, loss of righting reflex and death within 0.5 hours.

In a pharmacokinetic study, mean plasma concentrations of CP-526,555 in mice after oral administration were determined at 1, 2, 4 and 6 hours post dose. Maximal plasma concentrations occurred one hour post dose with mean values of 62.3, 149.7, 455.3 and 749.0 ng/mL, at doses of 0.32, 1.0, 3.2 and 10 mg/kg, respectively. The clinically efficacious plasma concentrations were projected to be in the range of 1 to 15 ng/mL. Therefore, CP-526,555 produced no behavioral effects in mice at plasma concentrations that were at least 30 times greater than the predicted clinical therapeutic level.

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Summary of Neurologic and Behavioral Assessments

Behaviors	Scoring	VEH Range	Dose (mg/kg)						
			0*	0.32	1	3.2	10	32	100
Tremors (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
Convulsions (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	67.0	67.0
Loss of Righting Reflex (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	33.0
Death (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	33.0
Salivation (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	50.0
Lacrimation (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Piloerection (%)	0-100	0	0.0	0.0	0.0	0.0	67.0	0.0	0.0
Disturbance of Gait	0-8	0	0.0	0.0	0.0	0.0	0.0	2.0	2.0
Positional Passivity	0-8	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Body Position	0-8	3-8	4.7	5.3	4.7	6.0	2.0	2.0	2.0
Locomotion	0-8	4-6	4.0	4.0	4.0	4.0	0.0	0.0	0.0
Respiration Rate	0-8	4	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Skin Color	0-8	4	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Transfer Arousal	0-8	4-6	4.7	4.0	4.0	6.0	4.0	2.0	3.0
Body/Abdominal Tone	0-8	4-6	4.7	4.7	5.3	5.3	4.0	4.0	3.0
Limb Tone	0-8	4-6	4.7	4.0	4.7	5.3	4.0	4.0	3.0
Provoked Biting	0-8	4-6	4.7	4.0	4.7	4.0	4.0	4.0	4.0
Tail Pinch	0-8	2-4	2.3	2.0	2.0	2.0	2.0	2.0	2.0
Toe Pinch	0-8	4-6	4.7	4.0	5.3	5.3	4.0	4.0	4.0
Corneal Reflex	0-8	4-6	4.0	4.0	4.7	4.0	4.0	4.0	4.0
Ptosis	0-4	0-1	0.0	0.0	0.0	0.0	0.0	0.0	0.5
Inverted Screen	0-2	1.7-2	2.0	2.0	2.0	2.0	2.0	2.0	1.5
Pupil Size	30 x mm	10-15	13.5	13.3	14.0	16.7	12.7	20.0	34.5

Behaviors	Scoring	VEH Range	Dose (mg/kg)						
			0*	0.32	1	3.2	10	32	100
Tremors (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	50.0
Convulsions (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Loss of Righting Reflex (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Death (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salivation (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lacrimation (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Piloerection (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Disturbance of Gait	0-8	0	0.0	0.0	0.0	0.0	0.0	0.7	2.0
Positional Passivity	0-8	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Body Position	0-8	2-4	3.0	3.3	2.7	4.0	2.7	2.0	2.0
Locomotion	0-8	0-3	1.7	2.7	0.7	2.0	0.7	0.0	0.0
Respiration Rate	0-8	4	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Skin Color	0-8	4	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Transfer Arousal	0-8	4	4.0	4.0	4.0	4.0	4.0	4.0	2.0
Body/Abdominal Tone	0-8	4-6	4.0	4.0	4.7	4.0	4.0	3.3	2.0
Limb Tone	0-8	4-6	4.7	4.7	4.7	4.0	4.0	3.3	2.0
Provoked Biting	0-8	4-6	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Tail Pinch	0-8	2-4	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Toe Pinch	0-8	4-6	4.3	4.0	4.7	4.0	4.7	4.0	2.0
Corneal Reflex	0-8	4-6	4.0	4.0	4.0	5.3	4.0	4.7	4.0
Ptosis	0-4	0-1	0.0	0.0	0.0	0.0	0.0	0.0	1.0
Inverted Screen	0-2	1.7-2	2.0	2.0	2.0	2.0	2.0	2.0	1.5
Pupil Size	30 x mm	10-15	11.8	11.0	13.0	17.3	13.0	18.3	34.0

Behaviors	Scoring	VEH Range	Dose (mg/kg)							
			0*	0.32	1	3.2	10	32	100	
Tremors (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
Convulsions (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Loss of Righting Reflex (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Death (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salivation (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lacrimation (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Piloerection (%)	0-100	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Disturbance of Gait	0-8	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Positional Passivity	0-8	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Body Position	0-8	2-4	2.7	2.0	2.0	2.0	3.3	2.7	2.0	2.0
Locomotion	0-8	0-2	0.7	0.0	0.0	0.0	1.3	0.7	0.0	0.0
Respiration Rate	0-8	4	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Skin Color	0-8	4	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Transfer Arousal	0-8	4	4.0	4.0	4.0	4.0	4.0	4.0	4.0	2.0
Body/Abdominal Tone	0-8	4-5	4.0	4.0	4.0	4.0	4.0	3.3	2.0	2.0
Limb Tone	0-8	4-5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	2.0
Provoked Biting	0-8	4-5	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.0
Tail Pinch	0-8	2-4	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Toe Pinch	0-8	4-6	4.3	4.0	4.0	4.0	4.0	4.0	4.0	2.0
Corneal Reflex	0-8	4-6	4.0	4.0	4.0	4.7	4.0	4.0	4.0	4.0
Ptosis	0-4	0-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5
Inverted Screen	0-2	1.7-2	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.5
Pupil Size	30 x mm	10-15	11.2	11.0	12.7	13.7	11.3	16.0	34.5	34.5

VEH=Vehicle, *n=6 mice.

Plasma concentrations of CP-526,555 in mice after oral administration of 0.32, 1.0, 3.2 or 10 mg/kg. Data are mean \pm SD of 3 mice per group.

Dose (mg/kg)	Plasma Concentration (ng/mL)				AUC _{0-6 hr} ** (ng.hr/mL)
	1 hr	2 hr	4 hr	6 hr	
0.32	62.3 \pm 14.3	44.7 \pm 6.0	18.8 \pm 1.9	13.1*	180
1.0	149.7 \pm 18.6	79.1 \pm 19.4	34.7 \pm 16.9	23.6 \pm 4.2	361
3.2	455.3 \pm 57.8	317.4 \pm 30.6	177.5 \pm 41.4	117.1 \pm 44.5	1404
10.0	749.0 \pm 152.3	569.9 \pm 250.3	268.3 \pm 152.6	420.0 \pm 168.3	2560

* n=2

** Calculated from mean plasma concentrations from 0 to 6 hours, time zero assumed to be zero

Proconvulsant/Anticonvulsant Effects in Mice:

CP-526,555 or vehicle (water) was orally administered to fasted male CD-1 mice (19-27 g, n=20-29 per group, 7 separate studies) at doses of 1, 3.2 and 10 mg/kg, at 15, 30, and 60 minutes prior to the administration of pentylenetetrazol (PTZ; 85 mg/kg, IP) to induce seizures. Mice were observed in individual cubicles for 30 minutes after PTZ and the occurrences of twitch, generalized myoclonus and tonic extension were recorded. Nicotine or vehicle (saline) was subcutaneously administered to fed male mice (23-30 g, n=9-20 per group, 5 separate studies) at doses of 0.32, 1 and 3.2 mg/kg, 15, 30 and 60 minutes prior to the administration of pentylenetetrazol (PTZ; 85 mg/kg, IP). Mice were

observed in individual cubicles for 30 minutes after PTZ and occurrences of twitch, generalized myoclonus and tonic extension were recorded. Differences in the incidence of seizure components between vehicle and CP-526,555 treated mice were analyzed using the Fisher exact test.

**Seizures induced in mice by 85 mg/kg PTZ administered
60 minutes after subcutaneous nicotine or oral CP-526,555**

		PTZ SEIZURE COMPONENTS		
		Twitch	Myoclonus	Tonic Extension
Treatment		(/n)	(/n)	(/n)
Vehicle		10/10	9/10	3/10
Nicotine	0.32 mg/kg	10/10	10/10	1/10
	1.0 mg/kg	9/9	8/9	1/9
	3.2 mg/kg	10/10	8/10	2/10

Vehicle = deionized, distilled water.

(/n) = number of mice showing the PTZ response/number tested.

		PTZ SEIZURE COMPONENTS		
		Twitch	Myoclonus	Tonic Extension
Treatment		(/n)	(/n)	(/n)
Vehicle		20/20	16/20	5/20
CP-526,555	1.0 mg/kg	20/20	20/20	8/20
	3.2 mg/kg	20/20	20/20	8/20
	10.0 mg/kg	20/20	16/20	8/20

Vehicle = deionized, distilled water.

(/n) = number of mice showing the PTZ response/number tested.

**Seizures induced in mice by 85 mg/kg PTZ administered
30 minutes after subcutaneous nicotine or oral CP-526,555**

		PTZ SEIZURE COMPONENTS		
		Twitch	Myoclonus	Tonic Extension
Treatment		(/n)	(/n)	(/n)
Vehicle		20/20	19/20	10/20
Nicotine	0.32 mg/kg	20/20	17/20	7/20
	1.0 mg/kg	19/19	14/19	3/19*
	3.2 mg/kg	20/20	16/20	7/20

*p<0.05 as determined by the Fisher exact test.

Vehicle = deionized, distilled water.

(/n) = number of mice showing the PTZ response/number tested.

PTZ SEIZURE COMPONENTS				
		Twitch	Myoclonus	Tonic Extension
Treatment		(/n)	(/n)	(/n)
Vehicle		20/20	19/20	6/20
CP-526,555	1.0 mg/kg	20/20	17/20	10/20
	3.2 mg/kg	20/20	19/20	8/20
	10.0 mg/kg	20/20	18/20	5/20

Vehicle = deionized, distilled water.

(/n) = number of mice showing the PTZ response/number tested.

**Seizures induced in mice by 85 mg/kg PTZ administered
30 minutes after subcutaneous nicotine or oral CP-526,555**

PTZ SEIZURE COMPONENTS				
		Twitch	Myoclonus	Tonic Extension
Treatment		(/n)	(/n)	(/n)
Vehicle		19/19	18/19	3/19
Nicotine	0.32 mg/kg	19/19	19/19	8/19
	1.0 mg/kg	18/18	18/18	7/18
	3.2 mg/kg	19/19	19/19	13/19*

*p<0.05 as determined by the Fisher exact test.

Vehicle = deionized, distilled water.

(/n) = number of mice showing the PTZ response/number tested.

PTZ SEIZURE COMPONENTS				
		Twitch	Myoclonus	Tonic Extension
Treatment		(/n)	(/n)	(/n)
Vehicle		29/29	28/29	14/29
CP-526,555	1.0 mg/kg	29/29	27/29	10/29
	3.2 mg/kg	29/29	26/29	13/29
	10.0 mg/kg	29/29	26/29	10/29

Vehicle = deionized, distilled water.

(/n) = number of mice showing the PTZ response/number tested.

Thermal Regulation:

CP-526,555 or vehicle (water) was orally administered to fasted male CD-1 mice (19-24 g, n=12-13 per group, 2 separate studies) at doses of 1, 3.2 and 10 mg/kg. Rectal temperatures were taken via rectal probe at -60, 0, 15, 30, 60, 120, and 180 minutes after dosing. Oral doses of 1 mg/kg CP-526,555 had no effect on rectal temperature, but doses of 3.2 and 10 mg/kg significantly decreased body temperature at 15 to 30 min post dose (p < 0.05). The maximum decrease in body temperature occurred at 15 minutes post dose. Both 3.2 mg/kg and 10 mg/kg CP-526,555 produced a 1°C decrease compared to vehicle-treated mice.

Cardiovascular effects:**Study Title: Effects of CP-526,555 on HERG-Encoded Potassium Current in Stably Transfected HEK-293 Cells****Study Number: CP-526,555 on HERG Report**

The potential for CP-526,555 to inhibit potassium currents involved in cardiac action potential duration and QT interval prolongation was studied electrophysiologically *in vitro* using a human embryonic kidney cell line (HEK293) that stably expressed human ether-a-go-related gene encoded potassium channel.

Key Study Findings:

- CP-526,555 was tested at 5 and 17 μM and inhibited the HERG current in a concentration dependent manner.

Effect of CP -526,555 on HERG current

Observation	% inhibition at 5 nM	% inhibition at 17 nM
1	5.2	13.6
2	5.8	16.2
3	7.2	19.3
4	6.6	16.2
5		17.8
MEAN \pm SD	6.2 \pm 0.9*	16.6 \pm 2.1**[#]

% inhibition was calculated based on the ratio of steady-state current measured in the presence of the drug relative to the control amplitude. Control data were corrected as described in the Materials and Methods section. * $p < 0.05$, ** $p < 0.01$ vs control; [#] $p < 0.001$ between the concentration groups.

Study Title: Effects of CP-526,555-18 on Action Potentials Recorded From Dog Isolated Purkinje Fibers *InVitro***Study Number: 555-18/IC/001/02**

This study examined the effects of CP-526,555-18 (1 μM , 3 μM and 10 μM) on evoked action potential characteristics and conduction in dog isolated Purkinje fibers *in vitro*. The parameters determined were resting membrane potential, action potential amplitude, maximum rate of depolarization (V_{max}) and the action potential duration at 50% and 90% repolarization (APD₅₀ and APD₉₀ respectively).

Key Study Findings:

- CP-526,555-18 (10 μM) statistically increased APD₅₀ and APD₉₀, compared to time-matched vehicle control recordings ($p < 0.05$ and $p < 0.01$, respectively). Therefore, at a concentration approximately 200-times the human efficacious plasma concentration, CP-526,555-18 significantly delayed cardiac repolarization.

- CP-526,555-18 (n=5) had no significant effect, at any concentration tested, on resting membrane potential, action potential amplitude or V_{max} when compared to time matched vehicle (n=5) control recordings.

Study Title: Cardiovascular Function in Conscious Monkeys

Study Number: 0705/GP

Adult *M. fascicularis* primates (3.3 - 6.8 kg) were anesthetized with isoflurane (2-2.5%) delivered in oxygen. Using sterile technique, a subcutaneous vascular access port was implanted in the descending thoracic aorta of each monkey via a carotid artery. At least one month after surgery, the primates were conditioned to sit quietly in a specially designed primate restraining chair. Arterial pressure and ECG signals were continuously recorded throughout the experiment. Six monkeys (3 males and 3 females) were orally gavaged with 0.1 mg/kg CP-526,555 (corrected for salt content) at a volume of 1 ml/kg or vehicle (deionized water) on separate days. All doses were flushed with 20 ml of water to assure complete delivery of the compound or vehicle. CP-526,555 and vehicle were presented to the same monkeys in random order with at least one week between administrations.

Oral administration of CP-526,555 at 0.1 mg/kg in conscious *M. fascicularis* primates did not significantly affect mean arterial pressure (MAP), heart rate (HR) or P-R interval when compared to vehicle control at plasma drug levels greater than the maximum projected efficacious plasma concentration. Baseline values prior to CP-526,555 administration were similar when compared to vehicle control for mean arterial pressure (108 ± 4 mmHg vs. 108 ± 4 mmHg, respectively), heart rate (182 ± 7 bpm vs 191 ± 8 bpm, respectively) and P-R interval (90 ± 6 ms vs. 92 ± 4 ms, respectively). CP-526,555 had no effects on MAP, HR or PR interval at any time point compared to corresponding vehicle administration in the same 6 primates. However, in one monkey, administration of CP-526,555 caused a 31% decrease in heart rate by 90 minutes that did not recover completely by 360 minutes post-dose. In addition, this primate had a slight increase in RR interval at +45, +60, and +90 minutes post-dose. These effects were not observed in any of the other primates studied. No signs of emesis were noted in any of the primates. Plasma concentrations of CP-526,555 were similar between primates with a mean maximal concentration (C_{max}) of 31 ± 5 ng/mL. The individual T_{max} values, however, were variable with a mean T_{max} of 3.2 ± 0.6 hours.

Pulmonary effects:

Study Title: Cardiopulmonary Function in Rats

Study Number: 0705/GP

CP-526,555 was tested for effects on heart rate, mean blood pressure, arterial blood gases and pH at doses of 0.3 and 3.0 mg/kg, p.o., in conscious rats. CP-526,555 produced no significant effect ($p > 0.05$) on heart rate, blood pressure, arterial PO_2 or pH at either dose. There was a significant ($p < 0.05$) effect on PCO_2 attributable to the small variance of the data. The magnitude of these PCO_2 changes were small, less than a 2 mmHg difference

between the vehicle and CP-526,555-treated groups. Therefore, the small change in PCO₂ is probably not an effect of CP-526,555.

Effect of oral CP-526,555 on blood gases, blood pH, arterial pressure, and heart rate in conscious rats

Dose (mg/kg)	Minutes after administration of CP-526,555						
	Baseline	20	40	60	80	100	120
Arterial PO ₂ (mm Hg)							
Vehicle	85±1.5	86±2.0	86±2.6	87±1.8	90±1.3	89±1.7	90±2.1
0.3	88±1.0	89±1.5	86±2.6	88±1.9	90±1.5	89±2.5	88±2.5
3.0	88±1.4	90±4.4	95±3.0	92±2.8	92±2.2	92±1.8	90±2.5
Arterial PCO ₂ (mm Hg)							
Vehicle	37±0.5	38±1.0	38±1.0	39±1.0	39±1.2	38±0.5	38±0.6
0.3*	38±0.4	37±0.4	38±0.4	38±0.3	38±0.6	38±0.5	38±0.5
3.0*	37±0.4	38±0.9	38±0.4	39±0.5	39±0.5	38±0.6	39±0.6
Arterial pH							
Vehicle	7.45±0.01	7.50±0.01	7.50±0.01	7.50±0.01	7.49±0.01	7.49±0.01	7.49±0.01
0.3	7.51±0.00	7.53±0.01	7.50±0.01	7.50±0.01	7.50±0.00	7.51±0.01	7.51±0.00
3.0	7.51±0.00	7.51±0.01	7.51±0.01	7.50±0.01	7.50±0.01	7.50±0.01	7.50±0.01
Mean Arterial Pressure (mm Hg)							
Vehicle	106±1.6	109±2.9	112±2.8	110±3.5	113±2.0	114±2.0	115±1.9
0.3	105±2.9	116±6.5	117±6.9	115±7.4	113±6.1	114±5.9	111±5.7
3.0	105±1.0	113±3.4	114±2.4	114±2.0	114±1.8	112±1.6	103±2.5
Heart Rate (beats/min)							
Vehicle	392±8.4	359±15	380±8.4	384±7.9	389±7.7	396±7.9	403±12
0.3	387±9.9	374±14	374±14	366±13	379±12	395±13	393±13
3.0	382±7.3	340±11	354±7.5	358±7.3	367±7.9	373±7.5	405±5.7

* p<0.05

Renal Function:

Eighteen-hour fasted male CD Sprague-Dawley rats (200-250 g) were orally dosed with either vehicle (0.5% methylcellulose) or CP-526,555 at 0.3, 3 or 30 mg/kg in a dose volume of 10 mL/kg. After dosing, rats were placed in metabolism cages and urine was collected for 5 hours. Urine volumes were recorded and samples frozen for subsequent analysis. CP-526,555 produced no effects on electrolyte excretion or on urine volume at oral doses of 0.3 and 3 mg/kg. The 30 mg/kg dose had no effect on the excretion of potassium, total osmoles, or urine volume, but significantly increased (p<0.05) sodium and chloride excretion by 138% and 173%, respectively, compared to the vehicle treated group.

In cardiovascular studies in anesthetized dogs, increased excretion of sodium and chloride was also observed.

Gastrointestinal Transit in Rats:

Male CD Sprague-Dawley rats (180-240 g) were fitted with tail cups to prevent coprophagy and fasted overnight. The next day the rats were orally administered either

vehicle (0.5% methylcellulose, 5 mL/kg) or CP-526,555 at doses of 0.3, 3, or 30 mg/kg. For comparison, in a separate parallel study, nicotine was given orally at the same doses. Sixty minutes later, the rats were given 1.0 mL PO of an evaporated milk solution containing 20,000 cpm of ^{51}Cr as sodium chromate. The rats were killed 20 minutes after administration of the radioactive marker. The gastroesophageal, pyloric, and ileocecal junctions were ligated, the stomach was removed and the small intestine was divided into ten equal lengths. The stomach and each length of intestine were assayed for radioactivity with a gamma counter. Gastric emptying was determined for each rat by comparing the amount of radioactivity in the intestine relative to the total in the intestine plus stomach. In addition, the geometric center of the distribution of the radioactive marker was used to measure the overall transit rate through the stomach and intestine. A 0.3 mg/kg dose of CP-526,555 had no effect on gastrointestinal transit. In rats orally dosed with 3 or 30 mg/kg, gastric emptying was reduced by 34% and 84%, and the mean geometric center was reduced by 53% and 90%, respectively, compared to the vehicle treated group. The effects of orally administered nicotine on gastrointestinal transit were examined in a comparative study. As with CP-526,555, nicotine had no effect at 0.3 mg/kg, but at doses of 3 or 30 mg/kg, inhibited gastric emptying by 69% and 95%, and reduced geometric centers by 81% and 98%, respectively. The reduction in the geometric center, which indicates a reduction in overall transport through the upper gastrointestinal tract, may be due entirely to the inhibitory effect of CP-526,555 on gastric emptying.

Abuse liability: See primary pharmacodynamic studies summarized above.

Other:

Study Title: Effects of CP-526,555P in the Ferret Emesis Model

Study Number: CP-526,555/GP/0705/FE

Both retching and emetic responses in ferrets involve peripheral mechanisms in the stomach and small intestine, the vagus nerve, and central pathways in the dorsal vagus nuclei. The neurotransmitters involved in the vomiting reflex in ferrets include serotonin (via 5-HT₃ receptors), acetylcholine (via muscarinic and possibly nicotinic receptors).

Key Study Findings:

- Oral administration of CP-526,555 to fasted ferrets produced dose-related retching and emesis. Retching and emesis were fast in onset (within 3.7 to 10 min postdose), had a short duration (approximately 20 min) and occurred at low doses (threshold dose of 0.025 mg/kg) and low plasma concentrations. Oral doses of 0.05, 0.1 and 0.3 mg/kg induced retching and emesis in 100% of fasted ferrets at plasma concentrations <10 ng/mL.
- The presence of food in the stomach reduced and delayed the occurrence of retching and emesis produced by oral administration of CP-526,555 at doses up to 0.1 mg/kg. A high dose of 0.3 mg/kg produced retching and emesis in 100% of

fed ferrets. The corresponding plasma concentrations were 108 ng/mL of CP-526,555.

- Subcutaneous administration of CP-526,555 at doses up to 0.3 mg/kg produced retching but no emesis, while maximum plasma concentrations were 77 ng/mL. A higher dose of 0.5 mg/kg, SC, produced retching and other nicotinic behaviors, but no emesis. Dilution of the effective CP-526,555 emetic dose (0.05 mg/kg) by 5-fold, reduced the incidence of retching and emesis given either orally or intraduodenally.
- Maximum plasma concentrations of CP-526,555 were similar after 0.05 mg/kg doses in 5 mL and 25 mL/kg dose volumes. Retching and emesis produced by oral CP-526,555 was effectively blocked by the Substance P antagonist, CP-99,994 or the 5-HT₃ antagonist, ondasetron and partially blocked (60%) by mecamlamine.

2.6.2.5 Pharmacodynamic drug interactions:

No nonclinical studies were submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

No pharmacology summary tables were provided by the Sponsor.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Absorption:

The CP-526,555 was absorbed fast (1 hr) after single dose oral administration in the rat and mouse. In primates, however, the T_{max} of the compound was between 3-5 hrs. The half-life in monkeys (24 h) was longer than for humans (16 h) under similar conditions. The total radioactivity excreted in urine after an oral dose of [¹⁴C]-CP-526,555 in mouse, rat, monkey, and human were 89, 75, 92, and 99%, respectively, indicating high absorption of the compound. In the Caco 2 cell permeation assay, the CP-526,555 permeation value was approximately 2/10⁶ cm/sec without affecting the intestinal transport protein supporting a high oral absorption profile. Clearance of total drug-related material in all species was low (36, 11, 3.6, and 1.9 in mouse, rat, monkey, and human respectively).

Absorption after Single Dose Administration of CP-526,555

PK Parameters	Mouse (3 mg/kg)	Rat (3 mg/kg)	Monkey (0.08 mg/kg)	Human (0.014 mg/kg)
C _{max} (ng/mL)	282/304	221/248	19.3	4.01
T _{max} (hr)	1.0/1.0	1.5/2.2	3	4.3
AUC _{0-∞} (ng·h/mL)	859/1070	-	232	93.9
CL/F (mL/min/kg)	58/47	25/18	5.7	2.5

kel (h ⁻¹)	0.463/0.501	-	0.031	0.043
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Distribution:

The percent of CP-526,555 binding to plasma proteins was 18, 45, 19, 41, and 20 in mouse, rat, dog, monkey, and human, respectively, indicating low plasma/serum distribution.

The tissue distribution was studied by whole body autoradiography after oral administration of [¹⁴C]CP-526,555 to Long Evans rats. The highest levels of the radioactive compound were located in the contents of the gastrointestinal tract (GIT) at 1 and 3 hours post dose for both males and females. This reflected the biliary elimination of CP-526,555 related compounds, since nearly most of the compound is absorbed in tissues. By 168 hours post dose, concentrations of radioactivity in GIT declined below detectable in both males and females. Exposure (based on tissue AUC) to CP-526,555 radioactivity was, however, found to be very high in the ciliary body. The Sponsor noted that the choroid, iris, and uvea had the next greatest exposures to CP-526,555 radioactivity; skin and vibrissal follicles, meninges, and tissues had exposures that were less than those noted for the melanin-rich components of the eye, but were greater than those exposures noted for all other tissues. Brain tissues that reside within the blood-brain barrier, except for the meninges, had exposures that were at least 3.4-fold greater than myocardial blood exposure. The pituitary, which lies outside of the blood-brain barrier, had an exposure that was at least 10-fold greater than myocardial blood. Blood and adipose tissues had the least amount of exposure to CP-526,555 radioactivity. All other tissues had exposures that were greater than the myocardial blood (212 nCi-hr/g) but less than nasal tissues (13,145 nCi-hr/g). The sponsor reported that the apparent t1/2 for the female and male melanin-rich components of the eye could not be determined because a definitive elimination phase was not noticeable. However, a fall in CP-526,555 radioactivity occurred between 18 and 168 hpd in these ocular tissues. The Sponsor's data showed that the apparent t1/2 for the meninges was 68 hr for the female rat and 52 hr for the male rat; all other brain tissues had a t1/2 that ranged from 3.8 to 7.6 hr; nasal tissues had the second longest measurable elimination half-life of 49 hr in the female rat and 45 hr in the male rat. All other female tissues where a measurable elimination half-life was determined had an apparent t1/2 less than 12 hr. For the male rat all other tissues where a measurable elimination half-life was determined had an apparent t1/2 less than 8.6 hr.

The tissue distribution of CP-526,555, a partial agonist for nicotine, is in conjunction with the pharmacology of the nicotine; both compounds are deposited in the melanin containing tissue, skin and eye. Therefore, the Sponsor postulates the pharmacological effect of the compound might be expected to differ based on human demographics which has clinical implications.

Summary of Tissue Distribution:

Tissue	Tissue Concentration (nCi/gm)
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	1 h	3 h	6 h	18 h	168 h
Male					
Nasal Tissues	150 ± 30	266 ± 67	266 ± 60	110 ± 37	18 ± 4
Skin	85 ± 8	52 ± 4	48 ± 8	23 ± 10	8 ± 3
Skin Follicles	442 ± 41	611 ± 246	1059 ± 333	1289 ± 536	596 ± 273
Meningis	638 ± 69	704 ± 141	937 ± 230	307 ± 155	70 ± 6
Pituitary	399 ± 95	456 ± 142	233 ± 43	36 ± 5	< LLOQ
Choroid	1349 ± 44	3296 ± 340	5838 ± 1343	7733 ± 1249	3959 ± 1438
Ciliary Body	3017 ± 600	7020 ± 1506	11667 ± 1770	16244 ± 3642	9682 ± 1905
Iris	1148 ± 115	2336 ± 520	3673 ± 732	12708 ± 3490	4814 ± 937
Uvea	1551 ± 174	3143 ± 499	5627 ± 879	10335 ± 1880	5102 ± 1495
Gastric Contents	7106 ± 2629	10857 ± 1232	8039 ± 211	59 ± 9	< LLOQ
Intestine Contents	6234 ± 2560	7059 ± 1277	2551 ± 844	483 ± 140	< LLOQ
Female					
Nasal Tissues	84 ± 2	334 ± 56	288 ± 33	123 ± 13	26 ± 8
Skin	53 ± 7	50 ± 5	40 ± 5	9 ± 3	< LLOQ
Skin Follicles	396 ± 79	1279 ± 138	652 ± 257	668 ± 265	338 ± 246
Meningis	298 ± 51	634 ± 218	363 ± 173	315 ± 182	69 ± 26
Pituitary	299 ± 52	236 ± 68	140 ± 28	24 ± 11	< LLOQ
Choroid	1682 ± 225	5470 ± 872	4290 ± 686	5085 ± 625	2663 ± 481
Ciliary Body	3148 ± 790	10632 ± 962	15580 ± 2706	8058 ± 1706	7485 ± 1766
Iris	1315 ± 129	3779 ± 960	5231 ± 459	5324 ± 1737	2976 ± 1042
Uvea	1824 ± 176	5490 ± 884	4587 ± 1746	4113 ± 1120	3118 ± 677
Gastric Contents	14732 ± 2801	4345 ± 3459	2713 ± 871	36 ± 3	< LLOQ
Intestine Contents	2920 ± 972	6398 ± 2167	3699 ± 874	236 ± 30	< LLOQ

Metabolism:

CP-526,555 was found to be metabolized extensively and the metabolites were found in both the circulation and in excreta in the non-clinical species. Thirteen metabolites were identified in the non-clinical species, however, none of the metabolites were considered to be major since their percentages in the systemic circulation was found to be less <10% as shown in the table below (except in rabbit). In excreta, there was no single metabolite comprising more than 5% of dose in any species. No oxidative metabolism of the CP-526,555 was detected in the rat, monkey, and human liver microsomes suggesting that it was not a good substrate for cytochrome P450 enzymes. Oxidative metabolites include ring-opened amino acids (M1 and M2), dihydroxy (M2a), putative lactam and carbonyl-containing metabolites (M3c and M3d), and 2- hydroxyvarenicline (M3b; CP-708,075). Conjugative metabolites include varenicline N-carbamoylglucuronide (M4), varenicline N-hydroxyglucuronide (M4a), N-formyl varenicline (M5), and N-glucosylvarenicline (M7). Three metabolites remain unidentified (designated as M3, M3a, and M6), but all were minor and were not detected in humans. The N-carbamoylglucuronide pathway could be observed in liver microsomes supported with UDPGA and conducted under a CO₂ atmosphere.

Table 4. Summary of Metabolites of Varenicline in Preclinical Species and Human

Metabolite/Identity	% of Dose Excreted				% Circulating Radioactivity*				
	Mouse†	Rat†	Monkey	Human	Mouse†	Rat†	Monkey	Rabbit	Human
Varenicline	93/92	88/84	75	81	85/85	80/82	80	17	91
M1 trans-carboxylic acid metabolite	0.43/0.62	0.95/0.82	1.1		1.5/1.4			4.2	
M2 cis-carboxylic acid metabolite	0.87/1.12		4.6		3.6/4.0	1.4/NQ‡	1.7	35	
M2a dihydroxy metabolite		1.01/0.47							
M3 glucuronidated cleavage product m/z 333		1.5/0.4							
M3a unknown	0.27/0.1		1.1		1.3/0.9		0.53		
M3b hydroxyquinoraline metabolite (CP-708,075)		0.59/0.43§		2.9					
M3c putative carbonyl metabolite (M+14 mass units)							0.93	6.7	1.1
M3d putative carbonyl metabolite (M+14 mass units)					7.0/6.6		2.1	6.4	
M4 N-carbamoyl glucuronide		2.4/1.6	3.6	3.6		13/7.9	8.7	26	3.8
M4a N-hydroxy glucuronide								5.6	
M5 N-formyl conjugate (CP-697,535)					2.4/2.6	4.9/5.7	2.6		0.9
M6 unknown						NQ‡/3.3			
M7 putative hexose conjugate						0.80/1.6	2.9		3.5

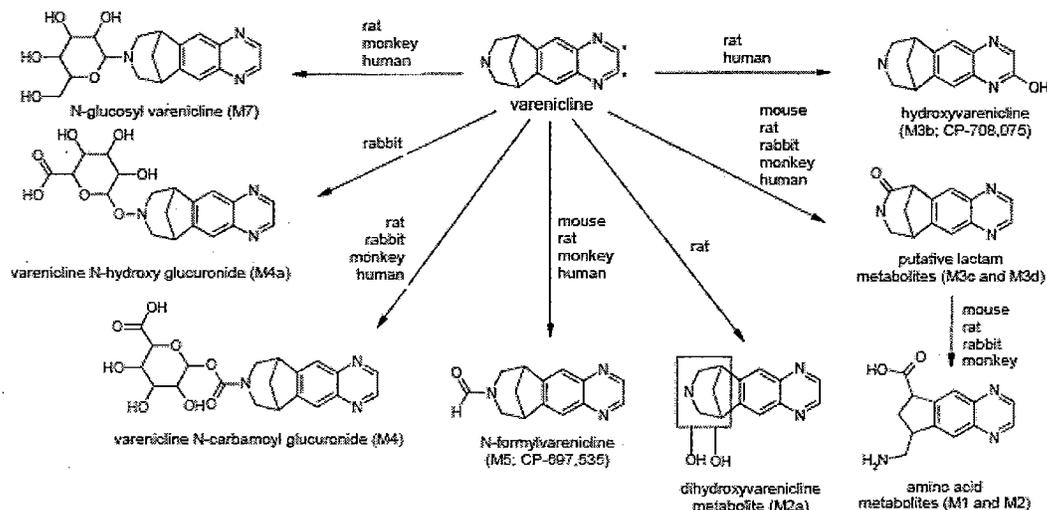
*Based on AUC (µg eq/h/mL)

†male/female

‡NQ = not quantitated, either not detectable or not able to be quantified due to low abundance

§Determined in a separate pharmacokinetic study

Figure 1. Structures of Metabolites of Varenicline.



Elimination:

The elimination profile of the CP-526,555 and its metabolites are shown in the following tables. In general, the major route of excretion in males and females from different species was through urine. In humans and probably other species, OCT2, a human organic cation transporter was involved in the urinary secretion of CP-526,555. Up to 87% of the dose of CP-526,555 as well as its metabolites were cleared between 0-168 h. In humans, a small amount of CP-526,555 was also found in feces (0.9%). The plausible route of clearance for the rest CP-526,555 was not determined or rationalized. Due to the distribution of the compound in the skin, evaporation might be suggested as another route

of excretion. However, the accumulation of the compound in the target tissues might prevent its elimination.

Summary of Elimination Profile in Different Species

Species: Human (Healthy Volunteers)	Urine	Feces	Bile	Serum
Total Time Period of Sample Collection	0-168 hr	0-96 hr		0-24 hr
Sample Analyzed for Metabolites	0-72 hr	ND ^a	NC ^b	0-24 hr
Compound (as % of Dose or Sample)	% of Dose	% of Dose		% of Sample
% of Total Dose Excreted (0-168 hr)	87.1 ± 5.5	0.9 ± 0.5		N/A ^c
CP-526,555	80.5 ± 4.8			90.8 ± 1.6
M3b (Hydroxyvarenicline; CP-708,075)	2.9 ± 0.9			-
M3c (Lactam-Putative)	-			1.1 ± 0.6
M4 (N-Carbamoyl Glucuronide)	3.6 ± 0.9			3.8 ± 0.9
M5 (N-Formylvarenicline; CP-697,535)	-			0.9 ± 0.5
M7 (N-glucosylvarenicline)	-			3.5 ± 0.4
Species: Monkey (Cynomolgus)	Urine	Feces ^a	Bile	Plasma
Total Time Period of Sample Collection	0-240 hr	0-240 hr	0-48 hr	0-72 hr
Sample Analyzed for Metabolites	0-24 hr	0-24 hr	0-48 hr	0-24 hr
Compound (as % of Dose or Sample)	% of Dose	% of Dose	% of Sample	% of Sample
% of Total Dose Excreted (0-240 hr)	74.1 ± 2.3	6.52 ± 2.54	ND ^b	N/A ^c
CP-526,555	68.5 ± 9.0	6.3	-	80.4 ± 3.2
M1 (Amino Acid)	1.1 ± 0.6	-	-	-
M2 (Amino Acid)	4.3 ± 1.4	0.3	-	1.7 ± 0.2
M3a	1.1 ± 0.5	-	-	0.5 ± 0.4
M3c (putative lactam)	-	-	-	0.9 ± 0.3
M3d (putative carbonyl metabolite)	-	-	-	2.1 ± 0.8
M4 (N-Carbamoyl Glucuronide)	3.6 ± 1.2	-	100	8.7 ± 2.5
M5 (N-Formylvarenicline; CP-697,535)	-	-	-	2.6 ± 0.3
M7 (N-glucosylvarenicline)	-	-	-	2.9 ± 0.8
Species: Mouse (CD-1)	Urine	Feces		Plasma
Total Time Period of Collection	0-192 hr	0-192 hr		0-12 hr
Sample Analyzed for Metabolites	0-24 hr	0-72 hr		0-12 hr
Compound (as % of Dose or Sample)	% of Dose	% of Dose		% of Sample
Male				
% of Total Dose Excreted (0-192 hr)	86.4 ± 8.8	8.15 ± 0.89		N/A ^a
CP-526,555	85.1 ± 8.6	7.9 ± 1.0		84.5
M1 (Amino Acid)	0.4 ± 0.1	-		1.3
M2 (Amino Acid)	0.9 ± 0.2	-		3.6
M3a (Unknown)	-	0.3 ± 0.2		1.3
M3d (Putative Carbonyl)	-	-		7.0
M5 (N-Formylvarenicline; CP-697,535)	-	-		2.4
Female				
% of Total Dose Excreted (0-192 hr)	79.9 ± 7.6	13.5 ± 6.5		N/A ^a
CP-526,555	78.1 ± 7.5	13.4 ± 6.7		84.5
M1 (Amino Acid)	0.6 ± 0.1	-		1.4
M2 (Amino Acid)	1.1 ± 0.1	-		4.0
M3a (Unknown)	-	0.1 ± 0.2		0.9
M3d (Putative Carbonyl)	-	-		6.6
M5 (N-Formylvarenicline; CP-697,535)	-	-		2.6

Species: Rat (Sprague-Dawley)	Urine	Feces	Bile
Total Time Period of Sample Collection	0-168 hr	0-168 hr	0-24 hr
Sample Analyzed for Metabolites	0-24 hr	0-24 hr	0-24 hr
Compound (as % of Dose or Sample)	% of Dose	% of Dose	% of Sample
Male			
% of Total Dose Excreted (0-168 hr)	69.9 ± 5.8	24.3 ± 5.7	NC ^a
CP-526,555	62.0 ± 5.1	24.3 ± 5.7	-
M1 (Amino Acid)	1.0 ± 0.2	-	-
M2a (Dihydroxy)	1.0 ± 0.3	-	-
M3 (Unknown)	1.5 ± 0.5	-	-
M4 (N-Carbamoyl Glucuronide)	2.3 ± 0.6	-	100
Female			
% of Total Dose Excreted (0-168 hr)	66.2 ± 15.0	20.3 ± 7.5	NC ^a
CP-526,555	61.1 ± 15.1	20.3 ± 7.5	-
M1 (Amino Acid)	0.8 ± 0.2	-	-
M2a (Dihydroxy)	0.5 ± 0.1	-	-
M3 (Unknown)	0.4 ± 0.1	-	-
M4 (N-Carbamoyl Glucuronide)	1.5 ± 0.4	-	100

2.6.4.2 Methods of Analysis

The detection methods, levels of quantization, and the internal standards for the mass spectrophotometric analysis of the test article in different non-clinical species are depicted below (Sponsor’s table). The method of analysis of the toxicokinetics and pharmacokinetics appeared to be reproducible and was found to be consistent in the single dose and multiple dose application in different species with this compound.

Summary Table for Method of Analysis:

Species:	Mouse	Mouse	Rat	Rat	Rat
Analyte:	CP-526,555	CP-526,555	CP-526,555	CP-526,555	CP-708,075
Sample:	Plasma	Serum	Serum	Plasma	Urine
Sample Volume:	0.2 mL	0.05 mL	0.1 mL	0.1 mL	0.02 mL
Processing Method:	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
Detection Method:	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS
Internal Standard:	CP-533,633	CP-533,633	CP-533,633	CP-533,633	CP-533,633
Dynamic Range	1.0-50 ng/mL	0.5-200 ng/mL	1.0-100 ng/mL	500-25000 ng/mL	500-25000 ng/mL
Study Number:	DM2001-526555-042	DM2004-526555-054	DM2000-526555-026	DM2001-526555-043	DM2001-526555-041
Species:	Monkey	Monkey	Monkey	Human	Rat, Dog, Human
Analyte:	CP-526,555	CP-526,555	CP-526,555	CP-526,555	CP-526,555
Sample:	Plasma	Plasma, Blood, and Ultrafiltrate	Serum	Plasma/Serum	Plasma, Blood, and Ultrafiltrate
Sample Volume:	0.1 mL	0.2 mL	0.1 mL	1.0	0.2 mL
Processing Method:	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
Detection Method:	HPLC-MS/MS	HPLC-MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS
Internal Standard:	CP-533,633	CP-532,543	CP-533,633	CP-533,633	CP-532,543
Dynamic Range	0.5-100 ng/mL	1.0-750 ng/mL	1.0-1000 ng/mL	0.1-50 ng/mL	2.5-1250 ng/mL
Study Number:	DM2000-526555-030	DM1998-526555-009	DM2004-526555-056	DM1999-526555-023 DM2000-526555-025 DM2000-526555-031	DM1998-526555-006
Species:	Rat, Monkey	Human			
Analyte:	CP-526,555	Metabolite M4			
Sample:	Liver Microsomes	Liver Microsomes			
Sample Volume:	0.15 mL	0.2 mL			
Processing Method:	XXXXXXXXXX	XXXXXXXXXX			
Detection Method:	HPLC-MS	HPLC-MS/MS			
Internal Standard:	CP-532,543	none			
Dynamic Range	5.0-500 ng/mL	2.5 - 1000 ng/mL			
Study Number:	DM1998-526555-008	DM2005-526555-076			

2.6.4.3 Absorption

Study Title: In Vitro Assessment of Human Intestinal Permeation of CP-526,555 Using Caco-2 Cell Monolayers

Study Number: DM2003-526555-053

Caco-2 cell monolayers, an *in vitro* tissue culture model of human intestinal epithelium, were used to determine the permeation (and mechanisms that determine the permeation) of CP-526,555 across intestinal epithelium.

Key Study Findings:

- CP-526,555 permeation across Caco-2 cell monolayers was high, did not exhibit concentration-dependency (over a concentration range of 5 to 50 μ M), and was not affected by efflux transport mechanisms. Increases in the luminal (apical) pH increased CP-526,555 rate of permeation; presumably mediated by changes in the ionization state of CP-526,555.

Study Title: Oral Pharmacokinetics of CP-526,555 in Organic Cation Transporter 2 (OCT2) Knockout and RT1490 Wild type Mice

Study Number: DM2004-526555-055

To determine whether CP-526,555 may be a substrate of the human organic cation transporter 2 (hOCT2), an uptake transporter found in the kidney, the pharmacokinetics of CP-526,555 in OCT2 knockout and RT1490 wild type (WT) mice were analyzed. Serum concentrations of CP-526,555 were measured at 0.25, 1, 4, 8, 12 and 24 hours following a 3 mg/kg oral (PO) dose of CP-526,555-24.(succinate salt)

Key Study Findings:

- The pharmacokinetics of CP-526,555 was similar between males and females in both the OCT2 KO and RT1490 WT mice. In KO mice, the estimated values for $t_{1/2}$, T_{max} , C_{max} , and AUC_{0-24h} were 2.76 h, 0.25 h, 529 ng/mL, and 1480 h•ng/mL, respectively. The corresponding PK values in the RT1490 WT mice were 2.39 h, 0.25 h, 490 ng/mL, and 1430 h•ng/mL, respectively. These were no apparent differences in the oral PK of CP-526,555 between genotypes, suggesting that CP-526,555 is not a substrate of the OCT2 transporter in mice.

2.6.4.4 Distribution

Study Title: Assessment of Blood Cell Partitioning and Plasma Protein Binding of CP-526,555 in Rat, Dog and Human Blood

Study Number: DM1998-526555-006

The blood/plasma (B/P) ratio of CP-526,555 in rat, dog and human blood was determined at concentration of 100 and 400 ng/mL. The B/P ratio of CP-526,555 at 100 ng/mL in rat, dog, and human blood was 0.55, 1.2 and 1.0, respectively.

Key Study Findings:

- The percent of drug bound to plasma proteins was 45%, 19% and 20% in rat, dog and human plasma, respectively. Thus, the fraction of drug unbound in rat, dog and human plasma was 0.55, 0.81 and 0.80, respectively.

Study Title: Plasma Protein Binding and Blood Cell Partitioning of CP-526,555 in Plasma and Whole Blood from Cynomolgous Monkeys

Study Number: DM1998-526555-009

Blood cell partitioning of by incubation of whole blood with either 100 and 400 ng/mL concentrations of CP-526,555. Binding of CP-526,555 to monkey plasma proteins was determined at a drug concentration of 100 ng/mL using the ultrafiltration technique.

Key Study Findings:

- The unbound fraction of CP-526,555 in monkey plasma was 0.59 ± 0.18 (mean \pm SD).
- The blood to plasma ratio was 1.42 ± 0.36 and 0.96 ± 0.14 at 100 and 400 ng/mL, respectively. Considering the variability in the blood to plasma ratio, CP-526,555 is roughly equally distributed in whole blood and plasma in monkeys.

Study Title: Serum Protein Binding For CP-526,555 in the Mouse

Study Number: DM2002-526555-050

The objective of this study was to determine the unbound fraction of CP-526,555 in mouse serum.

Key Study Findings:

- The unbound fraction of CP-526,555 in mouse serum was 0.82 ± 0.04 , similar to previously determined values in human and monkey, but higher than in rat.

Study Title: Tissue Distribution in Long-Evans Rats of a Neuronal Nicotinic Partial Agonist (CP-526,555) for Treatment of Nicotine Addiction

Study Number: DM2000-526555-012

CP-526,555 was orally administered to Long Evans rats at 3.4 mg/kg and 328 μ Ci/kg. One rat of each gender was euthanatized at 1, 3, 6, 18, and 168 hr post dose of [14 C]CP-526,555. All rats were prepared for whole-body autoradioluminography immediately

following euthanasia. The tables below show the tissue distribution of varenicline in males and females and the pharmacokinetics of the distribution. The autoradiograms below show the clearance of the compound from the different tissues in males and females.

Key Study Findings:

- The highest levels of [¹⁴C]CP-526,555 were located in the contents of the gastro intestinal tract (GIT) at 1 and 3 hours postdose (hpd) for both male and female rats. Since nearly most of the drug is absorbed, the majority of the radioactivity present in the GIT contents reflected biliary elimination. By 168 hpd, no radioactivity was seen in GIT. The presence of radioactivity in the renal pelvis and bladder indicated that CP-526,555-related material was also eliminated through the urine.
- The melanin-rich components of the eye contained the highest concentrations of CP-526,555-associated radioactivity at all sampling time points. The uvea appeared to have disproportionate distribution of [¹⁴C]CP-526,555 into its three substructures (e.g. choroid, ciliary body, and iris). The radioactivity present in the ciliary body was 2-fold higher than the radioactivity present in the choroid and iris at 1, 3, 6, and 168 hpd.
- Measurable concentrations of [¹⁴C]CP-526,555 were detected in hepatic, myocardial, and systemic blood for at least 6 hpd. Blood concentrations were similar at 1, 3, and 6 hpd regardless of the sampling region and gender. By 18 hpd (and at 168 hpd), blood concentrations declined below 3.6 nCi/g.
- Brain tissue radioactivity was highest at 1 hpd, and then concentrations decreased for all regions of the brain except for the meninges and pituitary. Maximum concentrations of [¹⁴C]CP-526,555 for the meninges and pituitary occurred between 3 and 6 hpd. At 168 hpd, the meninges had concentrations of [¹⁴C]CP-526,555 that were 19-fold higher than the assay LLOQ.
- The cerebral to myocardial blood ratio averaged 2.7 ± 0.2 over the time course of 1 to 6 hpd. The meninges and pituitary to myocardial blood ratios averaged 7 ± 2 and 15 ± 7 , respectively, over this same time course. Data obtained from other pharmacokinetic studies in the rat for CP-526,555 concentrations demonstrated a brain to plasma ratio of 4.6 at 2 hr after oral administration.
- Skin and vibrissal follicles, meninges, and nasal tissues had exposures that were less than those noted for the melanin-rich components of the eye but were greater than those exposures noted for all other tissues. Brain tissues that reside within the blood-brain barrier, except for the meninges, had exposures that were at least 3.4-fold greater than myocardial blood exposure. The pituitary, which lies outside of the blood-brain barrier had an exposure that was at least 10-fold greater than myocardial blood. Blood and adipose tissues had the least amount of exposure to CP-526,555 radioactivity. All other tissues had exposures that were greater than the myocardial blood (212 nCi-hr/g) but less than nasal tissues (13,145 nCi-hr/g). The apparent $t_{1/2}$ for the female and male melanin-rich components of the eye could not be determined because a definitive elimination phase was not evident although declines in CP-526,555 radioactivity were observed between 18 and 168

hpd in these ocular tissues. The apparent $t_{1/2}$ for the meninges was 68 hr for the female rat and 52 hr for the male rat. All other brain tissues had a $t_{1/2}$ that ranged from 3.8 to 7.6 hr. Nasal tissues had the second greatest measurable elimination half-life of 49 hr in the female rat and 45 hr in the male rat. All other female tissues where a measurable elimination half-life was determined had an apparent $t_{1/2}$ less than 12 hr. For the male rat all other tissues where a measurable elimination half-life was determined had an apparent $t_{1/2}$ less than 8.6 hr.

Table 3. Tissue Concentrations^a of Radioactivity (nCi/g) From Female Long-Evans Rats Administered An Oral Dose (3.4 mg/kg) of [¹⁴C]CP-526,555.

TISSUE	1 Hr	3 Hr	6 Hr	18 Hr	168 Hr
Liver	261 ± 23	193 ± 16	141 ± 16	16 ± 2	<LLOQ ^b
Lung	82 ± 8	60 ± 5	48 ± 4	7 ± 1	<LLOQ
Lymph Node	187 ± 20	146 ± 19	129 ± 19	16 ± 2	<LLOQ
Muscle	79 ± 7	74 ± 10	51 ± 7	6 ± 1	<LLOQ
Myocardium (Heart)	91 ± 9	65 ± 5	53 ± 11	7 ± 1	<LLOQ
Nasal Tissues	84 ± 2	334 ± 56	288 ± 33	123 ± 13	26 ± 8
Skin	53 ± 7	50 ± 5	40 ± 5	9 ± 3	<LLOQ
Skin Follicles	396 ± 79	1279 ± 138	652 ± 257	668 ± 265	338 ± 246
Spleen	339 ± 27	286 ± 27	197 ± 18	16 ± 3	<LLOQ
Vibrissal Follicles	1135 ± 322	3621 ± 1866	2650 ± 1138	1159 ± 576	ND ^c
Whole-Body	534 ± 309	329 ± 88	207 ± 20	58 ± 19	<LLOQ
ADIPOSE					
Multifolcular	64 ± 5	50 ± 7	29 ± 3	<LLOQ	<LLOQ
Subcutaneous	17 ± 3	21 ± 3	12 ± 2	<LLOQ	<LLOQ
BLOOD					
Hepatic	58 ± 7	43 ± 8	30 ± 6	<LLOQ	<LLOQ
Myocardial	48 ± 6	38 ± 4	30 ± 4	<LLOQ	<LLOQ
Systemic	41 ± 4	41 ± 6	31 ± 4	<LLOQ	<LLOQ
BONE MARROW					
Femur	190 ± 9	228 ± 18	110 ± 16	11 ± 2	<LLOQ
Humerus	236 ± 16	130 ± 17	142 ± 7	16 ± 3	<LLOQ
Pelvis	203 ± 26	183 ± 12	155 ± 7	8 ± 2	<LLOQ
Tibia	182 ± 46	315 ± 6	100 ± 8	10 ± 2	<LLOQ
Vertebra	187 ± 30	142 ± 22	119 ± 15	12 ± 2	<LLOQ
BRAIN					
Cerebellum	102 ± 10	74 ± 11	60 ± 8	8 ± 1	<LLOQ
Cerebrum	127 ± 13	112 ± 25	85 ± 9	8 ± 2	<LLOQ
Mechilla Oblongata	86 ± 6	66 ± 7	53 ± 4	9 ± 1	<LLOQ
Meningis	298 ± 51	634 ± 218	363 ± 173	315 ± 182	69 ± 26
Mesencephalon	100 ± 5	64 ± 5	63 ± 4	10 ± 2	<LLOQ
Olfactory Bulb	102 ± 9	87 ± 5	66 ± 7	8 ± 2	<LLOQ
Pineal Gland	165 ± 23	139 ± 33	105 ± 6	ND	<LLOQ
Pituitary	299 ± 52	236 ± 68	140 ± 28	24 ± 11	<LLOQ
Spinal Cord	103 ± 15	58 ± 6	52 ± 4	7 ± 2	<LLOQ
Thalamus	137 ± 11	102 ± 20	86 ± 8	10 ± 1	<LLOQ
GLANDS					
Adrenal	185 ± 17	196 ± 14	100 ± 7	15 ± 3	<LLOQ
Buccal	326 ± 41	376 ± 59	244 ± 23	30 ± 2	<LLOQ
Chitoidaan	336 ± 13	453 ± 21	253 ± 14	103 ± 11	<LLOQ
Exorbital Lacrimal	359 ± 35	300 ± 205	393 ± 30	33 ± 2	<LLOQ
Harderian	289 ± 26	335 ± 27	359 ± 33	27 ± 10	<LLOQ
Intraorbital Lacrimal	252 ± 18	195 ± 13	157 ± 15	19 ± 4	<LLOQ
Pancreas	183 ± 12	134 ± 12	117 ± 13	10 ± 2	<LLOQ

Table 3 continued:

TISSUE	1 Hr	3 Hr	6 Hr	18 Hr	168 Hr
GLANDS continued:					
Parotid	176 ± 9	118 ± 11	98 ± 7	11 ± 1	<LLOQ
Salivary	371 ± 29	376 ± 39	384 ± 39	27 ± 2	<LLOQ
Thymus	172 ± 14	141 ± 12	108 ± 15	10 ±	<LLOQ
Thyroid	322 ± 2	207 ± 26	195 ± 8	20 ± 7	<LLOQ
OCULAR					
Choroid	1682 ± 225	5470 ± 872	4290 ± 686	5085 ± 625	2663 ± 481
Ciliary Body	3148 ± 780	10632 ± 962	15580 ± 2706	8058 ± 1706	7485 ± 1766
Iris	1315 ± 129	3779 ± 960	5231 ± 459	5324 ± 1737	2976 ± 1042
Lens	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Uvea	1824 ± 176	5490 ± 884	4587 ± 1746	4113 ± 1120	3118 ± 677
Vitreous	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
UROGENITAL					
Kidney	443 ± 57	279 ± 21	261 ± 88	21 ± 6	<LLOQ
Renal Cortex	338 ± 45	230 ± 14	130 ± 14	ND	<LLOQ
Renal Medulla	712 ± 94	401 ± 21	308 ± 44	ND	<LLOQ
Renal Pelvis	1032 ± 188	661 ± 163	907 ± 182	73 ± 11	<LLOQ
Ovary	210 ± 47	213 ± 11	90 ± 9	10 ± 1	<LLOQ
Uterus	153 ± 14	114 ± 12	52 ± 6	ND	<LLOQ
Urinary Bladder	803 ± 135	ND	ND	ND	ND
GASTROINTESTINAL TRACT					
Cecum	31 ± 6	369 ± 93	1244 ± 268	367 ± 131	<LLOQ
Colon	188 ± 6	196 ± 36	1041 ± 231	751 ± 72	<LLOQ
Gastric	14732 ± 2801	4345 ± 3459	2713 ± 871	36 ± 3	<LLOQ
Intestine	2920 ± 972	6398 ± 2167	3699 ± 874	236 ± 30	<LLOQ
Rectum	19 ± 2	432 ± 53	1551 ± 379	810 ± 72	<LLOQ
Cecum Mucosa	ND	180 ± 21	ND	ND	<LLOQ
Gastric Mucosa	258 ± 6	94 ± 10	111 ± 24	18 ± 3	<LLOQ
Intestinal Mucosa	140 ± 15	185 ± 8	40 ± 6	ND	<LLOQ
Rectal Mucosa	265 ± 33	361 ± 47	ND	ND	<LLOQ

^a Mean (±SD) radioactivity values (nCi/g) were calculated by averaging tissue concentrations measured at different sectioning levels and/or from replicate cryosections obtained from the same sectioning level.

^b The lower limit of quantitation (LLOQ) was 3.6 nCi/g.

^c The radioactivity concentration was not determined because the tissue radioactivity declined below the LLOQ, the tissue was indistinguishable from other tissues, or the tissue was not sampled.

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Table 4. Tissue Concentrations^A of Radioactivity (nCi/g) From Male Long-Evans Rats Administered An Oral Dose (3.4 mg/kg) of [¹⁴C]CP-526,555.

TISSUE	1 Hr	3 Hr	6 Hr	18 Hr	168 Hr
Liver	343 ± 29	316 ± 18	151 ± 14	24 ± 3	<LLOQ ^B
Lung	100 ± 6	65 ± 10	52 ± 5	12 ± 2	<LLOQ
Lymph Node	230 ± 32	140 ± 10	104 ± 13	21 ± 3	<LLOQ
Muscle	84 ± 13	60 ± 4	52 ± 6	12 ± 1	<LLOQ
Myocardium (Heart)	94 ± 9	54 ± 5	49 ± 4	12 ± 3	<LLOQ
Nasal Tissues	150 ± 30	266 ± 67	266 ± 60	110 ± 37	18 ± 4
Skin	85 ± 8	52 ± 4	48 ± 8	23 ± 10	8 ± 3
Skin Follicles	442 ± 41	611 ± 245	1059 ± 333	1289 ± 536	596 ± 273
Spleen	344 ± 27	219 ± 22	156 ± 8	27 ± 4	<LLOQ
Vibrissal Follicles	ND ^C	ND	548 ± 196	2366 ± 1137	ND
Whole-Body	385 ± 126	348 ± 72	229 ± 38	73 ± 11	<LLOQ
ADIPOSE					
Multilocular	55 ± 3	32 ± 3	35 ± 7	<LLOQ	<LLOQ
Subcutaneous	15 ± 2	12 ± 2	7 ± 1	<LLOQ	<LLOQ
BLOOD					
Hepatic	81 ± 17	49 ± 8	33 ± 6	<LLOQ	<LLOQ
Myocardial	60 ± 7	40 ± 4	34 ± 5	<LLOQ	<LLOQ
Systemic	52 ± 6	34 ± 2	32 ± 5	<LLOQ	<LLOQ
BONE MARROW					
Femur	254 ± 32	174 ± 15	108 ± 6	23 ± 2	<LLOQ
Humerus	240 ± 42	129 ± 3	118 ± 10	ND	<LLOQ
Pelvis	217 ± 17	151 ± 24	74 ± 6	ND	<LLOQ
Tibia	263 ± 32	91 ± 4	117 ± 7	ND	<LLOQ
Vertebra	228 ± 31	126 ± 12	110 ± 12	19 ± 3	<LLOQ
BRAIN					
Cerebellum	119 ± 9	74 ± 9	65 ± 5	12 ± 2	<LLOQ
Cerebrum	151 ± 17	103 ± 17	90 ± 9	18 ± 3	<LLOQ
Medulla Oblongata	95 ± 16	65 ± 7	62 ± 6	14 ± 2	<LLOQ
Meningis	638 ± 69	704 ± 141	937 ± 230	307 ± 155	70 ± 6
Midbrain	116 ± 10	75 ± 10	64 ± 7	13 ± 3	<LLOQ
Olfactory Bulb	130 ± 18	67 ± 6	75 ± 7	18 ± 2	<LLOQ
Pineal Gland	162 ± 22	120 ± 21	109 ± 22	22 ± 8	<LLOQ
Pituitary	399 ± 95	456 ± 142	233 ± 43	36 ± 5	<LLOQ
Spinal Cord	107 ± 15	61 ± 22	58 ± 12	14 ± 2	<LLOQ
Thalamus	144 ± 20	107 ± 18	90 ± 12	18 ± 5	<LLOQ
GLANDS					
Adrenal	253 ± 22	126 ± 16	124 ± 15	27 ± 8	<LLOQ
Buccal	370 ± 84	386 ± 55	355 ± 104	33 ± 6	<LLOQ
Exorbital Lacrimal	316 ± 11	255 ± 17	251 ± 9	32 ± 3	<LLOQ
Harderian	361 ± 19	202 ± 13	295 ± 74	ND	<LLOQ
Intraorbital Lacrimal	417 ± 35	223 ± 20	197 ± 24	30 ± 4	<LLOQ
Pancreas	213 ± 23	140 ± 10	102 ± 13	19 ± 3	<LLOQ
Parotid	139 ± 11	133 ± 10	93 ± 8	16 ± 1	<LLOQ
Preputial	475 ± 51	369 ± 19	315 ± 41	343 ± 117	<LLOQ
Salivary	465 ± 31	319 ± 28	284 ± 27	37 ± 3	<LLOQ
Thymus	214 ± 20	130 ± 13	109 ± 17	21 ± 5	<LLOQ
Thyroid	287 ± 17	164 ± 18	162 ± 16	26 ± 0	<LLOQ

Table 4 continued:

TISSUE	1 Hr	3 Hr	6 Hr	18 Hr	168 Hr
OCULAR					
Choroid	1349 ± 44	3296 ± 340	5838 ± 1343	7733 ± 1249	3959 ± 1438
Ciliary Body	3017 ± 600	7020 ± 1506	11667 ± 1770	18244 ± 3642	9682 ± 1905
Iris	1148 ± 115	2336 ± 520	3673 ± 732	12708 ± 3490	4814 ± 937
Lens	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Uvea	1551 ± 174	3143 ± 499	5627 ± 879	10335 ± 1880	5102 ± 1495
Vitreous	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
UROGENITAL					
Kidney	471 ± 71	270 ± 64	262 ± 114	46 ± 11	<LLOQ
Renal Cortex	403 ± 36	204 ± 15	184 ± 5	ND	<LLOQ
Renal Medulla	692 ± 47	379 ± 31	372 ± 12	ND	<LLOQ
Renal Pelvis	1313 ± 214	955 ± 86	2670 ± 221	116 ± 12	<LLOQ
Epididymis		179 ± 16	106 ± 14	16 ± 1	<LLOQ
Prostate	566 ± 29	499 ± 75	170 ± 105	ND	<LLOQ
Seminal Vesicle	312 ± 22	162 ± 22	128 ± 9	26 ± 1	<LLOQ
Testis	91 ± 9	106 ± 9	91 ± 11	22 ± 3	<LLOQ
Urinary Bladder	1915 ± 369	7183 ± 997	4175 ± 1064	395 ± 75	<LLOQ
GASTROINTESTINAL TRACT					
Cecum Contents	38 ± 11	52 ± 7	661 ± 72	499 ± 61	<LLOQ
Colon Contents	25 ± 3	94 ± 14	1164 ± 1091	563 ± 55	<LLOQ
Gastric Contents	7106 ± 2629	10857 ± 1252	8039 ± 211	59 ± 9	<LLOQ
Intestine Contents	6234 ± 2560	7059 ± 1277	2551 ± 844	483 ± 140	<LLOQ
Rectum Contents	29 ± 7	64 ± 8	420 ± 145	697 ± 87	<LLOQ
Colon Mucosa	117 ± 17	122 ± 17	175 ± 11	<LLOQ	<LLOQ
Gastric Mucosa	172 ± 24	118 ± 12	68 ± 13	<LLOQ	<LLOQ
Intestinal Mucosa	234 ± 65	ND	156 ± 12	<LLOQ	<LLOQ
Rectal Mucosa	157 ± 16	124 ± 18	ND	<LLOQ	<LLOQ

* Mean (±SD) radioactivity values (nCi/g) were calculated by averaging tissue concentrations measured at different sectioning levels and/or from replicate cryosections obtained from the same sectioning level.

