

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**205422Orig1s000**

**205422Orig2s000**

**PHARMACOLOGY REVIEW(S)**

## Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**NDA:** 205422

**Submission date:** 7/11/2014

**Drug:** brexpiprazole

**Applicant:** Otsuka Pharmaceutical Development and Commercialization, Inc.

**Indication:** Treatment of schizophrenia (monotherapy) and MDD (adjunctive therapy to antidepressants)

**Reviewing Division:** Division of Psychiatry Products

### **Discussion:**

The pharmacology/toxicology reviewer and supervisor found the nonclinical information sufficient to support the approval of brexpiprazole for the indications listed above.

Brexpiprazole was tested in 2 year carcinogenicity studies in rats and mice using oral gavage administration. Both studies were found to be acceptable by the executive carcinogenicity assessment committee. In mice the combined incidences of mammary gland neoplasms in females were increased in all dose groups. These neoplasms may be related to elevated prolactin levels. No drug-related neoplasms were noted in rats.

Brexpiprazole was not teratogenic in rats and rabbits at doses that produced exposures that exceeded those in humans at the maximum recommended dose.

An appropriate established pharmacologic class for brexpiprazole is "atypical antipsychotic".

### **Conclusions:**

The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that this NDA may be approved for the above indication. I have provided comments on labeling to the Division separately.

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/s/  
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PAUL C BROWN  
07/09/2015

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

**PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION**

Application number: 205422  
Supporting document/s: N-0000 (SDN1); N-0003 (SDN3)  
Applicant's letter date: 07/09/2014 (SDN1); 08/07/2014 (SDN3)  
CDER stamp date: 07/11/2014 (SDN1); 08/07/2014 (SDN3)  
Product: Brexpiprazole, proposed trade name REXULTI  
(OPC-331, OPC-34712, Lu AF41156)  
Indication: Treatment of schizophrenia (monotherapy) and  
MDD (adjunctive therapy to antidepressants)  
Applicant: Otsuka Pharmaceutical Development and  
Commercialization, Inc.  
Review Division: DPP, HFD-130  
Reviewer: Violetta Klimek, Ph.D.  
Supervisor/Team Leader: Linda Fossom, Ph.D.  
Division Director: Mitchell V. Mathis, M.D.  
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## 1 Executive Summary

### 1.1 Introduction

This application is a 505(b)(1) NDA for brexpiprazole (also known as OPC-34712, OPC 331, and Lu AF41156) sponsored by Otsuka and Lundbeck. The proposed indications are: 1) monotherapy for treatment of schizophrenia and 2) adjunctive therapy for the treatment of major depressive disorder (MDD), both in adult patients. Brexpiprazole is thought to have modulatory activity at the serotonin-dopamine system that combines partial agonist activity at serotonergic 5-HT<sub>1A</sub> and dopaminergic D<sub>2</sub> receptors with antagonist activities at serotonergic 5-HT<sub>2A</sub> and noradrenergic  $\alpha_{1/2}$  receptors, and actions on several other central monoaminergic receptor subtypes.

### 1.2 Brief Discussion of Nonclinical Findings

Brexpiprazole was adequately assessed in nonclinical studies and there are no findings that would prevent the approval of this drug.

Brexpiprazole is a partial agonist at serotonergic 5-HT<sub>1A</sub> (K<sub>i</sub>=0.09/0.12nM; rat/human, respectively) and at dopaminergic D<sub>2</sub> (K<sub>i</sub>=0.35/0.30nM; rat/human) receptors and an antagonist at serotonergic 5-HT<sub>2A</sub> (K<sub>i</sub>=3.8/0.47nM; rat/human) receptors. It also has antagonist activity at noradrenergic  $\alpha_{1/2}$  receptors and a broad spectrum of binding affinities and actions on several other central monoaminergic receptor subtypes and transporters. The major metabolite of brexpiprazole DM-3411 (> 10% compared with total drug-related exposure) is present in all species studied (mice, rats, rabbits, dogs, monkey and humans) and has a pharmacological profile similar to brexpiprazole, but is generally less potent. DM-3411 was not detected in rat brain even with high doses of brexpiprazole, suggesting poor penetration into the brain.

Standard nonclinical studies were adequately conducted to support chronic use of brexpiprazole. Toxicities observed in rats, mice and monkeys were related to the exaggerated pharmacological activity of brexpiprazole which caused hypoactivity, tremors (monkey), hypothermia, increased serum prolactin (rats and mice), decreased blood pressure and prolonged QT/QT<sub>c</sub> interval (monkey and dog at 146 – 243 times, respectively the MRHD of 4 mg/day on mg/m<sup>2</sup> basis).

Brexpiprazole is considered to be not genotoxic. The two-year carcinogenicity study resulted in increased incidence of mammary gland adenocarcinoma at 0.9 to 6.1 times the MRHD and adenosquamous carcinoma at 2.4 and 6.1 times the MRHD in female mice. No drug-related tumors were seen in male mice. Elevated prolactin levels in mice were observed in a 2-week study of brexpiprazole at 6.1 times the MRHD. Increases in prolactin level are known to cause mammary tumors in rodents and this finding has been seen with similar antipsychotic drugs, like aripiprazole (ABILIFY). In the rat study, brexpiprazole was not carcinogenic in either sex at doses up to 73 times the MRHD.

Brexpiprazole was not teratogenic in rats or rabbits at doses up to 73 times or 730 times the MRHD, respectively, although in the pre- and postnatal study in rats, developmental toxicity in offspring of low birth weight, impaired viability, suppressed body weight gain, delayed pinna unfolding and decreased number of corpora lutea were noted at maternally toxic doses (73 times the MRHD) that included impaired nursing behavior attributable to pharmacologically-mediated effects on the CNS.

### **1.3 Recommendations**

#### **1.3.1 Approvability**

This application is recommended for approval from a Pharmacology/Toxicology perspective.

#### **1.3.2 Additional Non Clinical Recommendations**

None at this time

#### **1.3.3 Labeling**

The following are Reviewer's draft recommendations for sections: 8.1, 12.1, 12.2, and 13.1 of the labeling: Final labeling will be negotiated with the sponsor.

### **8.1 Pregnancy**

#### **Animal data:**

No teratogenic effects and no adverse developmental effects were observed in studies in which pregnant rats and rabbits were administered oral dose of brexpiprazole during the period of organogenesis at doses up to 30 mg/kg/day (73-fold and 146-fold for rats and rabbits, respectively of the 4 mg/day oral maximum recommended human dose (MRHD). In a rabbit embryo-fetal development study, decreased body weight, retarded ossification, and increased incidences of visceral and skeletal variations were observed in fetuses at 150 mg/kg/day (730- fold the MRHD), a dose that induced maternal toxicity.

In the study in which pregnant rats were administered oral dose of brexpiprazole up to 30 mg/kg/day (73 times the MRHD) during the period of organogenesis and lactation, impaired nursing of dams, low birth weight, impaired viability, suppressed body weight gain, delayed pinna unfolding and decreased number of corpora lutea in the offspring were observed at 73 times the MRHD but not at 24.3 times the MRHD.

### **12.1 Mechanism of action**

The mechanism of action of brexpiprazole, as with other drugs having efficacy in schizophrenia and major depressive disorders, is unknown. However it has been proposed that the efficacy of brexpiprazole is mediated through a combination of partial agonist activity at serotonergic 5-HT<sub>1A</sub> receptors, at dopaminergic D<sub>2</sub> receptors and antagonist activity at serotonergic 5-HT<sub>2A</sub> receptors. Brexpiprazole also has an antagonist activity at noradrenergic  $\alpha_{1/2}$  receptors and a broad spectrum of binding

affinities and actions on several other central monoaminergic receptor subtypes and transporters.

## 12.2 Pharmacodynamics

Brexpiprazole binds with high affinity to multiple monoaminergic receptors as it is shown in Table below. Brexpiprazole acts as a partial agonist at 5-HT<sub>1A</sub>, D<sub>2</sub>, and D<sub>3</sub> receptors and as an antagonist at 5HT<sub>2A</sub>, 5HT<sub>2B</sub>, 5HT<sub>7</sub>,  $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1D}$ , and  $\alpha_{2C}$  receptors.

Brexpiprazole exhibits a moderate affinity for histamine H<sub>1</sub> receptor ( $K_i$  = 19 nM) and inhibitory activity at serotonin transporter ( $IC_{50}$  = 29nM).

Table A: Brexpiprazole Human Receptor Affinities ( $K_i$ , nM)

Receptor	Binding affinity
Serotonin 5HT <sub>1A</sub>	0.12*
Serotonin 5HT <sub>2A</sub>	0.47#
Serotonin 5HT <sub>2B</sub>	1.88#
Serotonin 5HT <sub>7</sub>	3.66#
Dopamine D <sub>2</sub>	0.30*
Dopamine D <sub>3</sub>	1.14*
Adrenergic $\alpha_{1A}$	3.78#
Adrenergic $\alpha_{1B}$	0.17#
Adrenergic $\alpha_{1D}$	2.60#
Adrenergic $\alpha_{2C}$	0.59#

\* = partial agonist; # = antagonist

## 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

### Carcinogenesis

Carcinogenic potential of brexpiprazole was evaluated in a two year study in ICR mice at doses up to 5 mg/kg/day (6.1 times the maximum recommended human dose [MRHD] of 4 mg/day) and in rats at doses up to 30 mg/kg/day (73 times the MRHD).

In female mice, the incidence of mammary gland adenocarcinoma was increased at 0.9 to 6.1 times the MRHD and adenosquamous carcinoma at 2.4 and 6.1 times the MRHD. No increase in the incidence of tumors was observed in male mice. Elevated prolactin levels in mice were observed in a 2-week study of brexpiprazole administered at 6.1 times the MRHD. Increases in prolactin level are known to cause mammary tumors in rodents. In the rat study, brexpiprazole was not carcinogenic in either sex at doses up to 73 times the MRHD.

### Mutagenesis

Brexpiprazole was not mutagenic in the *in vitro* bacterial reverse mutation assay (Ames test). Brexpiprazole was mutagenic in the *in vitro* forward gene mutation assay in mouse

lymphoma cells and was clastogenic in the *in vitro* chromosomal aberration assay at doses that induced cytotoxicity. However, brexpiprazole was negative for clastogenic activity in the *in vivo* micronucleus assay in rats, and was not genotoxic in the *in vivo/in vitro* unscheduled DNA synthesis assay in rats.

### Impairment of Fertility

Treatment of rats with brexpiprazole at doses of 3 and 30 mg/kg/day (7.3 and 73 times the MRHD of 4 mg on mg/m<sup>2</sup> basis, respectively) caused impairment of female fertility with no effect on male fertility. A slight prolongation of mating phase and increased preimplantation loss were seen at 30 mg/kg/day dose.

## 2 Drug Information

### 2.1 Drug:

CAS Registry Number (Optional)

Generic Name: Brexpiprazole

Code Name: OPC-34712, OPC-331, Lu AF41156

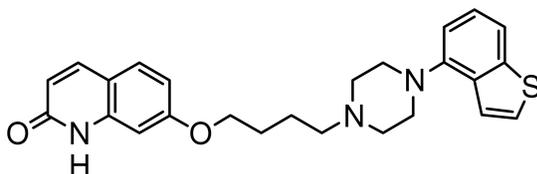
Chemical Name:

7-[4-(4-Benzo[*b*]thiophen-4-ylpiperazin-1-yl)butoxy]quinolin-2(1*H*)-one

Molecular Formula/Molecular Weight:

C<sub>25</sub>H<sub>27</sub>N<sub>3</sub>O<sub>2</sub>S/433.57

Structure:



Pharmacologic Class:

D<sub>2</sub>- and 5-HT<sub>1A</sub> partial agonist; antagonist at 5-HT<sub>2A</sub> and noradrenergic α<sub>1/2</sub> receptors

### 2.2 Relevant INDs, NDAs, BLAs and DMFs

INDs: 101,871; 103,958 (b) (4)

### 2.3 Drug Formulation

Tablets (immediate release) of 0.25-, 0.5-, 1-, 2-, 3-, and 4-mg strengths

### 2.4 Comments on Novel Excipients

Only commonly used (generally acceptable) excipients such as lactose monohydrate, corn starch, microcrystalline cellulose, low-substituted hydroxypropyl cellulose and hydroxypropyl cellulose, magnesium stearate were used to formulate tablets.

## 2.5 Comments on Impurities/Degradants of Concern

Several impurities (b) (4) have been identified in the drug substance. The mutagenicity of these manufacturing intermediates or impurities was investigated in the bacterial reverse mutation assay. Except for (b) (4), all of these impurities were not mutagenic in the bacterial reverse mutation assay with or without S9.

Impurity (b) (4) was identified as an impurity in the drug substance and *in silico* modeling found it has a structural alert for mutagenicity. When tested, (b) (4) was found to be mutagenic in *Salmonella typhimurium* TA1535 and TA100 strains with S9 as well as in TA1535 strain without S9. A specification of  $\leq$  (b) (4) ppm was established by the Sponsor. Based on a MHRD of 4 mg/d, the estimated dose of this impurity was calculated to be (b) (4)  $\mu$ g/day (a dose below the threshold of toxicological concern for a genotoxic impurity, (b) (4)  $\mu$ g/day).

During the review cycle, the CMC reviewer identified another impurity, (b) (4) and requested additional information from the Sponsor. (b) (4). In addition to being a process impurity and a possible degradation product, (b) (4) is a minor metabolite of brexpiprazole in animals and humans. In order to assess the potential genotoxicity of (b) (4), a DEREK *in-silico* analysis for mutagenicity supplemented with the Leadscape statistical-based QSAR models for 'Microbial in vitro – Salmonella' and 'Microbial in vitro - E. coli – Salmonella 102 A-T' mutation' were used and the information gathered allowed the conclusion that (b) (4) would not be predicted to be mutagenic. All issues with imp and deg have been resolved

## 2.6 Proposed Clinical Population and Dosing Regimen

For the treatment of patients with schizophrenia, the proposed starting dose of brexpiprazole is 1 mg/day. The dose should be increased to 2 mg after Day 4 and may subsequently be increased to 4 mg after Day 7 based on the patient's clinical response and tolerability. The proposed target dose for schizophrenia patients ranges from 2 to 4 mg/day. The proposed starting dose as adjunctive treatment for Major Depressive Disorder (MDD) is 0.5 mg/day or 1 mg/day. Dose titration to 1 mg/day and up to the target dose of 2 mg/day should occur at intervals of up to 1 week based on the patient's clinical response and tolerability. The proposed maximum recommended dose for patients with MDD is 3 mg/day.

## 2.7 Regulatory Background

Brexpiprazole has been studied for the treatment of schizophrenia under the IND 101,871, for adjunctive therapy in treatment of MDD under IND 103,958 (b) (4)

(b) (4)  
applications are cross referenced on the nonclinical sections within this NDA.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

All submitted pivotal studies, in various species, were reviewed in detail, except the juvenile animal studies (the indication for brexpiprazole under this NDA is for adults with schizophrenia and MDD)

#### 3.2 Studies Not Reviewed

Preliminary dose-range finding studies and non-pivotal studies for deciding nonclinical safety of brexpiprazole were not reviewed in detail. In addition, because the approval for Schizophrenia and MDD (adjunctive therapy to antidepressants) will be based on clinical trials in adults, the juvenile animal studies in rats and dogs were not reviewed for this NDA.

#### 3.3 Previous Reviews Referenced

Primary review of IND 101,871 (N000/03-22-2008) – reviewed by this Reviewer  
 Exec CAC Meeting Minutes – dated 10/22/2009  
 Statistical review (Biometrics – dated 12/29/2014) – Dr. Rahman  
 Exec CAC Meeting Minutes – dated 12/02/2014

### 4 Pharmacology

Brexpiprazole has modulatory activity at monoaminergic neurotransmitter systems in the brain. Brexpiprazole is a partial agonist at serotonin 5-HT<sub>1A</sub> and at dopamine D<sub>2</sub> receptors and antagonist at serotonin 5-HT<sub>2A</sub>, noradrenergic  $\alpha_{1/2}$  receptors with a broad spectrum of binding affinities and actions on several other central monoaminergic receptor subtypes. Brexpiprazole has been developed as a new therapy for the treatment of schizophrenia, adjunctive treatment for MDD and for other psychiatric disorders. The pharmacological profile of brexpiprazole is similar to that of aripiprazole, an antipsychotic developed by the current Sponsor (approved by the FDA in 2002).

#### 4.1 Primary Pharmacology

The primary pharmacodynamics of brexpiprazole are related to *in vitro* and *in vivo* dopaminergic, serotonergic and adrenergic receptor binding and functional activities in behavioral animal models relevant to the indication.

Table 1: Brexpiprazole binding affinity data for variety of receptors assessed in different cell line/tissue preparations *in vitro*

Receptor	Species	Cell line/Tissue preparation	Binding affinity K <sub>i</sub> (nM)	Relative affinity <sup>a</sup>
<b>Dopamine Receptors</b>				
D <sub>1</sub>	Human	Commercially available cloned human receptors	160	Not determined
D <sub>2</sub>	Rat	Striatum membrane preparation	0.35	-
D <sub>2L</sub>	Human	H-D2L-CHO cell line	0.30	-
D <sub>3</sub>	Human	CHO-ACV-hD3 cell line	1.1	Not determined
D <sub>4</sub>	Human	Commercially available cloned human receptors	6.3	Not determined

Serotonin Receptors				
5-HT <sub>1A</sub>	Rat	Hippocampal membrane preparation	0.09	Not determined
5-HT <sub>1A</sub>	Human	HeLa-h5-HT <sub>1A</sub> cell line	0.12	Not determined
5-HT <sub>1A</sub>	Human	Superior frontal cortex membrane preparation	0.15	Not determined
5-HT <sub>1B</sub>	Human	Commercially available cloned human receptors	32	Not determined
5-HT <sub>2</sub>	Rat	Frontal cortex membrane preparation	3.8	11
5-HT <sub>2A</sub>	Human	CHO-K1- h5HT <sub>2A</sub> cell line	0.47	1.6
5-HT <sub>2B</sub>	Human	Commercially available cloned human receptors	1.9	Not determined
5-HT <sub>2Cs23C</sub>	Human	Commercially available cloned human receptors	12	Not determined
5-HT <sub>2C(vsv)</sub>	Human	Commercially available cloned human receptors	34	Not determined
5-HT <sub>5A</sub>	Human	Commercially available cloned human receptors	140	Not determined
5-HT <sub>6</sub>	Human	Commercially available cloned human receptors	58	Not determined
5-HT <sub>7</sub>	Human	Commercially available cloned human receptors	3.7	Not determined
5HT <sub>7A</sub>	Human	CHO-h5-HT <sub>7A</sub> cell line	9.5	Not determined
Adrenergic Receptors				
α <sub>1</sub>	Rat	Cerebral cortex membrane preparation	18	51
α <sub>1A</sub>	Human	CHO-K1-hα <sub>1A</sub> cell line	3.8	13
α <sub>1B</sub>	Human	CHO cell line	0.17	0.64
α <sub>1D</sub>	Human	CHO cell line	2.6	9.4
α <sub>2</sub>	Rat	Cerebral cortex membrane preparation	120	Not determined
α <sub>2A</sub>	Human	CHO cell line	15	Not determined
α <sub>2B</sub>	Human	CHO cell line	17	Not determined
α <sub>2C</sub>	Human	CHO cell line	0.59	2.0
β <sub>1</sub>	Human	HEK293 cell line	59	Not determined
β <sub>2</sub>	Human	CHO cell line	67	Not determined
Histamine Receptors				
H <sub>1</sub>	Human	CHO-K1-hH <sub>1</sub> cell line	19	66

<sup>a</sup> Ratio to rat D<sub>2</sub> K<sub>i</sub> or human D<sub>2L</sub> K<sub>i</sub>.

Brexpiprazole had high binding affinities (inhibition constant [K<sub>i</sub>] < 5 nM) for cloned human dopamine D<sub>2L</sub> (D<sub>2L</sub>) and serotonin 5-HT<sub>1A</sub> (5-HT<sub>1A</sub>), serotonin 5-HT<sub>2A</sub> (5-HT<sub>2A</sub>) receptors in recombinant cell lines. The order of potency for the binding affinity of brexpiprazole for dopamine receptors was D<sub>2L</sub> > D<sub>3</sub> > D<sub>4</sub> > D<sub>1</sub> and for serotonin receptors as follows: h5-HT<sub>1A</sub> > h5-HT<sub>2A</sub> > h5-HT<sub>2B</sub> > h5-HT<sub>7</sub> > h5-HT<sub>7A</sub> ≥ h5-HT<sub>2Cs23C</sub> > h5-HT<sub>1B</sub> = h5-HT<sub>2C(vsv)</sub> ≥ h5-HT<sub>6</sub> > h5-HT<sub>5A</sub>. Brexpiprazole had a greater affinity for human D<sub>2L</sub> and 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, receptors compared with the reference compound aripiprazole. High affinities of brexpiprazole were also found for human α<sub>1B</sub><sup>-</sup>, α<sub>1D</sub><sup>-</sup>, and α<sub>2C</sub><sup>-</sup> adrenergic receptors and it also had moderate affinities for human α<sub>2A</sub><sup>-</sup>, α<sub>2B</sub><sup>-</sup>, β<sub>1</sub><sup>-</sup> and β<sub>2</sub><sup>-</sup> adrenergic receptors and low affinity for human β<sub>3</sub><sup>-</sup> adrenergic receptors.

Brexpiprazole also had an inhibitory effect on serotonin transporter (SERT;  $IC_{50}$  = 29 nM) and norepinephrine transporters (NET;  $IC_{50}$  = 140 nM), but weak on dopamine transporter (DAT;  $IC_{50}$  = 950 nM in the rat brain synaptosome). These inhibitory effects on SERT and NET were comparable to those of SNRI-antidepressants (Venlafaxine) as it is shown in the following Sponsor's table (*Report No. 020471*):

Table 2: Inhibitory effects of brexpiprazole and reference compounds on SERT, NET, and DAT uptake into rat brain synaptosomes.

Compounds	$IC_{50}$ (nM)		
	Serotonin	Norepinephrine	Dopamine
Brexpiprazole	29	140	950
Fluoxetine	11	170	> 3000
Sertraline	1.4	280	180
Venlafaxine	36	130	> 3000

$IC_{50}$  = concentration of drug producing 50% inhibition.

However, brexpiprazole had relatively weak binding affinities for cloned hSERT, hDAT, and hNET; at concentration of 10,000 nM, the ligand displacement was at 65%, 90%, and 0%, respectively. Positive reference compounds showed full ligand displacement at the respective transporter binding sites (*Report No. 020418*).

#### Binding affinities of DM-3411 (major metabolite)

The major metabolite, DM-3411 was tested for binding affinities and functional effects at different human targets including receptors and transporters at concentration of 1  $\mu$ M. This study results are shown in the following Sponsor's table (*Report No. 15700*):

Table 3: Receptor binding affinities of DM-3411, for cloned human receptors and transporters:

Receptor	Mean DM-3411 Binding Affinity, $K_i$ (nM)
hD <sub>1</sub>	190
hD <sub>2L</sub> (Cloned)	5.0
hD <sub>2S</sub>	10
hD <sub>3</sub>	9.7
hD <sub>4</sub>	130
h5-HT <sub>1A</sub>	8.5
h5-HT <sub>2A</sub>	2.6
h5-HT <sub>2B</sub>	1.0
h5-HT <sub>7</sub>	66
h $\alpha$ <sub>1A</sub>	64
h $\alpha$ <sub>1B</sub>	27
h $\alpha$ <sub>1D</sub>	135
h $\alpha$ <sub>2C</sub>	180
hH <sub>1</sub>	27
h5-HT transporter	64% at 1 $\mu$ M
hDA transporter	50% at 1 $\mu$ M

$K_i$  = inhibition constant

In the same study, further investigations of DM-3411 in functional assays showed that DM-3411 had no significant functional activity at the hD<sub>1</sub> and hD<sub>4</sub>, and acted as antagonists at hD<sub>2</sub> and hD<sub>3</sub> receptors with potencies of 4.1 nM and 38 nM, respectively. At the human serotonin receptors, DM-3411 behaved as a weak partial agonist at 5-HT<sub>1A</sub> receptors (22% stimulation at 100 nM) and as a moderate to weak partial agonist at rat 5-HT<sub>1D</sub> receptor with EC<sub>50</sub> of 47 nM and 55% efficacy. DM-3411 showed weak affinity for human SERT (64% inhibition at 1  $\mu$ M).

#### Functional effects at human receptors, *in vitro*:

##### **Dopamine receptors:**

In functional assays brexpiprazole showed D<sub>2</sub> receptor partial agonistic activity similar to aripiprazole. However, in 1) *in vitro* assay systems based on forskolin-induced adenosine 3',5'-cyclic monophosphate (cAMP) accumulation (*Report No. 019656*) and 2) calcium (Ca<sup>2+</sup>) mobilization in human dopamine D<sub>2L</sub> receptor-expressing cells (*Report No. 020505*), its intrinsic activity at the D<sub>2</sub> receptor was slightly lower than that of aripiprazole. Similarly, 3) brexpiprazole showed partial agonistic activity at hD<sub>3</sub> receptor (*Report No. 020192*) with lower than aripiprazole intrinsic activity (measured as percent of the maximum inhibition of forskolin-stimulated cAMP accumulation induced by 10  $\mu$ M dopamine).

- 1) Inhibition of Forskolin-stimulated cAMP Accumulation in Cells expressing Cloned hD<sub>2L</sub> Receptors

Table 4

Test Compounds	Agonist Potency (EC <sub>50</sub> , nM)	Emax (% of Dopamine)
Brexpiprazole	4.0	Approximately 40%
Aripiprazole	5.6	Approximately 60%
Dopamine	3.4	100%

EC50 = concentration of drug producing 50% of the maximum effect a drug produces

### 2) In Vitro Calcium Mobilization in Cells Cloned with hD<sub>2L</sub> Receptors

Table 5:

Test Compounds	Agonist Potency (EC <sub>50</sub> , nM)	Intrinsic Activity Relative to Dopamine (E <sub>max</sub> , %)
Brexpiprazole	52	15
Aripiprazole	140	50

Intrinsic activity = Emax effect the drug produces (a percent of the maximal effect of 10 µM dopamine)

### 3) Inhibition of Forskolin-stimulated cAMP Accumulation in Cells Cloned with hD<sub>3</sub> Receptors

Table 6:

Test Compounds	Agonist Potency (EC <sub>50</sub> , nM)	Intrinsic Activity Relative to Dopamine (E <sub>max</sub> , %)
Brexpiprazole	2.8	15
Aripiprazole	5.9	28
Dopamine	3.5	NA

Intrinsic activity = Emax effect the drug produces (a percent of the maximal effect of 10 µM dopamine)

### Serotonin receptors:

Partial agonist effect of brexpiprazole at h5-HT<sub>1A</sub> receptor was demonstrated in the functional activation of [<sup>35</sup>S]-GTPγS binding assay using cloned h5HT<sub>1A</sub> receptors and in the human superior frontal cortex membrane preparations. As shown in Table 7 (below), brexpiprazole potently increased binding of [<sup>35</sup>S]-GTPγS, with an E<sub>max</sub> of 60% of the effect of 10 µM serotonin. In this study, the effect of brexpiprazole (100 nM) was blocked by the selective 5-HT<sub>1A</sub> receptor antagonist WAY100635, demonstrating it is mediated through the 5-HT<sub>1A</sub> receptor. (*Report No. 020193*)

Table 7: Partial agonist effects of brexpiprazole on cloned h5-HT<sub>1A</sub> receptors:

Test Compounds	Agonist Potency (EC <sub>50</sub> , nM)	Intrinsic activity (% of 10 µM 5-HT)
Brexpiprazole	0.49	60
Aripiprazole	2.1	73
Serotonin	5.1	NA

Similarly, brexpiprazole showed partial agonist activity at h5-HT<sub>1A</sub> receptor in a [<sup>35</sup>S]-GTPγS binding assay using membranes prepared from human superior frontal cortex (*Report No. 020994*).

Table 8: Partial agonist effects of brexpiprazole on h5-HT<sub>1A</sub> receptors in human superior frontal cortex membrane preparations:

Test Compounds	Agonist Potency (EC <sub>50</sub> , nM)	Intrinsic activity (% of 10 μM (+)8-OH-DPAT)
Brexpiprazole	2.2	33
Aripiprazole	17	37
Serotonin	77	120

Brexpiprazole at concentration range from 0.010 to 1000nM had antagonized a serotonin-induced production of inositol monophosphate (IP<sub>1</sub>) in cells transfected with cloned h5-HT<sub>2A</sub> receptors (*Report No. 15701*). Brexpiprazole was an antagonist at 5-HT<sub>2A</sub> receptors and a calculated (c) IC<sub>50</sub> value was 6.5 nM. No agonist activity was observed at any concentration (up to 1000nM). In cells transfected with cloned h5-HT<sub>2B</sub> receptors (*Report No. 14562*), brexpiprazole had a potent antagonist activity on 5-HT-induced IP<sub>1</sub> production (IC<sub>50</sub> = 14 nM) and has devoid of any agonist activity at concentrations up to 10,000 nM. Brexpiprazole is an antagonist at 5-HT<sub>2B</sub> receptors, not an agonist there, therefore has no potential for being associated with serious cardiac side effects.

Functional characteristic at other 5-HT receptors revealed that brexpiprazole acted as a h5-HT<sub>2C(vsv)</sub> receptor partial agonist, with a very low intrinsic activity of approximately 10% of that produced by serotonin (*Report No. 023423*). Brexpiprazole was a weak h5-HT<sub>7A</sub> receptor antagonist (IC<sub>50</sub>: > 500 nM; *Report No. 023422*), which was in contrast with its high binding affinity shown in another study (*in vitro*). In addition, brexpiprazole showed potent antagonist activity (cIC<sub>50</sub> value of 6.8 nM) on histamine-induced Ca<sub>2+</sub> mobilization in cells transfected with cloned human histamine H<sub>1</sub> receptors (*Report No. 15701*).

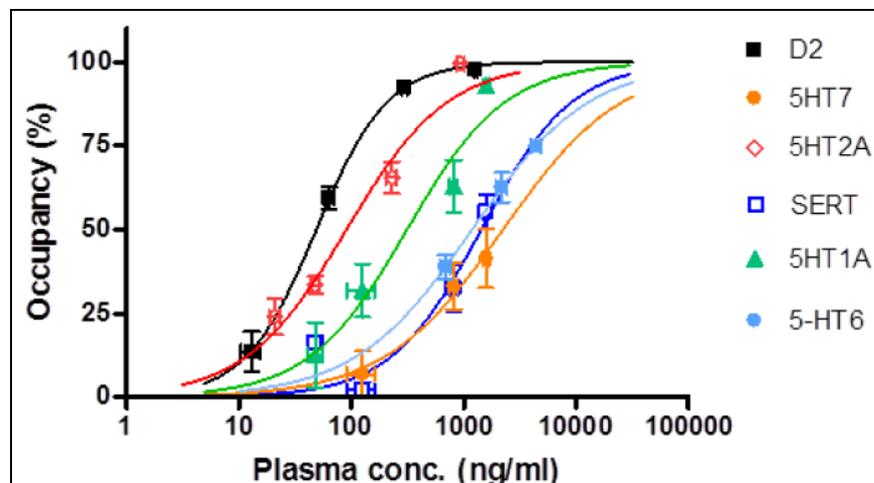
Brexpiprazole (concentration range: 0.01 to 1000 nM) had a potent antagonist activity on α<sub>1A</sub>- and α<sub>1B</sub>-adrenergic receptors, and a moderate to weak antagonist activity on α<sub>1D</sub>-, α<sub>2C</sub>-, and hβ<sub>1</sub>-adrenergic receptors. Much weaker effects were seen at the α<sub>2A</sub>-, α<sub>2B</sub>-, hβ<sub>2</sub>, or hβ<sub>3</sub>-adrenergic receptors, (i.e., IC<sub>50</sub> > 10000 nM; *Report No.14808*).

