# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

# 022454Orig1s000

# PHARMACOLOGY REVIEW(S)

# MEMORANDUM

DaTSCAN (Ioflupane $I^{123}$ injection)

**Date**: September 4, 2009
**To:** File for NDA #22-454
**From:** John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review and labeling provided by Dr. Awe and discussed with his team leader, Dr. Laniyonu. I concur with their conclusions that DaTSCAN may be approved. No additional pharmacology or toxicology studies are necessary for the proposed indication.

**----------**

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/s/

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JOHN K LEIGHTON
09/04/2009

**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: | **22-454** |
| SERIAL NUMBER: | **000** |
| DATE RECEIVED BY CENTER: | **03/09/09** |
| PRODUCT: | **DaTSCAN™: Ioflupane I 123 injection** |
| INTENDED CLINICAL POPULATION: | Patients with symptoms and signs suggestive of dopaminergic neurodegeneration. |
| SPONSOR: | **GE Healthcare Inc.** |
| DOCUMENTS REVIEWED: | **Electronic Submission** |
| REVIEW DIVISION: | **Division of Medical Imaging and Hematology Drug Products (HFD-160)** |
| PHARM/TOX REVIEWER: | **Sunday Awe, Ph.D.** |
| PHARM/TOX SUPERVISOR: | **Adebayo Laniyonu, Ph.D.** |
| DIVISION DIRECTOR: | **Rafel Rieves, MD** |
| PROJECT MANAGER: | **James Moore, Pharm.D.** |

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

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# EXECUTIVE SUMMARY

## I. Recommendations

**A. Recommendation on approvability:** The application is recommended for approval from Pharm/Tox perspective.

**B. Recommendation for nonclinical studies:** None

**C. Recommendations on labeling:**

## II. Summary of nonclinical findings:

[$^{123}$I]ioflupane (DaTSCAN™) is composed of N-ω-fluoropropyl-2β-carbomethoxy-3 β -(4-[$^{123}$I]iodophenyl)nortropane as the active substance. The compound is an intravenously administered diagnostic radiopharmaceutical intended for use with single-photon emission computed tomography (SPECT) imaging of the brain for detecting loss of nigrostriatal dopaminergic neurons. [$^{123}$I]ioflupane is intended for patients presenting with symptoms or signs suggestive of dopaminergic neurodegeneration. The proposed maximum dose of [$^{123}$I]ioflupane is 3-5 mCi and maximum mass dose of ≤0.325 µg.

### A. Brief overview of nonclinical findings:

1) **Efficacy Studies:** *In vitro* and *in vivo* studies were conducted to demonstrate the affinity and selectivity of [$^{123}$I]ioflupane for dopamine transporter (DaT). The *in vitro* studies showed that the compound has high affinity for human DaT. In studies using recombinant transporters, [$^{123}$I]ioflupane demonstrated 3-fold relative selectivity for human DaT compared to serotonin transporter (SERT) and over 110-fold relative selectivity for human DaT compared to noradrenergic transporter (NET). The *in vivo* imaging data from mouse, rat, monkey and baboon showed selective retention of [$^{123}$I]ioflupane in the striatum. For these imaging studies, significant correlation was demonstrated between the *in vivo* signal of [$^{123}$I]ioflupane to both *ex vivo* quantitative dissection data and neuropathology induced by a number of different treatment regimes. Thus, [$^{123}$I]ioflupane could potentially provide *in vivo* image that correlates with measurement of neurons of the striatal dopaminergic system.

2) **Pharmacokinetics, Distribution and Excretion:** [$^{123}$I]ioflupane is rapidly cleared from blood following an intravenous injection. The data from studies conducted in rats, baboons and Cynomolgus monkeys demonstrate rapid distribution and clearance of radioactivity from the blood followed by urinary and fecal elimination. The urinary and fecal excretion rates after administration were 68.9-71.0% and 12.6-17.6%, respectively. The metabolism of the compound is slow and [$^{123}$I]FP-CIT (ioflupane) acid, [$^{123}$I]nor-CIT (nor-ioflupane) acid and minor metabolites were formed. None of these metabolites

could cross the blood brain barrier. Therefore, there is no safety concern that the metabolites could have any effect on the central nervous system or complicate imaging.

3) **Behavioral Safety Studies:** Hyperactivity and stereotypic behavior could be expected due to similarity in the pharmacology of FP-CIT (ioflupane) to that of other DaT ligands like cocaine. However, studies conducted on behavioral safety of ioflupane indicates that such behavioral effects were observed only at very high doses which are approximately 3000-folds higher than the clinical dose of [$^{123}$I]ioflupane. In rat studies, FP-CIT (3 mg/kg-88440X MHD) or cocaine (10 mg/kg) significantly increased the number of rotations compared to the vehicle response. However, an intravenous injection of FP-CIT (0.03 mg/kg-884.4X MHD) did not cause any significantly increase in the number of rotations when compared to the vehicle treated controls. Comparable functional effect and efficacy of FP-CIT to cocaine was reported at doses in the order of 29000X MHD. In another study, FP-CIT at 2948X MHD induced enhanced stereotypical behavior in 1/6 rats while rats administered 300X MHD demonstrated no CNS effect. However, no effect on motor coordination was reported at all employed doses in this study.

4) **Cardiovascular Safety Studies:** The effect of FP-CIT on hERG tail current recorded from stably transfected HERK293 cells was evaluated. No statistically significant inhibitory effect was reported when the effect of ioflupane (0.03 μM) and vehicle treated groups on hERG tail current were compared. A concentration-dependent inhibition was observed at higher concentrations (0.1-10 μM; 1000X-3000X human plasma concentration). The estimated $IC_{25}$, $IC_{5O}$ and $IC_{75}$ obtained from the concentration-response curve were 0.070, 0.29 and 1.2 μM respectively and the positive control, E-4031 (100 nM) inhibited hERG tail current in the tested cells by 97.1%.

An *in vivo* cardiovascular assessment showed no cardiovascular effects in conscious telemetered dogs administered FP-CIT up to 983X MHD and no treatment related effect on PQ duration, QRS interval, and QTc duration at approximately 10000X MHD. However, increased blood pressure and heart rate were reported at this dose. It is not likely ioflupane would induce any cardiovascular adverse effect at the clinical dose.

5) **Other Safety Studies:** The possibility that combination with FP-CIT could enhance or reduce the pharmacological actions of therapeutic drugs used for treating Parkinson's disease was examined. Animal models of Parkinsonism (rats with substantia nigra bilaterally lesioned with 6-hydroxydopamine (6-OHDA) were used to evaluate the effect of FP-CIT on the pharmacological actions of L-DOPA, bromocriptine and amantadine. The combinations with FP-CIT 0.1 mg/kg and a DAT inhibitor, GBR-12935 (0.1 mg/kg), did not affect the stimulating activity of L-DOPA on locomotor activity in rats with substantia nigra bilaterally lesioned with 6-OHDA, but that combinations with FP-CIT (1

mg/kg) and GBR-12935 (1 mg/kg) prolong the actions of L-DOPA. Furthermore, combinations with FP-CIT (0.1 and 1 mg/kg) and GBR-12935 (0.1 and 1 mg/kg) did not affect the stimulating activity of bromocriptine and amantadine on locomotor activity. Thus, ioflupane did not affect the locomotor activity when administered in this experimental model of Parkinsonism in combination with L-DOPA, bromocriptine and amantadine

FP-CIT (0.65X – 648X MHD) was administered to male Sprague-Dawley rats and a modified Irwin-type Functional Observation Battery (FOB) conducted on the rats between 10 minutes to 8 days post dosing. There was no mortality. However, there were piloerection, labored respiration, increased defecation, touch reactivity, positional passivity, and alterations in muscle tone in FP-CIT treated animals. The reported alteration in respiration was dose- and treatment-related while significant alterations in muscle tone were observed at 648X MHD. In another study in a telemetered dog, FP-CIT (9828X MHD) induced an increased respiratory rate immediately after injection. However, no elevated respiratory rate was reported at lower doses.

6) **Toxicity Studies:** Single dose and repeat-dose toxicity studies were conducted in rats, rabbits and Cynomolgus monkeys. No treatment-related mortality was reported in any of these studies. A NOAEL of 10 µg/kg (294.8X MHD) was obtained in the rats. No change in body weight or clinical pathology was reported in rabbits following a single injection of 0.06 mg/kg (3604X MHD), the only dose employed in the study. Following a single dose injection to dogs, mydriasis, increased motility, and licking were reported during administration or immediately after administration of 29480X MHD FP-CIT to dogs and a NOAEL of 1 µg/kg (98.3X MHD) was obtained. Increased heart rate, blood pressure, respiratory rate, slight decrease in motility, mydriasis and restlessness were reported in Cynomolgus monkeys administered 5880X MHD and the NOAEL in the study was 0.3 µg/kg (17.7X MHD).

The 14-day repeat dose toxicity studies in rats revealed stereotype behavior, increased and violent physical activity, excessive sensitivity to external stimuli, and piloerection following a daily injection of 17688X MHD. However, no treatment-related clinical signs were reported in animals administered 0.006 mg/kg/day (176.88X MHD). There were scattered blood spots in the lungs of 1/5 female in the 8844X MHD group and histopathology of the lungs showed localized mild bleeding in males in the 294.8X MHD group and in males and females injected 8844X MHD. The NOAEL in this study was 10 µg/kg/day (294.8X MHD). There were stereotype and aggressive behaviors in rabbits treated with 360X and 36000X MHD for 2-weeks while increased responses to external stimuli, protruding eyes with dilated pupils, and fast or labored respiration occurred in rabbits administered 90000X MHD FP-CIT. No serious clinical sign was reported in Beagle dogs administered up to 98.3X MHD FP-CIT. However, there was mydriasis, congestion of the visible mucosa, flushing

of the pinnae, reddening of the skin, or panting after administration of 9828X MHD FP-CIT. The NOAEL in this study was 1 µg/kg/day (98.3X MHD).

7) **Reproductive Toxicity Studies:** No reproductive toxicity study was conducted on FP-CIT. The sponsor's request for a waiver for reproductive and developmental toxicity studies was granted.

8) **Genotoxicity Studies:** The standard ICH battery of tests including two *in vitro* assay covering the endpoints of gene mutation (in bacteria) and chromosomal effects (in cultured human lymphocytes and in mouse bone marrow) was evaluated. The tests were negative indicating that FP-CIT demonstrates no genotoxicity potential.

9) **Carcinogenicity Studies:** No carcinogenicity study was conducted on FP-CIT. The sponsor's request for a waiver for carcinogenicity studies was granted.

**B. Nonclinical safety issues relevant to clinical use:**

1) **Lack of evidence of local or systemic toxicity in rabbits, intravenously, intra-arterially or pervenously administered FP-CIT:** The intravenous, intra-arterial and perivenous tolerance of FP-CIT was assessed in the rabbit. No mortality or any evidence of local and systemic toxicity was reported following a single injection in the study.

The sponsor provided adequate preclinical data on the efficacy and safety of FP-CIT for the proposed indication. The data showed that:

1) [$^{123}$I]FP-CIT has high affinity and selectivity for DaT and this could provide *in vivo* image with high correlation to DaT distribution in the striatum.
2) FP-CIT metabolites did not cross the blood brain barrier. Thus, no CNS pharmacological effect is envisaged from metabolites.
3) Hyperactivity and stereotypic behaviors were produced at high doses, due to similarity in the pharmacology of FP-CIT to that of other DaT ligands like cocaine.

# *2.6 PHARMACOLOGY/TOXICOLOGY REVIEW*

## 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number**: 22-454

**Review number**: 001

**Sequence number/date/type of submission**: N000/March 9, 2009/Commercial

**Information to sponsor**: Yes () No (x)

**Sponsor and/or agent**: GE Healthcare Inc.,
101 Carnegie Center,
Princeton, NJ 08540-6231

**Manufacturer for drug substance**: GE Healthcare Inc.,
101 Carnegie Center,
Princeton, NJ 08540-6231

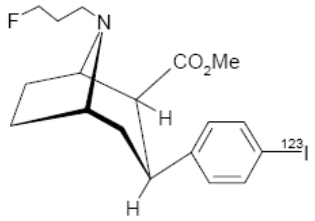
**Reviewer name**: Sunday Awe, Ph.D.
**Division name**: Medical Imaging and Hematology Products

**HFD #**: 160

**Review completion date**: August 30, 2009

**Drug**:

Generic name: [$^{123}$I]ioflupane
Code names: ioflupane, DaTSCAN, FP-CIT, CIT-FP, β-FP-CIT, β-CIT-FP or RTI-313
Chemical name: N-ω-fluoropropyl-2β-carbomethoxy-3 β -(4-[$^{123}$I]iodophenyl)nortropane
IUPAC name: Methyl (1R-2S-3S-5S)-8-(3-fluoropropyl)-3-(4-[$^{123}$I]iodophenyl)-8-azabicyclo[3.2.1] octane-2-carboxylate.
Molecular formula/molecular weight: $C_{18}H_{23}F[^{123}I]NO_2$ C/431.29
Structural formula:



**Relevant INDs/NDAs/DMFs**: IND 101,016

**Drug class**: Diagnostic radiopharmaceutical agent

**Indication:** Detection of loss of functional nigrostriatal dopaminergic neurons by single photon emission computed tomography (SPECT) imaging in patients presenting with symptoms or signs suggestive of dopaminergic neurodegeneration.

**Intended clinical population**: Patients with symptoms and signs suggestive of dopaminergic neurodegeneration.

**Clinical formulation**: DATSCAN is formulated for injection as a clear solution of [$^{123}$I]ioflupane in acetic acid/sodium acetate (b) (4) (pH (b) (4) and 5% (v/v) ethanol. Each ml contains 2mCi (74 MBq) at calibration time (0.07 to 0.13 μg of ioflupane).

**Dose:** Maximum human dose of 3-5 mCi and a mass dose of ≥0.325 μg).

**Route of administration**: Slow intravenous injection (not less than 15 to 20 seconds)

**Previous clinical experience:** Previous clinical studies include one phase 1 study (CY95.FP.I), two phase 2 studies (CY96.FP.II & PDT02005), six phase 3 studies (PDT301, PDT304, DP008-003, PDT03007, PDT408 and Walker). The data for DaTSCAN from 8 clinical studies (n=942 subjects) and post-marketing data from 2000-2008 reveal no significant safety signals for DaTSCAN administration. There have been no deaths or serious adverse events attributable to DaTSCAN as determined by the study investigators. In addition, data from clinical laboratory evaluations, vital sign monitoring and ECG assessments have produced no significant concerns regarding DaTSCAN use.

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

**Studies reviewed within this submission:** The studies reviewed in this submission are as follows:

Study title: 134103: Binding Properties of β-CIT-FP for Selected Rodent Receptors and Transporters.

Study title: PO882040: A Study to Investigate the Regional Brain Biodistribution of I123-Ioflupane in Male Wistar Rats.

Study title: DPO88R/002: [$^{123}$I]FP-CIT binding to the dopamine transporter as assessed by biodistribution studies in rats and SPECT studies in MPTP-lesioned Monkeys.

Study title: (b) (4)-155B: Behavioral Pharmacology Study of β-CIT-FP in Rats.

Study title: (b) (4)-51-80: Combinational Effects of FP-CIT on the Pharmacological Actions of L-DOPA, Bromocriptine, and Amantadine in Parkinsonism Model Rats.

Study title: 2838: FP-CIT: A single intravenous dose modified Irwin Screen test in rats.

Study title: 2001NM017: Safety Pharmacology Study of FP-CIT.

Study title: 2837: FP-CIT: A Non-Clinical Intravenous Cardiovascular Safety Pharmacology Study in Beagle Dogs.

Study title: B001002: Effect of Ioflupane on hERG Tail Current Recorded from Stably Transfected HEK293 Cells.

Study title: (b) (4)/PE9846: Study of the effects of the repeated administration of anti-Parkinson disease drugs on the binding of $^{125}$I-FP-CIT to the membrane fraction of the rat striatum.

Study title: DP0088R/066: Binding of [$^{123}$I]FP-CIT in rat brain *in vivo*: effects of acute and chronic medication of psychopharmaceuticals.

Study title: DP008R/002: [$^{123}$I]FP-CIT Binding to the Dopamine Transporter as assessed by biodistribution studies in rats and SPECT studies in MPTP-lesioned monkeys.

Study title: PO882039: A Study to Investigate the Whole Body Biodistribution of I123-Ioflupane in Male Wistar Rats.

Study title: PO20376: Single Intravenous dose Toxicokinetic Study of $^{125}$I-FP-CIT in Rats.

Study title: PO20377: Single Intravenous dose Pharmacokinetic Study of $^{125}$I-FP-CIT in Cynomolgus Monkeys

Study title: 9829: Excretion of Total radioactivity following Single Intravenous Administration of $^{125}$I-labelled FP-CIT to Rats.

Study title: 2001NM021: Single Dose Intravenous Toxicity Study FP-CIT in Rats.

Study title: 144552: Assessment of Acute Intravenous Toxicity with β-CIT-FP in the Rats.

Study title: 144574: Assessment of Acute Intravenous Toxicity with β-CIT-FP in the Rabbit.

Study title: 2001NM022: Single Intravenous Dose Toxicity Study FP-CIT in Dogs.

Study title: NM97076: Single Intravenous Dose Toxicity Study β-FP-CIT in Cynomolgus Monkeys.

Study title: 17094: FP-CIT: A Two-Week Intravenous Toxicity Study in the Rats.

Study title: 2001NM023: 2-Week Repeated Intravenous Dose Toxicity Study of FP-CIT in Rats.

Study title: 17095: FP-CIT: A Two-Week Intravenous Toxicity Study in the Rabbits.

Study title: 2001NM024: 2-Week Repeated Intravenous Dose Toxicity Study of FP-CIT in Dogs.

Study title: 17348: FP-CIT Mouse Micronucleus Test.

Study title: 25618: FP-CIT *In vitro* Mammalian Cytogenetic Test Performed with Human Lymphocytes.

Study title: 17349: FP-CIT *In vitro* Mammalian Cell Gene Mutation Test Performed with Mouse Lymphoma Cells (LY5178Y).

Study title: 2001NM020: Micronucleus Test of FP-CIT in Mice.

Study title: 17350: FP-CIT Mouse Micronucleus Test.

Study title: 144541: Assessment of Intravenous, Intra-arterial and Perivenous Tolerance of β-CIT-FP in the Rabbit after Single Administration.

**Studies not reviewed within this submission**:

(b) (4)

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary:

The efficacy, pharmacokinetics and safety pharmacology profile of FP-CIT (ioflupane) was evaluated in various species. The *in vitro* studies showed that the compound has high affinity for human dopamine transporter (DaT) with some affinity for serotonin transporter (SERT. However, studies using recombinant transporters showed

approximately 3-fold relative selectivity for the DaT than SERT and over 110-fold relative selectivity for DaT than NET. *In vivo* studies in rodents and non-human primates showed selective retention of [$^{123}$I]FP-CIT in the striatum. In addition, imaging studies demonstrate significant correlation between the *in vivo* signal of [$^{123}$I]FP-CIT to both *ex vivo* quantitative dissection data and neuropathology induced by a number of different treatment regimes. No clinical effects were observed in the safety pharmacology studies conducted FP-CIT. However, hyperactivity and stereotypic behavior were reported at high doses due to similarity in the pharmacology of FP-CIT to that of other DaT ligands like cocaine.

**2.6.2.2 Primary pharmacodynamics**

Data from several *in vitro* and *in vivo* primary pharmacodynamic studies demonstrating selective affinity of [$^{123}$I]FP-CIT for the DaT target were presented.

*In vitro* studies:

The summary data of the study conducted by Scheffel et al (1997) on binding affinities of five N-substituted phenyltropanes including FP-CIT for the DaT, serotonin transporter (SERT) and norepinephrine transporter (NET) was provided. In the study, [$^{3}$H]WIN 35,428 (0.5 nM) was used as the binding radioligand in homogenates of rat striatum, [3H]paroxetine (0.2 nM) in homogenates of rat brain stem, and [$^{3}$H]nisoxetine (0.5 nM) in homogenates of rat frontal cortex respectively.

**Table 2.6.2-1 Inhibitory potencies of cocaine and FP-CIT at DaT, SERT and NET from rat brain**

| | Inhibitory potency ($IC_{50}$; nM) n=3 to 4±SEM | | | DaT selectivity | |
|---|---|---|---|---|---|
| **Compound** | **DaT** | **SERT** | **NET** | **SERT/DaT** | **NET/DaT** |
| Cocaine | 89.1 | 1045 | 3298 | 11.7 | 37.1 |
| FP-CIT | 1.67 ± 0.12 | 16.3 ± 1.34 | 140 ± 13 | 9.76 | 83.8 |

SEM = Standard error of the mean

As shown in sponsor's Table 2.6.2-1, FP-CIT had a 53-fold greater affinity for the DaT in comparison to cocaine. However, both cocaine and FP-CIT showed similar selectivity for the DaT over the SERT and FP-CIT was more selective over the NET.

In a study conducted by Okada et al (1998) using human embryonic kidney cell (HEK293) lines permanently expressing rat DaT, rat SERT and human NET respectively, ligand uptake and inhibition were studied in the presence and absence of (-)cocaine or FP-CIT. The study showed that each transporter-transfected cell line demonstrates significant uptake of its respective ligand. $K_m$ values for [$^{3}$H]DA, [$^{3}$H]5-HT and [$^{3}$H]NE were 1406 nM, 1170 nM and 864 nM respectively compared with control nontransfected cells. The $K_i$ data obtained (sponsor's table 2.6.2-2) indicates that FP-CIT showed greater affinity for the DaT than cocaine and some selectivity for DaT relative to the other transporters.

**Table 2.6.2-2** ***$K_i$* values (nM) for inhibition of [$^3$H]DA, [$^3$H]5-HT and [$^3$H]NE uptake in transfected HEK293 cells**

| | DaT (45 nM [$^3$H]DA) | SERT (20 nM [$^3$H]5-HT) | NET (50 nM [$^3$H]NE) |
|---|---|---|---|
| **Inhibitor Compound** | ***$K_i$* (nM)** | ***$K_i$* (nM)** | ***$K_i$* (nM)** |
| (-)cocaine | 315.84 ± 126.33 | 580.73 ± 113.16 | 176.46 ± 24.84 |
| FP-CIT | 27.97 ± 7.36 | 113.39 ± 63.77 | 70.42 ± 15.37 |

Values shown are mean±Standard deviation (SD) (n=3)

It was also reported in a similar binding study using rat striatal or frontopariental membrane fraction homogenates, that FP-CIT had strong affinities for these three transporters, with a $K_i$ of 3.5 nM, 0.11 nM and 63 nM for DaT, SERT and NET respectively (Neumeyer et al, 1996).

**Study title**: **Binding properties of β-CIT-FP for selected rodent receptors and Transporters** (b) (4)

This study involved characterization of the binding of FP-CIT at 15 rodent cellular targets, and determination of the $K_i$ and $IC_{50}$.

The results showed that FP-CIT demonstrates significant binding to 8 target sites. Binding of [$^3$H]WIN 35,428 (specific ligand for the DaT) was inhibited with a $K_i$ of 3.3 nM and $IC_{50}$ of 3.7 nM. FP-CIT demonstrates very high selectivity for DaT over the adrenergic $\alpha_1$ receptor, choline uptake, the muscarinic receptor and the sodium channel. The selectivity for DaT over the adrenergic NET was considerably less, at 50 to 100 fold.

**Table 2.6.2-4** **Rodent targets where FP-CIT showed significant binding**

| Target | % inhibition at 100 µM | $IC_{50}$ | Ratio of target:DA uptake $IC_{50}$ | $K_i$ | Ratio of target:DA uptake $K_i$ |
|---|---|---|---|---|---|
| DA uptake | 93 | 3.7 nM | 1 | 3.3 nM | 1 |
| Adrenergic $\alpha_1$ | 56 | 74 µM | 20,000 | 20 µM | >6000 |
| Adrenergic NET | 101 | 549 nM | 148 | 176 nM | 53 |
| Choline uptake | 79 | 38 µM | >10,000 | 32 µM | >9600 |
| GABA transporter | -174 | 1.4 µM | 378 | 1.4 µM | 424 |
| Muscarinic | 66 | 37 µM | 10,000 | 12 µM | >3600 |
| SERT | 101 | 2.3 nM | 0.62 | 1.5 nM | 0.45 |
| $Na^+$ channel, site 2 | 93 | 2.4 µM | 649 | 2.2 µM | 667 |

FP-CIT has an approximate two-fold greater affinity for the SERT than the DaT in the rat brain.