^b The lower limit of quantitation (LLOQ) was 3.6 nCi/g.

^c The radioactivity concentration was not determined because the tissue radioactivity declined below the LLOQ, the tissue was indistinguishable from other tissues, or the tissue was not sampled.

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Table 5. Tissue Pharmacokinetics* of CP-526,555-Associated Radioactivity In Female and Male Long-Evans Rats Administered 328 µCi/kg of [¹⁴C]CP-526,555.

	Female Rats		Male Rats	
	AUC _(0-120h) (nCi-hr/g)	t _{1/2} (hr)	AUC _(0-120h) (nCi-hr/g)	t _{1/2} (hr)
Liver	2038	4.2	2331	4.7
Lung	875	4.8	775	6.0
Lymph Node	1789	4.7	1601	5.4
Muscle	723	4.1	758	6.2
Myocardium (Heart)	739	4.6	716	6.6
Nasal Tissues	15034	49	13145	45
Skin	559	5.9	3081	ND ^b
Skin Follicles	88140	ND	159242	ND
Spleen	2797	3.5	2596	4.9
Vibrissa	37584	9.4	19128	ND
Whole-Body	3524	6.1	3603	6.9
ADIPOSE				
Multilocular	265	4.3	215	8.6
Subcutaneous	96	ND	63	4.5
BLOOD				
Hepatic	240	5.3	294	3.9
Myocardial	212	7.5	241	6.4
Systemic	211	12	211	7.6
BONE MARROW				
Femur	1746	3.5	1764	5.2
Humerus	1840	4.5	860	5.2
Pelvis	1973	3.2	1378	5.1
Tibia	1621	3.4	798	4.9
Vertebra	1660	4.2	1596	5.0
BRAIN				
Cerebellum	836	4.6	923	5.3
Cerebrum	1156	3.8	1267	5.6
Medulla Oblongata	746	5.2	854	6.3
Meningis	35445	68	39862	52
Mesencephalon	843	5.2	920	5.6
Olfactory Bulb	914	4.2	1033	6.5
Pineal Gland	753	7.6	1493	5.9
Pituitary	2233	4.6	3702	4.2
Spinal Cord	732	4.5	832	6.2
Thalamus	1166	4.4	1267	5.7
GLANDS				
Adrenal	1608	4.1	1787	5.7
Buccal	3439	4.1	4381	4.0
Exorbital Lacrimal	4434	ND	3186	5.0
Harderian	4126	ND	1489	ND
Intraorbital Lacrimal	2157	4.5	2841	4.7
Pancreas	1547	4.6	1549	5.1
Parotid	1360	4.3	1335	4.9
Clitoridean/Preputial	4002	7.5	6056	ND
Salivary	3939	3.8	3847	4.7
Thymus	1481	3.8	1590	5.3
Thyroid	2583	4.3	2212	5.1

Table 5 continued:

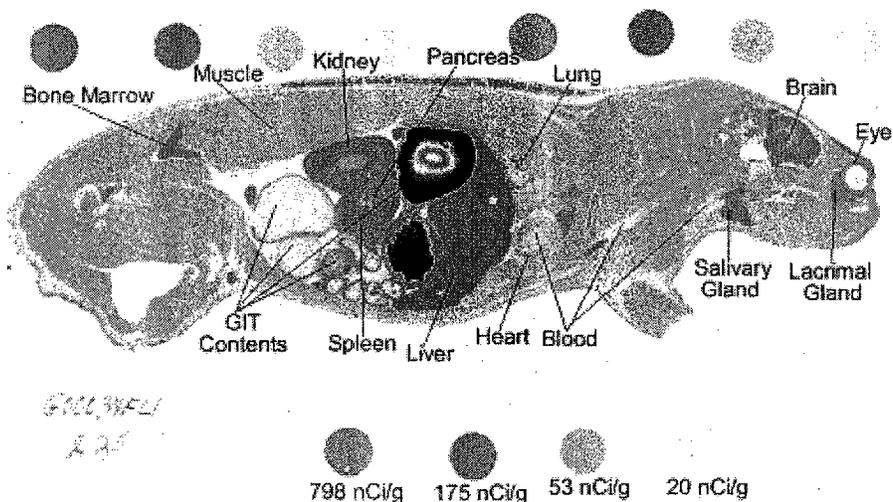
	Female Rats		Male Rats	
	AUC _(0-11hr) (nCi-hr/g)	t _{1/2} (hr)	AUC _(0-11hr) (nCi-hr/g)	t _{1/2} (hr)
OCULAR				
Choroid	659983	ND	977347	ND
Ciliary Body	1362225	ND	2151492	ND
Iris	705097	ND	1425508	ND
Uvea	617867	ND	1272172	ND
UROGENITAL				
Kidney	3446	3.9	3623	5.3
Renal Cortex	1277	3.6	1391	4.7
Renal Medulla	2533	4.3	2544	6.0
Renal Pelvis	10441	4.4	25078	ND
Epididymis	NP ^c		1428	4.3
Ovary	1583	3.5	NP	
Prostate	NP		2352	2.8
Seminal Vesicle	NP		1839	5.6
Testis	NP		1216	6.4
Uterus	593	3.2	NP	
GASTROINTESTINAL TRACT				
Gastric Mucosa	1563	4.9	655	3.7
Intestinal Mucosa	733	ND	743	ND

^a AUC values were calculated using linear trapezoidal approximation. The t_{1/2} was calculated as 0.693/K_{el}.

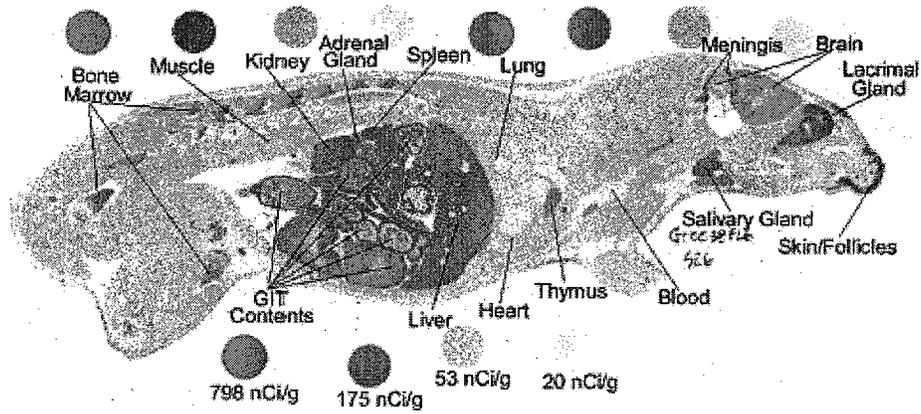
^b The t_{1/2} was not determined (ND) because a definitive elimination phase was not discernible.

^c Tissue was not present (NP) in this rat gender.

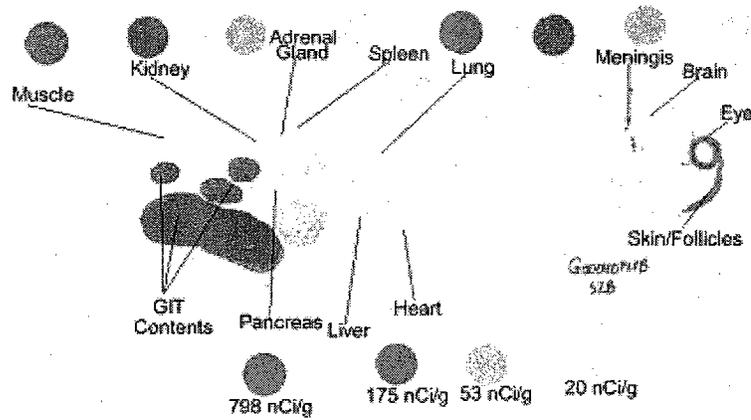
Autoradioluminogram 1. Female Rat: 1 Hr Post Oral Dose of [14C]CP-526,555.



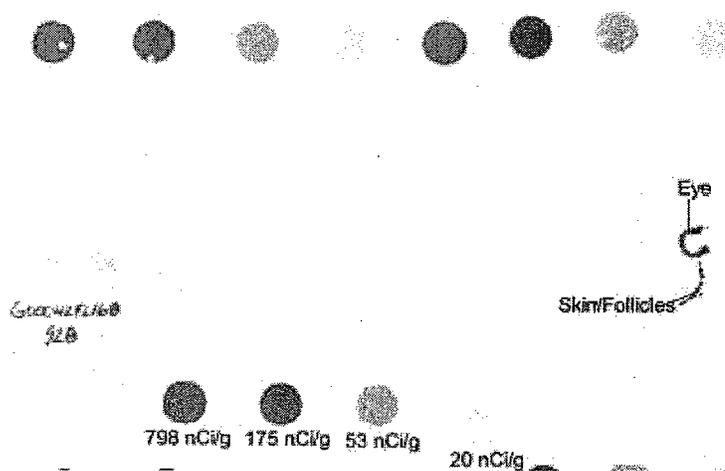
Autoradioluminogram 3. Female Rat: 6 Hr Post Oral Dose of [14C]CP-526,555.



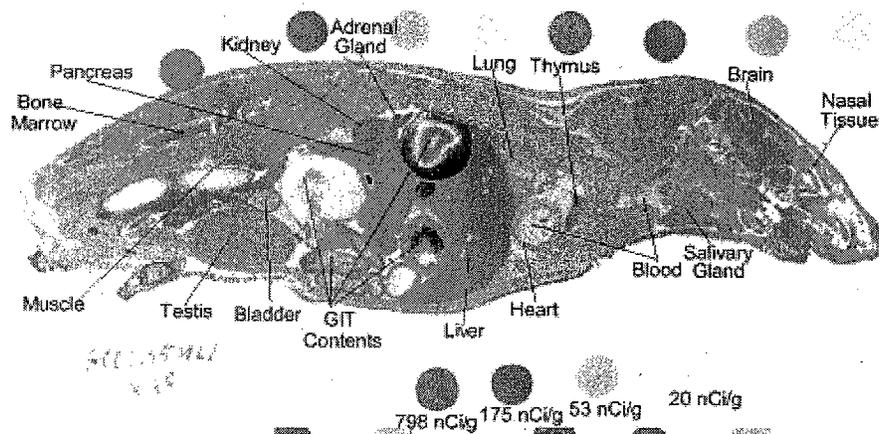
Autoradioluminogram 4. Female Rat: 18 Hr Post Oral Dose of [14C]CP-526,555.



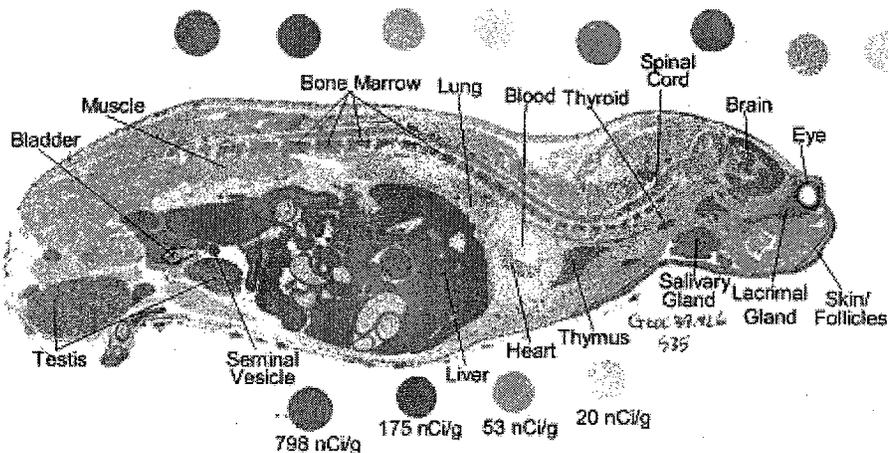
Autoradioluminogram 5. Female Rat: 168 Hr Post Oral Dose of [14C]CP-526,555.



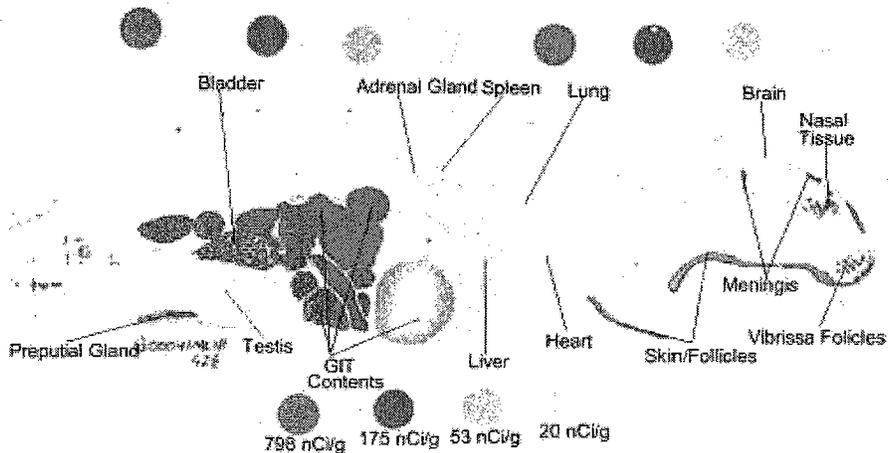
Autoradioluminogram 6. Male Rat: 1 Hr Post Oral Dose of [14C]CP-526,555.



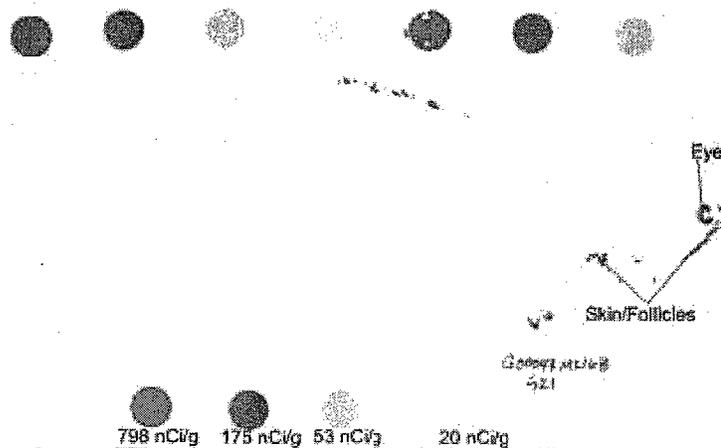
Autoradioluminogram 8. Male Rat: 6 Hr Post Oral Dose of [14C]CP-526,555.



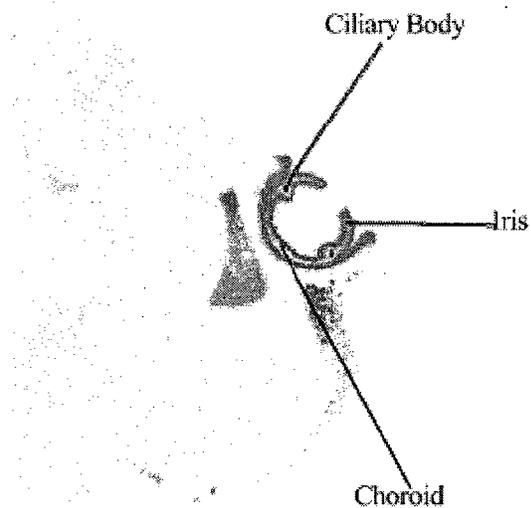
Autoradioluminogram 9. Male Rat: 18 Hr Post Oral Dose of [14C]CP-526,555.



Autoradioluminogram 10. Male Rat: 168 Hr Post Oral Dose of [14C]CP-526,555.



Autoradioluminogram 11. Magnification of the uvea.



Study Title: Skin Concentrations of Varenicline in Male Long-Evans Rats after Daily Administration for One or Three Days**Study Number:** DM2004-526555-066

Male Long-Evans rats (2/group) were orally dosed with [¹⁴C]varenicline at doses of approximately 91 mg/kg and 74 μCi/kg for either one or three days. Pigmented and non-pigmented skin samples were collected from each rat at 4 and 24 h post dose on Day 1 or 3. Pigmented skin samples were located between the cervical and thoracic vertebrae. Non-pigmented skin samples were located between the lumbar and sacral vertebrae.

Key Study Findings:

- There is a difference in the radioactivity distribution in the pigmented and in the non pigmented skin after single and multiple dose exposure of [¹⁴C]varenicline.
- In pigmented skin, increased mean radioequivalents from 3.4 to 1.9X occurred at 4 and 24 h, respectively, versus a single dose. A 1.6 to 1.2X increase in mean radioequivalents was observed in non-pigmented skin at 4 and 24 hr, respectively.
- 24 h after the third daily dose, the concentration of pigmented skin radioequivalents continued to increase (1.5X higher relative to 4 h concentrations) while non-pigmented radioequivalents remained similar (1.2X higher) relative to 4 h concentrations. At 24 h, following three consecutive daily doses, [¹⁴C] radioequivalents were 19X higher in pigmented skin relative to non-pigmented skin.

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Table 2. Pigmented and non-pigmented skin radioequivalents of [¹⁴C]varenicline in male Long-Evans rats after daily oral administration of 91 mg/kg

DM Study Number: DM2004-526555-066

Rat Id	Skin Sample	Frequency ^{a,b}	Hour	Mean±SD (µg eq/g)
M1	Pigmented	IX	4	14.8±4.0
M1	Non-Pigmented	IX	4	2.85±0.07
M2	Pigmented	IX	4	15.4±1.3
M2	Non-Pigmented	IX	4	2.96±0.31
M3	Pigmented	IX	24	44.9±7.7
M3	Non-Pigmented	IX	24	3.56±0.05
M4	Pigmented	IX	24	34.8±8.1
M4	Non-Pigmented	IX	24	2.95±0.09
M5	Pigmented	3X	4	56.8±14.0
M5	Non-Pigmented	3X	4	4.36±0.09
M6	Pigmented	3X	4	45.3±0.8
M6	Non-Pigmented	3X	4	4.97±0.09
M7	Pigmented	3X	24	75.2±10.3
M7	Non-Pigmented	3X	24	3.38±0.05
M8	Pigmented	3X	24	73.7±19.8
M8	Non-Pigmented	3X	24	4.69±0.14

^a Male rats M1, M2, M3, and M4 were administered one oral dose of [¹⁴C]varenicline prior to obtaining skin samples at 4 or 24 h post dose on day 1.

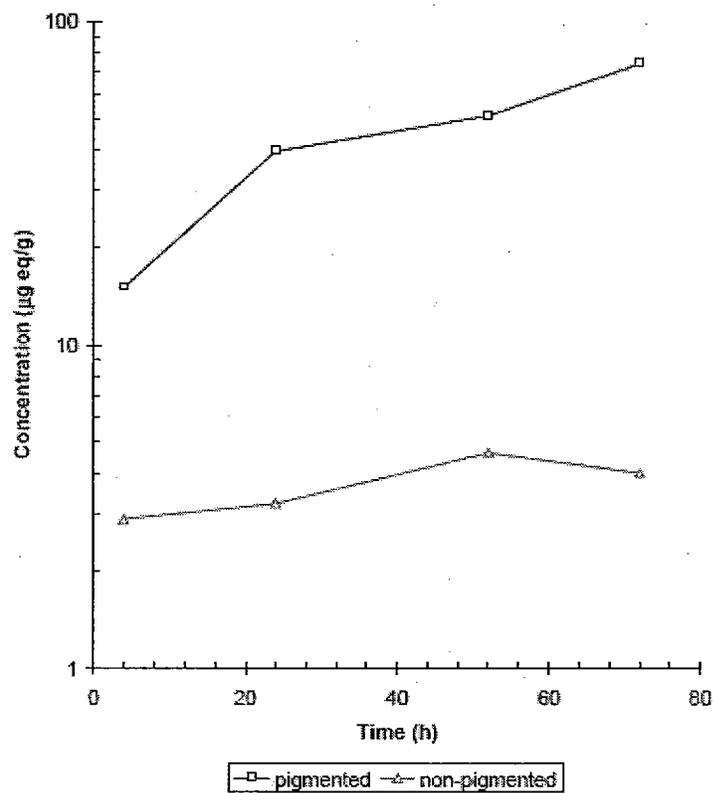
^b Male rats M5, M6, M7, and M8 were administered a daily oral dose of [¹⁴C]varenicline for three consecutive days prior to obtaining skin samples at 4 or 24 h after the third dose.

^c Mean±SD concentrations of [¹⁴C]varenicline radioequivalents were obtained by averaging data from triplicate skin samples.

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Figure 1. Radioequivalents of [¹⁴C]varenicline in pigmented and non-pigmented skin from male Long-Evans rats after daily oral administration of 91 mg/kg

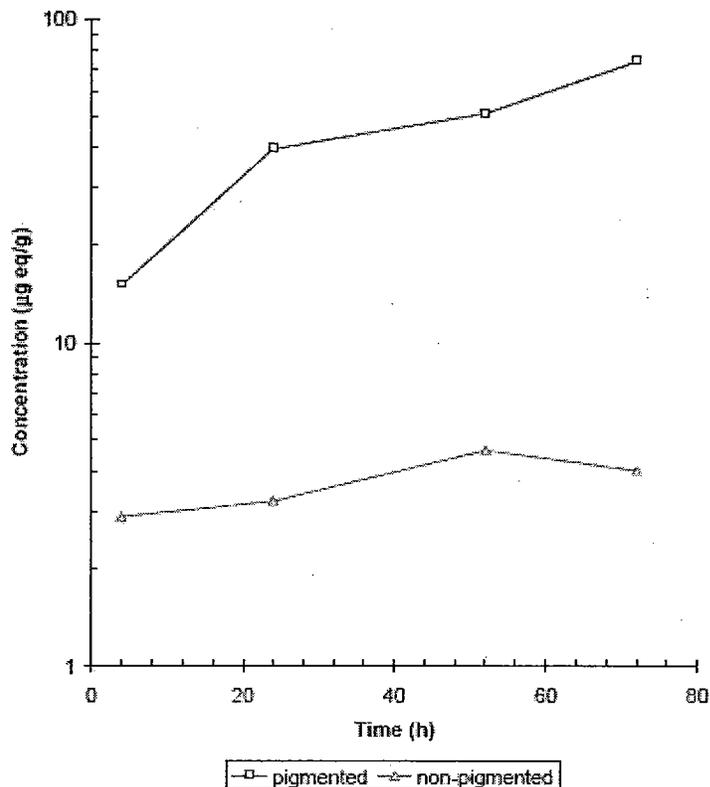
DM Study Number: DM2004-526555-066



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Figure 1. Radioequivalents of [14 C]varenicline in pigmented and non-pigmented skin from male Long-Evans rats after daily oral administration of 91 mg/kg

DM Study Number: DM2004-526555-066



2.6.4.5 Metabolism

Study Title: Metabolism and Excretion of CP-526,555 in Healthy Human Subjects after Oral Administration of 1.0 Mg [14 C]-CP-526,555 and Identification of Circulating and Excretory Metabolites

Study Number: DM2000-526555-031

Six healthy human male subjects were administered 1.0 mg [14 C]-CP-526,555 in water (240 mL). Three were non-smoker subjects and three were smokers. Blood samples (sufficient to provide 6 mL serum) were collected for pharmacokinetic evaluation of CP-526,555 and total radioactivity at time points of 0 (just prior to dosing), 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, and 192 hr post-dose. An additional blood sample was collected at 216 hr post-dose for subject #8. Additional blood sufficient to provide a minimum of 20 mL serum were collected at 1, 4, 8, 12, and 24 hr post-dose for profiling of metabolites by HPLC-MS. All blood samples were processed to obtain serum and stored frozen prior to analysis. Urine samples were collected prior to dosing and at intervals of 0-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hr post-dose. Feces were collected prior to dosing and at intervals of 0-24, 24-48, 48-72, and 72-96 hr post-dose.

Key Study Findings:

- The major mechanism of excretion of CP-526,555 was via the urine as unchanged drug, and almost no drug related material was excreted in feces.
- CP-526,555 had four minor routes of metabolism: N-carbamoyl glucuronidation, N-formylation, conjugation with a hexose sugar, and oxidation.
- Metabolites from these three routes were present in the circulation at low concentrations relative to parent drug. The N-carbamoyl glucuronide was present in the urine, however the other two circulating metabolites were not found in excreta, suggesting the possibility that these could be converted back to the parent compound *in vivo*. The hydroxyquinoxaline was very minor in abundance, and only detected in the urine.

Study Title: Metabolism and Excretion of CP-526,555 in Cynomolgus Monkeys After Oral Administration of 0.08 Mg/Kg [¹⁴C]-CP-526,555-24 and Identification of Circulating and Excretory Metabolites**Study Number: DM2000-526555-030**

Fasted male and female Cynomolgus monkeys (N=2/sex) were administered a single oral dose (0.08 mg/kg; 100-300 µCi/animal; 2 mL/kg body weight) of [¹⁴C]-CP-526,555-24 formulated in sterile water at 0.05 mg/mL. Whole blood (~3.0 mL) was collected into heparinized tubes prior to dosing and at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hours post dose. Urine and feces were quantitatively collected from animals for ten days (240 h after dosing) at intervals of 0-8 (urine only), 8-24 (urine only), 0-24 feces only, 24-28, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, and 216-240 hours after dosing. Two additional bile duct cannulated male Cynomolgus monkeys were also administered a single oral dose of (0.08 mg/kg; 100 µCi/animal; 2 mL/kg body weight) of [¹⁴C]-CP-526,555-24 formulated in sterile water at 0.05 mg/mL.

Key Study Findings:

- The half-life of total radioactivity was 30.4 hr whereas the half-life of unchanged CP-526,555 was 23.5 hr.
- Circulating metabolites included the N-carbamoyl glucuronide (M4, 8.7%), N-formyl conjugate (M5, 2.6%), putative N-hexose conjugate (M7, 2.9%), and an unidentified minor metabolite (M3d, 2.1%).
- After oral administration of radiolabel led CP-526,555 to monkeys, an average of 87.5% of the dose was recovered.
- Most of the CP-526,555-related material was recovered in the urine (>90% of radioactivity recovered). In the monkey, the major route of excretion of CP-526,555 was as unchanged drug in the urine, with some minor metabolites (M4, N-carbamoyl glucuronide; M2 and M1, putative dihydroxy metabolites). A small amount was excreted in feces, which was almost all unchanged CP-526,555.

- In the circulation, parent drug comprised about 60-80% of total CP-526,555-related material.

Study Title: Serum Concentrations of CP-526,555 and Total Drug Related Material after Oral Administration of [¹⁴C]-CP-526,555-24 to Male And Female Sprague-Dawley Rats and Identification of Circulating Metabolites
Study Number: DM2000-526555-026

Male and female Sprague-Dawley rats (N=3/sex) were administered a single oral dose (3 mg/kg; 100 μ Ci/kg; 2 mL/kg body weight) of [¹⁴C]-CP-526,555-24 formulated in sterile water at 1.5 mg/mL. Whole blood (~0.7 mL) was collected at 0.5, 1, 2, 4, 8, and 24 hours post dose. Serum was analysis for CP-526,555 by HPLC/MS/MS. An additional group of animals (N = 12/sex) were also administered with [¹⁴C]-CP-526,555-24. At time points of 1, 2, 4 and 8 hr post-dose, three animals were sacrificed by CO₂ asphyxiation, and blood samples were collected from the superior vena cava. Additionally, brains were removed from animals sacrificed at the 1 and 4 hr time points.

Key study Findings:

- The mean half-life of CP-526,555 was 4.0 hr. No apparent differences were noted between the sexes. The mean half-life of total drug-related material was 5.1 hr, slightly longer than that of parent drug. The mean C_{max} was approximately 1.8-fold greater than unchanged drug and the T_{max} occurred 30% later. By comparison of AUC values, unchanged drug represented approximately 55% of total drug-related material.
- In pooled serum samples, a total of five metabolites were readily observed in addition to CP-526,555. In this approach, CP-526,555 represented 80% of total drug-related material.
- Circulating metabolites include the N-carbamoyl glucuronide (M4, 8-13% of total AUC), an N-formyl conjugate (M5, \approx 5% of total AUC), an N-hexose conjugate (M7, \approx 1-2% of total AUC), a male specific metabolite with 32 amu higher than parent drug presumed to be a carboxylic acid (M2, 1.4% of total AUC), and one additional minor female specific metabolite of unknown structure (M6, 3% of total AUC).
- The N-carbamoyl glucuronide was previously identified in rat urine and bile. The N-hexose conjugate represented a metabolite not previously observed. The exact identity of the hexose (e.g. glucose, galactose, etc.) cannot be ascertained from the mass spectral data. Metabolite M5 was identified as an N-formyl conjugate, with an identical retention time as that observed for this metabolite in monkey urine. The metabolite eluting earliest possessed a molecular ion of *m/z* 244, an addition of 32 mass units to CP-526,555. This metabolite was also observed in excreta and eluted at an identical retention time and only observed in serum from male animals. An additional metabolite was observed in the radio chromatograms from female serum samples; however a mass spectral signal could not be detected for

this, excluding the possibility of proposing a structure. This metabolite represented 3% of the total AUC.

- Brain homogenates contained 2.2-fold the amount of total radioactivity as corresponding serum samples. A representative HPLC radiochromatogram of extracted brain homogenate showed 2 peaks: CP-526,555 and the N-formyl conjugate, with the former comprising the majority of the drug-related material (96%).

Study Title: Metabolism and Excretion of Cp-526,555 in CD-1 Mice after Oral Administration of 3.0 Mg/Kg [¹⁴C]-CP-526,555-24 and Identification of Circulating and Excretory Metabolites

Study Number: DM2001-526555-042

Fasted male and female CD-1 mice (n=9/sex) were administered a single oral dose (3.0 mg/kg; 16.4-21.8 µCi/animal; 10 mL/kg body weight) of [¹⁴C]-CP-526,555-24 formulated in sterile 0.9% saline at 0.3 mg/mL. Urine and feces were quantitatively collected from animals for eight days (192 h after dosing) at 24 h intervals; -24-0 (pre-dose), 0-24, 24-28, 48-72, 72-96, 96-120, 120-144, 144-168, and 168-192 hours after dosing. A second group of CD-1 mice (n=6/sex/timepoint) were administered a similar single oral dose and terminal blood samples were collected at 1, 4, and 12 hours postdose for quantification of CP-526,555 plasma concentrations.

Key Study Findings:

- The half-life of total radioactivity was 1.8 h whereas the half-life of CP-526,555 was 1.4 h. Circulating metabolites were minor and included the putative “dihydroxy” metabolites (M1 1.4% and M2, 3.8%), the N-formyl conjugate (M5, 2.5%), and two metabolites, which were not, identified (M3a, 1.1%; M3d, 6.8%).
- After oral administration of radiolabeled CP-526,555 to mice, an average of 94.4% of the dose was recovered. Most of the drug related material was recovered in the urine (>88% of the radioactivity recovered).
- In mice, the major route of excretion of CP-526,555 is as unchanged drug in the urine, with some minor metabolites (M1 and M2, putative dihydroxy metabolites, similar to monkey and rat). A small amount was excreted in feces (<12% of the radioactivity), which was predominately unchanged drug and one very minor metabolite which was not identified (M3a, 0.4% of recovered radioactivity). In the circulation, parent drug comprised about 84.5% of total CP-526,555-related material.

Study Title: Identification and Quantitation of Circulating Metabolites of CP-526,555 In Female New Zealand Rabbits After Oral Administration Of [¹⁴C]-CP-526,555

Study Number: DM2001-526555-044

Female New Zealand rabbits (N = 4) were administered a single oral dose (10 mg/kg; 85.7-93.5 $\mu\text{Ci}/\text{animal}$; 2.5 mL/kg body weight) of [^{14}C]-CP-526,555-24 formulated in sterile water. Blood samples were collected at 2, 4, 8, and 24 hours postdose.

Key Study Findings:

- Five radioactive peaks were observed in pooled rabbit serum samples. Two additional radioactive peaks were resolved using an additional isocratic LC-MS method. These were identified as parent compound (CP-526,555), metabolite M1 (carboxylic acid metabolite), metabolite M2 (carboxylic acid metabolite), metabolite M3c (lactam), metabolite M3d (unknown), metabolite M4 (N-carbamoyl glucuronide), and M4a (N-hydroxy glucuronide). M2, a carboxylic acid metabolite, comprised the major percentage of mean serum radioactivity (34.8%), followed by M4, unchanged drug, M3c, M3d, M4a, and M1 at 25.8%, 16.7%, 6.65%, 6.37%, 5.57%, and 4.18% of total radiolabel AUC, respectively.
- After oral administration of radiolabeled CP-526,555 to female rabbits, CP-526,555 and six metabolites were observed in the systemic circulation. Major routes of metabolism of CP-526,555 in the rabbit appear to be via oxidation of the alicyclic portion and subsequent ring opening, hydroxylation of the amino nitrogen (which is subsequently glucuronidated) and N-carbamoyl glucuronidation.

Study Title: Radiolabel led Mass Balance, Excretion, and Metabolite Identification of CP-526,555 in Urine, Feces, and Bile of Sprague-Dawley Rats after Oral Administration Of [^{14}C]-CP-526,555
Study Number DM2000-526555-011

The primary routes of CP-526,555 excretion and its metabolites were characterized. Male and female Sprague-Dawley rats ((180–325 g; n=3/gender) were administered a single oral dose of 3 mg/kg (free base equivalent) of [^{14}C]-CP-526,555 (specific activity 61.2 mCi/mmol, radiochemical purity []). Each animal received approximately 19-22 μCi . Urine and feces were quantitatively collected from animals for 168 hours at intervals of 0–8 (urine only), 8–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours postdose. Metabolites in bile from bile duct cannulated rats were also characterized.

Key Study Findings:

- The urinary profiles for both male and female rats were identical, with unchanged CP-526,555 accounting for 90.9 and 95.6% of the urinary radioactivity in males and females, respectively. The remaining radioactivity in urine was accounted for by four metabolites, designated M1-M4.
- Only a single radioactive peak corresponding to unchanged was detected in fecal samples that represented unchanged CP-526,555.

- In bile, only a single radioactive peak likely corresponding to N-carbamoyl glucuronide conjugate of CP-526,555 was detected.
- Four metabolites were identified:
 - **Metabolite M1:** M1 accounted for ~0.9% of the total dose. It had a HPLC retention time of ~8.5 minutes and showed a protonated molecular ion at m/z 244, 32 mass units greater than parent drug. This could represent either a dihydroxylated metabolite or a ring-opened carboxylic acid. Spectral data was limited, prohibiting further structural characterization.
 - **Metabolite M2a:** M2 accounted for ~0.7% of the total dose. It had a HPLC retention time of ~9 minutes and showed a protonated molecular ion at m/z 244. Following esterification to test for the presence of a carboxylic acid, there was no addition of 28 mass units to the molecule. An acetylation reaction confirmed the presence of two hydroxy groups on the molecule as observed through addition of three acetyl groups.
 - **Metabolite M3:** M3 had a HPLC retention time of ~17.5 min and showed a protonated molecular ion of m/z 338. Neutral loss scanning of loss of 176 mass units, indicative of a glucuronide conjugate, yielded a parent ion of m/z 338.
 - **Metabolite M4:** M4 had a HPLC retention time of ~22 minutes and showed a protonated molecular ion at m/z 432. Glucuronidase incubation resulted in a molecular ion of 212, reflecting the conversion back to the parent compound. This was confirmed with the carbamoyl glucuronide generated *in vitro* using liver microsomes incubated with UDPGA in carbonate containing buffer with continuous CO₂ exposure. The retention times of the *in vivo* glucuronide and the *in vitro* biosynthesized carbamoyl glucuronide metabolite provided confirmation. M4 accounted for ~2.0% of the total dose.

2.6.4.6 Excretion

Study Title: Urinary Excretion of CP-708,075 in Rats Following a Single Oral Dose of CP-526,555 at 30 mg/Kg

Study Number: DM2001-526555041

The objective of this study was to determine the percent of dose excreted as the hydroxyl metabolite, CP-708,075; in urine following a single oral dose of CP-526,555 at 30 mg/kg to rats. Six Sprague Dawley rats (3/sex) received a single oral gavage dose of CP-526,555-24 at 30 mg/kg. The dose was administered as a solution in water (3 mg/mL) under fed conditions. A urine sample was collected from each animal comprising the first 24 hrs after dosing.

Key Study Findings:

- After oral administration of CP-526,555 at 30 mg/kg to rats, the percent of dose excreted as the metabolite, CP-708,075 in urine over 24 hours ranged from 0.16 to

0.79% (mean = $0.51 \pm 0.26\%$). This corresponded to a range of 12.4 to 66.5 g CP-708,075 excreted during the first 24 h period.

- CP-708,075 was observed in human subjects after oral administration of 1.0 mg [^{14}C]-CP-526,555. The amount excreted in human over the urine collection period ranged from 17 to 38 g (approximately 0.24 to 0.54 g/kg body weight assuming a 70 kg body weight). In the rats in the present study, urinary excretion ranged from 12.4 to 66.5 g during the 24 h collection period (48 to 264 g/kg body weight).

2.6.4.7 Pharmacokinetic drug interactions

Study Title: Substrate Disappearance Studies of CP-526,555 in Rat, Dog, Monkey and Human Hepatic Microsomes

Study Number: DM1998-526555-008

This study was done to assess the metabolism of CP-526,555 (substrate consumption) in rat, dog, monkey and human hepatic microsomes. CP-526,555 was incubated with rat, dog, monkey or human hepatic microsomes and the loss of parent drug from the incubations was measured. No time-dependent loss of CP- 526,555 was detected in any of the species tested. This suggested that CP-526,555 was stable to metabolism by hepatic microsomal cytochrome P450 enzymes.

Key Study Findings:

- There was no loss of CP-526,555 from rat, dog or human microsomes that could be considered time-dependent under the experimental conditions.

Study Title: The Effect of Probenecid, Cimetidine, and NH_4Cl on the Pharmacokinetics of CP-526,555 in Rats Following Intravenous Administration

Study Number: DM2001-526555-043

The purpose of this study was to determine if exposure to compounds that can change renal clearance, such as probenecid, cimetidine, or NH_4Cl , affect CP-526,555 pharmacokinetics in rats.

Key Study Findings:

- Following the 1 mg/kg IV dose, the mean $\text{AUC}_{(0-\text{inf})}$ values for the control, probenecid, NH_4Cl , and cimetidine groups were 461, 725, 477, and 753 ng•h/mL, respectively. The $\text{AUC}_{(0-\text{inf})}$ values for the probenecid and the cimetidine animals were 1.6–fold greater than that of the control group. The $\text{AUC}_{(0-\text{inf})}$ in the NH_4Cl group were similar to those of the control group.
- After a 1 mg/kg IV dose, the plasma clearance of CP-526,555 (CL_p) in the control, probenecid, NH_4Cl , and cimetidine groups were 39.2, 23.3, 35.6, and 22.5 mL/min/kg, respectively.

- The pH of the urine of the NH₄Cl treated animals remained neutral during the course of the study.
- Therefore, the results from the NH₄Cl group are inconclusive, whereas, CLp the appears to be decreased by administration of probenecid or cimetidine. The V_{ss} and t_{1/2} values for CP-526,555 were similar among the four groups. The t_{1/2} for the NH₄Cl treated group was shorter than the other groups. The difference in the half-life is likely due to the lack of detectable terminal phase plasma concentrations in that group.

Study Title: Effect of CP-526,555 on Human Drug Metabolizing Enzymes *in Vitro*

Study Number: DM2001-526555-045

The study was done to determine the potential for CP-526,555 to inhibit human drug metabolizing enzymes *in vitro*.

Key Study Findings:

Based on the *in vitro* data presented in the Sponsor's table below, CP-526,555 should not demonstrate pharmacokinetic drug interactions with compounds for which CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A mediated metabolism constitutes the primary mechanism of clearance.

Summary of IC₅₀ Data for CP-526,555 in Human Liver Microsomes

Marker Substrate Activity	Enzyme	% of control at [I] = 30 μM	IC ₅₀ (μM) Mean ± SE
Phenacetin O-Deethylase	CYP1A2	100	>30
Coumarin 7-Hydroxylase	CYP2A6	110	>30
Bupropion Hydroxylase	CYP2B6	99	>30
Amodiaquine N-Deethylase	CYP2C8	99	>30
Diclofenac 4'-Hydroxylase	CYP2C9	93	>30
S-Mephenytoin 4'-Hydroxylase	CYP2C19	100	>30
Dextromethorphan O-Deethylase	CYP2D6	96	>30
Chlorzoxazone 6-Hydroxylase	CYP2E1	100	>30
Felodipine Oxidase	CYP3A	75	>30
Midazolam 1'-Hydroxylase	CYP3A	90	>30
Testosterone 6β-Hydroxylase	CYP3A	97	>30

Study Title: *In Vitro* Transport of CP-526,555

Study Number: DM2003-526555-052

This study was done to determine the mechanism of renal clearance of CP-526,555 *in vitro*.

Key Study Findings:

- The inhibition assays and kinetic profile of CP-526,555 indicated that this compound might act as a substrate and a weak inhibitor of hOCT2. CP-526,555 had a low to moderate affinity substrate of hOCT2; K_m was substantially greater than circulating concentrations associated with efficacy. Cimetidine, a known

inhibitor of hOCT2, partially inhibited CP-526,555 uptake by HEK cells expressing hOCT2.

Study Title: Determination of the Enzyme Kinetics and UGTs Involved in the Metabolism of Varenicline to the N-Carbamoylglucuronide Conjugate of Varenicline

Study Number: DM2005-526555-076

The objectives of this study were (1) to determine the enzyme kinetic parameters for the formation of the N-carbamoyl glucuronide metabolite of varenicline using human liver microsomes, (2) to identify the human recombinant UGT enzyme(s) responsible for the formation of the N-carbamoyl glucuronide, and (3) to determine the enzyme kinetic parameters for human recombinant UGT enzyme(s) that form the N-carbamoyl glucuronide metabolite of varenicline.

Key Study Findings:

- In pooled human liver microsomes, enzyme kinetics was studied up to a substrate concentration of 2 mM. The findings were such that an accurate determination of K_M and V_{max} could not be derived, since the K_M is >2 mM.
- Of the human recombinant UGT enzymes tested, only UGT2B7 demonstrated a capability to catalyze the formation of varenicline N-carbamoylglucuronide. The other UGT enzymes examined, UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B15, and 2B17 failed to significantly affect N-carbamoylglucuronide formation. The velocity vs. substrate concentration relationship for varenicline N-carbamoylglucuronidation by UGT2B7 was very similar to that observed for human liver microsomes.

Study Title: Induction Potential of Varenicline (CP-526555-18) on Cytochrome P450 1A2 and 3A4 in Human Hepatocyte

Study Number: RR 764-04912

To evaluate the potential for CP-526,555 to induce drug metabolizing enzymes, *in vitro* assays were performed to measure the induction of cytochrome P450 CYP3A4 and CYP1A2, enzyme activity and mRNA levels in freshly isolated human hepatocytes. These assays included 10 μ M rifampin as a positive control for CYP3A4 and 10 μ M lansoprazole as a positive control for CYP1A2.

Key Study Findings:

- Treatment of primary hepatocytes with multiple doses of varenicline indicated there was no significant increase in CYP3A4 or CYP1A2 activity at concentrations in excess of expected therapeutic concentrations (0.025-0.5 μ M).

- CYP3A4 activity reached a mean maximum of 10% rifampin control at 0.05 μ M while CYP1A2 activity was just below 1% of lansoprazole control.
- Mean maximum mRNA induction responses were 1.17-fold for CYP3A4 and 1.79-fold for CYP1A2 at 0.5 μ M of CP-526,555.

2.6.4.8 Other Pharmacokinetic Studies

Study Title: Monomethyl Tartrate (CE-157,254) Incubations at 37°C in Human Blood, Simulated Gastric Fluid and Buffer;

Study Number: DM2002-526555-051

The study examined the conversion of monomethyl tartrate (CE-157,254) to tartaric acid in human blood, simulated gastric fluid and buffer.

Key Study Findings:

- The lack of any measurable conversion of monomethyl tartrate to tartaric acid in buffer at pH 7.5 indicated that this conversion which occurred in human blood was due to an enzymatic process.
- These data also indicated that some conversion of monomethyl tartrate to tartaric acid occurred in gastric fluid incubations, and thus could potentially occur in the gastrointestinal tract.

2.6.4.9 Discussion and Conclusions

The major findings from the ADME profile of CP-526,555 are summarized in section 2.6.4.1. Extensive metabolism of the compound and interspecies variation of the metabolites were noted, however, none of the metabolites were considered major since <10% of the total metabolites were found in the systemic circulation. All metabolites found in the human were also found in at least one animal species. The potential for CP-526,555 to induce CYP isozymes was investigated. No oxidative metabolism of CP-526,555 was detected from incubations with the rat, monkey, and human liver microsomes, suggesting that it was not a good substrate for cytochrome P450 enzymes. The plasma protein binding was found to be low in all species studied (mouse, rat, dog, monkey, and human). Tissue distribution studies in rodents found that CP-526,555 was extensively deposited in the melanin-containing tissues like skin and eye. Therefore there is a potential for differential CP-526,555 effects dependent on human demographs. The T_{max} for CP-526,555 after oral administration was 3-4 hrs in the primates, with a half life of elimination of approximately 16-20 hrs. CP-526,555 is excreted mainly via urine (approximately 80-85%); feces accounted for approximately 5-6 % of the excretion. The fate of the rest of the compound administered was not determined.

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pharmacokinetics:

Absorption/Single Dose

Species: Gender (M/F) Number of Animals:	Mouse (CD-1) Males: 6/timepoint Females: 6/timepoint Fasted	Rat (Sprague-Dawley) Males: 3 Females: 3 Not Specified	Monkey (Cynomolgus) Males: 2 Females: 2 Fasted	Human Males: 6 (3 smokers; 3 non-smokers) Fasted
Vehicle/Formulation:	[¹⁴ C]CP-526,555 at 0.3 mg/mL in 0.9% saline	[¹⁴ C]CP-526,555 at 1.5 mg/mL in water	[¹⁴ C]CP-526,555 at 0.05 mg/mL in water	[¹⁴ C]CP-526,555 at 1.0 mg in 340 mL water
Method of Administration:	PO	PO	PO	PO
Dose (mg/kg):	3.0	3.0	0.08	0.014 ^a
Sample (eg, whole blood, plasma, serum):	Plasma	Serum	Plasma	Serum
Analyte:	CP-526,555 and ¹⁴ C	CP-526,555 and ¹⁴ C	CP-526,555 and ¹⁴ C	CP-526,555 and ¹⁴ C
Assay: CP-526,555	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS	HPLC-MS/MS
Assay: Total Radioactivity	LSQ ^a	LSQ ^a	LSQ ^a	LSQ ^a
PK Parameters: CP-526,555 ^b				
C _{max} (ng/mL)	282/304	221/248	19.3	4.01
T _{max} (h)	1.0/1.0	1.5/2.2	3	4.3
AUC(0-t _{max}) (ng·h/mL)	856/1070	2040/2740	210	90.3
AUC(0-∞) (ng·h/mL)	859/1070	-	232	93.9
CLF (mL/min/kg) ^d	58/47	25/18	5.7	2.5 ^e
k _e (hr ⁻¹)	0.463/0.501	-	0.031	0.043
T _{1/2} (h)	1.5/1.4	4.1/4.0	24	16.7
PK Parameters: Total Radioactivity ^b				
C _{max} (ng eq/mL)	357/415	447/378	29.0	4.57
T _{max} (h)	1.0/1.0	1.7/2.2	3	2.8
AUC(0-t _{max}) (ng eq·h/mL)	1240/1510	4110/4500	331	114
AUC(0-∞) (ng eq·h/mL)	1250/1520	-	369	124
CLF (mL/min/kg) ^d	40/33	12/11	3.6	1.9
k _e (hr ⁻¹)	0.377/0.397	-	0.023	0.040
T _{1/2} (h)	1.8/1.7	4.9/5.2	30	17.4

Absorption /Repeat dose included in the main text

Distribution tables included in the main text.

Metabolism in Human (healthy volunteers)

Species:	Human
Gender/Number of Subjects:	Male: 6 (3 smokers; 3 non-smokers)
Feeding Condition:	Fasted
Vehicle/Formulation:	[¹⁴ C]CP-526,555-24 (1.0 mg in 240 mL water)
Method of Administration:	PO
Dose (mg):	1.0
Radionuclide:	Carbon-14
Specific Activity:	21 mCi/μmol

Species: Human (Healthy Volunteers)	Urine	Feces	Bile	Serum
Total Time Period of Sample Collection	0-168 hr	0-96 hr		0-24 hr
Sample Analyzed for Metabolites	0-72 hr	ND ^a	NC ^b	0-24 hr
Compound (as % of Dose or Sample)	% of Dose	% of Dose		% of Sample
% of Total Dose Excreted (0-168 hr)				N/A ^c
CP-526,555	87.1 ± 5.5	0.9 ± 0.5		90.8 ± 1.6
M3b (Hydroxyvaranaciline; CP-708,875)	30.5 ± 4.8			-
M3c (Lactam-Putative)	-			1.1 ± 0.6
M4 (N-Carbamoyl Glucuronide)	3.6 ± 0.9			3.8 ± 0.9
M5 (N-Formylvaranaciline; CP-697,535)	-			0.9 ± 0.5
M7 (N-glucosylvaranaciline)	-			3.5 ± 0.4

^aND, not determined. Feces samples were not profiled for metabolites since less than 1% of dose was recovered in feces. ^bNC, bile was not collected. ^cN/A not applicable.

Metabolism in Monkey

Species:	Monkey (Cynomolgus)
Gender/Number of Subjects:	Male:2 Female:2 Bile Cannulated Male: 2
Feeding Condition:	Fasted
Vehicle/Formulation:	[¹⁴ C] ₃ CP-526,555-24 at 0.05 mg/mL in water
Method of Administration:	PO
Dose (mg/kg):	0.08
Radionuclide:	Carbon-14
Specific Activity:	119 mCi/μmol

Species: Monkey (Cynomolgus)	Urine	Feces*	Bile	Plasma
Total Time Period of Sample Collection	0-240 hr	0-240 hr	0-48 hr	0-72 hr
Sample Analyzed for Metabolites	0-24 hr	0-24 hr	0-48 hr	0-24 hr
Compound (as % of Dose or Sample)	% of Dose	% of Dose	% of Sample	% of Sample
% of Total Dose Excreted (0-240 hr)	74.1 ± 2.3	6.52 ± 2.54	ND ^b	N/A ^c
CP-526,555	68.5 ± 9.0	6.3	-	80.4 ± 3.2
M1 (Amino Acid)	1.1 ± 0.6	-	-	-
M2 (Amino Acid)	4.3 ± 1.4	0.3	-	1.7 ± 0.2
M3a	1.1 ± 0.5	-	-	0.5 ± 0.4
M3c (putative lactam)	-	-	-	0.9 ± 0.3
M3d (putative carbonyl metabolite)	-	-	-	2.1 ± 0.8
M4 (N-Carbamoyl Glucuronide)	3.6 ± 1.2	-	100	8.7 ± 2.5
M5 (N-Formylvaranacine; CP-697,535)	-	-	-	2.6 ± 0.3
M7 (N-glucosylvaranacine)	-	-	-	2.9 ± 0.8

*Metabolite profiles in feces were determined from a pool from all four animals. ^bND, not calculated. ^cN/A, not applicable.

Metabolism in Mouse

Species:	Mouse (CD-1)
Gender/Number of Subjects:	Male:9 Female:9 (Excreta) Male:18 Female:18 (Plasma)
Feeding Condition:	Fasted
Vehicle/Formulation:	[¹⁴ C]CP-526,555-24 at 0.3 mg/mL in 0.9% saline
Method of Administration:	PO
Dose (mg/kg):	3.0
Radionuclide:	Carbon-14
Specific Activity:	19.5 mCi/μmol

Species: Mouse (CD-1)	Urine	Feces	Plasma
Total Time Period of Collection	0-192 hr	0-192 hr	0-12 hr
Sample Analyzed for Metabolites	0-24 hr	0-72 hr	0-12 hr
Compound (as % of Dose or Sample)	% of Dose	% of Dose	% of Sample
Male			
% of Total Dose Excreted (0-192 hr)	86.4 ± 8.8	8.15 ± 0.89	N/A ^a
CP-526,555	85.1 ± 8.6	7.9 ± 1.0	84.5
M1 (Amino Acid)	0.4 ± 0.1	-	1.3
M2 (Amino Acid)	0.9 ± 0.2	-	3.6
M3a (Unknown)	-	0.3 ± 0.2	1.3
M3d (Putative Carbonyl)	-	-	7.0
M5 (N-Formylvaranacine; CP-697,535)	-	-	2.4
Female			
% of Total Dose Excreted (0-192 hr)	79.9 ± 7.6	13.5 ± 6.5	N/A ^a
CP-526,555	78.1 ± 7.5	13.4 ± 6.7	84.5
M1 (Amino Acid)	0.6 ± 0.1	-	1.4
M2 (Amino Acid)	1.1 ± 0.1	-	4.0
M3a (Unknown)	-	0.1 ± 0.2	0.9
M3d (Putative Carbonyl)	-	-	6.6
M5 (N-Formylvaranacine; CP-697,535)	-	-	2.6

^aN/A, not applicable.

Metabolism in Rat

Species:	Rat (Sprague-Dawley)
Gender/Number of Subjects:	Male:3 Female:3 Bile Cannulated: 2/sex
Feeding Condition:	Not Specified
Vehicle/Formulation:	[¹⁴ C]CP-526,555-24 at 0.56 mg/mL in water
Method of Administration:	PO
Dose (mg/kg):	3.0
Radioisotope:	Carbon-14
Specific Activity:	6.12 mCi/μmol

Species: Rat (Sprague-Dawley)	Urine	Feces	Bile
Total Time Period of Sample Collection	0-168 hr	0-168 hr	0-24 hr
Sample Analyzed for Metabolites	0-24 hr	0-24 hr	0-24 hr
Compound (as % of Dose or Sample)	% of Dose	% of Dose	% of Sample
Male			
% of Total Dose Excreted (0-168 hr)			
CP-526,555	69.9 ± 5.8	24.3 ± 5.7	NC*
M1 (Amino Acid)	62.0 ± 5.1	24.3 ± 5.7	-
M2a (Dihydroxy)	1.0 ± 0.2	-	-
M2b (Dihydroxy)	1.0 ± 0.3	-	-
M3 (Unknown)	1.5 ± 0.5	-	-
M4 (N-Carbamoyl Glucuronide)	2.3 ± 0.6	-	100
Female			
% of Total Dose Excreted (0-168 hr)			
CP-526,555	66.2 ± 15.0	20.3 ± 7.5	NC*
M1 (Amino Acid)	61.1 ± 15.1	20.3 ± 7.5	-
M2a (Dihydroxy)	0.8 ± 0.2	-	-
M2b (Dihydroxy)	0.5 ± 0.1	-	-
M3 (Unknown)	0.4 ± 0.1	-	-
M4 (N-Carbamoyl Glucuronide)	1.5 ± 0.4	-	100

*NC, not calculated.

Metabolism in Rabbit

Species:	Rabbit (New Zealand)
Gender/Number of Subjects:	Female: 4
Feeding Condition:	Not Specified
Vehicle/Formulation:	[¹⁴ C]CP-526,555-24 at 0.25 mg/mL in water
Method of Administration:	PO
Dose (mg/kg):	10
Radioisotope:	Carbon-14
Specific Activity:	0.53 mCi/μmol

Species: Rabbit (New Zealand)	Serum
Total Time Period of Sample Collection	0-24 hr
Sample Analyzed for Metabolites	0-24 hr
Compound (as % of Sample)	% of Sample
Female	
CP-526,555	16.7 ± 4.5
M1 (Amino Acid)	4.2 ± 2.0
M2 (Amino Acid)	34.8 ± 6.4
M3c (Putative Lactam)	6.7 ± 1.0
M3d (Putative Carbonyl Metabolite)	6.4 ± 1.5
M4 (N-Carbamoyl Glucuronide)	25.8 ± 4.7
M4a (N-Hydroxy Glucuronide)	3.6 ± 2.1

Pharmacokinetics: Comparative Metabolism

Species:	Mouse (CD-1)	Rat (Sprague-Dawley)	Rabbit (New Zealand)	Monkey (Cynomolgus)	Human
Gender/N:	Male:27 Female:27	Male: 20 Female: 20	Female: 4	Male:3 Female:3	Male: 6
Feeding Condition:	Fasted	Not Specified	Not Specified	Fasted	Fasted
Vehicle/	[¹⁴ C]CP-526,555-24 at	[¹⁴ C]CP-526,555-24 at 0.56,	[¹⁴ C]CP-526,555-24 at	[¹⁴ C]CP-526,555-24 at	[¹⁴ C]CP-526,555-24; 1.0
Formulation:	0.3 mg/mL in 0.9% saline	1.5, or 3.0 mg/mL in water	0.25 mg/mL in water	0.05 mg/mL in water	mg in 240 mL water
Route:	PO	PO	PO	PO	PO
Dose (mg/kg):	3.0	3.0 or 30	10	0.08	~0.014 ^a
Radionuclide:	Carbon-14	Carbon-14	Carbon-14	Carbon-14	Carbon-14
Specific Activity:	19.5 mCi/mmol	6.1 mCi/mmol	0.53 mCi/mmol	119 mCi/mmol	21 mCi/mmol
Study Number:	DM2001-526555-042	DM2000-526555-011 DM2000-526555-026 DM2001-526555-041	DM2001-526555-044	DM2000-526555-030	DM2000-526555-031
Location	Module 4, Section 4.2.2.4	Module 4, Section 4.2.2.4	Module 4, Section 4.2.2.4	Module 4, Section 4.2.2.4	Module 4, Section 4.2.2.4

Species/ Matrix	Sample Period		Parent	M1	M2	M2a	M3	M3a	M3b	M3c	M3d	M4	M4a	M5	M6	M7
Mouse ^b	Urine 0-24h	% Dose	85/78	0.4/0.6	0.9/1.1	-	-	-	-	-	-	-	-	-	-	-
	Feces 0-72h	% Dose	7.9/13	-	-	-	-	0.3/0.1	-	-	-	-	-	-	-	-
	Plasma 0-12h	% Sample	85/85	1.3/1.4	3.6/4.0	-	-	1.3/0.9	-	-	7.0/6.6	-	-	2.4/2.6	-	-
Rat ^b	Urine 0-24h	% Dose	64/64	1.0/0.8	-	1.0/0.5	1.5/0.4	-	0.6/0.4	-	-	2.4/1.6	-	-	-	-
	Feces 0-24h	% Dose	24/20	-	-	-	-	-	-	-	-	-	-	-	-	-
	Bile 0-48h	% Sample	-	-	-	-	-	-	-	-	-	100/100	-	-	-	-
	Serum 0-8h	% Sample	80/82	-	1.4/ND	-	-	-	-	-	-	13/7.9	-	4.9/5.7	ND/3.3	0.8/1.6
Rabbit	Serum 0-24h	% Sample	17	4.2	35	-	-	-	-	6.7	6.4	26	5.6	-	-	-
Monkey	Urine 0-24h	% Dose	69	1.1	4.3	-	-	1.1	-	-	-	3.6	-	-	-	-
	Feces 0-24h	% Dose	6.3	-	0.3	-	-	-	-	-	-	-	-	-	-	-
	Bile 0-48h	% Sample	-	-	-	-	-	-	-	-	-	100	-	-	-	-
	Plasma 0-24h	% Sample	80	-	1.7	-	-	0.5	-	0.9	2.1	8.7	-	2.6	-	2.9
Human	Urine 0-72h	% Dose	81	-	-	-	-	-	2.9	-	-	3.6	-	-	-	-
	Feces NP ^c	% Dose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Serum 0-24h	% Sample	91	-	-	-	-	-	-	1.1	-	3.8	-	0.9	-	3.5

^aMales/Females. ^bND, not detected. ^cNP, not profiled; only 0.9% of the dose was present in human feces. ^dAssumes 70 kg human

Metabolizing Enzymes

Type of Study: Inhibition of cytochrome P450 probe substrate metabolism in human liver microsomes

Method: Effect of CP-526,555 on metabolite formation from probe P450 substrates assessed by percent inhibition in human liver microsomes

Tabulated Results:

Marker Substrate Activity	Enzyme	% of control at	
		III = 30 μM	IC ₅₀ (μM)
Phenacetin O-Deethylase	CYP1A2	100	>30
Coumarin 7-Hydroxylase	CYP2A6	110	>30
Bupropion Hydroxylase	CYP2B6	99	>30
Amodiaquine N-Deethylase	CYP2C8	99	>30
Diclofenac 4'-Hydroxylase	CYP2C9	93	>30
S-Mephenytoin 4'-Hydroxylase	CYP2C19	100	>30
Dextrometorphan O-Deethylase	CYP2D6	96	>30
Chlorzoxazone 6-Hydroxylase	CYP2E1	100	>30
Felodipine Oxidase	CYP3A	75	>30
Midazolam 1'-Hydroxylase	CYP3A	90	>30
Testosterone 6β-Hydroxylase	CYP3A	97	>30

Excretion in Human

Species:	Human		
Gender/Number of Subjects:	Male: 6 (3 smokers; 3 non-smokers)		
Feeding Condition:	Fasted		
Vehicle/Formulation:	[¹⁴ C]CP-526,555-24 (1.0 mg in 240 mL water)		
Method of Administration:	PO		
Dose (mg):	1.0		
Analyte:	Total Radioactivity (¹⁴ C)		
Assay:	Liquid Scintillation Counting		
Excretion Route:	Urine	Feces	Total
Time	% of Dose	% of Dose	% of Dose
0 - 24 hours	49.4 +/- 7.1	0.28 +/- 0.25	49.7 +/- 7.1
24-48 hours	22.7 +/- 2.8	0.32 +/- 0.10	23.0 +/- 2.8
48-72 hours	8.67 +/- 1.11	0.31 +/- 0.17	8.93 +/- 1.12
72-96 hours	3.75 +/- 1.36	0.09 +/- 0.11	3.79 +/- 1.36
96-120 hours	1.60 +/- 0.74		1.60 +/- 0.74
120-144 hours	0.72 +/- 0.51		0.72 +/- 0.51
Total	87.1 +/- 5.5	0.9 +/- 0.5	88.0 +/- 5.7

Excretion in Monkey

Species:	Monkey (Cynomolgus)		
Gender/Number of Animals:	Male:2 Female:2		
Feeding Condition:	Fasted		
Vehicle/Formulation:	[¹⁴ C]CP-526,555-24 at 0.05 mg/mL in water		
Method of Administration:	PO		
Dose (mg/kg):	0.08		
Analyte:	Total Radioactivity (¹⁴ C)		
Assay:	Liquid Scintillation Counting		
Excretion Route: Males	Urine	Feces	Total
Time	% of Dose	% of Dose	% of Dose
0 - 24 hours	56.3	2.46	58.8
24-48 hours	4.28	1.30	5.58
48-72 hours	3.28	0.83	4.11
72-96 hours	2.53	0.42	2.95
96-120 hours	2.16	0.50	2.66
120-144 hours	1.30	0.29	1.59
144-168 hours	1.14	0.31	1.44
168-192 hours	0.92	0.24	1.16
192-216 hours	0.86	0.32	1.18
216-240 hours	0.84	0.24	1.08
Total	73.6	6.90	80.5
Excretion Route: Females	Urine	Feces	Total
Time	% of Dose	% of Dose	% of Dose
0 - 24 hours	60.0	0.79	60.8
24-48 hours	4.00	0.89	4.89
48-72 hours	2.91	NC*	2.91
72-96 hours	1.40	1.35	2.75
96-120 hours	1.66	0.79	2.44
120-144 hours	1.09	0.61	1.70
144-168 hours	1.07	0.29	1.35
168-192 hours	0.92	0.39	1.31
192-216 hours	0.85	0.32	1.17
216-240 hours	0.66	0.18	0.84
Total	74.5	6.14	80.7
Combined Total (Males+Females)	74.1 +/- 2.3	6.52 +/- 2.54	80.6 +/- 0.2

*NC, not calculated.