#### Occupancy studies *in vivo* and *ex vivo* (Report No.929-300-2013 065):

The binding occupancy of rat brain 5-HT<sub>1A</sub>, 5-HT<sub>7</sub>, and SERTs at 2 h after oral administration of brexpiprazole at doses of 1, 3, 10, or 30 mg/kg was determined by *ex vivo* autoradiography. Brexpiprazole administration lead to dose-dependent increases in occupancies at SERT, 5-HT<sub>1A</sub> receptors and 5-HT<sub>7</sub> receptors, with maximal occupancies of approximately 55%, 90% and 40%, respectively. Aripiprazole administration (reference compound) did not cause any meaningful changes in occupancy at SERT, but achieved maximal occupancies of approximately 55% at 5-HT<sub>1A</sub> receptors and 40% at 5-HT<sub>7</sub> receptors.

In this study, the relationship between plasma concentrations of brexpiprazole and receptor occupancy in rats is illustrated for comparison in the following Sponsor's figure:

Figure1. Relationship between plasma levels and receptor occupancy in rats:



#### Rat brain microdialysis studies *in vivo*:

Microdialysis was used to assess the effects of brexpiprazole administration on extracellular levels of neurotransmitters in the rat brain. The effects of a single oral dose of brexpiprazole at 1, 10, or 20 mg/kg on DA and its metabolites (DOPAC and HVA) were measured in dialysate of nucleus accumbens collected for 3 h post-dosing. The results of this study are summarized in the following Sponsor's table (*Report No. 020506*):

Table 9: Mean maximal effect of brexpiprazole and reference compounds on basal dopamine, DOPAC, and homovanillic acid (HVA) in rat nucleus accumbens

Test Compound	Dose (mg/kg, oral)	Mean E <sub>max</sub> (% Basal Concentration)		
		Dopamine	DOPAC	HVA
Brexpiprazole	1	83	96	97
	10	74**	120**	130
	20	82	120**	130*
Olanzapine	20	120***	220***	240***
Vehicle	0	87	96	92

E<sub>max</sub> = maximal effect as percent basal concentration; \*P < 0.05; \*\*P < 0.01; \*\*\*P ≤ 0.001.

Brexpiprazole moderately increased DOPAC and HVA at ≥ 10 mg/kg compared with vehicle-treated group. In contrast, the D<sub>2</sub> receptor antagonist antipsychotic olanzapine (20 mg/kg) produced a modest increase in basal DA and more than doubled the levels of DOPAC and HVA. A significant correlation was found between maximum effect on DOPAC ( $p = 0.0084$ ) and HVA ( $p = 0.0098$ ) and relative intrinsic activity at the cloned hD<sub>2L</sub> receptor *in vitro*. These results are consistent with partial agonist activity of brexpiprazole at the D<sub>2</sub> receptor in rat nucleus accumbens.

In another study, (*Report No. 025018*), the effects of brexpiprazole at single oral doses of 1, 3, or 10 mg/kg on extracellular DA and its metabolites (DOPAC and HVA), NE, and the serotonin metabolite (5-HIAA) in the medial prefrontal cortex (mPFC) of freely moving rats were assessed. The results of this study are consistent with partial agonist

activity of brexpiprazole at the D<sub>2</sub> receptor in rat mPFC as it is shown in the following Sponsor's table:

Table 10: Mean maximal effect of brexpiprazole on basal dopamine, DOPAC, HVA, norepinephrine (NE), and 5-HIAA in rat medial prefrontal cortex

Dose (mg/kg, oral)	Mean E <sub>max</sub> (% Basal Concentration)				
	Dopamine	DOPAC	HVA	NE	5-HIAA
0 (Vehicle)	120	120	110	68	110
1	120	120	120	76	110
3	150	140**	140*	67	120
10	110	210**	200**	78	110

DOPAC = 3,4-dihydroxyphenylacetic acid; E<sub>max</sub> = maximal effect as percent basal concentration; 5-HIAA = 5-hydroxy-indole acetic acid; HVA = homovanillic acid; NE = norepinephrine.

### Behavioral studies in animal models predictive of antipsychotic activity:

Apomorphine (APO)-induced hyperlocomotion: APO is a dopamine receptor agonist which induces hyperlocomotion and stereotyped behavior in rodents, which can be used to behaviorally assess an antipsychotic-like activity of tested compound. Brexpiprazole and haloperidol were administered 1 h and aripiprazole 2 h prior to APO. Brexpiprazole inhibited APO-induced hyperlocomotion (APO at 0.25 mg/kg SC) with the potency similar to that of aripiprazole as it is shown in the following Sponsor's table (*Report No. 020417*):

Table 11: Effect on APO-induced hyperlocomotion in rats

Compound	Dose (mg/kg PO)	n/Group	ED <sub>50</sub> Value (mg/kg)
Brexpiprazole	1 to 4	5	2.3
Aripiprazole	2 to 8	5	3.2
Haloperidol	0.1 to 0.4	5	0.20

ED<sub>50</sub> = dose producing a 50% response.

APO-induced stereotyped behavior in rats: APO (0.7 mg/kg SC)-induced stereotyped behavior in rats. Test compounds were administered 1 or 2 h prior to injection of APO. Stereotyped behavior was observed for 1 min at 10-min intervals for a total of 20 to 40 min; and the total score for 3 observations was calculated. Brexpiprazole (single oral dose of 0.3, 1, 3, or 10 mg/kg) significantly inhibited APO-induced stereotyped behavior in a dose-dependent manner. The effect of brexpiprazole was more potent than that of aripiprazole and similar to that of olanzapine and risperidone as it is shown in the following Sponsor's table (*Report No.019805*):

Table 12: Effect on APO-induced stereotyped behavior in rats

Compound	Dose (mg/kg PO)	n/Group	ED <sub>50</sub> Value (mg/kg)
Brexpiprazole	0.3 to 10	6	2.9
Aripiprazole	1 to 30	6	6.1
Bifeprunox	0.03 to 1	6	0.12
Olanzapine	0.3 to 10	6	2.5
Risperidone	0.3 to 10	6	4.7
Haloperidol	0.03 to 1	6	0.45

ED50 = dose producing a 50% response

**Conditioned Avoidance Response (CAR) in rats:** all clinically effective antipsychotics with D2 receptor antagonist activity are known to suppress CAR in rats trained to avoid foot shock after a warning tone (crossing to another compartment). In this test, brexpiprazole significantly inhibited the CAR in rats in a dose dependent manner, and the ED<sub>50</sub> value was 6.0 (4.3 to 9.7) mg/kg. The effect of brexpiprazole was more potent than that of aripiprazole and bifeprunox and was comparable to that of olanzapine and risperidone as it is shown in the following Sponsor's table (*Report No. 020320*):

Table 13: Inhibitory Effect of Brexpiprazole and Reference Compounds on Conditioned Avoidance Response

Compound	Dose (mg/kg PO)	n/Group	ED <sub>50</sub> Value (mg/kg)
Brexpiprazole	1.5 to 12	4 to 6	6.0
Aripiprazole	7.5 to 60	6	23
Bifeprunox	0.1 to 10	5	> 10
Olanzapine	0.3 to 10	6	4.1
Risperidone	1 to 10	6	3.3
Haloperidol	0.3 to 1.2	6	0.87

ED50 = dose producing a 50% response; Evaluation at 1 h postdose for brexpiprazole, olanzapine, risperidone, and haloperidol and 2 h for aripiprazole and bifeprunox

**Catalepsy and Ptosis** (animal model of extrapyramidal and sedative side effects):

Brexpiprazole's potential for extrapyramidal symptoms (EPS) and sedation was evaluated using the catalepsy and ptosis response in rats, respectively. Oral dosing of brexpiprazole (5, 10, 20, or 40 mg/kg) induced catalepsy and ptosis at high doses as it is shown in the following Sponsor's table (*Report No. 020244*):

Table 14: Induction of catalepsy and ptosis in rats by brexpiprazole and reference compounds

Test Compound	Catalepsy ED <sub>50</sub> at Maximum Time Point (mg/kg)	Ptosis ED <sub>25</sub> at Maximum Time Point (mg/kg)
Brexpiprazole	20, at 6 hours	40, at 2 hours
Aripiprazole	42, at 8 hours	≥ 120, at 6 hours
Risperidone	6.6, at 2 and 4 hours	2.2, at 2 hours

ED25 = dose producing a 25% response; ED50 = dose producing a 50% response

**Behavioral models of antidepressant-like activities in rodents:**

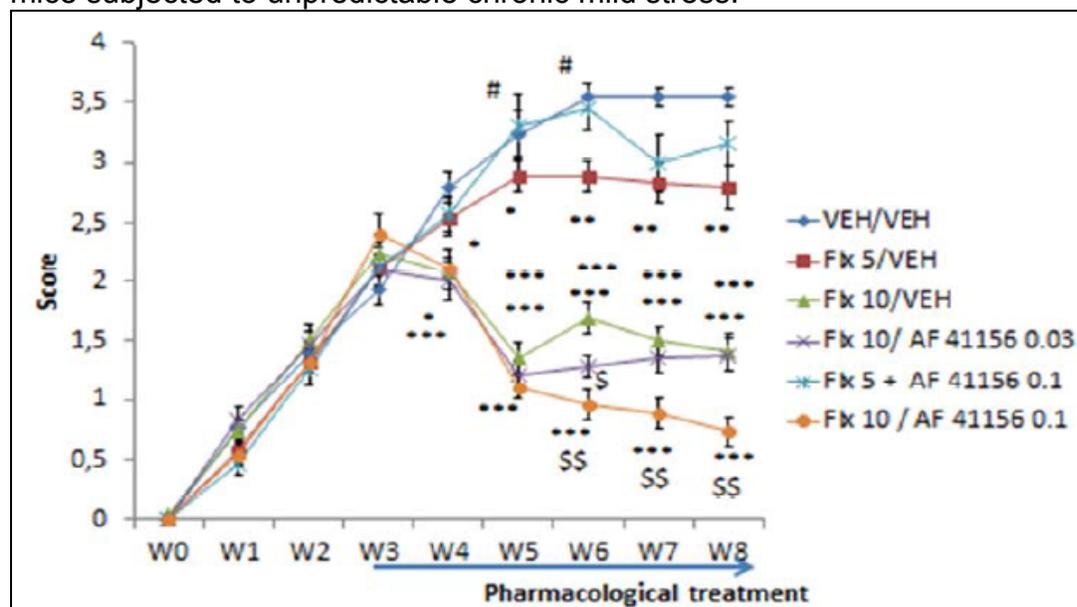
**Forced Swim Test (behavioral despair or Porsolt test):** the mouse or rat is placed in a water-filled container, from which it cannot escape. After an initial struggling period, the animal develops immobility (or behavioral despair), and the immobility time is measured as an index of depressive-like behavior. Antidepressant-like activity of brexpiprazole in combination with fluoxetine was evaluated by the ability of the treatment to decrease the immobility response in rats (*Report No. 15785*). Fluoxetine was administered IP at 24, 4 h and 60 min prior to test. Brexpiprazole was orally administered at 24 and 2 h prior to test. Brexpiprazole alone (0.3, 1 and 3 mg/kg) had no effect on immobility time, while

fluoxetine had a weak effect at 32 mg/kg, compared with the efficacy of the positive reference compound, imipramine. Brexpiprazole at 3 mg/kg significantly and markedly enhanced the effect of fluoxetine in this rat study.

In the forced swim test in mice, paroxetine and sertraline were orally administered 1, and brexpiprazole was administered IP 15 min before the start of test. Brexpiprazole (0.003 mg/kg), paroxetine (10 mg/kg), and sertraline (15 mg/kg) had no effect on immobility on their own. In contrast, the combination of brexpiprazole and paroxetine or sertraline significantly reduced the immobility time suggesting that their adjunctive treatment may have antidepressant potential (*Report No. 029383*):

Unpredictable Chronic Mild Stress Model in mice (a well validated model of stress-induced development of the core symptom of depression, anhedonia): three weeks after start of the stress protocol, a deterioration of coat state score was measured, indicative of development of an anhedonia-like stress response. Fluoxetine at 5 or 10 mg/kg/d, IP induced a gradual improvement of coat state scores, with significant effects being detected after 2 and 1 week, respectively. Adjunct administration of brexpiprazole (AF41156) at 0.1 mg/kg, oral, b.i.d. with fluoxetine (10 mg/kg/d, IP) further enhanced the effect of fluoxetine alone on coat state score at week 6-8 (i.e. after 3-5 weeks' drug treatment). Lower dose of brexpiprazole (0.03 mg/kg, oral, b.i.d.) transiently enhanced the fluoxetine response at week 6 as it is shown in the following Sponsor's figure (*Report No.15786*). The effect of brexpiprazole alone was not determined in this study.

Figure 2: Effect of brexpiprazole in combination with fluoxetine on coat state score in mice subjected to unpredictable chronic mild stress:



## 4.2 Secondary Pharmacology

In addition to identified affinities for the key receptors (primary pharmacology), brexpiprazole was tested at concentration of 10  $\mu$ M on several other targets and showed inhibition of  $\geq 50\%$  at:  $\alpha_2$ -adrenergic (nonselective),  $\beta$ -adrenergic

(nonselective), human DAT, muscarinic (nonselective), human muscarinic M1, sodium channel site 2, human neurokinin NK1, human neurokinin NK2, opiate (nonselective), human opiate  $\mu$ , oxytocin, human SERT, sigma (nonselective), and MAO-B.

These tests did not reveal any additional new targets; effects were more than 2 orders of magnitude less potent than the affinities for the key receptors and unlikely to play any significant role in the pharmacological profile of brexpiprazole.

### 4.3 Safety Pharmacology

Safety pharmacology studies of brexpiprazole were conducted to evaluate effects on the central nervous system (CNS), cardiovascular, and respiratory systems.

#### CNS Effects:

In the study entitled: “*Safety Pharmacology of OPC-34712: Effect of OPC-34712 on the General Symptoms and Behavior in Rats*” (Report No. 019187), brexpiprazole was administered orally at doses of 0 (vehicle), 10, 30, or 100 mg/kg to fasted male rats (6/group). General signs and behavior were observed according to the modified Irwin comprehensive observation assessment at pre-dose and 0.5, 1, 2, 4, 6, 8, and 24 h post-dose. Rectal body temperature was simultaneously measured prior and at 0.5, 1, 2, 4, 6, 8, and 24 h after drug administration.

OPC-34712 at oral doses  $\geq$  30 mg/kg induced CNS depression; decreased alertness, spontaneous activity, touch response, body tone, sedation, catalepsy, ptosis and flaccidity, dilatation of scrotum as well as a dose-dependent decrease in body temperature. OPC-34712 at 30 and 100 mg/kg significantly decreased the body temperature at 2 h and from 1 h to 4 h after administration, respectively. Risperidone produced similar changes in general condition, behavior and body temperature but at a lower dose ( $\geq$ 10 mg/kg). These findings indicate that brexpiprazole at 10 mg/kg had no effect on general symptoms, behavior, and body temperature in rats.

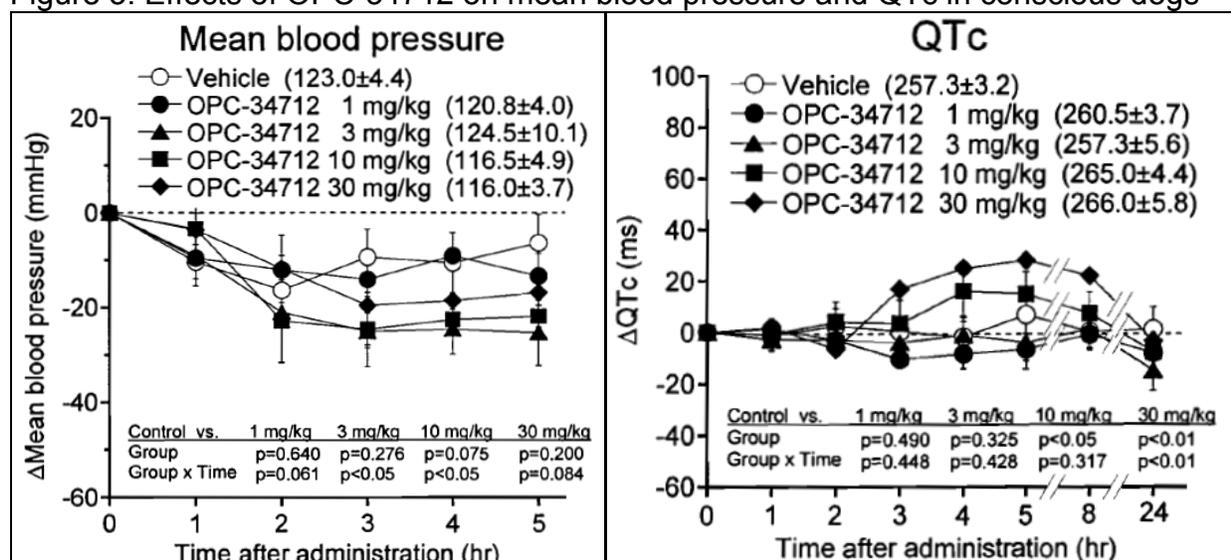
#### Cardiovascular and Respiratory Effects:

In the *in vitro* study entitled: “*Effect of OPC-34712 on hERG Currents in CHO-K1 Cells*” (study No. 024606; Report No. 019647), OPC-34712 significantly decreased the hERG current when compared with control (vehicle - 0.1% dimethyl sulfoxide) with the inhibition rate of 10.6%, 39.8% and 91.5% at concentrations  $10^{-8}$ ,  $10^{-7}$  and  $10^{-6}$  mol/l, respectively. The  $IC_{50}$  value was  $1.17 \times 10^{-7}$  mol/l. Olanzapine, risperidone and haloperidol used as comparators in this study also inhibited the hERG current with  $IC_{50}$  values of  $5.75 \times 10^{-6}$ ,  $2.45 \times 10^{-7}$  and  $2.88 \times 10^{-8}$  mol/l, respectively. Thus, the  $IC_{50}$  value of OPC-34712 was similar to that of risperidone in this study. In the *in vivo* study entitled: “*Safety Pharmacology of OPC-34712: Effects of OPC-34712 on Respiratory and Cardiovascular Systems in Conscious Dogs*” (Report No. 019648) OPC-34712 at oral doses of 0 (vehicle), 3, 0 (vehicle), 1, 10, 30 mg/kg was administered sequentially to each beagle dog (n = 4) at an interval of 7 or 8 days. The reference article risperidone at oral doses of 0 (vehicle), 0.3, 1, and 10 mg/kg was administered sequentially to each beagle dog at an interval of 7 days.

The  $C_{max}$  of OPC-34712 at 1, 3, 10, and 30 mg/kg was 91, 329, 1059, and 2048 ng/ml, respectively and  $t_{max}$  at 4 h post-dose.

No statistically significant changes in the respiratory and cardiovascular systems were observed in OPC-34712 treated groups (at all dose levels) when compared with those of the vehicle group. In comparison, risperidone induced a hypotensive effect at  $\pm 0.3$  mg/kg and a prolongation of QT and QTc intervals at  $\pm 1$  mg/kg. Although not statistically significant when compared to the control group, individually, decrease of the mean BP was observed in 2 animals of the 3-, 10-, and 30- mg/kg groups and prolongations of the QT interval and QTc were observed in 2 animals at 30-mg/kg between 2 and 8 h post-dosing (shown in the following Sponsor's figures).

Figure 3: Effects of OPC-34712 on mean blood pressure and QTc in conscious dogs



Each point represents mean  $\pm$  SE of 4 animals. Data in parenthesis indicate the pre-dose values. The QTc value was calculated using Van de Water's formula.

In conclusion, OPC-34712 at oral doses  $\geq 3$  mg/kg induced hypotensive effects and a prolongation of QT and QTc intervals at 30 mg/kg in the individual animals. Although these findings were not statistically significant compared with vehicle control group, they were supported by the fact that OPC-34712 inhibited hERG channel current with  $IC_{50} = 0.12 \mu M$ .

The mechanism of the hypotensive effect was investigated in the study entitled: "Effect of OPC-34712 on the Phenylephrine-induced Increase in Blood Pressure in Anesthetized Dogs" (Report No. 020054). Dogs were given the  $\alpha_1$ -adrenergic receptor agonist phenylephrine to induce hypertension. In these animals, OPC-34712 at 0.3 and 3 mg/kg IV significantly inhibited the phenylephrine-induced increase in BP (42.3% and 11.2% of control, respectively). Risperidone (a comparator) at 0.3 and 3 mg/kg also significantly inhibited phenylephrine-induced increase in BP (9.8% and 0.0% of control, respectively). Thus, OPC-34712 and risperidone may induce depressor responses by blockade of  $\alpha_1$ -adrenoceptors in peripheral blood vessels.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

The PKs of brexpiprazole were investigated in a number of *in vitro* and *in vivo* studies conducted in mice (*in vitro* only), rats, rabbits, dogs, and monkeys, including studies of brain concentration for brexpiprazole and its metabolites.

#### Absorption

Absorption of brexpiprazole after a single dose was studied in SD rats, cynomolgus monkeys and beagle dogs under fed and fasted conditions.

**Rats:** Brexpiprazole was administered at single oral doses of 1, 3, 10, and 30 mg/kg to fed and fasted SD rats (n=3) or at IV dose of 1 mg/kg to fed male SD rats. Plasma concentrations of brexpiprazole were measured and the results of this study are summarized in the following Sponsor's table (*Report No. 019394*):

Table 15: PK parameters of brexpiprazole in plasma after single oral or IV administration to fed and fasted SD rats:

Route	Dose (mg/kg)	Sex	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC <sub>∞</sub> (ng·h/mL)	t <sub>1/2,z</sub> (range) (h)
Oral (gavage)	1 (fed)	Male	10.2	2	58.61	2.922 (2 - 12)
	3 (fed)	Male	24.7	2	144.5	3.454 (6 - 12)
	10 (fed)	Male	107.2	2	535.9	3.084 (2 - 12)
	30 (fed)	Male	453.9	2	2139	3.126 (4 - 24)
	3 (fasted)	Male	43.9	4	281.7	5.247 (8 - 24)
	3 (fed)	Female	48.2	2	266.7	3.047 (8 - 24)
IV	1 (fed)	Male	674.6 <sup>a</sup>	-	431.3	0.8412 (4 - 8)

<sup>a</sup> Drug concentration at time 0; t<sub>1/2,z</sub> = terminal-phase elimination half-life

C<sub>max</sub> and AUC<sub>∞</sub> increased dose-dependently for doses of up to 30 mg/kg. The absolute bioavailability of brexpiprazole in fed male SD rats at oral dose of 1 mg/kg was low at 13.6%. Following the IV administration of brexpiprazole at 1 mg/kg, the total body clearance of the drug from plasma (CL) and apparent volume of distribution during the terminal phase (V<sub>z</sub>) were respectively 2.32 l/h/kg and 2.81 l/kg. The fasted-to-fed ratio was 178% for C<sub>max</sub> and 195% for AUC<sub>∞</sub>. The C<sub>max</sub> and AUC<sub>∞</sub> of the female rats were approximately 2 times higher than those in males at the same dose level.

**Monkeys:** Brexpiprazole was administered at single oral doses of 0.1, 0.3, 1, and 3 mg/kg to fed and fasted male Cynomolgus monkey or at IV dose of 1 mg/kg to fed male monkey. Plasma concentrations of brexpiprazole increased dose-proportionally and the t<sub>max</sub> ranged between 3 and 7 h post-dose. The results of this study are summarized in the following Sponsor's table (*Report No. 019595*):

Table 16: PK parameters of brexpiprazole in plasma after single oral or IV administration to fed and fasted Cynomolgus monkey:

Route	Dose (mg/kg)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (h)	AUC <sub>∞</sub> (ng·h/mL)	t <sub>1/2,z</sub> (h)
Oral (gavage)	0.1 (fed)	3.9 ± 0.9	7 ± 5	88.91 ± 36.14	10.10 ± 0.58
	0.3 (fed)	14.3 ± 8.2	3 ± 1	191.9 ± 36.5	11.23 ± 2.54
	1 (fed)	52.4 ± 10.6	5 ± 2	956.1 ± 92.4	8.021 ± 0.537
	3 (fed)	145.9 ± 40.7	5 ± 1	2954 ± 115	9.350 ± 2.086
	3 (fasted)	220.4 ± 48.0	5 ± 2	4036 ± 1392	7.887 ± 1.393
IV	1 (fed)	793.4 ± 171.2 <sup>a</sup>	—	3080 ± 210	3.839 ± 0.495

t<sub>1/2,z</sub> = terminal-phase elimination half-life

The absolute bioavailability of brexpiprazole in fed male monkeys at 1 mg/kg was found to be 31%. Following IV administration of brexpiprazole at 1 mg/kg, CL and V<sub>z</sub> were respectively 0.33 L/h/kg and 1.8 L/kg. The food effect was observed in fasted male monkey; fasted-to-fed ratio was 151% for C<sub>max</sub> and 137% for AUC<sub>∞</sub>.

In beagle dogs administered brexpiprazole at 30 mg/kg, (single oral capsule), the C<sub>max</sub> and AUC<sub>∞</sub> under the fed conditions were approximately 3.0- to 3.7-fold higher than those obtained under fasted conditions as it is shown in the following Sponsor's table (*Report No. 019824*):

Table 17: PK parameters of brexpiprazole in plasma after single oral administration to fed and fasted beagle dogs:

Route	Dose (mg/kg)	C <sub>max</sub> (µg/mL)	t <sub>max</sub> (h)	AUC <sub>∞</sub> (µg·h/mL)	t <sub>1/2,z</sub> (h)
Oral (capsule)	30 (fed)	3.487 ± 1.478	8.7 ± 3.1	73.47 ± 18.39	6.738 ± 0.606
	30 (fasted)	1.171 ± 0.408	10.0 ± 12.2	19.94 ± 3.08	7.391 ± 0.808

t<sub>1/2,z</sub> = terminal-phase elimination half-life

In dogs dosed with 1 mg/kg (IV), brexpiprazole was rapidly eliminated from the systemic circulation and the volume of distribution was larger than the volume of body fluid. The AUC<sub>∞</sub> value was 2181 ng·h/ml; t<sub>1/2,z</sub>, V<sub>z</sub> and CL values were 2.7 h, 2.0 L/kg, and 0.6 L/h/kg, respectively (*Report No. 028863*).

In conclusion, feeding affected absorption of brexpiprazole; absorption was higher under fasted conditions in rats and monkeys but was higher under the fed condition in dogs.

### Distribution:

Distribution studies were conducted in several species and tissues, including male and female rats, plasma and brain of individually and collectively housed male rats, and regions of the male rat brain. Plasma protein binding of brexpiprazole was assessed in mouse, rat, rabbit, dog, monkey and human sera, *in vitro* and *in vivo* in rat and monkey.

Tissue distribution (*in vivo*) of dual-label <sup>14</sup>C-brexiprazole-related radioactivity was assessed following a single oral dose of 3 mg/kg to fed male and female SD rats. The

concentration of radioactivity in all tissues peaked at 0.5 to 8 h after dosing and decreased thereafter. Distribution of radioactivity in tissues and organs with the highest concentrations is summarized in the following Sponsor's table (*Report No. 019834*):

Table 18: Tissue concentrations of radioactivity after single oral administration of <sup>14</sup>C-brexpiprazole at 3 mg/kg to male and female rats:

Tissue/Organ	Concentration (ng eq/mL or ng eq/g tissue) <sup>a</sup>			
	Male Rats		Female Rats	
	2 Hours	8 Hours	2 Hours	8 Hours
	(ng eq/mL)			
Plasma	66.62 ± 12.97	34.24 ± 8.265	44.86 ± 3.211	60.29 ± 12.69
	(ng eq/g tissue)			
Stomach	3183 ± 1152	923.6 ± 552.9	2679 ± 846.9	964.9 ± 260.2
Small Intestine	5861 ± 5160	1151 ± 526.1	3142 ± 594.6	1982 ± 254.2
Large Intestine	199.8 ± 70.39	947.5 ± 577.9	117.4 ± 60.27	1270 ± 286.5
Liver	1650 ± 627.4	738.6 ± 238.1	1282 ± 98.47	1215 ± 212.3
Adrenal Gland	818.0 ± 368.8	388.2 ± 32.00	688.9 ± 51.83	644.8 ± 78.14
Cerebrum	10.70 ± 18.54	Not detected	14.89 ± 25.78	8.647 ± 14.98
Cerebellum	24.18 ± 6.232	4.927 ± 7.442	16.23 ± 14.05	4.820 ± 8.349
Medulla Oblongata	35.03 ± 11.21	3.960 ± 6.859	31.36 ± 2.873	6.803 ± 11.78

Distribution of radioactivity was generally similar in male and female rats, except for sex organs (ovary, 214.6 and 183.1 ng at 2 and 8 h; uterus, 82.5 and 69.6 ng at 2 and 8 h; testis, 52.9 and 45.2 ng at 2 and 8 h, respectively). Concentrations in the brain regions were lower than those in plasma and were quantifiable up to 24 h post-dose in males and up to 8 h in females.

Brain distribution of radioactivity (brexpiprazole and its metabolites) was demonstrated in the study entitled: "Autoradiographic study of <sup>14</sup>C-OPC-34712 distribution in rat brain after single oral administration at 1000 mg/kg" (*Report No.020379*). Transversal and sagittal sections of the whole brain were imaged using a Fluoro-image analyzer at 8 and 24 h post-dose. This study showed that distribution of radioactivity was similar in the primary regions of the brain, including the olfactory bulb, cerebral cortex, striatum, hippocampus, hypothalamus, cerebellum, medulla oblongata, and cervical cord. The highest level of radioactivity was detected in the pineal gland and cerebral ventricles at both time points.

In the light microscopic autoradiography study, distribution of silver grains was evaluated in hematoxylin-andeosin- stained sections of the olfactory bulb, cerebral cortex, lateral olfactory tract, corpus callosum, striatum, hippocampal commissure, fimbria, hypothalamus, cerebellum including the cerebellar nucleus and paraflocculus, inferior olive, vestibular nucleus, and cervical cord. The results of this study demonstrated that overall, radioactivity was diffusively present in the gray matter containing neuronal cells and almost no radioactivity was observed in the white matter which contains myelinated fiber and oligodendrocytes (i.e., the lateral olfactory tract, corpus callosum, fibrous tract of the striatum, anterior commissure, and fimbria). In the

cerebellum, the highest radioactivity was observed in the granular cell layer, especially in neurites of the granule cell, followed by the molecular layer > Purkinje cell > cerebellar medulla. In the olfactory bulb, radioactivity was distributed equally in the granular cell layer, mitral cell layer, and internal plexiform layer.

In conclusion, OPC-34712 or its metabolites tend to be distributed in gray matter containing neuronal cells but not in matter containing myelinated fiber and oligodendrocytes. The radioactivity did not accumulate in the granule cells in the olfactory bulb and the Purkinje cells in the cerebellum. This distribution pattern did not correspond to the OPC-34712-related demyelination and necrosis of oligodendrocytes observed in the toxicity study which evaluated the effect of hypothermia observed in the individually housed rats on their brain lesions (see: Special Toxicity Study, Report No.: 020031).

To study further, the time-courses of blood, plasma and brain concentrations of radioactivity for individually or collectively housed male SD rats were investigated after a single oral dose of  $^{14}\text{C}$ -OPC-34712 at 1000 mg/kg. These data are summarized in the following Sponsor's table (*Report No. 020074*):

Table 19: Distribution of radioactivity to plasma and brain of rats housed individually or collectively:

Tissue/Organ	Concentration ( $\mu\text{g-eq/mL}$ or $\mu\text{g-eq/g tissue}$ ) <sup>a</sup>				
	Total	OPC-34712	DM-3411	OPC-3952	SFO-34318
<b>Individually Housed (N = 5)</b>					
Plasma	8.81 $\pm$ 1.88	4.25 $\pm$ 1.39	0.74 $\pm$ 0.35	0.55 $\pm$ 0.78	Not detected
Brain	28.31 $\pm$ 10.07	12.42 $\pm$ 9.03	Not detected	Not detected	16.29 $\pm$ 4.29
<b>Collectively Housed (N = 5)</b>					
Plasma	10.91 $\pm$ 2.25	5.92 $\pm$ 1.87	0.86 $\pm$ 0.41	1.46 $\pm$ 0.30	Not detected
Brain	40.43 $\pm$ 15.42	23.99 $\pm$ 14.25	Not detected	Not detected	19.43 $\pm$ 4.01

The concentration of radioactivity in the brain was higher for rats housed collectively than for rats housed individually (these rats also developed strong hypothermia). This study showed concentrations of the parent and metabolites DM-3411 and OPC-3952 in plasma and brain were higher for collectively housed rats compared with individually housed rats. Metabolites DM-3411 and OPC-3952 were detected only in plasma, and concentrations were higher in collectively housed rats. SFO-34318 was the only metabolite detected in the brain.