**Study title**: **Binding properties of β-CIT-FP for selected human receptors and Transporters** (b) (4)

In this study, similar protocol as employed above was used to evaluate the binding of FP-CIT at recombinant human DaT, NET, and SERT targets. It was reported that FP-CIT inhibited binding at the human recombinant DaT with a $K_i$ of 623 pM and an $IC_{50}$ of 701

pM, a 5-fold greater affinity than previously shown for the DaT in the rat striatum. FP-CIT demonstrates 3- to 4-fold selectivity for the DaT over the SERT. Selectivity over the adrenergic NET was also slightly greater in the human recombinant studies (between 100- and 300-fold) than for the previous rodent studies.

As shown in Table 2.6.2-5, FP-CIT has high affinity for the DaT (0.6 nM for the human recombinant transporter) and against human targets, has a 3- to 4-fold selectivity for the DaT over the SERT.

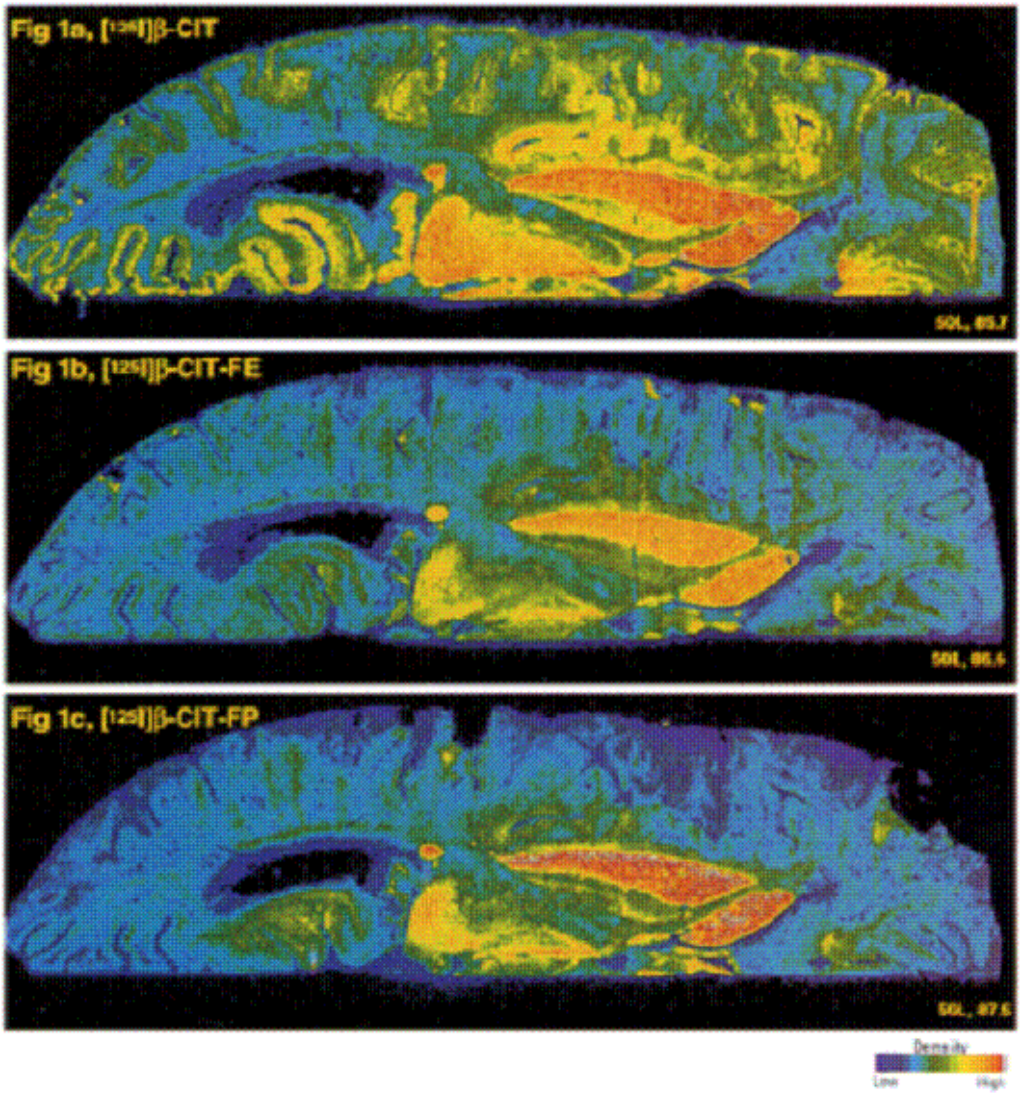
**Table 2.6.2-5 Human recombinant targets where FP-CIT shows significant binding**

| Target | % inhibition (at conc.) | $IC_{50}$ | Ratio of target:DA transport $IC_{50}$ | $K_i$ | Ratio of target:DA transport $K_i$ |
|---|---|---|---|---|---|
| DaT | 52 (1 nM) | 701 pM | 1 | 623 pM | 1 |
| Adrenergic NET | 75 (1 µM) | 229 nM | 327 | 73 nM | 117 |
| SERT | 79 (10 nM) | 2.9 nM | 4.14 | 1.9 nM | 3.05 |

**Autoradiography studies:**
Summary data of autoradiography study of [$^{125}$I]FP-CIT in human post-mortem brain slices conducted by Gunther et al (1997) was provided. Whole hemisphere macroautoradiography of post-mortem human brains was employed in studying the regional binding characteristics of [$^{125}$I] β-FP-CIT along with [$^{125}$I]β-CIT and [$^{125}$I] β -CIT-FE in 3 subjects, 53 -59 years old who died of heart failure. As shown in sponsor's Figure 2.6.2-2, the autoradiograms of the three ligands showed dense labeling of the caudate nucleus and putamen that is consistent with the suggestion that these compounds bind to the DaT. The labeling of the DaT in the striatum (caudate nucleus and putamen) was intense and homogeneous for each of the three ligands. The ratio of striatum to neocortex for [$^{125}$I] β-CIT-FP was 3.9±0.47 and striatum to thalamus was 2.7±0.44. These ratios were considerably greater than those observed for [$^{125}$I] β-CIT and [$^{125}$I] β-CIT-FE.

Furthermore, the specificity of the binding of the radioligands was studied by conducting competition studies with DaT inhibitor, GBR 12909, the SERT inhibitor, citalopram and the NET inhibitor, desipramine. In the study, citalopram (1 µM) reduced binding in the neocortex and thalamus and minor effects were reported in the striatum indicating that the binding in the cortex and thalamus is mainly to the SERT. However, desipramine (10 µM) showed no effect on the level of striatal binding of the radiolabeled analogs, but reduced extrastriatal binding by 60 to 85%. High concentrations of GBR 12909 (100 µM), abolished the binding of all three radioligands to the striatum (Figure 3 below). Inhibition by GBR 12909 indicates selectivity of [$^{125}$I]FP-CIT binding to DaT.

**Figure 2.6.2-2** **Color coded autoradiograms of human whole hemispheres labelled with (a) [I125]β-CIT (25.5 pM), (b) [I125]β-CIT-FE (24.6 pM) and (c) [I125]β-CIT-FP ([I125]FP-CIT) (28.7 pM)**



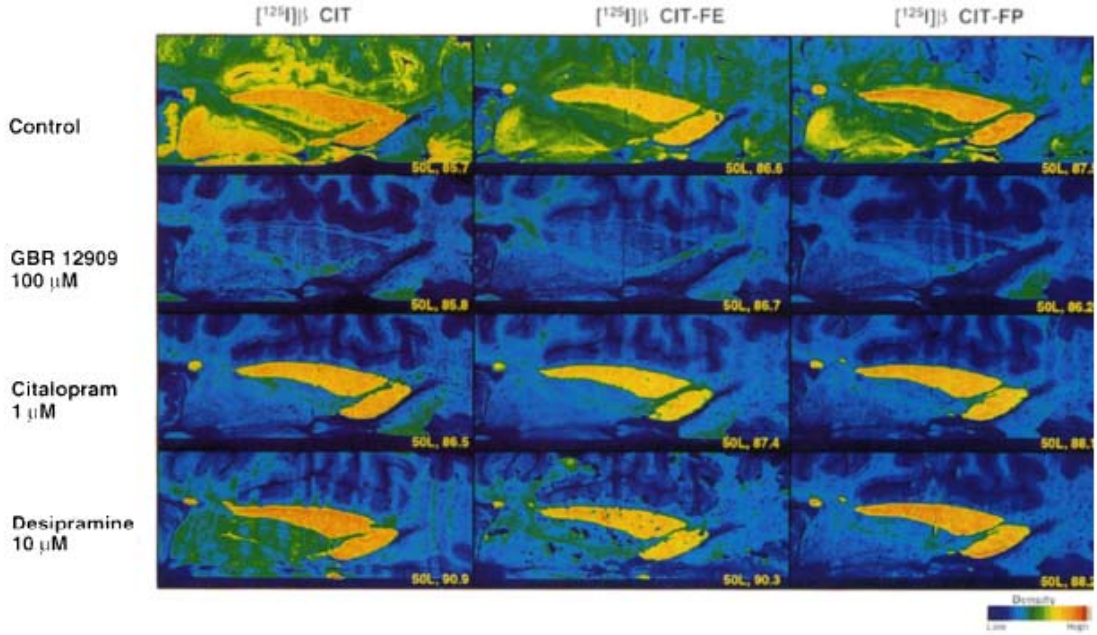



FIG. 3. Details of color-coded autoradiograms of human whole-hemispheres labeled with [$^{125}$I]β-CIT (25.5 pM), [$^{125}$I]β-CIT-FE (24.6 pM), and [$^{125}$I]β-CIT-FP (28.7 pM) showing basal ganglia region without and with the addition of 100 μM GBR 12909 (dopamine and serotonin uptake inhibitor), 1 μM citalopram (serotonin uptake inhibitor), or 10 μM desipramine (noradrenaline uptake inhibitor). Numbers in lower right corner are the internal brain numbers and the levels in millimeters from vertex.

*In vivo* studies:
The following *in vivo* brain biodistribution data was provided.

**Study title: PO882040: A Study to Investigate the Regional Brain Biodistribution of I123-Ioflupane in Male Wistar Rats.**

**Key study findings**: The regional brain distribution of $^{123}$I-Ioflupane in the rat was determined. $^{123}$I-Ioflupane was rapidly biodistributed with 4% retained within the brain within 2 min post injection (pi). The retention of $^{123}$I-Ioflupane within the brain decreased over time such that by 4 hours, only 0.6% was present. The selective retention of $^{123}$I-Ioflupane in the striatum relative to the cerebellum peaked by 20 minutes pi (2.45) and remained constant at approximately 2.20 from 1 hour pi through to 7 hours pi. This study indicates that $^{123}$I-Ioflupane was selectively retained within the striatum, an area in which the DAT is highly expressed.

**Study no.**: PO882040

**Volume # and page #:** Volume 1 pages 1- 42

**Conducting laboratory and location** (b) (4)

**Date of study initiation**: November 6, 2002

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: [$^{123}$I]-Ioflupane, Batch # 02L05D and purity 97%.

**Study Design:**
Doses: 0.5 mL of 74 MBq/ml (37 MBq per animal)

Species/strain: Male Wistar outbred rats/ (Crl: (WI)BR)

Weight: 180-220 g

Number/sex/group or time point: 3males/group.

Route of administration: Each rat was injected with a 0.5 ml bolus (approximately, 37 MBq $^{123}$I-Ioflupane) of the test item via the lateral tail vein.

**Main Study:** This study involved studying the compound at 6 sacrifice time points post injection (2 minutes, 20 minutes, 1, 2, 4 and 7 hours) with 3 rats per group. The animals were kept in the metabolic cages where the urine and feces were separately collected. Upon sacrifice, the animals were dissected and the blood (5 ml), brain, carcass and injection site were removed. The brain from each animal was further dissected into the following regions striatum, cerebellum, cortex, hippocampus and remainder for determination of the regional brain distribution of $^{123}$I-Ioflupane. All the samples were then assayed for radioactivity in an automatic twin-crystal gamma counter

**Results:** $^{123}$I-Ioflupane was rapidly distributed and only 3 percent of the injected radioactive dose (% id) present in the blood by 2 minutes pi. Retention of $^{123}$I-Ioflupane within the brain decreased over time such that by 20 minutes, 1, 2, 4, and 7 hours pi, approximately 1.6% id, 1.0% id, 0.7% id, 0.6% id and 0.5% id, respectively, was present within the brain. The regional brain biodistribution data was expressed as target (striatum) to non-target (e.g. cerebellum, cortex or hippocampus) ratios. Striatum to cerebellum ratio peaks at 20 minutes pi at 3.45 while the striatum to hippocampus ratio peaks by 1 hour pi at 2.56 and remains constant thereafter at approximately 2.10. The striatum to cortex ratio peaks at 4.76 by 2 minutes pi and decreases steadily over the 7 hour duration of the study such that the ratio has decreased to 2.19 by 7 hours pi.

Reviewer's Comments: The reviewer agrees with the result of this study which shows rapid biodistribution of the compound. The study demonstrates selective distribution and retention of ioflupane in the striatum regions of the brain where DAT is highly expressed.

Other *in vivo* studies:

1) Neumeyer et al (1994) - In this study, SPECT imaging was conducted for 5 hours following an intravenous administration of [$^{123}$I]FP-CIT (290 MBq; 7.8 mCi) to a

10 kg adult female baboon under isoflurane anesthesia. A significant uptake was reported in the striatal region with a peak at 90 minutes post-injection. A lower uptake in the midbrain region (rich in SERT) with a peak in less than 60 minutes post-injection and more rapid washout rate of 16% per hour over the same period were observed. The ratio of uptake in striatum to the occipital cortex was 3.4 at peak striatal uptake.

2) Baldwin et al (1995) - An intravenous injection of four [$^{123}$I]β-CIT analogues, including [$^{123}$I]FP-CIT (190 to 460 MBq; 5.2 to 12.6 mCi) was administered to adult female baboons (11.4 to 13.6 kg). The regional brain SPECT imaging showed that target to-background ratio for [$^{123}$I]FP-CIT was 3 to 4 at peak uptake (occurring at 30 to 90 minutes post-injection) and increased to 6 to 10 after 5 hours due to the more rapid washout from extrastriatal sites.

3) Lundkvist et al (1995) - A single male monkey (approximately 7 kg) was injected with [$^{11}$C]FP-CIT using different parameters of doses and observation time. The study demonstrates a marked displacement of [$^{11}$C]FP-CIT from the striatum with little or no effect on the thalamus, neocortex or cerebellum.

4) Lundkvist et al (1997) – The regional brain radioactivity and the specificity of [$^{18}$F]βFP-CIT for DAT was evaluated using a selective DAT antagonist, GBR 12909. In this study, [$^{18}$F]βFP-CIT was intravenously injected into two cynomolgus monkeys. About 2.5% of the radioactivity was present in the brain at 6 min. The cerebellum, a region with negligible density of DAT, was used as the reference region for comparison. The highest accumulation of radioactivity was found in the striatum with a striatal-to-cerebellum ratio of 4.5 to 5.0 at 70 minutes after injection. In this study, pre-treatment with GBR 12909 (5 mg/kg), in the third monkey induced about 50% reduction in the ratio in comparison with the two control animals. Thus, demonstrating the specificity of [$^{18}$F]βFP-CIT binding to the DAT target. No evident effect of GBR 12909 pre-treatment on the time curve for the cerebellum, the thalamus or the frontal cortex was reported.

**Study title: DPO88R/002: [$^{123}$I]FP-CIT binding to the dopamine transporter as assessed by biodistribution studies in rats and SPECT studies in MPTP-lesioned Monkeys.**

**Key study findings**: $^{123}$I-Ioflupane was evaluated as an agent for the *in vivo* labeling of dopamine (DA) transporters by biodistribution studies in rats and using SPECT studies in unilateral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys. Striatal uptake of radioactivity after injection of [$^{123}$I]FP-CIT was displaced significantly by GBR12,909, a DaT inhibitor, but not by fluvoxamine, a SERT inhibitor. However, less pronounced uptake was seen in the brain areas with high densities of serotonergic uptake sites. This indicates that [$^{123}$1]FP-CIT binds specifically to the striatal DA transporter *in vivo*.

**Study no.**: DPO88R/002

**Volume** 1 pages 1- 13

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: Not provided

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: [$^{123}$I]-Ioflupane, Lot # and purity: Not provided.

**Main Study:**
*In vivo* distribution studies in rats:
Male Wistar rats were administered approximately 1.85 MBq of [$^{123}$I]FP-CIT via tail vein under ether anesthesia. In the first study to investigate the time course of uptake in rat brain and assess the optimal time point for quantitative measurement of DA transporter binding, 3 rats were sacrificed at several time points after injection of [$^{123}$I]FP-CIT. The second study involved prior intravenous injection of blocker (GBRI2,909 or fluvoxamine; 5 mg/kg body weight dissolved in 0.4 ml buffer) to 4 rats while a control group of rats (n = 4 - 6) received an i.v. injection of 0.4 ml buffer 5 minutes before [$^{123}$I]FP-CIT injection. Blood, several brain regions (striatum, hypothalamus, occipital cortex, and cerebellurn) and other peripheral tissues (lung, heart, liver, kidney, spleen, fat, and muscle), were rapidly removed and weighed. The tissues and organs were then assayed in a gamma counter.

**Results:**
There was high absolute uptake of $^{123}$I in the striatum in comparison with other brain areas following [$^{123}$I]FP-CIT injection. [$^{123}$I]FP-CIT also rapidly accumulated in liver, spleen, kidney, and lung (sponsor's Table 1). Pretreatment with GBR 12909 significantly decreased the uptake of [$^{123}$I]FP-CIT to the striatum, but not to other brain regions. Similarly, administration of GBR 12909 after [$^{123}$I]FP-CIT injection displaced striatal [$^{123}$I]FP-CIT uptake significantly.

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*Table 1. Biodistribution of $^{123}$I radioactivity and striatal as well as hypothalamic uptake ratios at different times after intravenous injection of [$^{123}$I]FP-CIT in mature male rats[1].*

| | 15 min | 30 min | 1 h | 2 h | 3 h | 4 h | 24 h |
|---|---|---|---|---|---|---|---|
| Striatum | 0.533 ± 0.059 | 0.441 ± 0.067 | 0.335 ± 0.058 | 0.153 ± 0.008 | 0.102 ± 0.013 | 0.097 ± 0.013 | 0.014 ± 0.001 |
| Hypothalamus | 0.268 ± 0.041 | 0.193 ± 0.016 | 0.123 ± 0.039 | 0.077 ± 0.005 | 0.062 ± 0.012 | 0.066 ± 0.008 | 0.010 ± 0.001 |
| Occ. cortex | 0.236 ± 0.016 | 0.167 ± 0.014 | 0.088 ± 0.012 | 0.059 ± 0.004 | 0.045 ± 0.009 | 0.033 ± 0.007 | 0.008 ± 0.001 |
| Cerebellum | 0.145 ± 0.008 | 0.102 ± 0.005 | 0.057 ± 0.004 | 0.039 ± 0.002 | 0.028 ± 0.006 | 0.028 ± 0.004 | 0.008 ± 0.000 |
| Blood | 0.055 ± 0.000 | 0.055 ± 0.005 | 0.042 ± 0.001 | 0.035 ± 0.006 | 0.029 ± 0.004 | 0.031 ± 0.001 | 0.012 ± 0.001 |
| Fat | 0.049 ± 0.016 | 0.105 ± 0.041 | 0.136 ± 0.045 | 0.163 ± 0.053 | 0.151 ± 0.028 | 0.131 ± 0.030 | 0.017 ± 0.004 |
| Muscle | 0.037 ± 0.003 | 0.039 ± 0.004 | 0.023 ± 0.002 | 0.019 ± 0.003 | 0.016 ± 0.002 | 0.016 ± 0.000 | 0.005 ± 0.000 |
| Kidney | 0.246 ± 0.008 | 0.248 ± 0.012 | 0.206 ± 0.009 | 0.131 ± 0.020 | 0.114 ± 0.016 | 0.109 ± 0.008 | 0.037 ± 0.003 |
| Liver | 0.671 ± 0.047 | 0.577 ± 0.040 | 0.538 ± 0.012 | 0.498 ± 0.102 | 0.457 ± 0.083 | 0.453 ± 0.012 | 0.333 ± 0.033 |
| Spleen | 0.295 ± 0.018 | 0.213 ± 0.008 | 0.151 ± 0.026 | 0.081 ± 0.016 | 0.124 ± 0.012 | 0.058 ± 0.013 | 0.012 ± 0.001 |
| Lung | 0.589 ± 0.039 | 0.374 ± 0.076 | 0.219 ± 0.014 | 0.139 ± 0.013 | 0.114 ± 0.028 | 0.111 ± 0.014 | 0.020 ± 0.001 |
| Heart | 0.112 ± 0.014 | 0.097 ± 0.007 | 0.060 ± 0.005 | 0.050 ± 0.013 | 0.036 ± 0.008 | 0.035 ± 0.004 | 0.011 ± 0.001 |
| Str./Cereb | 3.677 ± 0.399 | 4.324 ± 0.515 | 5.824 ± 0.643 | 3.949 ± 0.260 | 3.642 ± 0.341 | 3.495 ± 0.309 | 1.762 ± 0.114[2] |
| Str./Occ. cort | 2.251 ± 0.190 | 2.638 ± 0.215 | 3.780 ± 0.149 | 2.597 ± 0.243 | 2.278 ± 0.189 | 2.772 ± 0.662 | 1.614 ± 0.073[2] |
| Hyp./Cereb | 1.843 ± 0.182 | 1.896 ± 0.070 | 2.155 ± 0.635 | 1.971 ± 0.056 | 2.191 ± 0.093 | 2.385 ± 0.086 | 1.318 ± 0.125[3] |

[1]*Data are given as the %ID x kg/g and represent the mean ± SD of three rats.*
[2]*ANOVA revealed no significant time trend for the striatum-to-cerebellum and striatum-to-occipital cortex ratios obtained at 2, 3, and 4 h p.i., in contrast to those obtained at 15 and 30 min and 1 h p.i.*
[3]*ANOVA revealed no significant time trend for the hypothalamus-to-cerebellum ratios obtained at 15 and 30 min and 1, 2, 3, and 4 h p.i.*

Displacement studies with fluvoxamine did not significantly change the striatal uptake of [$^{123}$I]FP-CIT, but did reduce the uptake in the hypothalamic and occipital cortical regions. This data shows that [$^{123}$I]FP-CIT uptake in rat striatum is mediated *via* the DaT since only GBR 12909 and not fluvoxamine was able to block and displace the uptake. This study shows that while [$^{123}$I]FP-CIT is selective for both dopamine and serotonin transporters, overall uptake in serotonergic regions of the brain is much lower than that in dopaminergic areas.

SPECT studies in monkey brain:

This study involved 10 male rhesus monkeys (*Maccaca mulatta*; 4-13 kg body weight). A unilateral 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion was induced by an intracarotid infusion of MPTP-RCI in the left carotid artery of 8 of the monkeys. SPECT studies were performed 8 to 10 weeks or approximately 4 years post-lesioning in 3 ("short-term lesioned") and 5 ("long-term lesioned") monkeys, respectively. The remaining 2 monkeys were employed as controls. Monkeys received an intravenous injection of 95-127 %1Bq [$^{123}$I]FP-CIT..For analysis of striatal [$^{123}$I]FP-CIT uptake, the striatum-to-occipital cortex ratio of uptake was calculated in the slice with the most intense striatal uptake, using a standard ROI template.

**Results:**

The [$^{123}$I]FP-CIT uptake ratio on the side of the unilateral MPTP treatment (left striatum) was approximately one, whereas on the non-lesioned side (right striatum) seems to have slightly increased in all MPTP-treated monkeys. However, in the control animals, there was a symmetrical striatal uptake of [$^{123}$I]FP-CIT 3 hours after an injection of [$^{123}$I]FP-CIT, as shown in sponsor's Figure 3, Thus, in MPTP-lesioned Rhesus monkeys, there is severe loss of specific striatal uptake of radioactivity which is consistent with loss of all indicators for the dopaminergic system, including the DaT.

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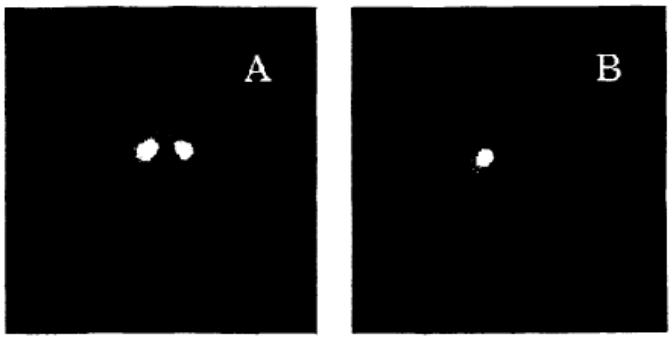
**FIGURE 3.** [$^{123}$I]FP-CIT SPECT images in a control and a unilateral MPTP-treated monkey.