Excretion in Rat

Species:	Rat (Sprague-Dawley)		
Gender/Number of Animals:	Male:5 Female:3		
Feeding Condition:	Not Specified		
Vehicle/Formulation:	[¹⁴ C]CP-526,555-24 at 0.56 mg/mL in water		
Method of Administration:	PO		
Dose (mg/kg):	3.0		
Analyte:	Total Radioactivity (¹⁴ C)		
Assay:	Liquid Scintillation Counting		
Excretion Route: Males	Urine	Feces	Total
Time	% of Dose	% of Dose	% of Dose
0 - 24 hours	68.3 +/- 5.4	21.5 +/- 5.5	89.8 +/- 1.2
24-48 hours	1.1 +/- 0.2	3.4 +/- 0.5	3.5 +/- 0.5
48-72 hours	0.2 +/- 0.1	0.2 +/- 0.1	0.3 +/- 0.1
72-96 hours	0.1 +/- 0.0	0.1 +/- 0.1	0.2 +/- 0.1
96-120 hours	0.1 +/- 0.1	0.1 +/- 0.0	0.2 +/- 0.1
120-144 hours	0.1 +/- 0.0	0.0 +/- 0.1	0.1 +/- 0.1
144-168 hours	0.1 +/- 0.1	0.0 +/- 0.1	0.1 +/- 0.2
Total	69.9 +/- 5.8	24.3 +/- 5.7	94.2 +/- 1.3
Excretion Route: Females	Urine	Feces	Total
Time	% of Dose	% of Dose	% of Dose
0 - 24 hours	63.8 +/- 15.7	12.5 +/- 1.5	76.3 +/- 16.2
24-48 hours	1.7 +/- 0.4	4.8 +/- 4.1	6.5 +/- 4.4
48-72 hours	0.3 +/- 0.3	0.8 +/- 0.9	1.1 +/- 1.1
72-96 hours	0.2 +/- 0.1	0.2 +/- 0.2	0.4 +/- 0.3
96-120 hours	0.1 +/- 0.0	1.8 +/- 2.8	1.9 +/- 2.8
120-144 hours	0.1 +/- 0.1	0.1 +/- 0.1	0.3 +/- 0.1
144-168 hours	0.1 +/- 0.1	0.1 +/- 0.0	0.2 +/- 0.1
Total	66.2 +/- 15.0	20.3 +/- 7.4	86.6 +/- 7.5
Combined Total (Males+Females)	68.1 +/- 10.3	22.3 +/- 6.3	90.4 +/- 6.4

Excretion in Mice

Species:	Mice (CD-1)		
Gender/Number of Animals:	Male:9 Female:9		
Feeding Condition:	Fasted		
Vehicle/Formulation:	[¹⁴ C]CP-526,555-24 at 0.3 mg/mL in 0.9% saline		
Method of Administration:	PO		
Dose (mg/kg):	3.0		
Analyte:	Total Radioactivity (¹⁴ C)		
Assay:	Liquid Scintillation Counting		
Excretion Route: Males	Urine	Feces	Total
Time	% of Dose	% of Dose	% of Dose
0 - 24 hours	82.3 +/- 8.5	5.51 +/- 1.38	87.8 +/- 9.9
24-48 hours	1.66 +/- 0.37	0.87 +/- 0.43	2.53 +/- 0.60
48-72 hours	0.99 +/- 0.33	0.79 +/- 0.47	1.78 +/- 0.80
72-96 hours	0.65 +/- 0.05	0.09 +/- 0.01	0.74 +/- 0.04
96-120 hours	0.31 +/- 0.06	0.48 +/- 0.08	0.79 +/- 0.14
120-144 hours	0.33 +/- 0.05	0.10 +/- 0.03	0.43 +/- 0.07
144-168 hours	0.14 +/- 0.05	0.24 +/- 0.03	0.38 +/- 0.07
168-192 hours	0.08 +/- 0.02	0.07 +/- 0.01	0.15 +/- 0.02
Total	86.4 +/- 8.8	8.15 +/- 0.89	94.6 +/- 9.6
Excretion Route: Females	Urine	Feces	Total
Time	% of Dose	% of Dose	% of Dose
0 - 24 hours	72.6 +/- 7.9	7.87 +/- 3.04	80.5 +/- 4.9
24-48 hours	3.19 +/- 1.72	2.33 +/- 1.43	5.53 +/- 2.22
48-72 hours	1.03 +/- 0.71	0.47 +/- 0.29	1.50 +/- 0.99
72-96 hours	0.90 +/- 0.54	0.12 +/- 0.09	1.02 +/- 0.60
96-120 hours	0.61 +/- 0.34	1.63 +/- 1.05	2.25 +/- 1.23
120-144 hours	1.06 +/- 0.59	0.17 +/- 0.14	1.22 +/- 0.67
144-168 hours	0.23 +/- 0.20	0.78 +/- 0.70	1.01 +/- 0.88
168-192 hours	0.21 +/- 0.13	0.11 +/- 0.08	0.32 +/- 0.19
Total	79.9 +/- 7.6	13.5 +/- 6.5	93.4 +/- 4.3
Combined Total (Males+Females)	83.1 +/- 8.2	10.8 +/- 5.1	94.4 +/- 6.7

*includes cage wash

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The systemic toxicity of the test article was studied in different non-clinical species such as mice, rats, dogs, and monkeys.

In a single dose oral toxicity study in rat, the test article was administered by the esophageal intubations. The doses used were 0, 30, 100, and 300 mg/kg. The high dose was not tolerated (clonic and tonic convulsions were observed). Clinical signs such as labored/noisy respiration, decreased activity, uncoordinated/unsteady gait, spayed hind limbs, tremors, ptosis, loose stool, hunched posture, and rough hair coat were also noted at the high dose. Clinical signs occurred on the day of dosing and were resolved by Day 2. However, ptosis, decreased activity, loose stool, and uncoordinated/unsteady gait were noted in a few animals throughout the observation period with all doses. Decrease in mean body weights (~ 0.78 - 0.92 X control) were noted at high dose from Day 5 through the end of the observation period in males; a decrease in mean body weight gain was also noted over the same time course with mean values at study end reaching ~ 0.49 - 0.58 X control. Females showed transient decrease in mean body weights at 300 mg/kg (Day 5 only); mean body weight gain in female was decreased at high dose on Days 5, 8, and 11. On Day 2, a decrease in mean total WBC counts (~ 0.49 - 0.73 X control) and in mean lymphocyte counts (~ 0.41 - 0.44 X control) were noted in males and females at high dose. All changes returned toward control by the end of the study. Plasma drug concentrations were elevated significantly by 1 hour post dose and the elevated level was maintained over the course of the day; T_{max} occurred at 1, 4, 8, or 24 hours post dose. Mean systemic exposure parameters (plasma C_{max} and AUC) increased with increasing dose, except for AUC in males, which appeared to reach a plateau between 200 and 300 mg/kg. There were no gender differences in mean exposure parameters at the 30 or 100 mg/doses. In another single dose toxicity study in the rats, the test article (tartrate salt form) was administered as a single dose by oral gavage at doses of 3 or 30 mg/kg. The purpose of this study was to bridge the previous studies which used the succinate salt form of the test article. On Day 1, mean C_{max} values were 362 and 767 ng/mL and mean AUC₀₋₂₄ values were 2330 and 15,500 ng.h/mL at 3 and 30 mg/kg, respectively. The exposure of varenicline using the tartrate salt form was similar to that observed in the previous 3-month rat toxicity study, which used the succinate salt form. The test article (succinate salt) administered to rats by oral gavage for 10 days at dose levels of 1, 10, and 100 mg/kg/day showed clinical signs like salivation, loose stool, distended abdomen, rough fur, alopecia (abdomen and/or hind limb), penile erection, urogenital staining, hunched posture, dehydration, decreased activity, labored respiration, tremors, cold-to-the-touch, and death at high dose. Decrease in mean body weights >15 % were observed at 100 mg/kg/day in males. Food consumption in these animals was decreased from Day 2 onward. The 100 mg/kg/day animals had serum chemistry changes with increases in ALT, AST, gammaglutamyl transferase, total bilirubin (TB), and blood urea nitrogen (BUN), and a decrease in total protein, albumin, globulin, cholesterol, potassium, and calcium. Hematology changes included increase in RBC, hemoglobin, hematocrit, and appearance of hypersegmented neutrophils. WBC, lymphocytes, and reticulocytes were decreased. There was a decrease in relative liver, heart, and kidney weights. Gross necropsy findings consisted of enlarged cecum and dilated stomach. Microscopic findings were slight to mild single-cell necrosis of hepatocytes and slight to marked lymphoid depletion in the spleen. The changes in the hematological parameters and histopathology might be due to the stress induced by the test article. Such changes

were not noted at low and mid dose. Mean C_{max} and AUC increased with dose, and there were no consistent gender differences in exposure parameters. At 100 mg/kg/day, mean C_{max} was 5580 ng/mL and mean AUC was 65,500 ng•h/mL. In a 6-week toxicity study in rats with the test article administered by oral gavage at doses of 0.3, 3, or 30 mg/kg/day clinical sign, decrease in body weight, and changes in the erythroid parameters were noted at high dose. Treatment-related clinical signs included salivation at the 3 and 30 mg/kg/day groups and increased incidence of alopecia at the 30 mg/kg/day group. At 30 mg/kg/day mean body weights decreased (>10%). At high dose, in females, changes in mean erythroid parameters included an increase in hemoglobin, hematocrit, and MCV counts together with a decrease in mean corpuscular hemoglobin concentration (MCHC). At mid dose in females, hematological changes consisted of an increase in MCV and a decrease in MCHC parameters. These effects returned towards control by Day 42. An increase in serum ALT was also noted at high dose in females. Cecal/colonic dilatation was also noted at necropsy at high and mid dose in males and females. C_{max} and AUC increased with dose, slight accumulation of the test article was noted at high dose. Based on increase in serum ALT, sustained decrease in body weight, and food consumption parameters at 30 mg/kg/day, 3 mg/kg/day (mean C_{max} of 334 ng/mL and mean AUC of 1730 ng•h/mL) was identified as a NOAEL in this study.

In a 3-month toxicity study in rats with the test article administered by oral gavage at doses of 3, 10, and 30 mg/kg/day clinical signs like salivation, loose stool hypoactivity etc, were observed at 10 and 30 mg/kg/day. At high dose a statistically significant decrease in the body weight, and food/ water consumption was reported; mean body weights decreased >10% of the control values, food consumption decreased >20 % of the control values during the first week and thereafter attenuated approximately 0.6-1.0 times the control values; water consumption decreased on Day 6 (>30 % of the control values). At high dose an increase in bilirubin (1.6-fold), alkaline phosphatase (2.4-fold), and ALT (2.7-fold) and a decrease in plasma proteins (~ 0.9 times of controls) were noted. Microscopic changes at high dose included cecal/clonic dilatation and minimal to mild cellular depletion of the bone marrow. The bone marrow findings were consistent with the decreases in food consumption and body weight. C_{max} and AUC increased with dose, the exposures increased between Days 1 and 47, but were comparable on Days 47 and 82. There were no gender differences. Based on the decreases in the clinical signs, body weights, food /water consumption, and effects on the hepatobiliary system at 30 mg/kg/day, 3 mg/kg/day (mean C_{max} of 906 ng/mL and mean AUC of 14,252 ng. h/mL) was established as the NOAEL in this study.

In a 6- month toxicity study in rat with the test article administered by oral gavage at 3, 10, and 30 mg/kg/day, clinical signs (reduced and/or soft feces, chromodacryorrhea, and urine staining) were noted at high dose (18/21 females and 10/21 males). At mid and low dose these clinical signs were still observed but only sporadically. Although no change in body weight gain was noted, decrease in body weight was observed in both males and females the decrease in body weight was higher on Week 1 compare to that in Week 26. The decrease in body weight might be related to the decrease in food consumption, which was also found to decrease more at Week 1 compare to Week 26. Hematological findings included changes in WBC counts at Week 13, suggesting an intrinsic stress response. The

intensity of these changes was decreased on Week 26 indicating recovery. Several changes in the serum chemistry parameters were noted. These include increase in ALT and ALKP levels which might be related to the changes in the liver function; however, no histopathological changes were noted in the liver. Increased Phosphorous levels were also noted which might be related to the kidney function, however, no changes in the histopathology of the kidney was observed. Histopathological findings are limited to jejunal epithelial vacuolation (5/15 males and 1/15 females), retinal dysplasia (1/15 males), and infiltration of foamy macrophages in the lung at high dose (2/15, 6/15, 5/15 in control, male, and females respectively). All of these changes might be related to the pharmacological effect of the compound, toxicological significance of the findings is not known. Increase in the liver weight >10% were noted in females at high dose which can be correlated with the enlarged liver observed in the macroscopic evaluation in the females from the same dose group. Increase in the exposure was noted with increasing dose, however, the increase was less than dose proportional, no gender differences were noted, accumulation was observed at all doses with increasing time. A NOAEL of 10 mg/kg/day is established for this 6-month rat study based on the decrease in body weight gain and hepatobiliary parameters (in concurrence with the Sponsor). At 10 mg/kg, C_{max} and AUC at Day 182 (end of the study period) were 906 ng/mL and 14,000 ng•hr/mL respectively.

In a single dose toxicity study in the Cynomolgus monkeys (2/sex) with the test article administered by oral gavage at doses of 0, and 3 mg/kg/day clinical signs including emesis were noted in all animals (4/4 animals, which occurred at ~1-4 hours post dose) Additionally, recumbency, decreased activity, and tremors were observed in 3/4, 2/4, and 1/4 animals, respectively, and generally were manifested for ~1-4 hours post dose. Reduced heart rates (0.80-0.84X pre-study values) were observed in 3/4 monkeys, and there were changes in electrocardiogram parameters (increased PRQ interval in 3/4, increased P wave width in 2/4, and decreased QT interval in 1/4) on day 1. Parameters returned to pre-study baseline by day 9. Systemic exposure as defined by C_{max} and AUC was 54.4 ng/mL and 670.1 ng•hr/mL, respectively in males and 50.6 ng/mL and 477.6 ng•hr/mL, respectively in females.

In single dose toxicity study the Cynomolgus monkeys (1/sex) were administered by oral gavage with a 0.1 mg/kg/day CP-526, 5554-24 and CP-526,555-01, the succinate salt and C J , respectively of CP-526,555, to compare pharmacokinetics. The 0.1 mg/kg dose was used because it did not induce emesis. The PK data obtained in the monkey was comparable for both of the salt forms.

To attain the maximal tolerated dose in the Cynomolgus monkeys, the test article (tartrate salt) was administered by intravenous infusion for 4 hours. At 50 $\mu\text{g}/\text{kg}/\text{hr}$ (Day 2), the females had emesis approximately 3.5 hours after the initiation of the infusion. At 4 hours after initiation, both animals showed tremors that were generalized in the male, but involved only the hind limbs in the female. At the same time, the male was hypoactive and showed muscle rigidity discernable as clenched fists and an inability to extend the digits. This muscle rigidity was present for about 15 minutes. At approximately 6 hours after the initiation of the 4-hour infusion 2 hours after it was discontinued the male was

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still hypoactive, although tremor and muscle rigidity were no longer present. The female continued to show hind limb tremors, but was also hypoactive and had muscle rigidity. In the females, muscle rigidity was manifested as a clenching of the feet. The feet would clench, relax, and clench again. All signs of hypoactivity, tremor, or muscle rigidity had resolved in both animals by 24 hours post initiation of infusion. At 75 µg/kg/hr (Day 3), the male showed emesis and generalized tremors at 3 hours post initiation of infusion. Because the signs were expected to increase in severity if the infusion was allowed to continue, dosing was stopped. Hypoactivity was the only drug-related sign at 4 and 6 hours after infusion initiation. The female had episodes of emesis at approximately 2, 2.5, 3, and 3.5 hours post-initiation, and bruxism (grinding of the teeth) at 3.5 hours post-initiation. Bruxism may have been an indication of abdominal pain. At 4 hours post-initiation there were no drug-related signs in the female; however, hypoactivity, tremors, and muscle rigidity were present at 6 hours post-initiation. Similar to Day 2 in the female, the tremors involved the hind limbs, and muscle rigidity was described as foot clenching. At 50 µg/kg/hr, all signs of hypoactivity, tremor, or muscle rigidity had resolved in both animals by 24 hours post-initiation of infusion. Red blood cells (RBC), hematocrit, and hemoglobin were decreased 12% to 15% in the male and 22% to 24% in the female. Changes in morphology of RBC were also noted (poikilocytosis and few Burr cells). The female had a 48% increase in white blood cells (WBC) due primarily to increases in absolute neutrophils (73%), lymphocytes (27%), and monocytes (3.1-fold). The changes in WBC parameters may be a response to temporary placement of an indwelling catheter. Aspartate aminotransferase (AST) activity increased 67% and 176% in the male and female, respectively. The male also had a minor increase in alanine aminotransferase (3-fold). Decreases in total protein (15% - 20%) and albumin (23% - 28%) were seen in both animals, consistent with the observed emesis and reduced food consumption. Results of serum concentration analyses showed CP-526,555 levels increased with increasing dose and with time post-initiation of infusion. The levels achieved 4 hours after the initiation of infusion at 50 µg/kg/hr ([] ng/mL in the male and female, respectively) were close to the target serum concentration of 60 ng/mL.

The intravenous infusion study in the Cynomolgus monkey was repeated with a lower dose (0.25 mg/kg) to find out a well tolerated dose for the primates. In this study, serum chemistry parameters were found to alter in the monkeys. Mean CK values were increased approximately 48- and 57-fold compared with pretest in drug-treated males and females, respectively, compared with 2-fold and 7-fold increases in male and female controls. Elevations in CK, AST and ALT after chair restraint have been reported in rhesus monkeys. CK activity was within the normal historical range for the laboratory in all animals evaluated at Day 15. One male demonstrated elevated AST and ALT on Day 2 representing 9.9- and 9.4-fold above pretest sample values, respectively. One drug-treated female had milder elevations of AST and ALT on Day 2 (2.2-fold and approximately 1-fold, respectively). The male was euthanized Day 2 and reversal information was not available, but in the female, AST and ALT values returned to normal range at Day 15. The mean C_{max} was 31.7 ng/mL and the mean AUC₍₀₋₂₄₎ was 379 ng. hr/mL. The systemic exposure returned to base line at 24 hrs.

In a dose escalation study the Cynomolgus monkeys were administered by oral gavage with 0.1, 0.2, 0.3, 0.4 and 1 mg/kg/day doses for 5 days. Emesis and loose stool were identified as the main clinical signs related to the test article. These clinical signs were observed at the dose 0.3 mg/kg and above. The toxicokinetics analysis showed accumulation (1.5-fold-3-fold) in all doses. The timing of emesis coincided with the Cmax indicating a direct treatment related effect of the compound.

In a dose range finding study the Cynomolgus monkeys were dosed with 0.25, 0.5, and 1 mg/kg/day with the test article (succinate salt) for 10 consecutive days by oral gavage. The animals were then observed for another 5 days. All treated animals showed multiple incidence of emesis. The incidence of emesis increased with increasing dosages. Hunched postures were noted at high dose. Loose stool were also noted in all treated animals. The observed clinical signs were clearly dose related, therefore, may be considered as treatment related. Toxicokinetics data showed increased accumulation (2-5-fold) of the compound at Day 10 with all dosages, exposure increased with increasing dose, no gender differences were noted. The test article was not well tolerated even at low dose (0.25 mg/kg).

In a one month oral dose range finding study with 14-Day recovery phase in the Cynomolgus monkeys, the test article (tartrate salt) were not well tolerated at doses 0.4 and 0.6 mg/kg/day for 28 days as indicated by the occurrence of emesis, loose stool, hypothermia, and dehydration. Modulation of WBCs were observed at intermediate dose suggesting a stress response, an increase in fibrinogen level was also noted which is indicative of an inflammatory response. Toxicokinetics studies showed dose related increase in exposure; accumulation was noted at all dosages with the increase in the duration of the study.

In a six week oral toxicity study with the test article (succinate salt) the Cynomolgus monkeys, showed changes in clinical signs and hematological parameters. Emesis and loose stool were observed at all dosages in this study (0.1, 0.05, and 0.2 mg/kg/day administered daily by oral gavage for 6 weeks), therefore no NOEL could be established. WBC related hematological changes and histopathological changes related to mononuclear cell infiltrations in different organs were noted at high dose. However, due to lack of dose response, microscopic findings could not be positively correlated as a toxicological finding. A NOAEL of 0.2 mg/kg/day is established for this study which is in concurrence with the Sponsor's proposal.

Cynomolgus monkeys administered with the succinate salt of the test article at different dosages (0.01, 0.05, 0.2 mg/kg) by oral gavage showed treatment related clinical signs. The clinical signs include loose stool in 2/3, 3/3, 1/3 females at low mid and high dose respectively. Emeses were also noted, however, the incidence of emeses were sporadic. No NOEL could be established. One female at mid dose showed 1.5-fold dilation of the colon (no histopathological correlation); the biological significance of the finding can not be determined due to the lack of the dose response. However, the same animal was found to have multiple incidences of loose stool and emesis, therefore, the finding may be treatment related. Toxicokinetics analysis showed dose related increase in the Cmax and

the AUC, no gender difference was noted. Accumulation up to 2-fold was noted with all different dosage when the AUC from the Day 1 was compared to the AUC from day 78. The clinical observations in this 3-month study were found to instigate around Day 30. Higher exposure due to accumulation of the test compound may be suggested as one of the reasons for this late initiation of the toxic findings. Changes in the WBC related hematological parameters were noted. The changes in the blood cell counts were within the historical control range, however, the trend in WBC changes suggest a stress response and may be related to inflammatory response. A decrease (31%) in the blood urea level was noted in females at high dose, the biological significance of this finding needs to be further evaluated. The dose related histopathological findings in the animals were limited to the infiltration of the mononuclear cells in different organs indicating an inflammatory response. At high dose myocardial degeneration were noted in 1/3 males; severity index for this finding was designated as minimal. A NOAEL of 0.2 mg/kg/day (HED=3.8 mg) is established for this study as proposed by the Sponsor.

No NOEL could be determined for the 9-month toxicity study in the monkey in which the test article CP-526, 555 (tartrate salt) was administered by oral gavage at the dose of 0.2, 0.4, 1.2 mg/kg/day due to the clinical observations of increased incidence of emesis and loose stools in the treatment groups. The test article was not tolerated at the high dose that was tested under this experimental condition. All animals at high dose were either euthanized due to >15 % loss in body weight by Day 55 or the treatment was terminated for observing the animals for recovery. At mid dose one female was found dead at Day 126, megacolon was observed in this animal after macroscopic and microscopic observation. Sponsor believes that this finding, although rare in monkey, may be an incidental observation. However, megacolon is related to volvulus which might be related to GIT retention and related problems. Loose stools and inherent pharmacological effect of this test article. Increased incidence of loose stools were observed in this animal, therefore, this observation may be treatment related. Hematological changes compared to control (within the historical control range) were noted in this study at mid and high dose. Infiltration of the mononuclear cells in males (2/3, 1/3, and 3/3 at control, 0.2, and 0.4 mg/kg respectively) was the only dose related histopathological finding observed in this study. Biological significance of this finding is not known. A NOAEL of 0.2 mg/kg/day was established for the monkey under this experimental condition based on the nature of the toxicity finding (Sponsor established a NOAEL of 0.4 mg/kg/day based on their conclusion that the megacolon observed in one female was an incidental finding).

No NOEL could be established for the test article CP-526,555 (tartrate salt) in monkeys (nasogastral intubations) due to the sporadic incidence of emesis and loose stools in all dose groups (0.01, 0.05, 0.2 (0.1 bid) mg/ kg/ day for 39 weeks). Lymphocytic infiltrations were noted in different tissues (trachea, pancreas, thyroid, salivary gland), this effect was dose related. Other histopathological findings include increase incidence of chronic inflammation in females (2/4 in control vs 3/4 at high dose), increase incidence of mineralization in ovary, and increased incidence of cyst formation in the pituitary and parathyroid in females at high dose. Modulation of the hematological parameters (increase in the monocytes and the lymphocyte counts etc. compared to those of the

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controls) The hematological changes were within the historical control range, however, the increased incidence compare to the respective control suggests a stress response. Dose related increase in the exposure were noticed, no gender differences were found, approximately 1.5-fold accumulation of the test article was noted at the steady state. Based on the nature of the toxicity findings a NOAEL of 0.2 mg/kg/day (HED=3.8 mg) is established for 9-month monkey study (this is in concurrence with the Sponsor's NOAEL).

CD 1 mice treated with the test article (tatrane salt) by oral gavage at the doses of 0, 1, 10, 100 mg/kg / day for 2 weeks showed clinical signs of labored breathing and sternal recumbence at high dose. One male and two females died at low and high dose respectively, the cause of death is not known. Transient decrease in body weights gains was noted around Day 4; a concomitant decrease in food consumption was reported at the same time. Hematology parameters were measured on Day 15. In the two 100 mg/kg/day females that had sample quantities sufficient for analysis, there were changes in mean erythroid parameters that included decreases in mean red blood cell count (~0.70X control), hemoglobin (~0.75X control), and hematocrit (~0.74X control) and increases in mean corpuscular volume (~1.06X control), mean corpuscular hemoglobin (~1.07X control), and mean reticulocyte count (~4.5X control). One of these animals showed a stress leukogram characterized by decreases in total white blood cell and lymphocyte counts (values were ~0.43 and 0.29X mean control values, respectively). Changes in hematology parameters in 100 mg/kg/d females were considered treatment-related. No changes were observed at low and mid dose. Clinical chemistry parameters were measured on Day 15. In 100 mg/kg/day females, there was a decrease (~0.76X control) in mean serum potassium concentration that was considered treatment-related, although mean values remained within the historical control range for this laboratory. No changes were observed at low and mid dose. A NOAEL of 10 mg/kg (HED= 48.8 mg) was established for the mouse in this study.

CD 1 mice treated with the test article (tatrane salt) by oral gavage at the doses of 0, 1, 10, 100 mg/kg / day for 2 weeks showed clinical signs of labored breathing and sternal recumbence at high dose. One male and two females died at low and high dose respectively, the cause of death is not known. Transient decrease in body weights gains was noted around Day 4; a concomitant decrease in food consumption was reported at the same time. Hematology parameters were measured on Day 15. In the two 100 mg/kg/day females that had sample quantities sufficient for analysis, there were changes in mean erythroid parameters that included decreases in mean red blood cell count (~0.70X control), hemoglobin (~0.75X control), and hematocrit (~0.74X control) and increases in mean corpuscular volume (~1.06X control), mean corpuscular hemoglobin (~1.07X control), and mean reticulocyte count (~4.5X control). One of these animals showed a stress leukogram characterized by decreases in total white blood cell and lymphocyte counts (values were ~0.43 and 0.29X mean control values, respectively). Changes in hematology parameters in 100 mg/kg/d females were considered treatment-related. No changes were observed at low and mid dose. Clinical chemistry parameters were measured on Day 15. In 100 mg/kg/day females, there was a decrease (~0.76X control) in mean serum potassium concentration that was considered treatment-related, although mean values remained within the historical control range for this laboratory. No

changes were observed at low and mid dose. A NOAEL of 10 mg/kg (HED= 48.8 mg) was established for the mouse in this study. On Day 15, Cmax and AUC were 566 ng/mL and 5,460 ng•h/mL respectively at NOAEL dose.

CD-1 mice administered with the test article (ttrate salt) at doses of 3, 25, 75, or 150 mg/kg/day for 92 consecutive days showed treatment-related clinical signs included decreased activity at 25 mg/kg/day, cold to touch and tremors at 75 mg/kg/day, and labored respiration, convulsions, and hunched posture at 150 mg/kg/day. Death occurred at doses 25 mg/kg/day and the incidence of treatment-related deaths increased with dose (1/23, 3/24, and 18/24 at 25, 75, and 150 mg/kg/day, respectively). There were sustained, treatment-related decreases in mean body weights in 150 mg/kg/day animals. There were treatment-related changes in erythroid parameters (decreases in RBC count, hemoglobin, hematocrit, and MCHC; increases in MCH, MCV, and reticulocyte count) at the 150 mg/kg/day dose on Day 44, and they were consistent with increased RBC turnover. There also were treatment-related decreases in mean total WBC and lymphocyte counts on Day 44, effects consistent with a stress response. Changes were reversible as parameters returned toward control on Day 93, except for mean lymphocyte counts in females, which remained decreased. Increases in serum potassium and chloride in one 150 mg/kg/day female were considered treatment-related. Treatment-related microscopic findings included hepatocellular (single-cell) necrosis in 4 animals at 150 mg/kg/day. Treatment-related necropsy findings included an enlarged stomach with or without an enlarged pylorus in two 150 mg/kg/day animals; there was no microscopic correlate for this finding. Systemic exposure as defined by Cmax and AUC₀₋₂₄ increased with increasing dose, and there were no consistent gender differences in exposure. A NOAEL of 3 mg/kg/day was established in this 3-month study in mice with based on the mortality. Mean Cmax and AUC₀₋₂₄ were 358 ng/mL and 1560 ng•h/mL, respectively, at the NOAEL on Day 91.

An exploratory oral dose escalation study was done in the Beagle dogs with of CP-526,555-01. Emesis were noted in dogs after the oral administration of the test article at different doses (0.05, 0.1, 0.3, and 1 mg/kg/day). Slight decrease in body weight, 4-5 % was noted in the dogs at the high dose group. Hematological changes (increased neutrophils and monocytes 1.6-3.9x) were noted in the high dose group animals at Day 2, indicating a stress response. No non emetic dose for this test article could be determined under this experimental condition in the dogs.

Genetic toxicology:

The test article was studied for clastogenic activity in vitro in human lymphocyte cultures. Chromosome damage was evaluated after 3 hours with and without metabolic activation at concentrations ranging from 630 to 1230 µg/mL and 984 to 1540 µg/mL respectively. Chromosome damage was evaluated after 24 hours without metabolic activation at concentrations ranging from 322 to 600 µg/mL. In all the tests, the highest concentrations produced a marked (43% to 56%) reduction of the mitotic index. No increase in chromosome damage was observed at any concentration evaluated

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The test article was studied for the induction of mutation in the bacterial strains TA 1535, TA 1537, TA 98, and TA 100 and *Escherichia coli* strain WP2uvrA pKM101. Varenicline was dissolved in dimethylsulfoxide (DMSO) and tested in either the presence or absence of a metabolic activation system using liver S9 fraction from Aroclor-1254-induced rats. No evidence of dose-related increases in the number of revertant colonies compared to the negative controls with any of the strains tested in either the absence or presence of S9 metabolic activation were observed.

CP,-526,555 tested in the gene mutational activity in the CHO/HGPRT assay. Definitive tests were conducted over concentrations ranging from 500 up to the maximum applied dose of 5000 µg/mL in the absence and presence S9 metabolic activation. No dose-dependent mutagenic response was induced by the test article either in the absence or presence of metabolic activation.

The test article was studied for the induction of micronuclei in male and female rat bone marrow cells. Male and female rats were administered with the test article (0, 25, 50, and 100 mg/kg/day) by oral gavage for 3 days. Bone marrow smears were evaluated for Dose-dependent decreases in polychromatic erythrocytes (PCE) and PCE were further evaluated for induction of micronuclei. There were no significant increases in the numbers of PCE with micronuclei in either males or females the mean male/female C_{max} (1 hour) and AUC₀₋₂₄ exposures at 100 mg/kg/day were 5,569/6,169 ng/mL and 65,187/68,884 ng•h/mL, respectively. The test article was not clastogenic in rats when tested up to an MTD of 100 mg/kg/day.

Carcinogenicity:

A 2 years carcinogenicity study was done in SD rats (oral gavage 1, 5, 15 mg/kg/day). The study was adequate for carcinogenicity evaluation There was a slight (not statistically significant) decrease in the survivability for the high dose group males compared to that in the control group (35% in high dose vs. 47 % in control). Survivability of females in the high dose group was approximately similar to that in the controls. This indicates that the compound do not have any effect on the survivability. The mean body weight gain decreased (statistically significant) in males for the 1, 5, and 15 mg/kg/day by 10, 13 and 17 % respectively at termination relative to the mean control body weights. The mean body weight gain in females was found to be decrease by 2 and 16% at termination for the mid and the low dose group respectively relative to the mean control body weights. The decrease in body weight might be correlated with the food consumption as observed in this experiment. The >15 % decrease in body weight suggests that the MTD might have been reached under this experimental condition. No statistically significant neoplastic or non neoplastic lesions were observed in this study. Hibernoma (a neoplasm of the brown adipose tissue) was observed in 1/65 and 2/65 males in the mid and high dose group respectively. Hibernoma in the high dose group was found to be malignant. All tumors were found in the mediastinum of the thorax. Due to the rarity of the tumor in the rats, the finding is considered treatment related. The P-value = 0.03 for the hibernoma noted in this study; for the rare tumor findings according to the ICH guidelines statistical significance should be ≤0.25,

therefore this finding can not be considered as statistically significant. The gross findings and the histopathology at the time of necropsy correlated to incidental neoplastic and nonneoplastic microscopic changes in pituitary gland and skin (tail and paws) of males and females and the mammary gland of females. The above mentioned incidental findings were determined to be the major cause of the death under this experimental condition. There were adequate dose related exposure in all dose groups, the compound was found to increase in exposure with increasing dose in a less than dose proportional manner, accumulation was noted in all dose groups, and no gender differences were noted. The AUC values at 15 mg/kg were 673.9 ng•hr/mL, approximately 5.7-fold human exposure based on MRHD of 1mg/day with AUC value of 116.8 ng•h/mL.

A 2 year mouse toxicity study was completed by the Sponsor (1, 5, 20 mg/kg/day, oral gavage). The study is reviewed and evaluated to be adequate. Survival decreased in males for the 20 mg/kg/day as compared to the male control group ($P < 0.001$). This decrease in survival was initially noted at approximately Week 27. This indicates that the MTD might have been exceeded slightly for the 20 mg/kg/day group of males; there were 33 of 65 (51%) surviving in the group at Week 80. The dosing for the high dose group was terminated at Week 93 (as per guidance from the Division), 23% of the mice from the high dose group males survived the rest of the study period (105 weeks). Slight increase in the survival of females was noted at study termination (105 weeks). No statistically significant, dose-related trend in the neoplastic findings at the 0.005 level (for common neoplasms) or the 0.025 level (for rare neoplasms) was observed in this study. The most common fatal neoplasm across all groups was malignant lymphoma. The most common identifiable nonneoplastic causes of unscheduled death in males were inflammation of the urinary tract, sepsis, and systemic amyloidosis; systemic amyloidosis was the most common nonneoplastic cause of unscheduled death for females. No significant changes in the body weight gains and food consumption were noted at termination. Slightly increased incidences of rough hair coat in males and swollen abdomen was noted in female, these observations might be related to the pharmacological effect of the compound. Amyloidosis was observed in several organs in both males and females. The incidence of systemic amyloidosis was greater in treated animals than in controls; however, the increase was not dose-related. The cause of this increase is unclear. Systemic amyloidosis is known to occur commonly as a spontaneous lesion with variable incidence in CD-1 mice of both sexes as noted by the sponsor and confirmed by the reviewer. Inflammation was also observed in different tissues. Amyloidosis induced by the inflammatory mediators is a well-known biological phenomenon. Considering the effect of the compound in the hematological parameters, treatment related effect on the amyloidosis could not be eliminated. There were no statistically significant benign tumor findings in male and female mice. The non neoplastic tumors found in the treated mice (and not in the control) is the sub capsular cell adenoma in females (1/65 in low and high dose female) and Leydig cell adenoma in the epididymis in 1/65 male in the mid dose group. The tumors are not considered as rare tumor and the findings are not statistically significant. However, the findings may be treatment related due to the increase in the incidence in the treatment group compared to those of the controls. The biological significance of the findings is unknown. The neoplastic tumors found in the treated female mice (and not in the control) were the jejunum adenocarcinoma (1/65 in mid

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dose), mast cell neoplasm (1/65 in mid dose), adenosarcoma in cervix (1/65, 2/65, 1/65 in low, mid and high dose respectively), basal cell carcinoma in skin (1/65 in mid dose) and ovarian carcinoma (1/65 in high dose). The ovarian carcinoma due to its occurrence at high dose may be considered as treatment related, however, the biological significance of this finding is not known. The other tumors mentioned above might not be treatment related due to the lack of dose response in those findings. In males, leukemia was seen in one male at high dose which might be treatment related, however, the biological significance of the finding is unknown. Adenosarcoma of the stomach was noted in one male from the mid dose group, no such finding was noted at high dose group, therefore, this finding might not be treatment related. Exposures generally increased with dose in a dose proportional manner. No apparent gender difference was noted. The AUC values at 20 mg/kg were 1790 ng•hr/mL, approximately 15-fold human exposure based on MRHD of 1mg/day with AUC value of 116.8 ng•h/mL.

Reproductive toxicology:

In the Segment I reproductive toxicity study with female Sprague -Dawley rats, the test article (0, 0.3, 3, 15 mg/kg/day) was administered by oral gavage for 14 days. The compound was administered prior to mating, during cohabitation, and through gestation day 7. The result show decreased body weight and increased clinical signs in dams at high dose group. A statistically significant decrease in the maternal body weight gain was observed in the high dose group females between Days 0-6. This observation is considered treatment related, no change in body weight gains was observed in the low and the mid dose group females. In high dose females, post dose salivation, irregular respiration, decreased activity and ptosis were noted. These observations were noted in toxicity studies with the compound and are considered treatment related. The females from the low and mid dose group and the males did not show any abnormal clinical signs. A NOAEL of 3 mg/kg/day (HED=29 mg) was determined for the maternal toxicity. A decrease in the total estrus cycle, average estrous cycle length was noted in the females from the high dose groups as compared to those of the controls. This effect was also seen in low dose group, but not in the mid dose group. The number of females with the insufficient number of the estrous cycles, increased for the low and the high dose group animals but not in the mid dose group. Although there were no changes in the copulatory rate in the treated compared to those in controls, the rate of the pregnancy decreased in the treated group. The decrease in pregnancy rate might be related to the changes in the estrous cycle observed in the animals from the low and high dose group. A NOAEL of 15 mg/kg/day (HED=290 mg) was established for the early embryonic toxicity for the test article in this study, but due to the decrease in the fertility rate related to the changes in the estrus cycle, no NOAEL for fertility could be established in this study. Serum level at the NOAEL dose was found to be approximately 540 ng/mL in rats (MRHD=4.5x). Dose proportional increase (approximately 10-fold) in the serum levels of the test article was noted from 0.3 (21±4.8 ng/mL) to 3.0 (198 ±40 ng/mL) mg/kg/day. A less than dose proportional increase (approximately 2.5-fold) in the serum levels of the test article was noted from 3.0 (198 ± 40 ng/mL) to 15 (500 ± 40 ng/mL) mg/kg/day.

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In a Segment II reproductive study in the pregnant rabbits, the test article was administered by oral gavage (1, 10, 30 mg/kg/day) from the Gestation Day 7- 19. The result showed decreased body weight gain in dams and fetus at high dose. One animal from high dose group was found dead on Day 7 of gestation within 10 minutes of dosing. The death was related to the acute cardiac and respiratory failure (uncollapsed lung with multiple dark areas, epicardial pale areas, dark areas in the thymus and skeletal muscles were noted). This incident might be related to the error in the oral gavage but the cause of the death could not be confirmed. Soft feces, thinning of the fur, and red staining of the fur were noted in the dams in the treated groups. These clinical signs were found to increase in a dose dependent manner and were determined to be treatment related. A statistically significant loss in the body weight gain was noted on gestation Day 19 and 20 for the high dose group females ($P < 0.01$). Significant loss of body weight was noted in the high dose group females from gestation Day 9. This trend continued throughout the treatment period and was observed in the mid dose group animal in a lesser extent. No such changes were noted for the low dose group animals. A NOAEL of 1 mg/kg was determined for the maternal toxicity based on the clinical findings and decrease in the body weight gain. There were no statistically significant major malformations, minor external visceral or skeletal anomalies or skeletal variants for any of the fetuses that were considered to be treatment-related. One fetus from the high dose group showed gastrochisis, this incidence might be related to the pharmacological effect of the test article. However, the low incidence ($< 1\%$ fetus affected) and due to the fact that the incidence was within the historical control range (0-2.8%), the effect is considered incidental. Fetal (13-15 %) and placental weights ($> 12\%$) were significantly lowered at 30 mg/kg/day in animals from the high dose group. There was no treatment related effect on fetal weight at 1 or 10 mg/kg/day. A decreasing trend in the placental weight was observed at low and mid dose; this decrease, however, was not statistically significant. A NOAEL of 10 mg/kg (HED= 193 mg) was determined for the fetal toxicity in rabbit based on the decrease in the body weight gain in fetus from the high dose group. Serum concentration of the dams at NOAEL dose was found to be 4400 ng·h/mL (MRHD=37 x) Serum exposure of the test article was determined in the dams after 13 days of dosing (Day 7-Day19). Exposure in rabbit was found to be more than dose proportional at low doses; a 10-fold increase in dose (1-10mg/kg/day) resulted in approximately 16-fold increase in AUC; however increase in Cmax at this time was 6-fold only. Increase in AUC with 5-fold increase (10-15 mg/kg/day) in dose was approximately 2-fold and increase in Cmax at this time point was approximately 3-fold. Placental crossing of the test article was confirmed by the fetal absorption of the test article. The serum level of the compound was higher in fetus than maternal serum concentration at low and mid dose. However, at high dose, the serum concentration of the test article was found to be similar in dams and fetus.

In a Segment II reproductive toxicity study in the Sprague-Dawley rats the test article was administered by oral gavage (0, 0.3, 5, 15 mg/kg/day) from Gestation Day 6-17. The result showed a decrease in the maternal body weight ($> 10\%$) and food consumption significantly ($P < 0.01$). Increased clinical signs of salivation were also noted in dams from high dose group. Decrease in body weight and salivation was also noted in the mid dose group. A NOAEL of 0.3 mg/kg was determined for maternal toxicity based on the

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above findings. No change in the reproductive parameters was noted in females. No changes in fetal body weight were noted. Following changes were noted in fetuses. One fetus from the 0.3 mg/kg/day group had anophthalmia. One fetus from the 15 mg/kg/day group showed anal atresia and acaudia, and another fetus from this same group had sites inverses. These incidences were within the historical control range. A small number of fetuses in the 0.3 mg/kg/day group showed one of the following findings oval shaped lens (5/149), absence of the innominate artery (2/149) or dilated ureters (2/149). No such changes were noted in animals from the mid and high dose group. The incidence of fetuses with reduced ossification of the hyoid bone was significantly increased for the 0.3, and 15 mg/kg/day groups (percent affected was 51 % and 49% for the 0.3 and 15 mg/kg/day groups, respectively). These values, however, fell within the historical control data range (percent fetuses with reduced ossification of hyoid bone ranged from 0 to 71 %), the number of litters affected was not significantly different between the control and treated groups. There were no significant differences between the control and treated groups for common visceral and skeletal findings. A NOAEL of 15 mg/kg was established for rat teratology in this study. Exposure in rat was found to be dose proportional at low doses; a 10-fold increase in dose (1-10 mg/kg/day) resulted in approximately 11 and 10-fold increase in serum levels at Day 17 and 20 respectively. Increase in serum levels with 3-fold increase (10-15 mg/kg/day) in dose was approximately 2 and 1.5-fold at Day 17 and Day 20 respectively. Fetus absorbed the test article. The serum level of the compound was higher in fetus than maternal serum concentration at all doses. The result suggests that the placental transport of the test article is not concentration dependent.

In a Segment III reproductive toxicity studies with CP-526,555 was administered to pregnant rats via oral gavage (0, 0.3, 3, and 15 mg/kg) from Gestation Day 6-Lactation Day 20. Decreased body weight and food consumption and increased clinical signs (salivation respiratory stress) in the dams for 3 and 15 mg/kg indicating maternal toxicity, however <4% body weight loss was seen in the mid dose; therefore a NOAEL of 3 mg/kg was established for the maternal toxicity (not in concurrence w/Sponsor). No reproductive toxicity was reported for the F₀ females. F₁ fetuses from the high dose group showed significant reduction in body weight. Auditory startle response significantly decreased in F₁ fetus. Increase in latency time (unknown biological significance) was noted in F₁ fetus. Fertility in F₁ fetus reduced (60-80%) significantly at high dose. A NOAEL of 3 mg/kg was established in F₁ fetus based on physical behavior noted and fertility (in concurrence w/Sponsor). CP-526,555 serum concentration in F₁ was found to be 21 ng•h/mL at Day 6 of lactation (MRHD 0.4x) with the NOAEL dose. The test article was passed to the fetus via milk, dose proportionately and continuously (no Tmax observed for fetuses at least up to 8 hrs post dose) in the same amount.

Local Tolerance:

Photo toxicity: In a phototoxicity study, the test compound was administered orally for 3- days, with the MTD dose (100 mg/kg/day). No evidence of cutaneous phototoxicity was observed at termination or on any of the three days of observation after the dose administration and UVR exposure. The rats administered with the positive control

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lomefloxacin or 8-methoxypsoralen once followed by a single UVR exposure had cutaneous responses of erythema and/or edema in the lightly and/or darkly pigmented skin sites that were exposed to UVR. These responses are indicative of a phototoxic response to these comparator articles indicating that the experiment was valid.

Acute Dermal Toxicity (Limit Test): Sprague-Dawley rat were administered with 2000 mg/kg of the test article formulated in the deionized water dermally. A piece of surgical gauze was placed over the treatment area and semi-occluded with a piece of self-adhesive bandage. After removal of the dressings and subsequently once daily for fourteen days, the test sites were examined for evidence of primary irritation and scored according to the following Draize scale. There were no signs of erythma indicating no dermal irritation.

Acute Eye Irritation in the Rabbit: Three New Zealand white rabbits were treated with a volume of 0.1 mL of the test material, (45 mg) by an adapted syringe that was placed into the conjunctival sac of the right eye, formed by gently pulling the lower lid away from the eyeball. The left eye remained untreated and was used for control purposes. Immediately after administration of the test material, an assessment of the initial pain reaction was made according to the six point scale shown below. Assessment of ocular damage/irritation was made approximately 1 hour and 24, 48 and 72 hours following treatment, according to the numerical evaluation given below (Draize scale). An additional observation was made in one treated eye on Day 7 to assess the reversibility of the findings.

The test article was determined to be a mild irritant at 45 mg in rabbits, conjunctivae (chemosis, redness, and discharge) and iridial irritation was noted in all of the three rabbits those were treated.

Acute Dermal Irritation in the Rabbit: Three New Zealand white rabbits were used for this study. At each test site a quantity of 0.5 gm of the test material was introduced onto 2.5 cm x 2.5 cm cotton gauze patch, moistened with 0.5 ml of distilled water and then placed in position on the shaven skin. One patch was applied to the back of each rabbit, and was allowed to remain in contact with the skin for a period of four hours. Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the Draize scale. The test material produced a primary irritation index of 0.2 and was classified as a mild irritant to rabbit skin according to the Draize classification scheme. No corrosive effects were noted.

Skin Sensitization in the Guinea Pig -Magnusson and Klingman Maximization

Method: Ten test and five control male albino Dunkin Hartley guinea pigs were used. Intradermal injections (0.1 mL/injection site) were made on the clipped shoulder of one guinea pig using a concentration of 5% w/w in distilled water. The degree of erythema at the injection sites was assessed approximately 24, 48, 72 hours and 7 days after injection according to the Draize scale. Two phases were involved in the main study; an induction of a response by intradermal injection and topical application and a topical challenge of that response. Based on the results of sighting tests, the concentrations of test material for the induction and challenge phases were selected as follows: intradermal induction of

5% w/w in distilled water; topical induction with 50% w/w in distilled water; topical challenge 25% and 10% w/w in distilled water. The test material produced a 0% (0/9) sensitization rate and was classified as a non-sensitizer to guinea pig skin under the conditions of the test. The local toxicity studies indicate that the CP-526,555 have less probability of effecting eye and skin when taken orally, however, dermal or ocular contact with the compound might cause irritation in human.

Special toxicology: No special toxicity studies were done with the test article.

2.6.6.2 Single-dose toxicity

Study Title and Study Number: Exploratory Single Dose Oral Pharmacokinetic Study of CP-526, 555-18 in Rats; 00-1545-26

Rats (CD (SD) IGS BR) were administered with CP 526,555-18 (Lot 50452-17-5MS) as a single dose by oral gavage. Two dosages: 3 and 30 mg/kg were used. 3-animals/sex/dose groups were used. Clinical signs were observed at Day 1 and 2 of the experiment and body weight was measured and body weights were recorded twice pre-treatment and on Day 1. Serum CP-526, 555 concentrations were measured with each dose a different time points on Day 1 (at 1, 4, 8, and 24 hours post-dose).

Key Study Findings:

- On Day 1, mean C_{max} values were 362 and 767 ng/mL and mean AUC 0-24h values were 2330 and 15,500 ng-h/mL at 3 and 30 mg/kg, respectively. No gender difference was noted.
- No changes in the clinical signs were noticed during this acute experiment.

This experiment was done with the tartarate salt. Sponsor mentioned that these values were similar to those of the previous 3-month rat toxicity study using the succinate salt form in which mean C_{max} values were 286 and 751 ng/mL and mean AUC_{0-24h} values were 3240 and 14500 ng-h/mL on Day 1 at 3 and 30 mg/kg, respectively. The 3-month study with the succinate salt is being reviewed under this submission and the reviewer made similar observation.

Study Title and Study Number: Exploratory Single Dose Oral Pharmacokinetic Study of CP-526, 555-18 in Monkeys; 1545-27

Cynomolgus monkeys were administered with CP-526, 555-18 (Lot 50452-17-5MS) by oral gavage as a single dose at 0.01 mg/kg or at a dose of 0.2 (0.1 BID; 6 hours between doses) mg/kg for 1 day. Clinical signs were observed at day 1 and 2 of the experiment and body weight was measured and body weights were recorded twice pre-treatment and

on Day 1. Serum CP-526, 555 concentrations were measured with each dose a different time points on Day 1 (at 3, 6, 9, and 24 hours post-dose).

Key Study Findings:

- C_{max} values were 1.5 and 19.2 ng/mL at 0.01 and 0.2 (0.1 BID) mg/kg, respectively and the mean AUC_{0-24h} value was 333 ng-h/mL at 0.2 (0.1 BID) mg/kg. *Mean AUC_{0-24h} for 0.01 mg/kg could not be calculated because most samples had serum concentrations below the limit of quantitation.* No gender difference were noted
- No changes in the clinical signs were noted.

This experiment was done with the tartarate salt. Sponsor mentioned that these toxicokinetic values were similar to those of the previous 3-month monkey toxicity study using the succinate salt form in which Mean C_{max} values were 1.87 and 16.8 ng/mL at 0.01 and 0.2 (0.1 BID) mg/kg, respectively and the mean AUC_{0-24h} value was 230 ng-h/mL at 0.2 (0.1 BID) mg/kg on Day 1. The study with the succinate salt is being reviewed under this submission and the reviewer made similar observation.

Study Title and Study Number: CP-526, 555-01 Single-Dose Oral Toxicity Study in Rats; 97-1545-06

CP-526, 555-01 [] was administered to Sprague-Dawley rats as a single oral (esophageal intubation) dose. The dosages used were 0, 30, 100, 300, 200 mg/kg; 3 animals/sex/group were used for the study. The animals were observed for clinical signs of toxicity for 14 days after dosing. Body weights were recorded on the Day of dosing (day 1) and on Days 2, 5, 8, 11, and 15. Clinical pathology was done on Days 2 and 14 (day 13 for the 200 and 300 mg/kg groups). The animals were sacrificed at Day 15 and examined for gross organ and tissue changes.

Key Study Findings:

- **Clinical signs:** At 200 mg/kg, animals showed labored/noisy respiration, decreased activity, uncoordinated/unsteady gait, splayed hind limbs, tremors, ptosis, loose stool, hunched posture, and rough hair coat. At 300 mg/kg, clonic and tonic convulsions were observed in addition to the signs observed at 200 mg/kg. Clinical signs occurred on the day of dosing and were resolved by day 2. However, ptosis, decreased activity, loose stool, and uncoordinated/unsteady gait were noted in a few animals throughout the observation period; 100-mg/kg doses were well tolerated.
- **Body weight:** Decrease in mean body weights (~0.78-0.92X control) were noted at 200 mg/kg from Day 5 through the end of the observation period in males; decrease in mean body weight gain was noted over the same time course with mean values at study end reaching ~0.49-0.58X control. Females showed transient decreases in mean body weights at 300 mg/kg (Day 5 only); mean body

weight gain in female decreased at doses >200 mg/kg on Days 5, 8, and/or 11 with recovery toward control by study end.

- **Hematology:** On day 2, there were decreases in mean total WBC counts (~0.49-0.73X control) in males at doses >200 mg/kg and in mean lymphocyte counts (~0.41-0.44X control) in males and females at 300 mg/kg. All changes returned toward control by study end.
- **Serum chemistry:** There were elevations in mean glucose concentration (~1.6-2.1X control) in males at 300 mg/kg and in females at doses >200 mg/kg on day 2. All changes returned toward control by study end.
- **Plasma drug concentrations:**
 - Plasma drug concentrations were elevated significantly by 1 hour post dose and generally were maintained near this initial concentration over the course of the day;
 - Tmax occurred at 1, 4, 8, or 24 hours post dose. Mean systemic exposure parameters (plasma Cmax and AUC) increased with increasing dose, except for AUC in males, which appeared to reach a plateau between 200 and 300 mg/kg.
 - There were no gender differences in mean exposure parameters at the 30 or 100 mg/doses; however, at doses >200 mg/kg, females had ~1.9-2.9X increases in mean Cmax and AUC values compared to males.

Study Title and Study Number: 5-Day Exploratory Bridging Study in Cynomolgus Monkeys; 98-1545-09

CP-526, 5554-24 and CP-526, 555-01, the succinate salt and [] respectively of CP-526, 555, were administered to Cynomolgus monkeys (1/sex) at a dose level of 0.1 mg/kg/day to compare pharmacokinetics after a single oral dose. The 0.1 mg/kg dose was used because it did not induce emesis. Both animals received a single oral dose of 0.1 mg/kg/day CP-5269555-24 on day one, followed by a two-day washout period. On day four both animals received a single oral dose of 0.1 mg/kg/day CP-526, 555-01.

Key Study Findings:

- Food consumption decreased by 75% for the female animal on the day following administration of CP-5269555-24 (day 2).
- Systemic exposure was slightly lower following administration of the succinate salt as compared to the [] whether assessed by AUC₀₋₂₄ (217 vs. 307 ng-hr/mL) or Cmax (16.1 vs. 24.1 ng/mL). Additionally, the succinate salt had a longer Tmax (5 hours) as compared to the [] (2 hours). Finally, the variability in plasma concentration between the two animals was slightly greater with the -24 salt (succinate) as compared to the -01 [] It should be noted that in a previous oral exploratory toxicology study (DSE #97-1545-07) in Cynomolgus monkeys administered 0.1

mg/kg CP-526,555-01, the mean AUC₀₋₂₄ and C_{max} were 201 ng•hr/mL and 19.9 ng/mL, respectively. These values are almost identical to the values obtained with the succinate salt in this study. These data suggest that inter animal variability in CP-5269555 exposure could be as great as the differences observed between the two salt forms. Therefore, the pharmacokinetic data taken together indicate that the two salt forms of CP-5269555 (succinate [-24] vs, [-01]) given in this study had generally similar pharmacokinetics in the Cynomolgus monkey.

Study Title: CP-526,555-24 Acute Oral Toxicity Study in Cynomolgus Monkeys; 98-1545-14

Cynomolgus monkeys (2/sex) were given CP-526,555-24 as a single dose (0, 3 mg/kg) by oral gavage. All animals were observed daily for clinical signs. Food consumption, and body weights were recorded pre-dose and on days 1, 8, and 14. Other measurements and observations included: physical examinations (pre-study and day 14); vital signs, blood pressure, and electrocardiograms (pre-study, on day 1 pre-dose and 4 hours post dose, and on day 9; an additional body temperature measurement was taken on day 1 at 1 hour post dose) hematology and serum chemistry (pre-study and days 2 and 14). Plasma CP-526, 555 concentrations were measured on day 1 at 1, 4, 8, and 24 hours post dose.

Key Study Findings:

- Emesis was noted in all animals (4/4 animals), which occurred at ~1-4 hours post dose. Additionally, recumbence, decreased activity, and tremors were observed in 3/4, 2/4, and 1/4 animals, respectively, and generally was manifested for ~1-4 hours post dose.
- Reduced heart rates (0.80-0.84X pre-study values) were observed in 3/4 monkeys, and there were changes in electrocardiogram parameters (increased PRQ interval in 3/4, increased P wave width in 2/4, and decreased QT interval in 1/4) on day 1. Parameters returned to pre-study baseline by day 9.
- Systemic exposure as defined by C_{max} and AUC was 54.4 ng/mL and 670.1 ng•hr/mL, respectively in males and 50.6 ng/mL and 477.6 ng•hr/mL, respectively in females.

Study Title: Escalating Dose Toxicity Study of CP-526,555-18 in Monkeys; RR 745-03502

One male and 1 female, approximately 10 and 11 years old and weighed 8.3 and 5.7 kg, respectively were used for this study. CP-526, 555-18 was administered by continuous intravenous infusion to one male and one female cynomolgus monkey over a 4-hour period. The monkeys (chair-restrained) were infused at a rate of 0.25 mL/kg/hr (dosages: 0, 20, and 80 µg/kg/hr at day 1; 50 and 200/150 µg/kg/hr at day 2 and 75 and 300 µg/kg/hr at day 3) through a temporary indwelling catheter in the vein. On Day 3,

infusion was discontinued in the male 3 hours after the infusion was begun due to the severity of clinical signs (total dose received = 225 µg/kg).

Key Study Findings:

- Reduced food consumption was noticed at all dosages. At 50 µg/kg/hr (Day 2), the female had emesis approximately 3.5 hours after the initiation of the infusion. At 4 hours after initiation, both animals showed tremors that were generalized in the male, but involved only the hind limbs in the female. At the same time, the male was hypoactive and showed muscle rigidity discernable as clenched fists and an inability to extend the digits. This muscle rigidity was present for about 15 minutes. At approximately 6 hours after the initiation of the 4-hour infusion 2 hours after it was discontinued the male was still hypoactive, although tremor and muscle rigidity were no longer present. The female continued to show hind limb tremors, but was also hypoactive and had muscle rigidity. In the female muscle rigidity was manifested as a clenching of the feet. The feet would clench, relax, and clench again. All signs of hypoactivity, tremor, or muscle rigidity had resolved in both animals by 24 hours post initiation of infusion.
- At 75 µg/kg/hr (Day 3), the male showed emesis and generalized tremors at 3 hours post initiation of infusion. Because the clinical signs were expected to increase in severity if the infusion was allowed to continue, dosing was stopped. Hypoactivity was the only drug-related sign at 4 and 6 hours after infusion initiation. The female had episodes of emesis at approximately 2, 2.5, 3, and 3.5 hours post-initiation, and bruxism (grinding of the teeth) at 3.5 hours post-initiation. Bruxism may have been an indication of abdominal pain. At 4 hours post-initiation there were no drug-related signs in the female; however, hypoactivity, tremors, and muscle rigidity were present at 6 hours post-initiation. Similar to Day 2 in the female, the tremors involved the hind limbs, and muscle rigidity was described as foot clenching. As at 50 µg/kg/hr, all signs of hypoactivity, tremor, or muscle rigidity had resolved in both animals by 24 hours post-initiation of infusion.
- Red blood cells (RBC), hematocrit, and hemoglobin were decreased 12% to 15% in the male and 22% to 24% in the female. Changes in morphology of RBC were also noted (poikilocytosis and few Burr cells). The female had a 48% increase in white blood cells (WBC) due primarily to increases in absolute neutrophils (73%), lymphocytes (27%), and monocytes (3.1-fold).
- Aspartate aminotransferase activity increased 67% and 176% in the male and female, respectively. The male also had a minor increase in alanine aminotransferase (3-fold). Decreases in total protein (15% - 20%) and albumin (23% - 28%) were seen in both animals, consistent with the observed emesis and reduced food consumption.
- Results of serum concentration analyses showed CP-526, 555 levels increased with increasing dose and with time post-initiation of infusion. The levels achieved 4 hours after the initiation of infusion at 50 µg/kg/hr (60 ng/mL in the male and female, respectively) were close to the target serum concentration of 60 ng/mL.

Study Title: Acute Intravenous Toxicity Study of CP-526,555-18 in Monkeys; RR 745-03516

Male and female Cynomolgus monkeys, approximately 3- to 5-years old and weighing 2.9 to 4.6 kg were used for the study. CP-526, 555-18 (4 animals/sex /group) was given IV as a single 4-hour infusion at either 0 or 45 µg/kg/hr (180 µg/kg).

All animals received a dose volume of 0.25 mL/kg/hr. Two animals per group were euthanized approximately 24 hours after the initiation of the drug or vehicle infusion. The remaining animals were euthanized 2 weeks after infusion (Day 15).

Key Study Findings:

- Mean creatinine kinase (CK) values were increased approximately 48- and 57-fold compared with pretest in drug-treated males and females, respectively, compared with 2-fold and 7-fold increases in male and female controls. In all samples evaluated, increases were primarily due to CK-MM, the skeletal muscle-specific isozyme.
- Elevations in CK, AST and ALT after chair restraint have been reported in rhesus monkeys. CK activity was within the normal historical range for the laboratory in all animals evaluated at Day 15.
- One male drug-treated animal (2230) demonstrated elevated AST and ALT on Day 2 representing 9.9- and 9.4-fold above pretest sample values, respectively. One drug-treated female (2241) had milder elevations of AST and ALT on Day 2 (2.2-fold and approximately 1-fold, respectively). The male was euthanized Day 2 and reversal information was not available, but in the female, AST and ALT values returned to normal range at Day 15.
- The mean C_{max} was 31.7 ng/mL and the mean AUC₀₋₂₄ was 379 ng·hr/mL. The systemic exposure returned to base line at 24 hrs.

Mean Serum Concentrations (ng/mL) (45 µg/kg/hr)

Time (hr)	Males	Females	Both Sexes
	Mean ±SD	Mean ±SD	Mean ±SD
1.0a	8.4	7.96 ±0.81	8.14 ±5.97
4.0	27.6 ±4.38	33.7 ±12.2	30.6 ±9.1
7.0	20.1 ±5.65	27.9 ±4.4	24.0 ±6.3
24	3.34 ±1.35	4.28 ±1.49	3.81 ±1.41

Mean Toxicokinetic Parameters

	Males	Females	Both Sexes
	Mean ±SD	Mean ±SD	Mean ±SD
C _{max} (ng/mL)	27.6 ±4.4	35.8 ±10.3	31.7 ±8.6

tmax (hr)	4.0 ±0.0	5.5 ±1.7	4.8 ±1.4
AUC ₍₀₋₂₄₎ (ng·hr/mL)	328 ±88	431 ±60	379 ±89

2.6.6.3 Repeat-dose toxicity

Study title: CP-526,555-01 Exploratory Escalation/Toleration Study In Monkeys

Key study findings:

- In this preliminary dose escalation (0.1, 0.2, 0.3, 0.4 and 1 mg/kg/day) study emesis and loose stool were identified as the main clinical signs related to the test article. These clinical signs were observed at the dose 0.3 mg/kg and above.
- The toxicokinetics analysis showed accumulation of the test article (1.5-fold-3-fold) at all doses.
- The timing of emesis coincided with the C_{max} indicating a direct treatment related effect of the compound.

Study no.: 97-1545-07

Volume # and page #: Volume: 1; Page 1-24

Conducting laboratory and location: Pfizer, Inc, Groton, CT

Date of study initiation: Not mentioned.

GLP compliance: No

QA report: No

Drug, lot #, and % purity: CP-526,555-01, Lot #38712-174-19, [] The administered doses were adjusted for purity.

Methods

Doses: 0.1, 0.2 (0.1 BID), 0.3, 0.4 (0.2 BID), 1.0, or 1.0 (0.5 BID) mg/kg/day

Species/strain: Cynomolgus monkey

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

Route, formulation, volume, and infusion rate: Oral gavage, deionized water, 1mL/kg

Satellite groups used for toxicokinetics or recovery: No recovery study done, this is a dose escalation study, animals were used for TK analysis and no histopathology was done.

Age: Not mentioned.

Weight: Not mentioned.

Sampling times: The blood samples for TK analysis were collected at the time outlined in the table below in the metabolism time points.

Treatment Group	Animal #	Day of Test	Dose (mg/kg/day)	Metabolism Timepoints (hours post-dose)
I	1, 3	1	0.1	0, and ~1
		2 and 8	1.0	~1, 4, 8, and 24
II	2, 4	1 and 5	0.1	~1, 4, 8, and 24
		7	1 (0.5 b.i.d.)	pre, ~1, and 9 post first-dose

Unique study design or methodology (if any):

Four Cynomolgus monkeys were distributed into 2 treatment groups (1/sex/dose). Group I received a single dose of CP-526,555 at 0.1 mg/kg/day and then 1 mg/kg/day for 7 consecutive days. Group II received 0.1 mg/kg/day for 5 consecutive days. Due to emesis observed at 1 mg/kg/day, Group I received a single dose at 0.3 and 0.4 (0.2 BID) mg/kg/day and Group II received 1 (0.5 BID) mg/kg/day for 3 consecutive days and a single dose of 0.2 (0.1 BID) mg/kg/day. Doses were administered by oral gavage (1 ml/kg) and BID doses were divided into two doses given 6 hours apart.