While  $^{14}\text{C}$ -brexpiprazole (3 mg/kg) was administered to pregnant rats on GD 16, almost no radioactivity was found in the amniotic fluid however the levels of radioactivity in fetuses were similar or lower than those of dams' blood (*Report No. 021886*). In another study (*Report No. 027301*),  $^{14}\text{C}$ -brexpiprazole was orally administered to pregnant rats on GD 18 (single dose of 3 mg/kg) to examine the fetal-placental transfer and to examine excretion of the radioactivity in maternal milk when the same oral dose of  $^{14}\text{C}$ -brexpiprazole was administered to lactating SD rats on Postpartum Day 12. The results of this study demonstrated that the radioactivity concentrations in most of the tissues reached  $C_{\text{max}}$  at 4 h post-dose and decreased with time. Concentrations of

the radioactivity in the studied tissues at 2 and 4 h post-dose are shown in the following Sponsor's table:

Table 20: Radioactivity concentrations in tissues after single oral administration of <sup>14</sup>C-OPC-331 to non-fasting pregnant rats on Day 18 of pregnancy at 3 mg/kg:

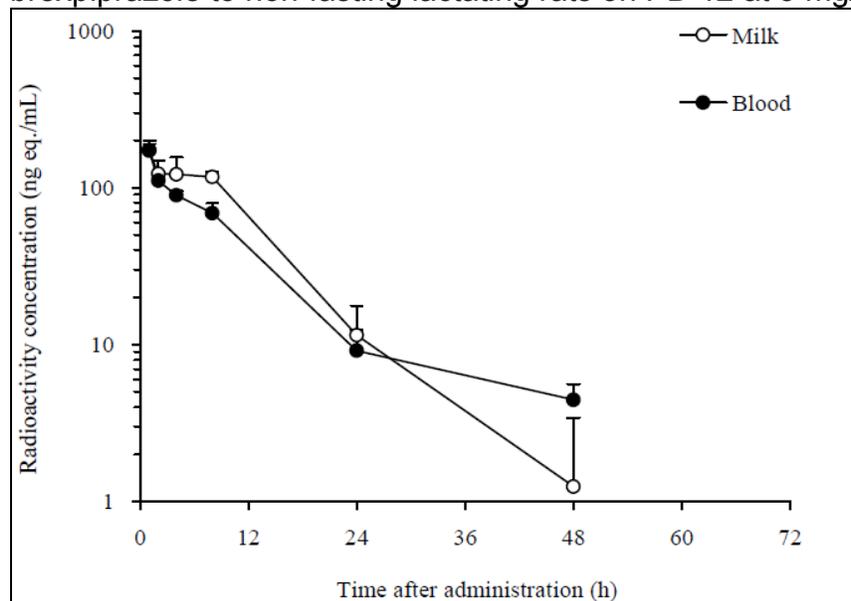
Animal	Tissue	Radioactivity concentration, ng eq./mL or g (Tissue/plasma ratio) [% of C <sub>max</sub> ]			
		2 h		4 h	
Dam	Blood	101.2 ± 19.1	( 0.677 ± 0.018 ) [ 100.0 ]	99.55 ± 22.83	( 0.729 ± 0.103 ) [ 98.4 ]
	Plasma	149.7 ± 29.5	( 1.00 ± 0.00 ) [ 100.0 ]	139.3 ± 40.2	( 1.00 ± 0.00 ) [ 93.1 ]
	Cerebrum	64.96 ± 9.63	( 0.437 ± 0.035 ) [ 95.6 ]	67.97 ± 36.79	( 0.472 ± 0.132 ) [ 100.0 ]
	Cerebellum	53.01 ± 10.02	( 0.357 ± 0.056 ) [ 99.9 ]	53.07 ± 27.43	( 0.370 ± 0.096 ) [ 100.0 ]
	Medulla oblongata	60.37 ± 8.80	( 0.407 ± 0.046 ) [ 91.7 ]	65.82 ± 34.47	( 0.458 ± 0.122 ) [ 100.0 ]
	Eyeball	48.69 ± 5.91	( 0.329 ± 0.042 ) [ 92.3 ]	52.76 ± 23.05	( 0.373 ± 0.072 ) [ 100.0 ]
	Harderian gland	871.5 ± 140.2	( 5.85 ± 0.20 ) [ 56.7 ]	1537 ± 399	( 11.2 ± 1.3 ) [ 100.0 ]
	Submaxillary gland	1072 ± 134	( 7.22 ± 0.49 ) [ 47.7 ]	1856 ± 733	( 13.2 ± 2.2 ) [ 82.6 ]
	Heart	248.3 ± 29.8	( 1.68 ± 0.22 ) [ 94.6 ]	262.4 ± 120.8	( 1.86 ± 0.44 ) [ 100.0 ]
	Lung	979.7 ± 43.8	( 6.70 ± 1.25 ) [ 96.6 ]	1014 ± 483	( 7.09 ± 1.52 ) [ 100.0 ]
	Liver	3307 ± 326	( 22.5 ± 3.6 ) [ 95.8 ]	3453 ± 989	( 25.8 ± 8.5 ) [ 100.0 ]
	Adrenal	1698 ± 251	( 11.4 ± 0.6 ) [ 94.7 ]	1793 ± 620	( 12.7 ± 0.8 ) [ 100.0 ]
	Kidney	839.3 ± 103.2	( 5.69 ± 0.88 ) [ 91.5 ]	917.1 ± 375.2	( 6.54 ± 1.31 ) [ 100.0 ]
	Spleen	777.0 ± 172.9	( 5.18 ± 0.27 ) [ 100.0 ]	775.4 ± 428.1	( 5.39 ± 1.62 ) [ 99.8 ]
	Stomach	2529 ± 676	( 17.3 ± 5.7 ) [ 89.6 ]	2822 ± 349	( 21.9 ± 9.0 ) [ 100.0 ]
	Small intestine	7875 ± 2227	( 53.8 ± 18.6 ) [ 100.0 ]	7196 ± 1103	( 54.1 ± 15.1 ) [ 91.4 ]
	Large intestine	169.4 ± 14.6	( 1.15 ± 0.16 ) [ 10.1 ]	1684 ± 958	( 14.3 ± 12.2 ) [ 100.0 ]
	Ovary	735.8 ± 198.1	( 4.98 ± 1.49 ) [ 99.4 ]	740.4 ± 452.8	( 5.04 ± 1.73 ) [ 100.0 ]
	Uterus	154.9 ± 8.7	( 1.06 ± 0.19 ) [ 72.1 ]	214.7 ± 102.8	( 1.50 ± 0.32 ) [ 100.0 ]
	Placenta	242.9 ± 33.1	( 1.66 ± 0.36 ) [ 79.5 ]	305.5 ± 134.9	( 2.14 ± 0.37 ) [ 100.0 ]
Amniotic fluid	8.817 ± 1.200	( 0.0595 ± 0.0072 ) [ 81.0 ]	10.88 ± 6.18	( 0.0736 ± 0.0310 ) [ 100.0 ]	
Cerebrospinal fluid	2.322 ± 4.021	( 0.0380 ) [ 100.0 ]	N.D.	( N.C. ) [ N.C. ]	
Fetus	Whole-body	76.95 ± 9.26	( 0.524 ± 0.099 ) [ 87.0 ]	88.42 ± 51.53	( 0.613 ± 0.205 ) [ 100.0 ]
	Blood	82.90 ± 10.70	( 0.559 ± 0.049 ) [ 66.2 ]	125.2 ± 65.2	( 0.872 ± 0.229 ) [ 100.0 ]
	Brain	66.86 ± 4.41	( 0.456 ± 0.074 ) [ 90.5 ]	73.91 ± 36.67	( 0.515 ± 0.120 ) [ 100.0 ]
	Heart	75.11 ± 17.68	( 0.500 ± 0.041 ) [ 80.8 ]	93.01 ± 41.41	( 0.658 ± 0.140 ) [ 100.0 ]
	Lung	79.01 ± 7.98	( 0.539 ± 0.102 ) [ 86.9 ]	90.89 ± 44.92	( 0.636 ± 0.152 ) [ 100.0 ]
	Kidney	76.85 ± 3.93	( 0.522 ± 0.070 ) [ 80.6 ]	95.37 ± 50.72	( 0.662 ± 0.177 ) [ 100.0 ]
	Liver	166.1 ± 12.7	( 1.13 ± 0.14 ) [ 86.3 ]	192.5 ± 94.0	( 1.34 ± 0.30 ) [ 100.0 ]

Values in parentheses ( ) are expressed as the ratio of tissue concentration to plasma; Values in parentheses [ ] are expressed as the ratio of radioactivity concentration in tissue relative to the maximum radioactivity concentration; N.D.: Not detected (<2-fold the background value); N.C.: Not calculated

At 4 h after administration, the highest radioactivity concentration among the maternal tissues was detected in the small intestine, which was 54.1-fold higher than that in the plasma, followed by the liver, stomach, submaxillary gland, adrenal, large intestine, Harderian gland, lung, kidney, spleen, ovary, placenta, heart, and uterus. The radioactivity concentrations in the CNS (cerebrum, cerebellum, and medulla oblongata) and amniotic fluid were low and significantly low compared with that in the plasma, respectively. The radioactivity concentration-time profile of the tissues related to pregnancy was similar to those of the main tissues. In the fetuses, the highest radioactivity concentration was detected in the liver, which was 1.3-fold that in the maternal plasma. The radioactivity concentrations in the whole-body and tissues of

fetuses were at the same level or lower than that in the maternal plasma at all of the measuring point, suggesting that the parent and its metabolites distributed into fetuses from their maternal animals via placenta to some extent.

Figure 4: Radioactivity concentrations in milk and blood after a single oral dose of  $^{14}\text{C}$ -brexpiprazole to non-fasting lactating rats on PD 12 at 3 mg/kg



The ratio of milk to maternal blood concentrations of radioactivity ranged from 1.0 to 1.7 over 24 h of measurement. The  $t_{\max}$  of radioactivity was 1 h post-dose for both milk and maternal blood. The  $C_{\max}$ ,  $t_{1/2}$ , and  $AUC_{\infty}$  of radioactivity were 173.0 ng eq./mL, 6.2 hours, and 2129 ng eq·h/mL, respectively, in maternal milk and 172.3 ng eq/mL, 9.2 h, and 1575 ng eq·h/mL, respectively, in maternal blood. The  $t_{1/2}$  values of the radioactivity indicated a slightly faster clearance of the parent and its metabolites from maternal milk than from the blood.

*In vitro* protein binding of  $^{14}\text{C}$ -brexpiprazole to mouse, rat, rabbit, dog, monkey, and human sera was  $\geq 98.8\%$  and was independent of  $^{14}\text{C}$ -brexpiprazole concentration. Protein binding was high to human serum and was bound predominantly to albumin (90.3% to 97.2%) and  $\alpha_1$ - acid glycoprotein (95.6% to 98.0%). *In vivo* protein binding (Report No. 019833) ranged from 91.8% to 95.5% in rat and monkey plasma after oral dosing and was lower than *in vitro* protein binding in these species. This finding suggests that protein binding of metabolites formed *in vivo* may be lower than that of unchanged brexpiprazole. Therefore in another study protein binding of main metabolite DM-3411 in animal sera was determined *in vitro* and the results are summarized in the following Sponsor's table (Report No. 023510):

Table 21: Protein Binding of DM-3411 in Serum

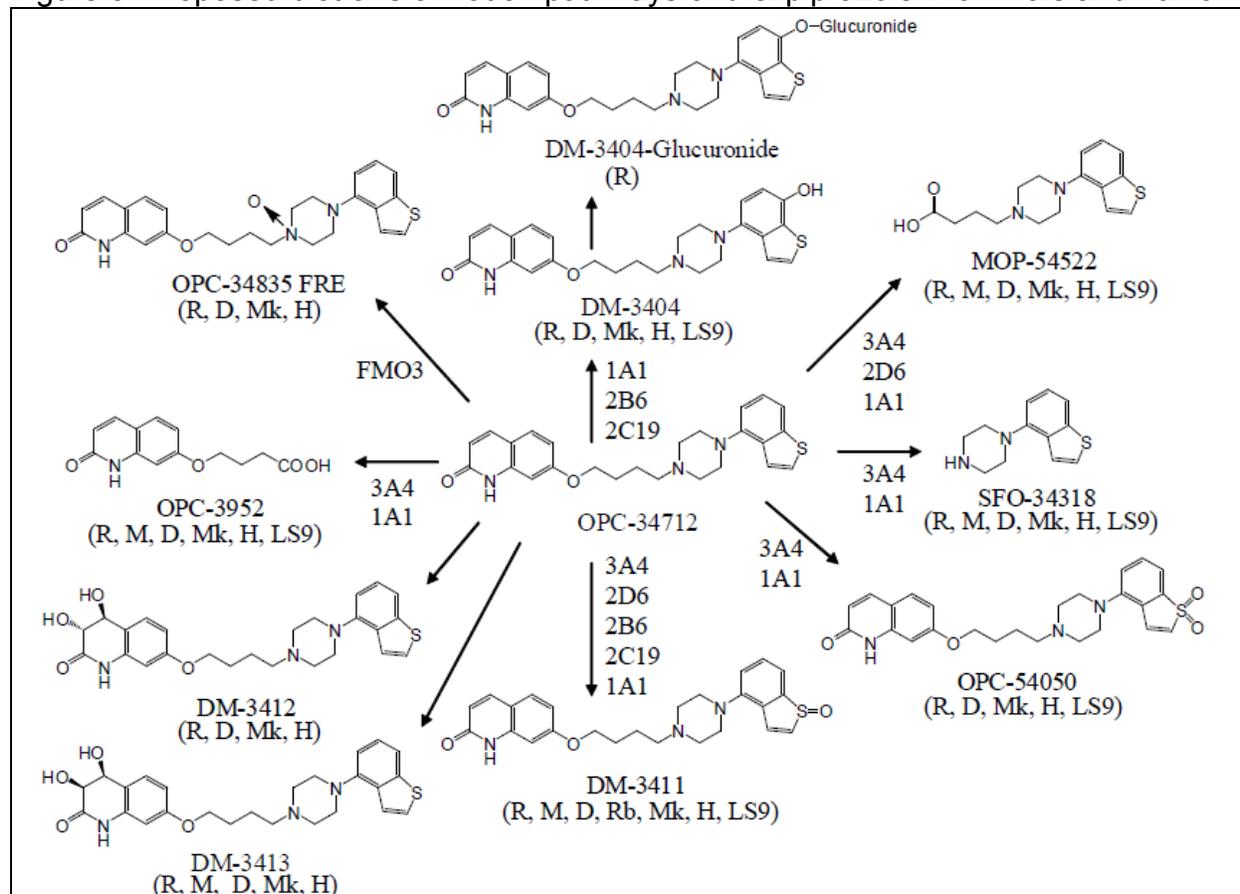
Species	Concentration	Protein Binding (%)		
		0.05 µg/mL	0.5 µg/mL	5 µg/mL
Mouse		93.4 ± 0.6	93.3 ± 0.8	93.5 ± 0.5
Rat		93.1 ± 0.6	92.8 ± 1.2	92.6 ± 0.8
Monkey		94.2 ± 0.0	94.3 ± 0.3	89.8 ± 0.3

As it is shown in the above table, the protein binding of main metabolite DM-3411 in mouse and rat sera was independent of DM-3411 concentration and ranged from 93.3% to 93.5% and from 92.6% to 93.1%, respectively.

### Metabolism:

Chemical structures of brexpiprazole and 10 identified metabolites (DM-3411, OPC-3952, SFO-34318, OPC-54050, MOP-54522, DM-3404, DM-3412, DM-3413, OPC-34835FRE, and DM-3404-glucuronide), which were identified across species: mouse, rat, rabbit, dog, monkey and human subjects, are shown in the Sponsor's figure 5:

Figure 5: Proposed biotransformation pathways of brexpiprazole in animals and humans



In an *in vitro* metabolism study of OPC-34712 (Reports No. 019521 and 019171) metabolites DM-3411, OPC-3952, and DM-3404 were produced by liver S9 from all

species tested (mouse, rat, rabbit, dog, monkey, human). Metabolite DM-3411 was produced at the highest rate in all species, except female rats, where DM-3404 was the predominant metabolite. Brexpiprazole, DM-3404, DM-3411, OPC-3952, and OPC-34835FRE were detected in plasma from male rats whereas only brexpiprazole, DM-3404, and DM-3411 were detected in female rats.

The predominant human CYP450s involved in the metabolism of brexpiprazole were CYP3A4 and CYP2D6 (*Report No. 019018*). The rank order of activity to produce brexpiprazole metabolite was CYP3A4 > CYP2D6 > CYP1A1 > CYP2B6 > CYP2C19. No metabolites were detected with CYP1A2, CYP2A6, CYP2C8, CYP2C9, or CYP2E1.

*In vivo* metabolism studies confirmed DM-3411 to be the main metabolite produced at the highest rate in all species studied (mice, rats, rabbits, dogs, monkey and humans). After a single oral dose of 3 mg/kg dual-label <sup>14</sup>C-brexiprazole to fed male and female SD rats, brexpiprazole, DM-3411, DM-3404-glucuronide, OPC-3952, and other minor metabolites were detected in plasma at time points up to 24 h post-dose (*Report No. 020545*). The ratios of brexpiprazole, DM-3411, DM-3404-glucuronide, and OPC-3952 to total radioactivity in male plasma samples at 2, 4, and 8 h post-dose were 39.2% to 59.4%, 7.0% to 12.7%, 3.5% to 24.4%, and 1.1% to 4.6%, respectively. The ratios of brexpiprazole, DM-3411, DM-3404-glucuronide, and OPC-3952 to total radioactivity in female plasma samples at 2, 4, and 8 hours were 38.2% to 71.1%, 7.1% to 12.4%, 2.0% to 30.1%, and 0.0% to 4.3%, respectively.

Table 22: PK parameters of brexpiprazole (OPC-34712) and its metabolites in rat plasma after a single oral dose of dual-label <sup>14</sup>C-Brexiprazole at 3 mg/kg

Parameters	Analytes <sup>a</sup>			
	OPC-34712	DM-3411	DM-3404-Glu	OPC-3952
<b>Males</b>				
t <sub>max</sub> (hours)	1.0	4.0	8.0	1.0
C <sub>max</sub> (ng eq/mL)	35.00	5.90	9.30	4.60
AUC <sub>t</sub> (ng eq·h/mL)	284.8	36.65	109.5	29.00
t <sub>1/2,z</sub> (hours)	4.52	Not calculated	24.60	2.46
<b>Females</b>				
t <sub>max</sub> (hours)	2.0	2.0	8.0	8.0
C <sub>max</sub> (ng eq/mL)	41.30	6.60	9.50	2.20
AUC <sub>t</sub> (ng eq·h/mL)	272.0	47.70	69.60	15.10
t <sub>1/2,z</sub> (hours)	4.38	7.97	Not calculated	Not calculated

Similar study was conducted in monkeys where brexpiprazole and its metabolites were measured following a single oral administered of brexpiprazole at 3 mg/kg to fed male monkeys. Brexpiprazole, DM-3404, DM-3411, DM-3412, OPC-3952, OPC-54050, SFO-34318, and MOP-54522 were detected in the plasma, and the PK parameters are shown in the following Sponsor's table (*Report No. 023634*):

Table 23: PK parameters of brexpiprazole (OPC-34712) and its metabolites in plasma from male monkeys after a single oral dose of brexpiprazole at 3 mg/kg

Parameters	Analytes <sup>a</sup>							
	OPC-34712	DM-3404	DM-3411	DM-3412	OPC-3952	OPC-54050	SFO-34318	MOP-54522
t <sub>max</sub> (hours)	8.0	6.0	4.3	16.0	3.0	8.0	16.0	20.0
C <sub>max</sub> (ng/mL)	186.2	0.759	154.0	59.65	17.24	7.11	6.29	5.20
AUC <sub>t</sub> (ng·h/mL)	3438	1.46	2860	1609	240.9	120.7	164.3	159.9
AUC <sub>∞</sub> (ng·h/mL)	3493	NC	2922	1181	290.6	113.0	76.97	66.37
t <sub>1/2,z</sub> (hours)	7.4	NC	8.0	13.1	11.2	9.4	16.5	17.4

In the clinical study (331-08-205), the exposure to brexpiprazole and its metabolites in the plasma on study Day-14 at oral dose of 4 mg/kg was summarized in the following Sponsor's table:

Table 24: PK parameters of brexpiprazole (OPC-34712) and its metabolites in plasma from male monkeys after a single oral dose of brexpiprazole at 3 mg/kg

Dose	4 mg		
Parameters (Day-14)	Mean C <sub>max</sub> (ng/mL)	Mean AUC <sub>24h</sub> (ng·h/mL)	AUC Ratio (%) of Metabolite to Total Drug-related
OPC-34712	199	3950	72.8
DM-3411	64.3	1280	23.6
DM-3412	8.74	174	3.2
MOP-54522	ND	ND	-
OPC-34835	ND	ND	-
OPC-3952	0.489	9.69	0.2
OPC-54050	0.808	15.4	0.3
SFO-34318	ND	ND	-
Total	-	5429	-

ND = not determined

Based on the calculated ratio of each metabolite to the total brexpiprazole-related exposure, DM-3411 metabolite is shown to be a major human metabolite.

In mice dosed with brexpiprazole at 1, 5, and 10 mg/kg for 28 days (toxicity study; *Report No. 029061*), the systemic exposure to the parent and its metabolites (OPC-3952, OPC-34835FRE, OPC-54050, SFO-34318, MOP-54522, DM-3404, DM-3411, DM-3412, and DM-3413) were evaluated. Among the 9 metabolites, plasma concentrations of DM-3411 and OPC-3952 were the highest. These tendencies did not change after repeated dosing.

### Excretion:

Excretion studies were conducted in male and female SD rats and male Cynomolgus monkeys. The main route for excretion of <sup>14</sup>C-OPC-34712-related radioactivity in rats

(single oral dose of 3 mg/kg) was feces as it is shown in the following Sponsor's table (*Report No. 019834*):

Table 25: Urinary and fecal excretion of radioactivity within 168 h after single oral administration of dual-label <sup>14</sup>C-brexpiprazole at 3 mg/kg in fed rats

Sex	Cumulative Excretion (% of Administered Dose)		
	Urine	Feces	Total
Male	1.728 ± 0.356	95.26 ± 1.391	96.99 ± 1.146
Female	1.567 ± 0.753	93.82 ± 5.671	95.39 ± 5.388

Excretion was almost complete by 168 h post-dose, with 97% and 95.4% of dosed radioactivity accounted for in the urine and feces of male and female, respectively. No sex differences in excretion of OPC-34712 were noted in SD rats.

Biliary and urinary excretion and enterohepatic circulation of radioactivity was investigated after a single oral dose of 3 mg/kg <sup>14</sup>C-OPC34712 to bile duct cannulated SD rats. Mean biliary and urinary excretion of radioactivity accounted for 73.3% and 3.6% of the administered dose in male rats, respectively, and 66.2% and 4.8% in female rats, respectively (0 to 48 h postdose). Biliary excretion was the main pathway for absorbed radioactivity in the rats, and no sex differences were observed. Also, the mean absorption of radioactivity in male and female rats was approximately 76.9% and 71.0% (0 to 12 h post-dose), respectively (*Report No. 020049*).

In monkeys administered single oral dose (3 mg/kg) of <sup>14</sup>C- OPC-34712 (*Report No. 019600*), 15.4% and 63.4% of dosed radioactivity was excreted (168 h post-dose) in urine and feces, respectively. HPLC analysis of radioactivity in plasma, urine and feces after single oral administration of dual-label <sup>14</sup>C-OPC-34712 at 3 mg/kg to male monkeys (*Report No. 020276*) showed that the parent and DM-3404 were the predominant substances detected in feces collected for 0 - 96 h post-dose; brexpiprazole and DM-3404 accounted for 22.7% and 11.1% of the administered dose, respectively.

## 5.2 Toxicokinetics

The reviews and evaluation of TK studies which were conducted during general toxicology studies are included in the toxicology studies reviews. In general, the exposure to brexpiprazole (AUC and C<sub>max</sub>) was confirmed in toxicology studies lasting up to 26 weeks in rats, 39 weeks in monkeys and up to 13 days in rabbits. In rats, at doses of 3 to 100 mg/kg/d and in monkeys at 1 to 30 mg/kg/d, exposure to brexpiprazole increased in a dose- and treatment duration-related manner. In rabbits, at doses of 10 to 150 mg/kg/d, exposure to brexpiprazole increased in an approximately dose- and duration-related manner.

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

A single oral dose of braxpiprazole was administered to rats or monkeys on study Day 1 and the animals were necropsied on Day 15 after an observation period. The results of single-dose toxicity studies are summarized in the following Sponsor's table:

Table 26: The list of single dose toxicity studies I rays and monkey

Species (Sex)	Approximate Lethal Dose	Results
Rat (M, F)	M > 2000 mg/kg F 2000 mg/kg	Mortality: 2000 mg/kg (2 F) In survivors, body weight decreased on Day 4; food consumption decreased during the first 3 days. Decreased adipose tissue only notable change at necropsy. Clinical observations: Closed eyes, hypoactivity, abnormal postures, fixed stare, loss of voluntary motion, hypothermia and lacrimation (M, F), flaccidity and dilation of the scrotum, and prolapse of the penis (M); paleness of auricle (F). Recovery: Day 4 (M), Day 5 (F).
Rat (F)	> 800 mg/kg	No mortality. Body weight suppressed on Day 4. Clinical observations: Closed eyes, hypoactivity, abnormal postures, hypothermia, and lacrimation. Recovery: Day 3 (320 mg/kg), Day 5 (800 mg/kg). The approximate minimum lethal dose of OPC-34712 in female rats was above the highest dose tested (> 800 mg/kg).
Monkey (M, F)	> 100 mg/kg	No mortality. Weight loss on Day 3 (M) and Days 3 and 7 (F) at 100 mg/kg; food consumption reduced on Days 2 to 6 (M), and Days 2 to 14 (F) at 100 mg/kg; Days 2 (M) and Days 2 to 4 (F) at 30 mg/kg. Clinical observations: Eyelid closure, prone/crouching position, sopor (deep sleep), hypothermia, decreased heart rate, limb tremors, hypoactivity, muddy stools, no feces. The approximate lethal dose was above the highest dose tested (> 100 mg/kg) in both sexes.

In conclusion, the single-dose oral toxicity studies showed that the minimum lethal dose in male rats was  $\geq 2000$  mg/kg and between 800 - 2000 mg/kg in female rats. In male and female monkeys, the minimum lethal dose was > 100 mg/kg, the highest dose tested. Clinical signs observed in these studies were related to the pharmacological effects of brexpiprazole on the CNS and included hypoactivity, closed eyes, abnormal postures, and hypothermia. Overall, these data showed that brexpiprazole has low acute toxicity in rats and monkeys.

## 6.2 Repeat-Dose Toxicity

Repeat-dose toxicity studies were conducted in CD-1 mice, SD rats, and cynomolgus monkeys. Non-pivotal oral gavage toxicity studies of one and four week in duration were conducted in rats and monkeys as a dose range-finding studies for the pivotal repeat-dose toxicity studies which are listed in the following Sponsor's table:

Table 27: The list of pivotal repeat dose toxicity studies in mouse, rat, and monkey

Study	Species/Strain (Number/Sex/ Group)	Route	Dose (mg/kg or mg/kg/day)	Key Results	Report Number
<b>Repeat-dose Toxicity</b>					
13-week Preliminary Carcinogenicity Including TK	Mouse/CD-1 (10/sex/group)	Oral (gavage)/ 13 weeks	0 (vehicle), 1, 5, 10	Mortality and decreased body weight: M 10 mg/kg/day	023553
13-week with a 4-week Recovery Including TK	Rat/Sprague Dawley (10 or 15/sex/group)	Oral (gavage)	M 0 (vehicle), 3, 10, 100, 300 F 0 (vehicle), 3, 10, 30, 100	NOAEL: M - 3 mg/kg/day F - 10 mg/kg/day	020367
26-week with a 13-week Recovery Including TK	Rat/Sprague Dawley (15 or 20/sex/group)	Oral (gavage)	0 (vehicle), 3, 10, 30, 100	NOAEL: M - 3 mg/kg/day F - 10 mg/kg/day	023880
13-week with a 4-week Recovery Including TK	Monkey/ cynomolgus (3 or 5/sex/group)	Oral (gavage)	0 (vehicle), 1, 3, 30	NOAEL: 1 mg/kg/day	020474
39-week Including TK	Monkey/ cynomolgus (4/sex/group)	Oral (gavage)	0 (vehicle), 1, 3, 30	NOAEL: 1 mg/kg/day	024584

### Summary of Repeated-Dose Toxicity Studies in Rats:

Repeat-dose toxicity studies of 1-week, 4-weeks and 13 weeks in duration in rats were reviewed by this Reviewer under the IND 101,871 (N000/03-22-2008) and are summarized below:

#### One-week Repeated Oral Dose Toxicity Study of OPC-34712 in Rats (Study No.: 024288, Report No. 019075)

OPC-34712 at 300 or 1000 mg/kg/d by oral gavage was administered to rats (10/sex/group) for 1 week in order to determine the MTD for a subsequent 4-week repeat-dose toxicity study.

During the dosing phase, 1 male at 1000 mg/kg/d died. Seven females given 300 mg/kg/d died or were euthanized due to deteriorating condition, and 8 females at 1000 mg/kg/d died or were euthanized (Day 2 to Day 6). Mean BW and food consumption decreased continuously throughout dosing. The body temperatures of

male and female rats were significantly reduced after the first dose although males partially recovered by Day 3. Low-dose females showed no recovery until Day 7, demonstrating a sex difference. The clinical findings of hypoactivity, closed eyes or incomplete eyelid closure, abnormal postures as hunchback position or creeping, hypothermia as cold to the touch at the body surface, flaccidity and dilatation of the scrotum occurred at relatively high incidences in males and/or females and observed general deteriorating condition of these animals suggested severe overdosing at 300 and 1000 mg/kg/d.

Clinical pathology findings included: decreases in WBC (principally due to the decrease of lymphocytes count), hemoglobin, hematocrit, red blood cell, platelet, and decrease or increase of reticulocyte, decreases of alkaline phosphatase, triglyceride, glucose, total protein, calcium, inorganic phosphorus, and chloride, and increases of total cholesterol, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, creatine kinase, blood urea nitrogen and creatinine. Most of these changes were seen in both males and females at 300 and 1000 mg/kg.

At necropsy, except for the changes attributable to the poor physical conditions, notable changes were observed in the brain, liver, kidneys and testes as follows; necrosis of the oligodendrocytes in the white matter, demyelination, necrosis of the Purkinje cells, chromatolysis of the neurons and vacuolation of the white matter and/or gray matter in the brain, enlargement of the hepatocellular nuclei with enlargement of its nucleous, and granular degeneration of the hepatocytes in the liver, necrosis and regeneration of the epithelial cells of cortical tubules in the kidneys, degeneration / necrosis of the germ cells, decrease of germ cells and multinucleated giant cell formation in the testes. Additionally, enlargement of mitochondria and increase of the electron density of mitochondrial matrix were seen in the hepatocytes by the electron microscopy. Therefore it was concluded that both doses selected in this study were too high for longer duration of treatment.

#### **Four-week Repeated Oral Dose Toxicity Study of OPC-34712 in Rats (Study No.: 024495; Report No. 019254)**

Sprague-Dawley [CrI: CD(SD)] rats (10/sex/group) were dosed at: 0, 10, 30, 100, 300 mg/kg/d (males) and 0, 10, 30, 100 mg/kg/d (females). Toxicities and TKs were assessed.