A: SPECT image at 3 h p.i., showing symmetric binding in the left and right striatum of a control monkey. B: SPECT image at 3 h p.i., showing lack of striatal uptake at the side of MPTP treatment.

Reviewer's Comments: The reviewer agrees with the result of this study which shows that [$^{123}$I]FP-CIT binds specifically to the striatal DaT *in vivo*.

**Additional referenced studies:** The sponsor made reference to the following published studies to support the efficacy of DaTSCAN.

1) Andriga et al (2005) – This study involved pre-treated mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce varying degrees of dopaminergic neurodegeneration. The result demonstrates a strong correlation between *in vivo* striatal [$^{123}$I]FP-CIT uptake and *ex vivo* striatal DaT immunoreactivity. As shown in sponsor's Figure 2.6.2-4, the striatal binding determined by [$^{123}$I]FP-CIT positively correlated with the level of DaT immunoreactivity ($R_2$=0.7844).

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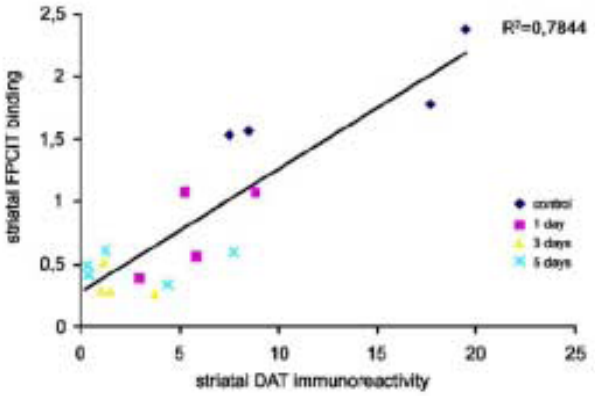


Figure 2.6.2-4 Correlation analysis of [123I]FP-CIT SPECT imaging and DaT immunohistochemistry in the striatum of MPTP-treated mice

2) Alvarez-Fischer et al (2007) – Quantitative [$^{123}$I]FP-CIT pinhole SPECT in two mouse models of parkinsonism was employed in this study. Male mice (24 to 26 g) were injected intraperitoneally with MPTP hydrochloride (25 mg/kg) once daily for 5 days to induce a bilateral mild lesion of the nigrostriatal system. Another group of animals were unilaterally injected with 6-hydroxydopamine (6-OHDA; 4 µg), directly into the right striatum. The brains were processed for determination of DA concentration and numbers of neurons in the substantia nigra by tyrosine hydroxylase immunostaining. As shown in sponsor's Figure 2.6.2-5A, the [$^{123}$I]FP-CIT binding ratio in both striata of MPTP-treated mice was significantly lower compared with saline-treated controls (1 week: 43±2.7%; 2 weeks: 52±0.5%; 4 weeks: 52±6.8%). This indicates a significant linear positive correlation between the striatal DA concentration and the striatal [$^{123}$I]FP-CIT binding ratio. A severe unilateral lesion was induced in the 6-OHDA model. Intense [$^{123}$I]FP-CIT binding was observed in the intact striatum but the binding ratio on the lesioned side was significantly lower compared with the unlesioned contralateral side (1 week: 50.5±6.2%; 2 weeks: 42.8±10.2%; 4 weeks: 34.9±5.2%) (Figure 2.6.2-5B). This study showed that [$^{123}$I]FP-CIT binding remains correlated with a loss of function of the neuron as a whole.

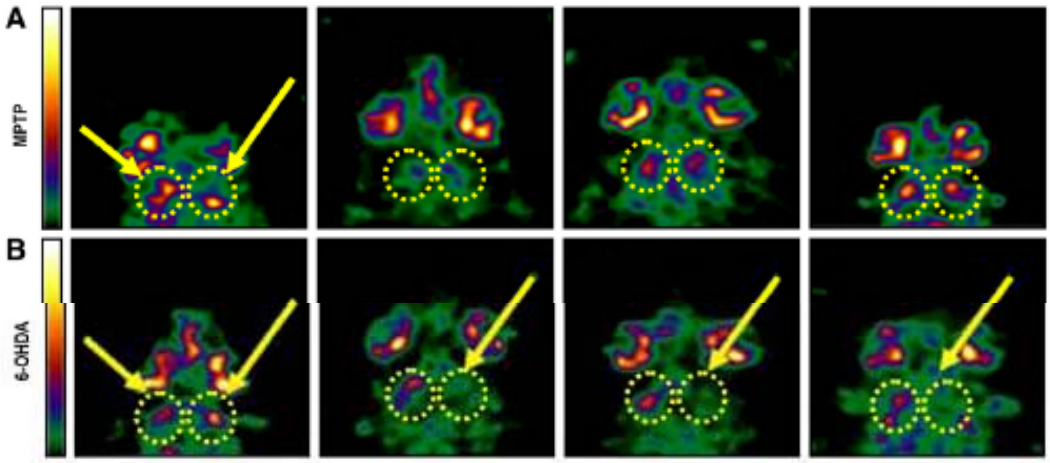


Figure 2.6.2-5 Iodine-123 FP-CIT SPECT in parkinsonian mice:
Horizontal SPECT images showing striatum (yellow circles) of both hemispheres in representative control mice (saline) or in parkinsonian mice at different time points (1, 2 or 4 weeks) after intraperitoneal injection of MPTP (A) or after injection of 6-OHDA into the right striatum (B).

**Reviewer's Comment:** The sponsor provided study reports and published articles on *in vitro* and *in vivo studies* to establish the efficacy and the pharmacology of this compound. The available *in vitro* data from a variety of preparations indicate that FP-CIT has high affinity, and also demonstrates some selectivity and relative regional transporter densities for the DaT. Radiolabelled FP-CIT showed a high selectivity in distinguishing between DaT-rich and deficient regions of the post-mortem human brain. The sponsor presented data from *in vivo* studies performed in a range of animal species evaluating FP-CIT as a

selective ligand for, or marker of, the DaT. [$^{123}$I]FP-CIT demonstrates a correlation between *in vivo* imaging data and both *ex vivo* quantitative dissection data and the degree of loss induced by a number of different drug-treatment regimes including the administration of the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), to mice, cynomolgus monkeys and rhesus monkeys. The *in vitro* and *in vivo* data indicates that radiolabeled FP-CIT is a potential marker for dopaminergic neuronal density in the striatum.

### 2.6.2.3 Secondary pharmacodynamics

**Study title: (b) (4)-155B: Behavioral Pharmacology Study of β-CIT-FP in Rats**

**Key study findings**: The sponsor provided the summary translation of this study. The study compared β-CIT-FP with cocaine with respect to its discrimination-stimulating effect in normal rats (cocaine appropriate responses). The rotatory behavior-inducing activity with unilaterally destroyed substantia nigra was also evaluated. No significant increased cocaine appropriate responses was induced by an intravenous injection of CIT-FP at 884.4X MHD while dose dependent cocaine-appropriate responses were 43.3, 93 and 100% were reported at higher doses of 2948X, 8844X and 29480X MHD respectively. In assessing functional specificity of FP-CIT for DaT, there was unilateral increase in locomotor activity as expressed by circling behavior due to disrupted dopaminergic pathway on the lesioned side of the brain. FP-CIT (88440X MHD) and cocaine significantly increased the number of rotations compared to the vehicle response. This study showed that CIT-FP induces antagonistic physiological effect on DaT. However, the discrimination choice test and apormorphine-induced circling indicates comparable functional effect and efficacy of FP-CIT to cocaine at doses in the order of 29000X MHD

**Study no.**: (b) (4)-155B

**Volume # and page #**: Volume 1 pages 1- 3

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: August 7, 1996

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: β-CIT-FP, Lot # NMP-1035 and purity: not provided.

**Study Design:**

Doses: 0.03, 0.1, 0.3, 1 and 3 mg/kg – 884.4X, 2948X, 8844X, 29480X and 88440X MHD respectively (CIT-FP) and 10 mg/kg (cocaine).

Species/strain: Sprague Dawley rats

Weight: Not provided

Number/sex/group or time point: 5 or 6 rats per group.

Route of administration: Intravenous injection

**Main Study:** The rats were taught to discriminate (as assessed by correct lever pressing) between the presence and absence of effects induced by the DaT inhibitor, cocaine (10 mg/kg i.p.). The discrimination-stimulating effect was used as a measure of cocaine appropriate responses in this study. The rats were then intravenously administered CIT-FP 0.03, 0.1, 0.3, 1 and 3 mg/kg, cocaine 10mg/kg and saline vehicle for the choice test. Functional specificity of FP-CIT for the DaT was assessed by the ability of the ligand to increase apomorphine-induced circling movement in rats with a unilateral lesion in the substantia nigra. Prior to the intravenous administration of cocaine (0.3 to 3.0 mg/kg, iv; n = 6 to 8), FP-CIT (0.03 to 1.0 mg/kg, iv; n = 6 to 8) or vehicle (n=29) one substantia nigra was lesioned with 6-OHDA (8 µg/kg). The rotational movement of the animals was evaluated.

**Results:**
The sponsor provided translated version of the final study report in form of a summary with a declaration of translation authenticity. In the vehicle controls, cocaine-appropriate lever pressing was 18.3% of the total lever pressings and FP-CIT (0.03 mg/kg) did not significantly increase the percentage of the cocaine-appropriate responses. At 0.1, 0.3 and 1.0 mg/kg doses, CIT-FP induced a dose-related effect of 43.3, 93 and 100% correct (cocaine-appropriate) responses respectively. Similarly, cocaine induced 30, 25 and 75% cocaine appropriate responses at doses of 0.1, 0.3 and 1.0 mg/kg respectively. FP-CIT (1 mg/kg) increased the vehicle circling movement response from 106±80 to 90±320% while cocaine significantly increased rotational movement at 1 and 3 mg/kg (from 92±63% of the apomorphine response in vehicle animals to 98±63 and 159±52% in animals administered 1 and 3 mg/kg respectively).

**Reviewer's Comments:** The study indicates that CIT-FP demonstrates cocaine-like pharmacological activity on DaT at extremely high doses of over several ten thousands folds. Therefore, it is highly unlikely that β-CIT-FP would exhibit cocaine-like effects when administered at the clinical dose for this indication.

**Study title: (b) (4)-51-80: Combinational Effects of FP-CIT on the Pharmacological Actions of L-DOPA, Bromocriptine, and Amantadine in Parkinsonism Model Rats**

**Key study findings**: This study examined the possibility that combination with FP-CIT could enhance or reduce the pharmacological actions of therapeutic drugs used for treating Parkinson disease. The effect of FP-CIT on the pharmacological actions of L-DOPA, bromocriptine and amantadine was evaluated. Rats with substantia nigra bilaterally lesioned with 6-OHDA were used in this study to measure locomotor activity and to observe clinical signs of stereotypical behaviors and convulsions. The effect of FP-CIT on spontaneous locomotor activity in the presence or absence of various Parkinson disease (PD) therapeutics was evaluated. The combinations with FP-CIT 0.1 mg/kg (2948X MHD) and GBR-12935 0.1 mg/kg do not affect the stimulating activity of L-DOPA on locomotor activity in rats with substantia nigra bilaterally lesioned with 6-hydroxydopamine (6-OHDA), but that combinations with FP-CIT 1 mg/kg (29480X MHD) and GBR-12935 1 mg/kg prolong the actions of L-DOPA. Furthermore, combinations with FP-CIT (2948X and 29480X MHD) and GBR-12935 (0.1 and 1 mg/kg) were shown not to affect the stimulating activity of bromocriptine and amantadine on locomotor activity.

**Study no.**: (b) (4)-51-80

**Volume # and page #**: Volume 1 pages 1- 44

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: June 1, 1996

**GLP compliance**: No

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: β-CIT-FP, Lot # NMP-1035 and purity: 99.4%.

**Study Design:**
Doses: 0.1 and 1 mg/kg (2948X and 29480X MHD) as shown in the Table below

Species/strain: Male Sprague Dawley rats/Crj:CD(SD)

Weight: Not provided

Number/sex/group or time point: 8 rats per group.

Route of administration: Intravenous injection

**Main Study:** The rats were fixed on the brain stereotaxic apparatus under diethyl anesthesis. A microsyringe was inserted into both substantia nigra and 6-OHDA was injected at a rate of 1 μL/min. Two days after the injection, the locomotor activity was measured and animals with reduced locomotor activity were selected for the study. Rats

with bilaterally lesioned substantia nigra were then dosed as in the Table below. An automatic locomotor activity measuring device was used to measure extent of motion for every 15-minute period over the 240-minute period after study article administration, and the actions of study article were examined.

**Dose levels and numbers of animals**

| Group | Test article (dose level) | Number of animals |
|---|---|---|
| 1 | Vehicle* + vehicle** | 8 |
| 2 | Vehicle* + FP-CIT (0.1 mg/kg) | 8 |
| 3 | Vehicle* + FP-CIT (1 mg/kg) | 8 |
| 4 | Vehicle* + GBR-12935 (0.1 mg/kg) | 8 |
| 5 | Vehicle* + GBR-12935 (1 mg/kg) | 8 |
| 6 | L-DOPA (10 mg/kg)*** + vehicle** | 8 |
| 7 | L-DOPA (10 mg/kg)*** + FP-CIT (0.1 mg/kg) | 8 |
| 8 | L-DOPA (10 mg/kg)*** + FP-CIT (1 mg/kg) | 8 |
| 9 | L-DOPA (10 mg/kg)*** + GBR-12935 (0.1 mg/kg) | 8 |
| 10 | L-DOPA (10 mg/kg)*** + GBR-12935 (1 mg/kg) | 8 |
| 11 | Bromocriptine (2 mg/kg) + vehicle** | 8 |
| 12 | Bromocriptine (2 mg/kg) + FP-CIT (0.1 mg/kg) | 8 |
| 13 | Bromocriptine (2 mg/kg) + FP-CIT (1 mg/kg) | 8 |
| 14 | Bromocriptine (2 mg/kg) + GBR-12935 (0.1 mg/kg) | 8 |
| 15 | Bromocriptine (2 mg/kg) + GBR-12935 (1 mg/kg) | 8 |
| 16 | Amantadine (50 mg/kg) + vehicle** | 8 |
| 17 | Amantadine (50 mg/kg) + FP-CIT (0.1 mg/kg) | 8 |
| 18 | Amantadine (50 mg/kg) + FP-CIT (1 mg/kg) | 8 |
| 19 | Amantadine (50 mg/kg) + GBR-12935 (0.1 mg/kg) | 8 |
| 20 | Amantadine (50 mg/kg) + GBR-12935 (1 mg/kg) | 8 |

*: The 0.5% tragacanth gum solution
**: The 1% ascorbic acid solution
***: Administer benserazide (2.5 mg/kg) concurrently.

**Results:**
The sponsor provided translated version of the final study report and provided a declaration of translation authenticity. Over the 240-minute period after treatment, there was no intrinsic activity of vehicle or FP-CIT (2948X MHD) on spontaneous movement as shown in sponsor's Figure 1. However, at 29480X MHD, FP-CIT induced a significant, but small increase in locomotor activity of 2.6-fold greater than baseline. This suggests that FP-CIT increases the availability of the small amount of endogenous DA released from those nerve terminals remaining after 6-OHDA lesioning. This represents only 6% of the observed increase in locomotor activity induced by L-DOPA (10 mg/kg). The GBR-12935 (0.1 and 1 mg/kg) alone administration groups showed no significant difference in locomotor activity. Co-administration of L-DOPA (10 mg/kg) with FP-CIT (2948X MHD) did not further increase spontaneous movement over the 240 minutes observation period. However, ten fold increase in dose of FP-CIT (29480X MHD) significantly increased locomotor activity over the period of study to 157% that of L-DOPA alone. This further show that FP-CIT could function on DaT. FP-CIT did not affect the locomotor activity of bromocriptine while bromocriptine alone induced an elevation in activity that did not decrease within the period of observation. Thus, FP-CIT had no opportunity to demonstrate prolongation of action. The study showed that amantadine alone did not induce any significant elevation of locomotor activity. This is presumably due to the low levels of endogenous DA, post-lesion. Similarly, lack of

response was observed with animals treated with FP-CIT in combination with amantadine.

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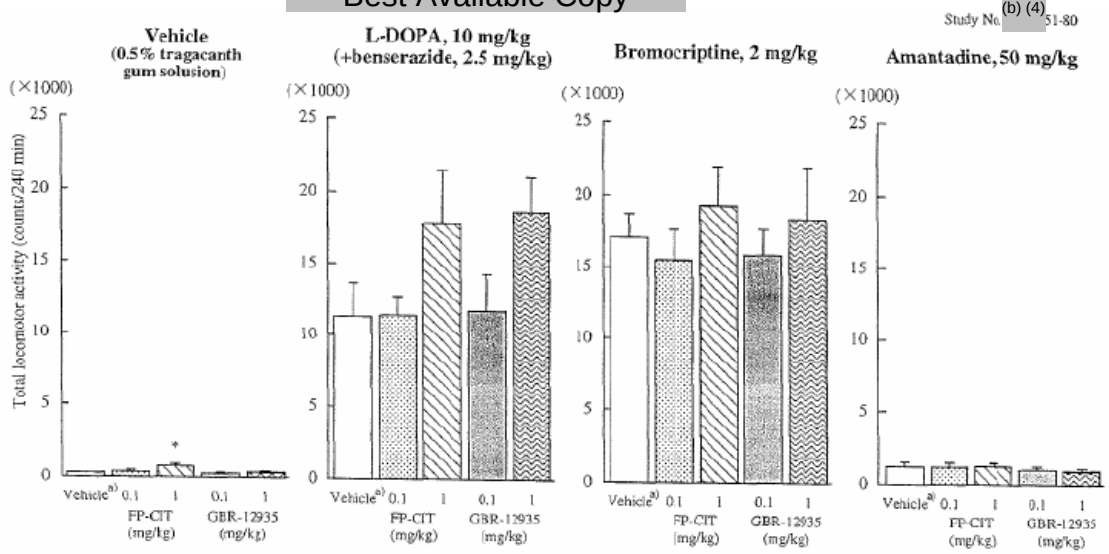


**Fig. 1 Effects of FP-CIT and GBR-12935 combined with anti-parkinson drugs on the locomotor activity in rats with bilaterally nigrostriatal lesion induced by 6-OHDA.**

Each column represents the mean±S.E. (n=8).
* : Significantly different from the vehicle at p<0.05.
a) : 1% ascorbic acid solution

**Reviewer's Comments:** The sponsor provided detailed translated data of this study in form of Tables, graphs and raw data from the original version of the study report. The study indicates that:

1) No stereotypical behaviors and convulsions affecting locomotor activity developed in any group indicating a successful evaluation of the study using locomotor activity parameter.
2) Combination of FP-CIT and GBR-12935 (0.1 mg/kg) does not affect the increasing effect of L-DOPA on locomotor activity in rats with both substantia nigra lesioned with 6-OHDA.
3) Combination of FP-CIT and GBR-12935 1 mg/kg prolongs the actions of L-DOPA.
4) Combinations with FP-CIT (0.1 and 1 mg/kg) and GBR-12935 (0.1 and 1 mg/kg) did not affect the stimulating activity of bromocriptine and amantadine on locomotor activity.

The data obtained from this study could not ascertain that the combination with ioflupane will actually not induce pharmacological effects on the rats treated with antiparkinson medications employed in this model as broadly claimed by the sponsor. The data only showed that ioflupane when administered in combination with L-DOPA, bromocriptine and amantadine might not affect locomotor activity. The study failed to assess any possible effect on the SPECT imaging with ioflupane in combination with any of the antiparkinson medications.

**2.6.2.3 Secondary pharmacodynamics**

**2.6.2.4 Safety pharmacology**

**Study title: 2838: FP-CIT: A single intravenous dose modified Irwin Screen test in rats.**

**Key study findings**: FP-CIT was evaluated in a modified Irwin test in Sprague-Dawley rats. Male rats were administered intravenous doses (0.06 – 60 µg/kg) of the compound and a modified Irwin-type Functional Observation Battery (FOB) was performed at the following times post-dosing: 10, 30, 60 minutes, 4 hours and 2, 4 and 8 days. No mortality was reported in the rats; however, there were dose-related alterations in respiration in all treated animals and alterations in muscle tone and traction were reported in rats administered 60 µg/kg dose.

**Study no.**: 2838

**Volume # and page #:** Volume 1 pages 1- 26

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: March 10, 1998

**GLP compliance**: Yes

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: [$^{123}$I]FP-CIT, Lot # and purity : Not provided.

**Study Design:**

Doses: 0.06, 6.0 and 60 µg/kg (1.8X, 176.9X and 1769X MHD)

Species/strain: Male Sprague Dawley rats (Tac:N(SD)fBR)

Age: 6 weeks

Weight: 149 -153.5g

Number/sex/group or time point: 3 male rats per group.

Route of administration: Intravenous administration at a volume of 0.6 mL/kg.

Vehicle: Sodium acetate buffer (50 mL) diluted with 2.5 mL ethyl alcohol (5% ethyl alcohol; 95% sodium acetate buffer).

Control article: 0.9% sodium chloride injection.

**Main Study:** FP-CIT labeled with non-radioactive $^{123}I$ was administered intravenously (via lateral tail vein) to three groups of three male rats per group at (1.8X, 176.9X and 1769X MHD. Modified Irwin-type Functional Observation Battery (FOB) in a blinded fashion was performed at the following times post-dosing: 10, 30, 60 minutes, 4 hours and 2, 4 and 8 days. The body weights were measured on the day before dosing and on days 1, 2, 4 and 8.

**Results:** No mortality or treatment-related body weight changes was reported. As shown by the modified Irwin scores provided by the sponsor, there were piloerection, labored respiration, increased defecation, touch reactivity, positional passivity, and alterations in muscle tone in the FP-CIT treated animals. The reported alteration in respiration was dose-related while significant alterations in muscle tone were observed at 1769X MHD.

**Reviewer's Comments:** This study shows that FP-CIT (1.8X - 1769X MHD) induced alterations in respiration following a single intravenous injection during a 7-day observation period with FOB. Reports of respiratory effect at a dose as low as 0.06 µg/kg (1.8X MHD) is probably a cocaine-like pharmacologic effect demonstrated by this compound at a relatively low dose. Potentially, FP-CIT could induce labored respiration in humans when administered at the clinical dose. Thus, extra caution should be exercised in administering this compound to patients with underlying respiratory diseases. It is however unlikely this compound would exhibit any alterations in muscle tone when administered to humans at the planned dose since the muscle tone alterations were reported at 1769X MHD. The reviewer agrees with the result of this study which shows that despite the report of no mortality and no treatment-related change in body weight, there were functional effects at all employed doses.

**Study title: 2001NM017: Safety Pharmacology Study of FP-CIT.**

**Key study findings**: This study involved safety pharmacology evaluation of FP-CIT on the cardiovascular, respiratory and central nervous systems (CNS) using rats and dogs. FP-CIT demonstrated no effect on the motor coordination, locomotor activity and body temperature in rats intravenously administered up to 2948X MHD. However, there were enhanced stereotypical behaviors in 1 of 6 rats administered 2948X MHD FP-CIT. FP-CIT induced no cardiovascular and respiratory effects in dogs administered up to 294.8X MHD. However, FP-CIT at high dose (2948X MHD) caused increased blood pressure, heart rate and respiratory rate.

**Study no.**: (b) (4) Study No. 2001NM017

**Volume # and page #**: Volume 1 pages 1- 73

**Conducting laboratory and location**: (b) (4)

(b) (4)

**Date of study initiation**: June 18, 2002

**GLP compliance**: Yes

**QA report**: yes (x) no ( )

**Drug, lot #, and % purity**: FP-CIT, Lot # FSL03 and purity is stated to be 98.8%.

**Study design:**
(1) General pharmacology study of β-CIT-FP (the same substance as FP-CIT) in rats (study No. (b) (4)-155C).
(2) Effects of β-CIT-FP on the respiratory and cardiovascular systems of unanesthetized dogs (study No.: NM97154).

Study 1- Species/strain: Male Sprague Dawley rats /Crj:CD (SD) IGS

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

Age: 6-7 weeks

Weight: 202-279g

Route of administration: Intravenous infusion via tail vein at 1 mL/minute and a dosing volume of 1 mL/kg.