Study design is provided below:

Dosing Calendar

Treatment Group	Animal #	Day(s) of Test	Dose (mg/kg/day)
I	1, 3	1	0.1
		2-8	1.0
		9-11	washout period
		12	0.3
		13-14	washout period
II	2, 4	15	0.4 (0.2 b.i.d.)
		1-5	0.1
		6	washout period
		7-9	1 (0.5 b.i.d.) ^a
		10-11	washout period
		12	0.2 (0.1 b.i.d.)

^a Dosing of the Group II animals on day 9 was suspended after the a.m. dose.

Observations times and Results:

Mortality: The animals were monitored daily. There was no mortality in this study.

Clinical signs: Clinical signs were recorded once daily pre-study and at least twice daily during dosing. Emesis was seen in ½ animals at 0.3 mg/kg/day, ½ animals in 0.4 mg/kg (emesis started immediately post dos) and 2/2 monkeys at 1 mg/kg. At the high dose loose stool was also observed in ½ animals. The emesis was observed at the high dose in the animal post dosing and the following morning prior to dosing indicating increase in the severity of the incidence.

Body weights: Body weights were obtained once pre-study and then daily throughout the dosing period. No test article related changes in body weight were seen.

Food consumption: Food consumption was recorded 1 week prior to treatment initiation and then daily throughout the dosing period. Food consumption was found to be decreased at all dose groups.

Ophthalmoscopy: Not conducted.

EKG: Electrocardiograms (leads I, II, III, and V) were performed once prior to study start and on days 1 (0.1mg/kg/day) and 2 (1 mg/kg/day s.i.d.) (pre-dose and ~1 hour post-dose) for Group I.

Hematology: Hematology blood samples (~9 ml) were collected and analyzed once pre-study and on days 2 and 8 for Group I and days 1 and 5 for Group II. Regular battery of the hematological parameters was tested. At high dose about 2-fold increase in neutrophil count was observed.

Clinical chemistry: Clinical chemistry blood samples (~9 ml) were collected and analyzed once pre-study and on days 2 and 8 for Group I and days 1 and 5 for Group II. Regular battery of the clinical chemistry parameters was tested. No treatment related changes were observed.

Urinalysis: Not conducted.

Gross pathology: Not conducted

Organ weights: Not done

Histopathology: Not done

Toxicokinetics: Samples were collected at different days as described above for Group I and II. No major gender difference was noted. Slight accumulation was noted at low dose (0.1 mg/kg, 1.5-fold) at Day 5 (males and females combined). AUC₀₋₂₄ were found to be 201 vs 322.1 ng•hr/mL at Day1 and Day 5 respectively. At high dose, about 2-3-fold accumulation was noted.

CP-526,555 Plasma Concentrations on Days 1 and 5 From a Male and Female Monkey Receiving Single Daily Doses of 0.1 mg/kg/day by Oral Gavage for 5 Consecutive Days.

Dose Level Day of Dosing	0.1 mg/kg Day 1		0.1 mg/kg Day 5	
	2/M ng/ml	4/F ng/ml	2/M ng/ml	4/F ng/ml
Time (hr)				
1	1.3	20.3	12.9	7.0
4	19.4	17.8	32.7	24.3
8	11.3	8.2	17.6	11.8
24	3.3	2.3	8.9	6.2
Parameter				
AUC ₀₋₂₄ (ng•hr/ml)	209.3	193.2	381.0	263.2
C _{max} (ng/ml)	19.4	20.3	32.7	24.3
T _{max} (hour)	4	1	4	4
Emetic Episodes Hours Post Dose	0	0	0	0
	-	-	-	-

CP-526,555 Plasma Concentrations on Days 1, 2 and 8 From a Male and Female Monkey Receiving a Single Dose of 0.1 mg/kg by Oral Gavage on Day 1 Followed on Day 2 by Seven Consecutive Daily Doses at 1.0 mg/kg/day.

Dose Level Day of Dosing Time (hr)	0.1 mg/kg Day 1		1.0 mg/kg Day 2		1.0 mg/kg Day 8	
	1/M (ng/ml)	3/F (ng/ml)	1/M (ng/ml)	3/F (ng/ml)	1/M (ng/ml)	3/F (ng/ml)
0	8.6*	0.0				
1	18.8	12.8	28.7	31.5	110.2	25.9
4			51.0	31.5	223.0	63.0
8			33.2	16.6	105.9	40.2
24			15.2	0.0**	84.4	27.5
Parameter						
AUC _{0-24h} (ng•hr/ml)	-	-	675.1	323.2	2680	881.4
C _{max} (ng/ml)	-	-	51.0	31.5	223.0	63.0
T _{max} (hour)	-	-	4	4	4	4
Emetic Episodes Hours Post Dose	0 -	0 -	1 2.2	1 1.7	0 -	1 4

* Determined to be carryover from a previous analysis, therefore the value is considered invalid.

** Value assumed to be zero because no analyte was detected. However, no internal standard was detected either presumably because it was inadvertently not added.

Other: Physical exams and vitals (respiration, heart rate and body temperature) were performed once prior to study start and on days 1 (0.1mg/kg/day) and 2 (1 mg/kg/day s.i.d.) (pre-dose and ~1 hour post-dose) for Group I. There were no treatment related changes.

Study title: 10-Day Oral Dose Range-Finding Study of CP-526,555-24 In Monkeys

Key study findings:

- Cynomolgus monkeys were dosed with 0.25, 0.5, and 1 mg/kg/day with the test article (succinate salt) for 10 consecutive days by oral gavage. The animals were then observed for another 5 days. All treated animals showed multiple incidence of emesis. The incidence of emesis increased with increasing dosages. Hunched postures were noted at high dose. Loose stool were also noted in all treated animals. The observed clinical signs were clearly dose related, therefore, may be considered as treatment related.
- Toxicokinetics data showed increased accumulation (2-5-fold) of the compound at Day 10 with all dosages, exposure increased with increasing dose, no gender differences were noted.
- The test article was not well tolerated even at low dose (0.25 mg/kg).

Study no.: 00-1545-23

Volume # and page #: Volume 1, Page 1-63

Conducting laboratory and location: Pfizer Inc, Groton, CT

Date of study initiation: 02/10/04

GLP compliance: No

QA report: No

Drug, lot #, and % purity: CP-526,555-24, Lot: 27506-110-1F, \bar{C} } The administered doses were adjusted for purity.

Methods

Doses: 0.25, 0.5, and 1mg/kg/day for 10 days.

Species/strain: Cynomolgus monkeys were used in this experiment.

Number/sex/group or time point (main study): 2/sex/dose

Route, formulation, volume, and infusion rate: Oral gavage, formulated in deionized water, volume used was 1mL/kg

Satellite groups used for toxicokinetics or recovery: None

Age: Not mentioned

Weight: Individual body weights ranged from 6 kg to 9 kg for males, and 2.4 kg to 4.5 kg females.

Sampling times: Blood samples were collected for determination of the plasma CP- 526,555 concentrations on Days 1 and 10 at ~ 3, 6, 9, and 24 hours post dose.

Unique study design or methodology (if any): see the study design below:

Study Design

Daily Dose* (mg/kg)	Dose Volume (mL/kg)	Drug Concentration (mg/ml)	Animal Numbers	
			Males	Females
Control (vehicle)	1	0	1-2	9-10
0.25	1	0.25	3-4	11-12
0.5 (0.25 BID)	2 (1 BID)	0.25	5-6	13-14
1	1	1	7-8	15-16

*All dose levels are expressed as mg of active moiety per kg of body weight.

Monkeys were dosed for 10 consecutive days.

Observations times and Results:

Mortality: The animals were observed daily for mortality; no mortality occurred under this experimental condition.

Clinical signs: Clinical signs were observed daily. Loose stools were observed sporadically at 0.25 mg/kg in 2/4 animals and at 0.5 (0.25 BID) mg/kg in 3/4 animals. Emesis was observed in treated animals (3/4, 4/4, and 4/4 at low, mid and high dose). The incidence of emesis was noted within 3 mins after the administration of the high dose, and with 12 minutes of the administration of the mid dose. Hunched posture

(within Days 6-9) and salivation (within Days 4-7) were observed sporadically at 1 mg/kg in 3/4 animals (immediately postdose).

Body weights: Body weights were recorded every 3 days beginning Day 1. There was a treatment-related decrease in body weight in the 1 mg/kg males (12-9 % decrease in males and 12% decrease in females) at Day 10. The decrease in body weight was associated with decreased food intake.

Food consumption: Food intake was observed daily semi-quantitatively. The 0.25 mg/kg animals, and 0.5 (0.25 BID) mg/kg males consumed ~ 75%-100% of their daily ration which is similar to those of the controls. The 0.5 (0.25 BID) mg/kg females consumed ~ 25%-50% within Days 1-11. The 1 mg/kg animals consumed <25%-25% within Days 7-11 and Days 1-11, males and females, respectively. Food intake increased with cessation of dosing.

Ophthalmoscopy: Not conducted.

EKG: Leads I, II, III, aVR, aVL, aVF, and V, heart rate (HR, beats per minute), and indirect systolic blood pressure (BP, mm Hg) data from conscious monkeys. Data was obtained from monkeys once pretreatment and then on Days 9 or 10 (1/sex/dose/day) predose and at ~ 2-4 hours postdose. There were changes in the ECG parameters under this experimental condition.

Hematology: Blood sample were collected from predose and at Day 11. Regular battery of the hematological parameters was tested. The hematological assessment from the individual animal was submitted. The data was too erratic to be analyzed.

Clinical chemistry: Blood sample were collected from predose and at Day 11. Regular battery of the clinical pathological parameters was tested. There was no summary data for the serum chemistry for this experiment. The data is too inconsistent and can not be evaluated.

Urinalysis: Not conducted.

Gross pathology: This is a dose range finding study, histopathology was not done.

Organ weights: This is a dose range finding study, gross pathology was not done.

Histopathology: This is a dose range finding study, histopathology was not done.

Toxicokinetics: Blood samples were collected for determination of plasma CP-526,555 concentrations on Days 1 and 10 at ~ 3, 6, 9, and 24 hours postdose. The exposure increased with increasing dose; however, at Day 1 no dose proportionality was seen. Dose proportion increase in exposure was observed at Day 10 (see table below). Accumulation was observed at all dosages. Two-fold accumulations were noted at low dose and about 5-fold accumulations were noted at high dose.

Summary of Toxicokinetic Parameters:

Dose (mg/kg)	Day	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) ng•hr/mL
0.25	1	31.0 ± 3.4	3.8 ± 1.5	455 ± 123
	10	43.8 ± 11.0	4.5 ± 1.7	750 ± 252
0.5	1	34.4 ± 6.	4.5 ± 3.0	665 ± 95

	10	75.8 ± 19.0	6.0 ± 3.5	1460 ± 270
0.1	1	35.4 ± 10.5	6.0 ± 3.5	513 ± 198
	10	146 ± 65	7.5 ± 3.0	2500 ± 770

Other: A physical examination was performed once pretreatment and on Day 10 to assess the animals' general condition. Vital signs, including respiration rate (RR, respirations per minute) and temperature body temperature, were collected from monkeys once pretreatment and then on Days 9 or 10 (1/sex/dose/day) predose and at ~ 2-4 hours post dose. Respiration rate was obtained manually and rectal body temperature. No treatment related changes were observed.

Study title: 1-Month Oral Dose Range-Finding Study With 14-Day Recovery Phase of CP-526,555-18 In Monkeys

Key study findings:

- The test article (tartrate salt) were not well tolerated at doses 0.4 and 0.6 mg/kg/day for 28 days as indicated by the occurrence of emesis, loose stool, hypothermia, and dehydration.
- Modulation of WBC were observed at intermediate dose suggesting a stress response, an increase in fibrinogen level was also noted which is indicative of an inflammatory response.
- Toxicokinetics studies showed dose related increase in exposure; accumulation was noted at all doses with the increase in the duration of the study.

Study no.: 04-1545-34

Volume # and page #: Volume 1; Page: 1-83

Conducting laboratory and location: Pfizer Inc, Groton, CT

Date of study initiation: Not mentioned.

GLP compliance: No

QA report: No

Drug, lot #, and % purity: CP-526,555-18 (tartrate salt); 42698-117-11F, [J]. The administered doses were adjusted for purity.

Methods

Doses: The test article was administered at doses of 0.2 (0.1 BID) escalated (on Day 8) to 0.6 (0.3 BID), 0.4 (0.2 BID), and 0.6 (0.3 BID) mg/kg for 1 month

(followed by a 14-day recovery period and 1.2 (0.6 BID) mg/kg for 12 consecutive days followed by a 30-day recovery period.

Species/strain: Cynomolgus monkeys were used.

Number/sex/group or time point (main study): 2/sex/dose

Route, formulation, volume, and infusion rate: Route of administration was oral gavage; the compound was formulated in deionized water, volume used was 1mL/ kg/day.

Satellite groups used for toxicokinetics or recovery: None

Age: Not mentioned.

Weight: Not mentioned.

Sampling times: Blood samples were collected for determination of serum and/or plasma CP-526,555 concentrations on Days 1, 7, 14, and 30 prior to dosing and at ~ 3, 6, 9, and 24 hours after the first dose for all dose groups except the 1.2 (0.6 BID) mg/kg group. Blood samples from the 1.2 (0.6 BID) mg/kg dose group were collected on Days 1, 5, and 12 prior to dosing and at ~ 3, 6, 9, and 24 hours after the first dose, and on Day 28 .

Unique study design or methodology (if any):

Study Design

Daily Dose* (mg/kg)	Dose Volume (mL/kg)	Drug Concentration (mg/mL)	Animal Numbers	
			Males	Females
Control (vehicle)	2 (1 BID)	0	1	10
0.2 (0.1 BID) (Days 1-7)	2 (1 BID)	0.1	2-3	11-12
0.6 (0.3 BID) (Days 8-30)	2 (1 BID)	0.3		
0.4 (0.2 BID)	2 (1 BID)	0.2	4-5	13-14
0.6 (0.3 BID)	2 (1 BID)	0.3	6-7	15-16
1.2 (0.6 BID)	2 (1 BID)	0.6	8-9	17-18

* All dose levels are expressed as mg of active moiety per kg of body weight.

Observations times and Results:

Mortality: Animals were observed daily for mortality. No death occurred under this experimental condition.

Clinical signs: Animals were observed for clinical signs. Loose stool and emesis were seen at all doses. The severity and the frequency of the incidence of emesis and loose stool increased at high dose, the animals at this had to receive subcutaneous and/or IV fluid intermittently. Heat lamps were provided since some animals were cold to touch. This indicates that this dose in monkeys may be the maximum tolerated dose. The incidence of emesis and its frequency were almost similar at 0.4 and 0.6 mg/kg. Animals in the dose group 0.2 escalated to 0.6 mg/kg showed 2 isolated incidents of reflux on Day 8. After escalation, reflux was seen intermittently in 3/4 animals throughout treatment. At high dose, reflux was observed in 2 animals. Hypothermia (1 animal even at low dose) and dehydration were also noted in mid and low dose group indicating that the compound was not well tolerated under this experimental condition.

Body weights: Body weights were recorded generally every 3 days beginning on Day 1. A decrease (18%) in body weight gain was seen in female monkeys at mid dose at Day 28. Similar changes were observed at high dose in females. At the end of the 14 day

recovery period the body weight of the females did not reach normal (16-10 % decrease). Male body weight decreased at high dose but was less than 10% even at high dose.

Food consumption: Animals were observed daily for food intake. At mid dose about 25-50% decrease in food intake was noted and at high dose similar changes were noted. Food intake was found to return to normal at the end of the recovery period.

Ophthalmoscopy: Not conducted.

EKG: Not conducted.

Hematology: Hematology parameters (regular battery) were analyzed from pretreatment and Days 7, 14, and 30 for all dose groups except the 1.2 mg/kg. For 1.2 mg/kg/day group hematology was done at pretreatment and Days 5, 9, 13, and 28. At high dose, in females, neutrophil and monocytes increased 5X and 4X respectively. Lymphocytes and eosinophil in females, at high dose, decreased 0.4X and 0.3X respectively. Changes in hematology parameters in males were less severe. At mid dose, in females, similar trend (less severe than that of the high dose) in WBC changes as that of the high dose group was noted. The changes in the hematology parameters were not consistent at different days of the treatment. The changes in WBCs were indicative of a stress response and may be correlated to the body weight changes observed in females. Changes in the WBC returned to the normal level at the end of the recovery period. There was a dose dependent increase (1.5-2X) in the fibrinogen level (3/4, 1/4 and 1/4 monkeys at high, mid and low dose respectively) in monkeys indicating an inflammatory response. No histopathology was done in this experiment, so the significance of the modulation of the blood cell parameters could not be confirmed. After 2 weeks of the dosing cessation, 1 male and 1 female from the high dose group showed an activated prothromboplastin time (< 4 sec). Toxicological significance of this finding is unknown. No morphological changes in RBCs were noted, however at the mid and the high dose a decreasing trend in the RBC parameters were noted. The maximum changes noted were 20-15 % decrease. This change was observed at Day-9 and may be correlated with the increase amount of fluid administration in the animals. An increase in the reticulocyte count at high dose (3X was noted) which may be due to the compensatory mechanism in the RBC parameters.

Clinical chemistry: Serum chemistry parameters (regular battery) were analyzed at pretreatment and Days 14, and 30 for all dose groups except the 1.2 mg/kg group. For 1.2 mg/kg group serum chemistry was done at pretreatment and Days 9, 13, and 28. A decrease in blood urea nitrogen and loss of electrolytes (Na and Cl) were observed at high dose. This may be related to loose stool and decrease food intake. Loss of P and K were also noted at high dose. Due to the absence of histopathology the biological significance of the findings can not be determined. Increase in total bilirubin (upto 10X) was noted at high dose animals which might be related to emesis, reduced food intake and body weight loss as suggested by the Sponsor.

Urinalysis: Not conducted.

Gross pathology: Not conducted.

Organ weights: Not conducted.

Toxicokinetics: Blood samples were collected for determination of serum and/or plasma CP-526,555 concentrations on Days 1, 7, 14, and 30 prior to dosing and at ~ 3, 6, 9, and 24 hours after the first dose for all dose groups except the 1.2 (0.6 BID) mg/kg group. Blood samples from the 1.2 (0.6 BID) mg/kg dose group were collected on Days 1, 5, and

12 prior to dosing and at ~ 3, 6, 9, and 24 hours after the first dose, and on Day 28 . A dose related increase in the exposure was noted as observed by increasing AUC and C_{max} (see Sponsor's table below). No gender differences were observed. Accumulation up to 2-3-fold was observed in all dose groups.

Summary of Toxicology Findings:

Species/ Strain/ Study No./ Lot No.	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	AUC ₍₀₋₂₄₎ (ng·h/mL)	C _{max} (ng/mL)	Gender and No. per Group	Noteworthy Findings
Monkey/ Cynomolgus 04-1545-34 42698-117- 11F	Oral Gavage (Deionized Water)	12 or 30 Days ^a	0 0.2 (0.1)→ 0.6 (0.3) ^b 0.4 (0.2); 0.6 (0.3); 1.2 (0.6)	ND <u>Day 30:</u> 1960 1320 2590 2680	ND <u>Day 30:</u> 97.6 71.1 129 128	1M, 1F (Controls only) 2M, 2F	≥0.2 (0.1 BID): ↑ Neutrophils, monocytes, and fibrinogen, ↓ lymphocytes and eosinophils, ≥0.4 (0.2 BID): Emesis, liquid/loose stools, dehydration, cold to touch; ↓ body weight and food intake; ↓ body temperature; ↓ red blood cell count, hemoglobin, hematocrit; ↓ blood urea nitrogen, sodium, chloride, potassium, and phosphorous, cholesterol, and glucose; ↑ total bilirubin

ND = Not Determined; M = Male, F = Female.

^aAnimals in the 1.2 (0.6 BID) mg/kg group were dosed for 12 consecutive days followed by a 30-day recovery period. Animals in the remaining groups were dosed for 30 consecutive days followed by a 14-day recovery period.

^bAnimals were dosed at 0.2 (0.1 BID) mg/kg on Days 1-7 and escalated to 0.6 (0.3 BID) mg/kg for the remainder of the dosing period.

Other: Physical examinations (pretreatment) and the body temperature measurements were done at Days 11, 15, 17, and 18 at ~ 1 hour post dose for all dose groups except the 1.2 mg/kg group. Physical examinations (pretreatment) and the body temperature measurements were done at Days 9, 13, 15, and 16 mg/kg. Decrease in body temperature (1-2° C) was noted in high dose animals.

Study title: CP-526,555-24 Six-Week Oral Toxicity Study in Cynomolgus Monkeys

Key study findings:

- Emesis and loose stool were observed at all dosages in this study (0.1, 0.05, 0.2 mg/kg/day administered daily by oral gavage for 6 weeks), therefore no NOEL could be established.
- WBC related hematological changes and histopathological changes related to mononuclear cell infiltrations in different organs were noted at high dose. However, due to lack of dose response, microscopic findings could not be positively correlated as a toxicological finding.
- A NOAEL of 0.2 mg/kg/day is established for this study in the absence of any major toxicity findings, which is in concurrence with the Sponsor's proposal.

Study no.: 98-1545-10

Volume # and page #: Volume 1; Page 1-147

Conducting laboratory and location: Pfizer Inc, Groton, CT

Date of study completion: 5/13/1998

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: CP-526,555-24, Lot: 27506-110-1F [] The administered doses were adjusted for purity.

Methods

Doses: 0, 0.01, 0.05, or 0.2 (0.1 BID ~6 hours apart) mg/kg/day for 42 consecutive days.

Species/strain: Cynomolgus monkeys were used in this study

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume, and infusion rate: Oral gavage, deionized water, 1mL/kg

Satellite groups used for toxicokinetics or recovery: None

Age: Not mentioned.

Weight: ~4.2 kg and ~2.9 kg for males and females, respectively.

Sampling times Blood samples were collected at 3, 6, 9, and 24 hours post dose on days 1 and 42.

Unique study design or methodology (if any):

Study Design

Group	Daily Dose ^a (mg/kg)	Dose Volume (ml/kg)	Drug Concentration (mg/ml)	Animal Numbers	
				Males	Females
Control (deionized water)	0 (0.0 b.i.d.) ^b	1	0	1-3	13-15
Low-dose (CP-526,555-24)	0.01	1	0.01	4-6	16-18
Intermediate-dose (CP-526,555-24)	0.05	1	0.05	7-9	19-21
High-dose (CP-526,555-24)	0.2 (0.1 b.i.d.) ^b	1	0.1	10-12	22-24

^a All dose levels are expressed as mg active moiety per kg of body weight.

^b Doses given ~6 hours apart.

Observations times and Results:

Mortality: All animals were observed twice for viability. There was no death in this experiment.

Clinical signs: All animals were observed twice daily for clinical signs. Loose stool and emesis were noted sporadically in all dose groups, the incidence of emesis were higher in the high dose group.

Body weights: Body weights were recorded weekly. No treatment-related effects on body weight parameters were seen.

Food consumption: All animals were observed daily for food consumption. No treatment related effects on food intake was observed.

Ophthalmoscopy: Ophthalmoscopic examinations was done once pre study and during dose-week 5). No treatment related effect was observed.

EKG: Indirect systolic blood pressure and electrocardiograms was done (once pre-study and during dose-weeks 5/6. No treatment related effect was observed.

Hematology: Hematology was done once pre-study and during dose-weeks 3 and 6. Regular battery of the hematological parameters was tested. Profound changes in the hematological parameter were noted at high dose in males and females; similar trend was noted at mid low dose. At Week -3 an increase in monocytes and lymphocytes were observed at all doses which was found to be recovered at Week-6. All changes tabulated below were within the historical control range. However, the changes that were observed (see table below) in regards to the different WBC parameters at Day 42 in this experiment is similar to those of the changes observed in the other toxicity studies with the same test article. Therefore, the findings may be related to the test article related stress response indicating inflammatory changes.

Summary of Hematological Findings at Week -6:

Parameters (%)	Male/ Dose	Female/Dose
	0.2 mg/kg	0.2 mg/kg
WBC count	31↓	18↓
Lymphocyte count	8↓	28↓
Neutrophil count	62↓	28↓
Monocytes count	20↓	16↑
Eosinophil counts	47↑	32↑
RBC counts	NC*	8↓
Reticulocyte counts	37↓	23↑

*NC=No Change

Clinical chemistry: Serum chemistry was done once pre-study and during dose-weeks 3 and 6. Regular battery of the clinical chemistry parameters was tested. At high dose, 14-16% decrease in BUN content was noted in both males and females. TB content in females decreased 57% at high dose compare to that of the pretest levels. Similar changes were noted in males but to a lesser extent. The changes in the BUN and TB might be related to the stress response as observed by the changes in the WBC counts.

Urinalysis: Urinalysis once pre-study and during dose-weeks 3 and 6. There were no treatment-related findings in this study.

Gross pathology: At necropsy, Day 42, gross pathology was examined.

Organ weights: Selected organs namely liver, kidneys, adrenals, heart, testes, ovaries, brain, and pituitary were examined. A 14% increase in absolute kidney weight was observed in female; at the same dose a decrease in heart weight (15%) was noted.

Histopathology: Adequate Battery: Yes; peer review: Yes.

Some of the histopathological findings are listed below. The findings are limited mainly to mononuclear cell infiltration. At high dose 1/3 males showed cytoplasmic vacuolation

and lung inflammation. Cyst formation in thyroid was noted which may be related to changes in WBC related parameters. The age of the monkeys were not mentioned, the immaturity of the testes observed may be related to the use of juvenile males in this experiment. The increase in kidney weight at high dose was observed in females, however, no histopathological correlation was noted, and therefore, the organ weight change might not be toxicologically significant.

Summary of Histopathological Findings

Incidence/Tissue	Male /Dosages mg/kg/day				Female /Dosages (mg/kg/day)			
	0	0.01	0.05	0.2	0	0.01	0.05	0.2
Inflammation/Foamy cell/Lung	0	0	0	1	0	1	0	0
Mononuclear cell infiltration / kidney	1	1	1	0	1	0	2	0
Mononuclear cell infiltration/ heart	0	0	1	0	0	0	0	0
Colloidal cyst/ thyroid	2	1	0	0	0	2	0	1
Cytoplasmic vacuolation/liver	0	0	0	1	0	0	0	0
Immaturity/testes	1	1	1	2				
Mononuclear cell infiltration /prostate	0	1	0	0				
Cyst/pituitary	0	0	0	0	0	0	0	1

Toxicokinetics: Plasma CP-526,555 concentrations were measured at 3, 6, 9, and 24 hours post dose on days 1 and 42. Dose proportional increase in C_{max} and AUC were noted. Exposure was higher at Day 42 compared to Day 1 with all dosages indicating accumulation of the test article. At high dose 2.5-fold accumulation of the test article was noted.

Summary of Toxicokinetic Parameters:

Dose (mg/kg)	Day	C _{max} (ng/mL)	T _{max}	AUC (0-24 hr) ng•hr/mL
0.01	1	2.6±0.5	3±0	12.4±2.5
	42	2.9±0.4	3±0	14.9±3.4
0.5	1	12±1.3	3±0	104±17
	42	22.5±2.8	3.5±1.2	242±71
0.2	1	25±2.2	NR*	320.9±144
	42	62.7±12.8	NR*	1059.6±322.62

NR= not reported

Other: Physical examinations and vital signs (heart rate, respiration rate, and body

Temperature) was done once pre-study, day 1 and dose-week 3 [body temperature only], and during dose-week 5); There were no treatment-related effects on physical examination, heart rate, respiration rate, or body temperature.

Hepatic microsomal enzyme determinations: Liver tissue samples were obtained at necropsy, and microsomal fractions were prepared for measurement of cytochrome P-450 and P-420 contents and NADPH cytochrome c reductase, 7-ethoxyresorufin O-deethylase, 7-methoxyresorufin O-deethylase, ethylmorphine N-demethylase, p-nitroanisole O-demethylase, and p-nitrophenol hydroxylase activities. There were no treatment-related changes in the microsomal enzymes in this study.

Study title: 3-Month Oral Toxicity Study In Cynomolgus Monkeys

Key study findings:

- Cynomolgus monkeys administered with the succinate salt of the test article at different dosages (0.01, 0.05, 0.2 mg/kg) by oral gavage showed treatment related clinical signs. The clinical signs include loose stool in 2/3, 3/3, 1/3 females at low mid and high dose respectively. Emeses were also noted, however, the incidence of emeses were sporadic. No NOEL could be established.
- One female at mid dose showed 1.5-fold dilation of the colon (no histopathological correlation); the biological significance of the finding can not be determined due to the lack of the dose response. However, the same animal was found to have multiple incidences of loose stool and emesis, therefore, the finding might be treatment related.
- Toxicokinetics analysis showed dose related increase in the Cmax and the AUC, no gender difference was noted. Accumulation up to 2-fold was noted with all different dosage when the AUC from the Day 1 was compared to the AUC from day 78.
- The clinical observations in this 3-month study were found to instigate around Day 30. Higher exposure due to accumulation of the test compound may be suggested as one of the reasons for this late initiation of the toxic findings.
- Changes in the WBC related hematological parameters were noted. The changes in the blood cell counts were within the historical control range, however, the trend in WBC changes suggest a stress response and may be related to inflammatory response. A decrease (31%) in the blood urea level was noted in females at high dose, the biological significance of this finding needs to be further evaluated.
- The dose related histopathological findings in the animals were limited to the infiltration of the mononuclear cells in different organs indicating an inflammatory response. At high dose myocardial degeneration were noted in 1/3 males; severity index for this finding was designated as minimal. A NOAEL of 0.2 mg/kg/day (HED=3.8 mg) was established for this study as proposed by the Sponsor.

Study no.: 00-1545-22

Volume # and page #: Vol 1; Page 1-147

Conducting laboratory and location: Pfizer Inc, Groton, CT

Date of study initiation: 05/16/00

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: CP-526,555-24; Lot # 27506-110-1F [] The administered doses were adjusted for purity.

Methods

Doses: 0.01, 0.05, 0.2 mg/kg/day

Species/strain: Cyanomolgus monkeys were used for the study.

Number/sex/group or time point (main study): 3 animals/sex/group

Route, formulation, volume, and infusion rate: Oral gavage, formulated in deionized water, volume administered 1 mL/kg

Satellite groups used for toxicokinetics or recovery: None

Age: Not mentioned.

Weight: 3.8 ± 1.2 kg and 3.0 ± 0.3 kg for males and females respectively.

Sampling times: Days 1, 41, and 78

Unique study design or methodology (if any): None

Study Design:

Group	Daily Dose* (mg/kg)	Dose Volume (ml/kg)	Drug Concentration (mg/ml)	Animal Numbers	
				Males	Females
Control (Deionized water b.i.d.)**	0	1	0	1-3	13-15
Low-dose (CP-526,555-24 s.i.d)	0.01	1	0.01	4-6	16-18
Intermediate-dose (CP-526,555-24 s.i.d)	0.05	1	0.05	7-9	19-21
High-dose (CP-526,555-24 b.i.d.)**	0.2	1	0.1	10-12	22-24

*All daily dose levels are expressed as mg active moiety per kg of body weight.

** Doses 6 hours apart.

Observations times and Results:

Mortality: Animals were observed daily for the signs of mortality and morbidity. All animals survived under this experimental condition.

Clinical signs: Animals were observed up to seven times daily. Loose stool was observed in 2/3, 3/3, and 1/6 monkeys from low, mid and high dose respectively. The incidence was higher in females than in males. Loose stool was observed in one high

dose female at Day 31 and then in females (one each) from low and mid at Day 32 and 33 respectively. Emeses were also observed sporadically across all test groups. Loose stool was observed sporadically thereafter in females from all dose groups sporadically. Loose stool was seen in the 1/3 males from the mid dose group at Day 62 and 1/3 males from the low dose group at Day 90. Loose stool was not observed in any of the control animals. At Day 56, emesis were seen in all dose groups in females (2/3, 3/3, 1/3, 2/3 in control, low, mid, and high dose group). At Day 86, emesis were seen in both male and female monkeys (1/3 and 2/3 males at low and high dose respectively; 2/3, 3/3, 1/3, and 1/3 females at control, low, mid, and high do se respectively). Although

Body weights: Body weights were measured pretest and weekly. No treatment related changes in the body weight were noticed.

Food consumption: Food consumption was estimated daily. No treatment related changes in the food consumption were noticed.

Ophthalmoscopy: Ophthalmoscopic examinations were performed by a clinical veterinarian twice pre-test and on study day 86. No treatment related changes in the ophthalmoscopic analysis were noticed.

EKG: Electrocardiograms (leads I, II, aVR, aVL, aVF, V1, and V2) and indirect systolic blood pressure recordings were obtained on all monkeys once prior to treatment initiation and then on days 50 and 83 (prior to the first daily dose and then ~2-4 hours later).

No treatment related changes in EKG were noticed.

Hematology: Blood samples for hematology determinations were collected by inguinal venipuncture from all monkeys once pre-test and on days 37, 44 and 91. Regular battery of the hematological parameters was tested. A decrease in the WBC and the eosinophil counts and an increase in the lymphocytes, neutrophil, and monocyte counts were noted in the high dose males. Hematological parameters of the male animals from the mid dose group, showed similar trend. Changes in the hematological parameters in the treated females were less prominent than that of the males. As observed in the other sub chronic studies increase in the WBC counts were observed earlier in the study and a recovering trend was observed at study termination.

Summary of Hematological Findings Day-91:

Parameters	Males	Females
	0.2 mg/kg	0.2 mg/kg
WBC count	18↓	4↓
Lymphocyte count	24↑	17↓
Neutrophil count	22↑	11↑
Monocytes count	28↑	7↑
Eosinophil counts	70↓	22↑
RBC counts	8↓	5↓
Reticulocyte counts	NC	30↓
Platelets	16↑	12↑

Clinical chemistry: Blood samples for serum chemistry determinations were collected by inguinal venipuncture from all monkeys once pre-test and on days 44 and 91. Regular battery of the clinical chemistry parameters was tested.

A decrease (31 %) in the blood urea nitrogen was noted at high dose in females. Similar trend was noted in females at mid dose. BUN level did not change in the treated males.

Urinalysis: Urine was collected for urinalysis (over a ~5 hour collection period) on days 43 and 90. All the urinalysis parameters from the standard battery were analyzed (for detail see Appendix).

No treatment related changes in the urinalysis parameters were noted.

Gross pathology: At necropsy all animals were observed for identifying macroscopic lesions. The colon from a mid dose female animal was observed to be dilated to approximately 1.5 times the normal size. There was no histological correlate. This finding lacked a dose response relationship, and found in one of the females at mid dose. The same animal was noted to have loose stools and emeses at multiple times. This macroscopic finding might be related to the clinical observation; however, in the absence of the dose relationship, the biological significance of the observation can not be ascertained.

Organ weights At necropsy selected organs (liver, kidneys, adrenals, heart, testes, ovaries, brain, and pituitary) were weighed.

Histopathology: Adequate Battery: Yes. Peer review: Yes

All of the tissues from the standard tissue battery were collected and analyzed for histopathologically. Increase incidence of the infiltration of the mononuclear cells was noted in the kidney at high dose (0/3 in control vs 1/3 at high dose in males and 1/3 in control vs. 2/3 at high dose in females). Other histopathological findings include increased incidence of the mononuclear cell infiltration in heart at high dose in male and myocardial degeneration of heart in 1/3 males.

Summary of Histopathological Findings

Incidence/Tissue	Male /Dosages mg/kg/day				Female /Dosages (mg/kg/day)			
	0	0.01	0.05	0.2	0	0.01	0.05	0.2
Infiltration of mononuclear cells / kidney	0	0	0	1	1	1	0	2
Infiltration of mononuclear cell/ heart	1	1	0	2	0	0	1	0
Myocardial degeneration /heart	0	0	0	1	0	0	0	0
Abscess/skin	0	0	0	1	0	0	0	0

Toxicokinetics: Blood samples (~2 ml) were collected via inguinal venipuncture from all drug-treated animals on days 1, 41, and 78 at ~3, 6, 9, and 24 hours after the first daily dose. Mean C_{max} and AUC values on day 78 were 2.06 ± 0.54 ng/mL and 13.7 ± 9.86 ng·hr/mL, respectively, at the low dose, 11.5 ± 1.6 ng/mL and 129 ± 28 ng·hr/mL, respectively, at the mid dose, and 26.9 ± 7.1 ng/mL and 449 ± 146 ng·hr/mL, respectively, at the high dose showing a dose related increase in the systemic exposure. No gender differences were observed. Accumulation was noted (approximately 2-fold) with all dosages when the AUC and the C_{max} were compared from different days.

Summary of Toxicokinetic Parameters:

Dose (mg/kg)	Day	C _{max} (ng/mL)	T _{max} (hr)	AUC (0-24 hr) ng·hr/mL
0.01	1	1.87 ± 0.57	3 ± 0	7.69 ± 4.12
	41	2.43 ± 0.26	3.5 ± 1.2	23.5 ± 9.2
	78	2.06 ± 0.54	3 ± 0	13.7 ± 9.86
0.1	1	8.03 ± 2.31	3 ± 0	61.0 ± 20.0
	41	12.3 ± 2.4	3 ± 0	136 ± 48
	78	11.5 ± 1.6	3 ± 0	129 ± 28
0.2	1	16.8 ± 4.8	NR	230.61 ± 61
	41	26.2 ± 7	NR	420.7 ± 70
	78	26.9 ± 7.1	NR	449 ± 146

Other: A scheduled physical examination was performed on all animals once prior to treatment initiation and then on days 41 and 86. Vital signs measurements (heart rate, respiratory rate, and rectal temperature) were obtained from all monkeys once prior to treatment initiation and then on days 50-52 and 83-85 (prior to the first daily dose and then ~2-4 hours later); no treatment related changes were observed.

Study title: 9-Month Oral Toxicity Study With 5-Week Recovery Phase of CP-526,555-18 In Monkeys

Key study findings:

- No NOEL could be determined for the 9-month toxicity study in the monkey due to the clinical observations of increased incidence of emesis and loose stools in the treatment groups. CP-526, 555 was administered by oral gavage at the dose of 0.2, 0.4, 1.2 mg/kg/day in this study.

- The test article was not tolerated at the high dose that was tested under this experimental condition. All animals at high dose were either euthanized due to >15 % loss in body weight by Day 55 or the treatment was terminated and the animals were observed for recovery.
- At mid dose one female was found dead at Day126, megacolon was observed in this animal after macroscopic and microscopic observation. Sponsor believes that this finding, although rare in monkey, might be an incidental observation. However, megacolon is related to volvulus which in turn is related to loose stools. Loose stools and inherent pharmacological effect of this test article. Increased incidence of loose stools were observed in this animal, therefore, this observation may be treatment related.
- Hematological changes compared to control (within the historical control range) were noted in this study at mid and high dose.
- Infiltration of the mononuclear cells in males (2/3, 1/3, and 3/3 at control, 0.2, and 0.4 mg/kg respectively) was the only dose related histopathological finding observed in this study. Biological significance of this finding is not known; however, no correlation with the clinical signs (like the redness of eye indicating inflammation) was noted.
- A NOAEL of 0.2 mg/kg/day was established for the monkey under this experimental condition based on the nature of the toxicity finding (Sponsor established a NOAEL of 0.4 mg/kg/day based on their conclusion that the megacolon observed in one female was an incidental finding).
- At NOAEL dose of 0.2 mg/kg (HED=3.8 mg), a C_{max} of 48.3±6.34 ng/mL and an AUC of 869 ± 111 ng·h/mL (MRHD=7.6x) were noted.

Study no.: 04-1545-35

Volume # and page #: Volume1, Page 1-315

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study initiation: July 27, 2004

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: CP-526,555-18, (Lot 04944002; Batch 3000054) L J The administered doses were adjusted for purity.

Methods

Doses: 0, 0.2 (0.1 BID), 0.4 (0.2 BID), and 1.2 (0.6 BID) mg/kg/day for 274 day (except 22 and 55 days for females and males respectively at high dose).

Species/strain: Cynomolgus monkeys

Number/sex/group or time point (main study): 4/sex/group for low dose and 6/sex/group for all other dose groups.

Route, formulation, volume, and infusion rate: Oral gavage, formulated in deionized water, 1mL/kg

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Satellite groups used for toxicokinetics or recovery: 2 animals/sex/group from mid dose group and 1 animal/sex /group were utilized for recovery for another 6 weeks.

Age: 2-4 years old

Weight: 2.9 to 4.2 kg for males and 2.7 to 3.9 kg for females

Sampling times: Blood samples (~ 2 mL) were collected on Days 1, 14, 30, 91, 148, 210, and 266 for the 0, 0.2 (0.1 BID), and 0.4 (0.2 BID) mg/kg dose groups predose and at ~ 3, 6, 9, and 24 hours post the first dose with the 6-hour sample collected immediately prior to the second dose. For the 1.2 mg/kg group, blood samples were obtained on Treatment Days 1, 14, and 30 (males only) at the time points mentioned above.

Unique study design or methodology (if any): Surviving monkeys were dosed for 9 months (274 consecutive days). At the conclusion of the treatment period, a cohort of monkeys in the control (Male 2 and Female 28) and 0.4 (0.2 BID) mg/kg dose groups (Males 11 and 12; Females 35 and 36) were placed in a recovery period for 36-37 days (females-males). Remaining animals in the 0, 0.2 (0.1 BID), and 0.4 (0.2 BID) mg/kg dose groups were necropsied.

Study Design:

Daily Dose ^a (mg/kg)	Dose Volume (mL/kg)	Drug Concentration (mg/mL)	Animal Numbers	
			Males	Females
0 (deionized water)	1 BID	0	1-6	23-28
0.2 (0.1 BID)	1 BID	0.1	7-10	29-32
0.4 (0.2 BID)	1 BID	0.2	11-16	33-38
1.2 (0.6 BID) ^b	1 BID	0.6	17-22	39-44

^a All dose levels are expressed as mg of active moiety per kg of body weight.

^b Animals in this dose group received deionized water until the 1.2 (0.6 BID) dose level was established and treatment was initiated (Study Day 65; Day 1 of treatment of CP-526,555-18).

Observations times and Results:

Mortality: All high dose animals were sacrificed either due to decrease in body weight >15% or dehydration or inappetance for >5 days. 2/6, 1/6 and 1/6 males were euthanized at Day 34, 50, 55 respectively. Rest of the males (2/6) animals was dosed upto 55 days and was placed for the recovery for another 29 days. 1/6 and 3/6 females were euthanized at Day 21 and 22 of the dosing period and the rest were dosed upto 22 days and then placed for recovery. Recovery animals from the high dose group were necropsied for histopathological evaluation. A mid dose female died at Day 126 Death was treatment related, histopathological finding from this animal revealed megacolon. Sponsor stated that the megacolon might be the result of the clonic volvulus related to gastrointestinal dysfunction (diarrhea, abdominal distention), however, since megacolon

was not observed in other animals from the high dose group or the animals from the mid dose group according to the Sponsor, the megacolon is not treatment related.

Clinical signs: Clinical signs were observed at least 4 times daily. Emesis and liquid/loose stool were observed in all dose groups. The incidence of emesis increased with duration and clearly dose related. Additional clinical signs including salivation, trembling/shaking, and hunched stance were seen at doses ≥ 0.2 mg/kg. Other than salivation, these signs were sporadically seen at the 2 lowest dose groups. The incidences of trembling/shaking and hunched stance were increased at 1.2 (0.6 BID) mg/kg. Hunched posture was observed in all monkeys at 1.2 (0.6 BID) mg/kg.

Assessment of skin turgor as an index of dehydration was performed. Animals 1/6 male and 3/6 females showed mild dehydration. All animals at 1.2 (0.6 BID) mg/kg had an abnormal skin turgor response that was observed during dosing. After ~ 2 weeks of dose administration, all animals at 1.2 (0.6 BID) mg/kg were severely dehydrated.

Body weights: Body weights were measured at pretest, every 3 days up to 30 days and the weekly during the rest of the treatment period. No decrease in body weight gain was noticed in animals from mid and low dose group at termination. At high dose group all animals loose weight and 4/6 males and females were sacrificed at different days as mentioned above due to the $>15\%$ decrease in body weight. The males and females undergoing recovery treatment from the high dose group gained up to 27% body weight at the end of the recovery period.

Food consumption: Food intake was semi-quantitatively determined daily. There were no treatment related changes at the low and the mid dose group, however, at high dose group 75 % reduction in the food consumption were noted. For animals in the 1.2 mg/kg group that were allowed to recover, food intake returned to normal during the recovery period.

Ophthalmoscopy: Ophthalmologic examinations were performed once pretreatment and on Days 78, 169, and 260 for the 0, 0.2, and 0.4 mg/kg dose groups. For the 1.2 mg/kg dose group, examinations were performed once pretreatment and on Day 20 for Female 41; Day 23 for Females 39, 40, 42, 43, and 44; Day 35 for Males 20 and 21; and Day 55 for Males 17, 18, and 22. There were no treatment-related effects noted during ophthalmologic examinations.

EKG: EKG with the Leads I, II, aVR, aVL, aVF, V1, and V2, heart rate (HR, beats per minute), and indirect systolic blood pressure (BP, mm Hg) data from conscious monkeys were obtained once pretreatment and on Day 1 for all dose groups, and on Days 87, 178, and 269 for the control, low and the mid dose groups (predose and at ~ 2-4 hours post the first dose). There were no treatment-related effects noted during EKG examinations.

Hematology: Blood samples were collected for the assessment of the hematology parameters at Days 16, 84, 176, and 273. Regular battery of the hematological parameters was tested. Increased neutrophils and decreased lymphocytes were observed in males at high dose group. Animals at high dose group showed increase fibrinogen values on Day 20 of treatment (1.5 xs and 2.1 xs in males and females respectively). The change in the WBC parameters and fibrinogen levels relates to stress response and inflammation and is consistent with the finding from the toxicity studies with the compound with shorter duration. At mid and low dose group increased lymphocyte counts were noted. Dose dependent changes in the monocytes, eosinophils and

neutrophils counts were also noted and considered to be treatment related. The significance of the changes in the WBC related parameters can not be conclusively determined but may be indicative to a stress related inflammatory response. It is to be noted that the monocytes, lymphocytes and neutrophil counts in this chronic study were found to increase at the termination which is in contrast with the subchronic studies where a recovering trend for these hematological parameters were observed.

Summary of Hematological Findings:

Parameters (%)	Male/Dosage(mg/kg)		Female/Dosage(mg/kg)	
	0.2	0.4	0.2	0.4
WBC count	10↑	10↑	5↑	10↑
Lymphocyte count	21↑	17↑	20↑	29↑*
Neutrophil count	25↑	31↑	26↓	24↓
Monocytes count	4↓	8↓	9↑	19↑
Eosinophil counts	30↓	41↓	12↑	9↑
RBC counts	10↑	12↑	9↑	12↑
Reticulocyte counts	5↓	7↓	8↓	10↓

*statistically significant change

Clinical chemistry: Blood samples were collected for the clinical chemistry assessment in Days 16, 84, 176, and 273. Regular battery of the clinical chemistry parameters was tested. In females, at mid dose, 40% decrease in the total bilirubin content (compared to control) was noted at Day 273, no such changes were observed in males. Several changes in the serum chemistry parameters were noted from the high dose group at Day 16 (the only time when blood could be collected from this group after treatment) some of which include changes in Phosphorous (↓), A/G ratio (↓), ALP (↑), AST(↑), GGT(↓) in There were no significant treatment-related serum chemistry at low dose findings.

Urinalysis: Urine was collected for urinalysis once pretreatment for all dose groups; on Day 21 or 22 of treatment for the 1.2 (0.6 BID) mg/kg dose group; and on Days 85; 177, 178, or 179; and 267 for the 0, 0.2 (0.1 BID), and 0.4 (0.2 BID) mg/kg dose groups. There were no treatment-related urinalysis findings.

Gross pathology: Gross pathology finding was limited to one female at mid dose (0.4 mg/kg) which was found dead in her cage on Day 126 of the treatment period. Sponsor reported segmental twisting, severe dilatation, and red discoloration of the colon that corresponded to microscopic acute diffuse mucosal hemorrhage and necrosis. The clinical history of chronic diarrhea and intermittent bloating combined with the necropsy and microscopic findings indicated that this monkey had megacolon with secondary colonic torsion, shock, and death. Megacolon is an uncommon idiopathic condition in female cynomolgus monkeys, and megacolon is associated with colonic torsion and death, however, in the absence of the similar findings in the rest of the animals from the same dose group and/or from the high dose group, Sponsor believed that this finding is

not treatment related. The high dose group animals were terminated after > 15 % body weight decrease so might not have long enough exposure to show megacolon. Because the incidence of megacolon is rare and also due to the fact that megacolon is related to the volvulus which in turn related to loose stool which has been observed frequently with this test article, the finding may be treatment related.

Organ weights (specify organs weighed if not in histopath table): Brain, liver, heart, adrenal, thymus, spleen, kidney, lung, ovary and testis were noted. About 30 % decreases in thymus were noted at mid dose in males. There was no histopathological correlation of this finding at mid dose at termination. High dose group was terminated at different days. 50 % decrease in the mean thymus weight was seen compared to that of the control in males. Lymphoid depletion was seen in the thymus at high dose in all males. Similar changes were seen in females at high dose group.

Summary of Organ Weight Changes (Relative Weight):

Parameters	Male	Female
	0.4 mg/kg	0.4 mg/kg
Heart	13↓	NC
Kidney	8↓	NC
Liver	NC	NC
Spleen	12↑	NC
Thymus	30↓	NC

Histopathology: Adequate Battery: Yes; peer review: Yes

Several changes were noted at high dose group which was sacrificed due to >15 % changes in body weight. Some of these changes include fibrosis in kidney 1/4 males, degeneration in liver in 1/4 males, vacuolation in heart 1/4 females, foam cell focus in lung in 1/4 males, lymphoid depletion in thymus 4/4 males and 4/4 females, atrophy in ileum 1/4 males, germ cell depletion in testis 2/4. No changes in the control group were seen for the above mentioned tissues. No dose related histopathological changes were seen at mid and high dose. However, necrosis of colon (1/4) and mineralization of the ovary (2/4) at mid dose were observed at termination. Increased infiltration of the mononuclear cells were also observed at mid dose in eye and heart, however, no inflammation were noted in these tissues

Summary of Histopathological Findings

Incidence/Tissue	Male /Doses mg/kg/day			Female /Doses (mg/kg/day)		
	0	0.2	0.4	0	0.2	0.4
Infiltration of mononuclear cell/ heart	3	3	4	0	1	0
Inflammation/lung	1	2	1			
Necrosis/colon						1

Infiltration of mononuclear cell/ eye	2	1	3	1	2	0
Mineralization/ovary						2

Toxicokinetics: Blood samples (~ 2 mL) were collected on Days 1, 14, 30, 91, 148, 210, and 266 for the 0, 0.2, and 0.4 mg/kg dose groups predose and at ~ 3, 6, 9, and 24 hours post the first dose with the 6-hour sample collected immediately prior to the second dose. For the 1.2 mg/kg group, blood samples were obtained on Treatment Days 1, 14, and 30 (males only) at the time points mentioned above.

Systemic exposure increased with increase in doses, six-fold increase in dose (0.2- 1.2) resulted in about 3.5-fold increase in C_{max} (44 vs 154 ng/mL at Day 1 and 14 respectively). Accumulation was observed 3-4-fold at all doses. Overall mean C_{max} and AUC₀₋₂₄ values on subsequent days (30, 91, 148, 210, and 266) were similar to the values observed on Day 14.

Summary of Toxicokinetic Parameters:

Dose (mg/kg)	Day	C _{max} (ng/mL)	T _{max} (hr)	AUC (0-24 hr) ng•hr/mL
0.2	1	25.2 ±4.5	9±0	379 ±54
	14	44.3±9.6	5.2±3.1	804 ±202
	30	51.3±13	7.5±2.7	933±236
	91	43.8±7.8	5.2±3.1	800±159
	148	47.7±9.4	6.7±3.1	847±214
	210	45.5±5.9	7.5±2.7	831±129
	266	48.3±6.34	6.75±3.1	869 ±111
0.4	Day	C _{max} (ng/mL)	T _{max} (hr)	AUC (0-24 hr) ng•hr/mL
	1	39.7±4.5	8.5±1.7	691±64
	14	68.2±11.5	8.5±1.7	1280±228
	30	85±16	7.5±3	1550±425
	91	79.4±16.2	6±3	1450±396
	148	101±41.3	9.8±5	1710±393

	210	69.6±11.9	7.3±2.8	1280 ±226
	266	79.5±7.8	8.4±1.8	1440±177
1.2*	Day	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) ng·hr/mL
	1	47.5±12	10.8±6.4	803±224
	14	154±45	7.7±2.0	2550±753

* Dose group discontinued due to toxicity findings

Other: A physical examination to assess the animals' general condition was performed from surviving animals once pretreatment for all dose groups; on Days 178 and 273 for the 0, 0.2, and 0.4 mg/kg dose groups; and on Recovery Day 36/37 (females/males) for the 0.4 mg/kg recovery animals. There were no treatment-related changes in the physical examinations.

Vital signs, which included respiration rate (RR, respirations per minute) and body temperature (BT, °C), were collected once pretreatment and on Day 1 for all dose groups, and on Days 87, 178, and 269 for the 0, 0.2 (0.1 BID), and 0.4 (0.2 BID) mg/kg dose groups predose and at ~ 2-4 hours post the first dose. Hypothermia was noted in all treatment groups (- 1.3° C in ¼ animals in the low dose group, -2.5° C in 1/6 animals in the mid dose group and upto 2.5 ° C in all animals at high dose group).

Study title: Nine-Month Oral Toxicity Study of CP-526,555-18 in Cynomolgus Monkeys

Key study findings:

- No NOEL could be established for the test article CP-526,555 (tatarate salt) in monkeys (nasogastral intubations) due to the sporadic incidence of emesis and loose stools in all dose groups (0.01, 0.05, 0.2 (0.1 bid) mg/ kg/ day for 39 weeks).
- Lymphocytic infiltrations were noted in different tissues (trachea, pancreas, thyroid, salivary gland), this effect was dose related. Other histopathological findings include increase incidence of chronic inflammation in females (2/4 in control animals vs ¾ at high dose animals), increase incidence of mineralization in ovary, and increased incidence of cyst formation in the pituitary and parathyroid in females at high dose.
- Modulation of the hematological parameters (increase in the monocytes and the lymphocyte counts etc. compared to those of the controls) The hematological changes were within the historical control range, however, the increased incidence compare to the respective control suggests a stress response.

- Dose related increase in the exposure were noticed, no gender differences were found, approximately 1.5-fold accumulation of the test article was noted at the steady state.
- Based on the nature of the toxicity findings a NOAEL of 0.2 mg/kg/day (HED=3.8 mg) is established for 9-month monkey study (this is in concurrence with the Sponsor's NOAEL).
- At the NOAEL of 0.2 (0.1BID) a Cmax of 15.4±2.9 ng/mL and an AUC of 265±45 ng•h/mL (MRHD=2.3x) were obtained.

Study no.: 00-1545-28

Volume # and page #: Volume: 1; Page 1-372

Conducting laboratory and location: ζ

Date of study initiation: 02/ 27/01

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: CP-526,555-18, Lot No. 50452-31-2B, ζ The administered doses were adjusted for purity.

Methods

Doses: 0, 0.01, 0.05, 0.2 (0.1 bid) mg/ kg/ day for 39 weeks

Species/strain: Cynomolgus monkeys were used for this experiment.

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: Nasogastral intubations were used as the route of administration; 1mL/kg volume was administered /dosing; crystal spring water was used for formulation.

Satellite groups used for toxicokinetics or recovery: None

Age: 2 years approximately

Weight: Body weights ranged from 1.9 to 2.5 kg for the males and 1.8 to 2.1 kg for the females.

Sampling times: Blood (2 mL target volume) was collected from the femoral vein of all surviving monkeys on Day 1 and once during Weeks 5, 13, and 39 at 3, 6, 9, and 24 hours after the first daily dose. The 6-hour post dose sample was collected prior to the second daily dose.

Unique study design or methodology (if any): None

Study Design:

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Group Designations and Dose Levels

Group No. & (Description)	No. of Animals		No. Daily Doses	Dose Level*		Conc. In serum**		Dose Volume mL/kg/dose
	Male	Female		mg/kg/dose	mg/kg/day	base	salt	
1 (Control)	4	4	2	0	0	0	0	1
2 (Low)	4	4	1	0.01	0.01	0.01	0.017	1
3 (Mid)	4	4	1	0.05	0.05	0.05	0.085	1
4 (High)	4	4	2	0.10	0.20	0.10	0.171	1

* Dose levels are expressed relative to the free base (CP-526,555). Concentrations (Conc) are expressed relative to the free base (CP-526,555) and as the tartrate salt (CP-526,555-18) by adjusting for the active moiety (55.4%).

Observations times and Results:

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Mortality: Twice daily (a.m. and p.m.), each monkey was observed for mortality and moribundity. There was no unscheduled death under this experimental condition.

Clinical signs: Four times daily, cage side observations were made for each monkey: at approximately 1 hour prior to the first daily dose, immediately after all monkeys were dosed, approximately 5 hours after the first daily dose, and approximately 2 hours after the second daily dose. No treatment related effects were observed. Increased incidence of vomitus (yellow color, containing food) was noted in treated animals. Similarly, increased incidence of mucoid feces and non-formed feces excretion was observed in all compound treated groups. These clinical observations were not dose related, however, similar findings were noted with this compound in other toxicity studies.

Body weights: Individual body weights were recorded at least twice prior to treatment, on the first day of treatment, and weekly thereafter. No treatment related effects were seen on the body weight gain.

Food consumption: Food consumption was measured qualitatively, no changes were observed.

Ophthalmoscopy: Ophthalmoscopic examinations were performed once prior to the treatment and during Weeks 20 and 40. No visible lesions were identified.

EKG: Electrocardiograms were measured twice prior to initiation of treatment and once during Weeks 13, 26, and 39. Electrocardiographic examinations were recorded using 10 leads (I, II, III, aVR, aVL, aVF, V1, V2, V6, and V10). One control and one high dose animal showed changes in QTc prolongation 22-26 ms at Week 26, no changes were seen for the same animals at Week 39. The observation was not considered treatment related due to the lack of dose relationship (in agreement with Sponsor).

Hematology: Hematology sampling was conducted once prior to treatment and once during Weeks 13, 26, and 39. Regular battery of the hematological parameters was tested.

Summary of Hematological Findings:

Parameters (%)	Male/Dosage(mg/kg)	Female/Dosage(mg/kg)
	0.2	0.2
WBC count	16↑	13↓
Lymphocyte count	33↑	31↑
Neutrophil count	NC	77↓
Monocytes count	58↑	20↑
Eosinophil counts	50↑	100↑
RBC counts	NC*	8↓
Reticulocyte counts	37↓	23↑

Clinical chemistry: Clinical chemistry sampling was conducted once prior to treatment and once during Weeks 13, 26, and 39. Regular battery of the clinical chemistry

parameters was tested. Slight increase in sodium, potassium and chloride levels was noted in both males and females when compared to those of the control levels. The levels of the increase were, however, higher at Week 13 for all the parameters mentioned above compared to the levels observed at Week 39 indicating tolerance.

Urinalysis: Urinalysis sampling was conducted once prior to treatment and once during Weeks 13, 26, and 39. There were no treatment related findings.

Gross pathology: At necropsy gross pathological examination was performed to evaluate macroscopic lesions. No test article related macroscopic lesions were observed

Organ weights: At necropsy and after gross examination, selected organs (liver, kidneys, adrenals, heart, testes, ovaries, brain, and pituitary) were weighed. Relative kidney weight changes in males were noted 16 & 18 % at mid and high dose respectively. Changes in kidney weight are less than 5% at high dose in females. About 10 % increases in the relative weight of heart in males were noted at mid and high dose, no such changes were noted in females. In males, relative weight of the pituitary decreased 26% at high dose; no changes in female were noted.

Histopathology: Adequate Battery: Yes. Peer review: Yes.

Lymphocytic infiltration was seen in different tissues, in a dose dependent way, the significance of the findings is unknown. Changes in liver, kidney and heart were not always dose related. Organ weight changes were noted in males (kidney and heart) at high dose but due to the lack of the dose response the biological significance of the histopathological findings could not be assessed. Higher incidence of mineralization in the ovary, at high dose, was noted (see table below).

Summary of Histopathological Findings

Incidence/Tissue	Male /Dosages mg/kg/day)				Female /Dosages (mg/kg/day)			
	0	0.01	0.05	0.2	0	0.01	0.05	0.2
Lymphocytic infiltration / trachea	1	0	0	1	1	1	2	2
Lymphocytic infiltration / kidney	2	1	2	2	1	3	2	1
Lymphocytic infiltration/ pancreas	0	0	0	1	0	0	0	0
Lymphocytic infiltration/ thyroid	1	1	0	2	0	1	2	2
Lymphocytic infiltration/salivary gland	3	3	2	4	1	2	2	3
Mineralization/ovary					1	0	0	2
Mineralization/salivary gland	0	0	0	1	0	0	0	0
Cytoplasmic vacuolation/liver	1	2	2	1	3	2	2	3
Chronic inflammation/heart	3	0	2	1	2	1	4	3
Cyst/parathyroid	0	0	0	0	0	0	0	1

Cyst/pituitary	0	0	0	0	0	0	0	2
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Toxicokinetics: Blood was collected from the femoral vein of all surviving monkeys on Day 1 and once during Weeks 5, 13, and 39 at 3, 6, 9, and 24 hours after the first daily dose. The 6-hour postdose sample was collected prior to the second daily dose. Exposure as defined by the AUC and the Cmax attained the steady state by Week 14. Dose related increase in Cmax and AUC were noted at all time point studies (see table below). An increase in Cmax and AUC were noted for each dose at Day 14 compared to those at Day 1. Exposure did not change significantly after Day 14 indicating that a steady state for the exposure might have reached at this time point.

Summary of Toxicokinetic Parameters:

Dose (mg/kg)	Time	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) ng•hr/mL
0.01	Day1	1.74±0.21	3	NC*
	Week 5	1.92±0.31	3	NC
	Week 13	2.32±0.21	33	NC
	Week 39	0.57±0.66	3	NC
0.05	Day1	7.36±0.57	3	55.3±6.9
	Week 5	9.69±1.76	3	81.4±16.6
	Week 13	11.9±1.2	3	116±22
	Week 39	5.59±0.88	3	65.3±15
0.2	Day1	13.1±1.8	5.3	183±35
	Week 5	23.4±4.9	5.3	365±51
	Week 13	28.7±4.2	5.3	497±62
	Week 39	15.4±2.9	5.3	265±45

*BLQ

Other: Physical examination and vital signs (respiration rate, heart rate, rectal body temperature), and blood pressures, were measured twice prior to initiation of treatment and once during Weeks 13, 26, and 39. No changes of the above mentioned parameters were noted.

Study title: CP-526,555-18 2-Week Oral Range-Finding Study In CD-1 Mice

Key study findings:

- CD 1 mice treated with the test article (ttrate salt) by oral gavage at the doses of 0, 1, 10, 100 mg/kg / day for 2 weeks showed clinical signs of labored breathing and sternal recumbence at high dose. One male and two females died at low and high dose respectively, the cause of death is not known.
- Transient decrease in body weights gains was noted around Day 4; a concomitant decrease in food consumption was reported at the same time.

- Hematology parameters were measured on Day 15. In the two 100 mg/kg/day females that had sample quantities sufficient for analysis, there were changes in mean erythroid parameters that included decreases in mean red blood cell count (~0.70X control), hemoglobin (~0.75X control), and hematocrit (~0.74X control) and increases in mean corpuscular volume (~1.06X control), mean corpuscular hemoglobin (~1.07X control), and mean reticulocyte count (~4.5X control). One of these animals showed a stress leukogram characterized by decreases in total white blood cell and lymphocyte counts (values were ~0.43 and 0.29X mean control values, respectively). Changes in hematology parameters in 100 mg/kg/d females were considered treatment-related. No changes were observed at low and mid dose.
- Clinical chemistry parameters were measured on Day 15. In 100 mg/kg/day females, there was a decrease (~0.76X control) in mean serum potassium concentration that was considered treatment-related, although mean values remained within the historical control range for this laboratory. No changes were observed at low and mid dose.
- A NOAEL of 10 mg/kg (HED= 48.8 mg) was established for the mouse in this study. On Day 15, C_{max} and AUC were 566 ng/mL and 5,460 ng•h/mL (MRHD=47x) respectively at NOAEL dose.