Mortality was observed at HD, one female (main study) at 100 mg/kg/d died and one male at 300 mg/kg/d and two females at 100 mg/kg/d (TK study) also died early. In the observation of the general conditions, extremely low body temperature (mainly during first 7 days of dosing) hypoactivity, closed eyes or incomplete eyelid closure were principal findings in this study. In addition, flaccidity and dilatation of the scrotum in the males, abnormal postures as hunchback position or creeping were observed in males and females at HD (with the highest incidences in the early period of dosing). Decreased BW/BW gains were attributable to the severe depression of the food consumptions throughout the dosing period in males at all dose groups and in the females at 100 mg/kg/d.

Decrease of blood glucose and disturbances of protein fractions particularly increase of  $\gamma$ -globulin were detected in males and females of all OPC-34712 treated groups.

Histopathological changes included, atrophy of pituitary (pars intermedia) in both males and females at all doses. Hypertrophy of adrenal, foamy cells accumulation in the alveoli of lungs and swelling of the acinar cells of salivary glands such as submaxillary and sublingual glands were observed in males at  $\geq 100$  mg/kg/d. Hypertrophy of adrenal, mucification of uterus and swelling of the acinar cells of submaxillary gland were observed in females at 100 mg/kg/d. Lobular hyperplasia with secretion of milk in the mammary gland and the changes as pseudopregnancy in the reproductive tissues in females and feminization of the mammary gland of males were observed in all OPC-34712 - treated groups. These findings were a consequence of drug-related pharmacologically mediated (D2- receptor antagonism) hyperprolactinemia similar to those changes described for the other antipsychotics. In males, the depressed spermatogonia such as degeneration/necrosis of the germ cells, decrease of the germ cells, multinucleated giant cell formation, atrophy of the seminiferous tubules and/or retention of step 19 spermatid as well as atrophic changes of the epididymis, prostate and seminal vesicle were observed mainly at HD.

The nutrient starvation or disturbance of the hormones including prolactin was assumed as one possible cause of these lesions in rats. Additionally, the decrease of body temperature might play a role in causing such testicular findings.

#### **Thirteen-week Repeated Oral Dose Toxicity Study of OPC-34712 with a 4-week Recovery Test in Rats (Study No.: 024963; Report No. 020367)**

Rats [CrI: CD(SD)] were dosed at 0, 3, 10, 100, 300 mg/kg/day (males); 0, 3, 30, 100 mg/kg/day (females) for 13 weeks. Toxicities and TKs were assessed.

In this 13-week oral repeat-dose toxicity study in rats, 4 males at 300 mg/kg/d were found dead and one female at 100 mg/kg/d was sacrificed in moribund condition. In the TK groups, one male at 300 mg/kg/d, and 2 males and 2 females at 100 mg/kg/d died.

The most prominent toxicity included extremely low mean body temperatures early in the dosing period and severe decrease in BW and food consumption in males at 100, and 300 mg/kg/d (-26% and -46%, respectively at the end of dosing) and in females at 100 mg/kg/d (-10% at the end of dosing). Hematology changes included increased reticulocytes in HD males and decreased RBC and Ht in HD females; biochemical changes included increased creatine kinase, AST, ALT, GTP, total cholesterol, phospholipid, and sodium, and decreased potassium and urinary excretion of creatinine in males at  $\geq 100$  mg/kg/d. In addition, in females, increases in PL and decreases in TG were observed at  $\geq 30$  mg/kg/day. Histopathologic examination (surviving animals) revealed brain lesions (2/6) and depressed spermatogenesis that included degeneration/necrosis of germ cells (4/6) in males at 300 mg/kg/d. Swollen acinar cells in sublingual gland, microgranuloma in spleen, hepatocyte vacuolation, increases of myelin-like structure in hepatocytes and Kupffer's cells were also noted.

Lobular hyperplasia with secretion of milk in the mammary gland and changes in reproductive tissues related to pseudopregnancy were observed in females at all dose levels; feminization of the mammary glands in males was observed at doses  $\geq 10$  mg/kg/d. These changes were considered to be induced by pharmacologically mediated ( $D_2$  antagonism) increases in serum prolactin.

At the end of 4-week recovery, drug-related changes either returned to normal or decreased in severity or incidence, except for the following: pigment granule deposition, vacuolation of hepatocytes in males and females and depressed spermatogenesis and microgranuloma in spleen (including controls) in males.

The NOAEL was at 3 mg/kg/d for males and 10 mg/kg/d for females.

**Supplementary (special) study:**

In repeat-dose toxicity studies in rats, brain and testicular lesions were observed along with severe decrease in body temperature. In this special study, the effect of hypothermia alleviation on brain and testis histopathology in male rats was investigated.

20 male rats were divided into two groups. The first group was housed individually in cages without bedding material and the second group was housed collectively (5 rats/cage) with paper pulp bedding. All rats were treated with OPC-34712 at 1000 mg/kg orally by gavage for 7 days.

Housing condition had no notable effects on TK parameters, incidence and time of death (4/group died), clinical signs other than hypothermia (no extremely low temperatures in rats housed collectively), and BW changes at least during the first 4 days of administration.

Lesions in the brain (necrosis of granule cells in the olfactory bulb, oligodendrocytes, and Purkinje cells in the cerebellum, vacuolation in the cerebrum, cerebellum and medulla oblongata, demyelination) and testis (nuclear vacuolation of round spermatids, degeneration/necrosis of germ cells, multinucleated giant cells, depressed spermatogenesis in testes and exfoliated germ cells in epididymal duct) were observed frequently in the individually housed rats, but not in the collectively housed rats. Exfoliated germ cells also observed in the collectively housed group were limited and considered within normal histological variation limits.

**In conclusion:** collective housing with paper pulp bedding prevented the decrease in body temperature induced by OPC-34712 treatment, as well as it prevented brain and testicular toxicity in rats. Therefore, these toxicities (brain and testicular lesions) were considered to be related to pharmacologically-mediated extreme hypothermia in rats rather than a direct toxic effect of the test article.

## Chronic Toxicity Study in Rats

### Study title: Twenty-six-week Repeated Oral Dose Toxicity Study of OPC-331 with 13-week Recovery Test in Rats

Study no.: B-6604 (Otsuka study No. 028625;  
Report No. 023880)  
Study report location: EDR  
Conducting laboratory and location:  (b) (4)  
Date of study initiation: December 26, 2008  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: OPC-331, lot# C07F70M, 99.8% purity

### Key Study Findings

OPC-331 was administered by oral gavage to male and female rats at 0, 3, 10, 30, and 100 mg/kg/d for 26 weeks. The following treatment-related findings were observed:

- Death of 2 HDF (Day 5 and Day 11)
- Clinical signs at HD (M + F) of incomplete eyelid closure, hypoactivity, lacrimation, staggering gait, flaccidity and dilatation of the scrotum, clonic convulsion (2 M and 4 F on several occasions)
- Lower body temperature at the beginning of dosing (up to Day 21 in HDM and up to Day 35 in HDF)
- Significant reduction in BW/BW gain and remarkable decrease in food consumption in males at  $\geq 10$  mg/kg/d and females at  $\geq 30$  mg/kg/d
- Deposition of pigment granules in the adrenal gland (cortical cells and macrophages); liver (hepatocytes and Kupffer cells); thyroid (follicle cells); and macrophages in lung, submandibular lymph node, ovary, or spleen of males or females at  $\geq 30$  mg/kg/d
- Atrophy in intermediate lobe of the pituitary in male and female rats at all dose levels; in mammary glands, minimal to mild feminization in males at all doses and increased incidence of lobular hyperplasia in females at  $\geq 3$  mg/kg/d
- An increased degree of milk secretion in females at  $\geq 30$  mg/kg/d and vaginal mucification at doses  $\geq 3$  mg/kg/d
- Atrophy of seminiferous tubules, prostate, and seminal vesicles in males at  $\geq 30$  mg/kg/d
- The NOAEL 3 mg/kg/d (males) and 10 mg/kg/d (females) was based on decreases in BW/BW gain at higher doses

**Methods**

Doses: 0, 3, 10, 30, and 100 mg/kg/day  
 Frequency of dosing: Once a day  
 Route of administration: Oral gavage  
 Dose volume: 5 ml/kg  
 Formulation/Vehicle: 5 w/v% GA solution  
 Species/Strain: Rats/Crl:CD(SD)  
 Number/Sex/Group: 15/sex/group  
 Age: 6 weeks old  
 Weight: Males: 197 - 241g; Females: 152 – 187g  
 Satellite groups: TK - 8/sex/group; recovery 5/sex/control and HD  
 Unique study design: None  
 Deviation from study protocol: N/A

**Observations and Results****Mortality**

Test article-related death occurred during the dosing phase in two HD females; one female (main study) died on Day 5 (food consumption was 0 g on Day 4) and one female of satellite group died on Day 11 of drug administration (BW decreased -44%). Histopathological findings in these animals included erosion in the glandular stomach, atrophy of several lymphatic tissues (follicles in the submandibular and mesenteric lymph nodes, thymus, and white pulp in the spleen) and swelling of the submandibular gland. All findings revealed undernourishment before death in both animals.

**Clinical Signs**

All animals were observed for clinical signs such as external appearance, nutritional condition, posture, behavior and appearance of feces. During the administration period, examination was done 3 times (before dosing, immediately after dosing and 4 to 6 hrs after dosing). During the recovery period, examination was done once daily.

Incomplete eyelid closure was observed at 30 mg/kg (M + F), flaccidity and dilatation of the scrotum in males at 30 mg/kg. At HD (M + F), incomplete eyelid closure, flaccidity and dilatation of the scrotum, hypoactivity, lacrimation, creeping, staggering gait, clonic convulsion (in 2 M and 4 F on several occasions), hyperreactivity (in a few M + F) and aggressiveness in a few HD-M were also observed during dosing period.

Clonic convulsions were observed in 2 males and 3 females at 100 mg/kg on the following days of dosing:

Male 1	before dosing on Days 176 and 182
Male 2	before dosing on Days 133, 135, 141, 163, 171, 172 and ~10 or ~15min after dosing on Day 176 and Day 177, respectively
Female 1	before dosing on Days 155, 168, 173, 175 and immediately after dosing on Days 176-179
Female 2	~10 or ~20 min after dosing on Day 82 and Day 112, respectively
Female 3	before dosing on Days 91 and 119

All these clinical signs were no longer present during the recovery phase of the study except hyperreactivity of one HD-M.

### Body Temperature

Rectal temperature was measured (not anesthetized rats) before and approximately 6 hrs after dosing on Days 1 to 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 119, 147, 175 and 182 and during the recovery period on Days 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91.

Results: In HD groups, body temperature was low at 6 h after dosing from Day 1 to 21 in males (the lowest mean value was 32.4°C vs 36.6°C in controls) and from Day 1 to 35 in females (the lowest mean value was: 29.8°C vs 37.1°C in controls). At doses  $\leq 30$  mg/kg, body temperature was also measured lower on several occasions, however these body temperature changes were recognized by the Sponsor as minimal and temporary changes and were thought to be within the range of physiological variations.

### Body Weights

During the administration period, all animals were weighed before dosing, between on Days 1, 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 119, 147, 175 and 182.

Results: Decreased BW and suppressed BW gain were observed in males at  $\geq 10$  mg/kg and in females at  $\geq 30$  mg/kg groups. The enhancement of BW gain was observed in females in 3 and 10 mg/kg groups which were considered by the sponsor to be related to the activated luteal function induced by an increased serum prolactin level. Terminal BW at the end of dosing was lower than controls in HDM by 34% and in HDF by 17%. The results are shown in the following sponsor's figures:

Figure 6: Twenty-six-week repeated oral dose toxicity study of OPC-331 with 13-week recovery test on *BW in Males*:

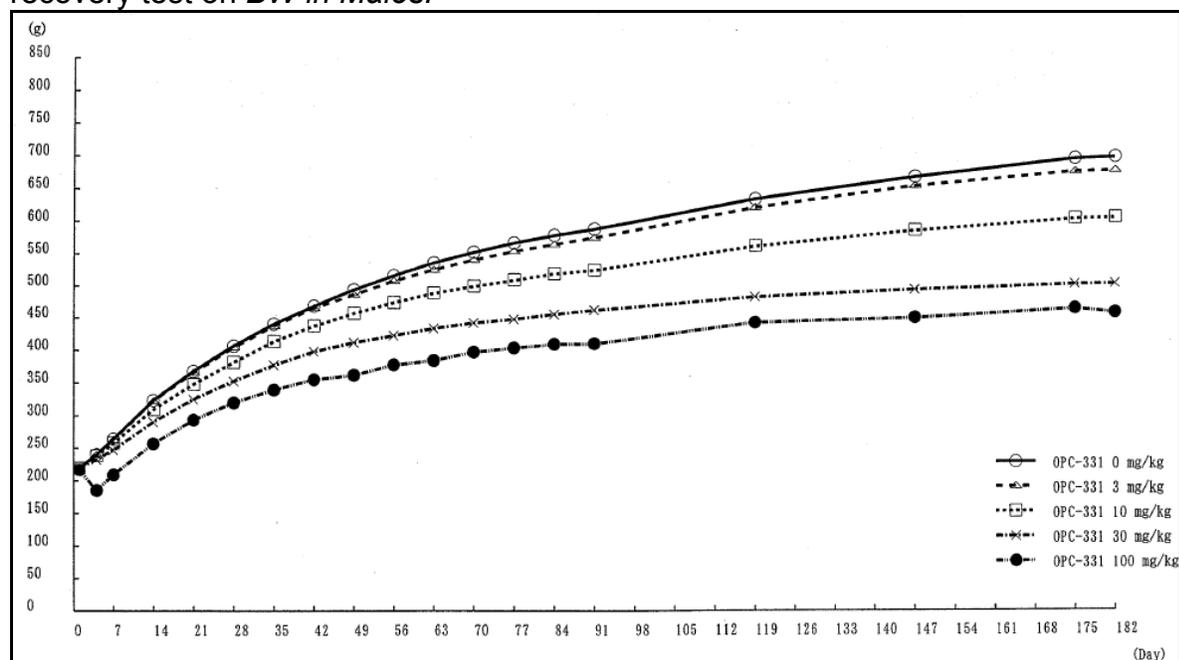
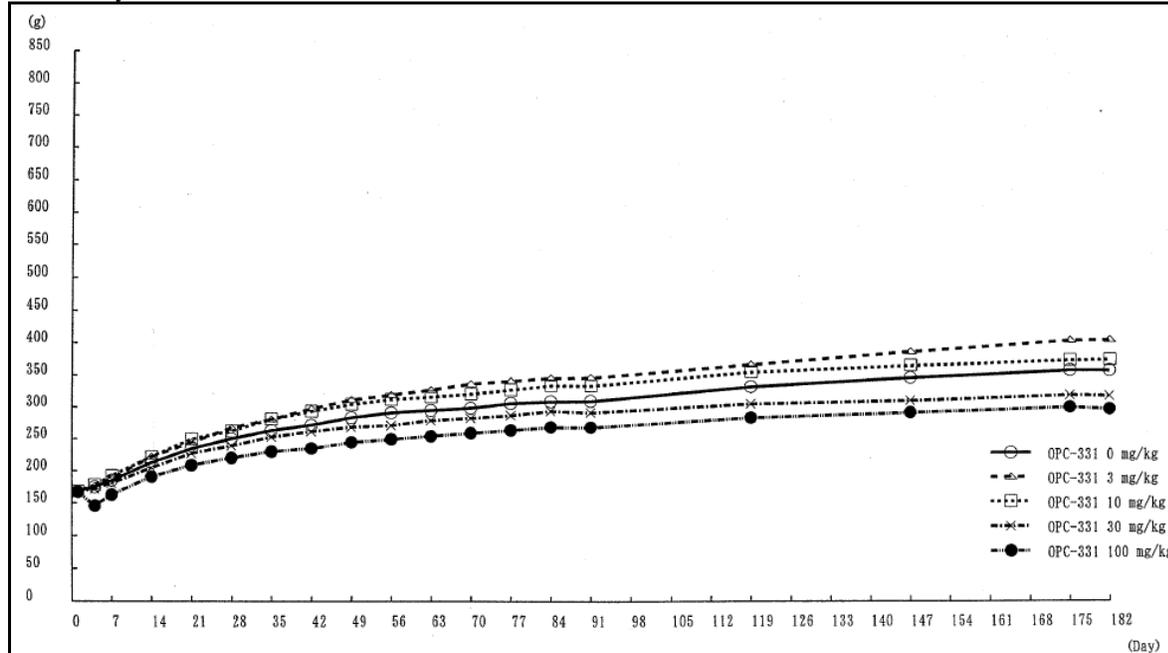


Figure 7: Twenty-six-week repeated oral dose toxicity study of OPC-331 with 13-week recovery test on *BW in Females*:



There was slightly higher BW gain in HDM, but not in HDF during the recovery period as compared to their controls (144 g in HDM vs 79 g in control) indicating some reversibility. However, terminal BW after the recovery period was lower than controls in HDM by 32% and in HDF by 17%.

### Feed Consumption

1-, 3-, or 7-day cumulative food consumption was measured (before dosing) weekly, except 2 times in Week 1.

**Results:** Remarkably lower food consumption was observed in HDM (from Day 4), and HDF (from Days 21-42). Lower food consumption was also observed in males and females at 30 mg/kg, but significantly higher values were noted in LDF.

### Ophthalmoscopy

Examinations were performed; 1) before the start of dosing; 2) in Week 13 (Day 85); 3) in Week 26 (Day 176) and in Week 4 (Day 27) of recovery.

**Results:** No abnormalities were detected at any time point of ophthalmic examination.

### Auditory Function Test – [at pre-dosing, Day 84, Day 175 and Day 26 of recovery]

The auditory functions were normal in all treated animals at any time point of testing.

### ECG

N/A

### Hematology

At the time of scheduled sacrifice (day following the end of dosing and recovery period) animals were subjected to laparotomy under ether anesthesia after fasting overnight and blood samples were collected from the abdominal aorta. The following parameters were evaluated: Hemoglobin (Hb), Hematocrit (Ht), Red Blood Cell Count (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Count (Plat), White Blood Cell Count Differential White Blood Cell Count (WBC), Reticulocyte Ratio (RET), Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT).

Results: At the end of dosing, there were increases in the neutrophil count, monocyte count and large unstained cell count in HDF females at the end of dosing. In addition, a statistically significant increase in red blood cells parameters in males at  $\geq 30$  mg/kg without any dose-dependency was observed. However, those increases were minimal and were considered to be within the range of physiological variation therefore without toxicological significance. These results are summarized in the following Sponsor's table:

Table 28: Summary of hematology (end of administration)

Sex	Males					Females				
	Dose (mg/kg)	0	3	10	30	100	0	3	10	30
No. of animals	15	15	15	15	14	15	15	15	15	14
HGB (g/dL)	15.3	N	N	16.4	16.1	-	N	N	N	N
	-			+7%**	+5%**					
HCT (%)	42.8	N	N	45.4	44.5	-	N	N	N	N
	-			+6%**	+4%*					
MCV (fL)	47.9	N	N	50.2	49.9	-	N	N	N	N
	-			+5%**	+4%**					
MCH (pg)	17.0	N	N	18.2	18.1	-	N	N	N	N
	-			+7%**	+6%**					
MCHC (g/dL)	35.6	N	N	36.1	36.2	-	N	N	N	N
	-			+1%**	+2%**					
PLT (10E4/ $\mu$ L)	110.7	N	N	95.4	96.6	-	N	N	N	N
	-			-14%**	-13%*					
WBC (10E2/ $\mu$ L)	-	N	N	N	N	50.5	N	74.1	71.5	76.5
						-		+47%**	+42%**	+51%**
LYMP (10E2/ $\mu$ L)	-	N	N	N	N	34.1	N	52.5	55.3	52.0
						-		+54%**	+62%**	+52%**
NEUT (10E2/ $\mu$ L)	-	N	N	N	N	13.1	N	N	N	19.6
						-				+50%*
MONO (10E2/ $\mu$ L)	-	N	N	N	1.6	1.7	N	N	N	2.9
					-24%*	-				+71%**
LUC (10E2/ $\mu$ L)	-	N	N	N	N	0.4	N	N	N	0.7
						-				+75%**

N: no significant difference

\*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  (significant difference from the control mean)

At the end of recovery, significantly increased red blood cell count, hemoglobin and hematocrit and significantly decreased white blood cell count accompanying decreased lymphocyte count were noted in HDM. These findings are small, occurring only in males, therefore they are of limited toxicological significance (see the following sponsor's table:

Table 29: Summary of hematology (end of recovery)

Sex	Males		Females	
	0	100	0	100
Dose (mg/kg)				
No. of animals	5	5	5	5
RBC (10E4/ $\mu$ L)	869	908	-	N
	-	+4%*		
HGB (g/dL)	14.9	15.7	-	N
	-	+5%*		
HCT (%)	43.5	45.5	-	N
	-	+5%*		
WBC (10E2/ $\mu$ L)	84.4	64.6	-	N
	-	-23%*		
LYMP (10E2/ $\mu$ L)	60.2	39.9	-	N
	-	-34%**		
NEUT (10E2/ $\mu$ L)	20.1	21.8	-	N
	-	+1%		

### Clinical Chemistry

At the same time as hematological examination; the following parameters were examined: Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total bilirubin (TBI), Alkaline phosphatase (ALP),  $\gamma$ -glutamyl transpeptidase (GTP), Total cholesterol (CHO), Triglyceride (TG), Phospholipid (PL), Glucose (GLU), Total protein (TP), Protein fraction A/G ratio, Blood urea nitrogen (BUN), Creatinine (CRE), CPK, Sodium (Na), Potassium (K), Calcium (Ca), Inorganic phosphorus (P), Chloride (Cl).

Results: Changes in clinical chemistry that were observed in males and females at HDs included decreased triglyceride, glucose and potassium; increased AST, inorganic phosphorus, and gamma-globulin. In addition, increased CPK and ALP and lower albumin were noted in females at HD and MD. There were no toxicologically significant changes observed at the end of the recovery period.

All changes observed at the end of dosing are summarized in the following Sponsor's table.

Table 30: Summary of blood chemistry

Sex	Males					Females				
	Dose (mg/kg)	0	3	10	30	100	0	3	10	30
No. of animals	15	15	15	15	14	15	15	15	15	14
AST (IU/L)	83	N	N	N	135	77	N	N	N	127
	-				+63%*	-				+65%**
CPK (IU/L)	-	N	N	N	N	44	N	54	N	60
	-					-		+23%*		+36%**
ALP (IU/L)	-	N	N	N	N	89	N	121	N	135
	-					-		+36%*		+52%*
$\gamma$ -GTP (IU/L)	1	N	N	2	2	1	N	1	N	N
	-			+100%*	+100%**	-		+0*		
T-CHO (mg/dL)	-	N	N	N	N	97	81	77	74	90
	-					-	-16%*	-21%**	-24%**	-7%
TG (mg/dL)	75	N	N	33	21	57	N	N	28	25
	-			-56%**	-72%**	-			-51%**	-56%**
PL (mg/dL)	106	124	N	N	127	179	146	139	135	155
	-	+17%*			+20%*	-	-18%**	-22%**	-25%**	-13%*
GLU (mg/dL)	148	N	N	125	115	129	N	N	N	105
	-			-16%**	-22%**	-				-19%**
BUN (mg/dL)	12	N	N	14	15	15	N	N	16	N
	-			+17%*	+25%**	-			+7%*	
CRNN (mg/dL)	0.27	N	0.31	0.33	0.35	-	N	N	N	N
	-		+15%*	+22%**	+30%**	-				
K (mmol/L)	4.8	N	N	4.3	4.2	4.1	N	N	N	3.6
	-			-10%**	-13%**	-				-12%**
Ca (mg/dL)	10.3	N	N	N	9.9	10.6	N	N	10.2	N
	-				-4%**	-			-4%*	
P (mg/dL)	5.8	N	N	6.7	6.8	4.9	N	N	5.8	6.1
	-			+16%*	+17%*	-			+18%*	+24%**
TP (g/dL)	-	N	N	N	N	7.3	6.9	6.9	6.9	7.2
	-					-	-5%*	-5%*	-5%*	-1%
ALB (%)	48.5	N	N	N	53.3	61.7	N	55.9	57.5	56.2
	-				+10%**	-		-9%**	-7%*	-9%**
Alpha 1 (%)	22.2	N	N	17.0	15.4	-	N	N	N	N
	-			-23%**	-31%**	-				
Gamma (%)	5.5	N	N	8.6	9.0	6.5	N	N	N	10.0
	-			+56%*	+64%**	-				+54%**
A/G	1.0	N	N	N	1.2	1.6	N	1.3	1.4	1.3
	-				+20%**	-		-19%**	-13%*	-19%**
ALB (g/dL)	3.1	N	N	N	3.4	4.5	4.1	3.9	4.0	4.0
	-				+10%**	-	-9%**	-13%**	-11%**	-11%**
Alpha 1 (g/dL)	1.5	N	N	1.1	1.0	-	N	N	N	N
	-			-27%**	-33%**	-				
Gamma (g/dL)	0.4	N	N	0.6	0.6	0.5	N	N	N	0.7
	-			+50%**	+50%**	-				+40%**

N: no significant difference

\*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  (significant difference from the control mean)

## Urinalysis

Urinalysis was done in Week 12 (Day 82 to 84), and in Week 25 (Day 173 to 174) of dosing and in Week 4 (Days 22 to 23) of recovery. The following parameters were measured: pH, protein, ketones, glucose, occult blood, bilirubin, urobilinogen, color, sediment, urine volume, osmolarity, NA, K, Cl and creatinine.

Results: There were no findings during Week 12 of administration, but decrease in potassium (-34%) and creatinine (-35%) were observed in HDM (but not in HDF) during Week 25 testing.

### Gross Pathology – [all surviving animals at necropsy]

Development of the mammary gland was observed at the end of dosing in 5 females at 10 mg/kg and all females at  $\geq 30$  mg/kg. Dark red focus in the glandular stomach was observed in 3 HDM and 2 HDF. At the end of recovery, development of the mammary gland was observed in all HD. However several other changes were also observed, they were considered to be incidental from the incidence of their occurrences and their pathological properties.

### Organ Weights – [all necropsied animals; organs/tissues list in Appendix 1]

The data were somewhat difficult to evaluate due to BW changes observed in both male and female rats. However, the observed changes in organ weights related to treatment with OPC-331 at the end of dosing are summarized in the following Sponsor's table:

Table 31: Summary of organ weight (end of administration)

Sex	Males					Females				
	0	3	10	30	100	0	3	10	30	100
Dose (mg/kg)										
No. of animals	15	15	15	15	14	15	15	15	15	14
Liver										
Absolute weight (g)	15.94	N	13.14	10.27	9.54	-	N	N	N	N
Relative weight (g/100 g)	2.41	N	2.25	2.14	2.13	2.34	N	N	N	2.91
	-		-7%*	-11%**	-12%**	-				+24%**
Adrenal										
Absolute weight (mg)	60	N	N	N	74	70	N	N	N	81
Relative weight (mg/100 g)	9	N	10	14	16	21	N	N	N	29
	-		+11%*	+56%**	+78%*	-				+38%**
Ovary										
Absolute weight (mg)	-	-	-	-	-	65.2	88.1	85.3	86.5	69.2
Relative weight (mg/100 g)	-	-	-	-	-	19.5	22.8	23.9	28.5	24.8
						-	+17%	+23%	+46%**	+27%*
Uterus										
Absolute weight (mg)	-	-	-	-	-	663	468	427	444	478
Relative weight (mg/100 g)	-	-	-	-	-	195	123	120	147	172
						-	-37%**	-38%**	-25%**	-12%

N: no significant difference; \*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  (significant difference from the control mean)

Otherwise, significant differences were observed in the multiple organs, but they were changes only in the absolute or relative weight or unrelated to dose levels. They included: decreased absolute kidney weights in males (12%, 19%, and 23% at 10, 30, and 100 mg/kg) and increased relative kidney weights (135 and 15% at 30 and 100 mg/kg); increased relative brain weight in males (15%, 41%, and 53% at 10, 30, and 100 mg/kg and in females (13% and 25% at 30 and 100mg/kg) and decreased relative

weight in LDF (10%); decreased absolute weight of the pituitary in HDM (13%) and increased relative weight in males at 30, and 100 mg/kg (20% and 25%, respectively) and HD females (31%), and decrease in relative weight in LDF (24%). All these organ weight changes appeared to be related to changes in BW observed in test article-treated male and female rats.

At the end of recovery phase, the BW remained lower in HDM and HDF rats which were reflected in organ weight changes as shown in the following Sponsor's table:

Table 32: Summary of organ weight (end of recovery)

Sex	Males		Females	
	0	100	0	100
Dose (mg/kg)				
No. of animals	5	5	5	5
<b>Adrenal</b>				
Absolute weight (mg)	55	62	-	N
Relative weight (mg/100 g)	7	11	-	N
	-	+57%**	-	-
<b>Ovary</b>				
Absolute weight (mg)	-	-	47.1	80.9
Relative weight (mg/100 g)	-	-	12.3	24.8
	-	-	-	+102%**
<b>Uterus</b>				
Absolute weight (mg)	-	-	897	569
Relative weight (mg/100 g)	-	-	232	176
	-	-	-	-24%

N: no significant difference

\*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  (significant difference from the control mean)

**Histopathology** – [tissues examined microscopically (controls and HD) are listed in the histopathology inventory table in Appendix 1]

Moreover, examination of H.E.-stained sections revealed yellowish brown pigments in several tissues (possibility of lipofuscin), sections from adrenal, lung, spleen, and thyroid (1 control and 2 HDM), liver and ovary (1 control and 2 HDF), and submandibular lymph node (1 control and 1 HDM) were also examined with periodic acid-Schiff (PAS), Schmorl's, and Berlin staining. The left lateral lobes of the liver and right adrenal cortex from 2 rats/sex/control and HD groups were collected for possible examination by TEM; only the liver was subsequently examined.