Vehicle control: 1% (w/v) ascorbic acid solution

Positive control: None was employed

Study 2- Species/strain: Male Beagle dogs

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

Age: 8-9 months

Weight: 10.70-12.80 kg

Route of administration: Intravenous infusion via the cephalic vein at 3 mL/minute rate and a dosing volume of 1 mL/kg.

Vehicle control: 1% (w/v) ascorbic acid solution

Positive control: None was employed

Doses: 1, 10, and 100 µg/kg (98.3X, 982.8X and 9828X MHD respectively).

**Main Study:** Study 1-The effects on the central nervous system was evaluated using the following tests:

1) Functional observational battery (FOB): This involved administration of the test article or vehicle intravenously to 6 rats per group. The animals were observed based on items of the FOB before and at 0.25, 0.5, 1, 1.5, 3, and 6 hours after administration.

2) Effects on extent of locomotor activity: This involved using a locomotor activity extent-measuring device (ACTY303, Biomedica) to measure extent of locomotor activity of the test article- or vehicle-administered rats (8 rats per group). This parameter was assessed in each animal before and at various time intervals after administration.

3) Effects on motor coordination: In this study, test article or vehicle was administered intravenously to 10 animals per group. Rotor rod (6 cm in diameter, Rota-rod, BRR-R04, Biomedica) was used to count the number of animals that fell from the rotor rod within 1 minute at the various (14, 10 and 7) rotation numbers/minute before administration and at 0.25, 0.5, 1, 1.5, 3, and 6 hours after administration. The maximal rotation number at which all 4 selected animals did not fall was employed as rotation number in this study. From the preliminary trial, 4 animals fell at 14 rotations/minute, 3 animals fell at 10 rotations/minute, and none of the animals fell at 7 rotations/minute.

4) Effects on body temperature: This study involved intravenous administration of the test article or vehicle to 8 rats per group. A thermistor thermometer was used to measure the rectal temperature before and at 0.25, 0.5, 1, 1.5, 3, and 6 hours after administration.

Study 2-The effects on the cardiovascular and respiratory systems was assessed in the dogs as below:
Effects on respiration, blood pressure, heart rate, and electrocardiogram of conscious animals: The dogs were anesthetized and telemeter (TL11M2-D70-PCT, Data Sciences International Inc.) was subcutaneously implanted in the left abdomen of dogs. The catheter for blood pressure measurement was inserted to the abdominal aorta via the left femoral artery while the vital potentiometric electrodes for the measurement of electrocardiogram (lead II) were also subcutaneously implanted in the right thorax and the left abdomen of the animals. After a week of recovery, the respiratory rate, heart rate, blood pressure (diastolic, systolic, mean), and electrocardiogram (PQ duration, QRS interval, and QTc duration) were measured before and at 0.1, 0.25, 0.5, 1, 1.5, 3, and 6 hours after intravenous administration of the test article or vehicle.

**Results:**

FOB: A rat exhibited sniffing and rearing as stereotypical behaviors at 0.25 and 0.5 hours after administration of 9828X MHD FP-CIT. However, no significant FOB change was reported at the employed doses of FP-CIT in this study compared to the control group.

Effect on extent of locomotor activity: No significant change was observed.

Effect of motor coordination: No effect on motor coordination was observed.

Effect on body temperature: There was no significant change in the body temperature.

Effects on heart rate: No effect on heart rate was induced by FP-CIT. However, at 9828X MHD dose, there was increased heart rate from a preadministration value of 68.4 beats/minute to 84.4 beats/minute at 0.1 hour after the completion of administration, followed by a gradual decrease to 72.8 beats/minute at 0.5 hours after the completion of administration, nearly reaching the preadministration value.

Effects on blood pressure: No effect on blood pressure was demonstrated by FP-CIT in this study. There was s significant elevated blood pressure in the 9828 MHD group in comparison to the controls.

Effects of respiratory rate: No effect on respiratory rate was reported. However, there was increased respiration in 9828X MHD group when compared to the controls. Respiratory rate increased gradually after administration and exhibited a higher value of 8 breaths/minute than the preadministration value at 0.5 hours after the completion of administration. The respiratory rate decreased gradually thereafter and nearly reached the preadministration value at 1.5 hours after the completion of administration.

Effects on $SpO_2$: No effect on $SpO_2$ was reported at the employed doses in this study.

Effects on electrocardiogram: No electrocardiography effect was reported in all treated animals.

**Reviewer's Comments:** The sponsor provided detailed translated data of this study in form of Tables, graphs and raw data from the original version of the study report. The declaration of translation authenticity was also provided. This reviewer agrees with the result of this study which showed that FP-CIT at 9828X MHD had no effect on the central nervous system of rats and on the cardiovascular and respiratory systems of dogs. At 100 µg/kg dose, 1 of 6 rats showed enhanced stereotypical behavior and dogs showed increase in blood pressure, heart rate, and respiratory rate. The sponsor's justification for the lack of a positive control article in this study was based on the good record in conducting experiments at the facility. However, this justification is not acceptable to this reviewer in that it would be difficult to detect any flaw in the experimental procedure which may lead to an underestimation of the cardiovascular, respiratory or CNS effects of FP-CIT. This study indicates that FP-CIT (10 µg/kg) had no effect on the central nervous system of rats and on the cardiovascular and respiratory systems of dogs. However, in dogs, FP-CIT at 9828X MHD induced slight symptoms (enhanced stereotypical

behaviors), an increase in blood pressure, an increase in heart rate, and an increase in respiratory rate. Based on the multiples of the human dose at which the effects occurred, it is highly unlikely that FP-CIT would cause any CNS, respiratory or cardiovascular adverse effects at proposed dose.

**Study title: 2837: FP-CIT: A Nonclinical Intravenous Cardiovascular Safety Pharmacology Study in Beagle Dogs.**

**Key study findings**: In this study, the effects of FP-CIT (up to 5897X MHD) on the cardiovascular and respiratory systems were monitored in conscious telemetered dogs. No morbidity, deaths or alterations in clinical signs and no statistically significant changes in heart rate, arterial blood pressure, systolic or diastolic blood pressure, ECG and respiratory rate following administration of vehicle or the escalating doses of FP-CIT as compared with saline was reported in this study. Thus, an intravenous administration of FP-CIT at doses up to 590X MHD had no effect on the cardiovascular system of normal conscious telemetered beagle dogs.

**Study no.**: 2837

**Volume # and page #:** Volume 1 pages 1- 91

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: March 10, 1998

**GLP compliance**: No.

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: FP-CIT, Lot # LMP-1036 and purity: Not provided.

**Study Design:**

Doses: 0.06, 6.0 and 60 µg/kg (5.9X, 589.7X and 5897X MHD)

Species/strain: Male and female Beagle dogs/*Canis familiaris*

Age: 8 to 10 months

Weight: 8.5 to 9.9 kg

Number/sex/group or time point: 2 male and 2 female dogs per group.

Route of administration: Intravenous administration at a volume of 0.06 mL/kg.

Vehicle: 5% ethanol, sterile sodium acetate buffer, pH 4.7.

Control article: Sodium chloride injection, USP.

**Main Study:** The dogs were surgically implanted telemeter and blood pressure catheter. After 2 weeks recovery, the dogs were administered a single injection of the controls and escalating drug product during the study, spaced by 72-96 hours. The dogs were conscious throughout the treatment and observation period. The heart rate, arterial blood pressure, systolic and diastolic blood pressure, and ECG were monitored prior to initiation of the study, prior to dosing and at 10 minute intervals for approximately one hour post-dosing and hourly through 24 hours. The animals were also monitored for appearance, behavior and mortality.
**Results:** No mortality or treatment related body weight change was reported. There were no statistically significant changes in heart rate, body weight, body temperature, arterial blood pressure, systolic or diastolic blood pressure following administration of vehicle or the escalating doses of FP-CIT as compared to saline. Respiratory rate was also similar among all groups. ECG showed normal sinus rhythm without any apparent prolongation of QT-interval.

**Reviewer's Comments:** The sponsor provided the raw and analyzed data of this study. The data indicates that intravenous administration of FP-CIT at a dosage of up to 589X MHD caused no effect on the cardiovascular system of normal conscious Beagle dogs assessed by remote telemetry. The reviewer agrees with the result of this study.

**Study title**: **B001002: Effect of Ioflupane on hERG Tail Current Recorded from Stably Transfected HEK293 Cells.**
**Key study findings**: In this study, the potential of FP-CIT to inhibit hERG tail current was evaluated in human ether-a-go-go-related gene (hERG)-encoded channel tail current recorded from HEK293 cells stably transfected with hERG cDNA. FP-CIT inhibited hERG tail current in a concentration-dependent manner. The estimated nominal $IC_{25}$, $IC_{5O}$ and $IC_{75}$ values for FP-CIT inhibition of hERG tail current were 0.12, 0.49 and 2.0 1μM, respectively. The estimated $IC_{25}$, $IC_{5O}$ and $IC_{75}$ were 0.070, 0.29 and 1.2 μM, respectively.

**Study no.**: B001002

**Volume # and page #**: Volume 1 pages 1- 65

**Conducting laboratory and location**: GE Healthcare AS,
Nycoveien 1-2, P.O. Box 4220, Nydalen, N-0401
Oslo, Norway.

**Date of study initiation**: 3 May, 2002

**GLP compliance**: Yes

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: FP-CIT, Batch # FFC00051071-03 and purity is stated to be 99.7%

**Study Design:**

Doses: 0.03, 0.1, 3, and 10 µM.

Number/sex/group: Ioflupane: n=4 cells per concentration; DMSO: n=4 cells and E-4031: n=2 cells.

Vehicle: Dimethyl sulfoxide (DMSO); 0.1%

Reference substance: E-4031 (100nM)

Control article: Sodium chloride injection, USP.

**Main Study:** Groups of cells were treated with vehicle, reference substance, or a range of concentrations of ioflupane as below:
a) loflupane (nominal concentrations 0.03, 0.1, 0.3, 3 and 10 µM); n = 4 cells per concentration
b) Vehicle (0.1% DMSO); n = 4 cells and
c) Reference substance (100 nM E-4031); n = 2 cells (vehicle treated).

The cells were covered with coverslips and transferred to the recording chamber and continuously perfused (1-2 mL/min) with bath solution at room temperature. The recordings were performed in the voltage-clamp mode, with the membrane voltage initially clamped at -80 mV, after a stable patch was achieved. The concentration-response relationship for ioflupane at 0.03, 0.1, 0.3, 3 and 10 µM was assessed on 4 cells per concentration. The effect of the vehicle (0.1 % DMSO) was examined in a separate set of 4 cells, E-4031 (100 nM) applied to 2 cells was used as positive control. The $IC_{25}$, $IC_{5O}$ and $IC_{75}$ values for inhibition of hERG tail current were estimated.

**Results:** Ioflupane statistically significantly inhibited hERG tail current in a concentration-dependent manner at 0.1, 0.3, 3 and 10 µM concentrations. No statistically significant inhibitory effect was reported at 0.03 µM iofluopane and vehicle treated groups on hERG tail current were compared. The estimated nominal $IC_{25}$, $IC_{5O}$ and $IC_{75}$ values for ioflupane inhibition of hERG tail current were 0.12, 0.49 and 2.0 µM, respectively. The estimated $IC_{25}$, $IC_{5O}$ and $IC_{75}$ obtained from the concentration-response curve plotted using the measured concentrations in the recording chamber samples were 0.070, 0.29 and 1.2 µM, respectively. The positive control, E-4031 (100 nM) inhibited hERG tail current by 97.1 %.

**Reviewer's Comments:** The reviewer agrees with the result of this study as presented above. The positive control, E-4031 (100 nM), induced up to 91% inhibition on the hERG cells. No significant inhibition was induced on the hERG cells by ioflupane at 0.03 µM concentration (100 X the calculated human plasma concentration of DaTSCAN immediately after intravenous injection) and by 0.1% DMSO treated group. However, a concentration-dependent inhibition was observed at higher concentrations (0.1 - 10 µM - 1000X - 3000X human plasma concentration). It is therefore very unlikely for ioflupane to pose any risk with respect to QT interval prolongation at clinical relevant dose.

### 2.6.2.5 Pharmacodynamic drug interactions

**Study title: (b) (4)/PE9846: Study of the effects of the repeated administration of anti-Parkinson disease drugs on the binding of $^{125}$I-FP-CIT to the membrane fraction of the rat striatum**

**Key study findings**: This study examined the effect on the striatal binding of [$^{125}$I]FP-CIT of repeated dosing with drugs used in the management of Parkinson's disease. The changes in the $K_d$ and $B_{max}$ of [$^{125}$I]FP-CIT to rat striatal membrane preparations were assessed following chronic treatment with the DA receptor agonists L-DOPA administered in combination with benserazide (12.5 mg/kg), bromocriptine (4 mg/kg), amantadine (50 mg/kg) (DA releasing agent), trihexiphenidyl (10 mg/kg) (anti-cholinergic agent) or vehicle (0.75% carboxymethyl cellulose solution). In this study, repeat administration of the above anti-Parkinson drugs for 21-day did not affect the binding profile of FP-CIT to the membrane fractions of the striatum of the treated rats.. This shows that chronic treatment with therapeutics commonly used for the management of Parkinson disease might possibly not affect the binding characteristics of FP-CIT.

**Study no.**: (b) (4)PE9846

**Volume # and page #**: Volume 1 pages 1-138

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: October 1, 1998

**GLP compliance**: No

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: $^{123}$I-FP-CIT, Lot # IFP-C9365) and purity: approximately 100%.

**Study Design:**

Doses: As shown in the Table below: Best Available Copy

| Study group | Dose level (mg/kg/day) | Concentration of the dosing solution (mg/mL) | Dosing volume (mL/kg/day) | Sex | Drug withdrawal period (day) | Number of animals | Animal number |
|---|---|---|---|---|---|---|---|
| Vehicle | 0 | 0 | 5 | Female | 0 | 6 | 301-306 |
| | | | | | 1 | 6 | 307-312 |
| | | | | | 3 | 6 | 313-318 |
| L-DOPA + benserazide | 50 + 12.5 | 10 + 2.5 | 5 | Female | 0 | 6 | 319-324 |
| | | | | | 1 | 6 | 325-330 |
| | | | | | 3 | 6 | 331-336 |
| Bromocriptine | 4 | 0.8 | 5 | Female | 0 | 6 | 337-342 |
| | | | | | 1 | 6 | 343-348 |
| | | | | | 3 | 6 | 349-354 |
| Amantadine | 50 | 10 | 5 | Female | 0 | 6 | 355-360 |
| | | | | | 1 | 6 | 361-366 |
| | | | | | 3 | 6 | 367-372 |
| Trihexyphenidyl | 10 | 2 | 5 | Female | 0 | 6 | 373-378 |
| | | | | | 1 | 6 | 379-384 |
| | | | | | 3 | 6 | 385-390 |

Species/strain: Female Sprague Dawley rats/Crj:CD(SD)

Age: 7 weeks

Weight: Not provided

Number/sex/group or time point: 6 female rats per group.

Route of administration: Intraperitoneally injection

**Main Study:** Female rats were intraperitoneally administered L-DOPA (50 mg/kg) + benserazide (12.5 mg/kg), bromocriptine (4 mg/kg), amantadine (50 mg/kg), trihexyphenidyl (10 mg/kg), or vehicle (0.75% carboxymethylcellulose sodium) repeatedly for 21-days. The pharmacological actions of anti-Parkinson disease drugs, locomotor activity was measured once every day during the administration period and during the drug withdrawal period (3 days) after the end of the administration period in a total of 30 rats (5 groups × 6 animals) that had been allocated to the 3-day drug withdrawal group in each study group. The cerebral striatum was excised at 2 hours (day 0 after drug withdrawal) and on days 1 and 3 after the last administration to prepare the membrane fraction before conducting the binding assay. The $K_d$ and $B_{max}$ values of the high and low affinity sites were calculated using Scatchard analysis to determine whether or not the binding profile of FP-CIT shows changes.

**Results:** The available data indicates an initial lack of increase in body weight immediately after the onset of repeated administration in the amantadine group. However, body weights increased uneventfully on day 4 of administration and later in all groups. There was no significant difference between the $K_d$ and $B_{max}$ values of the low and high affinity binding site of any of the treated groups and the vehicle group on day 0 after withdrawal. Similarly, on Day 3 none of the drug administration groups showed a significant difference of the $K_d$ and $B_{max}$ values of the low and high binding sites against the vehicle group. Thus, indicating that no significant change was found in the binding

parameters of the membrane fraction of the rat striatum immediately after the administration and after the drug withdrawal in all the treated groups.

**Reviewer's Comments:** This reviewer agrees with the result of this study which shows that long-term administration of anti-Parkinson drugs will not affect binding of FP-CIT to DaT. The sponsor provided detailed translated data of this study in form of Tables, graphs and raw data from the original version of the study report. Thus, the above results showed that the repeated administration of anti-Parkinson disease drugs did not affect the binding profile of FP-CIT to the membrane fraction of the rat striatum immediately after the end of administration and after drug withdrawal. However, the sponsor did not provide the reason for the selection of Day 3 for assessment of after drug withdrawal on the binding profile.

**Study title: DP0088R/066: Binding of [$^{123}$I]FP-CIT in rat brain *in vivo*: effects of acute and chronic medication of psychopharmaceuticals**

**Key study findings**: In this study, the potential effects of psychotropic pharmaceutical compounds on the binding of [$^{123}$I]FP-CIT (DaTSCAN) to the DaT was investigated in rats. Single and repeated (2 weeks) doses of L-DOPA (dopaminomimetic), selegiline (MAO-B inhibitor), fluvoxamine (SSRI antidepressant), haloperidol (antipsychotic) and risperidone (antipsychotic) were administered in this study. The blood and several brain regions and samples of peripheral tissues were collected, weighed and assayed for radioactivity. Following acute and chronic treatments, none of drugs cause any significant change in binding in the striatum at the employed dosages in this study.

**Study no.**: DP0088R/066

**Volume # and page #:** Volume 1 pages 1- 9

**Conducting laboratory and location**: (b) (4).

**Date of study initiation**: Not provided

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: [$^{123}$I]FP-CIT, Lot # and purity : Not provided.

**Study Design:**

Doses: As shown in the Table below:

| Drug | Acute Dosage mg/kg i.v. (n=4 per group) | Repeated Dosage mg/kg per day s.c. (n=4 per group) |
|---|---|---|
| L-DOPA | 100 | N/A |
| Haloperidol | 5 | 1 |
| Fluvoxamine | 10 | 2 |
| Selegiline | 5 | 1 |
| Risperidone | 5 | 1 |
| Control | 0 | 0 |

Species/strain: Male Wistar rats

Age: Not provided

Weight: 250-400g

Number/sex/group or time point: 4 male rats per group.

Route of administration: Intravenous (acute study) and subcutaneous (chronic study).

**Main Study:**
Single and repeated (2 weeks) doses of L-DOPA (dopaminomimetic), selegiline (MAO-B inhibitor), fluvoxamine (SSRI antidepressant), haloperidol (antipsychotic) and risperidone (antipsychotic) were administered in this study (sponsor's Table 2.6.2-11). In acute study, [$^{123}$I]FP-CIT was injected intravenously 5 minutes after injection of the above drugs while the same dose of [$^{123}$I]FP-CIT was injected one day after the last dose of psychotropic drug for the chronic study. Male Wistar rats were administered approximately 1.85 MBq of [$^{123}$I]FP-CIT via tail vein under ether anesthesia. The blood, several brain regions (striatum, hypothalamus, occipital cortex, and cerebellum) and pieces of various peripheral tissues (lung, heart, liver, kidney, spleen, fat, and muscle), were rapidly excised and weighted. The $^{123}$I radioactivity was then assayed in a gamma counter. The amount of radioactivity was expressed as percentage of the injected dose, multiplied by the body weight in kilograms, per gram of tissue or blood.

**Table 2.6.2-11 Dose and route of drugs used to evaluate the binding of [I123]FP-CIT in rat brain *in vivo* after psychopharmaceutical medication**

| | Chronic s.c. | Acute i.v. | Therapeutic human dosage Oral |
|---|---|---|---|
| L-DOPA* | N/A | 100 mg/kg | 200 to 800 mg/day, <3mg/kg |
| Risperidone | 1 mg/kg | 5 mg/kg | 4 to 8 mg/day, <0.11mg/kg |
| Haloperidol | 1 mg/kg | 5 mg/kg | 1 to 20 mg/day, <0.3mg/kg |
| Fluvoxamine | 2 mg/kg | 10 mg/kg | <300 mg/day, <5 mg/kg |
| Selegiline | 1 mg/kg | 5 mg/kg | 5 to 10 mg/day, <0.15mg/kg |

*L-DOPA was not studied after chronic administration due to difficulties in repeated subcutaneous administration of the formulated drug.

**Results:** None of the drugs had any significant influence on [$^{123}$I]FP-CIT binding in brain regions except fluvoxamine which induced decreased binding in the hypothalamus (brain region with high density of SERT) but did not cause any significant change in binding in the striatum. Similarly, in the chronic study, none of the drugs, including fluvoxamine, had any significant effect on uptake of [$^{123}$I]FP-CIT in any brain areas examined. The analysis of the organ to cerebellum ratios for any medication during the acute and chronic treatments showed no significant difference from the controls.

**Reviewer's Comments:** The reviewer agrees with the result of this study which shows that none of the examined psychopharmaceutical medications interfered with [$^{123}$I]FP-binding. A single injection at high dosage did not reduce [$^{123}$I]FP-CIT binding in the striatum. However, in the case of fluvoxamine, a reduction of [$^{123}$I]FP-CIT binding in brain regions known to contain serotonergic terminals was observed. In the chronic study, none of the drugs, including fluvoxamine, had any significant effect on uptake of [$^{123}$I]FP-CIT in any brain areas examined. This study indicates that the examined drugs at human therapeutic dosage levels would probably not interfere with [$^{123}$I]FP-CIT imaging of striatal DaT.

## 2.6.3 PHARMACOLOGY 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]

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

The available data of the biodistribution and excretion studies conducted in rats, baboons and cynomolgus monkeys show rapid distribution and clearance of radioactivity from the blood followed by urinary and hepatobiliary elimination in approximately equal proportions. Following the metabolism of FP-CIT, [$^{123}$I]FP-CIT acid, [$^{123}$I]nor-CIT acid

and other minor metabolites were formed. However, none of the metabolites is sufficiently lipophilic to cross the blood brain barrier.

**2.6.4.2 Methods of Analysis**

[see under individual study reviews]

**2.6.4.3 Absorption**

Not applicable

**2.6.4.4 Distribution**

There were two biodistribution studies of [$^{123}$I]FP-CIT (DaTSCAN) in rats.