Study no.: 01-1545-29

Volume # and page #: Volume: 1; Page 1-71

Conducting laboratory and location: Pfizer Inc, Groton, CT

Date of study initiation: 01/24/01

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: CP-526,555-18, 50452-17-5MSL J The administered doses were adjusted for purity.

Methods

Doses: 0, 1, 10, 100 mg/kg / day for 2 weeks

Species/strain: CD-1 Mice were used for this dose range finding study.

Number/sex/group or time point (main study): 10/sex/group

Route, formulation, volume, and infusion rate: Oral gavage, formulated in deionized water, dose volume was 10 mL/kg

Satellite groups used for toxicokinetics or recovery: _____ No recovery group, 3 animals /sex/group/ time point were used for toxicokinetic analysis.

Age: 39 days old approximately

Weight: At treatment initiation, individual body weights ranged from approximately 15 to 31 g for males and 20 to 27 g for females (~39 days old, both sexes).

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Sampling times: Blood samples were collected via the vena cava from satellite groups of animals at ~1, 4, 8, and 24 hours post-dose (3 different animals/sex/dose group were used at each time point) on Days 1 and 15.

Unique study design or methodology (if any):

Study Design:

Group	Daily Dose* (mg/kg)	Dose Volume (ml/kg)	Drug Concentration (mg/ml)	Animal Numbers	
				Males	Females
Control (deionized water)	0	10	0.0	1-10	41-50
Low-dose (CP-526,555-18)	1	10	0.1	11-20	51-60
Mid-dose (CP-526,555-18)	10	10	1	21-30	61-70
High-dose (CP-526,555-18)	100	10	10	31-40	71-80

* All daily dose levels are expressed as mg of active moiety per kg of body weight.

Observations times and Results:

Mortality: All animals were examined daily for mortality and morbidity. Mortality occurred in 1/10 control male, died at Day 11, 1/10 male at low dose at Day 15, and 2/10 females at Day 4 and Day 6. Cause of death in the treated animals was not clear. Labored respiration was noted in one female at 100 mg/kg/day female and may be associated with morbidity.

Clinical signs: All animals were examined daily for clinical signs. Clinical signs observed at 100 mg/kg/d included decreased activity (10/10 males and 10/10 females), tremors (8/10 females), sternal recumbency (1/10 males and 5/10 females), ataxia (4/10 females), labored respiration (2/10 females), and ptosis (2/10 males). All these clinical signs are treatment related. Clinical signs were not observed after Day 5, except for decreased activity and sternal recumbency which occurred throughout the study period. No clinical signs were observed at low and mid dose.

Body weights: Body weights were recorded pre-study and on Days 1, 4, 7, 10, and 14. At 100 mg/kg/day transient decrease in mean body weight gain (Day 4 in males; Days 4 and 7 in females) and mean absolute body weight (Days 4 and 7 in females); parameters returned to control values by Days 7 and 10 in males and females, respectively. No changes were seen at low and mid dose

Food consumption: Food consumption were recorded pre-study and on Days 1, 4, 7, 10, and 14. At 100 mg/kg/day, there were transient decrease in food consumption in male and female animals (~0.61X and 0.37X control, respectively) on Day 4; mean values returned toward control by Day 7. No changes were seen at low and mid dose.

Ophthalmoscopy: Not done

EKG: Not done

Hematology: Hematology parameters were measured on Day 15. Only two 100 mg/kg/day females had sample quantities sufficient for analysis. Regular battery of the hematological parameters was tested. There were changes in mean erythroid parameters that included decreases in mean red blood cell count (~0.70X control), hemoglobin (~0.75X control), and hematocrit (~0.74X control) and increases in mean corpuscular volume (~1.06X control), mean corpuscular hemoglobin (~1.07X control), and mean reticulocyte count (~4.5X control). One of these animals showed a stress leukogram characterized by decreases in total white blood cell and lymphocyte counts (values were ~0.43 and 0.29X mean control values, respectively). Changes in hematology parameters in 100 mg/kg/d females were considered treatment-related. No changes were observed at low and mid dose.

Clinical chemistry: Clinical chemistry parameters were measured on Day 15. Regular battery of the clinical chemistry parameters was tested. In 100 mg/kg/d females, there was a decrease (~0.76X control) in mean serum potassium concentration that was considered treatment-related, although mean values remained within the historical control range for this laboratory. No changes were observed at low and mid dose.

Urinalysis: Not done.

Gross pathology: Not done

Organ weights: Not done

Histopathology: Not done

Toxicokinetics: Serum drug concentrations were measured in groups of animals on Days 1 and 15 at 1, 4, 8, and 24 hours post-dose. Dose related increase in the exposure was noted, in general there was no gender difference. No accumulation was noted. On Day 15, at mid dose Cmax and AUC were 566 ng/mL and 5,460 ng•h/mL respectively.

Summary of Toxicokinetic Parameters:

Dose (mg/kg)	Day	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) ng•h/mL
1.0	1	98.0 ±0.21	1±0	415 ±14.2
	15	141 ±012.6	1±0	437 ±12.1
10	1	471 ± 9.1	1±0	3,390±127.6
	15	566±81.6	1±0	5,460±193.2
100	1	4,275±1000.5	6±1	56,000±7007.8
	15	3,205±708.9	6±1	42,700±800.5

Other: None

Study title: CP-526,555-18 3-Month Oral Toxicity Study in CD-1 Mice; Study # 01-1545-31

This study was reviewed for the dose selection for the carcinogenicity study.

Study title: Exploratory Oral Escalation/Toleration Study of CP-526,555-01 In Beagle Dogs

Key study findings:

- Emesis was noted in dogs after the oral administration of the test article at different doses (0.05, 0.1, 0.3, and 1mg/kg/day).
- Slight decrease in body weight, 4-5 % was noted in the dogs at the high dose group.
- Hematological changes (increased neutrophils and monocytes 1.6-3.9x) were noted in the high dose group animals at Day 2, indicating a stress response.
- No nonemetic dose for this test article could be determined under this experimental condition in the dogs.

Study no.: 97-1545-05

Volume # and page #: Volume 1, Page 1-34

Conducting laboratory and location: Pfizer Inc., CT

Date of study initiation: Not mentioned.

GLP compliance: No

QA report: No

Drug, lot #, and % purity: CP-526,555-01, lot # 38712-174-19, 100% The administered doses were adjusted for purity.

Methods

Doses: 0.05, 0.1, 0.3, and 1mg/kg

Species/strain: Beagle dogs

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

Route, formulation, volume, and infusion rate: Oral, deionized water, 5 mL/kg

Satellite groups used for toxicokinetics or recovery: None

Age: Not mentioned

Weight: Not mentioned

Sampling times: Blood samples were collected on Day 1 at 1, 4, 8, and 24 hrs post dose.

Unique study design or methodology (if any):

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Dosing Calendar			
Escalation/Toleracion Phase			
Treatment Group	Animal No.	Day(s) of Test	Dose (mg/kg)
1	1M, 6F	1-6	1*
2	2M, 7F	1	1**
3	3M, 8F	1-7	0.1
4	4M, 9F	1	0.05
5	5M, 10F	1-7	0.3

*Dose was administered at a volume of 5 mL/kg on Day 2 only.

**Dogs were dosed ~ 1 hour post feeding.

Observations times and Results: (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: The animals were observed daily for the signs of morbidity, all animals survived the dosing period.

Clinical signs: Clinical signs were noted twice daily, emesis, body tremors, decreased activity, loose stool, and salivation were observed in all dose groups. In addition to these findings dehydration was noted in the high dose group.

Body weights: Body weights were recorded pretreatment and daily thereafter, a decrease in body weight were noted in animals at the high dose group.

Food consumption: Food consumption was noted pretreatment and daily during treatment. A decrease in food consumption was noted in the high dose group animal.

Ophthalmoscopy: Not conducted.

EKG: Not conducted.

Hematology: Blood samples were collected on Day 2 and Day 7 for hematological assessment. Increased counts of neutrophils and monocytes were noted at high dose on Day 2 indicating a stress response.

Clinical chemistry: Blood samples were collected on Day 2 and Day 7 for serum chemistry assessment. Increase in ALT (1.8-4.4-fold) were noted in animals from all dose groups on Day 2; increase in the ALT levels were 2.2-5.5-fold on Day 7 suggesting treatment related changes in the liver function.

Urinalysis: Not conducted.

Gross pathology: Not conducted.

Organ weights (specify organs weighed if not in histopath table): Not conducted.

Histopathology: Not conducted.

Toxicokinetics: Dose related increase in AUC and Cmax were noted, no apparent gender difference was observed. Accumulation noted at high dose.

Other: None

Study title: CP-526,555-24: Exploratory 10-Day Oral Toleracion Study In Sprague Dawley Rats; Study # 97-1545-08

This study was reviewed for the dose selection for the carcinogenicity.

Study title: CP-526,555-24 3-Month Oral Toxicity Study in Sprague-Dawley Rats; Study # 0054

This study was reviewed for the dose selection for the carcinogenicity.

Study title: CP-526,555-24 6-Week Oral Toxicity Study In Sprague-Dawley Rats. 98-1545-11

This study was reviewed for the dose selection in the carcinogenicity study.

Study title: 6-Month Oral Toxicity Study of CP-526,555-18 In Rats**Key study findings:**

- A NOAEL of 10 mg/kg/day is established for this 6-month rat study (oral gavage administration of 3, 10, 30 mg/kg/day).
- Clinical signs (reduced and/or soft feces, chromodacryorrhea, and urine staining) were noted at high dose (18/21 females and 10/21 males). At mid and low dose these clinical signs were still observed but only sporadically.
- Although no change in body weight gain was noted, decrease in body weight was observed in both males and females the decrease in body weight was higher on Week 1 compare to that in Week 26. The decrease in body weight may be related to the decrease in food consumption, which was also found to decrease more at Week 1 compare to Week 26.
- Hematological findings: changes in WBC counts were observed at Week 13, suggesting an intrinsic stress response. The intensity of these changes was decreased on Week 26 indicating recovery.
- Several changes in the serum chemistry parameters were noted. these includes in crease in ALT and ALKP level which might be related to the changes in the liver function, however, no histopathological changes were noted in the liver. Increased Phosphorous levels were also noted which might be related to the kidney function, however, no changes in the histopathology of the kidney was observed.
- Histopathological findings are limited to jejunal epithelial vacuolation (5/15 males and 1/15 females), retinal dysplasia (1/15 males), and infiltration of foamy macrophages in the lung at high dose (2/15, 6/15, 5/15 in control, male, and females respectively). All of these changes might be related to the pharmacological effect of the compound, toxicological significance of the findings is not known.
- Increase in the liver weight >10% were noted in females at high dose which can be correlated with the enlarged liver observed in the macroscopic evaluation in the females from the same dose group.
- Increase in the exposure was noted with increasing dose, however, the increase was less than dose proportional, no gender differences were noted, accumulation was observed at all doses with increasing time.
- At the NOAEL dose of 10 mg/kg/day (HED=96 mg) a C_{max} of 906 ng/mL and an AUC of 14,400 ng•h/mL (MRHD=124) were determined.

Study no.: RR 745-03536

Volume # and page #: Volume 1; Page 1-288

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Conducting laboratory and location: Pfizer Inc, Ann Arbor, MI

Date of study initiation: 07-17-01

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: CP-526, 555-18, Lot # 53650-5-5B, [] The administered doses were adjusted for purity.

Methods

Doses: 3, 10, and 30 mg/kg/day for 26 weeks

Species/strain: Sprague-Dawley CD(SD)IGS BR rats

Number/sex/group or time point (main study): 15/sex/group

Route, formulation, volume, and infusion rate: Oral gavage, deionized water, 10 mL/kg

Satellite groups used for toxicokinetics or recovery: 6/sex/group was used for toxicokinetic analysis

Age: 7 to 8 weeks

Weight: 162 to 219 g

Sampling times: Blood samples were collected on Day 1, during Week 6, and during Week 26 at approximately 1, 4, 8, and 24 hours postdose

Unique study design or methodology (if any):

Study Design

Group	Males	Dose (mg/kg)	Group	Females
1	99814-99828	Vehicle Control ^a	5	99892-99906
2	99829-99849	3	6	99907-99927
3	99850-99870	10	7	99928-99948
4	99871-99891	30	8	99949-99969

^a Deionized water

Observations times and Results:

Mortality: Animals were observed predose and approximately 4 hours postdose daily for mortality and morbidity. One animal from the low dose group was euthanized for humane reason. Necropsy finding showed error in oral gavage.

Clinical signs: Animals were observed predose and approximately 4 hours postdose daily for clinical signs. At high dose 18/21 rats showed reduced and/or soft feces, chromodacryorrhea (or red staining), and urine staining were seen (females showed higher incidences than males). Similar findings but sporadic in nature were also noted in animals from the low and mid dose group.

Body weights: Body weights were recorded pretest, prior to dosing on Day 1 and weekly thereafter. Decrease in body weight were noted >15% in males at all different time points at which the body weight were recorded. 25% decrease in body weight was seen in females at high dose up to Week 4 after that decrease body weight gain was 9-11

%. However, the change in body weight gains did not decrease with the increasing duration of the treatment.

Summary of Body Weight Changes

Group	Control	Low	Mid	High
N	15	15	15	15
Dose (mg/kg)	0	3	10	30
Body weight in gm/ % control: (Males)				
Baseline	200±1.5	211.9±1.4	205.0±2.4	196.9±1.8
Week 1	257.9±2.6	265.9±2.2	249.3±4.8 4%↓	220.5±3.4 15%↓
Week 4	388.5±8.1	387.2±5.4	357.8±8.9 8%↓	333.1±5.5 15%↓
Week 12	456.3±9.4	471.6±6.6	426.3±11.1 7%↓	384.4±7.3 15%↓
Week 24	645.2±21.6	660.5±11.4	593. ±19.6 9%↓	479.9±9.47 25%↓
Body weight in gm/ % control: (Females)				
Baseline	179.2 ±3	180.2±2.6	185.8±2.5	180.8±2.4
Week 1	240.9±4.6	201.1±3.0	198.1±2.6	179.5±3.2 25%↓
Week 4	240.9±4.6	236.4±4.6	239.6±3.5	220.9±4.6 9%↓
Week 12	266.6±5.6	256.3±5.5	259.5±3.3	239.6±4.68 11%↓
Week 24	324±6.3	311.6±7.7	327.6±5.7	278.4±5.5 15%↓

* Statistically significant

Change in Body Weight Gains (gms)

Group	Control	Low	Mid	High
N	15	15	15	15
Dose (mg/kg)	0	3	10	30
Body weight (Males)				
Week 1	57.8±1.9	53.9±1.5	44.3±3.0	23.6±2.73
Week 4	38.4±2.4	32.0±2.1	28.2±1.4	29.3±2.4
Week 12	22.7±2.2	23.9±1.7	20±1.1	10.6±3.1
Week 24	5.0±3.3	3.8±2.8	2.5±3.1	-7.2±1.5
Body weight (Females)				
Week 1	16.2±2.3	20.9±1.6	12.2±2.2	-1.2±1.6
Week 4	12.5±1.1	9.6±2.1	12.9±1.9	6.8±1.6
Week 12	7.1±1.9	6.1±2.2	5.2±1.7	0.8±0.9
Week 24	-5.9±1.6	-3±1.8	-3.4±1.9	-5.5±1.7

Food consumption: Individual food consumption was recorded weekly and at termination. A statistically significant decrease in food consumption was noted in females

at Week 1. No statistically significant changes noted at termination. In males statistically significant decrease in food consumption were noted for Week 1 (40%) and for Weeks 2-26 (between 9-17%).

Food Consumption (gms)

Group	Control	Low	Mid	High
N	15	15	15	15
Dose (mg/kg)	0	3	10	30
Body weight in gm/% control (Males)				
Week 1	157.5±2.7	155.7±2.6	145.5±4.4	94.2±3.9
Week 4	174.5±4.2	174.2±3.3	169.9±4.4	146.7±4.9
Week 12	178.4±4.0	182.5±2.9	171.5±3.8	159.6±4.9
Week 24	157±5.7	159.4±4.1	152.7±4.8	134.9±2.4
Body weight in gm/% control (Females)				
Week 1	111.5±2.8	107.7±1.6	98.7±1.9	58.6±2.8
Week 4	120.3±2.6	119.3±2.6	118.9±2.45	114.3±2.5
Week 12	120.3±2.6	118.1±2.2	119.3±2.1	110.4±3.1
Week 24	99.1±2.4	100.1±2.3	104.0±1.8	99.9±2.4

Ophthalmoscopy: Ophthalmic examinations were performed pretest and during Week 26. There were no treatment related changes.

EKG: Not conducted.

Hematology: Hematological parameters were evaluated at Week 13 and at termination. Regular battery of the hematological parameters was tested. Increase in monocytes, lymphocytes and neutrophils counts were noted in both males and females on Week 13 indicating a stress response. However, both the magnitude of the changes as well as nature of changes were much less severe on week 26, suggesting recovery.

Summary of Hematological Findings (% change):

Parameters	Male	Female
	30 mg/kg	30 mg/kg
WBC count	NC	17↑
Lymphocyte	5↓	19↑
Neutrophil	16↑	16↑
Monocytes	20↓	35↓
Eosinophil	11↓	47↓
Reticulocyte	NC	18↓

*NC; no change

Clinical chemistry: Serum chemistry parameters were evaluated at Week 13 and at termination. Regular battery of the clinical chemistry parameters was tested. Several changes in the serum chemistry parameters were noted in males and females at 30

mg/kg/day on Week 13 and Week 26. These changes include increase in Phosphorous (11 and 35% in males and females respectively), slight decrease in the Phosphorous level were noted on Week 26 for males. In crease in ALKP (33 and 35% in males and females respectively) were noted on Week 13, a decrease in the ALKP was noted in males at termination ALT (56 and 35% in males and females respectively). No changes were noted in ALT on Week 26. Phosphorus at 10 mg/kg was statistically higher than control at Week 13 in females by 27%, and at Week 26 in males and females by 11% and 22%, respectively. ALKP in males at 10 mg/kg was statistically higher than control by 27% at Week 13. Total bilirubin in males at 30 mg/kg was statistically higher than control by 30% and 41% at Weeks 13 and 26, respectively. Liver enzyme changes signify functional changes in liver. Changes in Phosphorous may be related to kidney function. However, the biological significance of the changes in the serum chemistry findings can not be determined due to its lack of correlation with the histopathological findings.

Summary of Serum Chemistry Findings % change:

Parameters	Male	Female
	30 mg/kg	30 mg/kg
Total bilirubin	41↑	9↑
Alkaline Phosphatase	11↑	39↑
Urea Nitrogen	16↑	6↑
Calcium	NC	5↑
Phosphorus	15↑	21↑

Urinalysis: Urinalysis was conducted at termination. There were no

Gross pathology: Macroscopic findings were limited to enlarged liver noted at necropsy in 6/15 females.

Organ weights (specify organs weighed if not in histopath table): Dose related statistically significant increase in liver weight was observed in females (11 and 14 % at 10 and 30mg/kg/day respectively). Decrease in the weight of thymus was noted (no statistical significance) at 30 mg/kg in both males and females.

Summary of Organ Weight Changes (Relative Weight):

Parameters	Male	Female
	30 mg/kg	30 mg/kg
Brain	30↑	16
Heart	9↓	NC
Kidney	13↓	NC
Liver	9↓	14↑
Spleen	8↓	14↑
Thymus	30↓	14↓

Histopathology: Adequate Battery: Yes. Peer review: Yes.

The histopathological findings were limited to the cytoplasmic vacuolation in 5/15 males and 1/15 females at high dose. Ultrastructural examination of jejunal mucosal epithelium revealed that the cytoplasmic vacuoles were separation of artifacts adjacent to whorls of smooth endoplasmic reticulum (SER). These whorls of SER are consistent with SER proliferation. Sponsor believes that

The finding is not toxicologically relevant. However, due to the abundance of the finding at high dose and due to the pharmacological relevance of the finding, according to the reviewer, the jejunal vacuolation might be related to the treatment. Retinal dysplasia was noted in 1/15 male rat, no such changes were noted in control animals. This finding might be related to the test article (pharmacologically nicotine is known to deposit in the melanin containing tissue, the test article is partial agonist of nicotine).

Summary of Histopathological Findings:

Incidence/Tissue	Male /Dosages (mg/kg/day)				Female /Dosages (mg/kg/day)			
	0	3	10	30	0	3	10	30
Hyperplasia/Heart	7	0	0	4	0	0	0	1
Vacuolation, erosion /Small intestine	0	0	0	5	0	0	0	1
Hyperostosis/Bone	0	0	0	0	0	0	0	1
Infiltrate, foamy macrophages, alveolus/Lung	2	1	0	6	2	0	0	5
Dysplasia, retina/Eye	0	0	0	0	0	0	0	1
Cyst/Uterus	0	0	0	0	2	0	0	7

Toxicokinetics: Exposure as defined by the Cmax and AUC (0-24) values increased with dose in a less than dose proportional manner. Mean Cmax at 3, 10, and 30 mg/kg was increased relative to Day 1 by 20%, 46%, and 89%, respectively, at Week 6, and by 26%, 108%, and 148%, respectively, at Week 26. Mean AUC (0-24) at 3, 10, and 30 mg/kg was increased relative to Day 1 by 29%, 34%, and 38%, respectively, at Week 6, and by 90%, 115%, and 105%, respectively, at Week 26. Accumulation of the compound was noted with increasing time at all doses. No gender differences were noted.

Summary of Toxicokinetic Parameters:

Dose (mg/kg)	Day	Cmax (ng/mL)	Tmax (hr)	AUC (0-24 hr) ng•hr/mL
3.0	1	229	1	1,870
	42	274	1	2,410
	182	289	1	3,550

10	1	435	4	6,500
	42	633	4	8,700
	182	906	8	14,000
30	1	755	4	15,900
	42	1430	4	22,000
	182	1870	8	32,000

Other: None

Histopathology inventory (optional)

Study	00-1545-34		RR745-03536	
Species	Cynomolgus Monkey @0.2 mg/kg (NOAEL)		Rat @ 10mg/kg/day (NOAEL)	
	Organ weight	Histopat hology	Organ weight	Histopat hology
Adrenals	*	x	*	x
Aorta		x		x
Bone Marrow smear		x		x
Bone (femur)		x		
Brain	*	x	*	x
Cecum		x		x
Cervix		x		x
Colon		x		x
Duodenum		x		x
Epididymis		x		x
Esophagus		x		x
Eye		x		x
Fallopian tube		x		x
Gall bladder		x		x
Gross lesions		x		x
Harderian gland				x
Heart	*	x	*	x
Ileum		x		x
Injection site		x		x
Jejunum		x		x
Kidneys	*	x	*	x
Lachrymal gland		x		x
Larynx		x		x
Liver	*	x	*	x
Lungs	*	x	*	x

Lymph nodes, cervical		x		x
Lymph nodes mandibular		x		x
Lymph nodes, mesenteric		x		x
Mammary Gland		x		x
Nasal cavity		x		x
Optic nerves		x		x
Ovaries	*	x	*	x
Pancreas		x		x
Parathyroid		x		x
Peripheral nerve		x		x
Pharynx		x		x
Pituitary		x		x
Prostate		x		x
Rectum		x		x
Salivary gland		x		x
Sciatic nerve		x		x
Seminal vesicles		x		x
Skeletal muscle		x		x
Skin		x		x
Spinal cord		x		x
Spleen	*	x	*	x
Sternum		x		x
Stomach		x		x
Testes	*	x	*	x
Thymus	*	x	*	x
Thyroid		x		x
Tongue		x		x
Trachea		x		x
Urinary bladder		x		x
Uterus		x		x
Vagina		x		x
Zymbal gland		x		x

X, histopathology performed

*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: CP-526,555-01 In Vitro Cytogenetic Studies

Key findings:

- The test article was not clastogenic in the human lymphocyte cultures in vitro with or without metabolic activation.

Study no.: 97-1545-03

Volume # and page #: Volume 1; Pages 1-31

Conducting laboratory and location: Pfizer, Gorton, CT

Date of study initiation: 07-30-98

GLP compliance: Yes

QA reports: No

Drug, lot #, and % purity: 38712-067-01 (Preliminary Tests); 38712-079-20 (Definitive Tests); 38712-074-35 (Definitive Tests), [] The administered doses were adjusted for purity.

Methods

Strains/species/cell line: The study was conducted with human peripheral lymphocytes collected by venipuncture from healthy donors. Cells were cultured in Williams medium E with the mitogen, M-phytohemagglutinin, at a final medium concentration of 1% (v/v). Approximately 47 to 49 hours after culture initiation, the cultures were pooled, resuspended in fresh culture medium and counted. Prior to treatment, the cell density of the suspension was adjusted to approximately 3.5 to 4.5 x 10⁵ cells/mL (Day 0 cell count), then aliquoted into 5 ml cultures.

Doses used in definitive study:

984 µg/mL, 1230 µg/mL, 1540 µg/mL without metabolic activation at 3 hrs
630 µg/mL, 984 µg/mL, 1230 µg/mL with metabolic activation at 3 hrs
322 µg/mL, 403 µg/mL, 504 µg/mL, 630 µg/mL without metabolic activation at 24 hrs-test 1
400 µg/mL, 450 µg/mL, 500 µg/mL, 550 µg/mL, 600 µg/mL, 650 µg/mL, without metabolic activation at 24 hrs-test 2

Basis of dose selection: A preliminary cytotoxicity test assay with the highest concentration of CP-526,555-01 tested (1000 mg/mL) was used as the basis for dose selection. The study showed a <10% reduction of the mitotic index in the 3 hour tests with and without metabolic activation, respectively; and a 68% reduction of the mitotic index in the 24 hour direct test.

Negative controls: DMSO

Positive controls: Mitomycin-C, a direct-acting clastogen, is tested as a positive control without the S9 metabolic activation system at concentrations of 0.05 mg/mL (24 hour) and 0.40 mg/mL (3 hour). Cyclophosphamide, a proclastogen, is used as a positive control in the presence of the S9 metabolic activation system at a concentration of 10.0 mg/mL.

Incubation and sampling times: 3 and 24 hr without metabolic activation and 3 hr with metabolic activation.

Results

Study validity: The study used duplicate culture, the cells were manually counted (200 cells were counted /culture), positive and negative controls gave acceptable results demonstrating the validity and appropriate sensitivity of the assay. There was no evidence of incomplete solubility of the test article in any of the 100X stock solutions prepared in DMSO.

Study outcome:

In the 3 hour test without metabolic activation there was no significant difference in the number of abnormal cells compared to concurrent negative controls with 984, 1230, and 1540 mg/mL concentration of the test article. 18 to 56% reductions in the mitotic were reported in the 3 concentrations evaluated.

In the 3 hour test with metabolic activation no significant difference in the number of abnormal cells compared to the controls with the three test concentrations (630, 984, and 1230 mg/mL) producing a 16 to 48% reduction in the mitotic index were observed. In the 24 hour test without metabolic activation three test concentrations (322, 403, and 504 mg/mL) producing a 21 to 43% reduction in the mitotic index were selected to evaluate for chromosome damage. There was no significant difference in the number of abnormal cells compared to the controls. The highest test concentration (504 mg/mL) available for analysis produced only a 43% reduction of the mitotic index compared to the assay acceptability criteria of a 50% reduction. A second 24 hour test without metabolic activation was conducted with a dose range of 350 to 650 mg/mL in order to achieve a higher level of mitotic suppression >50%. In this repeat test three test concentrations (450, 500, and 600 mg/mL) producing a range of mitotic reduction (19 to 56%) were evaluated for chromosome damage. There was no significant difference in the number of abnormal cells compared to negative controls.

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CP-526,555-01
HUMAN LYMPHOCYTE ABERRATION ASSAY: INDIVIDUAL DATA VALUES (TEST 4)

3 HOUR TREATMENT
WITHOUT METABOLIC ACTIVATION

Treatment	Day 1 Cells per ml (x10 ⁶) ^a	(%) Mitotic Index	No. Cells Analyzed	P	Gaps		Aberrations				No. Abnormal Cells	
					Cib	Csb	Cib	Csb	R	M		PV
Negative Control: DMSO												
1.0%	5.15	16.9	100	0	1	0	2	2	0	0	0	3*
	5.11	17.4	100	0	1	0	2	0	0	0	0	2
Test Article: CP-526,555-01 (µg/ml)												
630	4.53	--	--	--	--	--	--	--	--	--	--	--
	4.64	--	--	--	--	--	--	--	--	--	--	--
787	4.86	--	--	--	--	--	--	--	--	--	--	--
	4.01	--	--	--	--	--	--	--	--	--	--	--
984	3.70	13.8	100	0	0	0	1	0	0	0	0	1
	5.09	14.3	100	1	1	0	0	1	0	0	0	1
1230	4.30	12.2	100	0	2	0	1	0	0	0	0	1
	4.10	11.4	100	1	2	0	1	0	0	0	0	1
1540	4.20	6.7	100	0	2	0	5	0	0	0	0	4*
	3.65	8.3	100	1	2	1	2	1	0	0	0	3
1920+	3.21	--	--	--	--	--	--	--	--	--	--	--
	2.47	--	--	--	--	--	--	--	--	--	--	--
2400+	2.81	INS	--	--	--	--	--	--	--	--	--	--
	3.73	INS	--	--	--	--	--	--	--	--	--	--
3000+	3.70	INS	--	--	--	--	--	--	--	--	--	--
	3.32	INS	--	--	--	--	--	--	--	--	--	--
Positive Control: Mitomycin-C												
0.40	3.41	7.6	50	0	4	1	9	6	8	0	0	13*
	3.04	7.6	50	0	3	1	7	7	5	0	0	13*

a: Day 1 Cells/ml = Mean Coulter Counts (x) Dilution Factor (4-3).
--: Dashes indicate data not available or determined.
*: Some cells contain more than one aberration.
=: Precipitate was observed in the culture.

Abbreviations: INS: Insufficient numbers of mitotic cells for analysis; Cib-Chromatid; Csb-Chromosome; P-The number of polyploid cells (including endoreduplicated cells) observed during metaphase collection; Cib-Chromatid Break; Csb-Chromosome Break; R-Rearrangement; M-Multiple Aberrations (≥ 7); PV-Pulverized Chromosomes.

CP-526,555-D1
 HUMAN LYMPHOCYTE ABERRATION ASSAY: INDIVIDUAL DATA VALUES (TEST 3)
 3 HOUR TREATMENT
 WITH METABOLIC ACTIVATION

Treatment	Day 1 Cells per ml (x10 ⁶) ^a	(% Mitotic Index	No. Cells Analyzed	P	Gaps		Aberrations					No. Abnormal Cells
					Ctb	Csb	Ctb	Csb	R	M	PV	
Negative Control: DMSO												
1.0%	6.25	16.5	100	1	1	0	0	0	0	0	0	0
	5.91	16.0	100	0	0	0	1	0	0	0	0	1
Test Article: CP-526,555-D1												
(µg/ml)												
630+	5.05	13.4	100	0	1	0	0	0	0	0	0	0
	5.95	15.4	100	0	1	0	1	0	0	0	0	1
787+	4.94	13.0	--	--	--	--	--	--	--	--	--	--
	5.18	11.6	--	--	--	--	--	--	--	--	--	--
984+	5.04	12.6	100	1	1	0	0	0	0	0	0	0
	4.67	13.0	100	1	1	0	1	0	0	0	0	1
1230+	4.73	9.9	100	1	0	0	1	0	0	0	0	1
	4.28	7.6	100	0	1	0	1	0	0	0	0	1
1540+	4.93	--	--	--	--	--	--	--	--	--	--	--
	4.65	--	--	--	--	--	--	--	--	--	--	--
1920+	5.05	INS	--	--	--	--	--	--	--	--	--	--
	4.87	INS	--	--	--	--	--	--	--	--	--	--
2400+	5.91	T	--	--	--	--	--	--	--	--	--	--
	5.40	T	--	--	--	--	--	--	--	--	--	--
3090+	5.43	T	--	--	--	--	--	--	--	--	--	--
	5.68	T	--	--	--	--	--	--	--	--	--	--
Positive Control: Cyclophosphamide												
10.0	4.92	6.8	50	0	2	0	9	3	3	0	0	10*
	4.59	6.8	50	0	5	0	15	5	9	0	0	14*

a: Day 1 Cells/ml = Mean Coulter Counts (x) Dilution Factor (40).
 --: Dashes indicate data not available or determined.
 *: Some cells contain more than one aberration.
 †: Predplate was observed on the slides.

Abbreviations: INS: Insufficient numbers of mitotic cells for analysis; T-Toxic: No mitotic cells; Gap
 Ctb-Chromatid; Gap Csb-Chromosome; P-The number of polyploid cells (including endoreduplicated cells)
 observed during metaphase collection; Ctb-Chromatid Break; Csb-Chromosome Break; R-Rearrangement;
 M-Multiple Aberrations (≥ 7); PV-Fragmented Chromosomes.

Protocol No: 025

CP-526,555-01
HUMAN LYMPHOCYTE ABERRATION ASSAY: INDIVIDUAL DATA VALUES (TEST 5)

24 HOUR TREATMENT
WITHOUT METABOLIC ACTIVATION

Treatment	Day 1 Cells per ml (x10 ⁶) ^a	[%] Mitotic Index	No. Cells Analyzed	P	Gaps		Aberrations				No. Abnormal Cells	
					Cib	Csb	Cib	Csb	R	M		PV
Negative Control: DMSO												
1.0%	6.73	11.6	100	0	0	0	1	0	0	0	0	1
	7.59	12.0	100	0	1	0	0	0	0	0	0	0
Test Article: CP-526,555-01 (µg/ml)												
350	6.57	--	--	--	--	--	--	--	--	--	--	--
	6.43	--	--	--	--	--	--	--	--	--	--	--
400	5.91	--	--	--	--	--	--	--	--	--	--	--
	6.37	--	--	--	--	--	--	--	--	--	--	--
450	6.63	8.1	100	2	0	0	1	0	0	0	0	1
	6.48	9.7	100	0	1	0	1	0	0	0	0	1
500	6.42	9.6	100	0	1	0	0	0	0	0	0	0
	6.49	9.4	100	0	0	0	1	0	0	0	0	1
550	5.33	5.0	--	--	--	--	--	--	--	--	--	--
	5.76	4.0	--	--	--	--	--	--	--	--	--	--
600	5.31	5.0	100	2	2	0	1	0	0	0	0	1
	5.49	5.5	100	1	1	0	0	0	0	0	0	0
650	5.57	4.3	--	--	--	--	--	--	--	--	--	--
	5.59	5.0	--	--	--	--	--	--	--	--	--	--
Positive Control: Mitomycin-C												
0.05	6.46	9.0	50	0	4	1	7	1	0	0	0	7*
	6.37	9.3	50	0	5	0	3	0	2	0	0	5

a: Day 1 Cells/ml = Mean Coulter Counts (x) Dilution Factor (#0).

--: Dashes indicate data not available or determined.

*: Some cells contain more than one aberration.

Abbreviations: Gap Cib-Chromatid; Gap Csb-Chromosome; P-The number of polyploid cells (including endoreduplicated cells) observed during metaphase collection; Cib-Chromatid Break; Csb-Chromosome Break; R-Rearrangement; M-Multiple Aberrations (≥ 7); PV-Polyrized Chromosomes.

Study title: Microbial Reverse Mutation Assays

Key findings:

- The test article was not mutagenic under this experimental condition.

Study no.: 97-1545-04

Volume # and page #: Volume 1; Pages: 1-23

Conducting laboratory and location: Pfizer, Groton, CT

Date of study report: 03-26-98

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP 526,555 38712-174-19, The administered doses were adjusted for purity.

Methods

Strains/species/cell line: *Salmonella* or *E. coli* strain (TA1535 TA1537, TA95, TA100, WP2uvrA, pKM101) were used in the study.

Doses used in definitive study: 0.010, 0.050, 0.20, 1.0, 5.0 mg/plate was used with or without metabolic activation

Basis of dose selection: A pilot study was done using the plate incorporation technique, no cytotoxicity or insolubility was noted.

Negative controls: DMSO

Positive controls: Sodium Nitrite, 9-Aminoacridine, 2Nitrofluorene, Nitrofurantoin, ENNG, 2-Anthramine were used.

Incubation and sampling times: 48-72 hrs incubation time with or without metabolic incubation was used.

Results

Study validity: In definitive tests, five concentrations of CP-526,555 were tested in triplicate with each *Salmonella* or *E. coli* strain. Metabolic activation was indicated by a dose-related and reproducible three-fold increase in the average number of revertant colonies on the activation plates relative to the corresponding negative controls in the absence of a similar response on the non-activation plates.

Study outcome: There was no evidence of significant, dose-related increases in the number of revertant colonies per plate compared to the negative controls with any of the strains tested in either the presence or absence of S9 metabolic activation. Compound levels ranged from 0.050 to 5.0 mg/plate were tested in the definitive assay with all of the strains in both the absence and presence of S9 metabolic activation.

**CP-526,555-1
BACTERIAL MUTATION ASSAY: SUMMARY OF TEST RESULTS**

**PRELIMINARY PLATE INCORPORATION ASSAY
WITHOUT (-) AND WITH (+) S9 METABOLIC ACTIVATION**

Compound	mg/plate	S9	Number of Revertant Colonies Per Plate					WP2 <i>uvrA</i> pKM101
			TA1535	TA1537	TA98	TA100		
DMSO (0.1 ml/overlay)	---	-	18	13	22	93	75	
CP-526,555-1	0.010	-	12	9	21	93	84	
	0.050	-	15	13	15	88	93	
	0.20	-	18	19	14	92	92	
	1.0	-	17	18	25	80	97	
	5.0	-	13	21	45	162	116	
DMSO (0.1 ml/overlay)	---	+	13	14	17	117	96	
CP-526,555-1	0.010	+	13	12	32	124	111	
	0.050	+	9	12	19	87	114	
	0.20	+	4 F	10	19	111	114	
	1.0	+	12	13	30	116	108	
	5.0	+	13	10	36	125	109	
POSITIVE CONTROLS								
Sodium Nitrite	2.0	-	283					
9-Aminoacridine	0.15	-		1253				
2-Nitrofluorene	0.02	-			1674			
Nitrofurantoin	0.002	-				1755		
ENNG	0.005	-					1579	
2-Anthramine	0.005	+	143					
	0.01	+		459	1800	2208		
	0.1	+					1293	

F.... The plate was scored as Toxicity Type F because the revertant count was less than one-half the control but the background lawn was normal; this was not considered to be indicative of cytotoxicity since it was not dose-related and the background lawn was normal.

Test 1 (Salmonella): Day 0 = 09-19-1997

Test 2 (E. coli): Day 0 = 09-30-1997

CP-526,555-1
BACTERIAL MUTATION ASSAY: SUMMARY OF TEST RESULTS
DEFINITIVE MUTATION ASSAY WITHOUT (-) AND WITH (+) S9 METABOLIC ACTIVATION

Compound	mg/plate	S9	Average Number of Revertant Colonies Per Plate \pm S.D.				
			TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i> pKM101
DMSO (0.1 ml/overlay)	--	-	23 \pm 4	10 \pm 2	39 \pm 3	185 \pm 7	74 \pm 2
CP-526,555-1	0.050	-	23 \pm 5	12 \pm 3	38 \pm 13	183 \pm 2	79 \pm 5
	0.15	-	18 \pm 3	10 \pm 3	27 \pm 6	178 \pm 2	77 \pm 4
	0.50	-	22 \pm 7	9 \pm 1	39 \pm 11	185 \pm 15	76 \pm 12
	1.5	-	20 \pm 5	11 \pm 0	35 \pm 9	188 \pm 5	72 \pm 3
	5.0	-	22 \pm 4	17 \pm 3	52 \pm 8	268 \pm 23	90 \pm 18
DMSO (0.1 ml/overlay)	---	+	18 \pm 8	11 \pm 5	37 \pm 8	181 \pm 23	80 \pm 11
CP-526,555-1	0.050	+	20 \pm 6	11 \pm 2	40 \pm 9	158 \pm 8	100 \pm 13
	0.15	+	14 \pm 5	10 \pm 3	44 \pm 6	168 \pm 19	92 \pm 12
	0.50	+	17 \pm 4	8 \pm 2	41 \pm 6	191 \pm 7	90 \pm 19
	1.5	+	17 \pm 3	11 \pm 2	43 \pm 4	188 \pm 29	72 \pm 12
	5.0	+	17 \pm 8	11 \pm 2	49 \pm 8	187 \pm 9	70 \pm 13
POSITIVE CONTROLS							
Sodium Nitrite	2.0	-	204 \pm 29				
9-Aminoacridine	0.15	-		1869 \pm 69			
2-Nitrofluorene	0.02	-			1762 \pm 97		
Nitrofurantoin	0.002	-				1619 \pm 98	
ENNG	0.005	-					1811 \pm 146
2-Anthramine	0.005	+	170 \pm 4				
	0.01	+		491 \pm 38	1906 \pm 143	2075 \pm 236	
	0.1	+					1421 \pm 89

Study title: Mammalian Cell Gene Mutation Assays; CP-526,555-01

Key findings:

The test article did not induce forward mutation with or without metabolic activation at the X-linked hypoxanthine-guanine phosphoribosyl transferase locus in CHO cells under this experimental condition.

Study no.: 98-1545-12

Volume # and page #: Volume 1; Pages 1-29

Conducting laboratory and location: Pfizer, Inc, Groton, CT.

Date of study initiation: 11/20/98

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP-526,555; Lot # 38712-174-19. The administered doses were adjusted for purity.

Methods

Strains/species/cell line: The HGPRT⁺ subclone CHO-K1-BH4, in logarithmic growth, was used for the testing of CP-526,555-01.

Doses used in definitive study: In the definitive mutagenicity test conducted without metabolic activation, CP-526,555-01 was tested in duplicate at concentrations ranging from 500 mg/mL up to the maximum applied dose of 5000 mg/mL. In the test with metabolic activation CP-526,555-01 was tested in duplicate at concentrations ranging from 1000 mg/mL up to the maximum applied dose of 5000 mg/mL.

Basis of dose selection: A pilot study was done for dose selection. In preliminary cytotoxicity tests performed without and with S9, CP-526,555-01 was tested at concentrations ranging from 16 to 5000 mg/mL. CP-526,555-01 was insoluble in DMSO at all 100X stock concentrations 150 mg/mL, however; compound precipitation was not noted in the dosing medium at any concentration tested following a five hour incubation period. Cytotoxicity was observed in the test conducted without metabolic activation at 5000 mg/mL and at final concentrations 2500 mg/mL in the preliminary test conducted with metabolic activation.

Negative controls: DMSO

Positive controls: 3-Methylcholanthrene was used as positive control with metabolic activation and Ethylmethanesulfonate was used as positive control without metabolic activation.

Incubation and sampling times: All cultures were exposed to selected concentrations of CP-526,555-01 or to positive or negative controls (Dosage: 100 µl of stock into each 10 ml culture = final conc.). Following treatment, the cells were washed with D-PBS, trypsinized, counted, and 400 cells were plated in one four well plate (100 cells/well) in complete medium. Colonies were fixed, stained, and counted following a 7 to 8 day incubation period. Cytotoxicity was determined by a substantial reduction of the relative Day 0 or Day 2-3 cell survival and/or a reduction in colony survival at Day 0 to approximately 10-20% of the control values. To allow the expression of induced mutations, an additional 10⁶ cells per culture were plated into 150 mm plates after treatment and subcultured every 2 or 3 days in complete medium. Mutant selection was performed on Day 7 in the presence of 6-TG. Cells were plated for mutant selection at 2 x 10⁵ cells per 100 mm dish (5 dishes total) in selection medium containing 10 mM 6-TG, with the serum component at 5%. An additional 400 cells were plated into a 4 well plate (100 cells/well) in medium without 6-TG for viability assessment. All plates were incubated for 7 to 8 days, and the colonies were then fixed, stained, and counted.

Results

Study validity: The tests with or without metabolic activation provided acceptable data for the evaluation of CP-526,555-01 in the CHO/HGPRT assay. Positive and negative control data were within the range of historical controls.

Study outcome: In the definitive mutagenicity test conducted without metabolic activation, CP-526,555-01 was tested in duplicate at concentrations ranging from 500 mg/mL up to the maximum applied dose of 5000 mg/mL. Cytotoxicity was observed in only one of two replicates at 5000 mg/mL thus lowering the average relative viability counts of Day 2 cell survival and the Day 0 cloning efficiency to 37% and 56%, respectively. The mean number of spontaneous mutants per 10^6 survivors for the negative controls was 4.5 within the historical control range. Mean mutants per 10^6 survivors for the treated cultures ranged from 0 to 2.5. Mutants per 10^6 survivors for the positive control, EMS, were within acceptable limits of the historical control range. In the test with metabolic activation CP-526,555-01 was tested in duplicate at concentrations ranging from 1000 mg/mL up to the maximum applied dose of 5000 mg/mL. No precipitation in the media was observed following the 5 hour incubation. All cultures at concentrations ≥ 3500 mg/mL were terminated on Day 2 due to cytotoxicity. The mean number of spontaneous mutants per 10^6 survivors for negative controls was 4.5 within the historical control value. Mean mutants per 10^6 survivors for the individual treated cultures ranged from 0 to 6.0. Mutants per 10^6 survivors for the positive control, 3-MCA, were within acceptable limits of the historical control range.

Appears This Way
On Original

CP-526,555-01
CHO/HGPRT ASSAY: SUMMARY OF RESULTS

TEST WITHOUT METABOLIC ACTIVATION

Treatment	Replicate Number	DAY 9 CYTOTOXICITY		DAY 7 MUTANT SELECTION		Mutants per 10 ⁶ Survivors*	Mean Mutant Frequency
		Cloning Efficiency %Absolute	%Relative	Cloning Efficiency %Absolute	%Relative		
Negative Control: DMSO							
1%	1	88	100	105	100	1	4.5
	2	101	100	86	100	8	
Test Article: CP-526,555-01 (µg/ml)							
500	1	102	108	92	96	0	2.5
	2	104	110	80	84	5	
1000	1	114	121	86	90	0	0.0
	2	106	112	87	91	0	
2000*	1	104	110	94	98	0	1.0
	2	101	107	89	93	2	
3000*	1	98	104	86	92	0	2.0
	2	108	114	102	107	4	
4000*	1	107	113	104	109	3	1.5
	2	98	104	95	99	0	
5000*	1	0	0	100	105	0	0.0
	2	107	113	93	97	0	
Positive Control: Ethylmethanesulfonate (µg/ml)							
400	1	86	91	88	93	147	198.0
	2	82	87	79	83	249	

Abbreviations:

* Incomplete compound solubility in the 100X DMSO stock concentrations.

Day 0 = 2APR98

Appears This Way
On Original

CP-526,555-01
CHO/HGPRT ASSAY: SUMMARY OF RESULTS

TEST WITH METABOLIC ACTIVATION

Treatment	Replicate Number	DAY 0 CYTOTOXICITY		DAY 7 MUTANT SELECTION		Mutants per 10 ⁶ Survivors	Mean Mutant Frequency
		Cloning Efficiency %Absolute	%Relative	Cloning Efficiency %Absolute	%Relative		
Negative Control: DMSO							
1%	1	94	100	90	100	0	
	2	119	100	85	100	9	4.5
Test Article: CP-526,555-01							
(µg/ml)							
1000	1	130	122	90	103	6	
	2	109	102	93	106	5	5.5
2000*	1	87	82	106	123	6	
	2	110	103	101	115	6	6.0
2500*	1	72	68	85	97	5	
	2	95	89	87	99	5	5.0
3000*	1	1	1	68	78	0	
	2	1	1	69	79	0	0.0
3500*	1	0	0	T	T	T	
	2	0	0	T	T	T	NA
4000*	1	0	0	T	T	T	
	2	0	0	T	T	T	NA
4500*	1	0	0	T	T	T	
	2	0	0	T	T	T	NA
5000*	1	0	0	T	T	T	
	2	0	0	T	T	T	NA
Positive Control: 3-Methylcholanthrene							
(µg/ml)							
4	1	116	109	94	107	49	
	2	106	101	97	111	58	53.5

Abbreviations: T - excessive cytotoxicity, NA - not applicable
* Incomplete compound solubility in the 100X DMSO stock concentrations.

Day 0 - 5APR96
Study No.: 98-1545-12 Protocol No.: 031 Test No.: 4

Study title: Rat In Vivo Micronucleus: Oral Route CP-526,555-24

Key findings:

- The test article was not clastogenic under the experimental conditions.

Study no.: 98-1545-13

Volume # and page #: Volume: 1, Pages: 1-26

Conducting laboratory and location: Pfizer Inc., Groton, CT.

Date of study initiation: 07/16/99

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP 526,555; Lot # 27560-56-4H, ¹ The administered doses were adjusted for purity.

Methods

Strains/species/cell line: Rat [strain CDR BR]

Doses used in definitive study: 25, 50 and 100 mg/kg (10 animals/sex/group) were administered orally. All groups received single oral treatments at 24 hour (\pm 2 hour) intervals on each of three days

Basis of dose selection: Pilot toxicology (10day) study showed that the maximum tolerated dose for rat was 100 mg/kg which served as the basis of the dose selection. In that 10-day exploratory study in rats reduced body weight gain was noted by Day 2 at 10 and 100 mg/kg/day, and death occurred at 100 mg/kg/day on Day 9.

Negative controls: Negative control animals were administered vehicle (deionized water) at a dose volume of 10 ml/kg daily for three days by oral gavage.

Positive controls: For positive control animals, one group each of six male and female rats were treated with 1.25 mg/kg/day of mitomycin-C by intraperitoneal injection using a prepared 0.125 mg/mL dosing stock solution diluted in 0.9% saline and an injection volume of 10 ml/kg of body weight.

Incubation and sampling times: The test article was administered by oral gavage for 3 days. The animals were sacrificed 24 hrs after the final treatment.

Results

Study validity: Treatment-related clinical signs were noted among animals at 100 mg/kg and consisted of decreased activity, ptosis and tremors. A statistically significant ($p=0.0001$ for males and females) dose-related reduction in mean % body weight gain (25 mg/kg males and females; 50 mg/kg males) or weight loss (50 mg/kg females; 100 mg/kg males and females) was apparent in drug-treated animals compared to concurrent controls. Both sexes showed statistically significant (males $p=0.0100$, females $p=0.0050$) treatment-related reductions in mean % PCE suggestive of cytotoxicity to the bone marrow. The mean (male/female) maximum plasma CP-526,555 concentrations (1 hour) and AUC₀₋₂₄ hr exposures at the highest dose tested (100 mg/kg) were 5569/6169 ng/mL and 65187/68884 ng•hr/mL.

Study outcome: There were no treatment-related increases in the numbers of PCE with micronuclei in either sex which demonstrates that CP-526,555-24 does not induce micronuclei in the bone marrow cells of rats.

CP-526,555-24
 RAT MICRONUCLEUS ASSAY: SUMMARY OF TEST RESULTS

ORAL TEST 1
 MALES

COMPOUND	ANIMAL #	% Wgt gain ^a	% PCE ^b	% MNPCE ^c
Deionized Water 10 ml/kg	1			
	2			
	3			
	4			
	5			
	Mean	21.1	78.6	0.10
	(SD) ±	2.6	7.1	0.06
Mitomycin C 1.25 mg/kg/day	7			
	8			
	9			
	10			
	11			
	Mean	10.4	63.3	2.73
	(SD) ±	2.2	5.9	0.79
CP-526,555-24 25 mg/kg	13			
	14			
	15			
	16			
	17			
	Mean	12.2	66.6	0.08
	(SD) ±	2.1	9.3	0.08
CP-526,555-24 50 mg/kg	19			
	20			
	21			
	22			
	23			
	Mean	5.2	61.0	0.11
	(SD) ±	4.7	11.0	0.07
CP-526,555-24 100 mg/kg	25			
	26			
	27			
	28			
	29			
	Mean	-8.1	61.2	0.15
	(SD) ±	2.1	5.9	0.07

a. Statistical analysis of % body weight gain; p= 0.0001 (statistically significant decrease in mean % body weight gain)

b. Statistical analysis of % PCE; p= 0.0100 (Statistically significant reduction in mean % PCE)

c. Statistical analysis of % MNPCE; p= 0.2027
 Day 1 = 05/05/98

2.6.6.5 Carcinogenicity

Study title: Two-Year Oral Gavage Carcinogenicity and Toxicokinetic Study with CP-526,555 in Mice

Key study findings:

- Survival decreased in males treated with 20 mg/kg/day compared to the male control group (P < 0.001). This decrease in survival was initially noted at

approximately Week 27. This indicates that the MTD might have been exceeded slightly for the 20 mg/kg/day group of males; there were 33 of 65 (51%) surviving in the group at Week 80. The dosing for the high dose group was terminated at Week 93 (as per guidance from the Division), 23% of the mice from the high dose group males survived the rest of the study period (105 weeks). Slight increase in the survival of females was noted at study termination (105 weeks).

- No significant changes in the body weight gains and food consumption were noted at termination. Slightly increased incidences of rough hair coat in males and swollen abdomen was noted in female, these observations might be related to the pharmacological effect of the compound.
- **Histopathological:** Amyloidosis was observed in several organs in both males and females. The incidence of systemic amyloidosis was greater in treated animals than in controls; however, the increase was not dose-related. The cause of this increase is unclear. Systemic amyloidosis is known to occur commonly as a spontaneous lesion with variable incidence in CD-1 mice of both sexes as noted by the sponsor and confirmed by the reviewer. Inflammation was also observed in different tissues. Amyloidosis induced by the inflammatory mediators is a well-known biological phenomenon. Considering the effect of the compound in the hematological parameters, treatment related effect on the amyloidosis could not be eliminated.
- There were no statistically significant benign tumor findings in male and female mice. The non neoplastic tumors found in the treated are tabulated below. The tumors are not considered as rare tumors and the findings are not statistically significant and not dose related.
- The most common fatal neoplasm across all groups was malignant lymphoma. The most common identifiable nonneoplastic causes of unscheduled death in males were inflammation of the urinary tract, sepsis, and systemic amyloidosis; systemic amyloidosis was the most common nonneoplastic cause of unscheduled death for females.
- No statistically significant, dose-related trend in the neoplastic findings at the $P < 0.005$ level (for common neoplasms) or the $P < 0.025$ level (for rare neoplasms) was observed in this study. The neoplastic tumors found in the treated female mice (and not in the control) were as follows: Adenosarcoma in cervix (1/65, 2/65, 1/65 in low, mid and high dose respectively) and ovarian carcinoma (1/65 in high dose). These tumors may be treatment related due to their occurrence at the high dose, however, due to the absence of the statistical significance, the biological implication of this finding is not known. In males, lympho reticular organ leukemia and mesenteric node hemangiosarcoma were found (1/65 in each case) at high dose which might be treatment related; however, the biological significance of the finding is unknown.

- Exposures generally increased with dose in a dose proportional manner. No apparent gender difference was noted. The AUC values at 20 mg/kg were 1790 ng•hr/mL, approximately 15-fold human exposure based on MRHD of 1 mg/day with AUC value of 114 ng•hr/mL.

Adequacy of the carcinogenicity study and appropriateness of the test model:

CD-1 mice were dosed once daily by oral gavage with different dosage of the test article for 104 weeks. The dosages were in concurrence with eCAC. The test model (CD-1 mouse) is appropriate because the mouse is a universal model routinely used for evaluating the toxicity and carcinogenicity of various classes of chemicals and for which there is a large historical database. The study was adequate because the study duration met the regulatory required duration for carcinogenicity studies (104 weeks). The doses evaluated were adjudicated to have reached the MTD based on mortality cumulative mortality at the high dose groups.

Evaluation of tumor findings: There was no statistically significant neoplastic or non neoplastic tumor in this study. There were no statistically significant benign tumor findings in male and female mice. The non neoplastic tumors found in the treated are tabulated below. No statistically significant, dose-related trend in the neoplastic findings at the P<0.005 level (for common neoplasms) or the P<0.025 level (for rare neoplasms) was observed in this study. The neoplastic tumors found in the treated female mice (and not in the control) are tabulated below. Adenosarcoma in cervix (1/65, 2/65, 1/65 in low, mid and high dose respectively) and ovarian carcinoma (1/65 in high dose) may be treatment related due to their occurrence at the high dose due to the absence of the statistical significance, the biological implication of this finding is not known. In males, lympho reticular organ leukemia and mesenteric node hemangiosarcoma were found (1/65 in each case) at high dose which might be treatment related, however, the biological significance of the finding is unknown. The tumors are not considered as rare tumors and the findings are not statistically significant.

Study no.: 02-1545-32

Volume # and page #: Volume 1-6; Page 1-2717

Conducting laboratory and location: []

Date of study initiation: May 24, 2002

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: CP-526,555-18, Lot #s were 53650-5-5B, 54526-23-9B, and 42,698-95-9B were used. Purity in all batches were found to be between [] The administered doses were adjusted for purity.

CAC concurrence: Yes

Methods

Doses: 0, 0, 5, 25, 75 mg/kg/day for males and 0, 0, 1, 5, 25 mg/kg/day for females. After 22 days, the dosages were changed to 1, 5 and 20 mg/kg/day. The Agency was consulted in the decision making process for changing the dose.

Clinical exposure multiples were taken into consideration for lowering the dose.

Basis of dose selection (MTD, MFD, AUC etc.): CAC recommended the following doses based on deaths in males at 150 mg/kg/day and in females at 75 and 150 mg/kg/day in the 3-month dose-ranging study. The greater incidence of mortality in female mice was also noted in the 2-week study at 100mg/kg/day.

Male: 5, 25, 75 mg/kg/day

Female: 1, 5, 25 mg/kg/day.

The decision on dosing was made based on MTD on April, 30 and the AUC ratio was not taken into consideration due to the absence of the protein binding and the metabolism data. Sponsor resubmitted the mouse carcinogenicity protocol with the protein binding and the metabolism data on July 8, 2002. At this point CAC concurred with the sponsor's suggested doses 0,0, 1, 5, 20 mg/kg/day for both males and females based on AUC (>25x exposure) ratios, contingent on the clinical dose not increasing such that the AUC ratio would be decreased to less than 25-fold.

Species/strain: CD-1® (ICR) []

Number/sex/group (main study): 65/ sex/group

Route, formulation, volume: Oral gavage, the compound was formulated in clear water (from []) and 10 mL/kg/day was administered daily.

Frequency of dosing: Daily

Satellite groups used for toxicokinetics or special groups: 20/sex/group

Age: Approximately 6 weeks old

Animal housing: 1/sex/ in suspended stainless steel cage with bedding, temperature 18-26°C and relative humidity 30-70%, a minimum of 10 air changes/hour 10%. Lighting cycle 12 hours light/12 hours dark per 24 hours. Water and food (rodent diet [] pellets) were given *ad libitum*.

Restriction paradigm for dietary restriction studies: none

Drug stability/homogeneity: Dose formulations were prepared at concentrations of 0.04, 0.1, 10, and 50 mg/mL weekly. Stability of the compound in the dosing solutions was determined at intervals for at least 10 days of refrigeration (2 to 8°C) storage and 8 hours at room temperature. The analytical stability of dose formulations showed []

impurities did not exceed [] Dose analyses results indicated that all dose formulations were within [] of target appropriate for use on study.

Dual controls employed: Yes

Study Design:

Days 1 - 22

Group	Males			Females		
	Dose Level (mg/kg/day)	Concentration (mg/mL)		Dose Level (mg/kg/day)	Concentration (mg/mL)	
		As free base	Lot # 53650-5-5B (potency: []) Day 1-Day 22		As free base	Lot # 53650-5-5B (potency: []) Day 1-Day 22
Carcinogenicity Mice						
1 (Control)	-	-	-	-	-	-
2 (Control)	-	-	-	-	-	-
3 (Low)	5	0.5	0.885	1	0.1	0.177
4 (Mid)	25	2.5	4.425	5	0.5	0.885
5 (High)	75	7.5	13.274	25	2.5	4.425
Toxicokinetic Mice						
6 (Low TK)	5	0.5	0.885	1	0.1	0.177
7 (Mid TK)	25	2.5	4.425	5	0.5	0.885
8 (High TK)	75	7.5	13.274	25	2.5	4.425

Day 23 to End of Study

Group	Males and Females				
	Dose Level (mg/kg/day)	As free base	Concentration (mg/mL)		
			lot # 53650-5-5B (potency: []) Day 23-Week 37	lot # 54,526-23-9B (potency: []) Week 38-Week 72	lot # 42,698-95-9B (potency: []) Week 73-106
Carcinogenicity Mice					
1 (Control)	-	-	-	-	-
2 (Control)	-	-	-	-	-
3 (Low)	1	0.1	0.177	0.173	0.173
4 (Mid)	5	0.5	0.885	0.865	0.866
5 (High)	20	2	3.540	3.460	3.466
Toxicokinetic Mice					
6 (Low TK)	1	0.1	0.177	0.173	0.173
7 (Mid TK)	5	0.5	0.885	0.865	0.866
8 (High TK)	20	2	3.540	3.460	3.466

At initiation of treatment, the animals were approximately 6 weeks old. Body weights ranged from 23.8 to 33.9 g and 14.9 to 25.3 g for the carcinogenicity males and females and 24.2 to 33.2 g and 19.8 to 25.8 g for the toxicokinetic males and females, respectively. Animals not used on study were euthanized and discarded. The following found-dead animals (and their day of death) were replaced with an animal of similar body weight and same sex from the group of possible replacements: Group 5 Male A66464 (Day 11), Group 5 Male A66508 (Day 2), Group 2 Females A66649 and A66668 (Day 3), and Group 2 Female A66648 (Day 2).

Interim sacrifices: None.

Deviations from original study protocol: Dosing was altered on Day 23 as noted above.

Observation times:

Mortality: The animals were observed twice daily for mortality and moribundity.

Clinical signs: The animals were observed twice daily for clinical signs.

Body weights: Body weights were recorded once pre treatment (including

Week 1), weekly for Weeks 1-27, once every 4 weeks thereafter, and at the beginning of each week, during which blood was collected for toxicokinetics (Weeks 26, 52, 78, and 105).

Food consumption: Food consumption was measured individually and recorded weekly for Weeks 1-26 and once every 4 weeks thereafter.

Histopathology: Peer review: Yes; Animals found dead or sacrificed in a moribund condition during the study were subjected to a gross postmortem examination. All surviving mice were weighed and subjected to necropsy after 104 weeks. The necropsy included examination of the external features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues. All the tissues listed in the standard tissue battery were collected from each animal and were preserved in 10% neutral-buffered formalin. Tissues were processed in the Pfizer laboratory for the histopathology.

Toxicokinetics: Blood samples collected at approximately 1 hour post dose during Weeks 26, 52, 78, and 105. Five mice/sex were used for each time point.

Results

Mortality:

Survival decreased in males for the 20 mg/kg/day as compared to the male control groups ($P < 0.001$). This decrease in survival was initially noted at approximately Week 27. The MTD might have been exceeded slightly for the 20 mg/kg/day group of males; there were 33 of 65 (51%) surviving in the group at Week 80, The dosing for the high dose group was terminated at Week 93 (as per guidance from the Division), 23% of the mice from the high dose group males survived the rest of the study period (105 weeks). There was no identifiable cause (grossly or microscopically) for the majority of the unscheduled deaths in this group.

Survival was however, significantly increased for the 20 mg/kg/day females as compared to the combined female control groups ($P = 0.014$ based on sequential trend tests). This increase in survival was initially noted at approximately Week 78. Survival was similar in the control and remaining treated (1 and 5 mg/kg/day) male and female groups.

Percent Survival in Mice

Time	0	0	1 mg/kg	5 mg/kg	20 mg/kg
Week 1-25 : % survival in M& F $\geq 97\%$ in all dosages					
Week 52 (1 year)					
M	97	88	98	95	77
F	97	95	97	100	98
Week 80					
M	80	66	77	69	51

F	81	78	75	75	88
Week 90					
M	72	55	69	60	37
F	75	66	69	63	82
Week 95					
M	62	45	60	52	29
F	70	57	65	55	77
Week 100					
M	52	37	51	49	28
F	66	48	54	52	74
Week 104					
M	46	30	46	42	23
F	59	42	43	51	65

Clinical signs:

The treatment related clinical signs include the dose related increase incidence of in rough hair coat finding at 5 and 20 mg/kg/day males. This observation may be treatment related as the compound has been shown to be deposited in the skin. Similarly, slightly greater incidences of treated females exhibiting swollen ventral abdomen were observed compared to the control females.