Adequate Battery - yes

Peer Review - yes

### Histological Findings

Changes observed in surviving animals at the end of dosing are summarized in the following Sponsor's table:

Table 33: Summary of histopathology (survivors, end of dosing)

Sex	Male					Female				
Dose (mg/kg)	0	3	10	30	100	0	3	10	30	100
No. of animals	15	15	15	15	14	15	15	15	15	14
<b>Pituitary</b>										
Atrophy, intermediate lobe										
minimal	0	0	6	15	14	0	5	13	15	14
<b>Mammary gland, inguinal</b>										
<b>Feminization</b>										
minimal	0	5	9	9	7	0	0	0	0	0
mild	0	1	1	4	7	0	0	0	0	0
<b>Hyperplasia, lobular</b>										
minimal	0	0	0	0	0	2	9	7	1	0
mild	0	0	0	0	0	0	1	8	7	4
moderate	0	0	0	0	0	0	0	0	7	10
<b>Hyperplasia, focal, acinar</b>										
mild	0	0	0	0	0	0	0	0	0	1
<b>Milk secretion</b>										
minimal	0	0	0	0	0	2	2	3	5	5
mild	0	0	0	0	0	0	0	0	2	4
<b>Ovary</b>										
<b>Yellowish brown pigment, macrophage</b>										
minimal	-	-	-	-	-	15	15	15	13	1
mild	-	-	-	-	-	0	0	0	2	13
<b>Hypertrophy, corpus luteum</b>										
minimal	-	-	-	-	-	0	12	11	13	6
mild	-	-	-	-	-	0	0	0	1	4
<b>Uterus</b>										
<b>Atrophy</b>										
minimal	-	-	-	-	-	0	1	8	7	4
mild	-	-	-	-	-	0	0	1	1	0
<b>Mucification, cervical</b>										
minimal	-	-	-	-	-	0	13	9	10	8
<b>Vagina</b>										
<b>Mucification</b>										
minimal	-	-	-	-	-	0	8	8	8	4
mild	-	-	-	-	-	0	5	1	2	4
<b>Stomach</b>										
<b>Erosion, glandular stomach</b>										
minimal	0	0	0	0	3	0	0	0	0	1
mild	0	0	0	0	0	0	0	0	0	1
<b>Testis</b>										
<b>Atrophy, seminiferous tubular</b>										
minimal	0	0	0	1	5	-	-	-	-	-
<b>Prostate</b>										
<b>Atrophy, gland</b>										
minimal	0	0	0	1	4	-	-	-	-	-
mild	0	0	0	0	1	-	-	-	-	-
<b>Seminal vesicle</b>										
<b>Atrophy</b>										
minimal	0	0	0	4	11	-	-	1	-	-
mild	0	0	0	0	1	-	-	-	-	-
<b>Salivary gland, submandibular</b>										
<b>Swelling, acinar cell</b>										
minimal	0	0	0	1	4	0	0	0	1	2

Sex	Male					Female					
	Dose (mg/kg)	0	3	10	30	100	0	3	10	30	100
No. of animals		15	15	15	15	14	15	15	15	15	14
<b>Lung</b>											
Foamy cell, alveolar	minimal	4	3	7	6	7	4	3	4	6	5
	mild	0	0	1	0	4	0	0	0	0	2
Yellowish brown pigment, macrophage	minimal	0	0	0	0	2	0	0	0	0	0
	mild	0	0	0	0	1	0	0	0	0	0
<b>Lymph node, submandibular</b>											
Yellowish brown pigment, macrophage	minimal	0	0	0	0	1	0	0	0	0	0
	mild	0	0	0	0	1	0	0	0	0	0
<b>Lymph node, mesenteric</b>											
Small granuloma	minimal	13	13	13	12	8	12	12	14	14	10
	mild	0	1	0	0	6	1	0	1	0	4
<b>Thyroid</b>											
Yellowish brown pigment, follicular	minimal	0	0	0	0	10	0	0	0	0	11
	mild	0	0	0	0	3	0	0	0	0	3
<b>Spleen</b>											
Yellowish brown pigment, macrophage	minimal	13	12	11	8	4	0	0	0	0	0
	mild	2	3	4	3	8	6	5	6	6	1
	moderate	0	0	0	4	2	9	10	9	9	13
<b>Adrenal</b>											
Hypertrophy, zona fasciculata	minimal	0	0	0	2	3	0	0	0	4	5
	mild	0	0	0	1	5	0	0	0	0	9
Yellowish brown pigment, macrophage	minimal	6	10	10	13	0	8	10	10	12	3
	mild	0	0	0	2	9	0	0	0	0	9
	moderate	0	0	0	0	5	0	0	0	0	2
Yellowish brown pigment, cortical	minimal	0	0	0	3	1	0	0	0	7	0
	mild	0	0	0	0	10	0	0	0	0	9
	moderate	0	0	0	0	3	0	0	0	0	5
<b>Liver</b>											
Necrosis, single cell, hepatocyte	minimal	0	0	0	0	0	0	0	0	0	2
	mild	0	0	0	0	0	0	0	0	0	0
Microgranuloma	minimal	13	14	14	14	13	11	15	14	15	10
	mild	0	0	0	0	0	0	0	1	0	3
Yellowish brown pigment, hepatocyte	minimal	0	0	0	0	0	0	0	0	0	4
	mild	0	0	0	0	0	0	0	0	0	0
Yellowish brown pigment, Kupffer	minimal	0	0	0	0	5	0	0	0	0	9
	mild	0	0	0	0	0	0	0	0	0	0

Discussion on observed histopathological findings:

Histological changes related to treatment with test article were observed in the pituitary (minimal atrophy in the intermediate lobe in male and female rats at all dose levels) and in mammary glands (minimal to mild feminization in males at all doses, increased incidence of lobular hyperplasia in females at  $\geq 3$  mg/kg/d and focal hyperplasia of acinar cells in 1 HDF). In addition, an increased degree of milk secretion in females at  $\geq 30$  mg/kg/d and vaginal mucification at doses  $\geq 3$  mg/kg/d; these changes were considered to be related to the pharmacology of OPC-33; to its ability of blocking dopamine receptors (increased prolactin secretion).

Atrophy of seminiferous tubules, prostate, and seminal vesicles were observed in males at  $\geq 30$  mg/kg/d. These effects on male reproductive tissues may have been a consequence of pharmacologically-mediated effects on prolactin or low food consumption and decreased body temperature.

An increased incidence of deposition of pigment granules was observed in the adrenal gland (cortical cells and macrophages); liver (hepatocytes and Kupffer cells); thyroid (follicle cells); and macrophages in lung, submandibular lymph node, ovary, or spleen of males or females at  $\geq 30$  mg/kg/day. According to the Sponsor, "these pigment granules were considered similar to lipofuscin based on special staining procedures and EM examination. Although the toxicological relevance was not clear, it is possible the accumulation of the pigment was related to aging or due to a localized cytotoxic effect of the test article as a result of incomplete phagocytic removal of intracellular components (eg, lipids, membrane protein, DNA) that normally occurs from the intercellular action of superoxides or free radicals". In addition, alveolar foam cell and small granuloma in lung and mesenteric lymph node were noted in both sexes at HD which may represent a phagocytic response to foreign material. The relevance of this finding to humans is not known.

Histopathological changes observed at the end of administration period showed some reversibility (decreased degree of corpora lutea hypertrophy, decreased incidence of mucification of cervix and vagina) except for the pigment granules depositions in the adrenal, thyroid and ovary still present at the end of recovery as it is shown in the following Sponsor's table:

Table 34: Summary of histopathology (end of recovery)

Sex		Male		Female	
		0	100	0	100
	Dose (mg/kg)				
	No. of animals	5	5	5	5
Mammary gland, inguinal					
	Hyperplasia, lobular				
	minimal	0	0	3	4
	mild	0	0	0	1
	Milk secretion				
	mild	0	0	1	0
Ovary					
	Yellowish brown pigment, macrophage				
	minimal	-	-	5	1
	mild	-	-	0	4
	Hypertrophy, corpus luteum				
	minimal	-	-	0	3
Uterus					
	Mucification, cervical				
	minimal	-	-	0	2
Vagina					
	Mucification				
	minimal	-	-	0	2
Testis					
	Atrophy, seminiferous tubular				
	minimal	0	1	-	-
Seminal vesicle					
	Atrophy				
	mild	0	1	-	-
Lung					
	Foamy cell, alveolar				
	minimal	2	1	0	1
Lymph node, mesenteric					
	Small granuloma				
	minimal	3	3	4	3
	mild	2	2	1	2
Thyroid					
	Yellowish brown pigment, follicular				
	minimal	0	3	0	4
	mild	0	0	0	1
Spleen					
	Yellowish brown pigment, macrophage				
	minimal	2	1	0	0
	mild	2	3	2	1
	moderate	1	1	3	4
Adrenal					
	Yellowish brown pigment, macrophage				
	minimal	5	0	5	0
	mild	0	1	0	2
	moderate	0	4	0	3
	Yellowish brown pigment, cortical				
	minimal	0	3	0	1
	mild	0	2	0	3
	moderate	0	0	0	1
Liver					
	Microgranuloma				
	minimal	5	5	4	5

### Special Evaluation

Yellowish brown pigments suggestive of lipofuscin were observed in H.E.-stained sections of multiple organs, therefore, a special staining (Schmorl, PAS and Berlin blue staining) was performed (control [n=1] and HD [n=2] animals) for the following organs (adrenal, liver, lung, submandibular lymph node, ovary, spleen and thyroid).

Results of this evaluation showed that all yellowish brown pigments observed at HD tested positive in Schmorl reaction and PAS reaction (weekly-positive, partially) and only a part of macrophage (submandibular lymph nodes and spleen) tested positive in Berlin blue stain. However, most yellowish brown pigments (macrophage and cortical cells in the adrenal, hepatocytes and Kupffer cells in the liver, macrophage in the lung, macrophage in the ovary and follicular cells in the thyroid) observed at HD tested negative in Berlin blue stain. These results suggest that yellowish brown pigments observed in HD animals were waste pigments and macrophage in the submandibular lymph nodes and spleen included hemosiderin.

### Electron Microscopic Examination

Portions of the liver (left lateral lobe) and adrenal (right, cortex) were collected from 2/sex/HD and 2/sex/control of the main study and recovery groups and pre-fixed in a mixed solution of phosphate buffered 2.5 vol% glutaraldehyde/2 w/v% paraformaldehyde, post-fixed in 1 w/v% osmium tetroxide solution, and embedded in epoxy resin.

Since yellowish brown pigments were observed in the liver, ultrathin sections were made for 1 control female and 2 HD females and subjected to electron staining by uranyl acetate and lead for observation under a transmission electron microscope.

The electron microscopy showed increased number of secondary lysosomes in central and peripheral hepatocytes in the liver in 1 HD female, and increased number of secondary lysosomes in Kupffer cells in the liver in 2 HD females compared to control. The secondary lysosomes were considered by the sponsor, to correspond to the yellowish brown pigments observed under an optical microscope.

### Toxicokinetics

The days and time points of blood collection were 1, 2, 4, 8 and 24 hrs after dosing on Day 1, and pre dosing, 1, 2, 4, 8 and 24 hrs after dosing on Week 13 (Day 89) and Week 26 (Day 180). OPC-331 and its metabolites (OPC-3952, OPC-34835FRE, OPC-54050, SFO-34318, MOP-54522, DM-3404, DM-3411, DM-3412 and DM-3413) were measured in rat plasma at given time points. Summary of the TK parameters of OPC-331 and its main metabolite DM-3411 is shown in the following Sponsor's table:

Table 35: TK parameters of OPC-331 and its main metabolite DM-3411

		$C_{max}$ (ng/mL)							
		Male				Female			
		Dose (mg/kg/day)				Dose (mg/kg/day)			
Compound	Period	3	10	30	100	3	10	30	100
OPC-331	Day 1	66.61	157.4	830.0	1433	80.36	315.3	1274	2449
	Week 13	107.1	443.0	1696	3425	160.9	532.7	1476	3020
	Week 26	91.28	525.3	1619	2859	188.5	551.3	1457	2651
DM-3411	Day 1	26.76	80.01	295.2	566.6	19.11	99.01	307.2	570.1
	Week 13	26.10	83.48	350.4	580.3	31.97	140.2	303.2	487.4
	Week 26	16.92	75.58	303.8	605.0	24.69	87.40	296.0	491.3
		$AUC_{0-24h}$ (ng·h/mL)							
		Male				Female			
		Dose (mg/kg/day)				Dose (mg/kg/day)			
Compound	Period	3	10	30	100	3	10	30	100
OPC-331	Day 1	387.3	1247	5907	21880	495.4	1682	8632	41810
	Week 13	889.0	2666	20020	45930	1140	2659	16390	38030
	Week 26	590.9	3603	19350	44230	939.5	3285	15670	34420
DM-3411	Day 1	119.3	536.4	1715	6523	126.4	468.8	1730	8623
	Week 13	155.9	544.0	2829	4647	195.1	527.9	2123	3763
	Week 26	103.4	572.3	2407	6830	141.5	440.3	2147	3982

Each value represents the mean of 3 animals; The lower limit of quantification: 1 ng/mL

There was no gender difference in exposure to OPC-33; the exposure increased in dose-related manner in both genders. The  $C_{max}$  and  $AUC_{0-24h}$  of OPC-331 in Week 13 and Week 26 were similar to or slightly higher than those on Day 1 (0.8 to 3.4 times) in all treated groups. Time to reach maximum plasma concentration ( $t_{max}$ ) of all treated groups on Day 1, in Week 13 and Week 26 were independent of dose, sex, and dosing frequency for OPC-331.

Plasma levels of OPC-331 were higher than those of its 9 metabolites for both sexes at all dose groups. Among the 9 metabolites, the plasma levels of DM-3411 were highest, as the  $C_{max}$  and  $AUC_{0-24h}$  were 9.5% to 49.0% of those of OPC-331 in molar ratio. The  $C_{max}$  and  $AUC_{0-24h}$  of other metabolites were 17.8% or less of those of OPC-331.

OPC-331 was not detected in the any plasma of the control group.

### Dosing Solution Analysis

The dosing formulations for all concentrations analyzed during study (Week 1, 13, and 26 of administration) were within 97.7 to 103.8% of the indicated concentration and the coefficient of variation (CV) between 0.3 and 1.0%, both of which were within the acceptable range (100 ± 10% of the indicated concentration, and CV within 10%).

### Summary and evaluation:

In this 26-Week (with 13 weeks recovery) toxicity study in SD rats dosed with OPC-331 at 0, 3, 10, 30, and 100 mg/kg/d, death occurred in 2 HDF (study Day 5 and Day 11). Decreased BW with remarkable decrease in food consumption preceded deaths and necropsy revealed undernourishment as main cause of deterioration of their general condition.

Clinical signs of survivors showed high incidence of incomplete eyelid closure in males and females, and flaccidity and dilatation of the scrotum in males at  $\geq 30$  mg/kg. Clonic convulsions were observed in 3 HDM and 2 HDF; they have developed later during the treatment period (Day 85 for females and Day 135 for males) and were not observed in the previously conducted 13-Week toxicity study with the same dose of 100 mg/kg/day. Hyperreactivity also developed later during dosing (Week 19) and lasted until the end of the treatment in HD animals.

Significant decrease in BW gain and food consumption had started on Day 4 of dosing in HDM (-23%) and HDF (-17%) that continued throughout the treatment phase. At the end of treatment phase, the mean BW was lower than controls by: -34%, -28%, and -13% in males at 100, 30, and 10 mg/kg, respectively and by -17% and -11% in females at 100 and 30 mg/kg, respectively. Similar pattern of changes was also observed in the 13-Week toxicity study with the same doses.

Hematology changes included increased RBC parameters and decreased platelet count in males at  $\geq 30$  mg/kg which were also present at the end of recovery (HD, except decreases in platelets count). Increased WBC, mainly due to an increase in lymphocytes were observed in females at  $\geq 10$  mg/kg, however, WBC decreased in males (HD) at the end of recovery. Clinical chemistry changes included decreased triglyceride, glucose and potassium; increased AST, inorganic phosphorus, and gamma-globulin. In addition, increased CPK and ALP and lower albumin were noted in females at  $\geq 30$  mg/kg. There were no toxicologically significant changes observed at the end of the recovery period.

Histopathological examination of surviving animals revealed hypertrophy of corpus luteum and atrophy and mucification of cervical part of the uterus in females at all doses that were accompanied by increased ovary weights and decreased weights of uteri. Other findings included atrophy in the intermediate lobe of pituitary in males and females at  $\geq 10$  mg/kg, and in females at 3 mg/kg. This finding was considered to be pharmacologically mediated and secondary to the partial  $D_2$  agonistic activity of OPC-331. In addition, an increased incidence of lobular hyperplasia with secretion of milk in the mammary gland and the changes indicating the pseudopregnancy in the reproductive organs including the decreases of uterus weights in the females, and feminization of mammary gland in males of all OPC-331 treated groups, were consequences of drug-related pharmacologically mediated hyperprolactinemia similar to those changes observed with other antipsychotics.

In male reproductive organs, atrophy of testicular seminiferous tubules, atrophy of prostate gland and atrophy in seminal vesicle were observed at  $\geq 30$  mg/kg. The cause of these atrophies to occur is not known, however a nutrient starvation and/or disturbance of hormones including prolactin may be involved. Additionally, the decrease of body temperature might play a role in causing such testicular findings.

It has been demonstrated by the Sponsor that brain and testis toxicities caused by OPC-331 were related to a substantial body temp decrease in individually housed rats (stainless-steel wire-mesh cages without any bedding material [as it was the case in this 26-week toxicity study]) versus rats housed collectively (5 rats per polycarbonate cage with paper pulp bedding) in which no brain lesions and testicular abnormalities were developed (see review of the Study No. 025190 at the end of this review under “Special Toxicology Studies”)

Granules of pigment depositions were observed in many organs of males and females at  $\geq 30$  mg/kg. Depending on the special staining procedures and EM examination, these pigment granules were described by the Sponsor as same material to lipofuscin. Although the toxicological meaning of these findings could not be clarified, the Sponsor has offered the following explanations: “1) that lipofuscin pigment gradually accumulated with aging or 2) due to continuous slight cytotoxic effects of the compound as a result of incomplete phagocytic removal of the intracellular components (e.g. lipids, membrane protein, DNA) degenerated by superoxides or hydroxy radicals”.

Overall, these toxicological findings showed some reversibility (recovery period, HD and controls only), except for the ovary and uterus weights and the pigment granules depositions in the adrenal, thyroid and the ovary.

In conclusion, the main toxicology findings include decreased BW and food consumption in males and females at  $\geq 10$  and  $\geq 30$  mg/kg, respectively; clonic convulsions and low body temp at 100 mg/kg. Except for the pharmacologically mediated hyperprolactinemia-related mammary gland and generative organs or pseudopregnancy-related findings in females, and atrophy of the pituitary pars intermedia in males and females, no OPC-331 treatment-related changes were detected in males and females at 3 and 10 mg/kg, respectively.

The NOAEL was estimated to be 3 mg/kg/day (males) and 10 mg/kg/day (females).

### **Summary of Repeated-Dose Toxicity Studies in Monkeys:**

The study: **Four-week Repeated Oral Dose Toxicity Study of OPC-331 in Cynomolgus Monkeys** (Study no.: 024290, Report no. 019594) was reviewed by this Reviewer under the IND 101,871 (N000/03-22-208) and is summarized below:

Cynomolgus monkeys (3/sex/group) were dosed orally with OPC-331 at 0, 3, 10, and 30 mg/kg/d for 4 weeks. Toxicities, recovery and TKs were assessed. In this 4-week repeat-dose toxicity study in cynomolgus monkeys, NOAEL was not identified. No deaths occurred. Tremors in extremities, decrease in movement, crouching position, partially closed eyes and drowsiness were observed in males and females in all treatment groups. Differences from the control group (Day 1 observations) included decreased blood pressure in both sexes at doses  $\geq 3$  mg/kg/d, and prolonged QTc interval by 65, 84 and 99 ms at 4, 8, and 24 hrs, respectively in 1 HDF, and in 2 MDF (by  $\sim 50$  ms at 2 - 8 hrs post-dosing). On Day 1, decreased body temperature was

observed at all doses in both sexes; on Day 24, body temperature was decreased in males at doses  $\geq 10$  mg/kg/d and in HDF.

The study: **Thirteen-week Repeated Oral Dose Toxicity Study of OPC-331 in Cynomolgus Monkeys with a Four-week Recovery Test** (Study no.: 024962, Report no. 020474) was reviewed by this Reviewer under the IND 101,871 (N000/03-22-208) and is summarized below:

Cynomolgus monkeys (3/sex/LD and MD and 5/sex/control and HD) were dosed orally with OPC-331 at 0, 1, 3, and 30 mg/kg/d for 13 weeks. Toxicities, recovery (4 weeks) and TKs were assessed. Treatment-related clinical signs of slight tremors (limited to extremities), decreased movement, prone, crouching, or lateral position, partially closed eyes, and/or drowsiness were observed in males and females at all doses. All findings except tremors of the extremities were relating to pharmacologically mediated CNS depression, including decreased food consumption, and slight and transient decreases in BW (at 1 mg/kg signs were predominantly observed during first two weeks of treatment). Decreased mean BP at 30 mg/kg/d: M -34%, 22%, 30%, and 16% at 2, 4, 8, and 24 hrs after dosing on Day 1; at  $\geq 3$  mg/kg/d: F - 24% and 26% at 2 and 4 hrs after dosing on Day 1 and 27%, 26% at 4, 8 hrs after dosing during Week 13. Prolonged QTc interval (Day 1) at 30mg/kg/d: in males by 48 ms/69 ms (12%/18%), and by 48ms/68ms (12%/18%); and in females by 41ms/53ms (10%/14%) and by 61ms/50ms (16%/13%) at 4 and 8 hrs after dosing, respectively – as compared to the pretest/control values. Body temperature was decreased in male and female monkeys at HD of 30mg/kg/d. Other findings of toxicological significance such as: oral mucosa hyperemia, decreased lymphocyte count and increased phospholipids in males and decreased WBC count in males and females were noted at 3 and 30 mg/kg/d doses. All changes were reversed during the recovery period, except for tremors which persisted in females dosed at 30 mg/kg/d until day of necropsy.

The NOAEL in monkeys dosed for 13 weeks was 1 mg/kg/d for both sexes.

### **Supplemental study:**

A supplementary 2-week study (*Report No. 020820*) was conducted in Cynomolgus monkeys to evaluate effects at lower doses (0.03 and 0.1 mg/kg/d) because of the slight and transient decreases in food consumption and BW associated with clinical signs (primarily, the CNS depressant effect) observed at 1 mg/kg/d in the 13-week repeat-dose oral dose toxicity study. The dosing period of 2 weeks was selected because clinical signs at 1 mg/kg were observed at a relatively high frequency during early stages of dosing in the 13-week study. No deaths occurred, and no treatment-related abnormalities were noted in clinical observations, BWs, food consumption, hematology, or blood chemistry in both treatment groups (0.03 and 0.1 mg/kg/d). Slight tremors in the hindlimbs occurred during both pretest and treatment periods. The NOEL was considered to be 0.1 mg/kg/d for both sexes. At 0.1 mg/kg/d, plasma  $C_{max}$  and  $AUC_{0-24h}$  at Week 2 were 15.5 ng/ml and 156.2 ng·hr/ml in males and 10.5 ng/ml and 112.7 ng·hr/ml in females, respectively.

## Chronic Toxicity Study in Monkeys

### Study title: Thirty-nine-week Repeated Oral Dose Toxicity Study of OPC-331 in Cynomolgus Monkeys

Study no.: GH08354 (Otsuka study No.: 028626,  
Otsuka Report No.: 024026)

Study report location: EDR

Conducting laboratory and location:

(b) (4)

Date of study initiation: January 8, 2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: OPC-331, lot#: C07F70M, 99.8% purity

## Key Study Findings

OPC-331 was administered by oral gavage to male and female Cynomolgus monkey at 0, 1, 3, and 30, mg/kg/d for 39 weeks. The following treatment-related findings were observed:

- Two males and two females at 30 mg/kg/d and one female at 3 mg/kg/d died or became moribund between Day 8 and Day 61.
- In the surviving animals: decrease in movement in all animals of all dose groups;
- crouching, prone or lateral position in males and females  $\geq 3$  mg/kg/d;
- whole body tremors in all surviving animals with the highest degree of occurrence at 30 mg/kg (2M and 2F);
- Drowsiness, partially closed eyes or eyelid closure in the females  $\geq 3$  mg/kg/d and males at 30 mg/kg/d
- Decreased food consumption in all animals of all treated groups at the early stages of the dosing period which contributed to a severe decrease in BW on Day 8 in animals at doses  $\geq 3$  mg/kg. However, the BW of these animals gradually increased to the level of controls and slightly above.
- Decreased white blood cell and neutrophil counts in all females at 30 mg/kg/d
- Atrophic and stress-related changes, such as atrophy in the stomach, cecum, salivary glands, adrenals and in males and females at 30 mg/kg/d
- The NOAEL - 1 mg/kg/day

**Methods**

Doses: 0, 1, 3, 30 mg/kg  
 Frequency of dosing: Once a day  
 Route of administration: Oral gavage (using gastric catheter)  
 Dose volume: 5 ml/kg  
 Formulation/Vehicle: Suspension in 5 w/v% gum arabic solution  
 Species/Strain: Cynomolgus monkey (b) (4)  
 Number/Sex/Group: 4/sex/group  
 Age: 3 years old  
 Weight: 2.4 – 3.35 kg (M); 2.3 – 3.0 kg (F)  
 Satellite groups: n/a  
 Unique study design: n/a  
 Deviation from study protocol: Additional BW and rectal temp were measured for 5 animals (Animal Nos. 31, 34, 64, 71 and 73) during Days 6-60 for confirmation due to the deteriorated condition and low body surface temp.

**Group assignment:**

Group	No. of animals	Males		Females	
		Animal Nos.	No. of animals	Animal Nos.	No. of animals
Control	4	01-04	4	41-44	
Low	4	11-14	4	51-54	
Mid	4	21-24	4	61-64	
High	4	31-34	4	71-74	

**Observations and Results****Mortality and moribundity**

One HD male (No. 34) was found dead prior to dosing on Day 61. Findings prior to death included: partially closed eyes and drowsiness on Days 1-7, crouching position during Days 2-7, prone or lateral position on Days 4, 60 and 61, decrease in movement almost daily from Day 7, muddy or watery stools almost daily from Day 12, whole body tremors during Days 32-36 and subnormal body surface temperature (rectal temperature 33.3-35.0°C) and dark purple discoloration of the oral cavity on Day 60.

One HD male (No. 31) and 2 HD females (Nos. 71 and 73) were euthanized moribund on Days 8, 13 and 9, respectively. Findings prior to death included: decrease in movement or crouching/prone position and sporadically exhibited partially closed eyes, eyelid closure, drowsiness, lateral position and/or whole body tremors. They were euthanized moribund after exhibiting subnormal body surface temperature (rectal temperature 26.2-32.7°C).

One female (Animal No. 64) of 3 mg/kg group was euthanized moribund on Day 35.

Findings prior to death included: decrease in movement almost daily from Day 1, partially closed eyes on Days 2-4, crouching position occasionally from Day 2 and whole body tremors, prone position and subnormal body surface temperature (rectal temperature 26.4°C and 27.5°C) from Day 30

### **Clinical Signs**

All animals were observed for clinical signs twice daily (prior to dosing and at 6 hours (5-7 hours) post-dosing) during the dosing period and once daily (in the morning) during the other periods. Basically, cage-side observations were conducted for each animal.

Results: Findings at HD of 30 mg/kg/day included: decrease in movement almost daily from Day 1, drowsiness, partially closed eyes or eyelid closure during Days 1-9, crouching, prone or lateral position during Days 1-133 and whole body tremors during Days 2-143 in all surviving animals (2 M and 2 F). A small quantity of vomiting (undigested feed) or muddy stools were observed transiently.

At MD of 3 mg/kg/day, decrease in movement in all the surviving animals (4 M and 3 F) almost daily during Days 1-77, crouching position in all animals during Days 2-6, whole body tremors in 3 M and 2 F during Days 9-134, drowsiness or partially closed eyes in all MDF during Days 1-4 and prone and lateral positions in 1 F during Days 2-3.

At LD of 1 mg/kg/day, decrease in movement was observed in all 4 animals/sex almost daily from Day 2 until Day 14, and observed sporadically thereafter. Meanwhile whole body tremors were observed in 2 M and 1 F sporadically between Days 6 and 11 as well as in 1 LDF also occasionally on Days 52 and 245; these tremors were judged by the Sponsor to be incidental based on their properties and the incidence of their occurrence.

### **Body Weights**

Once weekly (until Day 91, including Day 1), than once every 4 weeks (from Day 92) Body weights (BW) were measured prior to dosing during the dosing period and prior to feeding during the other periods.

Results: Decreased BWs were noted in almost all surviving HDM and HDF on Day 8 (the first day of BW measurement after initiation of dosing). Decreased BW continued in HDM up to Day 29 but was not statistically significant different as compared to the controls. No statistically significant differences from the control group were noted at MD or LD. The patterns of BW changes are shown in the following Sponsor's figures.

Figure 8: Mean BW in males monkeys:

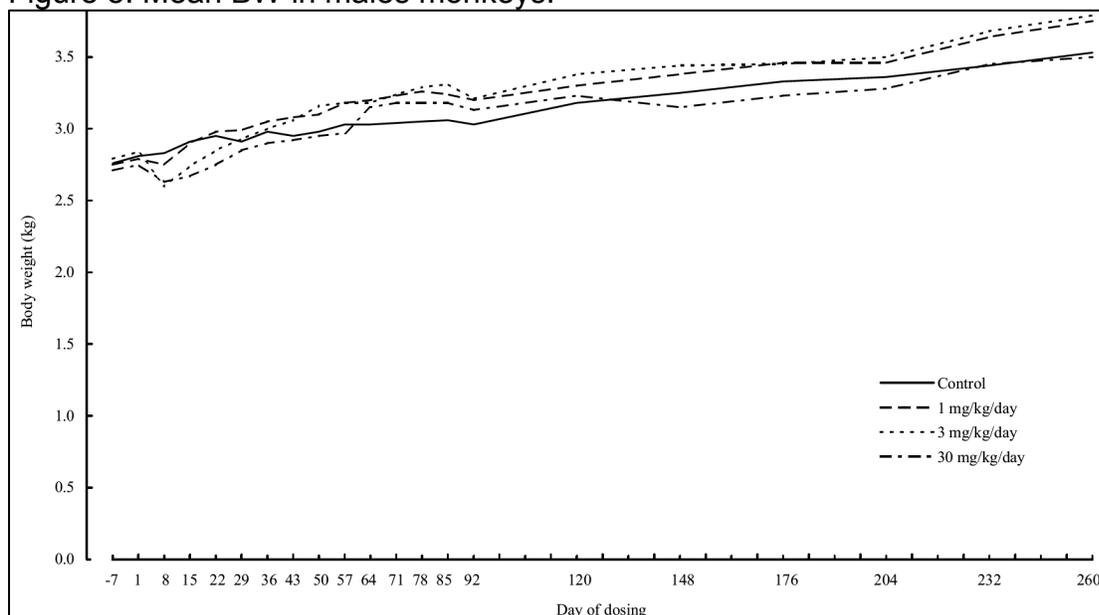
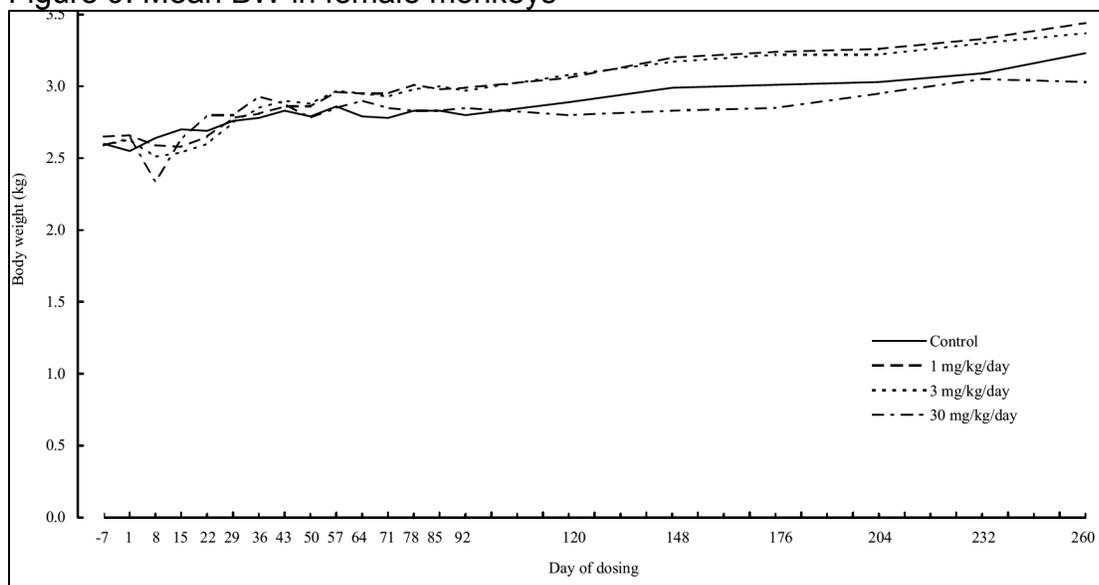


Figure 9: Mean BW in female monkeys



These decreases in BW were considered to be associated with decreased food consumption related to the clinical signs of decreased movements (CNS depression ascribed to the pharmacodynamics of the test article).

**Feed Consumption**

Daily from Day 7 to one day prior to necropsy

Results: Dose-related decrease in food consumption was observed in all treated males and females, excluding one LDF, from the early stage of the dosing period. Statistically significant decreases were sporadically noted in HD males during Days 2-61 and in HD females during Days 2-12 and a tendency to decrease in the mean food consumption

was noted in males during the other period. Especially marked decreases in food consumption were noted continuously in animals that died or were sacrificed moribund; of these animals, 3 at 30 mg/kg left the entire amounts of the provided pelleted feed uneaten for 3-11 days from Day 3. Although these animals were provided with bananas (half or whole per day) as supplementary food for 1-7 days, they left the bananas uneaten or ate only a quarter or half of a banana.

Since the decreased food consumption was observed nearly coincidentally with occurrences of clinical signs caused by CNS depression, such as decrease in movement, it was suggested that there was a relationship between the decreased food consumption and those clinical signs.

### **Ophthalmoscopy**

Evaluation was made in all animals at pretest and in Weeks 13, 26 and 39

Results: No treatment-related ophthalmological findings were noted in any animal during Week 13, 26 or 39.