**Study title: DP008R/002: [$^{123}$I]FP-CIT Binding to the Dopamine Transporter as assessed by biodistribution studies in rats and SPECT studies in MPTP-lesioned monkeys.**

This study was reviewed above along with the binding studies. The data obtained from male Wistar rats injected with DaTSCAN (37 MBq) followed by sacrifice and dissection with sampling at 0.033, 0.33, 1, 2, 4, 7, and 24 hour showed that radioactivity is rapidly accumulated into liver, spleen, kidney and lung. As shown in the Table below, in each peripheral organ studied, except for the adipose tissue, uptake of radioactivity declined gradually after 30 minutes post-injection.

| | Concentration (%ID, mean±SD; n=3) | | | | | | |
|---|---|---|---|---|---|---|---|
| Tissues/organs | 0.033 | 0.33 | 1 | 2 | 4 | 7 | 24 |
| Bone | 5.13±0.44 | 2.89±0.32 | 1.89±0.12 | 1.37±0.24 | 1.09±0.09 | 0.58±0.34 | 0.13±0.03 |
| Muscle | 19.81±1.82 | 17.86±0.61 | 15.25±1.49 | 12.00±1.20 | 9.95±1.03 | 8.07±2.05 | 1.47±0.28 |
| Blood | 2.64±0.22 | 2.40±0.10 | 2.78±0.23 | 2.40±0.20 | 1.90±0.10 | 1.79±0.26 | 0.43±0.09 |
| Kidneys | 5.53±0.69 | 1.97±0.23 | 1.88±0.10 | 1.42±0.11 | 1.06±0.07 | 0.86±0.22 | 0.20±0.06 |
| Bladder and Urine | 0.06±0.02 | 0.40±0.06 | 3.24±0.70 | 9.71±2.01 | 19.07±0.78 | 25.23±1.98 | 36.85±12.91 |
| Lung | 6.40±0.79 | 2.89±0.40 | 1.83±0.24 | 1.15±0.22 | 0.85±0.11 | 0.43±0.11 | 0.07±0.01 |
| Liver | 18.16±3.08 | 29.03±1.20 | 21.13±2.68 | 17.84±0.49 | 15.54±0.54 | 14.98±0.95 | 10.23±1.85 |
| Spleen | 1.36±0.17 | 0.90±0.15 | 0.58±0.15 | 0.36±0.06 | 0.28±0.06 | 0.16±0.07 | 0.02±0.01 |
| Stomach | 2.49±1.01 | 1.09±0.31 | 1.17±0.77 | 0.42±0.06 | 0.34±0.10 | 0.23±0.02 | 0.07±0.02 |
| Stomach content | 0.72±0.30 | 2.79±1.27 | 4.17±0.92 | 1.60±0.15 | 1.76±0.45 | 0.58±0.31 | 0.75±0.28 |
| Small Intestine | 8.84±1.68 | 6.57±1.95 | 7.29±1.99 | 8.72±4.43 | 5.87±1.87 | 3.91±1.17 | 0.56±0.26 |
| Small Intestine | 2.53±1.07 | 2.41±2.03 | 10.54±3.61 | 20.60±5.55 | 16.89±1.83 | 11.61±2.29 | 2.26±0.61 |

| | Concentration (%ID, mean±SD; n=3) | | | | | | |
|---|---|---|---|---|---|---|---|
| Tissues/organs | 0.033 | 0.33 | 1 | 2 | 4 | 7 | 24 |
| Contents | | | | | | | |
| Large Intestine | 2.12±0.32 | 0.97±0.05 | 0.93±0.15 | 0.66±0.09 | 1.61±0.19 | 1.52±0.11 | 0.20±0.03 |
| Large Intestine Contents | 0.54±0.15 | 0.36±0.04 | 0.54±0.21 | 0.94±0.53 | 10.74±2.26 | 20.46±2.98 | 3.58±0.55 |
| Heart | 0.82±0.19 | 0.32±0.03 | 0.26±0.04 | 0.20±0.01 | 0.14±0.01 | 0.11±0.02 | 0.02±0.01 |
| Brain | 2.94±0.08 | 1.57±0.19 | 0.97±0.19 | 0.62±0.15 | 0.59±0.06 | 0.32±0.10 | 0.05±0.01 |
| Salivaries | 0.57±0.11 | 0.59±0.07 | 0.44±0.06 | 0.37±0.09 | 0.25±0.04 | 0.13±0.04 | 0.02±0.01 |
| Skin | 7.95±0.65 | 15.16±0.73 | 16.54±0.45 | 11.20±0.79 | 7.37±0.12 | 4.93±1.04 | 1.32±0.39 |
| Faeces | 0.00±0.00 | 0.00±0.01 | 0.05±0.05 | 0.05±0.06 | 0.12±0.06 | 0.30±0.20 | 40.84±9.19 |
| Carcass[a)] | 11.40±3.02 | 9.83±1.89 | 8.50±0.60 | 8.37±0.86 | 4.59±0.95 | 3.80±0.79 | 0.93±0.24 |
| Injection site | 2.68±1.20 | 1.78±0.25 | 1.80±0.15 | 1.19±0.12 | 0.81±0.04 | 0.74±0.18 | 0.23±0.04 |

**Additional information:**
A total of 37% of the injected dose was excreted via the bladder and urine and 41% through the gastrointestinal tract to faeces.

[a)] After correction for bone, muscle, blood and skin contents.

**Study title: PO882039: A Study to Investigate the Whole Body Biodistribution of I123-Ioflupane in Male Wistar Rats**

**Key study findings**: In this study, the whole body distribution of $^{123}$I-Ioflupane at 2 minutes, 20 minutes, 1, 2, 4, 7 and 24 hours post-injection (pi) in normal male Wistar rats was determined. [$^{123}$I]-Ioflupane is rapidly biodistributed to the organs and tissues of the rat. Approximately 3% injected radioactive dose (id) initially retained in the brain fell to 0.6% id by 4 hours pi. Over 2% of the injected dose was taken up and retained in the bone, muscle, lung, stomach contents, skin, liver and kidneys. By 24 hours pi, over 1% id was only retained in muscle (1.5% id), liver (10.2% id) and skin (1.3% id). The study showed that approximately 40% of injected radioactivity was excreted via the faeces and 40% injected radioactivity via the bladder and urine.

**Study no.**: DPO882039

**Volume # and page #**: Volume 1 pages 1- 30

**Conducting laboratory and location** (b) (4)

**Date of study initiation**: November 22, 2002

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: [$^{123}$I]-Ioflupane, Batch # 02L 26D and purity: 97%.

**Study Design:**

Dose: 37 MBq [$^{123}$I]-Ioflupane

Species/strain: Wistar outbred rats (Crl: (WI)BR)

Weight: 180-220g (male)

Age: 9-13 weeks

Number/sex/group or time point: 3 males/group.

Route of administration: Single bolus intravenous injections were administered to the rats via the lateral tail vein, using a new needle and syringe for each animal.

**Main Study:**

The rats were anesthetized with halothane and injected approximately 37 MBq [$^{123}$I]-Ioflupane (sponsor's Table 1 below). The animals were kept in metabolic cages that allow separate collection of voided urine and faeces until the scheduled sacrifice times (2 minutes, 20 minutes, 1, 2, 4 and 7 hours) after injection. After dissection, all the organ and tissue were isolated and the samples were assayed for radioactivity in an automatic twin-crystal gamma counter using containers that ensured uniformity of sample counting geometry.

**Table 1 Study design**

| Group number | Sex | Number | Dosage $^{123}$I-Ioflupane (ml)[a] |
|---|---|---|---|
| 1M | Male | 3 | 0.5 |
| 2M | Male | 3 | 0.5 |
| 3M | Male | 3 | 0.5 |
| 4M | Male | 3 | 0.5 |
| 5M | Male | 3 | 0.5 |
| 6M | Male | 3 | 0.5 |
| 7M | Male | 3 | 0.5 |

[a] Animals administered with 0.5 ml of 74 MBq/ml $^{123}$I-ioflupane, approximating 37 MBq per animal.

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**Results:** The biodistribution of $^{123}$I-Ioflupane was rapid with approximately only 3% id present in the blood by 2 minutes pi. 2-3% of the injected radioactive dose was retained in the blood from 2 minutes pi through to 7 hours pi. By 2 minutes after injection, approximately 3% id was retained within the brain and the retention of $^{123}$Ioflupane within the brain decreased over time as shown in the biodistribution data provided by the sponsor. Initial uptake and retention of activity into non-target organs and tissues such as bone and muscle was approximately 5% id and 20% id, respectively. By 24 hours pi, approximately 0.1% id was retained in bone and 1.5% id retained in muscle. Approximately 6% of the injected dose was detected in the lung by 2 minutes pi. Pulmonary retention of radioactivity fell rapidly such that by 20 minutes 3% id was retained falling to 1% id by 2 hours pi and 0.07% id by 24 hours pi. Less than 1% id was detected in the heart and salivary glands. Approximately 1.4% id was retained in the spleen by 2 minutes pi, falling to 0.2% id by 24 hours pi. Initially, the uptake of activity into the liver and kidney was rapid with approximately 6% id and 18% id retained in the kidneys and liver, respectively. Liver retention of injected radioactivity peaked at 29% id by 20 minutes pi and slowly decreased to 10% id by 24 hours pi. Approximately 37% of the injected dose was excreted via the bladder and urine and 41% of the injected dose excreted in the feces.

**Reviewer's Comments:** The reviewer agrees with the result of this study which shows [$^{123}$I]ioflupane is rapidly distributed in all the major organs in the body and the compound is mostly excreted via urine and feces within 24 hours after injection.

**Study title: PO20376: Single Intravenous dose Toxicokinetic Study of $^{125}$I-FP-CIT in Rats**

**Key study findings**: The time-courses of unchanged FP-CIT and plasma metabolites after a single intravenous administration of $^{125}$I-FP-CIT (10, 30, and 1000 µg/kg) to rats were evaluated. There was no significant difference in the $AUC_{0-24\ h}$ of the plasma radioactivity concentrations obtained in male and female rats at these doses (45.5, 140, and 4858 ng eq.•h/mL – males; 47.4, 152, and 4156 ng eq.•h/mL – females for 10, 30, and 1000 µg/kg doses respectively). The compound was predominantly metabolized to nor-acid and no differences in dose level and sex was reported in the metabolism pattern.

**Study no.**: PO20376

**Volume # and page #**: Volume 1 pages 1- 74

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: Not provided

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: $^{125}$I-FP-CIT, lot #: not provided and purity: 100%.

**Study Design:**

Dose: 10, 30, and 1000 µg/kg (294.8X, 8844X and 29480X MHD respectively).

Route of administration: Single bolus intravenous injections were administered to the rats.

**Main Study:**
10, 30, and 1000 µg/kg doses of $^{125}$I-FP-CIT were given by a single intravenous administration to male and female rats. The plasma radioactivity concentrations at 5 minutes and various time intervals until 24 hour after administration were evaluated.

**Results:** The available summary translation of the original study report indicates following administration of 10, 30, and 1000 µg/kg to the rats, $AUC_{0-24\ h}$ of 45.5, 140, and 4858 ng eq.•h/mL, respectively were obtained in the males as against $AUC_{0-24\ h}$ values of 47.4, 152, and 4156 ng eq.•h/mL, respectively in the females. Within 5 minutes after administration, FP-CIT remained unchanged in both males and females. The unchanged FP-CIT was later metabolized into nor-acid which is predominantly present in the plasma. The $AUC_{0-3\ h}$ or $AUC_{0-24\ h}$ for unchanged FP-CIT were 2.35, 10.2, and 356 ng•h/mL, respectively, in male rats and were 2.71, 6.15, and 282 ng•h/mL, respectively, in female rats. The metabolism pattern indicates no dose level difference or sex difference in this study.

**Reviewer's Comment:** The sponsor provided only the summary of the translated data of this study and the declaration of translation authenticity. Thus, the available data on this study was grossly inadequate. The sponsor should have provided a complete detailed pharmacokinetic data including the tables, graphs and figures. It is therefore difficult to thoroughly review this study.

**Study title: PO20377: Single Intravenous dose Pharmacokinetic Study of $^{125}$I-FP-CIT in Cynomolgus Monkeys**

**Key study findings**: In this study, the plasma radioactivity concentrations, urinary and fecal excretions, tissue radioactivity concentrations, as well as metabolites in the plasma and urine following a single intravenous administration of $^{125}$I-FP-CIT to male Cynomolgus monkeys were examined. The study showed a $t_{1/2}$ of 4.85, 1.27, and 1.20 hours and $AUC_{0-\infty}$ of 1.80, 5.28, and 469 ng eq.•h/mL following 0.3, 1, and 100 µg/kg doses respectively. Urinary and fecal excretion rates after administration were 68.9-71.0% and 12.6-17.6%, respectively. Main metabolites found in the urine, were an unidentified metabolite RU8 and nor-acid and also small amount of nor-CIT, free $^{125}$I, unchanged FP-CIT, and acid were also present at not more than 4% of dose.

**Study no.**: PO20377

**Volume # and page #**: Volume 1 pages 1- 92

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: Not provided

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: $^{125}$I-FP-CIT, lot #: not provided and purity: 100%.

**Study Design:**

Dose: 0.3, 1, and 100 µg/kg (17.6X, 58.8X and 5880X MHD respectively)

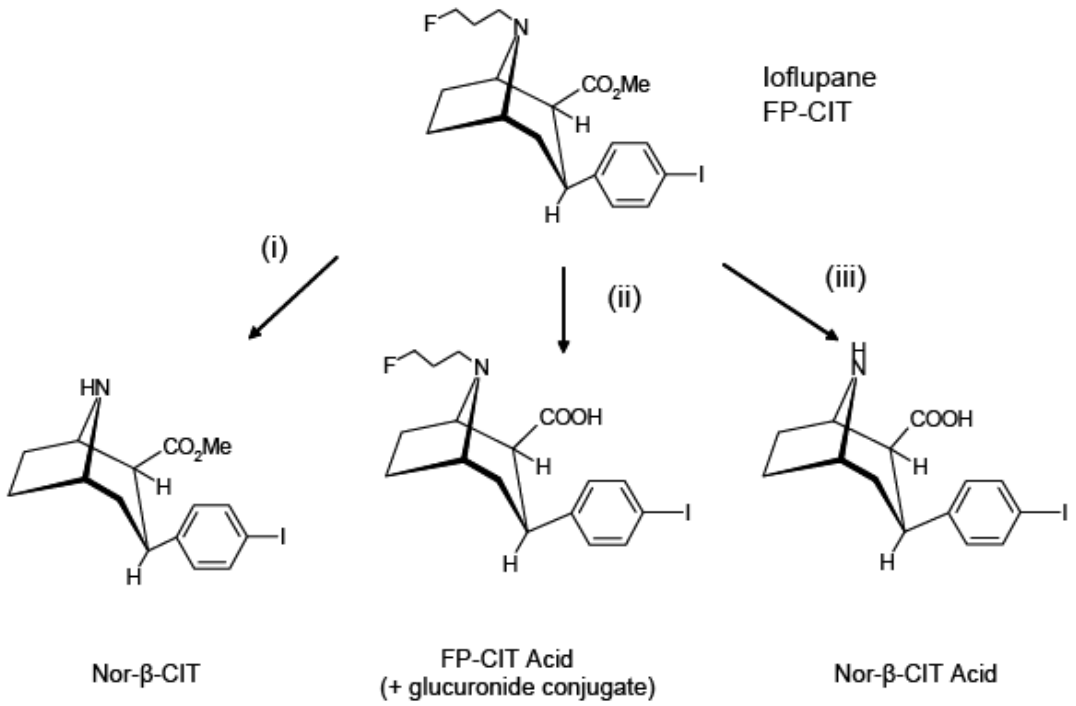
Route of administration: Single bolus intravenous injections.

**Main Study:**
0.3, 1, and 100 µg/kg doses of $^{125}$I-FP-CIT were given by a single intravenous administration to male Cynomolgus monkeys. The plasma radioactivity concentrations, urinary and fecal excretions, tissue radioactivity concentrations, as well as metabolites in the plasma and urine were evaluated.

**Results:** The available summary translation of the original study report indicates following administration of 0.3, 1, and 100 µg/kg doses to the monkeys, plasma radioactivity concentrations at 3 minutes after administration were 0.091, 0.483, and 36.0 ng eq./mL, respectively. The $t_{1/2}$ of 4.85, 1.27, and 1.20 hours and $AUC_{0-\infty}$ of 1.80, 5.28, and 469 ng eq.•h/mL following 0.3, 1, and 100 µg/kg (18X, 60X and 6000X MHD) doses respectively were reported. The urinary and fecal excretion rates (*of radioactivity*) at 0-168 hours or 0-336 hours after administration were 68.9-71.0% and 12.6-17.6%, respectively. The tissue radioactivity concentration at 336 hours after administration was highest (996 ng eq./g) in the thyroid glands, followed by the liver, skin, fat, and adrenals. The total accumulation in these tissues was 3.11% *(of the injected radioactivity).* In the urine, the main metabolites were an unidentified metabolite RU8 and nor-acid and also small amount of nor-CIT, free $^{125}I$, unchanged FP-CIT, and acid were also present at not more than 4% of dose. The $AUC_{0-\infty}$ for unchanged FP-CIT were 0.130, 0.526, and 45.4 ng•h/mL, following an administration of 0.3, 1, and 100 µg/kg doses respectively.

**Reviewer's Comment:** The data provided by the sponsor on this study was grossly inadequate. A more detailed data on the monkey study would have provided a better understanding of the metabolism of this compound in the monkey. The lack of a table showing the radioactivity concentrations in the tissue over the period of observation denied this reviewer the opportunity to assess any potential toxicity on any of the tissues or organs. However, the information that the total accumulation in the tissues was 3.11% was useful as it indicates that there is no significant accumulation of the compound within the body. Despite this, the accumulation level in each tissue was not provided. Thus, the provision of an inadequate data of this non human primate study is a major flaw of this study.

**Additional Information on Metabolism:**
Based on *in silico* (METEOR) analysis of the FP-CIT molecule, the metabolic pathways shown below was predicted by the sponsor.




**2.6.4.5 Excretion**

**Study title**: **9829: Excretion of Total radioactivity following Single Intravenous Administration of $^{125}$I-labelled FP-CIT to Rats.**

**Key study findings**: In this study, the whole body distribution of $^{123}$-IIoflupane at 2 minutes, 20 minutes, 1, 2, 4, 7 and 24 hours post-injection in normal male Wistar rats was determined. [$^{123}$I]-Ioflupane is rapidly biodistributed to the organs and tissues of the rat. Approximately 3% id initially retained in the brain fell to 0.6% id by 4 hours pi. Over 2% of the injected dose was taken up and retained in the bone, muscle, lung, stomach contents, skin, liver and kidneys. By 24 hours pi, over 1% id was only retained in muscle (1.5% id), liver (10.2% id) and skin (1.3% id). The study showed that approximately 40% of injected radioactivity was excreted via the feces and 40% injected radioactivity via the bladder and urine.

**Study no.**: 9829

**Volume # and page #**: Volume 1 pages 1- 23

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: May 8, 1998

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: [$^{123}$I]-FP-CIT, Lot # 98E18Fp and purity: 99.9%.

**Study Design:**

Dose: 1.85 MBq (50 µCi) [$^{123}$I]-FP-CIT/animal

Species/strain: Wistar outbred rats (Crl: (WI)BR)

Weight: 250-270 g (male), 190-200 g ( females)

Age: 6-7 weeks (males), 7-8 weeks (females)

Number/sex/group or time point: 5/sex/dose.

Route of administration: Single bolus intravenous injections were administered to the rats via the tail vein for each animal.

**Main Study:**
A total of 10 rats (5 males and 5 females) were employed in this study. Each of the rats were injected 1.85 MBq (50 µCi) [$^{123}$I]-FP-CIT. The urine samples were collected at 0 - 4h, 4 - 8h, 8 - 12h, 12 - 24h, 24 - 32h, 32 - 48h, 48 - 72h while the feces samples were collected at 0 - 12h, 12 - 24h, 24 - 48h, 48 - 72h periods. The samples were prepared for radioactivity measurement and the radioactivity levels in samples were assayed using a Wallac Wizard 1470 gamma counter (Pharmacia, Walla coy, Turku, Finland).

**Results:** The available data form this study indicates that $^{123}$1-FP-CIT was excreted rapidly during the first 24 hours, after 24 hours the mean excretion in both sexes was >68%, after 48 hours >77%, after 72 hours >80%. The data obtained from this study showed that the route of excretion of $^{123}$I-FP-CIT may be sex dependant. In the females, the main route of excretion is renal since 72 hours after administration, 56.66 ± 5.87 % was excreted with urine while only 24.22 ± 3.45 was excreted with the feces. A contrary result was obtained in males since 36.03 ± 5.02% was excreted with urine, and 45.71 ±7.53 % with feces as shown in sponsor's Figure 1. The sponsor concluded a possible sex difference in the route of excretion of $^{123}$I-FP-CIT and approximately 40% of injected radioactivity was excreted via the feces and 40% injected radioactivity via urine.

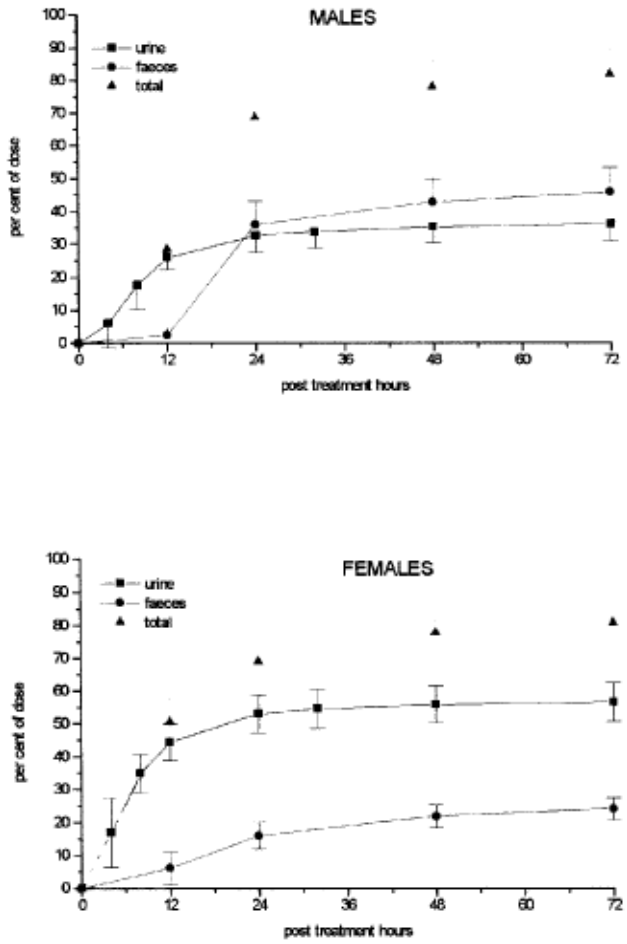



**Figure 1**
**Cumulative excretion of radioactivity (expressed in percent of dose) with urine and faeces from rats after administration of $^{123}$I-FP-CIT (upper half: males; lower half: females)**

**Reviewer's Comments:** This reviewer agrees with the result of this study which shows $^{123}$1-FP-CIT is excreted in the urine and feces and this accounts for approximately 90% of the administered dose. However, it seems the conclusion that the route of excretion is sex dependent as claimed by the sponsor is premature. It could be very difficult to convincingly establish sex differences in physiological effect using such a few numbers of animals per sex. An additional confirmatory study involving more animals is therefore necessary to ascertain this claim.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary:

The toxicity profile of FP-CIT was evaluated *in vivo* in rats, rabbits, dogs and monkeys. Single dose and repeat-dose toxicity studies were conducted in rats, rabbits and

Cynomolgus monkeys and no treatment related mortality was reported in any of these studies. In single dose toxicity studies, a NOAEL of 10 µg/kg (294.8X MHD) was obtained in the rats while 0.06 µg/kg (3604X MHD) was reported in rabbits. Mydriasis, increased motility, and licking were reported during administration or immediately after administration of 29480X MHD FP-CIT to dogs and a NOAEL of 1 µg/kg (98.3X MHD) was obtained. Increased heart rate, blood pressure, respiratory rate, slight decrease in motility, mydriasis and restlessness were reported in Cynomolgus monkeys administered 58800X MHD and the NOAEL in the study was 0.3 µg/kg (17.7X MHD).

During 14-day repeat dose toxicity study in rats, stereotype behavior, increased and violent physical activity, excessive sensitivity to external stimuli, and piloerection were reported in animals administered 3.6 mg/kg/day (106128X MHD) dose while less extreme stereotype behavior was seen in female rats at 0.6 mg/kg/day (17688X MHD). However, no treatment-related clinical signs were reported in animals administered 0.006 mg/kg/day (176.88X MHD). In another 2-week repeated dose toxicity study in rats, increased motility was reported in rats administered 300 µg/kg/day (8844X MHD) FP-CIT. There were scattered blood spots in the lungs of 1/5 females in the 8844X MHD group and histopathology of the lungs showed localized mild bleeding in males in the 294.8X MHD group and in males and females (2 or 3 animals each) in the 8844X MHD group. The NOAEL in this study was 10 µg/kg/day (294.8X MHD). Stereotype and aggressive behavior, increased responses to external stimuli, protruding eyes with dilated pupils, and fast or labored respiration occurred in rabbits administered 90000X MHD FP-CIT for 2-weeks. Stereotype behavioral effects were also observed in 360X and 36000X MHD treated rabbits. No clinical sign was reported in Beagle dogs treated with up to 98.3X MHD FP-CIT during a 14 day repeat-dose study. However, there was mydriasis, congestion of the visible mucosa, flushing of the pinnae, reddening of the skin, or panting after administration of 9828X MHD FP-CIT. The NOAEL in this study was 1 µg/kg/day (98.3X MHD).

The intravenous, intra-arterial and perivenous tolerance of FP-CIT was assessed in the rabbit. No mortality or any evidence of local and systemic toxicity was reported following a single injection in the study. The sponsor's request for a waiver for reproductive and developmental toxicity studies was granted.

The standard ICH battery of tests including two *in vitro* assay covering the endpoints of gene mutation (in bacteria) and chromosomal effects (in cultured human lymphocytes and in mouse bone marrow) was evaluated. The tests were negative indicating that FP-CIT demonstrates no genotoxicity potential.