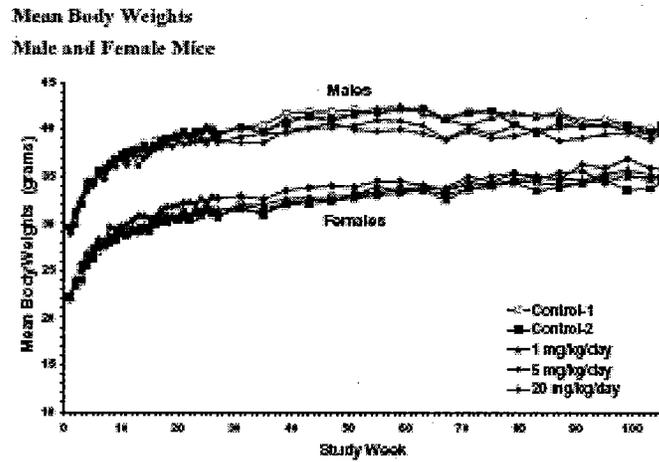
Clinical Signs

Incidence	0	0	1 mg/kg	5 mg/kg	20 mg/kg
Swollen/ Ventral abdomen/left					
M	16	12	11	12	9
F	12	12	16	20	23
Cloudy discharge / eye/right					
M					1
F					
Rough hair Coat					
M	22	23	22	29	29
F	27	21	27	29	13

Body weights:

There were no significant differences between in the mean body weight gain between the control and the treatment group at the study termination. The mean body weights at the 20 mg/kg/day dose level, relative to mean combined control body weights, tended to be significantly lower for males during Weeks 51 through 75 and significantly higher for

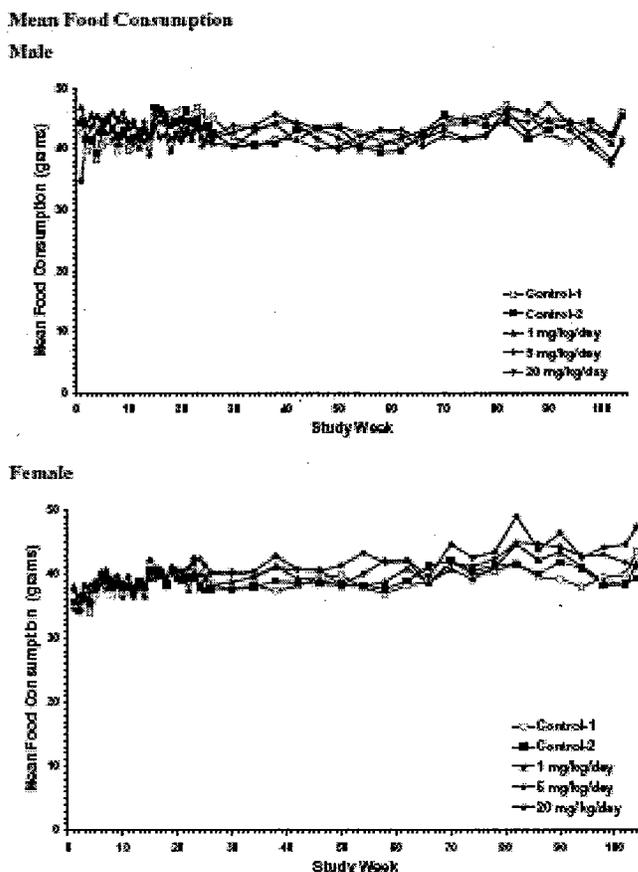
females during Weeks 3 through 55. The change was correlated to the decrease in food consumption and not of any toxicological significance.



Food consumption:

Slightly higher food consumption values were noted for the 20 mg/kg/day females during Week 23 through 62, and generally correlated to the increased mean body weights noted in this group during this same time period. The finding is not considered toxicologically significant.

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Gross pathology: Gross findings noted for male and female mice from control and treated groups correlated to incidental neoplastic and non neoplastic findings.

Histopathology:

Amyloidosis were observed in several organs in both males and females (see table below). The incidence of systemic amyloidosis was greater in treated animals than in controls, however the increase was not dose-related. The cause of this increase is unclear. Systemic amyloidosis is known to occur commonly as a spontaneous lesion with variable incidence in CD-1 mice of both sexes as noted by the sponsor and confirmed by the reviewer. Inflammation was also observed in different tissues. Amyloidosis induced by the inflammatory mediators is a well known biological phenomenon. Considering the effect of the compound in the hematological parameters, treatment related effect on the amyloidosis could not be eliminated.

The cause of death was identified for several mortalities. The most common fatal neoplasm across all groups was malignant lymphoma. The most common identifiable non neoplastic causes of unscheduled death in males were inflammation of the urinary tract, sepsis, and systemic amyloidosis; systemic amyloidosis was the most common nonneoplastic cause of unscheduled death for females. Other causes of unscheduled

deaths were from a variety of incidental nonneoplastic and neoplastic findings and occurred in control and dosed groups as noted in the Sponsor's table below:

Probable Cause of Death

<i>Palpable Masses</i>	<i>Control</i>	<i>High Dose</i>
Masses	1/130 (0.8%)	1/65 (1.5%)

<i>Probable Cause of Death/Endanosis</i>	<i>Control</i>	<i>High Dose</i>
Neoplasms*	1/10 (10%)	3/26 (12%)
Ascending Urinary Tract Infection**	4 (40%)	2 (8%)
Polyarteritis	1 (10%)	-
Sepsis	2 (20%)	1 (4%)
Amyloidosis	-	1 (4%)
Heart - Infarct	-	1 (4%)
Undetermined	2 (20%)	13/26 (69%)

* All neoplasms were Lymphoma or Leukemia, which are common neoplasms in aged mice.

** Pyelonephritis, cystitis, etc.

Best Possible Copy

Histopathological Findings:* NOTE: The incidence of findings in the control group is presented as the mean of the two control groups

Group/Dose (mg/kg)		0	5	20	60
Number of Animals		65*	65	65	65
Kidney					
Amyloid, glomeruli	M	6	7	3	6
	F	0	2	1	2
Hydronephrosis	M	3.5	12	15	9
	F	11.5	7	10	8
Liver					
Amyloid	M	2.5	6	5	3
	F	6	3	7	4
Gall Bladder					
Inflammation	M	0	0	0	1
	F	0	0	0	0
Dilation	M	0	0	0	1
	F	0	0	0	0
Amyloid	M	0	0	0	0
	F	0.5	0	2	0
Pancreas					
Atrophy, Acinar	M	0.5	0	0	0
	F	0.5	1	0	3
Inflammation, vascular	M	0	2	1	0
	F	0.5	1	0	2
Hyperplasia, Islets	M	0	0	0	
	F	0	0	0	1
Salivary glands					
Amyloid	M	0.5	2	3	0
	F	10.5	6	5	1

Urinary bladder					
Inflammation	M	2.5	6	7	7
	F				
Hemorrhage	M	3	2	4	8
	F	0	0	0	0
Lung					
Hyperplasia- bronchio alveolar cells	M				
	F	1	1	3	1
Heart					
Amyloid	M	4	11	11	9
	F	7.5	6	5	3
Thyroid					
Amyloid	M	1.5	5	8	4
	F	6	5	6	5
Inflammation ,vascular & infiltration of mononuclear cells	M	0	0	0	2
	F	0	0	2	
Parathyroid					
Amyloid	M	0.5	0	3	1
	F	2	3	3	2
Infiltration of mononuclear cells	M	0	0	0	0
	F	0	0	0	1
Adrenal					
Amyloid	M	2.5	8	14	8
	F	9	5	8	5
Spleen					
Amyloid	M	5	3	2	7
	F	8	12	9	8
Thymus					
Inflammation ,vascular	M	0	0	0	0
	F	0.5	0	2	
Lymphocytolysis	M	1	0	1	12
	F	0.5	0	2	1
Mesenteric node					
Angiectasis	M	3.5	4	6	6
	F	2.5	6	3	5
Amyloid	M	5	8	9	9
	F	5.5	7	5	5
Neutrophilia, sinus	M	0.5	1	2	2
	F	0	2	0	0
Ureter					
Dilatation and hyperplasia	M	0.5	0	0	3
	F	0	0	0	0
Stomach					
Hyperplasia, epithelium	M	0	1	0	0
	F	0	1	0	2
Dudoneum					
Amyloid	M	1	5	7	1
	F	5.5	2	3	3
Ileum					
Amyloid	M	6.5	17	16	9
	F	11.5	15	11	11

Testis/Amyloid	M	1.5	7	6	4
Prostrate/Lymphoreticular neoplasm	M	1.5	2	4	1
Ovary					
Granulosa	F	1	2	3	3
Hyperplasia	F	0.5	0	0	3
Uterus					
Hyperplasia	F	50	53	53	47
Bone, sternum					
Fibro-osseus change	M	0	1	0	1
	F	18	18	18	24

* Animal # in control =130; to compare the findings with the treatment group findings/65 animals are presented in the table.

Neoplastic: No statistically significant, dose-related trend in the neoplastic findings at the P<0.005 level (for common neoplasms) or the P<0.025 level (for rare neoplasms) was observed in this study. The neoplastic tumors found in the treated female mice (and not in the control) are tabulated below. Adenosarcoma in cervix (1/65, 2/65, 1/65 in low, mid and high dose respectively) and ovarian carcinoma (1/65 in high dose) may be treatment related due to their occurrence at the high dose due to the absence of the statistical significance, the biological implication of this finding is not known. In males, lympho reticular organ leukemia and mesenteric node hemangiosarcoma were found (1/65 in each case) at high dose which might be treatment related, however, the biological significance of the finding is unknown. The tables below were reproduced from the Statistical Review completed by Dr. Moh-Jee Ng.

Malignant Tumor Trends in Male Mice:

Organ Name	Tumor Name	CTR	LOW	MED	HIGH	P-Value
Harderian gland	ADENOCARCINOMA	0	0	1	0	0.3386
Lymphoreticular	LEUKEMIA, GRANULOCYTIC	0	0	0	1	0.1593
Mesenteric node	HEMANGIOSARCOMA	0	0	0	1	0.1290
Stomach	ADENOCARCINOMA	0	0	1	0	0.3386

Malignant Tumor Trends in Female Mice:

	Tumor Name	CTR	LOW	MED	HIGH	P-Value
Cervix	ADENOCARCINOMA	0	1	2	1	0.3572
Jejunum	ADENOCARCINOMA	0	0	1	0	0.4529
Liver	CARCINOMA, HEPATOCELLULAR	0	1	0	0	0.6027
Lymphoreticular	NEOPLASM, MAST CELL	0	0	1	0	0.4018
Ovary	CARCINOMA, NOS	0	0	0	1	0.2500
Skin and adnexa	CARCINOMA,	0	1	0	0	0.6046

	BASAL CELL					
Vagina	LEIOMYOSARCOMA	0	0	1	0	0.4036

Non Neoplastic: There were no statistically significant benign tumor findings in male and female mice. The non neoplastic tumors found in the treated are tabulated below. The tumors are not considered as rare tumors and the findings are not statistically significant and not dose related. The tables below were reproduced from the Statistical Review completed by Dr. Moh-Jee Ng.

Benign Tumor Trends in Male Mice:

Organ Name	Tumor Name	CTR	LOW	MED	HIGH	P-Value
Epididymis	ADENOMA, LEYDIG CELL	0	0	1	0	0.2692
Testis	ADENOMA, RETE TESTIS	0	0	1	0	0.2745

Benign Tumor Trends in Female Mice:

	Tumor Name	CTR	LOW	MED	HIGH	P-Value
Adrenal	ADENOMA, SUBCAPSULAR CELLS	0	1	0	1	0.2514
Liver	ADENOMA, HEPATOCELLULAR	0	3	2	2	0.2473
Liver	HEMANGIOMA	0	2	0	0	0.7179
Pancreas	NEOPLASM, ISLET CELL, BENIGN	0	1	0	0	0.6176
Skin and adnexa	PAPILLOMA, SQUAMOUS CELL	0	0	1	0	0.4529
Thyroid	ADENOMA, C-CELL	0	1	0	0	0.6213
Vagina	POLYP, STROMAL	0	2	1	0	0.7613

Toxicokinetics:

A dose related increase in the in the serum concentration of the test article was noted. The increase in exposure was less than dose-related. At Week 26 approximately 4-fold and 8-fold increase in exposure were noted with 5 and 25 mg/kg/day respectively when compared to that of the 1 mg/kg/day dose. CP-526,555 serum concentrations were approximately constant over at least 78 weeks for all dose groups suggesting that the steady state have been obtained. The mean CP-526,555 concentrations (males and females combined) at Week 105 in the 1, 5, and 20 mg/kg/day dose groups were approximately 1.4- to 2.0-fold of the Week 26, 52, and 78 values for all dose groups. These results indicate slight accumulation in exposure over the 104 weeks of once-daily oral administration of CP-526,555 to male and female mice.

Serum CP- 526,555 Exposures:

Dose	Day	Mean Serum
------	-----	------------

(mg/kg)		Concentration (ng/mL)
1.0	26	153 ± 31
	52	139 ± 52
	78	145 ± 35
	105	280 ± 39
5	26	595 ± 205
	52	519 ± 181
	78	491 ± 169
	105	986 ± 323
20	26	1240 ± 410
	52	999 ± 345
	78	1070 ± 200
	105*	1790 ± 198

*Male samples not received

Study title: Two-Year Oral Gavage Carcinogenicity and Toxicokinetic Study with CP-526,555 in Rats

Key study findings:

- In the 104-weeks carcinogenicity study with rats (0, 0, 1, 5, 15 mg/kg/day) there was a slight (not statistically significant) decrease in the survivability for the high dose group males compared to that in the control group (35% in high dose vs. 47% in control). Survivability of females in the high dose group was approximately similar to that in the controls. This indicates that the compound do not have any effect on the longevity.
- The mean body weight gain decreased (statistically significant) in males for the 1, 5, and 15 mg/kg/day by 10, 13 and 17% respectively at termination relative to the mean control body weights. The mean body weight gain in females was found to be decrease by 2 and 16% at termination for the mid and the high dose group, respectively, relative to the mean control body weights. The decrease in body weight might be correlated with decrease in the food consumption. The >15%

decrease in body weight suggests that the MTD might have been reached under this experimental condition.

- **Hibernoma** (a neoplasm of the brown adipose tissue) was observed in 1/65 and 2/65 males in the mid and high dose group respectively. The hibernomas in the high dose group were found to be malignant; whereas the hibernoma in the mid dose group was classified as benign. All tumors were found in the mediastinum of the thorax. Due to the rarity of the tumor in the rats, the finding is considered treatment-related. The P-value = 0.03 for the hibernoma noted in this study; for the rare tumor findings according to the ICH guidelines statistical significance should be ≤ 0.25 , therefore this finding was not considered to be statistically significant.
- Other malignant neoplastic findings which might be treatment related (since not observed in controls) were mesothelioma of heart, schwannoma of brain, schwannoma of pituitary, and hemangiosarcoma of spleen (1/ 65 in each case) at high dose males. In females, neoplasms were observed in the granulosa cell in ovary, adenocarcinoma in uterus, and basal cell carcinoma of the skin (1/65 in each case). None of the tumor findings in males and females were statistically significant.
- Dose related non neoplastic tumor findings in males were limited to hemangioma of spleen (1/65 in control vs. 1/65 at high dose) and keratocanthoma of skin (2/65 in control vs. 3/65 at high dose). In females, luteoma in ovary (1/65) and benign mammary mass (1/65) were noted at high dose. These findings were absent in the control group. None of these findings were found to be statistically significant.
- The gross findings and the histopathology at the time of necropsy correlated to incidental neoplastic and non neoplastic microscopic changes in pituitary gland and skin (tail and paws) of males and females and the mammary gland of females. The above mentioned incidental findings were determined to be the major cause of the death under this experimental condition.
- There were adequate dose related exposure in all dose groups, the compound was found to increase in exposure with increasing dose in a less than dose proportional manner, accumulation was noted in all dose groups, and no gender differences were noted. The AUC value at 15 mg/kg were 673.9 ng•hr/mL, approximately 5.9-fold human exposure based on MRHD of 1 mg/day with AUC value of 114 ng•h/mL.

Adequacy of the carcinogenicity study and appropriateness of the test model:

Sprague Dawley rats were dosed (0, 0, 1, 5, 15 mg/kg/day) once daily by oral gavage for 104 weeks. The doses were selected in concurrence with eCAC. The test model (SD rats) is appropriate because the rat is a universal model routinely used for evaluating the toxicity and carcinogenicity of various classes of chemicals and for which there is a large historical database. The study was adequate because the study duration met the regulatory required duration for carcinogenicity studies (104 weeks). The doses evaluated were found to have reached the MTD based on the cumulative mortality at the high dose

groups. The AUC value at 15 mg/kg were 673.9 ng•hr/mL, approximately 5.9-fold human exposure based on MRHD of 1 mg/day with AUC value of 114 ng•h/mL.

Evaluation of tumor findings:

Three treated males (1/65 and 2/65 at 5, and 15 mg/kg/day) were noted to have intrathoracic mass. These mass were composed of multivacuolated cells and were determined to be tumor cell positive by immunohistochemistry. Electron microscopical evaluation was done for these tumors. These mediastinal tumors were found to contain numerous mitochondria characteristics consistent with brown adipose tissue and thus the tumors were confirmed to be hibernomas (neoplasm of the brown fat). The hibernoma found in the mid dose group was classified as benign based on its morphology. The two hibernomas found in the high dose group were classified as malignant due to the variation of the nucleus and the cytoplasm; one of these two hibernomas were found to metastasize in the lung. The reviewer confirmed the Sponsor's observation 'the *hibernoma is a rare neoplasm of brown adipose tissue and only a few cases of hibernoma have been reported in control rats. In the Registry of Industrial Toxicology Animal-data (RITA) control animal database, there is one hibernoma in a male Sprague-Dawley rat but this did not occur in the mediastinum [Rita, Lesion-related Incidence Data, 2005]. An intrathoracic (mediastinal) hibernoma was reported in a control female F344 rat [Stefanski et al., Lab Anim Sci; 37(3):347-50, 1987] and a control female Sprague-Dawley rat [Port et al., Lab Anim Sci; 29(2):214-217, 1979]. Hibernoma has also been observed in one male Sprague-Dawley rat (adipose tissue), two male and four female Osborne-Mendel rats (mammary/subcutaneous tissue or prostate locations) in the control animal database of the National Toxicology Program [National Toxicology Program, Data Search Application = Hibernoma, 2005]'. No other hibernomas in rat have been reported. The incidence of hibernoma in male rats is confirmed to be very low. However, this finding does not meet the statistical requirement to be significant in the present study (rare tumor is considered to be statistically significant if the P-values are <0.025).*

Reviewer's Comment: Although, there is no statistical significance due to their rarity, the tumors might be test article related and should be labeled with the carcinogenicity findings.

Other malignant neoplastic findings which might be treatment-related (since not observed in controls) were mesothelioma of heart, schwannoma of brain, schwannoma of pituitary, and hemangiosarcoma of spleen (1/65 in each case) at high dose males. In females, neoplasms were observed in the granulosa cell in ovary, adenocarcinoma in uterus, and basal cell carcinoma of the skin (1/65 in each case). However, none of the tumor findings in males and females were statistically significant.

Dose-related non neoplastic tumor findings in males were limited to hemangioma of spleen (1/65 in control vs. 1/65 at high dose) and keratocanthoma of skin (2/65 in control

vs. 3/65 at high dose). In females, luteoma in ovary (1/65) and benign mammary mass (1/65) were noted at high dose. These findings were absent in the control group. None of these findings were found to be statistically significant.

Study no.: 02-1545-33

Volume # and page #: Volume: 1-6; Page1-2943

Conducting laboratory and location: []

Date of study initiation: 06/21/02

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: CP 526,555; Lot #s 53650-5-5B, 54526-23-9B, 42, 698-95-9B. The purity of all batches were between [] The administered doses were adjusted for purity.

CAC concurrence: Yes

Methods

Doses: 0, 0, 1, 5, 15 mg/kg/day for 105 weeks

Basis of dose selection (MTD, MFD, AUC etc.): The dose selection was based on AUC in concurrence with CAC. A high dose of 15 mg/kg/day should provide a 50- and 80-fold multiple of the human AUC and Cmax, respectively, and not cause body weight suppression greater than 10%, thereby providing sufficient longevity and data integrity for reliable analysis and interpretation. The dose was proposed based on a 3, 6 month toxicity study in rat.

Species/strain: CD (SD) IGS BR rats

Number/sex/group (main study): 65/sex/dose group

Route, formulation, volume: Oral gavage, formulated in water, 10 mL/kg

Study Design:

Group	Number of Rats		Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)
	Male	Female		
<u>Carcinogenicity Rats</u>				
1 (Control)	65	65	0	10
2 (Control)	65	65	0	10
3 (Low)	65	65	1	10
4 (Mid)	65	65	5	10
5 (High)	65	65	15	10
<u>Sentinel Rats</u>				
6 (Sentinel)	5	0	-	-

¹ Dose levels were expressed relative to the free base (CP-526,555). Concentrations were expressed (and presented below) both as the free base and as the tartrate salt (CP-526,555-18) by adjusting for the active moieties. []

Group	Dose Level (mg/kg/day)	Concentration (mg/mL)			
		As Free	Lot # 33630-3-1B	Lot # 34,326-23-9B	Lot # 41,698-94-9B
		Base	(potency: [] Day 1 - Week 38	(potency: [] Week 39 - Week 74	(potency: [] Week 75 - 105
1 (Control)	-	-	-	-	-
2 (Control)	-	-	-	-	-
3 (Low)	1	0.1	0.177	0.173	0.173
4 (Mid)	5	0.5	0.885	0.865	0.866
5 (High)	15	1.5	2.635	2.595	2.600

Frequency of dosing: Once daily.

Satellite groups used for toxicokinetics or special groups: Five animals/sex/group for TK analysis

Age: Approximately 6 weeks old

Body Weight: Body weights at the time of study initiation ranged from 136 to 187 g for the males and 119 to 167 g for the females

Animal housing: Rats were housed individually in polycarbonate cages.

Environmental controls were set to maintain the following animal room conditions: temperature range of 18 to 26°C, relative humidity range of 30 to 70%, 10 or greater air changes/hour, and a 12-hour light/12-hour dark cycle.

Restriction paradigm for dietary restriction studies: NA

Drug stability/homogeneity: The dose formulations were tested at concentrations of approximately 0.1, 10 and 50 mg/mL. The formulations were confirmed to be stable when stored for 10 days refrigerated (2 to 8°C) followed by 8 hours at room temperature.

The analytical stability determination of dose formulations (at concentrations of approximately 0.04, 10, and 50 mg/mL) showed that the % of the active moiety was between []. Both [] impurities were also determined analytically and were found to be within the range of the sponsor-specified criteria []

[] did not increase by ().

Dual controls employed: Yes

Interim sacrifices: None

Deviations from original study protocol: None

Observation times:

Mortality: The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity.

Clinical signs: The animals were observed twice daily (a.m. and p.m.), detailed observations were made for each animal once weekly. From Week 27 onwards, all grossly visible or palpable mass was recorded systematically. The time of onset of the

palpable mass, size of the mass [small (<1 cm³) or large (≥1 cm³)], location, and appearance of the mass was documented at Week 27, once every 4 weeks thereafter, and at Week 105.

Body weights: Individual body weights were recorded pretreatment, Day 1 prior to dosing, weekly for Weeks 2-27, once every 4 weeks thereafter, and at Week 105.

Food consumption: Individual food consumption was measured and recorded weekly for Weeks 1-26, once every 4 weeks thereafter, and at Week 104.

Histopathology: Peer review: Yes. All animals found dead or sacrificed in a moribund condition during the study were subjected to a gross postmortem examination and then necropsied. At the termination of the experiment all animals were subjected to necropsy. The necropsy included examination of the external features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues. All tissues from the standard tissue battery were collected and fixed in 10% formalin. Eyes with the optic nerve and Harderian gland was fixed in 3% gluteraldehyde and processed for the histopathological examination. In addition to the standard tissue batter, GALT was collected and processed for the microscopic analysis.

Toxicokinetics: Five rats/sex were bled at approximately 1 hour after dosing, during Weeks 26, 52, 79, and 104.

Results

Mortality:

The percent survival of the high dose males and females on Week 105 was 35 and 52 % respectively. The percent of survival is slightly lower in males at high dose compare to that of the controls (50%). The difference is however, not statistically significant. The decrease in survival (mortality>30%) in males were noted from Week 80 for the high dose males. The low and mid dose males did not show any changes in the survival rate compared to that of the controls. In females, the survival of the treated groups was always similar or even slightly higher than that of the control group. This indicates that the test article did not have any direct effect on the mortality in rats under the current experimental condition.

Percent Survival in Rat

Time	0	0	1 mg/kg	5 mg/kg	15 mg/kg
Week 1-25 : % survival in M& F	≥97% in all dosages				
Week 52 (1 year)	No change				
Week 80					
M	71	91	69	84	68
F	74	80	77	77	83

Week 90					
M	63	78	62	72	57
F	63	66	59	60	66
Week 95					
M	57	66	57	64	49
F	51	62	55	52	65
Week 100					
M	48	59	52	58	43
F	45	57	48	38	58
Week 105					
M	45	55	48	47	35
F	37	46	44	35	52

Accumulative Mortality% in Rat*

Sex varenicline (mg/kg/day)	Male				Female			
	CD 0	LD 1	MD 5	HD 15	CD 0	LD 1	MD 5	HD 15
Weeks 0 - 52	8.5	10.8	9.2	3.1	4.6	7.7	3.1	4.6
53-78	17.7	30.8	15.4	30.8	23.1	24.6	21.5	12.3
79-91	32.3	38.5	30.8	43.1	39.2	44.6	41.5	35.4
92-103	50.8	52.3	53.8	63.1	58.3	55.4	64.6	47.7

*table adapted from the statistical analysis data

Clinical signs: A dose related behavioral response related to the hunched posture and ataxia was seen in treated males. The incidences of hunched appearance and ataxic behavior were higher in males of treated males than for control males. These behaviors in males may be related to the discomfort in the stomach and in the intestine related to the pharmacology of the test article. In the treated females, similar clinical signs were observed; however, those behavioral responses were not dose related. Slight increase in convulsion was observed at high dose in males. In females, convulsion was seen in a slightly elevated number as compared to those of the control, however, the response was not dose related (see table below).

Summary of Clinical signs Findings

Incidence	0	0	1 mg/kg	5 mg/kg	15 mg/kg
Convulsions					
M	2	3	5	3	8
F	0	2	3	0	1
Hunched Posture					
M	5	3	14	16	19
F	13	15	16	9	8

Ataxic					
M	1	1	3	3	4
F	6	11	11	5	6

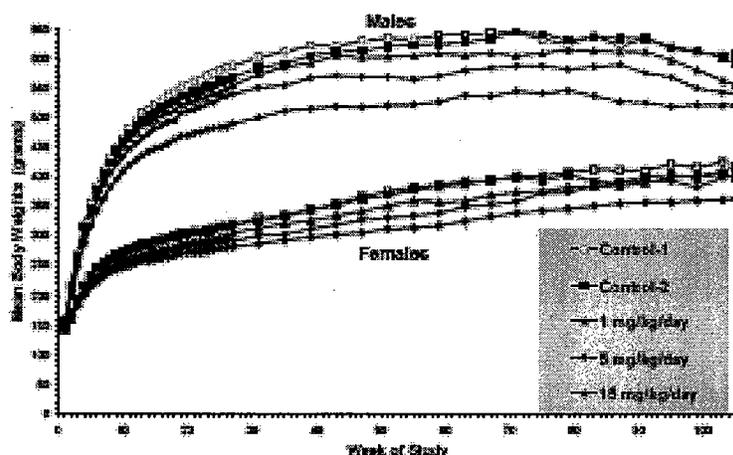
Body weights:

The mean body weight gain decreased (statistically significant) in males for the 1, 5, and 15 mg/kg/day by 10, 13 and 17 % respectively at termination relative to the mean control body weights. These decreases were noted within the first few weeks of the study and persisted throughout the study duration. The decreases noted at 5 and 15 mg/kg/day were significant most weeks throughout the study duration, whereas at 1 mg/kg/day, the decreases were significant less frequently. The mean body weight gain in females was found to be decrease by 2 and 16% at termination for the mid and the high dose group respectively relative to the mean control body weights. The decrease in the mean body weight gain in females was statistically significant for the high dose group at termination. Significant decrease in the mean body weight gain were noted for the high dose group female during Week 2 and reached a maximum of about 17 % decrease compared to that of the control at Week 55.

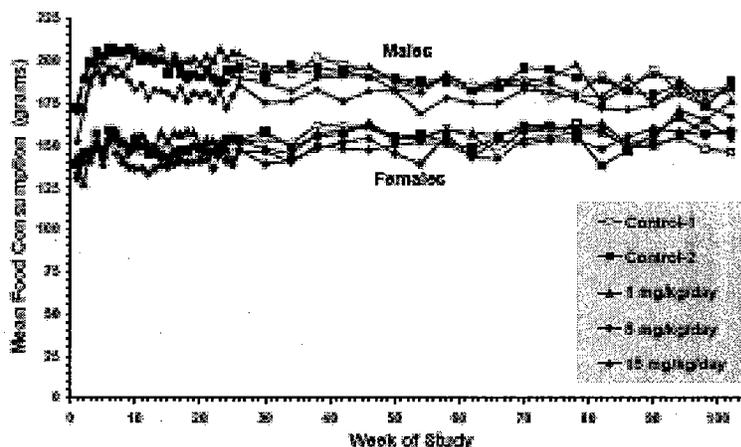
The decrease in the mean body weight gain (>15%) in both males and females at the high dose indicated that the maximum tolerated dose has been achieved for this experiment.

Mean Body Weight (%) for Rats

	Dose Groups	Mean Body Weight (grams)		Mean Body Weight Gain (MBWG)	% Differences in MBWG
		Beginning Study (week 1)	End of Study (week 105)		
Male	0 mg/kg/day	158.5	602	443.5	
	1 mg/kg/day	158	558	400	-10
	5 mg/kg/day	158	543	385	-13
	15mg/kg/day	157	523	366	-17
Female	0 mg/kg/day	143.5	409.5	266	
	1 mg/kg/day	143	418	275	4
	5 mg/kg/day	143	404	261	-2
	15mg/kg/day	142	365	223	-16



Food consumption: No change in food consumption was observed in the treated animal compare to that of the control group at the study termination. Food consumption in low dose group did not change out the experiment. Statistically significant decreases in mean food consumption values relative to control (Groups 1 and 2 combined) values were noted for the 5 mg/kg/day rats up through Week 46 and more frequently noted for the 15 mg/kg/day rats up through Week 86. The decrease in food consumption in the mid and high dose group may be correlated to the decrease in the body weight noted in these two dose groups.



Gross pathology: The gross findings other than mass from the unscheduled death at the time of necropsy are tabulated below. The major dose related findings as listed below were indented ventral surface in brain and enlarged, mottled and dark pituitary. Enlarged organs (like spleen, liver, distended organs (like uterus, colon, stomach), swollen and sore skin, and thickening of the mammary glands. The gross findings from the animals at termination did not show any major differences compared to those of the controls.

Summary of Gross Pathological Findings from Unscheduled Death:*

Group/Dose(mg/kg)		0	1	5	15
Number of Animals		65	65	65	65
Brain					
Indented ventral surface	M	9	12	13	16
	F	24	22	31	15
Pituitary					
Enlarged	M	9	14	15	17
	F	28.5	25	34	22
Mottled	M	6.5	7	10	9
	F	20.5	18	29	18
Dark	M	3	3	3	8
	F	6.5	7	5	5
Lung					
Mottled	M	7.5	9	6	6
	F	3.5	4	7	2
Dark	M	4.5	4	4	7
	F	3.5	1	8	4
Heart					
Thickened pericardial sac	M	0	0	0	
	F	0	0	0	1
Spleen					
Enlarged	M	2.5	1	2	3
	F	1.5	4	0	2
Liver					
Enlarged	M	2.5	4	1	5
	F	0	0	2	3
Colon					
Distended	M	1	1	1	2
	F	0	0	0	0
Stomach					
Dark Area	M	1.5	4	4	4
	F	5.5	1	2	2
Distended	M	1.5	1	3	3
	F	0	1	0	0
Testes					
Small	M	2.5	3	3	4
Soft	M	3	3	4	4
Seminal vesicle					
Small	M	6	4	8	9
Epididymis					
Small	M	1	0	1	2
Uterus					
Distended	M	1	2	0	3
Mammary Gland					
Thickened	M	5.5	5	2	8
Skin					
Sore	M	22	30	27	29
	F	16.5	5	15	10
Swollen	M	3	5	6	6
	F	3	0	1	1

* Animal # in control =130; to compare the findings with the treatment group findings/65 animals are presented in the table.

Histopathology:

Hyperplasia was one of the major observations in the different organs namely pituitary, adrenal, thyroid, thymus and urinary bladders. This finding is not always dose related, however, the increase in the incidence relative to control may indicate treatment related effect. Stress related changes in the hematological parameters observed in the earlier studies might be correlated with the changes in pituitary and adrenal gland observed under this experimental condition and related to the functional alterations in these endocrine glands. Thyroid changes observed in this experiment may also be related to the changes in the WBCs observed earlier and might also be accounted for a stress related response resulting from the test article administration. Changes in thymus and spleen might be directly related to the inflammatory response resulted from the test article which confirms earlier observation with this compound. Distension of stomach, colon, liver which has been observed in this experiment may be related to the known pharmacological class effect of the compound related to the gut retention and discomfort. Foamy macrophages in the lung have also been observed in the 6-month chronic rat study. Lung is a known target organ for this class of compound, presence of foamy macrophages might indicate phospholipidosis, the toxicological implication of the finding is not yet known. Atrophy of the male reproductive organs is also noted. This is a well-known pharmacological effect of nicotine. The compound being a partial agonist for nicotine is expected to show the same kind of response.

Summary of Histopathological Findings:*

Group/Dose(mg/kg)		0	1	5	15
Number of Animals		65	65	65	65
Brain					
Compression	M	8	19	25	19
	F	37.5	15	44	26
Pituitary					
Hyperplasia	M	19	15	8	18
	F	3	5	4	8
Testis					
Atrophy	M	10	9	10	14
Epididymis					
Aspermia	M	4.5	6	4	9
Prostrate					
Atrophy	M	1	0	1	2
Inflammation	M	49	51	52	53
Ovary					
Hyperplasia	F	7.5	8	12	8
Mammary gland					

Hyperplasia/atypical	F	2	3	3	3
Hyperplasia/lobular	F	20	17	24	14
Skin					
Inflammation in paw	M	41.5	50	43	40
	F	22	18	27	21
Stomach					
Ulcer/fore stomach	M	1.5	1	2	2
	F	1	2	1	2
Ulcer glandular	M	2	3	3	4
	F	1.5	4	2	1
Thymus					
Hyperplasia/ epithelial	M	0	0	0	0
	F	6.5	10	7	11
Spleen					
Increased Pigmentation	M	45	39	53	49
	F	40.5	40	46	45
Adrenal					
Hyperplasia/medulla	M	5.5	8	10	6
	F	7.5	5	8	6
Hyperplasia/medulla	M	3.5	6	3	8
	F	4.5	6	2	4
Thyroid					
Hyperplasia-C cells	M	3	3	5	5
	F	4.5	6	8	4
Heart					
Myofibrosis	M	59	57	52	54
	F	28	26	22	33
Lung					
Foamy cell foci	M	15	9	17	22
	F	11	12	12	10
Urinary Bladder					
Hyperplasia	M	0	2	4	2
	F	2	4	1	0

* Animal # in control =130; to compare the findings with the treatment group findings/65 animals are presented in the table.

Some of the major causes of death for the animals under this experimental condition are tabulated below. The most common cause of death was related neoplastic and non neoplastic microscopic changes in pituitary gland and skin (tail and paws) of males and females and the mammary gland of females. These findings were related to the macroscopic and microscopic observations. Inflammation of different organs causing death is also observed. Inflammation and stress related changes in the pituitary may be related to the pharmacological effect of the compound. However, under this experimental condition no dose relationship could be found for these findings.

Cause of Death

Group/Dose(mg/kg)		0	1	5	15
Terminal Sacrifice	M	34	29	30	23
	F	26	28	23	32

Undetermined	M	11.5	8	7	10
	F	4	2	3	5
Pituitary Neoplasia	M	12	9	7	10
	F	2	2	3	5
Mammary gland Neoplasia	M	12	16	18	20
	F	22	21	32	16
Inflammation of lung	M	0	0	0	0
	F	8	8	4	9
Inflammation of skin	M	2	0	0	0
	F	1	0	0	1
Inflammation of prostate	M	1	2	1	3
Calculi, urinary bladder	M	2	1	0	0
	F	0	0	0	0
Inflammation of mesentary	M	0	0	0	1
	F				
Inflammation of heart	M	1	1	0	0
	F	2	0	0	0
Fore stomach, erosion	M	1	1	0	0
	F	0	0	0	0

Neoplastic: Three treated males (1/65 and 2/65 at 5, and 15 mg/kg/day) were noted to have intrathoracic mass. These mass were composed of the multivacuolated cells and were determined to be tumor cell positive by immunohistochemistry. Electron microscopical evaluation was done for these tumors. These mediastinal tumors were found to contain numerous mitochondria characteristics of the brown adipose tissue and thus the tumors were confirmed to be hibernomas (neoplasm of the brown fat). The hibernoma found in the mid dose group was determined to be benign based on its morphology. The variation of the nucleus and the cytoplasm of the two hibernomas found in the high dose group and thereby considered as malignant; one of these two hibernomas were found to metastasize in the lung. The reviewer confirmed the Sponsor's observation 'the hibernoma is a rare neoplasm of brown adipose tissue and only a few cases of hibernoma have been reported in control rats. In the Registry of Industrial Toxicology Animal-data (RITA) control animal database, there is one hibernoma in a male Sprague-Dawley rat but this did not occur in the mediastinum [Rita, Lesion-related Incidence Data, 2005]. An intrathoracic (mediastinal) hibernoma was reported in a control female F344 rat [Stefanski et al., *Lab Anim Sci*; 37(3):347-50, 1987] and a control female Sprague-Dawley rat [Port et al., *Lab Anim Sci*; 29(2):214-217, 1979]. Hibernoma has also been observed in one male Sprague-Dawley rat (adipose tissue), two male and four female Osborne-Mendel rats (mammary/subcutaneous tissue or prostate locations) in the control animal database of the National Toxicology Program [National Toxicology Program, Data Search Application = Hibernoma, 2005]'. No other hibernomas in rat have been reported.

However, this finding does not meet the statistical requirement to be significant in the present study (rare tumor is considered to be statistically significant if the P-values are <0.025).

Reviewer's Comment: The incidence of hibernoma in male rats is confirmed to be very low. Although not statistical significant due to their

rarity, the tumors might be test article related and should be labeled with the carcinogenicity findings.

Other malignant neoplastic findings which might be treatment-related (since not observed in controls) were mesothelioma of heart, shwanoma of brain, shwanoma of pituitary, and hemangiosarcoma of spleen (1/ 65 in each case) at high dose males. In females, neoplasms were observed in the granulosa cell in ovary, adenosarcoma in uterus, and basal cell carcinoma of the skin (1/65 in each case). None of the tumor findings in males and females were statistically significant.

Non Neoplastic: Dose related non neoplastic tumor findings in males were limited to hemangioma of spleen (1/65 in control vs. 1/65 at high dose) and kearatoctanoma of skin (2/65 in control vs. 3/65 at high dose). In females, luteoma in ovary (1/65) and benign mammary mass (1/65) were noted at high dose. These findings were absent in the control group. None of these findings were found to be statistically significant. The tables below were reproduced from the Statistical Review completed by Dr. Moh-Jee Ng

Malignant Tumor Trends in Male Rats:

Organ Name	Tumor Name	CTR	LOW	MED	HIGH	P-Value
	*B-HIBERNOMA	0	0	1	0	0.3624
	*M-HIBERNOMA	0	0	0	2	0.0349
Heart	MESOTHELIOMA, ATRIOCAVAL, MA	0	0	0	1	0.1611
Liver	X-NEOPLASM, METASTATIC	0	0	0	1	0.2131
Pituitary	I-SCHWANNOMA	0	0	0	1	0.2131
Spleen	HEMANGIOSARCOMA	0	0	0	1	0.2131
Brain	SCHWANNOMA	0	0	0	1	0.1646

Malignant Tumor Trends in Female Rats:

Organ Name	Tumor Name	CTR	LOW	MED	HIGH	P-Value
Lymphoreticular	LYMPHOMA,	1	1	1	2	0.1631
Ovary	NEOPLASM, GRANULOSA CELL, MA	0	0	0	1	0.2429
Skin and adnexa	CARCINOMA, BASAL CELL	0	0	0	1	0.2407
Uterus	ADENOCARCINOMA	0	0	0	1	0.2429

Benign Tumor Trends in Male Rats:

Organ Name	Tumor Name	CTR	LOW	MED	HIGH	P-Value
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	*B-HIBERNOMA	0	0	1	0	0.3624
	*M-HIBERNOMA	0	0	0	2	0.0349
Adrenal	ADENOMA, CORTICAL	1	0	0	1	0.2527
Mammary gland	FIBROADENOMA	1	0	1	1	0.2870
Skin and adnexa	B-KERATOACANTHOMA	2	1	0	3	0.0592
Spleen	B-HEMANGIOMA	0	0	0	1	0.1611

Benign Tumor Trends in Female Rats:

Organ Name	Tumor Name	CTR0	LOW	MED	HIGH	P-Value
Mammary gland	MAMMARY MASS, NOT OTHERWISE	0	0	0	1	0.2429
Ovary	LUTEOMA, BENIGN	0	0	0	1	0.2429

Toxicokinetics: No apparent changes CP-526,555 serum concentrations (males and females combined) were noted from Week 26-Week 104, for each of the doses that was tested indicating that the steady state for the exposure had been reached at this time point. Increase in exposures with increasing doses was noted. At Week 26, a 2.3-fold and 5.3-fold increase in exposure was observed with 5 and 15 mg/kg/day doses respectively when compared to that of the 1 mg/kg/day dose indicating less than dose proportional increase for the compound. At Week 104, 1.9 and 3.3-fold increase were observed with 5 and 15 mg/kg/day doses respectively when compared to that of the 1 mg/kg/day dose. Higher than initial exposure was observed with all doses suggesting accumulation of the compound.

Serum CP- 526,555 Exposures:

Dose (mg/kg)	Study Week	Mean Serum Concentration (ng/mL)
1.0	26	144±25
	52	204±66
	79	122±47
	104	201±26
5	26	329± 76

	52	333±57
	78	288±65
	105	389±68
15	26	765±130
	52	531±55
	78	508±156
	105	673±93

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: CP-526,555-24 Reproductive Study I: Fertility and Early Embryonic Development Oral Gavage Study in Female Sprague-Dawley Rats

Key study findings:

- Female Sprague-Dawley rats administered with varenicline succinate (0, 0.3, 3, 15 mg/kg/day) by oral gavage for 14 days prior to mating, during cohabitation, and through gestation day 7 showed decreased body weight and increased clinical signs in dams at high dose group. This observation is considered treatment related, no change in body weight gains was observed in the low and the mid dose group females.
- Clinical signs in the high dose females included post dose salivation, irregular respiration, decreased activity, and ptosis. Similar observations were noted in general toxicity studies and are considered treatment related. The females from the low and mid dose group and the males did not show any abnormal clinical signs.
- A NOAEL of 3 mg/kg/day was determined for the maternal toxicity.

- The Sponsor concluded that the NOAEL for fertility and early embryonic development was 15 mg/kg day (HED=145 mg; AUC=500 ng•h/mL; 4.5x safety margin), the highest dose tested, reviewer agrees with this conclusion.
- Exposure data was only obtained at a single time point: 2 hours post dose on study day 8. Dose proportional increase (approximately 10-fold) in the serum levels of the test article was noted from 0.3 (21 ± 4.8 ng/mL) to 3.0 (198 ± 40 ng/mL) mg/kg/day. A less than dose proportional increase (approximately 2.5-fold) in the serum levels of the test article was noted from 3.0 (198 ± 40 ng/mL) to 15 (500 ± 40 ng/mL) mg/kg/day.

Study no.: 99-1545-20

Volume # and page #: Volume: 1; Page: 1-115

Conducting laboratory and location: Pfizer Inc., Groton CT

Date of study initiation: 10/11/99

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: Drug: CP-526,555-24, Batch: 27506-110-1F, Purity composition noted as follows: "No unspecified impurity ζ η detected (Purity by HPLC)."

Methods

Doses: 0, 0.3, 3, 15 mg/kg/day

Species/strain: CD (SD)IGS rats

Number/sex/group: 20/sex/group

Route, formulation, volume, and infusion rate: Oral gavage formulated in deionized water, 10 mL/kg

Satellite groups used for toxicokinetics: None

Study design: Male rats were not treated.

Group assignments, dose levels and animal numbers are tabulated below.

Group	Daily Dose (mg/kg)	Drug Concentration (mg/ml)	Dose Volume (ml/kg)	Animal Numbers	
				Males*	Females
Control (Deionized Water)	0.0	0.0	10.0	1-20**	81-100
Low-dose (CP-526,555-24)	0.3	0.03	10.0	21-40	101-120
Intermediate-dose (CP-526,555-24)	3.0	0.3	10.0	41-60	121-140
High-dose (CP-526,555-24)	15.0	1.5	10.0	61-80	141-160

Parameters and endpoints evaluated:

Toxicity parameters for F₀ male and female: Mortality and clinical signs were observed twice daily; body weight and food consumption was measured weekly.

Fertility parameters: Copulation, pregnancy rates, and changes in the estrus cycles were analyzed daily.

Reproductive parameters: Numbers of corpora lutea, implants, viable fetuses, dead fetuses, early resorptions, late resorptions, pre-implantation loss: $100 \times ((\# \text{ of corpora lutea} - \# \text{ of implantations}) / \# \text{ of corpora lutea})$, and post-implantation loss: $100 \times ((\# \text{ of implantations} - \# \text{ of viable fetuses}) / \# \text{ of implantations})$, were determined from gestational Day 14 dams and analyzed statistically.

Other parameters observed were the litter size and the maternal serum drug concentration.

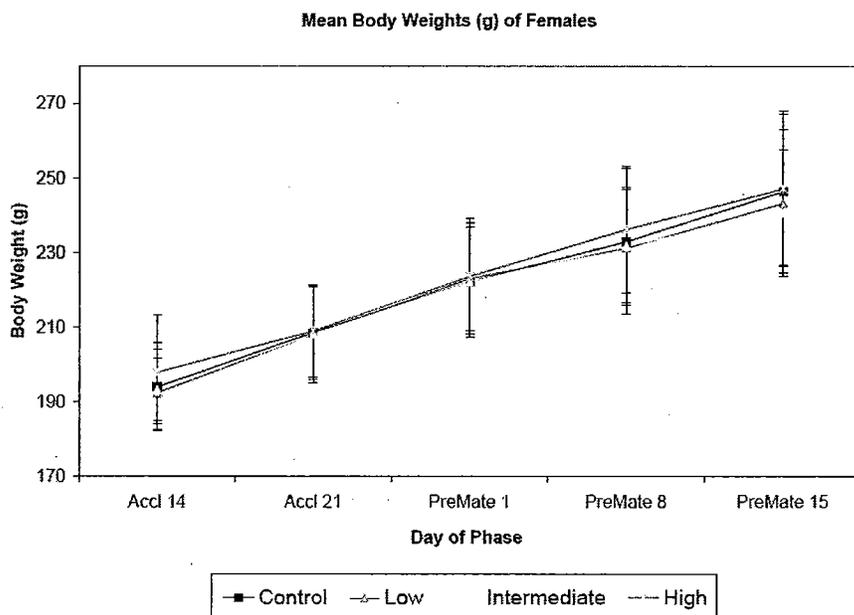
Results

Mortality: One high dose animal was sacrificed on Study Day 5; histopathological observation revealed evidence of gavage related injury (inflammation and irritation of the esophageal wall). There was no other mortalities in this study.

Clinical signs: In high dose females, post dose salivation, irregular respiration, decreased activity and ptosis were noted. These observations were noted in toxicity studies with the compound and are considered treatment related. The females from the low and mid dose group and the males did not show any abnormal clinical signs.

Body weight: A statistically significant decrease in the maternal body weight gain was observed in the high dose group females between Days 0-6. This observation is considered treatment related, no change in body weight gains was observed in the low and the mid dose group females.

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Food consumption: There were no treatment related changes in the food consumption in males and females. A decrease in food consumption was noted at Day 2 of gestation for low and high dose group female. This observation was not considered treatment related since the decrease in food consumption was noted in an isolated way and only on Day 2.

Necropsy:

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

A slight decrease in the total occurrence of estrus and average estrous cycle length was noted in the females from the high dose groups as compared to those of the controls. This effect was also seen in low dose group, but not in the mid dose group. Similarly, number of females with the insufficient number of the estrous cycles was increased for the low and the high dose group animals but not in the mid dose group. These changes are not dose dependent and therefore are not likely treatment-related.

A Summary of Estrous Cycle During the Dosing Period^a

Dose (mg/kg)	Control	0.3	3	15
Average Estrous Cycle Length \pm S.D.(Days) ^b	4.15 \pm 0.34 19 ^c	4.05 \pm 0.42 18 ^c	4.13 \pm 0.33 19 ^c	4.06 \pm 0.24 18 ^c
Number of Estrous Cycles \pm S.D.	3.32 \pm 0.58 19	3.33 \pm 0.77 18	3.53 \pm 0.51 19	3.28 \pm 0.57 18
Total Occurrences of Estrus	63	60	67	59
Number of Estrus (%) with each duration ^d				
1 day	53 (84)	53 (88)	55 (82)	53 (90)
2 days	1 (2)	0	1 (2)	0
3 days or more	0	0	0	0
No. Females with Insufficient Number of Estrous Cycles	1	2	1	2

^a Only Average Estrous Length and Number of Estrous Cycles were statistically analyzed.

^b Estrous cycle length (days) was determined from full cycles occurring during the observation period. The length of the entire estrous cycle was calculated by counting the day following the end of the first estrus to the end of the last observed estrus. The average length is the number of days in a cycle divided by the total # of occurrences - 1 (or - 2 if estrus falls on the last day of the observation period).

^c Females # 96 (0.0 mg/kg), 101, 104 (0.3 mg/kg), 132 (3.0 mg/kg) and 148, 160 (15.0 mg/kg) were excluded from calculations because of an insufficient # of cycles, during the observation period.

^d Not all occurrences of estrus could be used to calculate duration. Therefore, estrus (%) with each duration does not equal the total occurrence of estrus.

There were no dose related changes in the copulation rate or pregnancy rate between groups, as denoted in the summary table below, reproduced from the sponsor's submission:

Group	No. Sperm Positive	Number Gravid	Number Non-Gravid	Copulation Rate (%) ^a	Pregnancy Rate (%) ^b
Control	20/20	19/20	1/20	100	95
0.3 mg/kg	20/20	17/20	3/20	100	85
3.0 mg/kg	20/20	18/20	2/20	100	90
15.0 mg/kg	18/19	17/18	1/18	95	94

^a (number sperm positive/number bred) x 100

^b (number gravid/number sperm positive) x 100

There were no changes in the following reproductive parameters: mean numbers of corpora lutea, implants, viable fetuses, dead fetuses, early resorptions, late resorptions, pre-implantation loss: $100 \times ((\# \text{ of corpora lutea} - \# \text{ of implantations}) / \# \text{ of corpora lutea})$, and post-implantation loss: $100 \times ((\# \text{ of implantations} - \# \text{ of viable fetuses}) / \# \text{ of implantations})$, as determined from gestational Day 14 dams and analyzed statistically (see table below).

A SUMMARY OF MEAN CESAREAN SECTION VALUES FOR RATS

	CORPORA LUTEA (#)	IMPLANTS #	VIABLE #	DEAD #	ER #	LR #	PRE IMP LOSS (%)	POST IMP LOSS (%)
Control (0.0 mg/kg)								
MEAN:	14.9	13.2	12.0	0.0	1.2	0.0	13.45	12.08
STD DEV:	2.5	4.8	5.1	0.0	1.5	0.0	26.05	17.95
N:	19	19	19	19	19	19	19	19
Low (0.3 mg/kg)								
MEAN:	15.4	14.7	13.9	0.0	0.8	0.0	3.92	4.95
STD DEV:	1.7	1.3	1.2	0.0	1.0	0.0	5.32	6.30
N:	17	17	17	17	17	17	17	17
P:	TND	TND	TND	TND	TND	TND	TND	TND
Intermediate (3.0 mg/kg)								
MEAN:	14.6	13.6	13.1	0.0	0.6	0.0	7.09	3.96
STD DEV:	1.8	3.1	3.1	0.0	0.7	0.0	17.27	5.04
N:	18	18	18	18	18	18	18	18
P:	TND	TND	TND	TND	TND	TND	TND	TND
High (15.0 mg/kg)								
MEAN:	16.1	14.9	14.1	0.0	0.8	0.0	7.60	6.05
STD DEV:	1.8	2.5	3.1	0.0	1.5	0.0	12.91	11.19
N:	17	17	17	17	17	17	17	17
P:	0.257	0.414	0.345	1.000	0.138	1.000	0.799	0.156

TND=Test Not Done (+)=Statistically significant increase (-)=Statistically significant decrease

ER = early resorptions

LR = late resorptions

PRE-IMP Loss (%) (pre-implantation loss) = $(\# \text{ corpora lutea} - \# \text{ implantations sites} / \# \text{ corpora lutea}) \times 100$.

POST-IMP LOSS (%) (post-implantation loss) = $(\# \text{ implantation sites} - \# \text{ viable fetuses} / \# \text{ implantation sites}) \times 100$.

Toxicokinetics: Serum concentrations of drug were determined on study day 8, 2 hours after dose administration. Dose proportional increase (approximately 10-fold) in the serum levels of the test article was noted from 0.3 (21 ± 4.8 ng/mL) to 3.0 (198 ± 40 ng/mL) mg/kg/day. A less than dose proportional increase (approximately 2.5-fold) in the serum levels of the test article was noted from 3.0 (198 ± 40 ng/mL) to 15 (500 ± 40 ng/mL) mg/kg/day.

Serum Concentration at 2 hours post dose of CP-526,555 Following Oral Administration to Female Rats at 0.3, 3, and 15 mg/kg for Eight Days

Rat #	Dose (mg/kg)	Concentration (ng/mL)
103	0.3	26.0
114	0.3	27.9
115	0.3	17.5
117	0.3	17.9
	Mean	20.8
	SD	4.8
123	3	143
134	3	207
135	3	203
137	3	238
	Mean	198
	SD	40
143	15	496
154	15	522
155	15	544
157	15	438
	Mean	500
	SD	46

Study title: CP-526,555-24 Reproductive Study I: Fertility and Early Embryonic Development Oral Gavage Study in Male Sprague-Dawley Rats

Key study findings:

- Male Sprague-Dawley rats administered varenicline succinate by oral gavage (0, 0.3, 3, 15 mg/kg/day) for a period of 28 days prior to mating and during co-habitation through study days 65-66 showed decreased body weight and increased clinical signs in the high dose group. NOTE: Dose levels in this study report are expressed as mg of CP-526,555-24/kg bodyweight.
- A statistically significant decrease in the body weight gain was observed in the high dose males between Days 29-66. This observation is considered treatment related. There were no changes in body weight gain observed in the low and the mid dose group males.
- Clinical signs noted in high dose males included post dose salivation, irregular respiration, decreased activity and ptosis.

- A NOAEL of 3 mg/kg/day was determined for the male toxicity.
- A NOAEL of 15 mg/kg/day was established for the male fertility and early embryonic toxicity for the test article in this study.
- Dose proportional increase (approximately 10-fold) in the serum levels of the test article was noted from 0.3 (23.7 ± 5 ng/mL) to 3.0 (228 ± 37 ng/mL) mg/kg/day. A less than dose proportional increase (approximately 2.5-fold) in the serum levels of the test article was noted from 3.0 (228 ± 37 ng/mL) to 15 (581 ± 116 ng/mL) mg/kg/day.

Study no.: 99-1545-21

Volume # and page #: Volume: 1; Page: 1-213

Conducting laboratory and location: Pfizer Inc., Groton CT

Date of study initiation: 10/8/99

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: Drug: CP-526,555-24, Batch: 27506-110-1F, According to the study report, for this lot of drug substance, the assayed potency was [] with purity composition described as “no unspecified impurity [] detected (purity by HPLC).

Methods

Doses: 0, 0.3, 3, 15 mg/kg/day

Species/strain: CD (SD)IGS BR rats

Number/sex/group: 20/sex/group

Route, formulation, volume, and infusion rate: Oral, by gavage, formulated in deionized water, 10 mL/kg

Satellite groups used for toxicokinetics: None. Serum concentrations of varenicline were determined approximately one week prior to mating two hours after dosing.

Study design:

Parameters and endpoints evaluated:

Toxicity parameters for F₀ male and female: Mortality and clinical signs were observed twice daily; body weight and food consumption was measured weekly.

Fertility parameters: Copulation, pregnancy rates, and changes in the estrus cycles were analyzed daily.

Reproductive parameters: Numbers of corpora lutea, implants, viable fetuses, dead fetuses, early resorptions, late resorptions, pre-implantation loss: $100 \times ((\# \text{ of corpora lutea} - \# \text{ of implantations}) / \# \text{ of corpora lutea})$, and post-implantation loss: $100 \times ((\# \text{ of implantations} - \# \text{ of viable fetuses}) / \# \text{ of implantations})$, were determined from gestational Day 14 dams and analyzed statistically.

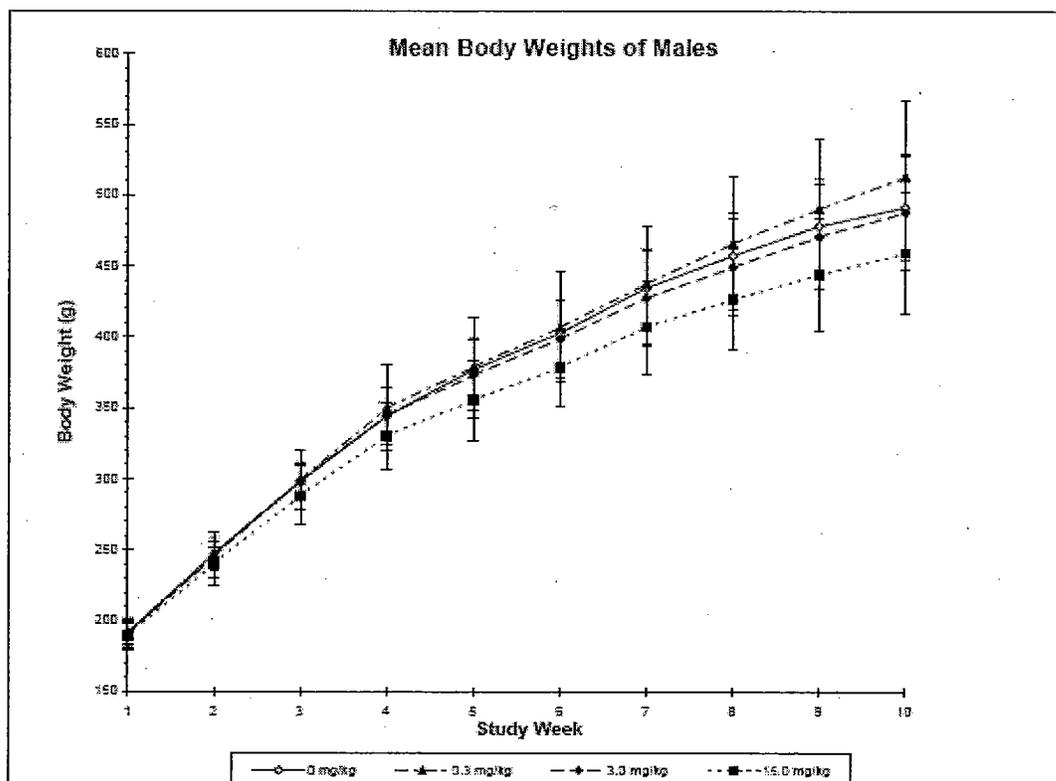
F₀ males were euthanized after 66 days of dosing and male reproductive tissues (epididymes and testes) were weighed and histopathologically examined. Sperm counts were obtained at the time of necropsy. Serum concentration of the compound was measured from Day 22 post dosing in males.

Results

Mortality: There was no mortality in this study.

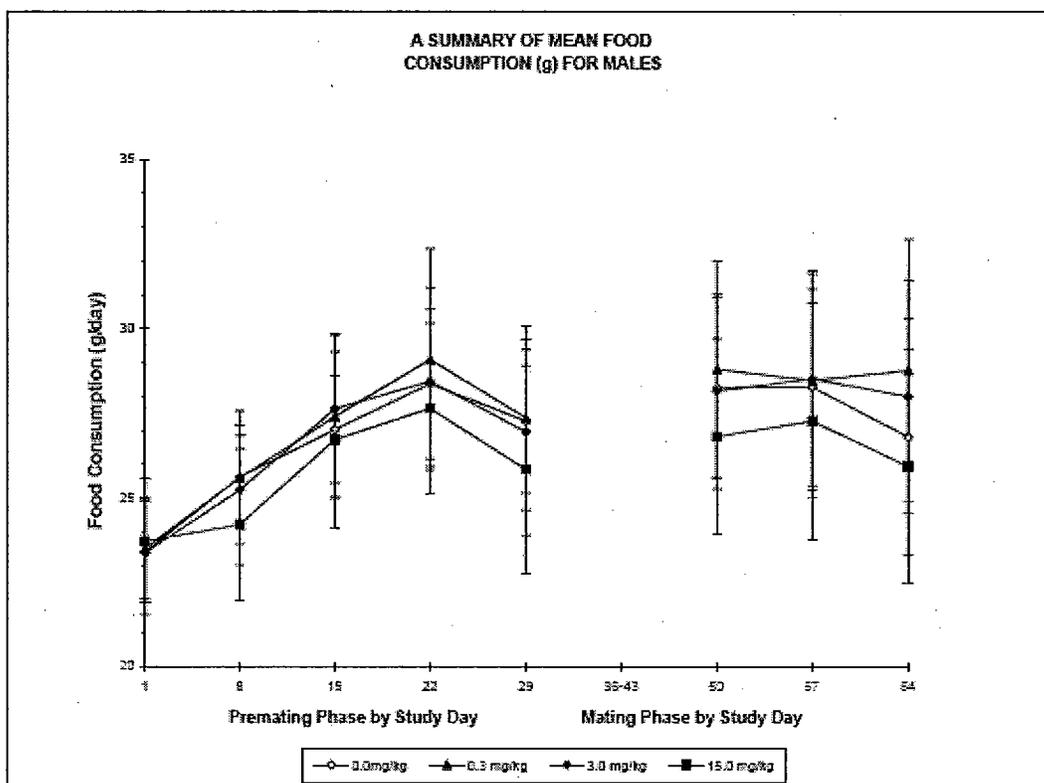
Clinical signs: In high dose males, post dose salivation, irregular respiration, and decreased activity were noted. These observations were also noted in toxicity studies with the compound and are considered treatment related. The males from the low and mid dose group and the females (females were not treated in this study) did not show any abnormal clinical signs.

Body weight: A statistically significant decrease ($P > 0.01$) in the body weight was observed in the high dose group males between Days 29-66. This observation is considered treatment related. There were no changes in body weight observed in the low and the mid dose group males compared to control animals.



Food consumption: There were no treatment related changes in food consumption in males and females. A statistically significant increase in food consumption was noted at Day 22 of dosing for all dose group males. This observation was considered

physiological and not treatment related since the decrease in food consumption was noted in an isolated way and only on Day 2.



Necropsy: There were no treatment-related differences in the weights of the testes or epididymes between treatment groups. Likewise, there were no treatment-related macroscopic changes. Microscopic observations of the testis and epididymis revealed minimal testicular hypoplasia in several animals distributed across the treatment groups, with the exception of one animal each in the low dose and mid dose groups which were reported as showing marked hypoplasia of the testis with abnormal content of the epididymis.

The histopathological diagnosis with comments for animal 27 in the low dose groups describes marked, diffuse testicular hypoplasia that correlated with gross macroscopic observations. The individual line listings noted that the “tubules are lined by sertoli cells with no evidence of spermatogenesis, consistent with congenital hypoplasia.” For the epididymis, the histopathologica description was “Abnormal content, present. Ducts contain scant proteinaceous material and variable numbers of sloughed cells. No spermatozoa are present.” Data from this animal were omitted from the sperm analysis evaluation.

The histopathological diagnosis with comments for animal 52 in the mid dose group reported marked, diffuse hypoplasia of the testis and the presence of abnormal content of

the epididymis with ducts containing “scant proteinaceous material and sloughed cells” and the absence of spermatozoa.