### **ECG and BP**

Evaluation was made in all animals at pretest and in Weeks 13, 26 and 39 (prior to dosing and at 4 and 8 hrs post-dosing)

Results: No treatment-related ECG abnormalities were noted in any animal during Week 13, 26 or 39. Low values in systolic, diastolic and mean blood pressures were observed in all surviving HD animals and in 1-3 animals/sex/group of LD and MD at 4 or 8 hrs post-dosing, during Weeks 13, 26 or 39. However these values were nearly within the range of the pretest values or within the range of variations.

### **Body Temperature**

Evaluation was made in all animals at pretest and in Weeks 13, 26 and 39 (prior to dosing and at 4 and 8 hrs post-dosing)

Results: No treatment-related changes in body temperature were noted in any of the surviving animal during Week 13, 26 or 39.

### **Auditory Examinations**

Auditory examinations included: immobility, pinna reflex, startle response, head turn toward sound, specific eye movement and were performed in all animals at pretest and in Weeks 13, 26 and 39.

Results:

No abnormalities were evident in the auditory responses in any animal during Week 13, 26, or 39.

### **Hematology**

Blood samples were collected from all unanesthetized animals at pretest (twice) and Weeks 13, 26 and 39. The following parameters were evaluated: Hemoglobin (Hb), Hematocrit (Ht), Red Blood Cell Count (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Reticulocytes (ratio and count), Platelet Count (Plat), White Blood Cell Count,

Differential White Blood Cell Count (WBC), Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT).

Results: Hematological examinations of the moribund animals revealed decreases in WBC, lymphocyte count, eosinophil ratio/count and reticulocyte ratio/count, increases in the RBC and Hb concentration and prolongation of the PT and APTT.

In the surviving animals, decreases in the WBC and neutrophil count were noted in 1 HDF (No. 74) during Weeks 13, 26 and 39 and in another HDF (No. 72) during Week 39. Although other hematological changes were sporadically noted in some animals at all dose levels during Week 13, 26 or 39, they were considered not to be treatment-related since the values were nearly comparable to the control or pretest values and no significant changes were evident at HD.

### **Clinical Chemistry**

Blood samples were collected from all unanesthetized animals at pretest (twice) and Weeks 13, 26 and 39. The following parameters were evaluated: Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total bilirubin (TBI), Alkaline phosphatase (ALP), lactate dehydrogenase (LD), Total cholesterol (CHO), Triglyceride (TG), Phospholipid (PL), Glucose (GLU), Total protein (TP), Protein fraction A/G ratio, Blood urea nitrogen (BUN), Creatinine (CRE), CPK, Na, K, Ca, Inorganic P, and Cl.

Results: Examinations of the moribund animals revealed changes in multiple parameters of clinical chemistry that were related to the cause of death, primarily, the starvation and deterioration of general condition. In the surviving animals, an increase in ALP was noted in 1 HDM (No. 32) during Weeks 26 (+ 31%) and 39 (+ 39%). Although other changes were also sporadically noted in some animals during Week 13, 26 or 39, they were considered not to be treatment-related since the values were nearly comparable to the control or pretest values and they were considered to be incidental since no significant changes were evident at HD.

### **Urinalysis**

Urine was collected at pretest and in Weeks 13, 26 and 39, the following parameters were evaluated: pH, protein, ketones, glucose, occult blood, bilirubin, urobilinogen, color, sediment, urine volume, NA, K, Cl and creatinine.

Results: No treatment-related changes were noted at any dose in Week 13, 26 or 39.

### **Gross Pathology**

GP was performed on all animals at termination of dosing (one day after the last dose)

Results: Gross pathology in the dead or moribund animals revealed a small thymus in all of these animals, dark reddish spots on the mucosa of the body, cardia or pylorus of the stomach in 1 HDM and 2 HDF, dark reddish foci on the mucosa or serosa of the colon or rectum in 1 LDF and 2 HDF, and large adrenals in 1 HDM and 1 female HDF with brownish discoloration of the adrenals and gelatinous marrow in this HDF.

At termination of dosing, discoloration of the liver in 1 HDM and 1 HDF (Nos. 32 and 74), a cyst in the ovary in 1 LDF and 1 MDF (Nos. 53 and 63), small or large amount of

granular substances in the bile in 1 LDM and 1 LDF (Nos. 14 and 52), 1 MDF (No. 62) and 1 HDM and 2 HDF (Nos. 32, 72 and 74) were observed.

### **Organ Weights**

At necropsy, organ weights listed in the histopathology inventory table (Appendix 1) were taken from all animals.

Results: No treatment-related changes were noted in any animal. The absolute and relative weights of the ovaries were increased in 1 LDF and 1 MDF (Nos. 53 and 63) corresponding to a gross pathological lesion of cyst formation in the ovary.

### **Histopathology**

Organs listed in the histopathology inventory table (Appendix 1) were routinely processed and stained with hematoxylin and eosin (HE); in addition the following staining was performed: periodic acid-Schiff (PAS) for specimens of the left kidney of 1 control male (No. 02), 1 MDF (No. 64) and 1 HDM (No. 31); PAS- and Alcian blue-stained specimens were prepared from the cerebrum (area including cortex of the parietal lobe), pons, medulla oblongata and spinal cord (thoracic) and Oil Red O-stained specimens were also prepared from the cerebrum (the same area as above).

Adequate Battery – yes; Peer Review - yes

Results: In the moribund animals, the following atrophic and stress-related lesions associated with the deteriorated condition were noted: atrophic changes in the adipose tissue of the coronary sulcus of the heart, thymus, spleen, lymph nodes, salivary glands, mucosal epithelium of the stomach/digestive tract, pancreatic acinar cells, zona glomerulosa of the adrenals, etc.; hypocellularity and gelatinous marrow in the bone marrow and hypertrophy of the zona fasciculata and decreased lipids in the adrenals; degeneration/necrosis of cardiac muscle fiber, single cell necrosis of hepatocytes, enlargement of the nuclei/nucleoli, increased mitotic hepatocytes and brownish pigmentation in the hepatocytes/Kupffer's cells in the liver; brownish pigmentation, dilatation and apoptotic bodies in the renal tubules of the kidneys; and apoptotic bodies in the bladder epithelium. In all moribund animals, chromatolysis of nerve cells was observed in the cortex of the parietal lobe of the cerebrum. Similar lesions were also observed in the pons and medulla oblongata in 1 HDM and 1 HDF (Nos. 31 and 71) and in the spinal cord in this male. These lesions were negatively stained by Alcian blue and Oil Red O stains.

At termination, atrophic and stress-related changes similar to those in the moribund animals were observed (to a slight degree) in the stomach and cecum of 1 HDM (No. 32), in the salivary glands and adrenals of another HDM (No. 33) and in the spleen of 1 HDF (No. 72). A cyst in the ovaries was also a cyst histopathologically. No histopathological lesions were observed in the gallbladder in which granular materials were noted.

### **Electron Microscopy**

The following organs/tissues were evaluated: Liver (left lateral lobe) and kidney (cortex of the left kidney) from 2/sex/controls and HD animals, and brain (cerebrum cortex of the parietal lobe) from 1 control male and 1 HDM.

Results: No appreciable lesions were noted in the liver or renal cortex in any animal at termination of the dosing period.

### Toxicokinetics

OPC-331 and its metabolites (OPC-3952, OPC-34835FRE, OPC-54050, SFO-34318, MOP-54522, DM-3404, DM-3411, DM-3412 and DM-3413) were measured in all OPC-331-treated animals and only parent was measured in controls. Blood samples were collected according to the following schedule: on Day 1 at 2, 4, 8, and 24 h post-dosing, on Days 91 and 273 prior to dosing and at 2, 4, 8 and 24 h post-dosing

Results: The TK parameters of OPC-331 and its major metabolites (DM-3411 and OPC-3952) are summarized in the following Sponsor's table:

Table 36: TK parameters of OPC-331, DM-3411 and OPC-3952 in monkeys

Compound		C <sub>max</sub> (ng/ml)					
		Male			Female		
		Dose level (mg/kg/day)			Dose level (mg/kg/day)		
		1	3	30	1	3	30
OPC-331	Day 1	138.7	142.1	510.6	102.5	267.0	798.0
	Day 91	145.0	348.4	639.8	145.8	368.4	1271
	Day 273	156.2	206.4	537.3	165.6	306.5	412.9
DM-3411	Day 1	58.06	63.29	261.1	56.85	142.0	405.0
	Day 91	66.03	195.1	492.0	86.31	257.2	685.1
	Day 273	78.11	132.7	320.9	91.55	178.3	330.9
OPC-3952	Day 1	12.40	25.16	90.03	17.62	29.88	92.49
	Day 91	20.93	61.56	540.4	19.88	45.80	567.1
	Day 273	22.76	49.92	381.8	28.22	51.61	248.2
Compound		AUC <sub>0-24h</sub> (ng·h/mL)					
		Male			Female		
		Dose level (mg/kg/day)			Dose level (mg/kg/day)		
		1	3	30	1	3	30
OPC-331	Day 1	1272	1698	9107	831.0	3037	14680
	Day 91	1636	3890	8863	1461	3658	13650
	Day 273	1680	2588	7193	1773	3211	5235
DM-3411	Day 1	445.4	623.2	4260	368.0	1222	6962
	Day 91	544.2	1840	5620	641.4	2062	7682
	Day 273	640.7	1116	3818	751.9	1427	3587
OPC-3952	Day 1	105.0	260.1	1313	117.5	230.5	1446
	Day 91	189.4	494.6	6258	188.0	356.4	5690
	Day 273	193.4	417.8	4135	236.8	424.1	2573

Each value represents the mean of 2 to 4 animals.

The TK parameters (C<sub>max</sub> and AUC) of OPC-331 generally increased dose-dependently. The C<sub>max</sub> and AUC<sub>0-24h</sub> for males and females were generally similar for OPC-331(0.7 to

2.0 times, female/male) and its major metabolites (0.6 to 2.2 times, female/male) during the 39-week administration. OPC-331 was not detected in any plasma of control group. The plasma levels of OPC-331 were higher than those of the 8 metabolites other than OPC-3952 for both sexes in all treated groups, however, those of OPC-3952 were nearly comparable to those of OPC-331 in both sexes at 30 mg/kg on Days 91 and 273 (73% to 148% of those of OPC-331 in molar ratio). Among the metabolites (except OPC-3952 mentioned above) the plasma levels of DM-3411 were the highest ( $C_{max}$  and  $AUC_{0-24h}$ ; 32% to 77% of those of OPC-331 in molar ratio), and  $C_{max}$  and  $AUC_{0-24h}$  of other 7 metabolites were 35% or less of those of OPC-331.

### Dosing Solution Analysis

Verifications of the concentrations were performed at the first preparation and the last preparations during Weeks 13, 26 and 39 of dosing.

Results: The mean measured concentrations were 97.6% to 101.3% of the nominal concentrations, which conformed to the criteria for acceptability.

### Summary

OPC-331 was administered orally to 24 Cynomolgus monkeys (4/sex/group) at doses of 0, 1, 3 and 30 mg/kg/d for 39 weeks to evaluate the potential toxicity of OPC-331. Two HDM and 2 HDF and one MDF died or became moribund between Day 8 and Day 61. These animals exhibited clinical signs of decrease in movement, crouching, prone or lateral position, partially closed eyes, drowsiness, whole body tremors and subnormal body surface temperature. In addition, extremely decreased food consumption was noted continuously prior to death or euthanasia. No severe gross pathological lesions were evident in the dead or moribund animals. Therefore, the deaths or deteriorated condition were considered to be due to poor food consumption caused by depression of the CNS (excessive pharmacodynamic action of OPC-331).

In the surviving animals, the following findings were observed: decrease in movement in all animals of all dose groups; crouching, prone or lateral position and whole body tremors in males and females  $\geq 3$  mg/kg/d; drowsiness, partially closed eyes or eyelid closure in the females at 3 mg/kg/d and males and females at 30 mg/kg/d.

Decreases in food consumption were noted in males and females of all treated groups at the early stages of the dosing period which contributed to a severe decrease in BW on Day 8 in animals at doses  $\geq 3$  mg/kg. However, the BWs of these animals gradually increased to the level of controls and slightly above.

Decreased blood pressure was noted in 1 HDF (4 and 8 hrs post-dosing) and decreases in the white blood cell and neutrophil counts in all females at 30 mg/kg/d during Week 13, 26 or 39.

In the pathological examination, atrophic and stress-related changes, such as atrophy in the stomach, cecum, salivary glands, adrenals and spleen were observed in males and females at 30 mg/kg/d.

The NOAEL was considered to be 1 mg/kg/d under the conditions of this study

Overall summary and evaluation of chronic studies:

Two toxicological studies with OPC-331; 26-Week oral dose toxicity study in rats (doses: 0, 3, 10, 30, and 100 mg/kg/d) and 39-Week oral dose toxicity study in monkeys (doses: 0, 1, 3, and 30 mg/kg/d) confirmed findings previously observed in toxicity studies of shorter duration in both species.

In rats, main toxicology findings include decreased BW and food consumption, in males and females at  $\geq 10$  and  $\geq 30$  mg/kg/d, respectively; convulsions and low body temp at 100 mg/kg/d. Convulsions were observed in 3 males and 2 females; they have developed later during the treatment period (Day 85 for females and Day 135 for males) and were not observed in the previously conducted 13-Week toxicity study with the same dose of 100 mg/kg/d. Lobular hyperplasia with secretion of milk in the mammary gland and changes in reproductive tissues related to pseudopregnancy were observed in females at all dose levels and feminization of the mammary glands in males at doses  $\geq 30$  mg/kg. Histopathological findings in male and female reproductive organs were attributed to pharmacologically mediated (D2 antagonism) increases in serum prolactin (not measured in this study). The mechanism of prolactin-mediated luteal function in rodents is well established and considered by many to be rodent-specific with uncertain toxicological significance.

Except for the pharmacologically mediated hyperprolactinemia-related mammary gland and generative organs or pseudopregnancy-related findings in females, and atrophy of the pituitary pars intermedia in males and females, no OPC-331-related changes were detected in males and females at 3 and 10 mg/kg/d, respectively. The NOAEL was at 3 mg/kg/d (males) and 10 mg/kg/d (females).

In the monkey study, two HDM and 2 HDF and one MDF died or became moribund between Day 8 and Day 61. These animals exhibited clinical signs of decrease in movement, crouching, prone or lateral position, partially closed eyes, drowsiness, whole body tremors and subnormal body surface temperature. These clinical signs were also observed in the surviving animals at doses  $\geq 3$  mg/kg and were considered to be related to pharmacologically mediated CNS depression, which also included decreased food consumption, and transient decreases in BW as well as decrease in body temperature.

Pathological examination revealed atrophic and stress-related changes, such as atrophy in the stomach, cecum, salivary glands, adrenals and spleen at 30 mg/kg/d.

Based on the clinical findings, the NOAEL was at 1 mg/kg/day.

## 7 Genetic Toxicology

Table 37: Summary of definitive genotoxicity studies which were reviewed below

Study	Species/Strain (Number/Sex/ Group)	Route/ Duration	Dose (mg/kg or mg/kg/day) or Concentration	Key Results	Report Number
Bacterial Reverse Mutation Assay	<i>S typhimurium</i> (TA98, TA100, TA102, TA1535, TA1537)	In vitro/ 48 hours <sup>a</sup>	0 (DMSO), 5-500 µg/plate ±S9 <sup>b</sup>	Negative	018913
Mammalian Cell Forward Mutation Assay	Mouse lymphoma L5178Y <i>Tk</i> <sup>+/-</sup> cells (2 cultures)	In vitro/ 3 hours ±S9, 24 hours -S9	3 hours: 0 (DMSO), 5, 10, 20, 50, 100, 200 µmol/L ±S9 (3 hours: 0 [DMSO], 10, 15, 20, 30, 50, 70, 100 µmol/L +S9, as an additional assay) 24 hours: 0 (DMSO), 1, 2, 5, 10, 20, 50, 100 µmol/L -S9	Positive (3 hours, +S9 at cytotoxic doses of 50 and 70 µmol/L)	018869
Chromosome Aberration Assay	CHO-K1 cells	In vitro/ 3 hours ±S9, 20 hours -S9	3 hours: 0 (DMSO), 30, 50, 70, 100 µmol/L +S9 0 (DMSO), 20, 30, 50, 70 µmol/L -S9 20 hours: 0 (DMSO), 5, 10, 20, 30 µmol/L -S9	Positive (3 hours, -S9 at a cytotoxic dose of 50 µmol/L)	019460
Bone Marrow Micronucleus Assay	Rat/Sprague Dawley (5/sex/group)	Oral (gavage)/ 2 days	M 0 (vehicle), 300, 1000, 2000 F 0 (vehicle), 30, 100, 300, 1000	Negative	019196
Unscheduled DNA Synthesis in Hepatocytes including TK <sup>c</sup>	Rat/Sprague Dawley (3/sex/group)	Oral (gavage)/ 1 day	M 0 (vehicle), 300, 1000, 2000 F 0 (vehicle), 30, 100, 300	Negative	019960

a) plate-incorporation method; b) the range shows the lowest and the highest doses where the number of revertant colonies were evaluated through all assays included in the study; c) the liver was perfused in all surviving animals in the negative and positive control groups and in the 2 highest brexpiprazole-dosed groups with a 100% survival rate (males at 1000 or 2000 mg/kg and females at 100 or 300 mg/kg).

## 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

### Study title: OPC-34712: Reverse Mutation Test in *Salmonella typhimurium* Using Male Rat Liver S9 by plate Incorporation Method

Study no.: 024424 (Report no. 018913)  
 Study report location: EDR  
 Conducting laboratory and location: Tokushima Research Institute Otsuka  
 Pharmaceutical Co., Ltd. 463-10  
 Kagasuno, Kawauchi-cho, Tokushima-shi  
 Tokushima, JAPAN  
 Date of study initiation: June 16, 2006  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: OPC-34712, lot# 05J72M, purity 99.6%

### Key Study Findings

OPC-34712 was not mutagenic in the bacterial reverse mutation test at concentrations up to 200 µg/plate

### Methods

Strains: Five strains of *Salmonella typhimurium* (TA100, TA1535, TA102, TA98, and TA1537)  
 Concentrations in definitive study: 500, 200, 100, 50, 20, 10 and 5 µg/plate  
 Basis of concentration selection: Growth inhibition effect at 500 µg/plate in every strain (with and without S9) in dose range finding study.  
 Negative control: DMSO, the solvent of the test article  
 Positive control:

Strains	With or without S9	Positive control article	Dose (µg/plate)
TA100	Without	AF-2	0.0100
	With	2AA	0.500
TA1535	Without	NaN <sub>3</sub>	0.100
	With	2AA	0.500
TA102	Without	MMC	0.500
	With	2AA	5.00
TA98	Without	AF-2	0.0500
	With	2AA	0.200
TA1537	Without	ACR	50.0
	With	2AA	2.00

Formulation/Vehicle: Solution in DMSO  
 Incubation & sampling time: Plate incorporation method, 48-hour incubation (+/- S9)

### Study Validity

Precipitation appeared at 200 and 500 µg/plate in all strains, and the precipitates at 500 µg/plate interfered with the colony count in all strains. The growth inhibition of background lawn was observed at 200 and 500 µg/plate in TA100, TA1535 and

TA1537, and at 500 µg/plate in TA98 and TA102. The negative and positive controls showed the appropriate values of the revertant colonies. Four and more treated dose groups of the test article could be analyzed without growth inhibition. Therefore, each treatment was considered valid.

**Results**

There was ~45% increase in mean revertant colonies in TA100 with metabolic activation (that was statistically significant different than control) which did not meet criteria for the positive results. A mean number of revertant colonies at any dose of OPC-34712 was lower than twice that of the negative control group and was not observed in dose-related manner, therefore the result of this test was considered to be negative.

The following Sponsor’s table shows the results of reverse mutation test in *Salmonella typhimurium*; TA100 with metabolic activation (+S9)

Table 38: Summary of the results of reverse mutation test in *Salmonella typhimurium*; TA100 with metabolic activation (+S9)

With Activation	DMSO	-	112+13	\$\$	
			$\frac{3}{3}$		
	OPC-34712	5.00		114+11	
				$\frac{3}{3}$	
		10.0		129+8	
				$\frac{3}{3}$	
		20.0		145+13	
				$\frac{3}{3}$	
		50.0		162+9	*
				$\frac{3}{3}$	
100		158+13	*		
		$\frac{3}{3}$			
		200	TL	115+9	
				$\frac{3}{3}$	
		500	P, TL	$\frac{\pm}{0}$	
<hr/>					
	2AA	0.500	417+30		
			$\frac{3}{3}$		

2AA: positive control; p: precipitation TL: Thin lawn \*; p<0.05, \*\*; p<0.01 : Significant difference from the control (one tailed) \$; p<0.05, \$\$; p<0.01 : Significant dose dependency (one tailed)

In conclusion, none of the treated doses of the test article showed two-fold or more increase in the revertant colonies compare to that of the negative control in any strains with and without S9. The negative results were obtained in the dose-range finding and main experiments, therefore OPC-34712 was judged to be non-mutagenic (negative) in these test systems.

## 7.2 *In Vitro* Assays in Mammalian Cells

### 7.2.1 Study title: OPC-34712: Forward Mutation Test at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells Using Male Rat Liver S9 by 96-well-plate Fluctuation Method

Study no.: 024414; Report No.: 018869  
 Study report location: EDR  
 Conducting laboratory and location: Tokushima Research Institute  
 Otsuka Pharmaceutical Co., Ltd.  
 463-10 Kagasuno, Kawauchi-cho,  
 Tokushima-shi, Tokushima, JAPAN  
 Date of study initiation: June 13, 2006  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: OPC-34712, lot# 05J72M, purity 99.6%

### Key Study Findings

OPC-34712 was mutagenic at 50 and 70  $\mu\text{M}$  (3 h exposure) with metabolic activation

### Methods

Cell line: L5178Y *Tk*<sup>+/-</sup> Mouse Lymphoma Cells  
 Concentrations in definitive study: 0, 5, 10, 20, 50, 70, 100, 200  $\mu\text{M}$

Basis of concentration selection:

OPC-34712 Dose ( $\mu\text{mol/L}$ )	Relative suspension growth		
	3 h; +S9	3 h; -S9	24 h; -S9
0 a	1.000	1.000	1.000
0.10	0.8935	0.6705	1.0861
1.0	0.8435	0.7725	0.9959
10	0.8316	1.2471	0.6407
100	0.1037	0.0928	0.0166 b
1000	c,d	c,d	c,d
10000	c,d	c,d	c,d

a: 10 ml/l DMSO; b: toxicity (significant decrease in RSG one day after exposure); c: precipitates were observed by naked eye in the medium at the end of exposure; d: heavy precipitates (microscopic evaluation)

Negative control: DMSO, the solvent of the test article

Positive control: N-Nitrosodimethylamine (DMN) – with metabolic activation;  
 Methylmethanesulfonate (MMS) –without metabolic activation

Formulation/Vehicle: Solution/DMSO

Incubation & sampling time:	Treatment	Doses of OPC-34712 ( $\mu\text{mol/L}$ )
3-hour exposure; in the absence of S9		0, 5.0, 10, 20, 50, 100, 200
		Doses of MMS ( $\mu\text{L/L}$ ): 10, 20
3-hour exposure; in the presence of S9 Experiment 1		0, 5.0, 10, 20, 50, 100, 200
		Doses of DMN ( $\mu\text{L/L}$ ): 100, 300
24-hour exposure; in the absence of S9		0, 1.0, 2.0, 5.0, 10, 20, 50, 100
		Doses of MMS ( $\mu\text{L/L}$ ): 5.0, 10
3-hour exposure; in the presence of S9 Experiment 2		0, 10, 15, 20, 30, 50, 70, 100
		Doses of DMN ( $\mu\text{L/L}$ ): 100, 300

### Study Validity

**Study validity:** When all data met the following criteria, this experiment was judged to be valid: 1) Survival in the negative control group of  $1 \pm 0.4$ . 2) Viability in the negative control group of  $1 \pm 0.3$ . 3) Total mutation frequency in the negative control group ranging from  $1 \times 10^{-5}$  to  $25 \times 10^{-5}$ . 4) Total mutation frequency in the positive control group of  $50 \times 10^{-5}$  or higher. 5) Total mutation frequencies in the test article groups were observed at four or more dose levels.

#### Criteria for positive results:

The test article was considered to be mutagenic under the present test conditions, if either a positive response was obtained, or one uncertain response and one positive response were obtained.

### Results

Each experiment by each exposure method was judged valid for negative control, positive control, and test article groups. In addition, MMS and DMN showed typical mutation induction as positive controls  $\pm$ S9, respectively.

Table 39: Summary of cell growth (relative survival and total growth) and total mutation frequency after 3h exposure in the presence of S9 in two experiments (Sponsor's tables)

Experiment 1:

Compound	Dose ( $\mu\text{mol/L}$ )	RS	RTG	MF
OPC-34712	0 a	1.00	1.00	13 <sup>###</sup>
	5.0	0.95	0.96	15
	10	0.82	1.10	10
	20	0.84	0.84	21
	50	0.63	0.55	23* (10) <sup>+</sup>
	100	0 <sup>b</sup>	not plated <sup>c</sup>	not plated <sup>c</sup>
	200	0 <sup>b</sup>	not plated <sup>c</sup>	not plated <sup>c</sup>
DMN	100	1.04	0.48	117 <sup>\$@</sup> (104) <sup>+</sup>
( $\mu\text{L/L}$ )	300	0.80	0.27	172 <sup>\$@</sup> (159) <sup>+</sup>

## Experiment 2:

Compound	Dose ( $\mu\text{mol/L}$ )	RS	RTG	MF
OPC-34712	0 a	1.00	1.00	12 <sup>###</sup>
	10	0.93	1.38	13
	15	0.95	1.35	17
	20	0.81	1.44	14
	30	0.79	1.24	15
	50	0.55	0.55	23* (11) <sup>+</sup>
	70	0.23	0.20	30* <sup>\$</sup> (18) <sup>+</sup>
	100	0.01 <sup>b</sup>	not plated <sup>c</sup>	not plated <sup>c</sup>
DMN	100	0.86	0.83	115 <sup>\$@</sup> (103) <sup>+</sup>
( $\mu\text{L/L}$ )	300	0.61	0.57	207 <sup>\$@</sup> (195) <sup>+</sup>

RS: relative survival; RTG: relative total growth; MF: total mutation frequency per  $10^5$  survivors. The parenthesized number means the induced mutation frequency (IMF).

a: 5.0 mL/L DMSO; b: extremely toxic as  $< 0.1$ .; c: extremely toxic as  $< 0.05$  in relative suspension growth; DMN: N-nitrosodimethylamine; ###: Linear regression test in mutation frequency ( $p < 0.001$ ; one-tailed); \*: Dunnett's test in mutation frequency ( $p < 0.05$ ; one-tailed); \$: outlier from the upper limit of the historical negative control in the mutation frequency ( $\geq 30$  per  $10^5$  survivors).

+: Biological significance in the induced mutation frequency ( $\geq 10$  per  $10^5$  survivors).

@: Biologically significant increase in the mutation frequency ( $\geq 50$  per  $10^5$  survivors).

Brexpiprazole was genotoxic in the forward mutation test in mouse lymphoma cells at 50 and 70  $\mu\text{M}$  after 3-h exposure with metabolic activation (S9). However, it was not genotoxic after 3-h exposure up to 50  $\mu\text{M}$  without metabolic activation or after 24-h exposure up to 20  $\mu\text{M}$  without metabolic activation. Induced mutation frequency was too low to classify the type of mutation as large or small colonies

Conclusion: OPC-34712 –induced weakly positive response by metabolic activation method (with male liver S9), therefore was judged to be mutagenic in mouse lymphoma L5178Y  $Tk^{+/-}$  cells.

### 7.2.2 Study title: **OPC-34712: Chromosome Aberration Test in Cultured Chinese Hamster Ovary (CHO) Cells**

Study no.: 024725; Report No.: 019460  
 Study report location: EDR  
 Conducting laboratory and location: Tokushima Research Institute  
 Otsuka Pharmaceutical Co., Ltd.  
 463-10 Kagasuno, Kawauchi-cho,  
 Tokushima-shi, Tokushima, JAPAN  
 Date of study initiation: October 12, 2006  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: OPC-34712, lot# 05J72M, purity 99.6%

### Key Study Findings

OPC-34712 induced chromosome aberrations in cultured CHO cells following 3-h treatment with 0.05 mM in the absence of S9.

### Methods

Cell line: Chinese hamster ovary (CHO) cells  
 Concentrations in definitive study: OPC-34712 at 0, 0.003, 0.005, 0.007, 0.010, 0.015, 0.02, 0.03, 0.05, 0.07, and 0.1 mM  
 Basis of concentration selection: Reduction in relative cell count (CC, % of control) and relative mitotic index (MI, % of control); the highest dose chosen was one at which a 50% to 80% reduction in CC occurred. However, a reduction in MI at the highest dose chosen was not to exceed 80%  
 Negative control: DMSO, the solvent of the test article  
 Positive control: Mitomycin C (MMC) – without metabolic activation and Cyclophosphamide (CP) – with metabolic activation  
 Formulation/Vehicle: Solution/DMSO  
 Incubation & sampling time: OPC-34712 was tested in duplicate cultures in three independent experiments as shown in the following Sponsor's table:

Treatment	Dose (mmol/L)	Number of cultures				
		Experiment 1		Experiment 2	Experiment 3	
		-S9 3+17h	+S9 3+17h	-S9 20h	-S9 3+17h	+S9 3+17h
OPC-34712	0	4	4	4	4	4
	0.003	—	—	2	—	—
	0.005	—	—	2	—	—
	0.007	—	—	2	—	—
	0.010	2	2	2	—	—
	0.015	2	2	2	2	—
	0.02	2	2	2	2	2
	0.03	2	2	2	2	2
	0.05	2	2	2	2	2
	0.07	2	2	—	2	2
	0.10	2	2	—	—	2
MMC	0.05 µg/mL	—	—	2	—	—
	0.1 µg/mL	2	—	2	2	—
	0.2 µg/mL	2	—	—	2	—
CP	5.0 µg/mL	—	2	—	—	2
	10 µg/mL	—	2	—	—	2

### Study Validity

The study was considered valid based on the following criteria: 1) the number of analyzed cells was not less than 200 in the negative control group, and the frequency of cells with structural and numerical aberrations in the negative control group fell within the normal range, and 2) the number of analyzed cells was not less than 50 in the positive control group and the positive control article induced statistically significant increases (one-tailed Fisher-Irwin test,  $p < 0.05$ ) in the number of cells with structural aberrations. The frequency of cells with structural aberrations exceeded the mean value + 3SD (SD: standard deviation) seen in the historical negative control data.

### Results

The frequency of cells with structural aberrations exceeded the mean value + 3SD seen in the historical negative control data. Thus, the validity of cytogenetic examination in each treatment was demonstrated.

In the 3h treatment in the absence of S9 (two experiments), there was a statistically significant increase in structural aberrations frequency at the highest dose analyzed (0.05 mM), and the frequency of cells with aberrations exceeded the normal range as shown in the following Sponsor's table:

Table 40: The frequency of cells with structural aberrations (SA, %) without metabolic activation (-S9)

Dose (mmol/L)	-S9, 3+17h			-S9, 3+17h (Repeat assay)		
	CC	MI	SA	CC	MI	SA
0	—	—	1.3%	—	—	1.5%
0.02	84%	105%	1.0%	76%	89%	1.0%
0.03	72%	92%	1.0%	70%	73%	1.0%
0.05	42%	48%	10.5%	48%	40%	10.0%
0.07	21%	5%		23%	3%	

In the 3 h treatment in the presence of S9, there were no statistically significant increases in the structural aberrations frequencies at any dose. However, at the highest dose, 0.07 mM, the frequency of cells with aberrations exceeded the normal range in the first assay and this increase was not reproduced as shown in the following table:

Table 41: The frequency of cells with structural aberrations (SA, %) in the presence of metabolic activation (+S9)

Dose (mmol/L)	+S9, 3+17h			+S9, 3+17h (Repeat assay)		
	CC	MI	SA	CC	MI	SA
0	—	—	4.0%	—	—	3.8%
0.03	111%	102%	2.0%	127%	113%	2.0%
0.05	106%	90%	3.5%	118%	108%	2.0%
0.07	84%	73%	8.5%	92%	84%	2.0%
0.10	47%	1%		53%	1%	

In the 20 h treatment in the absence of S9, there was no statistically significant increase in the SA frequencies at any dose as shown in the following table:

Table 42: The frequency of cells with structural aberrations (SA, %) in the 20 h treatment without metabolic activation

Dose (mmol/L)	-S9, 20h		
	CC	MI	SA
0	—	—	0.8%
0.005	90%	103%	2.5%
0.01	68%	54%	1.5%
0.02	51%	37%	3.0%
0.03	38%	11%	

Conclusion: OPC-34712 induced chromosome aberrations in cultured CHO cells following 3-h treatment with 0.05 mM in the absence of S9. In the presence of S9, the

frequency of cells with aberrations exceeded the normal range (3-h treatment) only in one assay but failed to be reproduced in the second one. After a 20-h treatment up to 20  $\mu$ M in the absence of metabolic activation, OPC-34712 was not genotoxic in CHO cells. Positive responses were generally slight and occurred at the doses of moderate cytotoxicity. Therefore, the result of this study is considered to be inconclusive.