**2.6.6.2 Single-dose toxicity**

**Study title: 2001NM021: Single Dose Intravenous Toxicity Study FP-CIT in Rats.**

**Key study findings**: The toxicity of single intravenous injection of FP-CIT at doses of 0, 10, 30, or 1000 µg/kg (0, 294.8X, 8844X or 29480X MHD respectively) to male and

female Sprague-Dawley rats was evaluated. No mortality was reported in any treated groups in this study. Rats administered 8844X and 29480X MHD demonstrated increased motility immediately after administration. No effects on body weight, hematology and histopathology. The NOAEL in this study was 10 µg/kg (294.8X MHD).

**Study no.**: Study No. 2001NM021

**Volume # and page #:** Volume 1 pages 1- 54

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: November 5, 2001

**GLP compliance**: Yes

**QA report**: yes (x) no ( )

**Drug, lot #, and % purity**: FP-CIT, Lot # NMP 1034 and purity is stated to be 99.5%

**Method:**
Doses: 0, 10, 30, or 1000 µg/kg (0, 294.8X, 8844X or 29480X MHD respectively)

Species/strain: Rats/Sprague-Dawley Crj:CD°(SD)IGS

Number/sex/group or time point (main study): 5/male and female/group: See table below.

Age: 5 weeks old

Weight: 141-163 g (males); 126-142 g (females).

Control article: 1% w/v ascorbic acid solution

Route, formulation, volume, and infusion rate: Intravenous injection via a tail vein using an infusion pump (b) (4) with a butterfly needle at a dose volume of 5 mL/kg of body weight and infusion rate of 1mL/minute,

The animals were assigned for this study as shown in the table below:

| Group | Name of substance administered | Dose level (µg/kg) | Concen-tration (µg/mL) | Dosing volume (mL/kg) | Animal No. | |
|---|---|---|---|---|---|---|
| | | | | | Male | Female |
| Control group | 1 w/v% ascorbic acid solution | 0 | 0 | 5 | 001-005 | 401-405 |
| Low dose level group | FP-CIT | 10 | 2 | 5 | 101-105 | 501-505 |
| Middle dose level group | FP-CIT | 30 | 6 | 5 | 201-205 | 601-605 |
| High dose level group | FP-CIT | 1000 | 200 | 5 | 301-305 | 701-705 |

The rats were observed before administration, immediately after administration, at 15 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours, and 6 hours after administration and at other timings as appropriate; and from the day after administration to day 14 after administration - once daily in the morning.

**Results:**
Mortality: None

Clinical signs: On the day of administration, 1/5 males and 5/5 females each in the 8844X MHD group and 4/5 males and 5/5 females each in the 1000 µg/kg (29480X MHD) group showed increased motility immediately or shortly after administration. However, this clinical sign disappeared by 15 minutes after administration in the 8844X MHD group and by 60 minutes after administration in the 29480X MHD group.

Body weights: No treatment related effect.

Hematology: No treatment related hematological effect

Clinical chemistry: There was a significant increase in cholesterase in the males administered 29480X MHD in comparison to the control animals.

Gross pathology: The liver showed mild solitary focal necrosis in 2 males in the 29480X MHD group; mild or moderate vacuolation of the hepatocytic cytoplasm in the lobular periphery of 2 females in the control group and of 1 male and 2 females in the 29480X MHD group; and mild mononuclear cell infiltration in 1 female in the 29480X MHD group. In 1 male and 1 female in the 8844X MHD group the kidney showed mild or moderate pyelectasis. One male in the 29480X MHD group showed mild cysts in the cortex, mild hyperplasia of the epithelium, and the mild focal dilatation of the uriniferous tubules involving the thickened basement membrane.

Organ weight: Males in the 29480X MHD group showed significant increases in the actual weight of the lungs and in the organ weight-to-body weight ratio of the lungs compared to the control group. Males in the 294.8X MHD group also showed a significant increase in the actual weight of the lungs, and females in the same group showed a significant increase in the organ weight-to-body weight ratio of the heart.

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

**Histopathology inventory (optional)**

| Study | Acute Study | | | |
|---|---|---|---|---|
| Species | Dog | | | |
| Adrenals | x | | | |

| | | | | |
|---|---|---|---|---|
| Aorta | x | | | |
| Bone Marrow smear | x | | | |
| Bone (femur) | x | | | |
| Brain | x | | | |
| Cecum | x | | | |
| Cervix | x | | | |
| Colon | x | | | |
| Duodenum | x | | | |
| Epididymis | x | | | |
| Esophagus | x | | | |
| Eye | x | | | |
| Fallopian tube | | | | |
| Gall bladder | x | | | |
| Gross lesions | x | | | |
| Harderian gland | | | | |
| Heart | x * | | | |
| Ileum | x | | | |
| Injection site | x | | | |
| Jejunum | x | | | |
| Kidneys | x * | | | |
| Lachrymal gland | | | | |
| Larynx | x | | | |
| Liver | x * | | | |
| Lungs | x* | | | |
| Lymph nodes, cervical | x | | | |
| Lymph nodes mandibular | x | | | |
| Lymph nodes, mesenteric | x | | | |
| Mammary Gland | x | | | |
| Nasal cavity | | | | |
| Optic nerves | x | | | |
| Ovaries | x* | | | |
| Pancreas | x | | | |
| Parathyroid | x | | | |
| Peripheral nerve | | | | |
| Pharynx | x | | | |
| Pituitary | x | | | |
| Prostate | x | | | |
| Rectum | x | | | |
| Salivary gland | x | | | |
| Sciatic nerve | x | | | |
| Seminal vesicles | | | | |
| Skeletal muscle | x | | | |
| Skin | x | | | |
| Spinal cord | x | | | |
| Spleen | x* | | | |
| Sternum | | | | |
| Stomach | x | | | |
| Testes | x * | | | |
| Thymus | x | | | |

| | | | | |
|---|---|---|---|---|
| Thyroid | x | | | |
| Tongue | x | | | |
| Trachea | x | | | |
| Urinary bladder | x | | | |
| Uterus | x | | | |
| Vagina | x | | | |
| Zymbal gland | | | | |

x, histopathology performed
*, organ weight obtained

**Reviewer's Comments:** Agreed with the results of this study.

**Study title: 144552: Assessment of Acute Intravenous Toxicity with β-CIT-FP in the Rats.**

**Key study findings**: The toxicity of an acute intravenous injection of β-CIT-FP at doses of 0, 0.06 and 17.5 mg/kg (0, 1768.8X or 515900X MHD respectively) to male and female Wistar rats was assessed. Mortality was reported in 8/10 rats (5 males and 3 females) in the 515900X MHD treated group within 24 hours post dosing. There were also clinical signs varying from clonic spasms, hunched posture, uncoordinated movement to labored respiration in the two surviving female rats in this group. No mortality occurred in 1768.8X MHD treated rats. However, there were reports of intense movements during treatment with 1768.8X MHD dose. The NOAEL was not determined.

**Study no.**: Study No. 144552

**Volume # and page #**: Volume 1 pages 1- 20

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: April 27, 1995

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: β-CIT-FP, Batch # 95028FP and purity not stated

**Method:**
Doses: 0, 0.06 and 17.5 mg/kg (0, 1768.8X or 515900X MHD respectively)

Species/strain: Rats/Wistar Crl:WI) BR (outbred, SPF-Quality)

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

Age: 6-7 weeks old

Control article: Isotonic sodium acetate buffer, pH 4.75 with 5% v/v ethanol

Route, formulation, volume, and infusion rate: Intravenous injection via lateral tail vein at an injection rate of 1mL/minute.

The rats were observed at periodic intervals before and after administration till day 15.

**Results:**
Mortality: All males and 3 females administered 515900X MHD died within 24 hours after treatment.

Clinical signs: No clinical signs were reported in vehicle- and 1768.8X MHD-treated rats. However, all the 1768.8X MHD β-CIT-FP dosed rats demonstrated intense movements during treatment. Several signs of toxicity including lethargy, clonic spasms, ventro-lateral recumbency, hunched posture, increased activity, uncoordinated movements, labored respiration, piloerection, shaking of the head, moving backwards and pushing away of the bedding material were reported in the rats administered 515900X MHD dose. These symptoms noted in the 2 surviving female rats subsided within 24-48 hours after treatment.

Body weights: No treatment related effect was reported on the body weight.

Hematology: No treatment related hematological effect.

Clinical chemistry: No data provided.

Gross pathology: No abnormal macroscopic findings reported.

Organ weight: No data provided.

**Reviewer's Comments:** The study showed that β-CIT-FP caused intense movement during treatment with 1768.8X MHD dose and the effect is probably not due to discomfort related to the injection but probably due to cocaine-like effect mediated by the β-CIT-FP. This is because no such clinical sign was reported in vehicle-treated group. This reviewer feels that doses employed in the study were not properly selected. One or two doses less than 1768.8X MHD should have been used in addition to the doses employed in this study to ensure determination of NOAEL.

**Study title: 144574: Assessment of Acute Intravenous Toxicity with β-CIT-FP in the Rabbit.**

**Key study findings**: The acute toxicity of a single intravenous injection of FP-CIT (0.06 mg/kg -3604X MHD) 5 males and 5 females New Zealand white albino rabbits was evaluated. No mortality and no signs of clinical signs of toxicity were reported.

**Study no.**: Study No. 144574

**Volume # and page #:** Volume 1 pages 1- 12

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: May 10, 1995

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: β-CIT-FP, Batch # NMP 1034 and purity not stated.

**Method:**
Dose: 0.06mg/kg (3604X MHD)

Species/strain: Rabbits/New Zealand White (SPF-Quality)

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

Age: 12 weeks old

Weight: Not provided.

Control article: Not employed

Route, formulation, volume, and infusion rate: Intravenous injection via the marginal ear vein in the direction of the ear base/formulated in an isotonic sodium acetate buffer, pH 4.75 with 5% ethanol/ dose volume of 2ml/kg and injection rate of 6ml/min.

The animals were injected 0.06mg/kg dose once on day 1, observed for mortality and clinical signs throughout the study. The animals were sacrificed on day 15 at the end of the study for necropsy.

**Results:**
Mortality: No mortality was reported.

Clinical signs: There were reports of shaking and withdrawal of the head and high tension in the paws and body of the first 3 rabbits injected the test substance. However, this

reaction subsided with a slight warming of the test substance before injection into the remaining animals.

Body weights: No biologically meaningful treatment related effect was reported

Hematology: No treatment-related hematological findings were reported.

Clinical chemistry: No treatment related findings was reported in the blood chemistry parameters examined.

Gross pathology: Macroscopic post mortem examination of the animals at termination did not reveal any abnormalities.

Organ weight: No treatment-related effect on organ weight was reported.

Histopathology: Adequate Battery: yes (), no (x)—Explain-Only the lungs, kidney, liver, heart and spleen were examined in this study.
Peer review: yes (), no (x)

Reviewer's Comments: This study indicates that an intravenous injection of β-CIT-FP at a dose level of 3604X MHD caused no mortality and no signs of toxicity were observed in the treated rabbits. However, this reviewer disagrees with the sponsor that this study is GLP-compliant. The planning and conduct of the study failed to meet the standard of a GLP study. There is no control group and scanty information is provided on the test substance, neither was the analysis of the test substance conducted. It is clear that the sponsor failed to conduct organ and tissue samples analysis during this study in addition vital histopathology data is not available. Thus, this study is not acceptable and might have to be repeated if a similar deficiency is noted in acute-dose study conducted on another species employed in this NDA submission.

**Study title: 2001NM022: Single Intravenous Dose Toxicity Study FP-CIT in Dogs.**

**Key study findings**: The acute toxicity effect of a single intravenous injection of FP-CIT at doses of 0, 0.3, 1 or 300 µg/kg (0, 29.5X, 98.3X or 29480X MHD respectively) to dogs was evaluated. No mortality was reported in any treated groups in this study. There were reports of mydriasis, increased motility, and licking during administration or immediately after administration of 29480X MHD (FP-CIT) dose to dogs. The NOAEL in this study was 1 µg/kg (98.3X MHD).

**Study no.**: Study No. 2001NM022

**Volume # and page #**: Volume 1 pages 1- 50

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: October 12, 2001

**GLP compliance**: Yes

**QA report**: yes (x) no ( )

**Drug, lot #, and % purity**: FP-CIT, Lot # NMP 1034 and purity is stated to be 99.5%

**Method:**
Doses: 0, 0.3, 1 or 300 µg/kg (0, 29.5X, 98.3X or 29480X MHD respectively)

Species/strain: Dogs/Beagle

Number/sex/group or time point (main study): 2/group: See table below.

Age: 5-6 months old

Weight: 9.15-10.20 kg.

Control article: 1% w/v ascorbic acid solution

Route, formulation, volume, and infusion rate: Intravenous injection via a 22-gauge indwelling needle via the cephalic vein an infusion pump (b) (4) with a butterfly needle at a dose volume of 3 mL/kg in the control group and the high dose level group, 0.3 mL/kg in the low dose level group, and 1 mL/kg in the middle dose level group and infusion rate of 3 mL/minute.
The animals were assigned for this study as shown in the table below:

[Dose levels and group composition]

| Group | Name of substance administered | Dose level (µg/kg) | Concen-tration (µg/mL) | Dosing volume (mL/kg) | Animal No. |
|---|---|---|---|---|---|
| Control group | 1 w/v% ascorbic acid solution | - | - | 3 | 11, 12 |
| Low dose level group | FP-CIT | 0.3 | 1 | 0.3 | 21, 22 |
| Middle dose level group | FP-CIT | 1 | 1 | 1 | 31, 32 |
| High dose level group | FP-CIT | 300 | 100 | 3 | 41, 42 |

The rats were observed before administration, immediately after administration, at 15 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours, and 6 hours after administration and at other timings as appropriate; and from the day after administration to day 14 after administration - once daily in the morning.

**Results:**
Mortality: No mortality was reported.

Clinical signs: On the day of administration, both animals in the 29480X MHD group showed mydriasis, increased motility, and licking during administration or immediately after the end of administration and later. In addition, there were reports of flushing of the oral mucosa, salivation and flushing of the pinnae 30 minutes post-injection. All these changes subsided within 4 hours after administration.

Body weights: No treatment related effect was reported on the body weight.

Hematology: No treatment related hematological effect.

Clinical chemistry: No treatment related effect.

Urinalysis: There were reports of a slight occult blood-positive reaction on day 1 or day 13 after administration in 29.5X and 98.3X MHD treated groups

Gross pathology: No treatment related effect. However, there was infiltration of mononuclear cells in the renal pelvic mucosa in one animal in the 29.5X MHD group and another animal in the 29480X MHD group showed the slight infiltration of macrophages, mononuclear cells, and neutrophils in the lungs.

Necropsy: No abnormalities were found in any animal.

Organ weight: No treatment related effect

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

**Histopathology inventory (optional)**

| Study | Acute Study | | | |
|---|---|---|---|---|
| Species | Dog | | | |
| Adrenals | x | | | |
| Aorta | x | | | |
| Bone Marrow smear | x | | | |
| Bone (femur) | x | | | |
| Brain | x | | | |
| Cecum | x | | | |
| Cervix | x | | | |
| Colon | x | | | |
| Duodenum | x | | | |
| Epididymis | x | | | |
| Esophagus | x | | | |
| Eye | x | | | |

| | | | | |
|---|---|---|---|---|
| Fallopian tube | | | | |
| Gall bladder | x | | | |
| Gross lesions | x | | | |
| Harderian gland | | | | |
| Heart | x * | | | |
| Ileum | x | | | |
| Injection site | x | | | |
| Jejunum | x | | | |
| Kidneys | x * | | | |
| Lachrymal gland | | | | |
| Larynx | x | | | |
| Liver | x * | | | |
| Lungs | x* | | | |
| Lymph nodes, cervical | x | | | |
| Lymph nodes mandibular | x | | | |
| Lymph nodes, mesenteric | x | | | |
| Mammary Gland | x | | | |
| Nasal cavity | | | | |
| Optic nerves | x | | | |
| Ovaries | x | | | |
| Pancreas | x | | | |
| Parathyroid | x | | | |
| Peripheral nerve | | | | |
| Pharynx | x | | | |
| Pituitary | x | | | |
| Prostate | x | | | |
| Rectum | x | | | |
| Salivary gland | x | | | |
| Sciatic nerve | x | | | |
| Seminal vesicles | | | | |
| Skeletal muscle | x | | | |
| Skin | x | | | |
| Spinal cord | x | | | |
| Spleen | x* | | | |
| Sternum | | | | |
| Stomach | x | | | |
| Testes | x | | | |
| Thymus | x | | | |
| Thyroid | x | | | |
| Tongue | x | | | |
| Trachea | x | | | |
| Urinary bladder | x | | | |
| Uterus | x | | | |
| Vagina | x | | | |
| Zymbal gland | | | | |

x, histopathology performed
*, organ weight obtained

**Reviewer's Comments:** Agreed with the results of this study.

**Study title: NM97076: Single Intravenous Dose Toxicity Study β-FP-CIT in Cynomolgus Monkeys.**

**Key study findings**: The acute toxicity effect of a single intravenous injection of FP-CIT at doses of 0, 0.3, 1 or 100 µg/kg (0, 17.7X, 58.8X or 5880X MHD respectively) to Cynomolgus monkeys was evaluated. No mortality was reported in any treated groups in this study. However, animals in 5880X MHD group demonstrated a slight decrease in motility and mydriasis immediately after administration and restlessness at 60 minutes after administration. There was a slight increase in creatinine phosphokinase (CPK) activity in 58.8X MHD group on day 2 after administration. Animals administered 5880X MHD dose demonstrated increased heart rate, blood pressure and respiratory rate within 1 hour after treatment. The NOAEL in this study was 0.3 µg/kg (17.7 X MHD).

**Study no.**: Study No. NM97076

**Volume # and page #**: Volume 1 pages 1- 57

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: May 29, 1997

**GLP compliance**: Yes

**QA report**: yes (x) no ( )

**Drug, lot #, and % purity**: β-FP-CIT, Lot # NMP 1035 and purity is stated to be 99.5%

**Method:**
Doses: 0, 0.3, 1 or 100 µg/kg (0, 17.7X, 58.8X or 5880X MHD respectively)

Species/strain: Monkeys/Cynomolgus monkeys

Number/sex/group or time point (main study): 8 male/group: See table below.

Age: 3-4 years old

Weight: 2.85-3.70 kg.

Control article: 1% w/v ascorbic acid solution

Route, formulation, volume, and infusion rate: Intravenous injection via a 25-gauge butterfly injection needle via the tail vein using an infusion pump (b) (4) at a

dose volume of 3 mL/kg in the β-CIT-FP 0.3 μg/kg group and 0.4 mL/kg of body weight in other groups and infusion rate of 3 mL/minute.

The animals were assigned for this study as shown in the table below:

[Dose levels and group composition]

| Group | Name of substance administered | Dose level (μg/kg) | Concentration (μg/mL) | Volume of the dosing solution (mL/kg) | Animal No. |
|---|---|---|---|---|---|
| I | 1 % ascorbic acid solution | - | - | 0.4 | 01, 02 |
| II | β-CIT-FP | 0.3 | 1.0 | 0.3 | 11, 12 |
| III | β-CIT-FP | 1 | 2.5 | 0.4 | 21, 22 |
| IV | β-CIT-FP | 100 | 250 | 0.4 | 31, 32 |

The monkeys were observed before administration and at various intervals after administration to day 14 after administration and the respiratory rate was measured using a stethoscope. Furthermore, electrocardiography data was obtained using an autoanalyzing electrocardiograph (Carisuny 501AX-D, Fukuda M-E Kogyo Co., Ltd) and the blood pressures (minimum and maximal) measured with sphygmomanometer (BP-203NP, Nihon-Korin Co.) before treatment and at various time intervals and on day 14 after administration.

**Results:**

Mortality: No mortality was reported in this study.

Clinical signs: The animals in the 5880X MHD group demonstrated mydriasis and a slight decrease in motility immediately after administration. The monkeys also exhibited restlessness at 60 minutes after administration. However, these changes disappeared by 2 hours after administration. No treatment-related clinical signs were reported in other treatment groups.

Respiratory rate: A slight increase in respiratory rate was reported in the 5880X MHD group immediately after administration. However, this effect subsided within 1 hour post dosing.

Blood pressure: An animal in the 5880X MHD group demonstrated an increase in blood pressure within the first hour after administration.

Electrocardiography: The available detailed ECG measured showed that one animal in the 5880X MHD group demonstrated increased heart rate at 1 hour after administration.

Body weights: No treatment related effect was reported on the body weight.

Hematology: No treatment related hematological effect

Clinical chemistry: There was a slight increase in creatinine phosphokinase (CPK) activity in one animal in the 58.8X MHD group. In the 5880X MHD group, there were marked increases in CPK activity as well as increases in glutamic-oxaloacetic transaminase (GOT) and lactate dehydrogenase (LDH) activity. Furthermore, all the animals in the 5880X MHD group and the control group demonstrated increased GPT activity.

Urinalysis: No treatment related effect

Gross pathology: No treatment related effect.

Necropsy: No abnormalities were found in any animal.

Organ weight: No treatment related effect

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

**Histopathology inventory (optional)**

| Study | Acute Study | | | |
|---|---|---|---|---|
| Species | Dog | | | |
| Adrenals | X | | | |
| Aorta | X | | | |
| Bone Marrow smear | X | | | |
| Bone (femur) | X | | | |
| Brain | X* | | | |
| Cecum | X | | | |
| Cervix | X | | | |
| Colon | X | | | |
| Duodenum | X | | | |
| Epididymis | X | | | |
| Esophagus | X | | | |
| Eye | X | | | |
| Fallopian tube | | | | |
| Gall bladder | X | | | |
| Gross lesions | X | | | |
| Harderian gland | | | | |
| Heart | X * | | | |
| Ileum | X | | | |
| Injection site | X | | | |
| Jejunum | X | | | |
| Kidneys | X * | | | |
| Lachrymal gland | | | | |
| Larynx | X | | | |
| Liver | X* | | | |
| Lungs | X* | | | |

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

x, histopathology performed
*, organ weight obtained

**Reviewer's Comments:** Agreed with the results of this study.

### 2.6.6.3 Repeat-dose toxicity

**Study title: 17094: FP-CIT: A Two-Week Intravenous Toxicity Study in the Rats.**

**Key study findings**: The toxicity of daily intravenous injection of β-CIT-FP at doses of 0, 0.006, 0.6 and 3.6 mg/kg/day (0, 176.9X, 17688X and 106128X MHD respectively) to male and female Wistar rats for 14 days was assessed. No treatment-related clinical signs were reported in animals administered 176.9X MHD dose. However, stereotype behavior,

increased and violent physical activity, excessive sensitivity to external stimuli, and piloerection were reported in animals administered 106128X MHD dose while less extreme stereotype behavior was seen in female rats at 17688X MHD dose. Rats treated with 106128X MHD dose also demonstrated decreased food intake, and this was probably responsible for effects on weight gain, serum protein concentrations, and organ weight. The NOAEL in this study was 0.006 mg/kg/day (176.9 X MHD).

**Study no.**: Study No. 17094

**Volume # and page #**: Volume 1 pages 1- 175

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: September 10, 1996

**GLP compliance**: Yes

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: FP-CIT, Lot # GT-IV-03 and purity not stated

**Method:**

Doses: 0, 0.006, 0.6 and 3.6 mg/kg/day (0, 176.9X, 17688X and 106128X MHD respectively) as in the table below.

Species/strain: Rats/Wistar Crl:WI) BR (outbred, SPF-Quality)

Number/sex/group or time point (main study): 10/male and female/group.

Age: 5-6 weeks old

Weight: 92-109 g (males); 94-110 g (females).

Control article: Acetate buffer solution/5% ethanol

Route, formulation, volume, and infusion rate: Intravenous injection via lateral tail vein at an injection rate of 1mL/minute and volume dosage of 2.5 mg/kg.

The groups, dose levels, animal numbers and colour codes were as follows:

| Group | Dosing (FP-CIT mg/kg body wt/day) | Animal Nos. | | Colour code |
|---|---|---|---|---|
| | | ♂ | ♀ | |
| 1 | 0 (vehicle) | 1 - 10 | 11 - 20 | White |
| 2 | 0.006 | 21 - 30 | 31 - 40 | Blue |
| 3 | 0.6 | 41 - 50 | 51 - 60 | Green |
| 4 | 3.6 | 61 - 70 | 71 - 80 | Red |

The rats were dosed daily for at least 14 days and until the day before necropsy.

**Results:**

Mortality: No mortality was reported.