Lesion Incidence	Males (mg/kg/day)			
	0	0.3	3.0	15.0
Testis				
Hypoplasia				
Minimal	2/20	1/20		2/20
Marked		1/20	1/20	
Epididymis				
Periarteritis	0/20	0/20	0/20	0/20
Abnormal content	0/20	1/20	1/20	0/20

The results of the sperm analysis are presented in the sponsor’s table below:

SPERM ANALYSIS SUMMARY FOR STUDY 99-1545-21, CP-526,555				
	0 mg/kg	0.3 mg/kg	3 mg/kg	15 mg/kg
Mean # sperm/gram testis (x10 ⁶)	109.8	112.2 ^a	119.2 ^b	118.5
SD	14.1	19.5	19.9	23.0
n	20	19	19	20
Mean # sperm/gram epididymis (x10 ⁶)	711.9	682.0 ^a	640.5 ^b	630.2
SD	163.6	118.4	117.6	145.5
n	20	19	19	20

^a Animal #27 excluded from the mean calculations due to marked diffuse bilateral hypoplasia.

^b Animal # 52 excluded from the mean calculations due to marked diffuse bilateral hypoplasia.

The data above suggest a trend toward a decrease in the mean number of sperm/gram epididymis with increasing dose of varenicline; however, there was only an 11% decrease in the mean value in the highest dose group compared to control animals which did not appear to reduce overall fertility.

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

No treatment related changes in the fertility parameters were noted in this study, as depicted in the sponsor’s table below reproduced from the study report:

Group	No. Sperm Positive	Number Gravid	Number Non-Gravid	Copulation Rate (%) ^a	Pregnancy Rate (%) ^b
Control	20	19	1	100	95
0.3 mg/kg	19	19	1	95	100
3.0 mg/kg	19	19	1	95	100
15.0 mg/kg	20	20	0	100	100

^a (number sperm positive/number bred) x 100

^b (number gravid/number sperm positive) x 100

No treatment related changes between the treated and control groups for the numbers of corpora lutea, implantation sites, viable fetuses or resorptions.

Toxicokinetics: Serum concentrations of CP-526,555 (varenicline base) two hours after dosing on treatment day 22 are depicted in the sponsor's table below. Dose proportional increase (approximately 10-fold) in the serum levels of the test article was noted from 0.3 (23.7 ± 5 ng/mL) to 3.0 (228 ± 37 ng/mL) mg/kg/day. A less than dose proportional increase (approximately 2.5-fold) in the serum levels of the test article was noted from 3.0 (228 ± 37 ng/mL) to 15 (581 ± 116 ng/mL) mg/kg/day.

Serum Concentration at 2 hours post dose of CP-526,555 Following Oral Administration to Male Rats at 0.3, 3, and 15 mg/kg for Twenty Two Days

Rat #	Dose (mg/kg)	Concentration (ng/mL)
25	0.3	29.4
28	0.3	19.1
29	0.3	19.9
32	0.3	26.5
	Mean	23.7
	SD	5.0
45	3	199
48	3	253
49	3	193
52	3	266
	Mean	228
	SD	37
65	15	434
68	15	574
69	15	601
72	15	716
	Mean	581
	SD	116

Embryofetal development: Rabbit

Study title: CP-526,555: A Oral Teratology Study Of CP-526,555-24 In the Rabbit

Key study findings:

- Pregnant rabbits administered varenicline succinate (1, 10, 30 mg/kg/day) by oral gavage from the Gestation Day 7- 19 resulted in decreased body weight gain in dams and reduced fetal weights only following the high dose.
- One animal from high dose group was found dead on Day 7 of gestation within 10 minutes of dosing. The death was related to the acute cardiac and respiratory failure (uncollapsed lung with multiple dark areas, epicardial pale areas, dark

- areas in the thymus and skeletal muscles were noted). This incident might be related to the error in the oral gavage but the cause of the death could not be confirmed.
- The incidence of soft feces, thinning of the fur, and red staining of the fur were increased in the dams in the treated groups. These clinical signs were found to increase in a dose dependent manner and were determined to be treatment related.
 - A statistically significant loss in the body weight gain was noted on gestation Day 19 and 20 for the high dose group females ($P < 0.01$). Significant loss of body weight was noted in the high dose group females from gestation Day 9. This trend continued throughout the treatment period and was observed in the mid dose group animals to a lesser extent. No such changes were noted in the low dose group animals.
 - A NOAEL of 1 mg/kg was determined for the maternal toxicity based on the clinical findings and decrease in the body weight gain.
 - There were no statistically significant major malformations, minor external visceral or skeletal anomalies or skeletal variants for any of the fetuses that were considered to be treatment-related. One fetus from the high dose group showed gastrochisis. Although this may be treatment-related, the incidence ($< 1\%$ fetus affected) was within the historical control range (0-2.8%).
 - Mean fetal body weight (13-15%) and placental weights ($> 12\%$) were significantly reduced in animals from the high dose group. There was no treatment related effect on fetal weight at either 1 or 10 mg/kg/day. A decreasing trend in the placental weight was observed at low and mid dose; this decrease, however, was not statistically significant.
 - A NOAEL of 10 mg/kg (HED= 193 mg; AUC for fetus at NOAEL = 399 ng•h/mL= 3.5x) determined for the fetal toxicity based on the decrease in the body weight gain in fetus from the high dose group.
 - Serum exposure of the test article was determined in the dams after 13 days of dosing (Day 7 - Day 19). Exposure in the rabbit was found to be more than dose proportional at low doses; a 10-fold increase in dose (1-10 mg/kg/day) resulted in approximately 16-fold increase in AUC; however only a 6-fold increase in Cmax. Increase in AUC with 5-fold increase (10-15 mg/kg/day) in dose was approximately 2-fold and increase in AUC and 3-fold increase in Cmax.
 - Varenicline crosses the placenta barrier. The fetal serum concentrations of the compound were higher than maternal serum concentration following administration of varenicline to the doe at low and mid dose. However, at high dose, the serum concentration of the test article was found to be similar in dams and fetus.

Study no.: 99-1545-18

Volume # and page #: Volume 1 and 2; Page: 1-216 and 1-214

Conducting laboratory and location: []

Date of study initiation: August 25, 1999

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: Drug: CP-526,555-24; Batch # 27506-110-1F, Purity [] (NOTE, this is the same batch of drug used in the Female fertility study in rats. That study report notes that the specs on this lot show the assayed potency as [] and notes that in reference to the purity composition, no unspecified impurity [], detected (purity by HPLC). The dose formulations were prepared daily and adjusted for purity. The accuracy of the preparations was confirmed and the dose formulations were considered acceptable when the mean value of individual analyses was [] of the nominal concentration.

Methods

Doses: 0, 1, 10, 30 mg/kg/day

Species/strain: New Zealand white rabbits

Number/sex/group: 20/ groups

Route, formulation, volume, and infusion rate: Oral gavage, formulated in deionized water, dose volume = 2 mL/kg

Satellite groups used for toxicokinetics: 4/sex/group

Study design:

Group No. Identification	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Number of Females	
			Main Study	T/K Phase
1 Vehicle control	0	2	20	4
2 CP-526,555-24	1	2	20	4
3 CP-526,555-24	10	2	20	4
4 CP-526,555-24	30	2	20	4

Parameters and endpoints evaluated: Clinical signs were observed twice daily and detailed examinations, individual body weight and food consumptions were measured daily from the Day 5 to 29 of gestation. On Day 29 of gestation, the uterine contents from each doe were examined at necropsy. The placentas and fetuses were weighed. Each fetus was examined in detail. The brain morphology and skeletal examination from each fetus was recorded.

Blood samples were collected from the dams of the toxicokinetic phase on Days 7 and 19 of gestation at 2, 4, 8 and 24 hours post-dosing. Fetal blood samples were collected from each fetus/uterine horn/doe in the litter by decapitation of the fetus.

Results

Mortality (dams): There were two mortalities in this study. One doe from the control group died; the cause of death was likely due to perforation in the trachea related to gavage error. Another animal from high dose group was found dead on Day 7 of gestation within 10 minutes of dosing. The death was attributed to acute cardiac and respiratory failure (uncollapsed lung with multiple dark areas, epicardial pale areas, dark areas in the thymus and skeletal muscles were noted). The Sponsor suggested that this death might be related to the error in the oral gavage; however, the clinical signs are consistent with acute nicotine toxicity and may be treatment related.

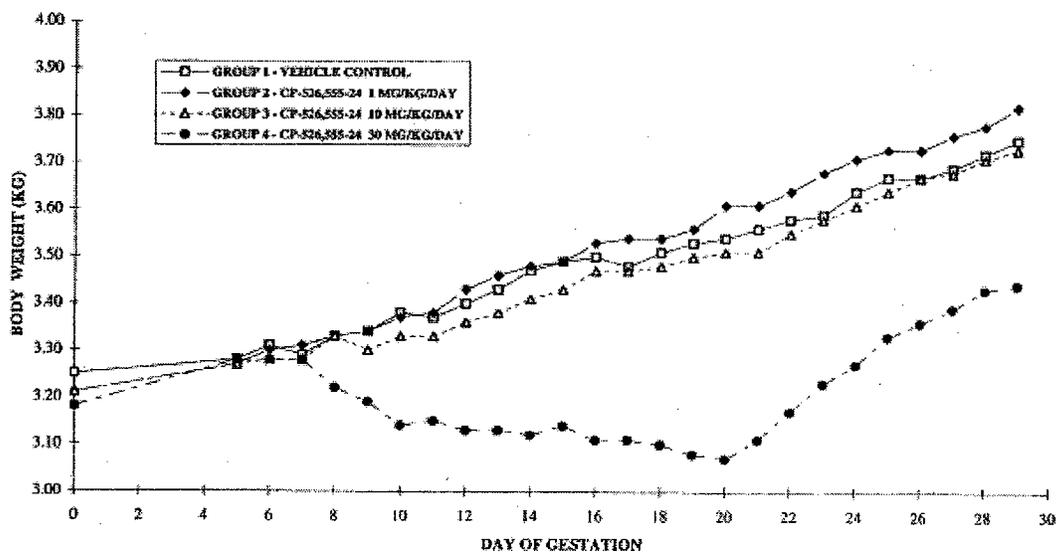
Clinical signs (dams): Soft feces, thinning of the fur, and red staining of the fur were noted in the dams in the treated groups. These clinical signs were found to increase in a dose dependent manner and were determined to be treatment related.

Summary of Clinical Observations: Incidence of selected clinical observations are presented below (n=20):

Parameters	Control	1 mg/kg	10 mg/kg	30 mg/kg
Fur, staining, red/yellow/brown, tail	4	6	6	8
Fur, thin cover, forepaw(s)/forelimb(s)/hindpaw(s)/hindlimb(s)	7	3	6	10
Fur, thin cover, axillary/inguinal/lumbar/ abdominal/dorsal thoracic/ventral cervical/ventral thoracic/urogenital region(s)	2	3	7	10
Feces, soft/absent/output decreased	6	7	14	20

Body weight (dams):

A statistically significant loss in the body weight gain was noted on gestation Day 19 and 20 for the high dose group females (P<0.01). Significant loss of body weight was noted in the high dose group females from gestation Day 9 as clearly depicted in the Sponsor’s graph reproduced below. This trend continued throughout the treatment period. No such changes were noted for low and mid dose group.



Food consumption (dams): There was a significant decrease in the food consumption (P<0.05 or P<0.01) lower from gestation Days 7 to 14 at 10 mg/kg/day and significantly (P<0.01) lower between gestation Days 7 and 21 at 30 mg/kg/day. These reductions of food intake were treatment-related and correlated with the change in body weight gain.

GROUP 1	VEHICLE CONTROL
GROUP 2	CP-526,555-24 1 MG/KG/DAY
GROUP 3	CP-526,555-24 10 MG/KG/DAY
GROUP 4	CP-526,555-24 30 MG/KG/DAY

SEX GROUP	FEMALES			
	1	2	3	4
5-6	164.8	178.2	173.7	176.1
6-7	166.7	178.2	173.4	171.8
7-8	170.3	178.8	146.5**	56.9**
D 8-9	171.7	178.2	121.4**	40.1**
9-10	170.6	179.1	123.9**	56.0**
10-11	161.9	178.2	129.7**	55.4**
A 11-12	162.7	177.8	119.4**	52.0**
12-13	162.3	174.7	125.1*	56.2**
13-14	161.3	174.7	128.9*	46.3**
Y 14-15	155.8	172.5	127.8	54.8**
15-16	144.8	170.1	137.1	38.1**
16-17	145.8	169.5	142.7	45.9**
17-18	155.7	172.8	155.4	41.1**
18-19	165.8	178.3	152.3	39.4**
19-20	170.8	180.0	152.4	50.7**

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: * P<0.05 ** P<0.01 (DUNNETT'S)

Toxicokinetics:

Serum exposure of the test article was determined in the dams after 13 days of dosing (Day 7- Day 19). Exposure in rabbit was found to be more than dose proportional at low doses; a 10-fold increase in dose (1-10 mg/kg/day) resulted in approximately 16-fold increase in AUC; however increase in C_{max} at this time was 6-fold only. Increase in AUC with 5-fold increase (10-15 mg/kg/day) in dose was approximately 2-fold and increase in C_{max} at this time point was approximately 3-fold.

Toxicokinetics was also done from the dams at Day 21. Fetal blood samples were also collected at Day 21. Fetus absorbed the test article. The serum level of the compound was higher in fetus than maternal serum concentration at low and mid dose. However, at high dose, the serum concentration of the test article was found to be similar in dams and fetus.

Summary of Toxicokinetics Parameters in Female Rabbits*:

Dose Groups mg/kg/day	AUC ₀₋₂₄ (ng•hr/mL)	C _{max} (ng/mL)	T _{MAX} (hr)
1	279 ± 62	58.5 ± 13.2	2.0
10	4400 ± 260	372 ± 63	2.0
30	9580 ± 4090	1230 ± 800	2.0

* Gestation Day 19

CP-526,555 Serum Levels in Rabbit Fetus:*

Dose Groups mg/kg/day	Maternal Serum Conc (ng/mL)	Fetal Serum Conc (ng/mL)	Maternal: Fetal Drug ratio
1	39.4 ± 13.2	58.8 ± 16.1	0.7
10	300 ± 50	399 ± 62	0.8
30	1430 ± 1330	1390 ± 1010	1.0

* Gestation Day 21

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

There were no treatment related changes in the numbers of corpora lutea, implantation sites, live fetuses, dead fetuses, numbers of resorptions, the sex ratio and the pre and post implantation losses as observed from the C-section of the dams at Day 29. Four dams aborted, one low dose doe (between Day 27-28); and three dams from the high dose (between Day 18-24). The abortion rate in high dose dams was within the historical control range, therefore not considered as treatment related.

Offspring (malformations, variations, etc.): There were no statistically significant major malformations, minor external visceral or skeletal anomalies or skeletal variants for any of the fetuses that were considered to be treatment-related. One fetus from the high dose group showed gastrochisis, this incidence might be related to the test article. However, the low incidence (<1% fetus affected) and due to the fact that the incidence was within the historical control range (0-2.8%), the effect is considered incidental. Fetal and placental weights were significantly lowered (13.5%) at 30 mg/kg/day in animals from the high dose group. There was no treatment related effect on fetal weights or placental weights at 1 or 10 mg/kg/day.

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MALES

	FETAL WEIGHT	PLACENTAL WEIGHT
GROUP 1 - VEHICLE CONTROL	46.50 6.819	6.17 1.096
GROUP 2 - CP-526,555-24 1 MG/KG/DAY	45.66 4.729	5.89 .791
GROUP 3 - CP-526,555-24 10 MG/KG/DAY	45.74 6.005	5.92 .807
GROUP 4 - CP-526,555-24 30 MG/KG/DAY	40.21 ** 5.185	5.29 * .672

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: * P<0.05 ** P<0.01 (DUNNETT'S)

FEMALES

	FETAL WEIGHT	PLACENTAL WEIGHT
GROUP 1 - VEHICLE CONTROL	44.16 5.735	5.84 1.000
GROUP 2 - CP-526,555-24 1 MG/KG/DAY	45.13 4.600	5.76 .760
GROUP 3 - CP-526,555-24 10 MG/KG/DAY	44.37 4.319	5.40 .543
GROUP 4 - CP-526,555-24 30 MG/KG/DAY	40.03 5.243	5.13 * .709

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: * P<0.05 (DUNNETT'S)

Embryofetal development: Rat

Study title: An Oral Teratology Study Of CP-526 .55-24 In the Rat

Key study findings:

- Pregnant Sprague-Dawley rats administered varenicline succinate by oral gavage (0, 0.3, 5, 15 mg/kg/day from Gestation Day 6-17 reduced maternal body weight (>10%) and food consumption significantly (P<0.01) with the mid and high dose. Increased clinical signs of salivation were also noted in dams from high dose group. Decrease on body weight and salivation was also noted in the mid dose

- group. A NOAEL of 0.3 mg/kg was determined for maternal toxicity based on the above findings. No change in the reproductive parameters was noted in females.
- No changes in fetal body weight were noted.
 - Following changes were noted in fetuses. One fetus from the 0.3 mg/kg/day group had anophthalmia. One fetus from the 15 mg/kg/day group showed anal atresia and acaudia, and another fetus from this same group had situs inversus. These incidences were within the historical control range. A small number of fetuses in the 0.3 mg/kg/day group showed one of the following findings: oval shaped lenses (5/149), absence of the innominate artery (2/149) or dilated ureters (2/149). No such changes were noted in animals from the mid and high dose group. The incidence of fetuses with reduced ossification of the hyoid bone was significantly increased for the 0.3 and 15 mg/kg/day groups (percent affected was 51% and 49% for the 0.3 and 15 mg/kg/day groups, respectively). These values, however, fell within the historical control data range (percent fetuses with reduced ossification of hyoid bone ranged from 0 to 71%), the number of litters affected was not significantly different between the control and treated groups. There were no significant differences between the control and treated groups for common visceral and skeletal findings. A NOAEL of 15 mg/kg (HED= 145 mg; Maternal AUC=734 ng•h/mL; 6x safety margin; fetal AUC=1041 ng•h/mL, 9x safety margin) was established for rat teratology in this study.
 - Fetus absorbed the test article. The serum level of the compound was higher in fetus than maternal serum concentration at all doses. The result confirmed the placental transport of the test article is not concentration dependent.

Study no.: 99-1545-19

Volume # and page #: Volume: 1 and 2; Page: 1-214 and 1-321

Conducting laboratory and location: []

Date of study initiation: August 26, 1999

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP 526,555, Batch # 27506-110-1F

Methods

Doses: 0, 0.3, 5, 15 mg/kg/day

Species/strain: CD (Sprague-Dawley) rats

Number/sex/group: 20/sex/group

Route, formulation, volume, and infusion rate: Oral gavage, formulated in deionized water, volume used was 10 mL/kg

Satellite groups used for toxicokinetics: 4/ group were used for toxicokinetics

Study design:

<u>Group No.</u> <u>Identification</u>	<u>Dose Level</u> <u>(mg/kg/day)</u>	<u>Dose Volume</u> <u>(mL/kg/day)</u>	<u>Number of Females</u>	
			<u>Main Study</u>	<u>T/K Phase</u>
1 Vehicle control	0	10	20	8
2 CP-526,555-24	0.3	10	20	8
3 CP-526,555-24	5.0	10	20	8
4 CP-526,555-24	15.0	10	20	8

Parameters and endpoints evaluated:

Mortality and clinical signs were noted twice daily. Body weight and food consumption was determined at Days 0, 3, 5, and daily from 6 to 20 of gestation, gross pathology and uterine examination of dams (Day 20 of gestation), fetal weight, sex and external/internal examination (Day 20 of gestation).

Toxicokinetic evaluations were conducted on 4 animals/group on gestation Day 17 and on another 4 animals/group on gestation Day 20

Results

Mortality (dams): One animal from the toxicokinetic group (high dose) died, the cause of the death could not be determined.

Clinical signs (dams): Salivation was noted post dosing in the high dose group female (14/20) at Day 13 and continued for at least 4 days. Salivation was also (2/20) noted in the mid dose group females. This finding is considered treatment related due to the fact that similar findings were noted in the treatment groups in the toxicity studies with this test article and the findings is believe to be stress associated with the tolerance of the compound.

Body weight (dams):

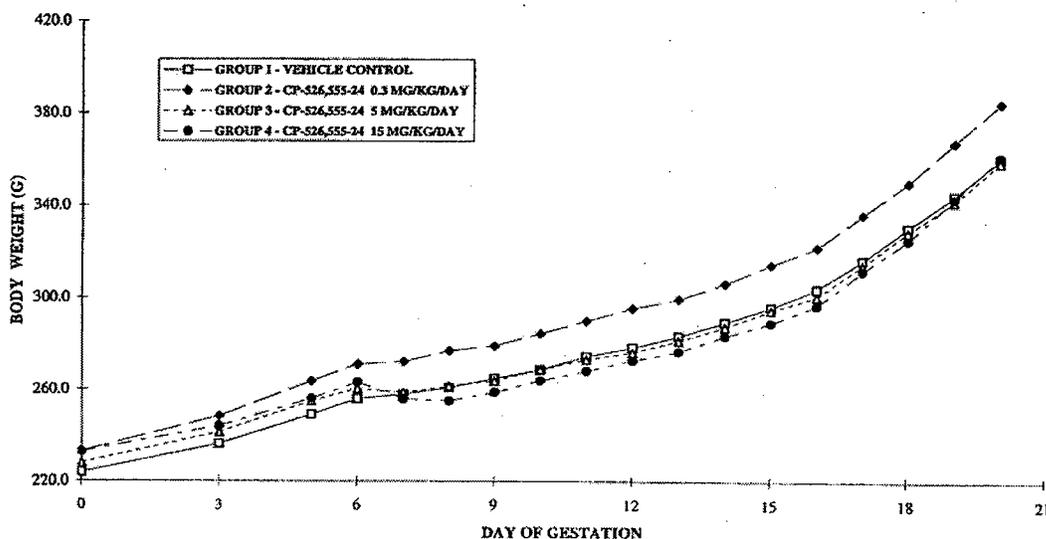
The corrected body weight gain (body weight gain from gestation Day 6 to 20 minus gravid uterus weight) was significantly lower for the 5 and 15 mg/kg/day groups. The cumulative corrected body weight gain for the high dose group animal decreased between 6-18 days significantly. At Day 18-20, after the treatment was terminated body weight gain in the high dose group female increased significantly.

Body Weight Gains:

GROUP 1	VEHICLE CONTROL	
GROUP 2	CP-526,555-24	0.3 MG/KG/DAY
GROUP 3	CP-526,555-24	5 MG/KG/DAY
GROUP 4	CP-526,555-24	15 MG/KG/DAY

SEX GROUP	FEMALES			
	1	2	3	4
D 6- 12	22.5	24.6	16.0*	9.9**
A 12- 18	52.3	54.7	52.4	52.2
Y 18- 20	30.1	34.7	30.5	36.6**
Y 6- 18	74.8	79.3	68.5	62.1**

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: * P<0.05 ** P<0.01 (DUNNETT'S)



Food consumption (dams):

There was a decrease in food consumption for the 15 mg/kg/day group (P<0 .01) decreased at gestation Day 7 to 18 which corresponds to the decrease in the body weight observed in the dams during this treatment period. A significant (P<0 .05) increase in food consumption occurred for the high dose group from gestation Day 18 to 19 and 19 to 20 which might be attributed to compensatory responses related to the cessation of treatment.

Summary of Food Consumption

GROUP 1	VEHICLE CONTROL
GROUP 2	CP-526,555-24 0.3 MG/KG/DAY
GROUP 3	CP-526,555-24 5 MG/KG/DAY
GROUP 4	CP-526,555-24 15 MG/KG/DAY

SEX GROUP	FEMALES			
	1	2	3	4
7 - 12	145.1	153.0	130.7	116.4**
D13 - 18	158.4	168.7	157.6	147.0
A 7 - 18	303.5	321.7	288.3	263.5**
Y19 - 20	53.9	63.1*	54.9	63.8*

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: * P<0.05 ** P<0.01 (DUNNETT'S)

Toxicokinetics:

Serum exposure of the test article was determined in the dams after 13 days of dosing (Day 7-Day17 and 20). Exposure in rat was found to be dose proportional at low doses; a 10-fold increase in dose (1-10 mg/kg/day) resulted in approximately 11 and 10-fold increase in serum levels at Day 17 and 20 respectively. Increase in serum levels with 3-fold increase (10-15 mg/kg/day) in dose was approximately 2 and 1.5-fold at Day 17 and Day 20 respectively.

Fetus absorbed the test article. The serum level of the compound was higher in fetus than maternal serum concentration at all doses. The result suggests that the placental transport of the test article is not concentration dependent.

CP-526,555 Serum Levels in Rat Fetus:*

Dose Groups mg/kg/day	Maternal Serum Conc (ng/mL) @ Day 17	Maternal Serum Conc (ng/mL) @ Day 20	Fetal Serum Conc (ng/mL) @ Day 20	Maternal: Fetal Drug ratio @ Day 20
0.3	33.1±2.0	40.6±5.8	57.1±7.2	0.7
5	370.2±26.2	429±37.5	612.2±58.9	0.7
15	800.2±123.1	734.2±76.4	1041.2±100.5	0.7

* 2hr post dose in gestation Day 17 and 20

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

There were no treatment related changes in the mean numbers of corpora lutea, implantation sites, live fetuses, dead fetuses, numbers of resorptions, the sex ratio or pre and post implantation losses as observed from the C-section of the dams at Day 20.

Offspring (malformations, variations, etc.):

There were no treatment related changes in the fetal body weights. One fetus from the 0.3 mg/kg/day group had anophthalmia. One fetus from the 15 mg/kg/day group showed anal atresia and acaudia, and another fetus from this same group had situs inverses. These incidences were within the historical control range (submitted from []). A small number of fetuses in the 0.3 mg/kg/day group showed one of the following findings: oval shaped lens (5/149), absence of the innominate artery (2/149) or dilated ureters (2/149). No such changes were noted in animals from the mid and high dose group. The incidence of fetuses with reduced ossification of the hyoid bone was significantly increased for the 0.3 and 15 mg/kg/day groups (percent affected was 51 % and 49% for the 0.3 and 15 mg/kg/day groups, respectively). These values, however, fell within the historical control data range percent fetuses with reduced ossification of hyoid bone ranged from 0 to 71 %), the number of litters affected was not significantly different between the control and treated groups. There were no significant differences between the control and treated groups for common visceral and skeletal findings.

Prenatal and postnatal development

Study title: Oral Pre- and Postnatal Development Study in Sprague-Dawley Rats with CP-526,555-24

Key study findings:

- CP-526,555 administered to pregnant rats via oral gavage (0, 0.3, 3, and 15 mg/kg) from Gestation Day 6-Lactation Day 20 decreased body weight and food consumption and increased clinical signs (salivation respiratory stress) in the dams for 3 and 15 mg/kg indicating maternal toxicity. Sponsor's NOAEL is 0.3 mg/kg, however due to <4% body weight loss for the F₀ females with 3 mg/kg dose, a NOAEL of 3 mg/kg (HED= 29 mg; AUC =229 ng•h/mL; 2x safety margin for human exposure) is established for the maternal toxicity.
- No reproductive toxicity was reported for the F₀ females.
- F₁ fetuses from the high dose group showed significant reduction in body weight. Auditory startle response significantly decreased in F₁ fetus. Increase in latency time (unknown biological significance) was noted in F₁ fetus.
- Fertility in F₁ females reduced (60-80%) significantly at high dose.
- A NOAEL of 3 mg/kg was established in F₁ (exposure 21 ng•h/mL at Day 6 of lactation) females based on physical behavior noted and fertility (in concurrence w/Sponsor).
- The test article was passed to the fetus via milk, dose proportionately.

Study no.: 01-1545-30

Volume # and page #: Volume 1, Page 1-766

Conducting laboratory and location: Pfizer Inc, Groton, CT

Date of study initiation: 02/07/01

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP-526,555-24, 27506-110-1F

Methods

Doses: 0, 0.3, 3, 15 mg/kg

Species/strain: CD (SD)IGS BR rats

Number/sex/group: 24/group

Route, formulation, volume, and infusion rate: Oral gavage, formulated in deionized water, 10 mL/kg

Satellite groups used for toxicokinetics: 3/group

Study design: F₀ dams were dosed from gestation day (GD) 6 to lactation day (LD) 20. Males were not treated. On post-natal day 21 (PND 21), two F₁ pups/sex/litter were randomly selected to continue on the study.

Dose group	^a Dose level (mg/kg/day)	Animal numbers per replicate				TK
		1	2	3	4	
Group 1 (vehicle)	0	1-6	7-12	13-18	19-24	97-100
Group 2 (CP-526,555-24)	0.3	25-30	31-36	37-42	43-48	101-104
Group 3 (CP-526,555-24)	3	49-54	55-60	61-66	67-72	105-108
Group 4 (CP-526,555-24)	15	73-78	79-84	85-90	91-96	109-112

^aAll dose levels are expressed as mg active moiety of CP-526,555-24 per kg of body weight.

Parameters and endpoints evaluated:

Animals were observed at least twice daily for clinical signs. Maternal body weights were recorded daily from gestation Day 6 through parturition and on lactation Day 1, 4, 7, 10, 14, 17 and 21. Maternal food consumption was recorded daily until parturition and on lactation Day 4, 7, 10 and 14. Dams were euthanized and received a gross necropsy examination.

Pups were weighed on postnatal Days (PND) 1, 4, 7, 10, 14, 17, 21 and then weekly thereafter. Physical and reflexive development was evaluated by the following endpoints: appearance of incisors, acquisition of the air-righting reflex, functional observational battery, motor activity, passive avoidance, straight channel water maze, Cincinnati water maze, and auditory startle response (ASR). Sexual development and reproductive competence was evaluated by the following endpoints: vaginal opening in females, preputial separation in males, estrus cycle, copulation and fertility parameters.

The F₂ offspring were evaluated for survival, clinical signs and body weights.

monitoring the age of incisor eruption and attainment of the air-righting reflex. Sexual development was assessed by monitoring the age of attainment of balanopreputial separation or vaginal opening for male and female offspring, respectively.

Animals were assessed by a Functional Observational Battery (FOB) on PND 24-25 and for motor activity on PND 58-65. Learning and memory was evaluated using a Passive Avoidance Test (PND 73-81) and in the Cincinnati Water Maze (PND 71-79), and the auditory startle response was assessed on PND 80-87.

Adult F₁ offspring were assessed for reproductive competency by monitoring estrous cyclicity, breeding performance and the ability to deliver and rear a litter. To further evaluate reproductive ability, F₁ females in the 15 mg/kg/day dose group that failed to deliver a litter were re-bred to proven male sires from the same dose group and F₁ males in the 15 mg/kg/day dose group failing to sire a litter were re-bred to proven females from the same dose group. For toxicokinetic evaluation, blood samples were taken from dams at 2 hr post-dose on PND 4 and 10. Blood samples were taken from pups (pooled for each litter, by sex) at 2 hr post-dose on PND 4 and at 0, 2, 4, and 8 hr post-dose on PND 10.

Results

F₀ in-life: There was no mortality in the F₀ females.. Clinical observations included salivation (20/24), rapid respiration (4/24) and gasping (1/24) at high dose. These observations are considered treatment related due to its occurrence at high dose. Decrease in body weight gain from gestation Day 7 to lactation Day 14 was upto 11% in animals treated with the high dose. A 4% decrease in body weight gain was noted in the mid dose group at the same period,. Decrease in the food consumption was noted in both mid and high dose group animals between gestation Day 6 and lactation Day 14 indication a correlation with the decrease body weight in animals during this time period. Reproductive parameters for the F₀ females did not show any treatment related changes.

F₀ necropsy: There were no treatment related necropsy findings for the F₀ females.

F₁ physical development: There was a significant (P> 0.01) decrease in body weight (11-12 %) in the body weights from postnatal Day (PND) 7 for both male and female offspring at high dose group. This decrease continued in males and females until PND 28 and 35 respectively.

F₁ behavioral evaluation: Both males and females in the 15 mg/kg/day group had a statistically significant reduction in the incidence of rearing (animal rises on hind legs). The Sponsor attributed this finding to biological variation as there were no treatment-related effects on other neuromuscular or sensorimotor measurements (gait, handling reactivity, mobility score or arousal). However, a treatment related effect cannot be ruled

out. Loss of energy due to body weight loss might be reflected in the decrease of rearing activity in these animals.

Varenicline treatment was associated with a decrease in the mean latency time observed in the learning and memory test (both straight channel water maize and Cincinnati water maize test). This finding is not considered adverse, as an increase in the latency time is usually considered a toxicological effect (decrease motor function etc.). However, the biological significance of increase in latency finding is not known. A statistically significant increase in the maximum amplitude of the auditory startle response (ASR) was reported for 15 mg/kg/day group males: There was no effect on mean amplitude of the ASR. Although, there were no such effects for the 15 mg/kg/day group females, a relationship to treatment could not be rejected. A decrease in total latency was noted in the 3 mg/kg/day female group, however, the effect was not deemed treatment-related because a similar effect due to lack of dose response.

F₁ reproduction: A reduction in fertility rate was observed in the high dose group. The fertility indices (number of mating pairs producing a litter/number of mating pairs x 100) were 95, 95, 94.75 and 80% for the control, 0.3, 3, and 15 mg/kg/day groups, respectively. The decrease in the fertility was due to the failure of mating pairs to produce litters (4/); 1 pair did not copulate and 3 pairs copulated but were non-gravid. To further evaluate this finding, Sponsor paired these non-performing F₁ males (4) and females (3, 1 female had been sacrificed per protocol on post mating day 25 and was not available for further evaluation) with proven breeders from the same dose group. Of the 4 mating pairs consisting of non-performing males and proven females, 3 pairs copulated resulting in 3 pregnancies (75%). Of the 3 mating pairs consisting of non-performing females and proven males, breeding was 57% (4/7). These findings confirmed the initial finding of reduced fertility in the breeding pairs. The reduced fertility could not be attributing to either gender.

F₂ findings: There were no treatment related clinical signs or body weight changes in the F₂ offspring.

Toxicokinetics: A dose proportional increase in the serum levels of the compound was noted at Day 6 and 10 of lactation in the lactating dams and males and females pups. The data shows that the test article is passed to the fetus via milk dose proportionately and continuously (no T_{max} observed for fetuses at least up to 8 hrs post dose) in the same amount.

Summary of Toxicokinetics on Lactation Day 6 (Values represent serum concentrations of varenicline (ng/mL):

	Dose (mg/kg/day)		
	0.3	3.0	15.0
Dams	34.4 ±5.2	229 ±24	521 ±92
Male pups	3.73 ±2.13	22.2 ±5.5	116 ±36
Female Pups	3.08 ±0.25	*16.7	114 ±43
Mean ±SD			
*N=2			

Summary of Toxicokinetics on Lactation Day 10 (Values represent serum concentrations of varenicline (ng/mL):

	Time (hr) post-dose	Dose (mg/kg/day)		
		0.3	3	15
Dams	2	26.7 ±5.4	234 ±57	513 ±96
Male pups	0	<LLOQ	3.67 ±2.15	43.4 ±18.9
	2	1.42 ±1.08	15.5 ±7.9	53.9 ±21.8
	4	2.56 ±0.50	22.5 ±7.2	42.0 ±6.8
	8	1.61 ±0.42	17.2 ±9.9	60.8 ±27.6
Female Pups	0	<LLOQ	4.05 ±2.81	35.1 ±9.5
	2	1.35 ±1.41	12.7 ±10.5	62.4 ±26.5
	4	2.99 ±0.48	23.1 ±7.1	61.1 ±14.8
	8	1.83 ±0.47	20.9 ±11.4	59.0 ±22.6
Mean ±SD				
<LLOQ: less than the lower limit of quantitation for the assay (1.0 ng/mL)				

2.6.6.7 Local tolerance

Study title: Three Day Dosage Tolerance and Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of CP-526,555 In Pigmented Rats

Key study findings:

- The test article did not show any phototoxic effect up to 100 mg/kg/day under this experimental condition.

Study no.: 04-1545-36

Volume # and page #: Volume: 1, Pages: 1-158

Conducting laboratory and location: []

Date of study initiation: 04/10/2004

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP-526,555, lot # 3000054, purity: not reported.

Formulation/vehicle: The test compound was formulated in deionized water. Lomefloxacin in carboxymethylcellulose at a dose of 800 mg/kg or 8-methoxypsoralen (8-MOP) in corn oil at 50 mg/kg was used as positive control.

Methods

Doses: 100 mg/kg/day for 3 days. The dose was selected based on an oral tolerance study in which the rats were administered with the 30 or 100 mg/kg/day CP-526,555 formulated in water. Anticipated treatment-related dose-dependent clinical responses (soft or liquid feces, scant feces, no feces, ptosis, decreased motor activity, labored breathing, gasping and body weight loss) based on the previous experience with CP-526,555 in Sprague Dawley rats were observed. Thus, 100 mg/kg/day CP-526,555 formulation was determined to be the maximum tolerated dose and chosen for inclusion in the phototoxicity phase of the study.

Study design: Five rats/group were administered the test article (0, 100 mg/kg) by oral gavage for three days. Approximately one hour after the final administration, rats were anesthetized and a lightly and darkly pigmented skin site exposed to solar-simulated ultraviolet radiation (UVR) from a long arc xenon arc lamp filtered to mimic mid-latitude summer sunlight. The comparator articles (lomefloxacin in carboxymethylcellulose, 800 mg/kg at a dose of 800 mg/kg and 8-methoxypsoralen (8-MOP) in corn oil at a dose of 50 mg/kg) were administered orally. One hour later a single exposure to UVR was also administered to these animals as described above. All rats were observed on the days of the test article administration at 30 minutes \pm 10 minutes of administration. On the day of UVR exposure observations were made within approximately 30 minutes of the end of UVR exposure and for three days following UVR exposure.

Results:

No evidence of cutaneous phototoxicity was observed at termination or on any of the three days of observation after the dose administration and UVR exposure. The rats administered with the positive control lomefloxacin or 8-methoxypsoralen once followed by a single UVR exposure had cutaneous responses of erythema and/or edema in the lightly and/or darkly pigmented skin sites that were exposed to UVR. These responses are indicative of a phototoxic response to these comparator articles indicating that the experiment was valid.

Study title: CP-5269555-18: Acute Dermal Toxicity (Limit Test) In The Rat

Key study findings:

- The test article did not show dermal toxicity up to 2000 mg/kg under the current experimental condition.

Study no.: 1131-550

Volume # and page #: Volume 1; Pages 1-28

Conducting laboratory and location: J

Date of study report: 02/02/2004

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP-526, 555; lot # 54526-10-10B, Purity: not reported.

Formulation/vehicle: Deionized water

Methods

Study design: Sprague-Dawley CD (SD) IGS BR) rats weighed 227 to 301 g, and eight to twelve weeks of age (5/sex/group). 2000 mg/kg of the test article formulated in deionized water was administered dermally. A piece of surgical gauze was placed over the treatment area and held in place with a self-adhesive bandage.

After the 24-hour contact period the bandage was carefully removed and the treated skin assessed for signs of irritation. The animals were returned to group housing for the remainder of the study period.

The animals were observed for deaths or overt signs of toxicity 2 and 4 hours after dosing and subsequently once daily for fourteen days. Morbidity/mortality inspections were conducted twice daily during normal working days and once daily on weekends.

The test sites were examined for evidence of primary irritation and scored according to the following Draize scale Immediately after removal of the dressings and subsequently once daily for fourteen days.

Results: There were no signs of erythema indicating no dermal irritation.

Study title: CP-5269555-18: Acute Eye Irritation in the Rabbit

Key study findings:

- The test article was determined to be a mild irritant at 45 mg in rabbits, conjunctivae (chemosis, redness, and discharge) and iridial irritation was noted in all of the three rabbits those were treated.

Study no.: 1131-552

Volume # and page #: Volume: 1; Pages 1-30

Conducting laboratory and location:

Date of study initiation: 07/18/2002

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP-526, 555, lot # 54526-10-10B, purity: not reported.

Formulation/vehicle: Deionized water

Methods

Doses: 45 mg in 0.1 mL

Study design: New Zealand White rabbits of either sex were obtained and used for this study. At the start of the study the animals weighed between 2.0 and 3.5 kg (approximately twelve to twenty weeks old). Three rabbits were treated with a volume of 0.1 mL of the test material, (45 mg) by an adapted syringe that was placed into the conjunctival sac of the right eye, formed by gently pulling the lower lid away from the eyeball. The left eye remained untreated and was used for control purposes. Immediately after administration of the test material, an assessment of the initial pain reaction was made. Assessment of ocular damage/irritation was made approximately 1, 24, 48 and 72 hours following treatment, according to the (Draize scale). An additional observation was made in one treated eye on Day 7 to assess the reversibility of the findings.

Results: The test article administered directly to one of the two eyes of three rabbits produced treatment related iridial inflammation and minimal to moderate conjunctival irritation. 1/3 rabbits presented with a score of 5. Chemosis was noted in 3/3 rabbits 1 hr post dose; in one of these rabbits the chemosis was still evident at the 24 hr time point. Discharge from the eye and redness of eye was noted in all rabbits at 1 hr post dose. The score for redness and discharge in 1/3 rabbits was 2 and it continued for 72 hrs in this rabbit. Treated eyes appeared normal at 7 days.

CP-526,555-18 : ACUTE EYE IRRITATION IN THE RABBIT

Table 2 Individual Total Scores And Group Mean Scores For Ocular Irritation

Rabbit Number and Sex	Individual Total Scores At				
	1 Hour	24 Hours	48 Hours	72 Hours	7 Days
112 Male	6	2	0	0	-
126 Male	13	8	6	2	0
128 Male	6	0	0	0	-
Group Total	27	10	6	2	0
Group Mean Score	9.0	3.3	2.0	0.7	0.0

Study title: CP-5269555-18: Acute Dermal Irritation in the Rabbit**Key study findings:**

- The test material produced a primary irritation index of 0.2 and was classified as a mild irritant to rabbit skin according to the Draize classification scheme at 500 mg dose.

Study no.: 1131-551**Volume # and page #:** Volume: 1; Pages: 1-25**Conducting laboratory and location:** J**Date of study initiation:** 07/04/02**GLP compliance:** Yes**QA reports:** Yes**Drug, lot #, and % purity:** CP-526, 555; lot # 54526-10-10B, purity: not reported.**Formulation/vehicle:** Deionized water**Methods****Doses:** 0.5 gm/rabbit

Study design: Three New Zealand White rabbits (12 to 16 weeks old) weighing 2.55 to 2.71 kg were used for this study. As part of a dose range-finding study, one rabbit was initially treated. Three suitable sites were selected on the back of the rabbit. At each test site a quantity of 0.5 gm of the test material was introduced onto 2.5 cm x 2.5 cm cotton gauze patch, moistened with 0.5 ml of distilled water and then placed in position on the shorn skin. Each patch was secured in position with a strip of surgical adhesive tape. To prevent the animal from interfering with the patches, the trunk of the rabbit was wrapped in an elasticated corset. One patch was removed at each of three time points: 3 minutes, 1 hour and 4 hours after application.

After consideration of the skin reactions produced in the first animal, two additional animals were treated with 0.5 g of test material moistened with 0.5 ml distilled water. One patch was applied to the back of each rabbit, and was allowed to remain in contact with the skin for a period of four hours.

Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the Draize scale and expressed as a primary irritation index.

Results: The test material produced a primary irritation index of 0.2 and was classified as a mild irritant to rabbit skin according to the Draize classification scheme (see table below). No corrosive effects were noted.

Primary Irritation Index	Classification of Irritancy
0	Non-irritant
> 0 to 2	Mild irritant
> 2 to 5	Moderate irritant
> 5 to 8	Severe irritant

Summary of Irritation findings in Rabbits:

CP-526,555-18 : ACUTE DERMAL IRRITATION IN THE RABBIT

Table 1 Individual Skin Reactions Following 4-Hour Exposure

Skin Reaction	Observation Time	Individual Scores – Rabbit Number and Sex			Total
		93 Male	9 Male	12 Male	
Erythema/Eschar Formation	1 Hour	0	1	0	(1)
	24 Hours	1	0	0	1
	48 Hours	1	0	0	(1)
	72 Hours	0	0	0	0
Oedema Formation	1 Hour	0	0	0	(0)
	24 Hours	0	0	0	0
	48 Hours	0	0	0	(0)
	72 Hours	0	0	0	0
Sum of 24 and 72-hour Readings (S)		1			
Primary Irritation Index (S/6)		1/6 = 0.2			
Classification		MILD IRRITANT			

Study title: CP-5269555-18: Skin Sensitization in the Guinea Pig - Magnusson and Kligman Maximization Method

Key study findings:

- The test material produced a 0 % (0/9) sensitization rate and was classified as a non-sensitizer to guinea pig skin under the conditions of the test.

Study no.: 1131/553

Volume # and page #: Volume 1; Page 1-40

Conducting laboratory and location: τ J

Date of study initiation: 02/09/2004

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: CP-526,555; Lot# 54526-10-10B, purity: not reported.

Formulation/vehicle: Deionized water

Methods

Doses: Intradermal induction of the test article 5% w/w in distilled water; topical induction with 50% w/w in distilled water; topical challenge 25% and 10% w/w in distilled water

Study design: Ten test and five control male albino Dunkin Hartley guinea pigs were used. At the start of the main study the animals were in the weight range of 254 to 307g, and were eight to twelve weeks old. Intradermal injections (0.1 mL/injection site) were made on the clipped shoulder of one guinea pig using a concentration of 5% w/w in distilled water. The degree of erythema at the injection sites was assessed approximately 24, 48, 72 hours and 7 days after injection according to the Draize scale. The degree of edema was not evaluated. Two phases were involved in the main study; an induction of a response by intradermal injection and topical application and a topical challenge of that response.

Based on the results of sighting tests, the concentrations of test material for the induction and challenge phases were selected as follows: intradermal induction of 5% w/w in distilled water; topical induction with 50% w/w in distilled water; topical challenge 25% and 10% w/w in distilled water.

Results:

The test material produced a 0% (0/9) sensitization rate and was classified as a non-sensitizer to guinea pig skin under the conditions of the test.

2.6.6.8 Special toxicology studies: None submitted

2.6.6.9 Discussion and Conclusions:

Compared to the full agonist nicotine, CP-526,555 (varenicline) functions as a partial agonist at neuronal nicotinic receptors. Receptor binding studies demonstrated that the

varenicline has high affinity for the $\alpha 4\beta 2$ neuronal nicotinic receptor subtype (rat and human cortex, $K_i \sim 0.2$ nM). The test article >500-fold $\alpha 3\beta 4$, >20,000-fold selective for $\alpha \beta \gamma \delta 1$ (muscle nicotinic receptor), and >3500-fold selective for $\alpha 7$ (nicotinic toxin) receptors. CP-526,555 had low affinity at the $5HT_{3A}$ (K_i 350 nM). The compound do not show any affinity for a variety of other neurotransmitter receptors (approximately 56 different receptors tested), modulatory binding sites, ion channels, and neurotransmitter uptake sites in membranes derived from relevant tissues and cell lines. The partial agonist efficacy of the compound was further justified by the dopamine release activity of the compound. In the in vivo assay in rats an ED_{50} of 0.032 mg/kg PO (maximal response 153%) was obtained which is approximately 63% of that of nicotine (maximal response 184%). The result shows that the test article reduced the peak dopamine release effect of the nicotine to approximately 60%. Rats were responsive to the substitution of the nicotine by the test article demonstrating efficacy for nicotine substitution in vivo. The test article was also compared to nicotine in the animal model to evaluate the withdrawal effect and physical dependence after self administration. Discontinuation of CP-526, 555, did not result in any behavioral disorder or body weight loss which might imply less liability for dependence with this test article.

In the safety pharmacology study mild tremors, decreases in locomotor activity, piloerection and hunched to flattened body posture was noted at single oral administration of 10 mg/kg dose in rat [human equivalent dose (HED) =96 mg]. C_{max} at this dose in rat was found to be 749.0 ng/mL at 1 hr. Plasma concentration at the human efficacious dose of 1mg is found to be 9.2 ng/mL, indicating that approximately 50--fold safety margin exists for CNS behavioral changes. In addition to the CNS related behavioral changes mentioned above, at 100 mg/kg dose in rat, convulsion, salivation, mild to moderate ptosis, decreases in response to toe pinch, decreases in exploratory behavior, disturbances in gait (splayed hind limbs) and decreases in body / limb tone were noted. No effects on the incidence of twitch, myoclonus or tonic extension induced by PTZ compared to the incidence in vehicle-treated mice were noted with the compound up to 10 mg/kg dose. A significant decrease in the body temperature in rat was noted with the compound at 10 mg/kg ($2^\circ C$); no change in body weight temperature was noted at 1 mg/kg dose.

A significant increase in the sodium (138%) and chlorine (173%) excretion was noted in rat with the test article at 30 mg/kg dose (HED=290 mg), no such changes were noted at 3 mg/kg (HED=29 mg). This is a pharmacological effect of nicotine, the compound being a partial agonist of nicotine might be showing a real manifestation of this pharmacological effect, however, in the repeat dose toxicity studies no toxicological effect related to this findings were observed.

A significant decrease in gastric emptying were noted with CP-526,555 at 3 (55% \downarrow) and (89% \downarrow) mg/kg, the reduction in geometric center of the gastrointestinal tract (GIT) indicating reduction in the gastro intestinal motility is a well known pharmacological effect of nicotine and thus might be a valid finding with this compound. This effect is dose related. In rat one hour after oral administration of CP-526,555 at 0.3 (plasma level=142 ng/mL), 3 (plasma level=656 ng/mL), and 30 mg/kg (plasma level= 1038

ng/mL) 3, 53, and 90% reduction in the geometric center was noted. This safety pharmacology observation was found to be manifested in all the toxicity studies described and reviewed under this submission.

The compound induced excessive retching and emesis in ferret at all dose studied (0.025-0.3 mg/kg) with different routes of administration (SC, oral). Mecamylalanine a nicotine receptor blocker only partially blocked this emetic effect. Ondasteron, a receptor antagonist effectively blocked the retching and emesis in ferrets. This suggest an inherent structural effect (the compound showed binding affinity to 5-HT₃, approximately 530 nM) of the compound for induction of emesis.

The ADME profile of CP-526, 555 was adequately studied. Extensive metabolism of the compound and interspecies variation of the metabolites were noted, however, none of the metabolites were considered as major metabolite since <10 % of the metabolites in total were found in the systemic circulation. All metabolites found in human were found to be present in at least one animal species, and therefore, deemed qualified. The potential for the varenicline to induce CYP isozymes have been investigated. No oxidative metabolism of the test article is reported in the rat, monkey, and human liver microsomes suggesting that it is not a good substrate for cytochrome P450 enzymes. The plasma protein binding was found to be low in all species studied (18, 45, 19, 41, and 20 in mouse, rat, dog, monkey, and human respectively). The test article was found to be absorbed fast (1 hr) after single dose oral administration in rat and mouse. In the primates, however, the T_{max} of the compound was found to be between 3-5 hrs. The T_{1/2} in monkeys (24 h) was higher after single dose than that in human (16 h) under similar conditions. The tissue distribution studies in rodents showed that the test article is extensively deposited in the melanin containing tissues like skin and eye. This indicates that the test article might have different effect on skin and eye demographically. The T_{max} for the test article after oral administration was found to reach within 3-4 hrs in the primates, the compound has a long T_{1/2} (approximately 16- 20 hrs in primates). The compound is excreted mainly via urine (approximately 80-85 %), feces accounted for approximately 5-6 % of the excretion. The fate of the rest of the compound administered, however, is not carefully studied.

Preclinical studies include toxicology studies in rats, dogs, mice and monkeys with duration of single dose to 12 months, 2-year carcinogenicity studies in mice and rats, genotoxic studies, reproductive toxicity studies in rats and rabbits, and special toxicology studies. The major target organs were brain/central nervous system (CNS), gastrointestinal tract (GIT), and lymphoid system.

Decreased food consumption and body weight gain or weight loss were noted in all species. The severity of the effects ranged from minimal to profound decreases in food consumption and marked body weight loss, associated with clinical signs suggestive of CNS effects and overall health deterioration at high dose levels.

CP-526, 555 were tested in two pivotal repeat dose toxicity studies in Cynomolgus monkey (9-month). In these studies, the compound was administered either by oral

gavage or via nasogastral intubation. The doses tested ranged from 0.01-1.2 mg/kg/day. The high dose (1.2 mg/kg/day) was not tolerated as observed by >15% decrease in the body weight within 3-weeks and the treatment with this dose was discontinued. One mortality was seen (1/4 females) at 0.4 mg/kg, the second highest dose tested. Sporadic incidences of the treatment related clinical signs were observed in animals with all doses. The major clinical signs include loose stool and emesis. Increase in monocyte and lymphocyte counts were noted at ≥ 0.2 mg/kg/day; these increases were within the historical control range. However, an increase in the lymphocyte infiltration in different tissues (trachea, thyroid etc) along with the chronic inflammation observed histopathologically in some tissues (heart) at the same dose range might implicate a treatment related effect of the compound on the lymphoid system. The changes in the monocytes/lymphocyte counts might be stress related. Increased fibrinogen level was observed in dose above ≥ 0.2 mg/kg/day suggesting underlying increased inflammatory processes, biological significance of the finding is not known. Megacolon a rear pathological finding in monkey was observed at 0.4 mg/kg /day (in 1/4 females), this finding might be related to the increased incidence of emesis and/or stress related effect in GIT and therefore might be considered as treatment related. A NOAEL of 0.2 mg/kg/day (HED=3.8 mg), exposure at this dose (by the end of the 9-month) as described by C_{max} and AUC were found to be 48 ng/mL and 869 ng•h/mL (MRHD=7.6x) respectively.

CP-526,555 tested in 6-month toxicity study in rat showed sporadic clinical signs of reduced and/or soft feces, chromodacryorrhea, and urine staining at all doses (3, 10, 30 mg/kg). A decrease in the body weight gain >10 % was observed at high dose which was correlated with a decrease in food consumption and considered as a treatment related effect. Changes in WBC counts were noted with all doses at Week -13 which was observed to be partially recovered at Week -26. Clinical pathological changes including increases in ALT and ALKP were noted at high dose; > 10% gain in liver weight in females was noted at the same dose suggesting treatment related functional changes in the liver. Histopathological findings were limited to jejunal epithelial vacuolation (5/15 males and 1/15 females), retinal dysplasia (1/15 males), and infiltration of foamy macrophages in the lung at high dose (6/15, 5/15 male, and females respectively compared to 2/15 in control animals). A NOAEL of 10 mg/kg/day (HED= 96 mg) by oral gavage is established for this 6-month rat study based on the decrease in body weight gain and hepatobiliary parameters. At NOAEL dose exposure as described by C_{max} and AUC were found to be 906 ng/mL and 14,000 ng•h/mL (MRHD=126x) respectively by the end of the 6-month toxicity study.

Single dose toxicity studies as well as subchronic toxicity studies in different non clinical species showed similar clinical signs as described above in the chronic toxicity studies in monkey and rat. These observations were not always dose related but consistent throughout the different doses that were tested and were found in all non clinical species studied. The test article showed low affinity for 5HT₃ receptor in the binding study and the emetic effect of the compound was found to be blocked in Ferrets by ondasterone (an antagonist of 5 HT₃ receptor) in the safety pharmacology studies. These results suggest that CP-526,555 might be acting as an agonist of the 5 HT₃ receptor. The emetic effect observed is considered as treatment related pharmacological effect of the compound and the effect is predicted to be manifested clinically. In addition, to the histopathological

observations mentioned above in the chronic toxicity studies dilatation of colons, jejunum, cecum, and ileum were noted at doses higher than the NOAEL dose in the single and repeat dose studies. The study results identify GI tract as the major target organ of toxicity for the compound. The safety pharmacology studies also confirmed the reduction in the geometric center of the GIT for the compound indicating inherent changes in the gastric mobility related to the pharmacology of the compound. In the distribution studies in rat the C¹⁴ labeled compound were noted in the GI tract within one hour after the administration of the test article. The compound was found to reside in the gastric content at least up to 18 hr. The exposure (AUC_{0-last} ng•h/mL) of the compound in GIT mucosa after single dose administration ranged between 633-1563, indicating a moderate exposure of the test article in this tissue. The results indicate that the chronic application of the test article in clinic may have compounded the effects at the GIT and might have pronounced pathological consequences in GIT, a dire treatment related effect on the digestive function might also be observed clinically. In the 6-month toxicity study in rat, phospholipidosis was observed in different tissues as indicated by vacuolation and foamy macrophage infiltration. The toxicological implication of the findings is not yet known. Interestingly, although the test article was found to be distributed in the melanin containing tissue in the skin and eye, only a few toxicological manifestations of these pharmacological effects of the test article were noted in any of the chronic toxicity studies. Rough hair coat was reported in rodents in the 2-years carcinogenicity study, however, the toxicological implication of the finding in that study is unclear. Retinal dysplasia was observed in 1/20 rats in the 6-month study at 30 mg/kg dose (HED=288 mg). At this dose a > 200-fold safety margin exist as compared to the clinical dose based on the body surface area.

Mutagenicity and Carcinogenicity: CP-526,555 was tested negative in Ames test, in vitro cytogenetics study human peripheral blood cells, mammalian cell gene mutation assay, and in vivo rat micronucleus test, suggesting it does not have mutagenic potential. 104-days carcinogenicity studies were completed in rat (1, 5, 15 mg/kg) and mice (1, 5, 20 mg/kg/day). In the 2-year carcinogenicity studies, drug-related neoplastic alterations were limited to hibernoma in male rats. 1/65 male rats showed benign hibernoma at 10 mg/kg dose; 2/65 male rats showed malignant hibernoma at 15 mg/kg dose. This finding is not statistically significant. The 'hibernoma' is a rare tumor finding in rodents. Therefore, it is considered treatment related and should be in the label. No apparent drug-related neoplastic findings were observed in female rats at doses up to 15 mg/kg (HED=145 mg, AUC=674, MRHD=6x) or in mice at doses up to 20 mg/kg (HED= 97.5 mg, AUC=1790 ng•h/mL, MRHD=15x).

Reproductive toxicity: Reproductive toxicology was assessed in both rats and rabbits following oral administration.

In the Segment I reproductive toxicity study in male rats with oral administration of 0, 0.3, 3, and 15 mg/kg a NOAEL of 3 mg/kg (HED=29 mg) was established for F₀ male ; serum levels at the NOAEL dose was approximately 228 ng/mL in males (MRHD=2x). In the Segment I reproductive toxicity study in female rats with oral administration of 0, 0.3, 3, and 15 mg/kg a NOAEL of 3 mg/kg was established for F₀ female; serum levels at the NOAEL dose was approximately 198 ng/mL in females(MRHD=1.8x). A NOAEL

of 15 mg/kg/day (HED= 290 mg) was established for the early embryonic toxicity for the test article in this study, serum level at the NOAEL dose was found to be approximately 581 and 500 ng/mL in males and females respectively (MRHD=9x).

In the Segment II reproductive toxicity studies in rat (0, 0.3, 5, 15 mg/kg) and rabbit (0, 1, 10, 30 mg/kg) NOAELs of 1 mg/kg (AUC= 279 ng•h/mL) and 3 mg/kg (AUC= 370 ng/mL) respectively were established for the maternal toxicity. No teratological effect was observed in rats; therefore, 15 mg/kg was established as the NOAEL for the teratology in rat. A significant decrease in the fetal weight was observed in the F₁ fetuses in rabbit at high dose, which might be related to the maternal body weight decrease. A NOAEL of 10 mg/kg (HED=193 mg, serum concentration in fetus = 399 ng/mL, MRHD=3.5x) was determined for rabbit teratology. The test article was found to cross the placental barrier, in fetus and dose dependent increase in the serum concentration of the test article was noted in the fetuses.

Segment III pre-/postnatal development toxicity was assessed in the female rats at doses of 0, 0.3, 3, and 15 mg/kg/day. The F₀ dams showed overall health deterioration. No reproductive toxicity was reported for the F₀ females. F₁ fetuses from the high dose group showed significant reduction in the body weight. Auditory startle response significantly decreased in F₁ fetus. Increase in latency time (unknown biological significance) in water maize test (for learning and memory) was noted in F₁ fetus. Fertility in F₁ fetus reduced (60-80%) significantly (P< 0.01) at high dose. A NOAEL of 3 mg/kg (HED=29 mg, MRHD=2x) was established in F₁ fetus based on physical behavior noted and fertility. The test article was quantifiable in the serum in fetuses at Day-6 (approximately 20 ng/mL). The result confirms the transport of the test article via milk.

Local toxicology: In the local toxicity studies, the compound was not found phototoxic in rat up to a dose of 100 mg/kg (HED=967 mg); no dermal toxicity was observed in rat up to 2000 mg/kg, and the compound was found negative in the skin sensitization study in the guinea pig up to a challenge dose of 500 mgs. These results indicate that the oral formulation of the compound do not cause irritation or phototoxic reaction in rat, predicting low clinical potential for dermal toxicity.

The intraocular administration of the test article showed mild ocular irritation at 45 mg (HED=4.8 mg). Conjunctivae (chemosis, redness, and discharge) and iridial irritation was noted in all of the three treated rabbits. The test article was also considered as a mild irritant in the dermal toxicity study in rabbit at 500 mg (HED=53 mg). The tissue distribution study in rats indicated drug deposition in skin and eye after single oral administration. Although, the level of the test article was found to be decreasing by Day 7 an acute effect indicated by irritation in skin and eye is considered to be treatment related, since the nicotine is also known to be deposited in the melanin containing tissue, this effect is considered pharmacologically related and predicted to manifest clinically only if dermal or ocular contact with the drug product occurs.

In the toxicology studies, different salt for ms namely [] tartrate, and succinate salts of the test article were tested. The pivotal repeat dose toxicity studies and the carcinogenicity studies were done with the tartrate salt which is the proposed commercial for. Succinate salts were used in the reproductive toxicity studies and mostly in the single dose toxicity studies and [] was used in the cytogenetics

studies. All of these salt forms of the test article were analyzed for systemic exposure and was found to have comparable Cmax and AUC in the different non clinical species. The active moiety in all of the salt forms were between [], and all the dose levels reported referred to as mg active moiety/kg body weight.

Impurity qualification:

In the toxicology studies, different salt forms namely [] tartrate, and succinate salts of CP-526,555 were tested. The pivotal repeat dose toxicity studies and the carcinogenicity studies were done with the tartrate salt which is the proposed commercial form. Succinate salts were used in the reproductive toxicity studies and mostly in the single dose toxicity studies and [] was used in the cytogenetics studies. All of these salt forms of the test article were analyzed for systemic exposure and was found to have comparable Cmax and AUC in the different non clinical species. The active moiety in all of the salt forms were between [], and all the dose levels reported referred to as ‘mg active moiety/kg’ body weight. The administered doses were adjusted for purity.

CP-526, 555- tartrate salt was developed [] for clinical use. [] tartaric acid was found to be the specified impurity that was found above the [] level on a consistent basis in the CP-526, 555- tartrate salt. This impurity [] are present. The range of values for [] found in the different batches of varenicline tartrate is tabulated below. The impurity concentration in batch #53650-5-5B was not determined during release. The concentration of the impurity was found to be [] after the date of manufacturing. This lot is tested in the pivotal toxicity testing (6- month toxicity study in rats and in part of the carcinogenicity studies in rat and mice). Therefore, CP-526,555 – tartrate salt which contains [] was qualified in toxicology upto six month with the specified impurity of [] In this toxicity study a NOAEL of 10 mg was established which contains the [] upto the limit of []. The Sponsor proposed the acceptance criterion for [] a tightening of the specification would depend on the Chemist’s discretion. The reproductive toxicity studies were done with the succinate salts; therefore [] is not qualified in any of the reproductive studies. In some of the non pivotal toxicity studies (like the single dose toxicity studies) with the tartrate salt of CP-526,555, [] was not tested at release but was found in the batches (– in batch# 50452-17-5MS) after [] This suggests that the formation of the impurity is []

Impurity Profile of the CP-526,555-tartrate salt:

Batch Number	Type of Pivotal Non-Clinical Study and Identification Number	Phase of Clinical Development and Identification Number	Impurities	Residual Solvents

27,506-110-1F	10-Day Monkey Toxicity Study/00054		T	
	3-Month Monkey Toxicity Study			
	3-Month Rat Toxicity Study/01-1545-23			
	Seg I Male Fertility/99-1545-21			
	Seg I Female Fertility/99-1545-20			
	Seg II Rat Teratology/99-1545-19			
	Seg II Rabbit/99-1545-18			
	Seg III Pre & Post natal Development in Rat/99-1545-30			
53650-5-5B*	6-Month Rat Toxicity Study/03536			
50-452-31-2B	9-Month Monkey Toxicity Study/00-1545-28	Cinical Phase 2B/ A1051007; A1051008; A1051009; A1051010; A1051012; A1051013; A1051014; A1051015; A1051016; A1051026;		
04944-002	9-Month Monkey Toxicity with 5-Week Recovery Study/04-1545-35	Clinical Phase 3 /A305104; A3051045;		
54-526-23-9B	2-Year Carcinogenicity Study in Rat/02-1545-33	Clinical Phase 2B/ A3051035; A3051043; A3051028; A3051036; A3051046;		
42-698-95-9B	2-Year Carcinogenicity Study in Mice/02-1545-32			

* This lot was also used in the rat and mouse carcinogenicity studies.