### 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

#### Study title: OPC-34712: Micronucleus test in rat bone marrow erythrocytes after oral administration for 2 consecutive days.

Study no: 024375; Report No.: 019196  
 Study report location: EDR  
 Conducting laboratory and location: Tokushima Research Institute  
 Otsuka Pharmaceutical Co., Ltd.  
 463-10 Kagasuno, Kawauchi-cho,  
 Tokushima-shi, Tokushima, JAPAN  
 Date of study initiation: August 2, 2006  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: OPC-34712, lot# 05J72M, purity 99.6%

#### Key Study Findings

OPC-34712 was not genotoxic in this clastogenicity assay following 2 days of oral doses up to 2000 mg/kg/d in male rats and up to 300 mg/kg/d in female rats

#### Methods

Doses in definitive study: Males: 0, 300, 1000, 2000 mg/kg  
 Females: 0, 30, 100, 300, 1000 mg/kg  
 Frequency of dosing: Once a day for 2 days  
 Route of administration: Oral gavage  
 Dose volume: 10 ml/kg  
 Formulation/Vehicle: Solution/5% gum arabic  
 Species/Strain: Rats/Crl:CD(SD)  
 Number/Sex/Group: 5/sex/group  
 Satellite groups: None  
 Basis of dose selection: In a single and 1-Week repeated oral dose toxicity studies in rats, OPC-34712 did not cause any death in males at doses up to 2000 mg/kg. Death in females at  $\geq$ 1000mg/kg.  
 Negative control: 5% gum-arabic  
 Positive control: Mitomycin C (MMC) single dose of 2 mg/kg IV

#### Study Validity

The study was considered valid if the following criteria were met: 1) the MNPCE frequency and the PCE ratio in the negative control article group were within the

following ranges specified by the in-house data for the negative control. The mean and standard deviation in the historical negative control of male and female rats were  $2.2‰ \pm 1.2‰$  and  $2.1‰ \pm 1.1‰$  in MNPCE frequency, and  $59\% \pm 9\%$  and  $61\% \pm 9\%$  in PCE ratio, respectively, collected from 142 males and 142 females. MNPCE frequency:  $\leq$  Mean + 2 standard deviations ( $\leq 4.6‰$  for males,  $\leq 4.3‰$  for females) and PCE ratio: Mean  $\pm$  3 standard deviations (32% to 86% for males, 34% to 88% for females); 2) the MNPCE frequency in the positive control group was significantly higher than that in the negative control group.

## Results

One female at 1000 mg/kg was found dead on Day 3 (day after the second administration), therefore this group was excluded from the MNPCE analysis. Hypoactivity in all animals and flaccidity and dilatation of scrotum in all males were observed in the OPC-34712 treated groups. Hypothermia was noted in males and females at all doses except females at 30 mg/kg. Body weight (BW) after dosing gradually decreased from the pre-dosing value in males and females at  $\geq 300$ -mg/kg, and statistically significant and dose-dependent decrease in the mean BW (compare to the negative control) was observed on study Day 3 (day after the second dosing).

The MNPCE frequency and PCE ratio in the concurrent vehicle control group were within the ranges specified by the in-house historical data for the negative control, and MMC was confirmed to induce micronuclei. The mean of PCE ratio in the test article groups indicated no statistically significant decreases when compared to the negative control article group. The MNPCE frequency in the test article groups showed no statistically significant increases compared with the concurrent negative control group.

In the micronucleus test, exposure (based on  $C_{max}$  and  $AUC_{0-24h}$ ) of OPC-34712 on Day 2 at the highest dose, 2000 mg/kg/d in males and 300 mg/kg/d in females, was 6097 and 3259 ng/ml and 86900 and 59410 ng•h/ml, respectively.

**In conclusion**, OPC-34712 did not induce micronuclei in bone marrow polychromatic erythrocytes in Sprague-Dawley rats under the experimental conditions of 2 days of oral dosing up to 2000 mg/kg/d in male rats and up to 300 mg/kg/d in female rats.

## 7.4 Other Genetic Toxicity Studies

### Study title: In vivo/in vitro Unscheduled DNA Synthesis (UDS) Test of OPC-34712 with Rat Hepatocytes

Study no: 024714; Report No.: 019960  
 Study report location: FDR  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: August 2, 2006  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: OPC-34712, lot# 05J72M, purity 99.6%

### Key Study Findings

OPC-34712 was not genotoxic in the unscheduled DNA synthesis test in male rats at oral dose up to 2000 mg/kg and in female rats up to 300 mg/kg

### Methods

Doses in definitive study: Males: 0, 300, 1000, 2000 mg/kg  
 Females: 0, 30, 100, 300 mg/kg  
 Frequency of dosing: Once a day for 2 days  
 Route of administration: Oral gavage  
 Dose volume: 10 ml/kg  
 Formulation/Vehicle: Solution/5% gum arabic  
 Species/Strain: Rats/Crl:CD(SD)  
 Number/Sex/Group: 4/sex/group; evaluation: 3/sex/group  
 Satellite groups: None  
 Basis of dose selection: The same as for/from the micronucleus test.  
 The mortality was observed in females at 1000 mg/kg, a dose of 300 mg/kg was selected as high dose for females (2000 mg/kg for males).  
 Negative control: 5% gum-arabic  
 Positive control: DMN (dimethylnitrosamine) at 10 mg/kg for the 2 h post-treatment group and 2-AAF (2-acetylaminofluorene) at 50 mg/kg for the 16 h post-treatment group (known to induce UDS).

Sampling time hepatocytes: Hepatocytes were isolated from the livers of the treated animals at 2 and 16 h after the administration according to the collagenase perfusion method and incubated under the presence of <sup>3</sup>H-thymidine. The incidence of UDS was detected by autoradiography. Since no deaths occurred, 1000 and 2000 mg/kg for males, 100 and 300 mg/kg for females were selected for UDS evaluated groups and 3 animals in descending order of cell viability in each group were subjected to the evaluation in the group.

## Study Validity

The study was considered valid if: (1) the cell viabilities of all animals in the negative control groups was 50% or more; (2) the individual data of net grain in the negative control groups was in the range of the historical control data (Mean $\pm$ 3SD) in the testing facility; (3) the positive control groups indicated clear positive responses.

The test substance was judged positive when the mean number of net grains in a group was  $\geq 5$  and the mean percentage of cells in repair was  $\geq 20\%$ .

## Results

The study was considered valid for the following reasons: the mean numbers of net grains were less than 5 and the mean percentages of cells in repair were less than 20% in the negative control groups. These values were within the range of the historical negative control data in the testing facility. In contrast, the positive controls increased the number of net grains and the percentage of cells in repair.

The results of BW assessment, clinical signs and TK analysis, indicate that rats were exposed to the test article. Since no deaths occurred, 1000 and 2000 mg/kg for males, 100 and 300 mg/kg for females were selected for UDS evaluation.

In the OPC-34712-treated groups:

- The mean numbers of net grains were less than 5 (Male: 1000 and 2000 mg/kg for the 2 h post-treatment were  $-0.67 \pm 0.15$  and  $-0.50 \pm 0.17$ , for the 16 h post-treatment were  $-0.58 \pm 0.26$  and  $-0.24 \pm 0.45$ , respectively. Female: 100 and 300 mg/kg for the 2 h post-treatment were  $-0.41 \pm 0.10$  and  $0.07 \pm 0.31$ , for the 16 h post-treatment were  $-0.27 \pm 0.26$  and  $-0.24 \pm 0.52$ , respectively.)
- The mean percentages of cells in repair were less than 20% (Male: 1000 and 2000 mg/kg for the 2 h post-treatment were  $2.7\% \pm 0.6\%$  and  $6.3\% \pm 0.6\%$ , for the 16 h post-treatment were  $4.7\% \pm 1.2\%$  and  $4.7\% \pm 0.6\%$ , respectively. Female: 100 and 300 mg/kg for the 2 h post-treatment were  $5.0\% \pm 2.6\%$  and  $8.7\% \pm 1.2\%$ , for the 16 h post-treatment were  $2.0\% \pm 0.0\%$  and  $5.3\% \pm 0.6\%$ , respectively.)

**In conclusion**, OPC-34712 ability to induce UDS in rat hepatocytes was negative under the test conditions of this study.

## Overall conclusion

OPC-34712 was not genotoxic in Bacteria Reverse Mutation Test with or without S9. A genotoxic signal was observed in the Forward Mutation Test in Mouse Lymphoma Cells at 50 and 70  $\mu\text{M}$  after 3-h exposure with S9 and in the Chromosome Aberration Test in CHO cells after 3-h treatment at 50  $\mu\text{M}$ , without S9. Positive responses observed in both tests were generally slight and occurred at the doses of moderate cytotoxicity, therefore this findings were considered to be rather inconclusive than positive. No genotoxicity was observed in two *in vivo* tests; in the Rat Bone Marrow Micronucleus Test at doses up to 2000 mg/kg in males and up to 300 mg/kg in females and in the Unscheduled DNA Synthesis Test in male rats at doses up to 2000 mg/kg and female rats up to 300 mg/kg.

## 8 Carcinogenicity

Table 43: Carcinogenicity studies reports submitted and reviewed by this Reviewer:

Study	Species/Strain (Number/Sex/ Group)	Route/ Duration	Dose (mg/kg/day)	Key Results	Report Number
2-year Carcinogenicity	Mouse/CD-1	Oral (gavage) M: Week 91 F: Week 99	0, <sup>a</sup> 0, <sup>b</sup> 0.75, 2, 5	M: No neoplastic lesions F: Neoplastic lesions in mammary gland and pituitary gland	028338
2-year Carcinogenicity	Rat/Sprague Dawley	Oral (gavage) Week 104	M: 0, <sup>a</sup> 0, <sup>b</sup> 1, 3, 10 mg/kg/d  F: 0, <sup>a</sup> 0, <sup>b</sup> 3, 10, 30 mg/kg/d	M, F: No neoplastic lesions	028376

### Study title: Oral Dose Carcinogenicity Study of OPC-331 in Rats

Study no.:	B-6796 (No. 0302240)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 27, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	OPC-331, lot # C09G83M, purity 99.74%
CAC concurrence:	Yes

### Key Study Findings

- Male (M) and female (F) SD rats were dosed orally with OPC-331 at 0, 0, 1, 3, 10 mg/kg (M) and 0, 0, 3, 10, 30 mg/kg (F) for 104 weeks from 6 weeks of age (doses are 2.3- to 68.6-fold the MRHD [4 mg] on a body surface area basis)
- Decreased BW in MD (-13%) and HD (-28%) females and HD males (-19%)
- No biologically relevant, drug-related increases in incidences of neoplasms at any dose level were observed in both male and female rats
- Non-neoplastic findings were limited to increases in incidence/severity of lesions in HD-males (pituitary) and MD- and HD-females (adrenal, lung and pituitary).

### Adequacy of Carcinogenicity Study

Overall, the study was adequate; it was conducted according to standard procedures based on recommendations of applicable guidelines. The doses used in the study were recommended by the Exec CAC (see Meeting minutes dated 10/22/09). Treatment required concentrations of all OPC-331 samples were within 98 to 105% of the

predefined acceptable range ( $100 \pm 10\%$  of the nominal concentration). Two controls were used as recommended by the Exec CAC; vehicle and negative (water for injections).

The MTD was achieved as decreased body weight (BW) gain associated with decreased food consumption was observed in HD groups. The final BW was lower by -19% in HD-males and by -13% (MD) and -28% (HD) in females.

The organs and tissues from all animals of all study groups were histologically examined.

The exposure to OPC-331 and its metabolite (DM-3411) during the study period was verified in TK groups of rats; the exposure ( $C_{max}$  and  $AUC_{0-24h}$ ) was dose-related in both genders. The plasma levels of the parent, OPC-331, were higher than those of its metabolite, DM-3411, in all dose groups for both genders. OPC-331 was not detected in any plasma sample of control groups.

### **Appropriateness of Test Models**

The rat is a standard species used for 2-year carcinogenicity bioassay

### **Evaluation of Tumor Findings**

The sponsor's analysis found no treatment-related neoplastic lesions in male and female rats at any dose and lack of positive dose-response relationships.

Independently conducted statistical review by the FDA reviewer, Mohammad Atiar Rahman, Ph.D. found only a statistical significance in dose response relationship for the incidences of malignant lymphoma in hemolymphoreticular (all sites) in male rats and adenoma in mammary gland of female rats. The pairwise comparison showed increased incidence of acinar cell adenoma in pancreas of MD-males compared to their vehicle control (but not to the negative control). These statistically significant changes were considered to be of limited toxicological significance within the context of this study. There were no statistically significant differences between two controls: vehicle and water controls.

Increased incidence and severity of non-neoplastic lesions were observed in HD-males (pituitary) and MD- and HD-females (adrenal, lung and pituitary). In the adrenal, increased incidence and severity of yellowish brown pigment in cortical cells (MD- and HD-females) and increased severity of yellowish brown pigment in macrophages at HD. In the lung, increased incidence and severity of alveolar foamy cells were observed in HD females. In the pituitary, increased incidence and severity of atrophy of the pars intermedia were observed in HD-males and females in all dose groups. This finding was considered to be associated with the pharmacology of test article (partial D2 agonistic activity of OPC-331).

## Methods

Doses: 0, 0, 1, 3, 10 mg/kg (M); 0, 0, 3, 10, 30 mg/kg (F)  
 Frequency of dosing: Once a day  
 Dose volume: 5 ml/kg  
 Route of administration: Oral gavage  
 Formulation/Vehicle: 5 w/v% gum arabic (GA) solution  
 Basis of dose selection: Mortality and body weight (BW) reductions in the 4-week and 13- week study were used as toxicity-based endpoints for high dose (HD) selection.  
 Species/Strain: Crl:CD(SD) SPF rats  
 Number/Sex/Group: 60/sex/group  
 Age: 6 weeks old at the beginning of dosing  
 Animal housing: Housed individually in bracket-type stainless-steel wire-mesh cages  
 Paradigm for dietary restriction: Food and water ad libitum  
 Dual control employed: Yes; 1) - water and 2) - 5 w/v% GA solution  
 Interim sacrifice: No  
 Satellite groups: 8/sex/drug group; 5/sex/control group 1 and 2  
 Deviation from study protocol:

Test group	Dose level (mg/kg/day)	Dose concentration (mg/mL)	Dose Volume (mL/kg)	Sex	Main group		Satellite group	
					No. of animals	Animal number	No. of animals	Animal number
Negative control	0	0	5	M	60	1001-1060	5	1201-1205
Vehicle control	0	0	5		60	2001-2060	5	2201-2205
Low dose	1	0.2	5		60	3001-3060	8	3201-3208
Middle dose	3	0.6	5		60	4001-4060	8	4201-4208
High dose	10	2.0	5		60	5001-5060	8	5201-5208
Negative control	0	0	5	F	60	1101-1160	5	1301-1305
Vehicle control	0	0	5		60	2101-2160	5	2301-2305
Low dose	3	0.6	5		60	4101-4160	8	4301-4308
Middle dose	10	2.0	5		60	5101-5160	8	5301-5308
High dose	30	6.0	5		60	6101-6160	8	6301-6308

Negative control group was received the negative control material (water for injection).

Vehicle control group was received the vehicle (5 w/v% GA solution).

M: Male, F: Female

## Observations and Results

### Mortality

A tendency toward increase in the survival rate was observed in HD males and statistically significant trend in females at all dose levels. A tendency toward decrease in survival was observed in males treated with water for injection (negative control). According to the sponsor, the increase in survival was incidental since there was a low surviving rate in the negative controls. One HD male (#5042) was sacrificed in Week 38 (Day 266) due to remarkable aggressiveness.

Table 44: Summary of mortality and survival rate:

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	1	3	10	0 <sup>a)</sup>	0 <sup>b)</sup>	3	10	30
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
No. of deaths										
1-26w	0 <sup>c)</sup>	0	0	0	0	0	0	1	0	0
1-52w	3	0	1	1	4	0	1	1	0	1
1-78w	11	8	12	8	8	15	13	13	7	3
1-105w	37	31	30	30	25	38	39	23	24	17
No. of survivors	23	29	30	30	35	22	21	37	36	43
Survival rate (%)	38.3	48.3	50.0	50.0	58.3	36.7\$	35.0	61.7*	60.0*	71.7*

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

c): Cumulative number of animals that died (including those sacrificed as moribund).

\$: p≤0.05 (statistically significant trend, Tarone's test)

\*: p≤0.05 (statistically significant difference from the negative control group, log-rank test)

### Clinical Signs

Observations were made 3x/day; palpation to detect superficial tumors 1x/week.

Results: Incomplete eyelid closure in females at HD was the only treatment-related clinical observation (at termination occurred in ~ 1/5 animals) which returned to normal before dosing on the next day. Palpable masses were at relatively high incidence (findings in at least 5rats/sex/group) as shown in the following Sponsor's table:

Table 45: Summary of palpable mass-bearers

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	1	3	10	0 <sup>a)</sup>	0 <sup>b)</sup>	3	10	30
Dose (mg/kg/day)										
No. of animals used	60(37)	60(31)	60(30)	60(30)	60(25)	60(38)	60(39)	60(23)	60(24)	60(17)
No. of palpable mass-bearers <sup>c)</sup>	13(8)	7(2)	12(6)	11(9)	8(2)	41(25)	35(20)	30(4)	34(9)	42(10)
No. of animals with neck mass	4(3)	0(0)	0(0)	2(2)	0(0)	10(6)	7(2)	1(0)	5(0)	3(0)
No. of animals with thoracic mass	0(0)	0(0)	1(0)	0(0)	0(0)	2(0)	1(1)	5(0)	1(0)	6(1)
No. of animals with axillary mass	2(1)	1(1)	1(1)	2(2)	0(0)	18(8)	17(10)	12(4)	8(1)	19(3)
No. of animals with abdominal mass	1(1)	1(0)	0(0)	0(0)	1(0)	14(8)	9(6)	5(0)	4(0)	6(2)
No. of animals with inguinal mass	0(0)	0(0)	2(2)	2(2)	1(1)	15(13)	11(5)	10(0)	11(3)	13(2)
No. of animals with pubic mass	1(0)	0(0)	3(1)	0(0)	1(0)	15(9)	13(8)	11(1)	18(6)	20(5)
No. of animals with callosity in limb planta	22(13)	15(9)	22(8)	22(9)	26(7)	1(1)	0(0)	3(0)	4(0)	2(1)

Numbers in the table indicate the number of animals with respective signs and numbers in parentheses the number of animals that died or were sacrificed as moribund.

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

c): Excluding animals with callosity in limb planta.

No treatment-related superficial masses indicating tumors were observed at any dose.

### Body Weights

Body weights were recorded on Day 1 and Day 7, thereafter, 1x/week up to Week 13, every 4 weeks up to Week 101 and in Week 104.

Results: Decreased BW was observed in HD males (-19% in Week 104) and in MDF and HDF (-13% and -28%, respectively in Week 104) as shown in the following sponsor's figures:

Figure 10: Rat carcinogenicity study - mean BWs in males:

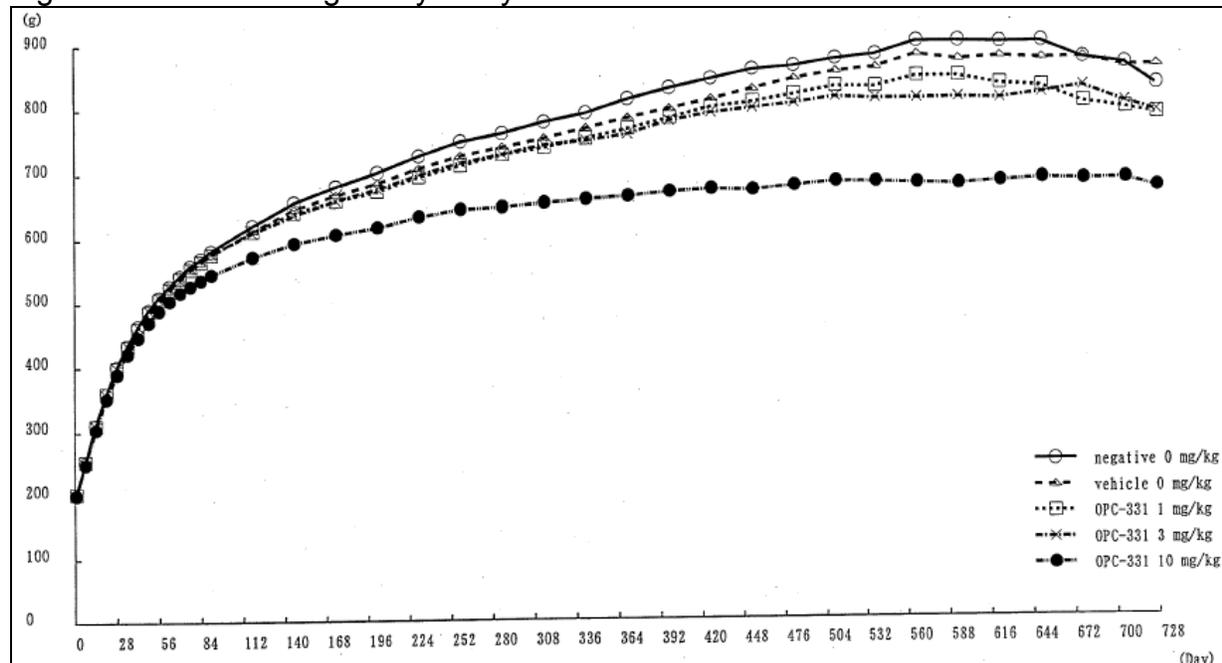
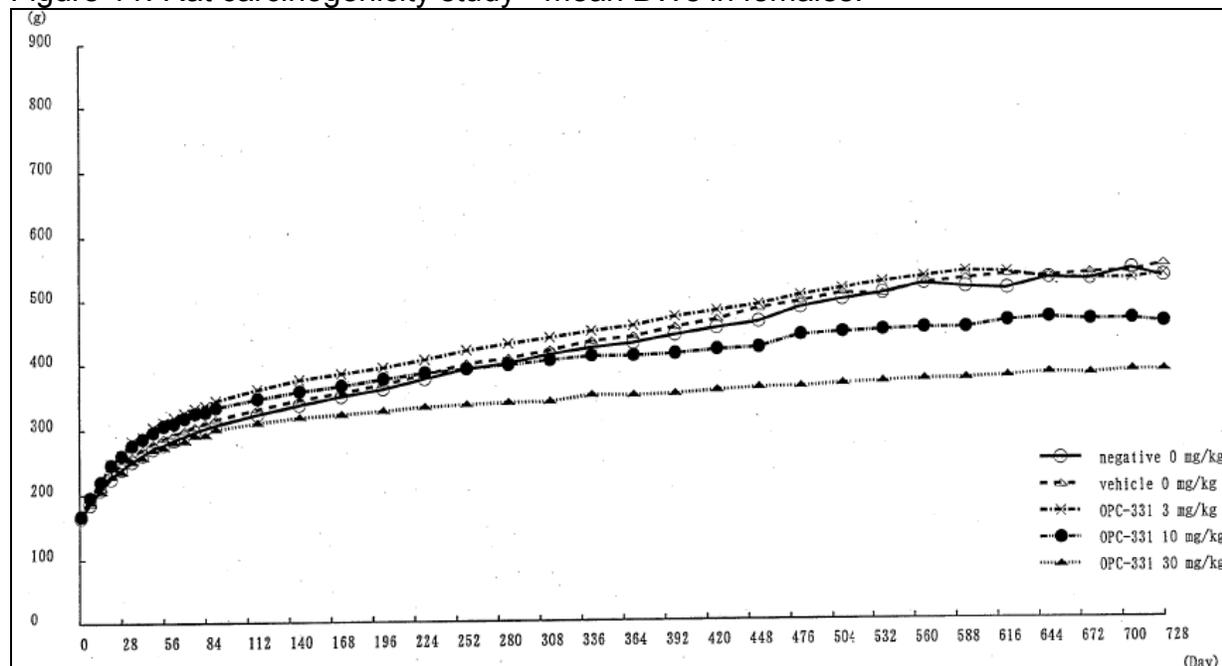


Figure 11: Rat carcinogenicity study - mean BWs in females:



## Feed Consumption

Feed consumption was recorded at the same days as BW measurements were taken.

Results: Significantly lower food consumption was observed among HDM in Weeks 10, 11 and 21 to 93; higher food consumption was observed among MDF in Weeks 1 to 25, then it became significantly lower in Weeks 53 to 77. In HDF food consumption was significantly lower in Weeks 4 and 37 to 97; all these changes in food consumption correlated with observed BW reduction in given groups of rats.

**Hematology** (at the time of necropsy; the following parameters were examined: RBC, HGB, HTC, MCV, MCH, MCHC, Retic, PLT, WBC, differential WBC, and microscopic examination).

Results: A statistically significant increase in WBC, lymphocytes and eosinophils was recorded in HDF. These significant increases were considered incidental caused by including two leukemic animals with remarkable increase in WBC in averaging the results.

## Organ Weights

The absolute weight of the thyroid (-19%), heart, lung, liver and kidney in HDM and statistically significant increases in the relative weight of the brain, pituitary, salivary gland, lung and testis in the same group of HDM as compared to the negative control group, they were considered to be changes associated with decreased BW in these animals (-19%). Similarly, decreases in the absolute weight of the kidney in MDF, thyroid, heart, liver and kidney in HDF and significant increases in the relative weight of the lung in MDF, brain, salivary gland, heart, lung, spleen, kidney, adrenal and ovary in HDF as compared to the negative control group, they were considered to be changes associated with low body weight.

## Gross Pathology

Results: There were no test article-related changes observed in males in any dose group. An increased incidence of nodule in the pancreas was noted however, it was considered an incidental change since it was not dose-related and there were no test article-related changes in histopathological examination.

In females, an increased incidence of white focus was observed in the lung (bronchus) in all dose groups.

In the vehicle control group for males and for females, there were no apparent differences from the negative control group.

The following is the Sponsor's summary of major gross lesions observed at necropsy:

Table 46: Incidence summary of major gross lesions

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	1	3	10	0 <sup>a)</sup>	0 <sup>b)</sup>	3	10	30
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
General descriptions										
Discoloration, pale skin	2	1	2	2	1	6	2	0	2	0
Undernourishment	2	3	3	1	1	4	7	8	11	5
Adrenal										
Large	3	2	3	1	2	6	8	3	1	5
Nodule	5	2	4	2	3	2	6	3	6	3
Liver										
Focus, dark red	8	7	5	6	5	6	10	10	5	6
Focus, white	7	5	2	1	1	1	5	0	2	4
Lung (bronchus)										
Nodule	2	2	2	3	0	8	2	2	0	1
Focus, white	4	2	4	4	4	0	3	16	15	25
Ovary										
Cyst	/	/	/	/	/	7	7	11	6	10
Pancreas										
Nodule	19	23	24	36	28	11	3	9	4	1
Pituitary										
Nodule	22	31	23	16	17	46	44	31	30	22
Focus, dark red	6	6	3	6	6	5	5	1	4	4
Skin + Subcutaneous										
Nodule	11	7	10	12	4	43	39	35	37	45
Spleen										
Large	12	8	8	12	16	10	5	5	5	7
Stomach										
Focus, dark red, glandular stomach	10	9	6	11	7	10	12	4	5	12
Testis										
Small	5	8	7	8	6	/	/	/	/	/
Uterus										
POLYP	/	/	/	/	/	4	1	2	6	6
Lymph node, nos <sup>c)</sup>										
Large	4	2	1	3	6	1	1	3	1	0
Planta										
Callosity	24	15	19	22	25	1	0	3	4	2

Number in the table indicates the number of animals with respective findings.

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

c): Lymph nodes other than the submandibular and mesenteric lymph nodes.

/: Not applicable

## Histopathology

Adequate Battery

Yes (see Appendix 1)

Peer Review

Yes, re-examination of all tissues and diagnoses from 10% of both control groups and HD male and female rats selected randomly, re-examination of all reported lesions and all potential target tissues identified by the study pathologist.

Neoplastic

The Sponsor has summarized the total number of tumors and tumor bearers in the following table:

Table 47: Total number of tumors and tumor bearers in male and female rats

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	1	3	10	0 <sup>a)</sup>	0 <sup>b)</sup>	3	10	30
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
No. of tumors	106	111	106	133	116	204	178	135	145	150
No. of benign tumors	76	90	79	100	75	113	110	87	87	83
No. of malignant tumors	30	21	27	33	41	91	68	48	58	67
No. of tumor bearing animals	53	50	51	51	52	60	60	55	54	57
No. of benign tumor bearers	40	48	43	44	41	57	55	47	48	45
No. of malignant tumor bearers	28	18	22	26	33	42	35	26	33	41
No. of multiple tumor bearers	26	34	26	37	33	49	50	36	35	41

Number in the table indicates the number of animals.

a): Negative control group (water for injection); b): Vehicle control group (5 w/v% GA solution)

The Sponsor’s analysis showed no treatment-related neoplastic lesions in either sex in any dose group.

Statistical review and evaluation of the results of this study was independently conducted by the statistical reviewer, Mohammad Atiar Rahman, Ph.D. (see Statistical Review for statistical methods and references). The only statistically significant finding was a dose response relationship for the incidences of malignant lymphoma in hemolymphoreticular (all sites) in male rats and adenoma in mammary gland of female rats. The pairwise comparison showed increased incidence of acinar cell adenoma in pancreas of MD-males compared to their vehicle control (but not to the negative control – n=3); see the following summary table excerpted from the statistical review:

Table 48: Summary table of tumor types with p-values ≤ 0.05 for dose response relationship or pairwise comparisons of treated groups and vehicle control in rats

Sex	Organ Name	Tumor Name	Veh				P_Value			
			Cont	Low	Med	High	Dose Resp	VC vs. L	VC vs. M	VC vs. H
Male	Hemolymphoreticular(all sites)	LYMPHOMA, MALIGNANT	0	1	0	4	0.0111*	0.4947	.	0.0638
	Pancreas	ADENOMA, ACINAR CELL	0	3	6	0	0.7974	0.1171	0.0142*	.
		ADENOMA, ISLET CELL	17	15	31	20	0.2695	0.5574	0.0103	0.3386
	Testis	LEYDIG CELL TUMOR	1	0	0	3	0.0489	0.4947	0.5052	0.3085
Female	Mammary gland	ADENOCARCINOMA	27	22	26	40	0.0077	0.8650	0.5867	0.1011
		ADENOMA	0	0	0	3	0.0214*	.	.	0.1792
		ADENOMA+ADENOCARCINOMA	27	22	26	40	0.0077	0.8650	0.5867	0.1011
	Vagina	GRANULAR CELL TUMOR	0	0	1	3	0.0279	.	0.5444	0.1792

These statistically significant changes were considered to be of limited toxicological significance within the context of this study.