Clinical signs: No clinical signs were reported in vehicle- and 176.9X MHD dose-treated rats. However, animals administered 106128X MHD β-CIT-FP demonstrated various types of stereotype behavior, and showed both increased and violent physical activity. These animals were excessively sensitive to external stimuli and they showed piloerection from the fourth day of treatment to the end of the study. The females treated with 176.9X MHD daily dose also showed stereotype behavior though with less magnitude than reported at 106128X MHD.

Body weights: No treatment related effect was reported on the body weight. However, mean body weight gain in 106128X MHD group was lower throughout the treatment period.

Food consumption: No treatment related effect was reported on the body weight. There were however lowered mean food consumption in males in 106128X MHD group.

Ophthalmology: No treatment related effect.

Hematology: No treatment related hematological effect.

Clinical chemistry: The mean serum concentrations of protein and albumin were significantly lower in the females in 106128X MHD treated group. The sponsor attributed this decrease to the lower food intake in this group. There was also a dose-related decreased mean concentration of bilirubin in both males and females administered 0.6 and 3.6 mg/kg/day dose. No other treatment related effects on the evaluated clinical parameters was reported in this study.

Urinalysis: No treatment related effect. There was however presence of blood in the urine for males in 17688X and 106128X MHD treated groups, as determined with Ames Multistix. This was not confirmed by the presence of an increased number of erythrocytes in the spun sediment.

Gross pathology: No treatment related effect.

Necropsy: No abnormalities were found in any animal.

Organ weight: No treatment related effect was reported at low doses employed in this study. However, there were increased relative weights of the brain in both sexes and increased relative weights of the kidneys, thymus and ovaries in females administered 106218X MHD dose.

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

**Histopathology inventory (optional)**

| Study | Acute Study | | | |
|---|---|---|---|---|
| Species | Dog | | | |
| Adrenals | x* | | | |
| Aorta | x | | | |
| Bone Marrow smear | x | | | |
| Bone (femur) | x | | | |
| Brain | x* | | | |
| Cecum | x | | | |
| Cervix | x | | | |
| Colon | x | | | |
| Duodenum | x | | | |
| Epididymis | x | | | |
| Esophagus | x | | | |
| Eye | x | | | |
| Fallopian tube | | | | |
| Gall bladder | x | | | |
| Gross lesions | x | | | |
| Harderian gland | | | | |
| Heart | x * | | | |
| Ileum | x | | | |
| Injection site | x | | | |
| Jejunum | x | | | |
| Kidneys | x * | | | |
| Lachrymal gland | | | | |
| Larynx | x | | | |
| Liver | x* | | | |
| Lungs | x | | | |
| Lymph nodes, cervical | x | | | |
| Lymph nodes mandibular | x | | | |
| Lymph nodes, mesenteric | x | | | |

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

x, histopathology performed
*, organ weight obtained

**Reviewer's Comments:** This reviewer agrees with the result of this study which indicates that intravenous administration of FP-CIT to rats for 14 days at a dose level of 176.9X MHD caused no signs of toxicity. Stereotype behavior was reported in females administered 17688X MHD that became more intense in both males sexes at 106128X MHD dose. This is due to a dose-dependent pharmacologic effect of FP-CIT. The NOAEL in this study was 0.006 mg/kg/day (176.9X MHD). Thus, indicating an adequate safety margin for using β-CIT-FP at the human dose of 0.325 µg.

**Study title: 2001NM023: 2-Week Repeated Intravenous Dose Toxicity Study of FP-CIT in Rats.**

**Key study findings**: The toxicity of daily intravenous injection of β-CIT-FP at doses of 0, 1, 10 and 300 µg/kg/day (0, 29.5X, 294.8X and 8844X MHD respectively) to Spraque Dawley rats for 14 days was assessed. No mortality was reported. There was increased motility after administration of 17688X MHD FP-CIT. Necropsy revealed scattered

blood spots in the lungs of 1/5 female in the 8844X MHD group and histopathology of the lungs showed localized mild bleeding in males in the 294.8X MHD group and in males and females in the 8844X MHD group. No other clinical signs were demonstrated in the treated groups. The NOAEL in this study was 10 µg/kg/day (294.8X MHD).

**Study no.**: Study No. 2001NM023

**Volume # and page #**: Volume 1 pages 1- 148

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: June 10, 2002

**GLP compliance**: Yes

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: FP-CIT, Lot # FSL03 and purity stated as 98.8%.

**Method:**
Doses: 0, 1, 10 and 300 µg/kg/day (0, 29.5X, 294.8X and 8844X MHD respectively) as in the table below.

Species/strain: Rats/Crj:CD (SD) IGS rats

Number/sex/group or time point (main study): 10/male and female/group.

Age: 6 weeks old

Weight: 186-218 g (males); 142-168 g (females).

Control article: 1 % (w/v) ascorbic acid solution

Route, formulation, volume, and infusion rate: Intravenous injection via a tail vein using an infusion pump (b) (4) with a 24-guage butterfly needle. Dosing volume of 1 mL/kg of body weight for 1 µg/kg/day group and 10 mL/kg body weight for other groups and infusion rate of 1mL/minute was employed.

The rats were dosed once daily for 14 consecutive days and the animals were observed daily from day 3 before administration to the day of necropsy. At the end of the administration period, the animals subjected to fasting for 16 or more hours were anesthetized with ether to collect blood samples and subsequent necropsy.

[Dose levels and group composition]

| Group | Name of substance administered | Dose level (μg/kg/day) | Concen-tration (μg/mL) | Dosing volume (mL/kg) | Animal No. | |
|---|---|---|---|---|---|---|
| | | | | | Male | Female |
| Control group | 1 w/v% ascorbic acid solution | 0 | 0 | 10 | 1M01-1M10 | 1F01-1F10 |
| Low dose level group | FP-CIT | 1 | 1 | 1 | 2M01-2M10 | 2F01-2F10 |
| Middle dose level group | FP-CIT | 10 | 1 | 10 | 3M01-3M10 | 3F01-3F10 |
| High dose level group | FP-CIT | 300 | 30 | 10 | 4M01-4M10 | 4F01-4F10 |

**Results:**

Mortality: No mortality was reported.

Clinical signs: No clinical signs were reported in vehicle- and FP-CIT-treated rats. However, animals administered 8844X MHD β-CIT-FP demonstrated increased motility after administration on days 1 to 14 of administration.

Body weights: No treatment related effect.

Food consumption: No treatment related effect.

Water consumption: No treatment related effect.

Ophthalmology: No abnormalities were reported.

Hematology: No treatment related hematological effect.

Clinical chemistry: High values of potassium in comparison to the control group were found in males administered 294.8X and 8844X MHD doses. The differences were not statistically significant. However, females in the 29.5X MHD group showed significantly high values of creatine phosphokinase. This effect was not regarded as treatment related based on lack of any relationship with the dose level of this compound. No other treatment related effects on the evaluated clinical parameters was reported in this study.

Urinalysis: No treatment related effect.

Gross pathology: No treatment related effect.

Necropsy: No abnormalities were found in any animal. However, one male in the 8844X MHD group showed a cyst-like change in the cecal apex, a female in the same group showed scattered blood spots in the right and left lungs. In addition 1/10 females in 294.8X MHD group demonstrated pyelectasis. This effect was incidental and not treatment related.

Organ weight: No treatment related effect was reported at low doses employed in this study. However, females in the 8844X MHD group showed significantly high values of the absolute weight and organ weight-to-body weight ratio of salivary glands in comparison to the control group.

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

**Histopathology inventory (optional)**

| Study | Acute Study | | | |
|---|---|---|---|---|
| Species | Dog | | | |
| Adrenals | x * | | | |
| Aorta | x | | | |
| Bone Marrow smear | x | | | |
| Bone (femur) | x | | | |
| Brain | x * | | | |
| Cecum | x | | | |
| Cervix | x | | | |
| Colon | x | | | |
| Duodenum | x | | | |
| Epididymis | x* | | | |
| Esophagus | x | | | |
| Eye | x | | | |
| Fallopian tube | | | | |
| Gall bladder | x | | | |
| Gross lesions | x | | | |
| Harderian gland | | | | |
| Heart | x* | | | |
| Ileum | x | | | |
| Injection site | x | | | |
| Jejunum | x | | | |
| Kidneys | x* | | | |
| Lachrymal gland | | | | |
| Larynx | x | | | |
| Liver | x * | | | |
| Lungs | x* | | | |
| Lymph nodes, cervical | x | | | |
| Lymph nodes mandibular | x | | | |
| Lymph nodes, mesenteric | x | | | |
| Mammary Gland | x | | | |
| Nasal cavity | | | | |
| Optic nerves | x | | | |
| Ovaries | x* | | | |
| Pancreas | x | | | |

| | | | | |
|---|---|---|---|---|
| Parathyroid | x* | | | |
| Peripheral nerve | | | | |
| Pharynx | x | | | |
| Pituitary | x | | | |
| Prostate | x* | | | |
| Rectum | x | | | |
| Salivary gland | x* | | | |
| Sciatic nerve | x | | | |
| Seminal vesicles | x* | | | |
| Skeletal muscle | x | | | |
| Skin | x | | | |
| Spinal cord | x | | | |
| Spleen | x* | | | |
| Sternum | | | | |
| Stomach | x | | | |
| Testes | x* | | | |
| Thymus | x* | | | |
| Thyroid | x* | | | |
| Tongue | x | | | |
| Trachea | x | | | |
| Urinary bladder | x | | | |
| Uterus | x* | | | |
| Vagina | x | | | |
| Zymbal gland | | | | |

x, histopathology performed
*, organ weight obtained

From the NOAEL of 1 µg/kg/day in this study:
**The safety margin for the maximum clinical dose of 0.325 µg is 29.5-folds.**

**Reviewer's Comments:** This reviewer agrees with the result of the studies as discussed under various sections above. No serious toxicity was demonstrated following 2 week repeat dose administration of this compound to rats and adequate safety margin was established for the clinical use of 0.325 µg/kg dose of the compound for this indication.

**Study title: 17095: FP-CIT: A Two-Week Intravenous Toxicity Study in the Rabbits.**

**Key study findings**: The toxicity of daily intravenous injection of β-CIT-FP at doses of 0, 0.006, 0.6 and 1.5 mg/kg/day (0, 360X, 36000X and 90000X MHD respectively) to New Zealand rabbits for 14 days was evaluated. No treatment-related mortality was reported in this study. Stereotype and aggressive behavior, increased responses to external stimuli, protruding eyes with dilated pupils, and fast or labored respiration occurred in animals administered 90000X MHD FP-CIT. Some stereotype behavioral effects were reported at 360X MHD dose while more pronounced behavioral effects were also reported at 36000X MHD dose. However, no signs of toxicity were demonstrated at these doses. The NOAEL was not determined in this study.

**Study no.**: Study No. 17095

**Volume # and page #:** Volume 1 pages 1- 175

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: November 27, 1996

**GLP compliance**: Yes

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: FP-CIT, Lot # NMP 1035 and purity not stated

**Method:**
Doses: 0, 0.006, 0.6 and 1.5 mg/kg/day (0, 360X, 36000X and 90000X MHD respectively) as in the table below.

Species/strain: Rabbits/New Zealand White rabbits

Number/sex/group or time point (main study): 4/male and female/group.

Age: 4-6 months old

Weight: 1.91-2.23 kg (males); 2.06-2.44 kg (females).

Control article: Acetate buffer solution/5% ethanol

Route, formulation, volume, and infusion rate: Intravenous injection via marginal ear vein at a volume dosage of 0.5 mg/kg.

| Group | Dosing (FP-CIT mg/kg body wt/day) | Animal Nos. | | Colour code |
|---|---|---|---|---|
| | | ♂ | ♀ | |
| 1 | 0 (vehicle) | 1 - 4 | 5 - 8 | White |
| 2 | 0.006 | 9 - 12 | 13 - 16 | Blue |
| 3 | 0.6 | 17 - 20 | 21 - 24 | Green |
| 4 | 1.5 | 25 - 28 | 29 - 32 | Red |

The rats were dosed daily for at least 14 days.

**Results:**

Mortality: No treatment related mortality was reported in the animals. However, the sponsor stated that the two rabbits (one male and female in 90000X MHD dosed group) found dead in this study died of underlying ailments.

Clinical signs: Clinical signs were reported in all treated animals irrespective of the dose level. Vocalization and violent reactions as well as stereotype and aggressive behavior, increased responses to external stimuli, protruding eyes with dilated pupils, and fast or labored respiration were reported at 90000X MHD dose. Less severe stereotype behaviors were reported at 0, 360X and 36000X MHD. The stereotype behavior included gnawing and biting the cage, gnawing the fore-paws, scratching, and excessive grooming around the mouth.

Body weights: No treatment related effect was reported on the body weight.

Food consumption: No treatment related effect was reported on the body weight at 0, 360X and 36000X MHD doses. There were however lowered mean food consumption in males in 36000X MHD group when compared to the controls.

Ophthalmology: No treatment related effect

Hematology: No treatment related hematological effect

Clinical chemistry: No significant treatment related effect reported in most clinical chemistry parameters of controls and treated groups. However, the mean concentration of urea, creatinine kinase and lactate dehydrogenase were higher at the end of the vehicle- or FP-CIT-treatment period than before treatment. The bilirubin concentrations were lower after treatment than before treatment. Statistically significant reductions were reported in the 36000X and 90000X MHD treated females when compared to the control group ($p<0.01$ and $p< 0.05$).

Urinalysis: No treatment related effect reported.

Gross pathology: No treatment related effect. However, there were local hemorrhages at the injection sites of both treated and control animals.

Necropsy: No abnormalities were found in any animal.

Organ weight: No treatment related effect was reported.

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

**Histopathology inventory (optional)**

| Study | Acute Study | | | |
|---|---|---|---|---|
| Species | Dog | | | |
| Adrenals | x* | | | |
| Aorta | x | | | |
| Bone Marrow smear | x | | | |
| Bone (femur) | x | | | |
| Brain | x* | | | |
| Cecum | x | | | |
| Cervix | x | | | |
| Colon | x | | | |
| Duodenum | x | | | |
| Epididymis | x* | | | |
| Esophagus | x | | | |
| Eye | x | | | |
| Fallopian tube | | | | |
| Gall bladder | x | | | |
| Gross lesions | x | | | |
| Harderian gland | | | | |
| Heart | x* | | | |
| Ileum | x | | | |
| Injection site | x | | | |
| Jejunum | x | | | |
| Kidneys | x* | | | |
| Lachrymal gland | | | | |
| Larynx | x | | | |
| Liver | x* | | | |
| Lungs | x* | | | |
| Lymph nodes, cervical | x | | | |
| Lymph nodes mandibular | x | | | |
| Lymph nodes, mesenteric | x | | | |
| Mammary Gland | x | | | |
| Nasal cavity | | | | |
| Optic nerves | x | | | |
| Ovaries | x* | | | |
| Pancreas | x | | | |
| Parathyroid | x* | | | |
| Peripheral nerve | | | | |
| Pharynx | x | | | |
| Pituitary | x* | | | |
| Prostate | x* | | | |
| Rectum | x | | | |
| Salivary gland | x* | | | |
| Sciatic nerve | x | | | |
| Seminal vesicles | x* | | | |
| Skeletal muscle | x | | | |
| Skin | x | | | |
| Spinal cord | x | | | |
| Spleen | x* | | | |

| | | | | |
|---|---|---|---|---|
| Sternum | | | | |
| Stomach | x | | | |
| Testes | x* | | | |
| Thymus | x* | | | |
| Thyroid | x* | | | |
| Tongue | x | | | |
| Trachea | x | | | |
| Urinary bladder | x | | | |
| Uterus | x* | | | |
| Vagina | x | | | |
| Zymbal gland | | | | |

x, histopathology performed
*, organ weight obtained

**Reviewer's Comments:** The sponsor did not provide any convincing evidence to prove that the mortality of the two rabbits in 90000X MHD group was not related to the treatment. The sponsor stated that the female rabbit died of diarrhea while the male rabbit died within 3 hours of the first dose. It is very likely that these rabbits died because of toxicity of this compound. The reported clinical signs at the employed doses in this study are indicative of dose dependent cocaine-like pharmacological effect of FP-CIT including at the lowest dose (360X MHD). Thus, the NOAEL was not established in this study.

**Study title: 2001NM024: 2-Week Repeated Intravenous Dose Toxicity Study of FP-CIT in Dogs.**

**Key study findings**: The toxicity of daily intravenous injection of β-CIT-FP at doses of 0, 0.3, 1 and 100 µg/kg/day (0, 29.5X, 98.3X and 9828X MHD respectively) to Beagle dogs for 14 days was assessed. No mortality was reported in this study. There was mydriasis, congestion of the visible mucosa, flushing of the pinnae, reddening of the skin, or panting after administration of 9828X MHD FP-CIT. The NOAEL in this study was 1 µg/kg/day (98.3X MHD)

**Study no.**: Study No. 2001NM024

**Volume # and page #**: Volume 1 pages 1- 88

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: June 10, 2002

**GLP compliance**: Yes

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: FP-CIT, Lot # FSL03 and purity stated as 98.8%.

**Method:**

Doses: 0, 0.3, 1 and 100 µg/kg/day (0, 29.5X, 98.3X and 9828X MHD respectively) as in the table below.

Species/strain: Dogs/Beagle

Number/sex/group or time point (main study): 3/male and female/group.

Age: 8 months old

Weight: 9.35-12.0 kg (males); 8.60-10.65 kg (females).

Control article: 1 % (w/v) ascorbic acid solution

Route, formulation, volume, and infusion rate: Intravenous injection via the cephalic vein using an infusion pump [redacted] (b) (4) with a 22-guage indwelling needle. Dosing volume of 1 mL/kg of body weight for 1 µg/kg/day group and 0.3 mL/kg body weight for other groups and infusion rate of 3mL/minute was employed.

The dogs were dosed once daily for 14 consecutive days and the animals were observed daily from day 3 before administration to the day of necropsy.

[Dose levels and group composition]

| Group | Name of substance administered | Dose level (µg/kg/day) | Concen-tration (µg/mL) | Dosing volume (mL/kg) | Animal No. | |
|---|---|---|---|---|---|---|
| | | | | | Male | Female |
| Control group | 1 w/v% ascorbic acid solution | - | - | 1 | 01, 02, 03 | 41, 42, 43 |
| Low dose level group | FP-CIT | 0.3 | 1 | 0.3 | 11, 12, 13 | 51, 52, 53 |
| Middle dose level group | FP-CIT | 1 | 1 | 1 | 21, 22, 23 | 61, 62, 63 |
| High dose level group | FP-CIT | 100 | 100 | 1 | 31, 32, 33 | 71, 72, 73 |

**Results:**

Mortality: No mortality was reported.

Clinical signs: All animals in the 9828X MHD group showed mydriasis, congestion of the visible mucosa, flushing of the pinnae, reddening of the skin, or panting after administration. Loose stool was reported in one control animal before dosing while vomiting and partial food consumption were reported before administration period in one

animal administered 29.5X MHD, partial food consumption were reported before administration period in two animals in 98.3X MHD group and also partial food consumption were reported after administration in one animals (9828X MHD).

Body weights: No treatment related effect.

Food consumption: No treatment related effect was reported on the body weight.

Water consumption: No treatment related effect.

Ophthalmology: No abnormalities were reported.

Hematology: No treatment related hematological effect.

Clinical chemistry: No treatment-related effect was reported. However, one female administered 29.5X MHD demonstrated an increased glutamic-pyruvic transaminase (GPT) activity during week 2 of the study.

Urinalysis: No treatment related effect.

Gross pathology: No treatment related effect reported. However, fibrous thickening of the alveolar septum involving the hypertrophy of alveolar cells was reported in one male while localized bronchitis was reported in one female in the 9828X MHD group. A male and a female in the 98.3X and 9828X MHD groups also demonstrated a mild cyst in the anterior lobe of the pituitary. These changes were sporadic, occurred in one animal per group and not treatment related.

Necropsy: There was coloration to dark red, coloration to red-brown, or bleeding that was localized subcutaneously at the site of administration in three animals in the control group, all females in the 29.5X MHD group, three animals in the 98.3X MHD group and one male in the 9828X MHD group. Presence of a cyst in the pituitary was reported in one female each in the 29.5X and 98.3X MHD groups. This effect was incidental and not treatment related.

Organ weight: No treatment related effect was reported.

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

**Histopathology inventory (optional)**

| Study | Acute Study | | | |
|---|---|---|---|---|
| Species | Dog | | | |
| Adrenals | x * | | | |

| | | | | |
|---|---|---|---|---|
| Aorta | x | | | |
| Bone Marrow smear | x | | | |
| Bone (femur) | x | | | |
| Brain | x * | | | |
| Cecum | x | | | |
| Cervix | x | | | |
| Colon | x | | | |
| Duodenum | x | | | |
| Epididymis | x* | | | |
| Esophagus | x | | | |
| Eye | x | | | |
| Fallopian tube | | | | |
| Gall bladder | x | | | |
| Gross lesions | x | | | |
| Harderian gland | | | | |
| Heart | x* | | | |
| Ileum | x | | | |
| Injection site | x | | | |
| Jejunum | x | | | |
| Kidneys | x* | | | |
| Lachrymal gland | | | | |
| Larynx | x | | | |
| Liver | x * | | | |
| Lungs | x* | | | |
| Lymph nodes, cervical | x | | | |
| Lymph nodes mandibular | x | | | |
| Lymph nodes, mesenteric | x | | | |
| Mammary Gland | x | | | |
| Nasal cavity | | | | |
| Optic nerves | x | | | |
| Ovaries | x* | | | |
| Pancreas | x | | | |
| Parathyroid | x* | | | |
| Peripheral nerve | | | | |
| Pharynx | x | | | |
| Pituitary | x | | | |
| Prostate | x* | | | |
| Rectum | x | | | |
| Salivary gland | x* | | | |
| Sciatic nerve | x | | | |
| Seminal vesicles | x* | | | |
| Skeletal muscle | x | | | |
| Skin | x | | | |
| Spinal cord | x | | | |
| Spleen | x* | | | |
| Sternum | | | | |
| Stomach | x | | | |
| Testes | x* | | | |
| Thymus | x* | | | |

| | | | | |
|---|---|---|---|---|
| Thyroid | x* | | | |
| Tongue | x | | | |
| Trachea | x | | | |
| Urinary bladder | x | | | |
| Uterus | x* | | | |
| Vagina | x | | | |
| Zymbal gland | | | | |

x, histopathology performed
*, organ weight obtained

From the NOAEL of 1 µg/kg/day in this study:
**The safety margin for the maximum clinical dose of 0.325 µg is 98.3-folds.**

**Reviewer's Comments:** This reviewer agrees with the result of the studies as discussed under various sections above. The sponsor established adequate safety margin for the clinical use of 0.325 µg dose of the compound for the indication.

**2.6.6.4 Genetic toxicology**

The genotoxicity potential of FP-CIT was evaluated using the standard ICH battery of tests. The tests include two *in vitro* assays covering the endpoints of gene mutation (in bacteria) and chromosomal effects (in cultured human lymphocytes and in mouse lymphoma cells).

**Study title**: **17348: FP-CIT Mouse Micronucleus Test.**

**Key findings**: FP-CIT did not induce mutation in *Echerichia coli* and five histidine-requiring strains of *Salmonella triphimurium* TA98, TA100, TA1535, TA1537 and TA102 when tested at concentrations up to 1.5 mg/ml of preincubation reaction mixture, in the absence and presence of a rat liver metabolic activation system.

**Study no.**: 17348

**Volume # and page #**: Volume 1 page 1-21

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: February 11, 1997.

**GLP compliance**: Yes

**QA report**: yes (x) no ( )

**Drug, lot #, and % purity**: FP-CIT Batch # 97A16FPHSA and purity: 99.7%.

**Methods**
This study was conducted based on the experimental materials and methodology described by Ames et al (1975).

Strains/species/cell line: Five histidine-requiring strains of *Salmonella triphimurium* – TA98, TA100, TA1535, TA1537 and TA102.

Doses used in definitive study: Doses tested in this study were 0.1, 0.2, 0.37, 0.75 and 1.5 mg of FP-CIT per plate of preincubation reaction mixture in both presence and absence of S9 mix.

Basis of dose selection: No information was provided on dose selection.

Vehicle controls: Phosphate buffer and 3% Human Serum Albumin

Positive controls: The following were used as positive control:
1) Cumene hydroperoxide was used for TA 102 without S-9 mix.

2) Na-azide for TA 100 and TA 1535 and 1.0 pg/plate 2-nitrofluorene (2-NF) for TA 98 and TA 1537 without S-9 mix.

3) 2-Aminoanthrazene (2-AA) for strain TA 102 and other four strains with S-9 mix.