The individual unspecified impurities in the varenicline tartrate drug substance batches ranged from [] In the batch #53650-5-5B [] unspecified impurities were observed; []

The total impurities (sum of specified and unspecified impurities) for a TDI of 2 mg - the maximal dosage for varenicline tartrate is in compliance with the ICH Guideline Q3A and is found to be below [] at this stage of development.

[

demonstrated that the levels of their analogs in varenicline tartrate drug substance are within the threshold of toxicological concern of NMT [] µg/day which is in accordance with draft CDER Guidelines on the 'Genotoxic Impurities'. The levels of the impurities in the varenicline tartrate drug substance and the structure of the genotoxic impurities are provided in the Sponsor's tables and depicted below for the future reference (in case manufacturing process changes and thereby the control for the genotoxic impurities changes). The total genotoxic impurities under quality controlled current process are observed to be [] ppm and therefore deemed qualified.

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 § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

 § 552(b)(4) Draft Labeling

2.6.6.10 Tables and Figures

Repeat Dose Toxicity studies:

Type of Study	Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	GLP Compliance	Testing Facility
Repeat-Dose Toxicity (Nonpivotal)						
	Mouse/CD-1	Gavage	14 Days	0, 1, 10, 100	No	PGRD Groton, CT USA
	Rat/ Sprague-Dawley	Gavage	10 Days	0, 1, 10, 100	No	PGRD Groton, CT USA
Escalation/Toleration	Dog/Beagle	Gavage	1, 6, or 7 Days ^d	0.05, 0.1, 0.3, 1 ^d	No	PGRD Groton, CT USA
Escalation/Toleration	Monkey/ Cynomolgus	Gavage	1, 3, 5, or 7 Days ^e	0.1, 0.2 (0.1 BID), 0.3, 0.4 (0.2 BID), 1, 1 (0.5 BID) ^g	No	PGRD Groton, CT USA
	Monkey/ Cynomolgus	Gavage	10 Days	0, 0.25, 0.5 (0.25 BID), 1	No	PGRD Groton, CT USA
	Monkey/ Cynomolgus	Gavage	12 or 30 Days ^f	0.2→0.6 (0.1→0.3 BID) ^f , 0.4 (0.2 BID), 0.6 (0.3 BID), 1.2 (0.6 BID)	No	PGRD Groton, CT USA

^d In the escalation phase, single doses of 0.05, 0.1, 0.3, and 1 mg/kg were administered. In the toleration phase, dosing continued for 6 more days in the 0.1 and 0.3 mg/kg/day dose groups and for 5 more days in the 1 mg/kg/day dose group. These animals were dosed in the fasted state and an additional group received a single dose of 1 mg/kg in the fed state.

^e Animals were divided into 2 groups. Group 1 received a single dose of 0.1 mg/kg, then 1 mg/kg/day for 7 days followed by a 3-day washout period, and then single doses of 0.3 and 0.4 (0.2 BID) mg/kg with a 2-day washout period in between doses. Group 2 received 0.1 mg/kg/day for 5 days followed by a 1-day washout period, then 1 (0.5 BID) mg/kg/day for 3 days (dosing was terminated following the first dose on the third day of this dosing regimen) followed by a 1-day washout period, and then a single dose of 0.2 (0.1 BID) mg/kg.

^f Doses of 0.2 (0.1 BID) escalated to 0.6 (0.3 BID) on Day 8, 0.4 (0.2 BID), and 0.6 (0.3 BID) mg/kg/day were administered for 30 days followed by a 14-day recovery period, and doses of 1.2 (0.6 BID) mg/kg/day were administered for 12 days followed by a 30-day recovery period.

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Type of Study	Species/Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	GLP Compliance	Testing Facility
Repeat-Dose Toxicity (Pivotal)						
	Rat/ Sprague-Dawley	Gavage	6 Weeks	0, 0.3, <u>3</u> , 30	Yes	PGRD Groton, CT USA
	Rat/ Sprague-Dawley	Gavage	3 Months	0, 3, <u>10</u> , 30	Yes	PGRD Amboise, France
	Rat/ Sprague-Dawley	Gavage	6 Months	0, 3, <u>10</u> , 30	Yes	PGRD Ann Arbor, MI USA
	Monkey/ Cynomolgus	Gavage	6 Weeks	0, 0.01, 0.05, <u>0.2 (0.1 BID)</u>	Yes	PGRD Groton, CT USA
	Monkey/ Cynomolgus	Gavage	3 Months	0, 0.01, 0.05, <u>0.2 (0.1 BID)</u>	Yes	PGRD Groton, CT USA
	Monkey/ Cynomolgus	Nasogastric Intubation	9 Months	0, 0.01, 0.05, <u>0.2 (0.1 BID)</u>	Yes	L
	Monkey/ Cynomolgus	Gavage	9 Months ^E	0, 0.2 (0.1 BID), <u>0.4 (0.2 BID)</u> , 1.2 (0.6 BID)	Yes	PGRD Groton, CT USA

^E Following 9 months of treatment, a cohort of animals in the control and 0.4 (0.2 BID) mg/kg/day was placed in an approximate 5-week recovery period. A cohort of animals in the 1.2 mg/kg/day dose group was placed in a 29-day recovery period following 22/55 days (females/males) of dosing.

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Overview of Toxicokinetics

Daily Dose (mg/kg/day)	AUC0-24 (ng·h/mL)												
	Mouse		Mouse	Rat		Rat	Rabbit	Dog		Monkey		Monkey	
	M	F	M&F	M	F	M&F	F	M	F	M	F	M&F	
0.01	-	-	-	-	-	-	-	-	-	-	-	-	50.5 ^h 13.7 ^h <LLOQ ^g <LLOQ ^g
0.05	-	-	-	-	-	-	-	118.6 ^g	105.1 ^g	-	-	-	242.1 ^h 129 ^h 65.3 ^c
0.08	-	-	-	-	-	-	-	-	-	f	f	-	-
0.1	-	-	-	-	-	-	-	257.3 ^g	180.8 ^g	381.0 ^h	263.2 ^h	-	-
0.18	-	-	-	-	-	-	-	-	-	336.5 ^h	198.4 ^h	-	-
0.2 (0.1 BID)	-	-	-	-	-	-	-	-	-	331.9 ^h	281.9 ^h	-	-
0.2	-	-	-	-	-	-	-	-	-	328 ^g	431 ^g	-	-
0.3	-	-	-	-	-	-	-	-	-	f	f	-	1059.6 ^h 449 ^h 265 ^h 333 ^g 332 ^h 869 ^h

- = No data; LLOQ = Lower limit of quantitation (LLOQ = 1.00 ng/mL).
- ^a 6-Week Toxicity (Day 42; Study 98-1545-10).
- ^b 3-Month Toxicity (Day 78; Study 00-1545-22).
- ^c 9-Month Toxicity (Week 39; Study 00-1545-28).
- ^d Single-Dose Pharmacokinetic (Study 00-1545-27).
- ^e Escalation/Toleration (Day 1; Study 97-1545-05).
- ^f Intravenous Escalation (Study 745-03502); AUC not determined.
- ^g Escalation/Toleration (Day 7; Study 97-1545-05).
- ^h Escalation/Toleration (Day 5; Study 97-1545-07).
- ⁱ Single-Dose Pharmacokinetic Bridging (varenicline succinate; Study 98-1545-09).
- ^j Single-Dose Pharmacokinetic Bridging (varenicline ; Study 98-1545-09).
- ^k Intravenous Single-Dose Toxicity (Study 745-03516).
- ^l 1-Month Toxicity With 14-Day Recovery (Day 1; Study 04-1545-34).
- ^m 9-Month Toxicity With 5-Week Recovery (Day 266; Study 04-1545-35).

Daily Dose (mg/kg/day)	AUC0-24 (ng·h/mL)												
	Mouse		Mouse	Rat		Rat	Rabbit	Dog		Monkey		Monkey	
	M	F	M&F	M	F	M&F	F	M	F	M	F	M&F	
0.25	-	-	-	-	-	-	-	-	-	-	-	-	750 ^g
0.3	-	-	-	-	239 ^g	190 ^g	-	540.6 ^g	718.3 ^g	f	f	-	-
0.4 (0.2 BID)	-	-	-	-	-	-	-	-	-	-	-	-	1320 ^h 1440 ^h
0.5 (0.25 BID)	-	-	-	-	-	-	-	-	-	-	-	-	1460 ^h
0.6 (0.3 BID)	-	-	-	-	-	-	-	-	-	-	-	-	2590 ^h
1	45 ^g	41 ^g	-	953 ^g	1080 ^g	-	2.79 ^g	693.0 ^h 3504.3 ^h	564.1 ^h 2047.9 ^h	2680 ^h	881.4 ^h	-	2500 ^h
1.2 (0.6 BID)	-	-	-	-	-	-	-	-	-	-	-	-	2680 ^h 2550 ^h

- = No data.
- ^a Escalation/Toleration (Day 1; Study 97-1545-05).
- ^b Intravenous Escalation (Study 745-03502); AUC not determined.
- ^c Escalation/Toleration (Day 7; Study 97-1545-05).
- ^d 9-Month Toxicity With 5-Week Recovery (Day 266; Study 04-1545-35).
- ^e 10-Day Toxicity (Day 10; Study 00-1545-23).
- ^f Teratology (Gestation Day [GD] 17; Study 99-1545-19).
- ^g 6-Week Toxicity (Day 42; Study 98-1545-31).
- ^h 1-Month Toxicity With 14-Day Recovery (Day 30; Study 04-1545-34).
- ⁱ 14-Day Toxicity (Day 15; Study 01-1545-29).
- ^j 10-Day Toxicity (Day 10; Study 97-1545-08).
- ^k Teratology (GD 19; Study 99-1545-18).
- ^l Animals were in the fed-state.
- ^m Animals were in the fasted-state.
- ⁿ Escalation/Toleration (Day 3; Study 97-1545-07).
- ^o 1-Month Toxicity With 14-Day Recovery (Day 12; Study 04-1545-34).
- ^p 9-Month Toxicity With 5-Week Recovery (Day 14; Study 04-1545-35).

Daily Dose (mg/kg/day)	AUC0-24 (ng·h/mL)											
	Mouse			Rat		Rat	Rabbit	Dog		Monkey		Monkey
	M	F	M & F	M	F	M & F	F	M	F	M	F	M & F
3	-	-	1380 ^g	-	-	1730 ^g	-	-	-	670.1 ^h	477.0 ^h	-
						4135 ^g						
						3550 ^g						
						2330 ^g						
5	-	-	-	-	4397 ^g	-	-	-	-	-	-	-
10	5100 ^g	5820 ^g	-	11300 ^g	10500 ^g	14252 ^g	4400 ^g	-	-	-	-	-
						14000 ^g						
15	-	-	-	-	10200 ^g	-	-	-	-	-	-	-
25	-	-	12600 ^g	-	-	-	-	-	-	-	-	-
30	-	-	-	19200 ^g	17200 ^g	20454 ^g	9680 ^g	-	-	-	-	-
						289800 ^g						
						32680 ^g						
						15560 ^g						
75	-	-	33700 ^g	-	-	-	-	-	-	-	-	-

- = No data.

^g Teratology (Gestation Day (GD) 17; Study 99-1545-19).

^h 6-Week Toxicity (Day 42; Study 99-1545-11).

ⁱ 14-Day Toxicity (Day 15; Study 01-1545-29).

^j 10-Day Toxicity (Day 10; Study 97-1545-08).

^k Teratology (GD 19; Study 99-1545-18).

^l 3-Month Toxicity (Day 91; Study 01-1545-31).

^m 3-Month Toxicity (Day 82; Study 00854).

ⁿ 6-Month Toxicity (Day 182; Study 745-03536).

^o Single-Dose Pharmacokinetic (Study 00-1545-26).

^p Single-Dose Toxicity (Study 98-1545-14).

^q Single-Dose Toxicity (Study 97-1545-06).

Daily Dose (mg/kg/day)	AUC0-24 (ng·h/mL)											
	Mouse			Rat		Rat	Rabbit	Dog		Monkey		Monkey
	M	F	M & F	M	F	M & F	F	M	F	M	F	M & F
100	38200 ^r	47400 ^r	-	24100 ^r	31000 ^r	-	-	-	-	-	-	-
				72400 ^r	58500 ^r							
				65187 ^r	68884 ^r							
				41500 ^r								
150	-	-	58100 ^r	-	-	-	-	-	-	-	-	-
200	-	-	-	46100 ^r	85400 ^r	-	-	-	-	-	-	-
300	-	-	-	49200 ^r	120200 ^r	-	-	-	-	-	-	-

- = No data.

^r 14-Day Toxicity (Day 15; Study 01-1545-29).

^s 10-Day Toxicity (Day 10; Study 97-1545-08).

^t Single-Dose Toxicity (Study 97-1545-06).

^u In Vivo Micronucleus (Day 3; Study 98-1545-13).

^v Phototoxicity (Day 3; Study 04-1545-36).

^w 3-Month Toxicity (Day 1; 01-1545-31).

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Daily Dose (mg/kg/day)	C _{max} (ng/mL)												
	Mouse		Mouse	Rat		Rat	Rabbit	Dog		Monkey		Monkey	
	M	F	M & F	M	F	M & F	F	M	F	M	F	M & F	
0.01	-	-	-	-	-	-	-	-	-	-	-	4.1 ^h 2.06 ^h <LLOQ ^g 1.50 ^g	
0.05	-	-	-	-	-	-	-	11.8 ^h	12.2 ^h	-	-	22.5 ^h 11.5 ^h 5.59 ^g	
0.08	-	-	-	-	-	-	-	-	-	16.2 ^h	24.7 ^h	-	
0.1	-	-	-	-	-	-	-	20.8 ^h	25.4 ^h	32.7 ^h	24.3 ^h	-	
0.18	-	-	-	-	-	-	-	-	-	20.56 ^h	11.55 ^h	-	
0.2 (0.1 BID)	-	-	-	-	-	-	-	-	-	26.74 ^h	21.39 ^h	-	
0.2	-	-	-	-	-	-	-	-	-	27.6 ^h	35.8 ^h	-	
0.2	-	-	-	-	-	-	-	-	-	53.7 ^h	70.6 ^h	62.7 ^h 26.9 ^h 15.4 ^h 19.2 ^h 22.5 ^h 48.3 ^h	

- = No data; LLOQ = Lower limit of quantitation (LLOQ = 1.00 ng/mL).

^a 6-Week Toxicity (Day 42; Study 98-1545-10).

^b 3-Month Toxicity (Day 78; Study 00-1545-22).

^c 9-Month Toxicity (Week 39; Study 00-1545-28).

^d Single-Dose Pharmacokinetic (Study 00-1545-27).

^e Escalation/Tolerant (Day 1; Study 97-1545-05).

^f Intravenous Escalation (Days 1 and 2 for 0.08 and 0.1 mg/kg, respectively; Study 745-03502).

^g Escalation/Tolerant (Day 7; Study 97-1545-05).

^h Escalation/Tolerant (Day 5; Study 97-1545-07).

ⁱ Single-Dose Pharmacokinetic Bridging (varenicline succinate; Study 98-1545-09).

^j Single-Dose Pharmacokinetic Bridging (varenicline succinate; Study 98-1545-09).

^k Intravenous Single-Dose Toxicity (Study 745-03516). ^l 1-Month Toxicity With 14-Day Recovery (Day 1; Study 04-1545-34).

^m 1-Month Toxicity With 14-Day Recovery (Day 1; Study 04-1545-34).

ⁿ 9-Month Toxicity With 5-Week Recovery (Day 266; Study 04-1545-35).

Daily Dose (mg/kg/day)	C _{max} (ng/mL)												
	Mouse		Mouse	Rat		Rat	Rabbit	Dog		Monkey		Monkey	
	M	F	M & F	M	F	M & F	F	M	F	M	F	M & F	
0.25	-	-	-	-	-	-	-	-	-	-	-	43.8 ^h	
0.3	-	-	-	-	33.1 ^o	68 ^h	-	51.3 ^h	109.6 ^h	86.9 ^h	90.3 ^h	-	
0.4 (0.2 BID)	-	-	-	-	-	-	-	-	-	-	-	71.1 ^h	
0.5 (0.25 BID)	-	-	-	-	-	-	-	-	-	-	-	79.5 ^h	
0.6 (0.3 BID)	-	-	-	-	-	-	-	-	-	-	-	75.8 ^h	
1	160 ^g	122 ^g	-	179 ^g	204 ^g	-	58.5 ^h	63.3 ^h ^h	82.3 ^h ^h	223.0 ^h	63.0 ^h	146 ^h	
1.2 (0.6 BID)	-	-	-	-	-	-	-	381.3 ^h ^h	247.9 ^h ^h	-	-	128 ^h 154 ^h	

- = No data.

^a Escalation/Tolerant (Day 1; Study 97-1545-05).

^b Intravenous Escalation (Day 3; Study 745-03502).

^c Escalation/Tolerant (Day 7; Study 97-1545-05).

^d 9-Month Toxicity With 5-Week Recovery (Day 266; Study 04-1545-35).

^e 10-Day Toxicity (Day 10; Study 00-1545-23).

^f Teratology (Gestational Day [GD] 17; Study 99-1545-19).

^g 6-Week Toxicity (Day 42; Study 98-1545-11).

^h 1-Month Toxicity With 14-Day Recovery (Day 30; Study 04-1545-34).

ⁱ 14-Day Toxicity (Day 15; Study 01-1545-29).

^j 10-Day Toxicity (Day 10; Study 97-1545-08).

^k Teratology (GD 19; Study 99-1545-18).

^l Animals were in the fed-state.

^m Animals were in the fasted-state.

ⁿ Escalation/Tolerant (Day 8; Study 97-1545-07).

^o 1-Month Toxicity With 14-Day Recovery (Day 12; Study 04-1545-34).

^p 9-Month Toxicity With 5-Week Recovery (Day 14; Study 04-1545-35).

Daily Dose (mg/kg/day)	Cmax (ng/mL)											
	Mouse		Mouse	Rat		Rat	Rabbit	Dog		Monkey		Monkey
	M	F	M & F	M	F	M & F	F	M	F	M	F	M & F
100	2550 ^f	3860 ^f	-	1400 ^g	1800 ^g	-	-	-	-	-	-	-
				6050 ^g	5100 ^g							
				5569 ^g	6169 ^g							
				2110 ^g								
150	-	-	6400 ^h	-	-	-	-	-	-	-	-	-
200	-	-	-	2600 ^h	5500 ^h	-	-	-	-	-	-	-
300	-	-	-	3000 ^h	8600 ^h	-	-	-	-	-	-	-

- = No data.
^f 14-Day Toxicity (Day 15; Study 01-1545-29).
^g 10-Day Toxicity (Day 10; Study 97-1545-08).
^h Single-Dose Toxicity (Study 97-1545-06).
ⁱ In Vivo Micronucleus (Day 3; Study 98-1545-13).
^j Phototoxicity (Day 3; Study 04-1545-36).
^k 3-Month Toxicity (Day 1; 01-1545-31).

Daily Dose (mg/kg/day)	Cmax (ng/mL)											
	Mouse		Mouse	Rat		Rat	Rabbit	Dog		Monkey		Monkey
	M	F	M & F	M	F	M & F	F	M	F	M	F	M & F
3	-	-	358 ^l	-	-	334 ^l	-	-	-	-	54.4 ^m	50.6 ^m
						304 ^l						
						289 ^l						
						362 ^l						
5	-	-	-	-	393 ^o	-	-	-	-	-	-	-
10	407 ^o	720 ^o	-	961 ^o	743 ^o	908 ^o	372 ^o	-	-	-	-	-
						906 ^o						
15	-	-	-	-	671 ^o	-	-	-	-	-	-	-
25	-	-	883 ^o	-	-	-	-	-	-	-	-	-
30	-	-	-	1200 ^o	1100 ^o	1380 ^o	1230 ^o	-	-	-	-	-
						1760 ^o						
						1870 ^o						
						767 ^o						
75	-	-	2570 ^o	-	-	-	-	-	-	-	-	-

- = No data.
^l Teratology (Gestation Day [GD] 17; Study 99-1545-19).
^m 6-Week Toxicity (Day 42; Study 98-1545-11).
ⁿ 14-Day Toxicity (Day 15; Study 01-1545-29).
^o 10-Day Toxicity (Day 10; Study 97-1545-08).
^p Teratology (GD 19; Study 99-1545-18).
^q 3-Month Toxicity (Day 91; Study 01-1545-31).
^r 3-Month Toxicity (Day 82; Study 00034).
^s 6-Month Toxicity (Day 182; Study 745-03536).
^t Single-Dose Pharmacokinetic (Study 00-1545-26).
^u Single-Dose Toxicity (Study 98-1545-14).
^v Single-Dose Toxicity (Study 97-1545-06).

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Daily Dose (mg/kg/day)	Serum Drug Concentrations (ng/mL)								
	Mouse		Rat		Rat Pup		Rat Fetal-Pooled	Rabbit	Rabbit Fetal-Pooled
	M&F	M	F	M & F	M	F	M & F	F	M & F
0.3	-	23.7 ^h	20.9 ^h /40.6 ^h 26.7 ^h	-	3.73 ^h	3.08 ^h	57.7 ^h	-	-
1	280 ^h	-	-	301 ^h	-	-	-	39.4 ^h	59.8 ^h
3	-	228 ^h	198 ^h /420 ^h 234 ^h	-	22.2 ^h	16.7 ^h	-	-	-
5	980 ^h	-	-	389 ^h	-	-	610 ^h	-	-
10	-	-	-	-	-	-	-	300 ^h	390 ^h
15	-	581 ^h	500 ^h /734 ^h 513 ^h	673 ^h	116 ^h	114 ^h	1041 ^h	-	-
20	1790 ^h	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	1430 ^h	1390 ^h

^h Male Reproduction and Fertility (~ 2 hours postdose on Day 22; Study 99-1545-21).
^j Female Fertility and Early Embryonic Development (~ 2 hours postdose on Day 8; Study 99-1545-20).
^k Teratology (~ 2 hours postdose on GD 20; Study 99-1545-19).
^l Pre- and Postnatal Development (~ 2 hours postdose on Postnatal Day 10; Study 01-1545-30).
^m 2-Year Carcinogenicity (Week 105; Study 02-1545-32).
ⁿ 2-Year Carcinogenicity (Week 104; Study 02-1545-33).
^o Teratology (~ 2 hours postdose on GD 21; Study 99-1545-18).

Single Dose Toxicity

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Rat Sprague-Dawley	Oral Gavage (Deionized Water)	0, 30, 100, 200, 300	3M, 3F	300	>300	≥200 mg/kg: Labored/ noisy respiration, ↓ activity, uncoordinated/unsteady gait, splayed hindlimbs, tremors, ptosis, loose stool, hunched posture, rough haircoat; ↓ mean body weight; ↓ mean white blood cell and lymphocyte count; ↑ mean serum glucose	97-1545-06
Toxicokinetic parameters (M/F):							
Dose (mg/kg)		30		100	200	300	
C _{max} (ng/mL)		1200/1100		1400/1800	2600/5500	3000/8500	
AUC(0-24)(ng·h/mL)		19200/17200		24100/31000	46100/85400	49200/120200	
Lot Number 38713-174-19. M = Male, F = Female.							

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Rat Sprague-Dawley	Oral Gavage (Deionized Water)	3, 30	6M, 6F	NA	NA	Exposure of CP-326,555 using the tartrate salt form was similar to that observed in the 3-month rat study, which used the succinate salt form (Study 00054).	00-1545-26
Toxicokinetic parameters (M and F combined):							
Dose (mg/kg)		3		30			
C _{max} (ng/mL)		363		767			
AUC(0-24)(ng·h/mL)		2330		15500			
Lot Number 50452-17-SMS. M = Male, F = Female, NA = Not applicable.							

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Monkey/ Cynomolgus	Oral Gavage (Deionized Water)	0, 3	2M, 2F	3	>1	3 mg/kg: Enesia, recumbency, ↓ activity, tremors, ↓ food intake, ↓ heart rate, ↑ PRQ interval and P-wave width, ↓ QT interval. All treatment-related findings were reversible.	98-1543-14
Toxicokinetic parameters (M/F):							
Dose (mg/kg)			3				
Cmax (ng/mL)			34.4/50.6				
AUC(0-24)(ng·h/mL)			670.1/477.6				
Lot Number 37560-56-4H M = Male; F = Female.							

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study Number
Monkey/ Cynomolgus	Intravenous 4-hour infusion (Aqueous Phosphate Buffer in NaCl)	0, 0.18 (0.045 mg/kg/h)	4M, 4F*	0.18	>0.18	0.18 mg/kg: ↓ Food consumption; ↑ creatine kinase, alanine aminotransferase, and aspartate aminotransferase	745-03516
Toxicokinetic parameters (M/F):							
Dose (mg/kg)			0.18				
Cmax (ng/mL)			37.6/35.8				
AUC(0-24)(ng·h/mL)			328/431				
Lot Number 50452-31-2B. M = Male; F = Female. * Two animals/group were euthanized ~ 24 hours after the initiation of the drug or vehicle infusion. The remaining animals were euthanized 2 weeks after infusion (Day 15).							

Repeat Dose Toxicity

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg/day)*	Duration of Dosing	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Mouse/ CD-1	Oral Gavage (Deionized Water)	0, 1, 10, 100	14 Days	10M, 10F	ND	10 mg/kg: 1/20 Died 100 mg/kg: 2/10 Died; ↓ activity, tremors, sternal recumbency, stasia, ptosis, labored respiration; transient ↓ body weight and food consumption; ↓ potassium, red blood cell count, hemoglobin, and hematocrit, ↑ mean corpuscular volume, mean corpuscular hemoglobin, and reticulocyte count; stressed-related ↓ white blood cell and lymphocyte count	01-3545-29
Toxicokinetic parameters (M/F):							
Dose (mg/kg/day)			1		10		100
Day 1							
Cmax (ng/mL)			98.5/97.4		479/463		1400/7150
AUC(0-24)(ng·h/mL)			394/437		3410/3370		22300/89700
Day 15							
Cmax (ng/mL)			160/122		407/726		2550/3860
AUC(0-24)(ng·h/mL)			457/417		5100/5820		38000/47400
Lot Number 50453-17-5MS. NOAEL = No-observed adverse effect level; M = Male, F = Female; ND = Not defined * All dose levels are expressed as mg of active moiety per kg of body weight per day.							

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg/day)*	Duration of Dosing	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Rat/Sprague-Dawley	Oral Gavage (Deionized Water)	0, 1, 10, 100 ^a	10 Days	5M, 5F	ND	10 mg/kg: Transient ↓ food consumption 100 mg/kg: 1/10 Died; salivation, loose stool, distended abdomen, ↓ activity, tremors, rough fur, hair loss, penile erection, urogenital staining, hunched posture, dehydration, labored respiration, cold to touch; ↓ body weight and food consumption; ↑ ALT, AST, GGT, TB, BUN, TP, albumin, globulin, cholesterol, potassium, calcium, RBC, hemoglobin, hematocrit, hypersegmented neutrophils; ↓ WBC, lymphocytes, reticulocytes; ↓ relative liver, heart, and kidney weight; gastric dilation, cecal enlargement; hepatocellular necrosis, splenic lymphoid depletion; stressed-related thymic lymphoid depletion and testicular degeneration in the dead male	97-1545-08

Toxicokinetic parameters (M/F):

Dose (mg/kg/day)	1	10	100
Day 1			
C _{max} (ng/mL)	363/389	838/728	1160/1510
AUC(0-24)(ng-h/mL)	2240/2180	8100/8130	24300/27900
Day 10			
C _{max} (ng/mL)	179/204	961/743	6050/5100
AUC(0-24)(ng-h/mL)	953/1080	11300/10500	72400/58500

Lot Number 27580-49-3.

NOAEL = No-observed adverse effect level; M = Male, F = Female; ND = Not defined; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; GGT = γ-Glutamyl transferase; TB = Total bilirubin; BUN = Blood urea nitrogen; TP = Total protein; RBC = Red blood cell; WBC = White blood cell.

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg/day)*	Duration of Dosing	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Dog/Beagle	Oral Gavage (Deionized Water)	0.05, 0.1, 0.3, 1 ^b	1, 6, or 7 Days ^c	1M, 1F	ND	≥0.05 mg/kg: Emesis ≥0.1 mg/kg: Tremors, ↓ activity, loose stool, salivation ≥0.3 mg/kg: ↑ alanine aminotransferase 1 mg/kg: Unsteady gait, prolapsed nictitating membrane, dehydration; ↓ body weight and food consumption; ↑ neutrophil and monocyte count	97-1545-05

Toxicokinetic parameters (M/F):

Dose (mg/kg/day)	0.05	0.1	0.3	1 (Repeat-dose, fasted) ^d	1 (Single-dose, fed)
Day 1					
C _{max} (ng/mL)	11.8/12.2	32.4/0.6	52.1/73.8	381.3/247.9	63.3/82.3
AUC(0-24)(ng-h/mL)	118.6/105.1	295.2/3.1	661.4/740.3	3504.3/2047.9	693.0/564.1
Day 2					
C _{max} (ng/mL)	NA	NA	NA	73.6/123.4	NA
AUC(0-24)(ng-h/mL)	NA	NA	NA	759.8/605.1	NA
Day 7					
C _{max} (ng/mL)	NA	20.8/25.4	51.3/109.6	NA	NA
AUC(0-24)(ng-h/mL)	NA	257.3/130.8	540.6/718.3	NA	NA

Lot Number 38712-174-19.

NOAEL = No-observed adverse effect level; M = Male, F = Female; ND = Not defined; NA = Not applicable.

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

^b In the escalation phase, single doses of 0.05, 0.1, 0.3, and 1 mg/kg were administered. In the toleration phase, dosing continued for 6 more days in the 0.1 and 0.3 mg/kg/day dose groups; and for 5 more days in the 1 mg/kg/day dose group; these animals were dosed in the fasted-state, and the dose volume was 1 mL/kg except for the 1 mg/kg/day dose group on Day 2, which was 5 mL/kg. An additional dose group received a single dose of 1 mg/kg (dose volume 1 mL/kg) in the fed-state.

^c Dose volume was 5 mL/kg on Day 3 only.

Species/Strain	Method of Administration (Vehicle/ Formulation)	Doses (mg/kg/day) ^a	Duration of Dosing	Gender and No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Study Number
Monkey Cynomolgus	Oral Gavage (Deionized Water)	0.1, 0.3 (0.1 BID), 0.3, 0.4 (0.2 BID), 1, 1 (0.5 BID) ^a	1, 3, 5, or 7 Days ^d	1M, 1F ^e	ND	≥0.3 mg/kg: Emesis 1 mg/kg: Loose stool, ↓ food intake	97-1545-07
Toxicokinetic parameters (M/F):							
Dose (mg/kg/day)			0.1 (Group 2) ^d	0.1 (Group 1) ^e	1 (Group 1) ^f		
Day 1							
C _{max} (ng/mL)			19.4/20.3	NC	NA		
AUC(0-24)(ng·h/mL)			209.3/193.2	NC	NA		
Day 2							
C _{max} (ng/mL)			NA	NA	51.0/31.5		
AUC(0-24)(ng·h/mL)			NA	NA	675.1/323.2		
Day 3							
C _{max} (ng/mL)			32.7/24.3	NA	NA		
AUC(0-24)(ng·h/mL)			381.0/263.1	NA	NA		
Day 8							
C _{max} (ng/mL)			NA	NA	223.0/63.0		
AUC(0-24)(ng·h/mL)			NA	NA	2880/381.4		

Lot Number 38712-174-19.

NOAEL = No-observed adverse effect level; M = Male, F = Female; ND = Not defined; NC = Not calculated; NA = Not applicable.

^aAll dose levels are expressed as mg of active moiety per kg of body weight per day.

^dFour animals were divided into 2 groups of 1/sex. Group 1 received a single dose of 0.1 mg/kg, then 1 mg/kg/day for 7 consecutive days followed by a 3-day washout period, and then single doses of 0.3 and 0.4 (0.2 BID) mg/kg with a 2-day washout period between doses. Group 2 received 0.1 mg/kg/day for 5 consecutive days followed by a 1-day washout period, then 1 (0.5 BID) mg/kg/day for 3 consecutive days (dosing was terminated following the first dose on the third day of this dosing regimen) followed by a 1-day washout period, and then a single dose of 0.2 (0.1 BID) mg/kg.

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Report Title: 6-Week Oral Toxicity Study in Sprague-Dawley Rats

Species/Strain: Rat/Sprague-Dawley Study Number: 98-1545-11
 Initial Age: ~ 7 Weeks
 Date of First Dose: 06 May 1998 Lot Number: 27360-56-4H
 Duration of Dosing: 6 Weeks (42 or 43 Days) Location in CTD: Module 4, Section 4.2.3.3
 Duration of Postdose: Not Conducted GLP Compliance: Yes
 Method of Administration: Oral Gavage (QD, 1 mL/kg)
 Vehicle/Formulation: Deionized Water
 Special Features:
 - Plasma drug concentrations were determined from a satellite group of animals (6/sex/dose group) at ~ 1, 4, 8, and 24 hours postdose on Days 1 and 42.
 - Body temperature was measured from the first 5 animals/sex/dose group once pretreatment and then on Days 1, 15, and 38 pre-dose and at ~ 1 hour postdose.
 - Hepatic microsomal enzyme activities were measured with liver samples collected from control and 30 mg/kg/day animals (4/sex) at termination.
 No Observed Adverse Effect Level: 3 mg/kg/day

Dose* (mg/kg/day)	0 (Control)		0.3		3		30	
	M	F	M	F	M	F	M	F
Sex (M/F)								
Number of Animals								
Toxicity	10	10	10	10	10	10	10	10
Toxicokinetics	6	6	6	6	6	6	6	6
Toxicokinetics: C _{max} (ng/mL)	Males and Females Combined		Males and Females Combined		Males and Females Combined		Males and Females Combined	
Day 1	ND		58		302		854	
Day 42	ND		68		334		1386	
Toxicokinetics: AUC(0-24) (ng·h/mL)	Males and Females Combined		Males and Females Combined		Males and Females Combined		Males and Females Combined	
Day 1	ND		194		1334		16671	
Day 42	ND		190		1730		20434	
Noteworthy Findings								
Died or Sacrificed Moribund	0	1 [§]	0	1 [§]	0	0	1 [§]	0
Body Weight (Day 40) [†]	413.15	255.73	1.0	0.98	1.0	0.95	0.86†	0.86†
Food Consumption (Day 40) [†]	26.73	18.73	1.0	1.0	1.0	1.0	0.80†	0.88*
Clinical Observations								
Salivation	0	0	1	0	5	5	10	10
Hair loss	2	0	1	0	0	1	1	4
Ophthalmology								
Body temperature	-	-	-	-	-	-	-	-
Hematology (Day 17)								
Hemoglobin (g/dL)	15.14	15.54	15.23	15.61	15.45	13.36	15.85	16.16†
Hematocrit (%)	46.28	46.58	46.56	47.00	47.49	47.55	48.57	49.87†
Mean Cell Volume (fL)	39.1	39.7	40.5	39.2	40.6	40.5†	39.6	40.5†
Mean Cell Hemoglobin Concentration (%)	32.73	33.38	32.71	33.22	32.55	32.28†	32.68	32.37†

Dunnett's Test: * p < 0.05, † p < 0.01; Modified t-test: ‡ p < 0.05, § p < 0.01.

M = Male; F = Female; ND = Not determined; - = No noteworthy findings.

* All dose levels are expressed as mg of active moiety per kg of body weight per day.

† Deaths were not considered treatment-related, and were caused by the following: The control female died spontaneously following body temperature collection; the 0.3 mg/kg/day female died due to blood sampling procedures; and the 30 mg/kg/day male died due to a urinary tract infection.

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Report Title: 3-Month Oral Toxicity Study in Sprague-Dawley Rats

Species/Strain: Rat/Sprague-Dawley
 Initial Age: ~7 Weeks
 Date of First Dose: 15 June 2000
 Duration of Dosing: 3 Months (91 or 93 Days)
 Duration of Postdose: Not Conducted
 Method of Administration: Oral Gavage (QD,
 1 mL/kg)
 Vehicle/Formulation: Deionized Water
 Special Features: Serum drug concentrations were determined from a satellite group of animals (6/sex/dose group) at ~1, 4, 8, and 24 hours postdose on Days 1, 47, and 82.
 No Observed Adverse Effect Level: 10 mg/kg/day

Study Number: 00054
 Lot Number: 27506-110-1F
 Location in CTD: Module 4,
 Section 4.2.3.2
 GLP Compliance: Yes

Dose* (mg/kg/day)	0 (Control)		3		10		30	
	M	F	M	F	M	F	M	F
Sex (M/F)								
Number of Animals								
Toxicity	15	15	15	15	15	15	15	15
Toxicokinetics	6	6	6	6	6	6	6	6
Toxicokinetics: Cmax (ng/mL)	Males and Females Combined		Males and Females Combined		Males and Females Combined		Males and Females Combined	
	Day 1		286		551		751	
	Day 82		304		906		1766	
Toxicokinetics: AUC(0-24) (ng•h/mL)	Males and Females Combined		Males and Females Combined		Males and Females Combined		Males and Females Combined	
	Day 1		3040		9179		14526	
	Day 82		4135		14252		28980	
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	1 ^a	1 ^b	0	0	0
Body Weight (Day 91) ^c	557.23	313.35	1.0	1.0	0.95	0.99	0.72†	0.92*
Food Consumption ^c								
Day 8	28.51	20.96	1.0	1.0	0.97	0.93	0.32†	0.49†
Day 91	27.41	20.20	1.1	1.0	1.0	0.97	0.92	1.0
Water Consumption (Day 6) ^c	32.71	28.13	1.1	0.89	1.1	0.89	0.36§	0.70†
Clinical Observations								
Salivation	0	0	0	0	8	7	8	8
Ophthalmology	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Plasma Chemistry								
Day 43 or 44 ^d								
Alanine Aminotransferase (IU/L)	25.1	20.3	27.5	27.9	29.3†	25.9	31.9§	42.7§
Total Bilirubin (mg/dL)	0.134	0.156	0.137	0.170	0.143	0.168	0.201§	0.235§
Alkaline Phosphatase (IU/L)	376.3	157.7	289.1	162.5	290.7	160.1	339.3†	301.4§

Dunnett's Test: * p < 0.05, † p < 0.01; Modified t-test: ‡ p < 0.05; § p < 0.001.

M = Male; F = Female; ND = Not determined; - = No noteworthy findings.

* All dose levels are expressed as mg of active moiety per kg of body weight per day.

^a The 3 mg/kg/day female was euthanized as moribund due to a gavage accident. The cause of death for the 10 mg/kg/day male could not be determined; however, due to the isolated incidence and absence of mortality at 10 mg/kg/day, the death was not considered treatment-related.

^c Group means in grams are shown for controls. Fold differences from controls are shown for treated groups. Statistical significance is based on actual data and not on the fold differences.

^d The first 6/sex/dose group were measured on Days 43 and 92, and the second 7/sex/dose group were measured on Days 44 and 93.

Report Title: 6-Month Oral Toxicity Study of CP-526,555-18 in Rats

Species/Strain: Rat/Sprague-Dawley
 Initial Age: ~ 7-8 Weeks
 Date of First Dose: 17 Jul 2001
 Duration of Dosing: 6 Months (182 Days)
 Duration of Postdose: None Conducted
 Method of Administration: Oral Gavage (QD, 10 mL/kg)
 Vehicle/Formulation: Deionized Water
 Special Features: Serum drug concentrations were determined from a satellite group of animals (6/sex/dose group) at ~ 1, 4, 8, and 24 hours postdose on Days 1, 43, and 182.
 No Observed Adverse Effect Level: 10 mg/kg/day

Study Number: 745-03536
 Lot Number: 53650-5-5B
 Location in CTD: Module 4, Section 4.2.3.2
 GLP Compliance: Yes

Dose* (mg/kg/day)	0 (Control)		3		10		30	
	M	F	M	F	M	F	M	F
Sex (M/F)								
Number of Animals	15	15	15	15	15	15	15	15
Toxicity	0	0	6	6	6	6	6	6
Toxicokinetics								
Toxicokinetics: C _{max} (ng/mL)	Males and Females Combined		Males and Females Combined		Males and Females Combined		Males and Females Combined	
Day 1	ND		229		435		755	
Day 42	ND		274		635		1430	
Day 182	ND		289		906		1370	
Toxicokinetics: AUC(0-24) (ng·h/mL)	Males and Females Combined		Males and Females Combined		Males and Females Combined		Males and Females Combined	
Day 1	ND		1870		6500		15900	
Day 42	ND		2410		8700		22000	
Day 182	ND		3550		14600		32600	
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	1 ^b	0	0	0	0	0
Body Weight (Day 182) ^c	645.5	324.1	1.0	0.96	0.92	1.0	0.74*	0.86*
Food Consumption ^d								
Day 3	157.5	111.5	0.99	0.97	0.92	0.89	0.60*	0.53*
Day 182	157.0	99.1	1.0	1.0	0.97	1.0	0.56*	1.0
Clinical Observations								
Reduced Feces	0	1	1	4	2	11	15	15
Soft Feces	0	1	0	0	4	2	15	15
Chromocopyrinitis	2	1	1	0	1	0	0	6
Red Staining (Nuzzle/Head)	1	0	2	2	6	1	15	14
Urine Staining	0	0	1	0	0	0	1	13
Allopecia	1	1	3	1	2	2	2	1
Ophthalmology	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-

Sequential trend test within one-factor analysis of variance: *p < 0.01.

M = Male; F = Female; ND = Not determined; - = No noteworthy findings.

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

^b The 3 mg/kg male was euthanized as moribund due to a gavage error.

^c Group means in grams are shown for controls. Fold differences from controls are shown for treated groups. Statistical significance is based on actual data and not on the fold differences.

^d Food consumption is presented as weekly data.

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Sex (M/F)	Dose ^a (mg/kg/day)		0 (Control)		3		10		30		
	M	F	M	F	M	F	M	F	M	F	
Serum Chemistry											
Day 91:											
	109.1	110.0	123.1	109.7	104.5	99.7	85.4*	92.0*			
Glucose (mg/dL)	37.8	37.3	64.9	32.8	56.3	38.3	24.5*	31.5			
Triglyceride (mg/dL)	5.79	6.23	6.07	6.40	6.17	7.63*	6.44*	7.51*			
Phosphorus (mg/dL)	45.6	42.8	49.0	40.3	55.7*	41.8	71.3*	57.6*			
Alanine Aminotransferase (U/L)	114.7	75.0	120.1	72.0	145.9*	73.3	152.1*	101.4*			
Alkaline Phosphatase (U/L)	0.10	0.12	0.11	0.13	0.11	0.14	0.13*	0.13			
Total Bilirubin (mg/dL)	Day 181:										
	194.0	152.7	179.9	152.5	166.6*	164.8	149.1*	135.7			
Glucose (mg/dL)	68.4	52.9	73.4	58.5	72.2	45.9	39.1*	67.4			
Triglyceride (mg/dL)	7.13	6.23	7.28	6.40	7.94*	7.63*	8.17*	7.51*			
Phosphorus (mg/dL)	61.0	45.3	47.4	41.8	61.3	33.7	70.7	48.1			
Alanine Aminotransferase (U/L)	109.7	54.1	95.6	49.1	98.6	53.5	121.4*	75.2*			
Alkaline Phosphatase (U/L)	0.22	0.33	0.26	0.35	0.26	0.32	0.31*	0.35			
Total Bilirubin (mg/dL)	Urinalysis										
	-	-	-	-	-	-	-	-	-	-	
Absolute Organ Weights ^{a,b}											
Brain	2.251	2.073	1.0	0.97	0.99	0.99	0.95*	0.97			
Kidney	4.090	2.256	1.1	0.95	0.97	1.0	0.83*	0.96			
Heart	1.780	1.690	1.1	1.0	1.0	1.1	0.87*	1.0			
Liver	16.142	7.727	1.1	1.0	0.97	1.1*	0.84*	1.1*			
Spleen	0.933	0.557	1.0	0.99	0.98	1.0	0.91	1.1			
Thymus	0.396	0.282	1.1	1.1	0.84	1.0	0.70*	0.84			
Relative Organ Weights ^{a,b}											
Brain	0.366	0.673	0.98	1.0	1.1	0.99	1.3*	1.2*			
Kidney	0.650	0.730	1.0	1.0	1.1	1.0	1.1*	1.2*			
Heart	0.289	0.353	1.0	1.0	1.1	1.1	1.2*	1.2*			
Liver	2.591	2.497	1.0	1.1*	1.1	1.1*	1.2*	1.4*			
Testes	0.594	NA	0.98	NA	1.1	NA	1.3*	NA			
Spleen	0.130	0.180	1.0	1.0	1.1	1.0	1.2*	1.4*			
Gross Pathology											
Number Examined	15	15	15	15	15	15	15	15			
Histopathology											
Number Examined	15	15	15	15	15	15	15	15			

Sequential trend test within one-factor analysis of variance: * p < 0.01.

M = Male; F = Female; - = No noteworthy findings; NA = Not applicable.

^a All dose levels are expressed as mg of active moiety per kg of body weight per day.

^b Group means in grams are shown for controls. Fold differences from controls are shown for treated groups. Statistical significance is based on actual data and not on the fold differences.

^c Group means (%) are shown for controls and are calculated as follows: absolute organ weight x 100 / terminal body weight. Fold differences from controls are shown for treated groups. Statistical significance is based on actual data and not on the fold differences.

^d Organ weight changes were due to decreased mean terminal body weight. The exception included increased absolute liver weight in the 20 mg/kg females, and was not considered biologically significant due to the small magnitude and lack of histopathologic correlates.

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Report Title: 9-Month Oral Toxicity Study With 5-Week Recovery Phase in Monkeys

Species/Strain: Monkey/Cynomolgus Study Number: 04-1545-35
 Initial Age: ~ 2-4 Years Lot Number: 04944602
 Date of First Dose: 27 Jul 2004 Batch Number: 3000054
 Duration of Dosing: 9 Months (274 Days) Location in CTD: Module 4, Section 4.2.3.3
 Duration of Postdose: 5-Week Recovery GLP Compliance: Yes
 Method of Administration: Oral Gavage
 Vehicle/Formulation: Deionized Water

Special Features:

- Based on the condition/response of the 0.4 (0.2 BID) mg/kg/day dose group in which minimal effects were observed, an additional group of monkeys received 1.2 (0.6 BID) mg/kg/day for up to 23/55 days (females/males); these animals initially received deionized water for 64 days until the dose was established.
- Due to treatment-related findings in the 1.2 (0.6 BID) mg/kg/day dose group, dose administration was terminated for this group. Surviving animals were either necropsied (in conjunction with a control of the same sex) or placed in a 29-day recovery period, and then necropsied at the end of recovery.
- At the end of treatment, 2 animals/sex in the 0.4 (0.2 BID) mg/kg/day dose group and 1 control/sex were placed on recovery for 36/37 (females/males) days, and then necropsied at the end of recovery. The 0.2 (0.1 BID) mg/kg/day dose group and remaining animals in the control and 0.4 (0.2 BID) mg/kg/day dose groups (not selected for recovery) were necropsied at the end of treatment.
- Doses were administered BID ~ 6 hours in between.
- Electrocardiography, blood pressure, and vital signs (respiration and body temperature) measurements were determined once pretreatment and Day 1 for all dose groups and Days 87, 178, and 269 for the 0, 0.2 (0.1 BID), and 0.4 (0.2 BID) mg/kg/day dose groups (predose and ~ 2-4 hours post the first dose). Beginning Day 4 (Study Day 68), additional body temperatures were measured to monitor health status from 1.2 (0.6 BID) mg/kg/day and control animals every 3 days until Day 55 (Study Day 119) and then on Recovery Day 8 (Study Day 126) for the males, and every 3 days until Day 50 (Study Day 114) for the females.
- Serum drug concentrations were determined on Days 1 and 14 for all dose groups; Day 30 for the 1.2 (0.6 BID) mg/kg/day males; and Days 30, 91, 148, 210, and 266 for the 0, 0.2 (0.1 BID), and 0.4 (0.2 BID) mg/kg/day dose groups predose and at ~ 3, 6, 9, and 24 hours post the first dose.
- Behavior assessment for possible withdrawal symptoms following discontinuation of varenicline tartrate treatment was performed the last 5 days of treatment through Recovery Day 5 on the control and 0.4 (0.2 BID) mg/kg/day recovery animals.

No Observed Adverse Effect Level: 0.4 (0.2 BID) mg/kg/day

Dose* (mg/kg/day)	0 (Control)		0.2 (0.1 BID)		0.4 (0.2 BID)		1.2 (0.6 BID)	
	M	F	M	F	M	F	M	F
Sex (M/F)								
Number of Animals	6	6	4	4	6	6	6	6
Toxicokinetics: C _{max} (ng/mL)	Males and Females Combined							
	NA		25.2		39.7		47.5	
	NA		44.3		68.2		154	
	NA		48.3		79.5		NA	
Toxicokinetics: AUC(0-24)(ng·h/mL)	Males and Females Combined							
	NA		379		691		803	
	NA		804		1280		2530	
	NA		869		1440		NA	

M = Male; F = Female; NA = Not applicable.

*All dose levels are expressed as mg of active moiety per kg of body weight per day.

Dose* (mg/kg/day)	0 (Control)		0.2 (0.1 BID)		0.4 (0.2 BID)		1.2 (0.6 BID)	
Noteworthy Findings								
Died or Sacrificed Moribund	0	0	0	0	0	1 ^b	4 ^c	4 ^c
Body Weight	-	-	-	-	-	-	0.75-0.84 ^d	0.77-0.84 ^d
Food Intake	-	-	-	-	-	-	≤25% ^e	≤25% ^e
Clinical Observations								
Emesis	2	3	4	4	6	6	6	6
Loose stools	3	4	1	3	3	3	6	5
Liquid stools	1	1	0	1	3	5	6	4
Salivation	0	0	0	2	6	6	3	6
Trembling/Shaking	0	0	0	1	0	2	4	1
Hunched stance	0	0	0	1	1	4	6	6
Decreased activity	0	0	0	0	0	0	2	0
Fur biting	0	0	0	0	0	0	0	2
Fur pulling/teasing	0	0	0	0	0	1	0	0
Withdrawal Behavior Assessment								
Ophthalmology	-	-	-	-	-	-	-	-
Vital Signs								
Body temperature (°C)	-	-	-	-	↓ ^a	-	↓ ^a	↓ ^a
Electrocardiography	-	-	-	-	-	-	-	-
Blood Pressure	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Clinical Chemistry	-	-	-	-	-	-	-	-
Urinalysis	-	-	-	-	-	-	-	-
Organ Weights								
Gross Pathology	-	-	-	-	-	-	-	-
Number Examined	6	6	4	4	6	6	6	6
Histopathology								
Number Examined ^b	5	5	4	4	4	4	5	4

M = Male; F = Female; - = No noteworthy findings; NA = Not applicable.

* All dose levels are expressed as mg of active moiety per kg of body weight per day.

^a Animal 33 was found dead on Day 126. Death was attributed to megacolon with secondary colonic torsion, and was not considered treatment-related.

^b Male Animals 18, 19, 20, and 21 were euthanized on Days 55, 51, 35, and 33 of treatment with vanilichine tartrate, respectively. Female Animals 39, 41, 42, and 43 were euthanized on Days 23, 21, 23, and 23 of treatment with vanilichine tartrate, respectively. These animals were euthanized due to decreased body weight, inappetence, or severe/prolonged dehydration.

^c For 1.2 mg/kg/day Male Animals 19, 20, and 21, the range of fold differences from pretreatment values is shown (differences between Study Day 59 [pretreatment] and Study Day 99 [Day 35 of vanilichine tartrate treatment] for Animals 20 and 21 and Study Day 114 [Day 50 of vanilichine treatment] for Animal 19).

^d For 1.2 mg/kg/day Female Animals 40-43, the range of fold differences from pretreatment values is shown (differences between Study Day 59 [pretreatment] and Study Day 84 [Day 20 of vanilichine tartrate treatment]).

^e Food intake was semi-quantitatively determined as either ~25%, ~25%, ~50%, ~75%, or ~100%. Food intake for those animals affected returned normal during the recovery period.

^a Decreased body temperature was observed intermittently during the treatment period in individual animals, and was generally associated with a decline in health status.

^b Histopathologic examination was not performed on the 0, 0.4 (0.2 BID), 1.2 (0.6 BID) mg/kg/day recovery animals; however, tissues from 1.2 (0.6 BID) mg/kg/day Males 17 and recovery control Animals 1 and 27 (animals necropsied in conjunction with the 1.2 mg/kg/day recovery animals) were invariably examined microscopically.

Local Tolerance

Species/Strain	Method of Administration	Dose (mg/kg)	Duration of Dosing	Gender and Number per Group	Noteworthy Findings
Acute Dermal Irritation					
Rabbit/New Zealand White	Dermal via Semi-occluded Patch	500 mg/animal	Single: 3-minute and 1-hour Applications (1 M); 4-hour Application (3 M)	3 M	Mild skin irritation following 4-hour application. Appeared normal 72 hours after dosing.
Acute Ocular Irritation					
Rabbit/New Zealand White	Ocular	45 mg/animal	Single	3 M	Iridial inflammation and minimal to moderate conjunctival irritation. Appeared normal 7 days after dosing.

Lot Number 54526-10-10B.
M = Male.

Species/Strain	Method of Administration (Vehicle/Formulation)	Dose (mg/kg/day)	Duration of Dosing	Gender and Number per Group	Noteworthy Findings	Study Number
Phototoxicity						
Rat/Long-Evans	Toleration Phase: Oral Gavage (Deionized Water)	30, 100	3 Days	3M	Toleration/Phototoxicity (Gavage): ≥30 mg/kg: Soft/liquid feces, scant feces, no feces, prosis, ↓ motor activity, labored breathing, gasping, ↓ body weight	04-1545-36
	Phototoxicity Phase: Gavage (Deionized Water)	0, 100	3 Days	5M	UVR Exposure: No evidence of cutaneous phototoxicity in lightly or darkly pigmented skin sites.	
	Oral Gavage (LOM ^a in CMC)	800	Single			
	Oral Gavage (8-MOP ^a in corn oil)	50	Single			
	UVR Exposure ^b	0.5 MED	Single			
Toxicokinetic parameters:						
Dose (mg/kg/dry)		100 ^c				
Day 3						
C _{max} (ng/mL)		2110				
AUC(0-24)(ng·h/mL)		41500				

Lot Number 04944002/Batch Number 3000054.

M = Male; LOM = Lomefloxacin; CMC = Carboxymethylcellulose; 8-MOP = 8-Methoxypropolene; UVR = Ultraviolet radiation; MED = Minimal erythema dose (MED refers to a UVR dose adequate to elicit a barely perceptible response in skin).

^a Comparator articles. UVR exposure to comparator articles had cutaneous responses of erythema and/or edema in the lightly or darkly pigmented skin sites indicative of phototoxicity.

^b UVR exposure ~ 1 hour following the final oral administration of CP-526,555-18 and comparator articles, LOM and 8-MOP.

^c Toxicokinetic parameters were determined from a separate satellite group of 6 males.

Species/Strain	Method of Administration	Dose (mg/kg)	Duration of Dosing	Gender and Number per Group	Noteworthy Findings	Study Number
Acute Dermal Irritation						
Rat/Sprague-Dawley	Dermal via Semi-occluded Bandage	2000	24-hour Application	5 M, 5 F	No signs of dermal irritation or systemic toxicity. The acute dermal lethal dose was >2000 mg/kg.	1131/550
Skin Sensitization Test						
Guinea Pig/Dunkin Hartley	Intradermal via Injection	Induction: 5% w/w in Distilled Water	3 Single Injections ^{a, b}	5 M ^c 10 M (main study) 5 M (control)	No evidence of skin sensitization (delayed contact hypersensitivity).	1131/553
	Topical via Occlusive Patch	Induction: 50% w/w in Distilled Water ^b	48-hour Application (7 days after intradermal injection)			
	Topical via Occlusive Patch	Challenge: 25% and 10% w/w in Distilled Water ^b	24-hour Application (21 days after intradermal injection)			

Lot Number 54526-10-10B.

M = Male; F = Female.

^a Single injections (0.1 mL each) included: 1) Freund's Complete Adjuvant plus distilled water ratio 1:1 (w/v); 2) 5% CP-526,555-18 in distilled water (w/w); and 3) 5% CP-526,555-18 in Freund's Complete Adjuvant plus distilled water (w/v).

^b The induction of the control animals was performed using an identical procedure to that used for the test animals except CP-526,555-18 was omitted from the intradermal injections. Similarly, the topical patches were identical to that used for the test animals except CP-526,555-18 was omitted.

^c Five males were used for selection of concentrations of intradermal induction (1 male), topical induction (2 males), and topical challenge (2 males).

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: The major toxicity findings and their clinical relevance are listed below:

In the tissue distribution study (where 1.30 μCi of ^{14}C was tagged with the compound to analyze the tissue distribution) in rat single administration of 3.4 mg/kg (HED = 33 mg considering 60 kg man) showed drug distribution in the different ocular tissues for a long period of time at least 7 days. A decreasing trend of the exposure of test article in all different tissues was noted after a peak exposure at 18 hrs. No major toxicity related to the skin and eye was observed in non clinical species, however, the melanin deposition is a pharmacological characteristic of nicotine. The compound being a partial agonist of nicotine might show related toxicity in the melanin containing tissues and the effect might vary demographically.

The CNS and GIT related clinical signs like emesis, loose stool, and salivation was noted sporadically in all dose groups in all non clinical species studied, therefore, similar treatment related clinical signs are predicted to be observed in human. Decrease in body weight and food consumption >10% is observed in the dose > NOAEL doses. The safety margins (comparison of NOAEL dose with the maximal proposed human dose) and the noteworthy findings are tabulated in the executive summary section.

In the reproductive toxicity studies a decrease in the pregnancy rate was observed in F₁. Decrease in behavioral pattern in the F₂ (like rearing, auditory startle) indicating a decrease in motor coordination and exploratory behavior is noted.

In a 2-year carcinogenicity study in rats, hibernoma, a rare tumor finding was noted in males at 10 and 15 mg/kg (HED= \geq 96 mg). The tumor findings are not statistically significant. This finding, however, is considered treatment related due to the rarity of the findings in rodents.

Unresolved toxicology issues (if any): None

Recommendations: From the nonclinical pharmacology and toxicology perspective, NDA 21-928 may be approved.

SUGGESTED LABELING: AS SENT TO SPO NSOR MAY 8, 2006.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Carcinogenesis. Lifetime carcinogenicity studies were performed in CD-1 mice and Sprague-Dawley rats. There was no evidence of a carcinogenic effect in mice administered varenicline tartrate by oral gavage for 2 years at doses up to 20 mg/kg/day (47 times the maximum recommended human daily exposure in terms of AUC). Rats were administered varenicline tartrate (1, 5, and 15 mg/kg/day) by oral gavage for 2

years. In male rats ($n = 65$ per sex per dose group), incidences of hibernoma (tumor of the brown fat) were increased at the mid dose (1 tumor, 5 mg/kg/day, 31 times the maximum recommended human daily exposure in terms of AUC) and maximum dose (2 tumors, 15 mg/kg/day, 1 times the maximum recommended human daily exposure in terms of AUC). The clinical relevance of this finding to humans has not been established. There was no evidence of carcinogenicity in female rats.

Mutagenesis. Varenicline was not genotoxic, with or without metabolic activation, in the following assays: Ames bacterial mutation assay; mammalian CHO/HGPRT assay; and tests for cytogenetic aberrations in vivo in rat bone marrow and in vitro in human lymphocytes.

Impairment of fertility. There was no evidence of impairment of fertility in either male or female Sprague-Dawley rats administered varenicline succinate up to 15 mg/kg/day (1 times the maximum recommended human daily exposure based on AUC at 1 mg BID) [SPONSOR TO PROVIDE AUC RATIO].

However, a decrease in fertility was noted in the offspring of pregnant rats who were administered varenicline succinate at an oral dose of 15 mg/kg/day (36 times the human AUC at 1 mg BID). This decrease in fertility in the offspring of treated female rats was not evident at an oral dose of 3 mg/kg/day (SPONSOR TO PROVIDE AUC RATIO).

PREGNANCY

Pregnancy Category C.

Varenicline was not teratogenic in rats and rabbits at oral doses up to 15 and 30 mg/kg/day, respectively (36 and 1 times the maximum recommended human daily exposure based on AUC at 1 mg BID, respectively). (SPONSOR Confirm AUC)

Nonteratogenic effects. Varenicline has been shown to have an adverse effect on the fetus in animal reproduction studies. Administration of varenicline to pregnant rabbits resulted in reduced fetal weights at an oral dose of 30 mg/kg/day (50 times the human AUC at 1 mg BID); this reduction was not evident following treatment with 10 mg/kg/day (1 times the maximum daily human exposure based on AUC). In addition, in the offspring of pregnant rats treated with varenicline there were decreases in fertility and increases in auditory startle response at an oral dose of 15 mg/kg/day (36 times the maximum recommended human daily exposure based on AUC at 1 mg BID).

There are no adequate and well-controlled studies in pregnant women. Varenicline should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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this page is the manifestation of the electronic signature.**

/s/

Mamata De
5/8/2006 08:06:17 PM
PHARMACOLOGIST

R. Daniel Mellon
5/8/2006 08:09:51 PM
PHARMACOLOGIST

I concur with Dr. De. From the nonclinical pharmacology
toxicology perspective, NDA 21-928 may be approved pending
agreement on final labeling.

Executive CAC

Date of Meeting: April 4, 2006

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Chuck Resnick, DCaRP, Alternate Member
Dan Mellon, Ph.D., DAARP, Team Leader
Mamata De, Ph.D., DAARP, Presenting Reviewer

Author of Draft: Mamata De, Ph.D., Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 21-928

Drug Name: Varenicline tartrate

Sponsor: Pfizer Inc.

Background: Varenicline tartrate is a partial subtype specific nicotinic agonist ($\alpha 4\beta 2$). It is a NME proposed to be an aid to smoking cessation. The exec-CAC had previously concurred with the selection of doses for the studies.

Mouse Carcinogenicity Study:

Varenicline tartrate was administered to CD-1 mice (1, 5, and 20 mg/kg/day in deionized water) for 2 years by oral gavage. The systemic drug exposure (AUC) at the high dose in mice was 54 times the human exposure at a dose of 1 mg BID. No evidence of carcinogenic potential was observed in mice.

Rat Carcinogenicity Study:

Varenicline was administered to Sprague-Dawley rats (1, 5, and 15 mg/kg/day in deionized water) for 2 years by oral gavage. The systemic drug exposure (AUC) at the high dose in rats was 36 times the human exposure at a dose of 1 mg BID. No evidence of carcinogenic potential was observed in female rats. In male rats (n = 65 per sex per dose group), incidences of hibernoma (tumor of the brown fat) were increased at the mid dose (1 tumor at an exposure in rats that was 12 times the human AUC at 1 mg BID) and high dose (2 tumors, at an exposure in rats that was 36 times the human AUC at 1 mg BID). The incidences were not statistically significant, but hibernomas are very uncommon in rats. In the Registry of Industrial Toxicology Animal-data (RITA) control animal database, there is one (1/1793) hibernoma in a male Sprague-Dawley rat but this did not occur in the mediastinum [RITA, Lesion-related Incidence Data, 2005. Due to the rarity of hibernoma in rodents, the finding is considered treatment related, and therefore, would be included in the label. The clinical relevance of this finding has not been established.

Executive CAC Recommendations and Conclusions:

Mouse:

- * The Committee concurred that the study was adequate.
- * The Committee concurred that the study was negative for drug-related neoplasms.

Rat:

- * The Committee concurred that the study was adequate.

- * The Committee concurred that although the finding of hibernomas in males did not reach statistical significance, hibernomas of brown fat are very uncommon and it is biologically plausible that they are drug related. The clinical relevance of the findings for humans is unclear.

The Committee agreed with the Sponsor and the Division that the findings should be included in the product labeling.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\n
/Division File, DAARP
/Dan Mellon/Team leader, DAARP
/Mamata De/Reviewer, DAARP
/Dominic Chiapperino/CSO/PM, DAARP
/ASeifried, OND IO

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this page is the manifestation of the electronic signature.**

/s/

David Jacobson-Kram
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