Incubation and sampling times: 0.3 ml of the test solution, 0.5 ml S-9 mix or 0.02M phosphate buffer pH 7.4 and 0.1 ml bacterial suspension (approx. 109 bact./ml). The test tube was incubated for I hour at 37$^{o}$C under gentle shaking. 2.0 ml top agar was added and after vortexing, the mixture was spread on a Vogel-Bonner agar plate. The culture was incubated for 48 to 72 hours at 37$^{o}$C and the number of colonies was counted.

**Results**

Study validity: The test substance would be considered to be mutagenic if the following criteria were met:

1. Statistically significant and dose related increase in the level of revertants on the test plates as compared to the control plates. The Analysis of Variance test was used to compare test and negative control groups.
2. The number of revertants at the dose level where the highest effect was found was more than twice the concurrent spontaneous level.

However, if 1 and not 2 was met in both test series, the test substance would be considered to be weakly mutagenic. Furthermore, sporadically occurring statistically significant increases in revertants which were not dose related (i.e. occurring at the lower dose levels when there was no increase at higher non-toxic doses) were considered incidental and not relevant for the evaluation.

Study outcome: No cytotoxicity was observed with any of the tester strains since no depression of the background growth was observed on the plates. However, a weak toxicity was observed especially in strain TA 102 which could be seen as a reduction in the number of revertants mainly at the maximum test article concentration. Generally in this study, the number of revertants in the test groups and the controls were similar. There was however small increases in revertant numbers in TA 100 and TA 98 in a few test groups. This was considered incidental and of no biological significance because the increases were weak and not dose related.

**Reviewer's Comments:** This study indicates that at the tested doses (up to 1.5 mg/plate), FP-CIT demonstrated no mutagenic effect in the Ames Salmonella assay. However, the maximum concentration employed in this study is over 3-fold lower than the recommended dose of 5000 µg/plate. The sponsor did not provide any explanation for failure to employ adequate concentration for this study. This study therefore failed to determine if FP-CIT could induce mutation on the Salmonella typhimurium strains at higher concentrations. Thus, this study failed to conclusively show that FP-CIT is non-mutagenic during Ames test.

**Study title**: **25618: FP-CIT *In vitro* Mammalian Cytogenetic Test Performed with Human Lymphocytes.**

**Key findings**: The potential chromosome damaging effect of FP-CIT was evaluated using mammalian cytogenetic test. FP-CIT was tested in primary human lymphocytes at up to 5 µg/ml concentration with or without S-9 mix using 24h sampling time in both tests and an additional 48 h sampling time in the second test. No biologically significant increase in the frequency of chromosomal aberrations was found in either test, or following either incubation period. Thus, FP-CIT was non-clastogenic in this study.

**Study no.**: 25618

**Volume # and page #**: Volume 1 page 1-20

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: October 7, 1997.

**GLP compliance**: Yes

**QA report**: yes (x) no ( )

**Drug, lot #, and % purity**: FP-CIT Batch #s 97K06FPDMF and 97103FPDMF and the purity was not provided.

**Methods**

Strains/species/cell line: Primary human lymphocytes were obtained from two healthy male volunteers from the Scantox laboratory.

Doses used in definitive study: Doses tested in this study were 0.63, 1.25, 2.5 and 5 µg/ml concentrations of FP-CIT per plate of preincubation reaction mixture in both presence and absence of S9 mix.

Basis of dose selection: The dose selection was based on limited solubility of FP-CIT. Thus, 5 µg/ml was the highest practicable treatment concentration of FP-CIT employed in this study.

Vehicle controls: Aqueous solution of 10% DMF in phosphate buffer (pH 7).

Positive controls: The following were used as positive control:

1) Daunomvcin C at 0.015 and 0.03 µg/ml was used as the positive control without S-9 mix.

2) Cyclophosphamide at 6 and 12 µg/ml was used as the positive control with S-9 mix.

Incubation and sampling times: The culture was centrifuged at 800 rpm, 5 minutes and the supernatant removed. The cell pellet was resuspended in 5 ml 0.075 M KCl for 10 minutes after which the suspension was centrifuged again and the supernatant removed. The cells were fixed by drop wise addition of methanol/acetic acid (3:1), washed twice with fresh fixative, dropped on to clean glass slides and air dried. The chromosome preparations were stained in 3 % Giemsa for 10 minutes and cover glasses were mounted in Damniarxylen®. The microscopic analysis involving 100 metaphases was then performed at 1000 x magnification on each culture.

**Results**

Study validity: The sponsor did not provide the criteria for consideration for chromosomal aberration in this study.

Study outcome: No marked differences were reported in the mitotic index or the frequency of metaphases with chromosomal aberrations was observed between the vehicle controls and the untreated controls. There were small reductions in mean mitotic index observed at the 24 hour sampling time in cultures treated with FP-CIT in the absence of S-9 mix at 5 µg/ml in both tests (32 % in the first test and 41 % in the second test) and at 1.25 µg/ml in the second test (36%), compared to the untreated control values. However, no marked reductions in mitotic index were observed in cultures treated in the presence of S-9 mix, or at the 48 hour sampling time. The frequency of metaphases with chromosome aberrations was within the normal range in all negative control culture indicating that no significant increased frequency of metaphases with aberration was

induced by FP-CIT at the employed concentrations in this study. However, in contrast there was increased frequency of metaphases with aberrations after treatment with the positive control agents. This demonstrates that the employed assay system is sensitive to detect any chromosomal aberration.

Based on absence of any biologically significant increase in the frequency of chromosomal aberrations during test and after incubation period, FP-CIT is non-clastogenic in this study.

**Reviewer's Comments:** Agreed with result of this study. The sponsor was not able to employ higher concentration of FP-CIT in this study due to limited solubility.

**Study title**: **17349: FP-CIT *In vitro* Mammalian Cell Gene Mutation Test Performed with Mouse Lymphoma Cells (LY5178Y).**

**Key findings**: The potential mutagenic effect of FP-CIT up to 330 µg/ml concentration was evaluated using mouse lymphoma cells (L5178Y) with or without S-9 metabolic activation. No increase in mutation frequency was reported in this study. Thus, FP-CIT is non-mutagenic in the i*n vitro* mammalian cell gene mutation test performed with mouse lymphoma cells with and without metabolic activation.

**Study no.**: 17349

**Volume # and page #**: Volume 1 page 1-18

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: August 4, 1997.

**GLP compliance**: Yes

**QA report**: yes (x) no ( )

**Drug, lot #, and % purity**: FP-CIT Batch # 97A16FPHSA and the purity was not provided.

**Methods**

This study involved adding varying concentrations (up to 0.33 mg/ml) of FP-CIT to cell cultures of L5178Y mouse lymphoma cells. The first test was conducted at 0.01, 0.02, 0.16 and 0.33 mg/ml concentrations with and without S-9 mix. In the second test, 0.04, 0.08, 0.16 and 0.33 mg/ml concentrations were employed with and without S-9 metabolic activation.

Strains/species/cell line: The assay was performed with the mouse L5178Y lymphoma cell line Ba 21:1 obtained from (b) (4) The cells were grown as suspension cultures under gentle mixing in RPMI 1640 medium supplemented with l0% horse serum, 200 µg/ml sodium pyruvate and 50 µg/ml gentamycin.

Doses used in definitive study: Doses tested in the presence and absence of S-9 metabolic activation are:
Test 1- 0.01, 0.02, 0.16 and 0.33 mg/ml
Test 2- 0.04, 0.08, 0.16 and 0.33 mg/ml.

Basis of dose selection: The dose selection was based on limited solubility of FP-CIT. The sponsor stated that 0.33 mg/ml was the highest practicable treatment concentration of FP-CIT in this study.

Vehicle controls: HSA/phosphate buffer.

Positive controls: The following were used as positive control:

1) Ethylnitrosourea (ENU) at 50 and 150 µg/ml concentrations was used as the positive control without S-9 mix.
2) Dimethylbenzanthracene (DMBA) at 3.3 and 10 µg/ml was used as the positive control with S-9 mix.

Incubation and sampling times: Before conducting the mutagenicity assay, the cells are grown for 1-2 days in medium containing thymidine (9 µg/ml), hypoxanthine (15 µg/ml), methotrexate (0.3 µg/ml) and glycine (22.5 µg/ml) (THIVIG-medium). The cell cultures were exposed to the various concentrations of the test article (as above) during each of the independent mutagenicity tests. Both vehicle and positive controls were included in the tests. After 10 days incubation (5% $CO_2$, 37$^{o}$C) the number of cell clones were counted and the mutation frequencies determined for each dose and for the negative and positive controls as shown in the flow diagram and summary protocol below.

Flow diagram and summary protocol

| | | |
|---|---|---|
| Day 0 | $4.5 \times 10^6$ cells per tube before dosing | |
| | ↓ | ↓ |
| | + S-9 mix | - S-9 mix |
| | + FP-CIT | + FP-CIT |
| | $2.25 \times 10^6$ cells per ml | $2.25 \times 10^5$ cells per ml |
| | (final volume: 3 ml) | (final volume: 15 ml) |
| | ↓ | ↓ |
| | After 3 hours | After 4 hours |
| | remove FP-CIT | remove FP-CIT |
| | resuspend to $3 \times 10^5$ per ml | resuspend to $3 \times 10^5$ per ml |
| | (15 ml totally) | (15 ml totally) |
| | Clone in non-selective medium | Clone in non-selective medium |
| | Incubate for 10 days | Incubate for 10 days |
| Day 1 | Count and subculture to $3 \times 10^5$ per ml | |
| Day 2 | Count and subculture to $3 \times 10^5$ per ml | |
| Day 3 | Count and clone in non-selective and selective (TFT) medium. Incubate for 10 days | |
| Day 10 | Count clones from day 0 | |
| Day 13 | Count clones from day 3 | |

**Results**

Study validity: The sponsor did not provide the criteria for consideration for chromosomal aberration in this study. However, during clone counting, gene mutations and chromosome deletions were distinguished by "large" clones representing gene mutations and "small" clones representing chromosome deletions.

Study outcome: The sponsor presented tables showing the results of these tests. No significant toxicity was induced by FP-CIT up to 0.33 mg/ml concentration in the tests. The tables indicate that the mutation frequency of the vehicle control was similar to that of FP-CIT at all concentrations with and without S-9 mix while there was elevated (8- to 11-folds) mutation frequency in the positive control in the presence and absence of S-9 mix. However, the mutation frequency of the vehicle and positive controls were within expected and acceptable ranges.

There was no significant increase in mutation frequency at all concentrations of FP-CIT employed in this study. Thus, FP-CIT was found to be non-mutagenic in the *in vitro* mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y).

**Reviewer's Comments:** Agreed with result of this study.

**Study title**: **2001NM020: Micronucleus Test of FP-CIT in Mice.**

**Key findings** The sponsor provided a summary of the translated version of the original full study in Japanese language. The potential of FP-CIT to induce chromosomal aberrations *in vivo* was examined using a micronucleus test. Smears of bone marrows obtained from male Crj:CD-1 (ICR) mice intravenously administered up to 300 µg/kg FP-CIT for two consecutive days were employed. No dose-dependent or significant induction of micronuclei was reported in any of the mice administered FP-CIT indicating that FP-CIT failed to induce aberrations in the chromosomes of mouse bone marrow cells.

**Study no.**: 2001NM020

**Volume # and page #**: Volume 1 page 1-2

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: Not Provided.

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: Not provided.

**Methods**
The sponsor did not provide detailed information on the method employed in this study. The only available information was extracted from a short summary of the translated version of the original summary. In this study, four doses of FP-CIT were intravenously injected to male mice for 2 consecutive days. Smears were prepared from their bone marrows at 18 to 24 hours after treatment. 2000 polychromatic erythrocytes (PCEs) per animal were observed to calculate the incidence of micronuclei. Furthermore, ≥ 1000 total erythrocytes (PCEs + NCEs) per animal were observed as an indicator of the inhibition of bone marrow cell growth, and the percentage of PCEs was calculated.

Strains/species/cell line: Crj:CD-1 (ICR) mice (males)

Doses used in initial study: 37.5, 75, 150 and 300 µg/kg.

**Results**
Very scanty summary result was provided. FP-CIT demonstrates no dose dependent and no significant micronuclei induction was reported. This shows that this compound did not induce any chromosomal aberration in the mouse bone marrow cells in this study.

**Reviewer's Comment:** This study indicates that FP-CIT failed to induce any chromosomal aberration in the mouse bone marrow cells. However, the sponsor provided inadequate data for proper review of this study. No raw data, table or information on the study validity was available for evaluation of this *in vivo* genotoxicity study and this is unacceptable. Therefore, the available data is inadequate to make a determination on whether or not FP-CIT could induce chromosomal aberration based on this study. However, from the literature, chromosomal aberrations were reported to cocaine at 0.5 – 5 mg/ml in the presence and absence of S9. Thus, it is very unlikely that FP-CIT would induce any chromosomal aberration when administered at microdose level for this proposed indication.

**Study title**: **17350: FP-CIT Mouse Micronucleus Test.**

**Key findings**: The *in vivo* clastogenic activity and/or disruption of the mitotic apparatus of FP-CIT was evaluated by detecting micronuclei in polychromatic erythrocyte (PCE) cells in bone marrow isolated from SPF mice. No statistically significant increase in polychromatic erythrocytes with micronuclei and no signs of toxicity on the bone marrow as indicated by the ratio of polychromatic/normachromatic erythrocytes were reported following an administration of up to 20 mg/kg FP-CIT to the mice. Thus, FP-CIT was negative in this bone marrow micronucleus test.

**Study no.**: 17350

**Volume # and page #**: Volume 1 page 1-21

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: November 5, 1996.

**GLP compliance**: Yes

**QA report**: yes (x) no ()

**Drug, lot #, and % purity**: FP-CIT Batch #NMP1035 and the purity was not provided.

**Methods**

This study was conducted using the method previously described by Schmid (1975). The mice were intravenously injected 5, 10 and 20 mg/kg doses of FP-CIT and the bone marrows were collected from the right femur for preparation of the smears at various intervals. The specimen slides were fixed in methanol and stained with Geimsa (Merck). The dosing scheme and sampling time point for the micronucleus study shown below was employed in the study:

| Group | No of animals | Dosing | Mg/kg | Bone marrow sampling |
|---|---|---|---|---|
| Control | 5 ♂ + 5 ♀ | Vehicle | - | 24 h after dosing |
| Control | 5 ♂ + 5 ♀ | Vehicle | - | 48 h after dosing |
| Test group | 5 ♂ + 5 ♀ | MTD | 20 | 24 h after dosing |
| Test group | 5 ♂ + 5 ♀ | MTD | 20 | 48 h after dosing |
| Test group | 5 ♂ + 5 ♀ | MTD | 20 | 72 h after dosing |
| Test group | 5 ♂ + 5 ♀ | 0.5 x MTD | 10 | 24 h after dosing |
| Test group | 5 ♂ + 5 ♀ | 0.25 x MTD | 5 | 24 h after dosing |
| Positive control | 2 ♂ + 2 ♀ | Cyclophosphamide | 20 | 24 h after dosing |
| Positive control | 2 ♂ + 2 ♀ | Cyclophosphamide | 20 | 48 h after dosing |

The chromosome damaging effect of FP-CIT was assessed by comparing the frequency of PCE with micronuclei in the bone marrow of the treated versus control mice.

Strains/species: Mice/SPF mice

Weight: 7 weeks

Age: 25-32 g

Number/sex/group or time point (main study): 5/male and female/group.

Doses used in definitive study: 5, 10 and 20 mg/kg

Basis of dose selection: The doses were selected after an initial range finding study.

Vehicle controls: Acetate buffer solution/5% ethanol.

Positive controls: The positive control chemical is cyclophosphamide.

Incubation and sampling times: FP-CIT-, positive- and vehicle-treated groups were sacrificed and the bone marrow extracted from femur bones. Slides of the bone marrows were prepared and this was followed by the following slide scoring for each mouse:

a) The number of polychromatic erythrocytes (PCE) per 1000 erythrocytes.
b) The number of micronuclei (MN) in 1000 normachromatic erythrocytes.
c) The number of MN in normachromatic erythrocytes observed during scoring of the 1000 PCE.

**Results**

Study validity: No information on study validity was provided in this study.

Study outcome: There were signs of clinical toxicity demonstrated by piloerection, hyperactivity and hyperventilation in all FP-CIT administered mice. In the 20 mg/kg group, 24 hours, 3 male mice died between 2 hours to 3 days after dosing. Two male mice were also found dead the day after dosing in test group 10 mg/kg, 24 hours- In test

group 20 mg/kg, 72 hours, and one male mouse was found dead after three days. The tables showing the mean number of micronuclei per 1000 PCE as well as PCE in percent of total erythrocytes for the harvest times of 24, 48 and 72 hours were provided. The data showed no effect on the ratio of polychromatic/normochromatic erythrocytes was observed indicating no toxic effect on the bone marrow. Furthermore, no statistically significant increases in the frequency of micronuclei were observed in the mice treated with FP-CIT at various dose levels at the employed harvesting time (24, 48 or 72 hours) in this study. The data obtained to the positive and negative controls were within acceptable ranges.

FP-CIT did not demonstrate any mutagenic/clastogenic effect in this micronucleus test at the employed experimental conditions.

**Reviewer's Comment:** Agreed with the study results. It is noted that the clinical signs of piloerection, hyperactivity and hyperventilation in all FP-CIT administered mice reported in this study is probably due to cocaine-like pharmacological effect of the administered FP-CIT at all doses used in this study.

### 2.6.6.5 Carcinogenicity

No carcinogenicity study was conducted. The sponsor requested for a waiver for carcinogenicity studies for [$^{123}$I]FP-CIT.
Based on the available information that:

a) This compound is a diagnostic imaging agent for a single once in a life time use at a tracer dose of 0.325 µg, or for an infrequent use with long intervals between treatment for monitoring disease progression.
b) There is no previous demonstration of any carcinogenicity potential in the product class that could be considered relevant to humans.
c) There is no structural-activity relationship suggesting carcinogenicity risk and
d) No pre-neoplastic lesions was reported in any of the available repeated-dose toxicity studies on this compound

This reviewer recommends that the sponsor's request for a waiver for the carcinogenicity studies for this compound be granted.

### 2.6.6.6 Reproductive and developmental toxicology

The sponsor requested for a waiver for reproductive and developmental toxicity and the waiver was granted.

### 2.6.6.7 Local tolerance

**Study title: 144541: Assessment of Intravenous, Intra-arterial and Perivenous Tolerance of β-CIT-FP in the Rabbit after Single Administration.**

**Key study findings**: The tolerance of a single intra-arterial, intravenous and perivenous injection of β-CIT-FP to rabbit ears was assessed. β-CIT-FP was administered to the left ear and the right ears were treated similarly with the vehicle as reference control. No mortality or any local and systemic toxicity was reported in this study and few macroscopic and microscopic findings were observed in both β-CIT-FP- and vehicle-treated ears. Thus, β-CIT-FP was well tolerated following a single intra-arterial, intravenous and perivenous injection to rabbit ears in this study.

**Study no.**: Study No. 144541

**Volume # and page #:** Volume 1 pages 1- 22

**Conducting laboratory and location**: (b) (4)

**Date of study initiation**: November 27, 1996

**GLP compliance**: No

**QA report**: yes () no (x)

**Drug, lot #, and % purity**: β-CIT-FP, Batch # 95E12FPL and purity not stated

**Method:**

Dose: Not provided. However, 0.2, 0.3 and 0.5 ml were administered for periverous, intravenous and intra-arterial injections respectively.

Species/strain: Rabbits/New Zealand White rabbits

Number/sex/group or time point (main study): 3 males used for the study

Age: >3 weeks

Weight: <3.5 kg.

Control article: Isotonic sodium acetate buffer/5% ethanol (pH 4.75)

Route, formulation, volume, and infusion rate: Intra-arterial injection via the central ear artery (0.5 ml); intravenous injection via the marginal ear vein (0.3 ml) and perivenous injection via puncturing of the vein following the intravenous injection (0.3 ml)/0.00017 mg/ml β-CIT-FP in isotonic sodium acetate buffer pH 4.75 with 5% ethanol.

The hair from the injection site was removed before treatment. In the study, 0.5 ml of β-CIT-FP was administered intra-arterially retrograde into the central ear artery of the left ear of 3 male rabbits; 0.3 ml was injected intravenously into the marginal vein in the direction of the ear base and after partial withdrawal of the needle 0.2 ml injected perivenously. The three injection sites per ear were marked with indelible ink for identification. The animals were observed daily for 5 days for mortality, toxicity, body weight and skin reactions. The injection sites were monitored after 4, 24, 48, 72 and 96 hours after administration. The necropsy of the sites was performed.

**Results:**
Mortality: No mortality was reported in the animals.

Clinical signs: Clinical signs reported in all treated and control animals include screaming during treatment and tachypnoea which were considered a reaction to injection of the vehicle and stress due to the injection procedure rather than a sign of toxicity. Erythema was observed at all injection sites of the animals on day 5.

Body weights: No treatment related effect was reported on the body weight.

Necropsy: Macroscopic and microscopic examination of the areas of injection did not reveal any changes attributable to an injection of β-CIT-FP.

**Reviewer's Comments:** This study demonstrates that an administration of β-CIT-FP to the rabbit ears in a single injection, intra-arterially, intravenously and perivenously, produced no evidence of local and systemic systemic toxicity. This reviewer agrees with the sponsor that none of the observed lesions and clinical signs could be considered treatment related. Thus, there is no evidence that a single dose injection of β-CIT-FP could cause any local and systemic toxicity via the injection site.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

**Conclusions:**
The sponsor provided adequate preclinical data on the efficacy and safety of [$^{123}$I]FP-CIT for the proposed indication. The data showed:

1) Adequate preclinical data has been provided to show that [$^{123}$I]FP-CIT has high affinity and selectivity for DaT and this could provide *in vivo* image with high correlation as a measure of neurons of the striatal dopaminergic system.

2) There is no evidence that the metabolites of FP-CIT would cross the blood brain barrier. Thus, the metabolites would not complicate imaging.

3) There is no evidence that that combination with FP-CIT would enhance or reduce the pharmacological actions of therapeutic drugs used for treating Parkinson disease.

4) The standard ICH battery of tests on FP-CIT was negative indicating that DaTSCAN demonstrates no genotoxicity potential.

5) There is no evidence of any local or systemic toxicity following intravenous, intra-arterial or perivenous injection of FP-CIT in rabbits.

6) However, there were reports of hyperactivity and stereotypic behavior at high doses, excess of the clinical dose, due to similarity in the pharmacology of FP-CIT to that of other DaT ligands like cocaine.

**Recommendations:**
There is no unresolved preclinical Pharm/Tox issue. The approval of this application therefore recommended from pre-clinical Pharm/Tox perspective.

**Suggested labeling:**

**Signatures (optional):**

**Reviewer Signature** _____

**Supervisor Signature**_____ **Concurrence Yes** ___ **No** ___

**APPENDIX/ATTACHMENTS**

**References:**

Alvarez-Fischer D, Blessmann G, Trosowski C, Béhé M, Schurrat T, Hartmann A, Behr TM, Oertel WH, Höglinger GU, Höffken H. (2007) Quantitative [(123)I]FP-CIT pinhole SPECT imaging predicts striatal dopamine levels, but not number of nigral neurons in different mouse models of Parkinson's disease. Neuroimage. 2007 38(1):5-12.

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Nikolaus S, Wirrwar A, Antke C, Arkian S, Schramm N, Müller HW, Larisch R. (2005) Quantitation of dopamine transporter blockade by methylphenidate: first in vivo investigation using [123I]FP-CIT and a dedicated small animal SPECT. Eur J Nucl Med Mol Imaging. 32(3):308-13.

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| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-22454 | ORIG-1 | GE HEALTHCARE INC | DA TSCAN |

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09/03/2009

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

**NDA/BLA Number:** 22-454 **Applicant:** GE **Stamp Date:**

**Drug Name:** DaTSCAN **NDA/BLA Type:** 505(b)(1)

On **initial** overview of the NDA/BLA application for filing:

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin? | X | | |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin? | X | | |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin? | X | | |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)? | X | | Carcinogenicity studies are not required for short-term use.<br>The sponsor's request for a waiver for the reproductive toxicity studies was granted. |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). | X | | |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route? | X | | |
| 7 | Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? | X | | |
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions? | X | | |

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 9 | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57? | X | | |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.) | X | | |
| 11 | Has the applicant addressed any abuse potential issues in the submission? | X | | |
| 12 | If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted? | | | N/A |

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __YES_____**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

NONE

Reviewing Pharmacologist — Date 04/16/08

Team Leader/Supervisor — Date 04/16/08

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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