## Non Neoplastic

Treatment-related non-neoplastic lesions were observed in the pituitary, adrenal and lung (bronchus). In the pituitary, a tendency toward increase in the incidence of pars distalis hyperplasia was observed in females in all dose groups as shown in the following Sponsor's table:

Table 49: Treatment-related tumors and hyperplastic lesions in rats given OPC-331

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	1	3	10	0 <sup>a)</sup>	0 <sup>b)</sup>	3	10	30
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
Pituitary										
Adenoma, pars distalis	24	35	27	25	22	51	50	32	32	22
Carcinoma, pars distalis	0	0	0	0	0	3	1	0	0	0
Hyperplasia, pars distalis, focal	15	15	18	21	15	3	5	15	12	23

Number in the table indicates the number of animals with respective tumors.

a): Negative control group (water for injection) b): Vehicle control group (5 w/v% GA solution)

Other tumors, considered by the Sponsor as non-treatment-related (occur spontaneously in aged rats) and which occurred at relatively high incidence are summarized in the following sponsor's table:

Table 50: Incidence summary of major tumors in rats

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	1	3	10	0 <sup>a)</sup>	0 <sup>b)</sup>	3	10	30
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
Adrenal										
Pheochromocytoma	6	9	7	12	7	0	3	4	2	6
Mammary gland										
Fibroadenoma	0	0	2	1	2	20	26	20	20	19
Adenocarcinoma	1	0	1	0	0	34	27	22	26	40
Pancreas										
Adenoma, acinar cell	3	0	3	6	0	0	0	0	0	0
Adenoma, islet cell	17	17	15	31	20	8	2	5	2	4
Carcinoma, islet cell	8	9	8	7	11	5	1	4	3	0
Thyroid										
Adenoma, c cell	6	6	7	3	5	7	5	4	4	5
Uterus										
Polyp, endometrial stromal	/	/	/	/	/	4	2	2	6	6

Number in the table indicates the number of animals with respective tumors.

a): Negative control group (water for injection) b): Vehicle control group (5 w/v% GA solution)

Treatment-related non-tumor lesions were observed in the adrenal, lung (bronchus) and pituitary as shown in the following sponsor's table:

Table 51: Incidence summary of treatment-related non-tumor lesions

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	1	3	10	0 <sup>a)</sup>	0 <sup>b)</sup>	3	10	30
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
<b>Adrenal</b>										
Yellowish brown pigment, cortical	0	0	0	0	0	10	8	12	33	60
minimal	0	0	0	0	0	10	8	12	32	6
mild	0	0	0	0	0	0	0	0	1	53
moderate	0	0	0	0	0	0	0	0	0	1
Yellowish brown pigment, macrophage	57	58	57	60	58	57	57	58	59	60
minimal	44	38	47	41	38	43	45	46	41	8
mild	13	20	10	19	20	14	12	12	18	35
moderate	0	0	0	0	0	0	0	0	0	17
<b>Lung (bronchus)</b>										
Foamy cell, alveolar	30	20	19	20	24	25	30	29	33	45
minimal	20	19	15	18	20	21	25	18	22	26
mild	10	1	4	2	4	3	5	11	11	15
moderate	0	0	0	0	0	1	0	0	0	4
<b>Pituitary</b>										
Atrophy, pars intermedia	0	0	0	0	10	0	0	8	18	38
minimal	0	0	0	0	2	0	0	0	0	0
mild	0	0	0	0	8	0	0	8	18	38

Number in the table indicates the number of animals with respective lesions.

a): Negative control group (water for injection) b): Vehicle control group (5 w/v% GA solution)

Increased incidence and severity of lesions were observed in HD-males (pituitary) and MD- and HD-females (adrenal, lung and pituitary).

In addition, an increase in the incidence and severity of retinal atrophy was observed in HD-male and HD-female rats (increased severity also in MD-females). These changes were considered by the sponsor "to be a spontaneous change observed in aged rats and there were no clear differences in the incidence or severity of retinal atrophy among the animals that were sacrificed as scheduled, it is considered to be related to the increase in survival rate in females and not related to administration of the test article".

Table 52: Incidence summary of non-treatment-related non-tumor lesions

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	1	3	10	0 <sup>a)</sup>	0 <sup>b)</sup>	3	10	30
Dose (mg/kg/day)										
No. of animals used	60(23)	60(29)	60(30)	60(30)	60(35)	60(22)	60(21)	60(37)	60(36)	60(43)
<b>Eye</b>										
Atrophy, retinal	13(6)	13(12)	10(8)	9(7)	18(12)	18(13)	15(10)	24(22)	20(18)	33(25)
minimal	5(3)	2(2)	3(3)	1(0)	9(7)	6(4)	2(2)	4(4)	3(3)	11(9)
mild	5(2)	5(5)	3(2)	4(4)	3(0)	5(5)	7(4)	13(12)	7(6)	8(7)
moderate	2(0)	4(3)	3(2)	2(1)	2(2)	6(4)	3(1)	1(1)	2(1)	4(2)
Severe	1(1)	2(2)	1(1)	2(2)	4(3)	1(0)	3(3)	6(5)	8(8)	10(7)

Numbers in parentheses indicate the number of animals that were sacrificed as scheduled.

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

Of note; however, retinal degeneration was also observed in rat chronic toxicity and carcinogenicity studies with aripiprazol (NDA 021-436) which has a similar pharmacological profile to OPC-331.

## Toxicokinetics

OPC-331 and its metabolite (DM-3411) in rat plasma 1, 2, 4, 8 and 24 h post dose on Day 1, Week 13 and Week 26 of the study were quantified by a validated LC-ESI-MS/MS method, to assess their systemic exposure.

Results: The following Sponsor's table shows the summary of TK data:

Table 53: TK parameters of OPC-331 and DM-3411 in rats

Compound	Period	$C_{max}$ (ng/mL)					
		Male			Female		
		Dose (mg/kg)			Dose (mg/kg)		
		1	3	10	3	10	30
OPC-331	Day 1	20.69	43.27	200.8	77.23	287.7	970.4
	Week 13	18.60	61.37	435.2	114.8	396.4	1104
	Week 26	20.37	65.09	418.5	139.2	431.7	1433
DM-3411	Day 1	3.101	8.474	69.25	14.47	72.04	221.5
	Week 13	4.802	16.23	124.4	20.88	87.82	253.3
	Week 26	5.362	19.49	104.3	23.79	81.62	259.7

Compound	Period	$AUC_{0-24h}$ (ng·h/mL)					
		Male			Female		
		Dose (mg/kg)			Dose (mg/kg)		
		1	3	10	3	10	30
OPC-331	Day 1	109.7	364.6	1265	501.4	1956	7808
	Week 13	123.6	390.7	3362	741.8	2506	12870
	Week 26	204.0	556.9	3912	867.1	3644	17400
DM-3411	Day 1	16.49	79.75	355.8	78.89	332.5	1404
	Week 13	36.32	95.02	766.1	130.3	417.4	1483
	Week 26	53.17	154.9	861.4	145.7	539.0	2081

Each value represents mean of 3 animals. The lower limit of quantification is 1 ng/ml

On Day 1, Week 13 and Week 26 of the administration period, the  $C_{max}$  and  $AUC_{0-24h}$  of OPC-331 and its metabolite, DM-3411, increased in a dose-related manner for both sexes. The  $t_{max}$  of OPC-331 (1 - 4h) and DM-3411 (1 – 2h) was independent of dose, sex and dosing frequency for OPC-331 in all dose groups. The exposure of OPC-331 in Week 13 and Week 26 were similar to or slightly higher than on Day 1 (0.9 to 3.1 times) in all dose groups.  $C_{max}$  and  $AUC_{0-24h}$  of DM-3411 were 11.1% to 33.3% of those of OPC-331 in molar ratio. Only slightly higher exposure (to the parent and its metabolite) was observed in females than in males.

## Dosing Solution Analysis

The dosing formulations used in the study were analyzed 7 times (at the first administration and every 4 months thereafter; months 4, 8, 12, 16, 20 and 24). As a result, all dose concentrations were within 98.0 to 105.0% of the nominal concentration and the coefficient of variation (CV) between 0.0 and 1.5%, both of which were within the acceptable range (100 ± 10% of the nominal concentration, and CV below 10%).

Table 54: The Sponsor's tabulated summary of carcinogenicity study in rats

Animal	CrI:CD(SD) rat, 6 weeks of age				
Test article	Negative control <sup>a)</sup>	Vehicle control <sup>b)</sup>	OPC-331		
Dosage (mg/kg/day) (M:F)	0:0	0:0	1:3	3:10	10:30
Dosage volume (mL/kg/day)	5	5	5	5	5
No. of animals (M:F) <sup>c)</sup>	60:60	60:60	60:60	60:60	60:60
Dosing period (week) (M:F)	104:104	104:104	104:104	104:104	104:104
Week of necropsy (M:F)	105:105	105:105	105:105	105:105	105:105
Mortality (M:F)	37:38	31:39	30:23	30:24	25:17
Survival rate (%) (M:F)	38.3 ↓:36.7 ↓	48.3:35.0	50.0:61.7 ↑	50.0:60.0 ↑	58.3 ↑:71.7 ↑
Clinical signs	-	-	-	-	Incomplete eyelid closure (F)
Palpation	-	-	-	-	-
Body weight	-	-	↑ (F: Week 1-57)	↑ (F: Week 1-25) ↓ (F: Week 69-104)	↓ (M: Week 6-104) ↓ (F: Week 21-104)
Terminal body weight	-	-	-	↓ (F: -13%)	↓ (M: -19%, F: -28%)
Food consumption	-	-	↑ (F: Week 1-29, 37)	↑ (F: Week 1-25) ↓ (F: Week 53-77)	↓ (M: Week 10, 11, 21-93) ↓ (F: Week 4, 37-97)
Hematology	-	-	-	-	-
Organ weight	-	-	-	-	-
Necropsy	-	-	Lung; ↑ Focus, white (F)		
Histopathology (Neoplastic lesions)	-	-	Pituitary; ↓ Adenoma and carcinoma, pars distalis (F) {thereby, ↑ Hyperplasia, pars distalis, focal (F)}		
Histopathology (Non-neoplastic lesions)	-	-	Pituitary; ↑ Atrophy, pars intermedia (F)		
			Adrenal; ↑ Yellowish brown pigment, cortical (F)		
			Adrenal; ↑ Yellowish brown pigment, macrophage (F)		
			Lung; ↑ Foamy cell, alveolar (F)		
			Pituitary; ↑ Atrophy, pars intermedia (M)		

a): Water for injection; b): 5 w/v% gum arabic solution; c): Additional 5 or 8 animals/sex/group were used as satellite groups for toxicokinetics. M: Male, F: Female  
 -: No treatment-related effects, ↑: Increase, ↓: Decrease

**Study title: Oral Dose Carcinogenicity Study of OPC-331 in Mice**

Study no.: B-6795 (Otsuka study/report numbrs:  
030239/028338)  
Study report location: EDR  
Conducting laboratory and location: (b) (4)  
Date of study initiation: November 16, 2009  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: OPC-331, lot # C09G83M, purity 99.74%  
CAC concurrence: Yes

**Key Study Findings**

- Male and female Crlj:CD1(ICR) mice were dosed orally at 0, 0, 0.75, 2, and 5 mg/kg/d. These dose levels are 0.9- to 5.8-fold the oral MRHD (4 mg) on a body surface area basis.
- Increased incidence of mammary gland adenocarcinomas in females of all dose groups and in adenosquamous carcinomas in MD and HD groups
- Increased incidence of pars distalis adenoma in the pituitary in all dose females
- Treatment-related non-neoplastic lesions were observed in the mammary gland, ovary, uterus and vagina.

**Adequacy of Carcinogenicity Study**

Overall, the study was adequate; it was conducted according to standard procedures based on recommendations of applicable guidelines. The doses used in the study were recommended by the ExecCAC (see Meeting minutes dated 10/22/09). The Committee recommended doses of 0.75, 2, and 5 mg/kg in male and female mice, based on mortalities in both sexes and the excessive decrease in BW in males at doses  $\geq 10$  mg/kg observed in the 4- and 13-week subchronic toxicity studies in mice which were reviewed by this Reviewer under IND 101,871(SDN 54, SDN 46, respectively). The exposure to OPC-331 during the study period was confirmed in TK groups. The organs and tissues from all animals of all study groups were histologically examined. Animal survival was sufficient for an adequate assessment of tumorigenic potential.

**Appropriateness of Test Models**

Mice Crlj:CD1(ICR) are standard species used for 2-year carcinogenicity bioassay.

**Evaluation of Tumor Findings**

The incidence of adenocarcinoma in mammary gland was increased in females in all dose groups and the incidence of adenosquamous carcinoma was increased in females in the 2 and 5 mg/kg groups. At the same time, increased incidence and severity of lobular hyperplasia were observed clearly in females in all dose groups. In the pituitary gland, the incidence of pars distalis adenoma was marginally increased in females in all dose groups.

The increased incidence of mammary gland tumors is likely being caused by hormonal alterations as a result of increased prolactin-mediated D<sub>2</sub>-receptor antagonism which is consistent with the pharmacology of OPC-331. Although serum prolactin was not measured in this carcinogenicity study the Sponsor demonstrated that single dose of OPC-331 (5 mg/kg) caused a significant increase in serum prolactin of female mice by about 60- to 20- fold (1- and 4-h post-dose, respectively). Similar findings were reported for other antipsychotics including aripiprazole, another drug from the same Sponsor, which has a very similar mechanism of action (NDA 021-436); [the results of this study are summarized in the Special Toxicology section of this review.]

## Methods

Doses: 0, 0, 0.75, 2, and 5 mg/kg/day  
 Frequency of dosing: Once a day, 7 days/week for 104 weeks  
 Dose volume: 10 ml/kg  
 Route of administration: Oral gavage  
 Formulation/Vehicle: Suspension/5 w/v% arabic gum solution  
 Basis of dose selection: Mortalities in both sexes and excessive decrease in BW in males at doses  $\geq$ 10 mg/kg (4- and 13-week studies)  
 Species/Strain: Mice/Crlj:CD1(ICR)  
 Number/Sex/Group: 60/sex/group  
 Age: Started at 6 weeks of age  
 Animal housing: Individually in stainless-steel wire-mesh cages  
 Paradigm for dietary restriction: Free access to pelleted diet and tap water  
 Dual control employed: Yes (1- water for injections and 2- vehicle; 5 w/v% arabic gum solution)  
 Interim sacrifice: No  
 Satellite groups: 11/sex (both control groups); 50/sex/group  
 Deviation from study protocol: Due to increased mortality with the progression of dosing, the surviving males were sacrificed in Week 91 and the surviving females in Week 99 (see survival rate table below).

Test group	Dose level (mg/kg/day)	Dose concentration (mg/mL)	Dose Volume (mL/kg)	Sex	Main group		Satellite group	
					No. of animals	Animal number	No. of animals	Animal number
Negative control	0	0	10	M	60	1001-1060	11	1201-1211
				F	60	1101-1160	11	1301-1311
Vehicle control	0	0	10	M	60	2001-2060	11	2201-2211
				F	60	2101-2160	11	2301-2311
Low dose	0.75	0.075	10	M	60	3001-3060	50	3201-3250
				F	60	3101-3160	50	3301-3350
Middle dose	2	0.2	10	M	60	4001-4060	50	4201-4250
				F	60	4101-4160	50	4301-4350
High dose	5	0.5	10	M	60	5001-5060	55	5201-5255
				F	60	5101-5160	55	5301-5355

Negative control group was received the negative control material (water for injection).

Vehicle control group was received the vehicle (5 w/v% GA solution).

M: Male, F: Female

## Observations and Results

### Mortality

All surviving males were sacrificed prematurely when negative control group reached critical number of 20 mice in Week 91 after administration for 90 weeks. All surviving females were also sacrificed prematurely in Week 99 after administration for 98 weeks (LD- and MD-mice fell below the critical number of 20/group). The Division agreed with the early termination of the study. The summary of mortality and survival rate is shown in the following Sponsor's table:

Table 55: Summary of mortality and survival rate

Sex	Male <sup>a)</sup>					Female <sup>b)</sup>				
	0 <sup>c)</sup>	0 <sup>d)</sup>	0.75	2	5	0 <sup>c)</sup>	0 <sup>d)</sup>	0.75	2	5
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
No. of deaths										
1-26w	1 <sup>e)</sup>	0	1	0	0	0	0	0	0	1
1-52w	4	9	9	8	5	3	3	3	5	10
1-78w	28	25	25	21	17	15	20	26	23	20
1-91w	40	31	29	32	25	23	28	35	34	30
1-99w	/	/	/	/	/	32	35	45	41	37
No. of survivors	20	29	31	28	35	28	25	15	19	23
Survival rate (%)	33.3\$	48.3	51.7	46.7	58.3*	46.7	41.7	25.0*	31.7*	38.3

a): All surviving males were sacrificed prematurely in Week 91 after administration for 90 weeks.

b): All surviving females were sacrificed prematurely in Week 99 after administration for 98 weeks.

c): Negative control group (water for injection)

d): Vehicle control group (5 w/v% GA solution)

e): Cumulative number of animals that died (including those sacrificed as moribund).

/: Not applicable

\$: p≤0.05 (statistically significant trend, Tarone's test)

\*: p≤0.05 (statistically significant difference from the negative control group, log-rank test)

The sponsor observed a trend of increase in the survival rate in the treatment groups of males versus negative control with statistical significance at the HD (5 mg/kg). However according to the FDA's independent statistical analysis performed by Mohammad Atiar Rahman, Ph.D. (see statistical review in Appendix 1) the tests did not show statistically significant dose-response relationship across vehicle control and treated groups or pairwise difference between any treated group and vehicle control in mortality in either sex of mice.

### Clinical Signs

No treatment-related clinical signs were observed at any dose and in either sex. There was lack of superficial masses in treated males. However, an increase in the number of females with palpable masses was observed; mainly in the neck and axillary regions in all dose groups and in the abdominal region at MD and dorsal region at LD. Except for one animal, the palpable masses were mammary tumors confirmed by histological examination. The incidence of palpable masses is summarized in the following Sponsor's table:

Table 56: Summary of palpable mass-bearers

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	0.75	2	5	0 <sup>a)</sup>	0 <sup>b)</sup>	0.75	2	5
Dose (mg/kg/day)										
No. of animals used	60(40)	60(31)	60(29)	60(32)	60(25)	60(32)	60(35)	60(45)	60(41)	60(37)
No. of palpable mass-bearers <sup>c)</sup>	2(2)	4(4)	5(5)	2(0)	4(3)	9(8)	8(4)	21(19)	21(17)	15(14)
No. of animals with neck region	1(1)	1(1)	2(2)	0(0)	1(1)	0(0)	2(1)	4(4)	5(5)	7(7)
No. of animals with axillary region	0(0)	1(1)	0(0)	1(0)	0(0)	0(0)	0(0)	4(3)	8(8)	5(5)
No. of animals with abdominal region	0(0)	1(1)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1)	5(3)	1(1)
No. of animals with public region	0(0)	0(0)	3(3)	1(0)	3(2)	5(5)	1(1)	2(2)	3(3)	2(2)
No. of animals with dorsal region	0(0)	0(0)	1(1)	1(0)	0(0)	1(1)	1(0)	7(6)	1(1)	2(1)

Numbers in the table indicate the number of animals with respective signs and numbers in parentheses the number of animals that died or were sacrificed as moribund.

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

c): Excluding animals with callosity in limb planta.

## Body Weights

The terminal BW in male and female mice of all treatment groups was comparable to respective negative and vehicle controls. During treatment phase of the study, BW was lower in males (-3 to -5%) and higher in females (+5 to +12%) in all dose groups as shown in the following Sponsor's table:

Table 57: Summary of mean BW in male and female mice

Sex	Dose (mg/kg/day)	Mean body weight (Unit: g)				
		Week 13 (Day 91)	Week 25 (Day 175)	Week 53 (Day 371)	Week 77 (Day 539)	Terminal <sup>a)</sup>
Male	0 <sup>b)</sup>	39.3	40.5	41.5	42.6	41.9
	0 <sup>c)</sup>	39.1	40.5	41.2	41.5	40.8
	0.75	38.1* (-3%)	39.7	41.1	41.5	41.2
	2	38.0* (-3%)	40.0	40.9	41.4	40.9
	5	37.5** (-5%)	39.3	39.9* (-4%)	40.3* (-5%)	40.2
Female	0 <sup>b)</sup>	32.3	33.9	36.5	38.3	39.2
	0 <sup>c)</sup>	32.7	33.9	36.1	37.7	39.9
	0.75	35.3** (+9%)	38.0** (+12%)	40.6** (+11%)	42.3** (+10%)	40.9
	2	35.0** (+8%)	37.7** (+11%)	39.7** (+9%)	40.6* (+6%)	38.8
	5	33.8** (+5%)	36.0** (+6%)	37.9	39.3	39.0

Values in parentheses indicate the percent changes against the mean value of the negative control group (+: increase; -: decrease).

a): Data in Week 90 (Day 632) for males and in Week 98 (Day 691) for females.

b): Negative control group (water for injection)

c): Vehicle control group (5 w/v% GA solution)

\*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  (significantly different from the negative control group)

## Feed Consumption

Food consumption was in line with observed BW gain in males and females; it was occasionally lower in males and higher in treated females as compared to their respective controls.

Of note: according to the sponsor, the enhanced BW gain and food consumption in females were attributed to increased serum prolactin levels via an endocrine mechanism.

**Hematology** (at the time of necropsy; the following parameters were examined: RBC, HGB, HTC, MCV, MCH, MCHC, Retic, PLT, WBC, differential WBC, and microscopic examination).

The following changes in the hematology parameters were observed in male mice only

Table58: Summary of hematology

Sex	Male				
	0 <sup>a)</sup>	0 <sup>b)</sup>	0.75	2	5
Dose (mg/kg/day)	0 <sup>a)</sup>	0 <sup>b)</sup>	0.75	2	5
No. of animals used	20	29	31	28	35
RBC	/	N	N	N	+9%**
HGB	/	N	+4%*	N	+12%**
HCT	/	N	+3%*	N	+13%**
MCV	/	N	N	N	+4%*
WBC	/	N	N	N	-32%*
LYMP %	/	N	N	-28%**	-28%**
NEUT %	/	N	N	+47%**	+45%**
LYMP count	/	N	-27%*	-29%*	-52%**
LUC count	/	N	N	N	-60%*

Values in the table indicate percentage of change against the mean value of the negative control group (+: increase, -: decrease).

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

\*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$  (significantly different from the negative control group)

N: No remarkable changes

/: Not applicable

These observed hematology changes in the absence of a clear dose-response, are considered incidental and not clearly related to test article administration.

## Organ Weights

Increased weight of salivary gland (absolute +18% and relative +20%) and kidney (absolute +14% and relative +16%) was observed in HD males. In females, decreased uterus weight (absolute and [relative]) was observed in all dosing groups (-78% [79%] at LD; -80% [79%] at MD; and -81% [80%] at HD, respectively).

## Gross Pathology

There were no test article-related changes observed in males. In females, a decrease incidence of nodule in the ovary, an increased incidence of nodule in the pituitary and in the skin + subcutaneous in all dose groups was observed. In addition, a decreased incidence of endometrial cyst in uterus was observed in all dose groups.

**Histopathology**

Adequate Battery

Yes (see Appendix 1)

Peer Review

Yes, re-examination of all tissues and diagnoses from 10% of both control groups and HD male and female rats selected randomly, re-examination of all reported lesions and all potential target tissues identified by the study pathologist (potential target tissues as identified by the study pathologist and reviewed by the reviewing pathologist were identified in the pituitary of male and females, and in the mammary gland, uterus, vagina, and ovaries of females).

Neoplastic

Tumors and hyperplastic lesions were observed in the mammary glands and pituitary of treated females as shown in the following Sponsor's table:

Table 59: Tumors and hyperplastic lesions in male and female mice

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	0.75	2	5	0 <sup>a)</sup>	0 <sup>b)</sup>	0.75	2	5
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
<b>Mammary gland</b>										
Adenocarcinoma	-	-	-	-	-	4	2	14**	12	12**
Carcinoma, adenosquamous	-	-	-	-	-	1	1	3	15*	6*
Total with mammary gland tumors	-	-	-	-	-	4	3	16**	23**	16**
Hyperplasia, lobular	-	-	-	-	-	16	14	42	42	47
minimal	-	-	-	-	-	12	12	25	25	26
mild	-	-	-	-	-	4	1	16	16	21
moderate	-	-	-	-	-	0	1	1	1	0
<b>Pituitary</b>										
Adenoma, pars distalis	0	0	0	0	0	2	0	5	7	7

Number in the table indicates the number of animals with respective tumor or hyperplastic lesion.

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

\* : P<0.05 (statistically different from the negative control, rare tumor, Peto's test)

\*\* : P<0.01 (statistically different from the negative control, common tumor, Peto's test)

-: Not examined

The FDA's independent statistical analysis (see Statistical Review and Evaluation by Mohammad Atiar Rahman, Ph.D.) showed similar results which are shown in the following table excerpted from Dr. Rahman's review:

Table 60: Summary table of tumor types with p-values  $\leq 0.05$  for dose response relationship and/or pairwise comparisons of treated groups and vehicle control in mice

Sex	Organ Name	Tumor Name	Veh				P_Value			
			Cont	Low	Med	High	Dose Resp	VC vs. L	VC vs. M	VC vs. H
//										
Male	Harderian gland	ADENOMA	1	2	3	7	0.0120	0.5089	0.3347	0.0448
	Hemolymphoretic	SARCOMA, HISTIOCYTIC	0	1	0	3	0.0446	0.5060	.	0.1523
Female	Mammary gland	ADENOCARCINOMA	2	14	12	12	0.0568	<0.001*	0.0039*	0.0034*
		ADENOMA+ADENOCARCINOMA	3	14	12	13	0.0522	0.0023*	0.0104	0.0055*
		ADENOMA+ADENOCARCINOMA+CARCINOMA	3	16	23	16	0.0164	<0.001*	<0.001*	<0.001*
		CARCINOMA,ADENOSQUAMOUS	1	3	15	6	0.0673	0.2707	<0.001*	0.0509
	Pituitary	ADENOMA, PARS DISTALIS	0	5	7	7	0.0261	0.0225*	0.0044*	0.0054*

The difference in the statistical significance of pairwise comparisons between sponsor’s analysis and the FDA’s statistical reviewer is caused by the use of different control groups for comparisons; negative control was used by the sponsor and the vehicle control by Dr. Rahman.

Non Neoplastic

Non neoplastic tumors were noted in multiple organs in males and females; these findings were summarized in the following Sponsor’s table:

Table 61: Incidence summary of major tumors

Sex	Male					Female				
	0 <sup>a)</sup>	0 <sup>b)</sup>	0.75	2	5	0 <sup>a)</sup>	0 <sup>b)</sup>	0.75	2	5
Dose (mg/kg/day)										
No. of animals used	60	60	60	60	60	60	60	60	60	60
Hemolymphoretic(all sites)										
Lymphoma, malignant	/	/	/	/	/	13	10	11	12	6
Harderian gland										
Adenoma	5	1	2	3	7	5	2	0	2	0
Liver										
Adenoma, hepatocellular	12	10	10	8	11	0	0	1	1	1
Lung (bronchus)										
Adenoma, bronchiole-alveolar	4	16**	8	13	5	6	9	9	9	9
Carcinoma, bronchiole-alveolar	2	2	3	3	5	3	4	4	7	7
Uterus										
Polyp, endometrial, stromal	/	/	/	/	/	3	5	1	1	1

Number in the table indicates the number of animals with respective tumors.

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

\*\* : p≤0.01 (significantly different from the negative control group, common tumor, Peto’s test)

/: Not applicable

In addition, treatment-related non-tumor lesions were observed in the mammary gland, ovary, uterus and vagina of females in all dosing groups as it is shown in the following Sponsor’s table:

Table 62: Incidence summary of treatment-related non-tumor lesions

Dose	0 <sup>a</sup>	0 <sup>b</sup>	0.75	2	5
<b>Number of Animals Used</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>	<b>60</b>
<b>Mammary Gland</b>					
Dilatation, ductal/acinar	11	17	35	42	36
Metaplasia, squamous	0	2	2	2	7
<b>Ovary</b>					
Cyst	45	44	37	36	29
<b>Uterus</b>					
Atrophy	4	12	21	18	15
Hyperplasia, endometrial, cystic	45	37	20	23	24
Adenomyosis	1	1	11	9	5
<b>Vagina</b>					
Atrophy, mucosal	24	31	49	48	46
Mucification	12	14	38	33	31

a): Negative control group (water for injection)

b): Vehicle control group (5 w/v% GA solution)

### Toxicokinetics

OPC-331 and its metabolite, DM-3411, were measured in mouse plasma at 1, 2, 4, 8 and 24 hrs post dose on Day 1 and in Week 13 and Week 26 and  $C_{max}$  and  $AUC_{0-24}$  of OPC-331 and DM-3411 are shown in the following Sponsor's table:

Table 63: TK parameters of OPC-331 and DM-3411 in mouse plasma

Sex	Dose (mg/kg/day)	$C_{max}$ (ng/mL)			$AUC_{0-24h}$ (ng·h/mL)		
		Day 1	Week 13	Week 26	Day 1	Week 13	Week 26
<b>OPC-34712</b>							
Males	0.75	113.1	93.51	129.6	1313	1120	1174
	2	295.0	260.3	360.0	3369	2748	4322
	5	512.9	746.8	1076	8663	5756	12430
Females	0.75	78.15	84.86	65.33	833.0	797.4	772.5
	2	219.9	218.7	287.9	2586	1833	2280
	5	368.1	549.4	412.0	4885	4195	4321
<b>DM-3411</b>							
Males	0.75	4.965	2.857	2.648	44.72	33.36	35.05
	2	14.02	8.320	8.091	163.6	79.52	96.19
	5	35.91	23.87	19.03	449.2	153.3	246.8
Females	0.75	6.511	5.690	6.175	67.22	49.46	58.56
	2	19.96	17.45	20.16	226.4	119.2	188.9
	5	37.46	46.26	45.18	442.0	323.8	465.5

Each value represents mean of 3 animals (LLQ is 1 ng/ml)

The exposure to OPC-331 increased in a dose –related manner for both sexes and it was significantly higher than those of its metabolite, DM-3411.  $C_{max}$  and  $AUC_{0-24h}$  of DM-3411 were 1.7% to 10.6% of those of OPC-331 in molar ratio. Time to reach maximum plasma concentration ( $t_{max}$ ) in all treated groups on Day 1 and in Week 13 and Week 26 were independent of dose, sex and dosing frequency for OPC-331 (1 – 2 hrs). OPC-331 was not detected in any plasma sample from the control group.

## Dosing Solution Analysis

The concentrations of dosing formulations were analyzed 7 times during the study (at the first administration and every 4 months thereafter) and were within 95.6 to 101.7% of the nominal concentrations which were within the acceptable range (100 ± 10%).

Table 64: The Sponsor's tabulated summary of the results of oral dose carcinogenicity study of OPC-331 in mice

Animal	Crj:CD1(ICR) mouse, 6 weeks of age				
Test article	Negative control <sup>a)</sup>	Vehicle control <sup>b)</sup>	OPC-331		
Dosage (mg/kg/day)	0	0	0.75	2	5
Dosage volume (mL/kg/day)	10	10	10	10	10
No. of animals (M:F) <sup>c)</sup>	60:60	60:60	60:60	60:60	60:60
Administration period (week) (M:F)	90:98	90:98	90:98	90:98	90:98
Week of necropsy (M:F)	91:99	91:99	91:99	91:99	91:99
Mortality (M:F)	40:32	31:35	29:45	32:41	25:37
Survival rate (%) (M:F)	33.3:46.7	48.3:41.7	51.7:25.0 ↓	46.7:31.7 ↓	58.3 ↑:38.3
Clinical signs	-	-	-	-	-
Palpation	-	-	Mass bearers (F), Neck region (F) and axillary region (F)		
			Dorsal region (F)	Abdominal region (F)	-
Body weight	-	-	↓ (M: Week 2-13) ↑ (F: Week 2-97)	↓ (M: Week 2-13) ↑ (F: Week 3-81)	↓ (M: Week 2-85) ↑ (F: Week 6-49)
Terminal body weight	-	-	-	-	-
Food consumption	-	-	↓ (M: Week 2-4, 90) ↑ (F: Week 2-89)	↓ (M: Week 1-8) ↑ (F: Week 4-85)	↓ (M: Week 1-10, F: Week 1) ↑ (F: Week 5-69)
Hematology	-	-	- - - - ↓ LYMP count (M)	- - - - ↓ LYMP % and count (M)	↑ RBC (M) ↑ HGB (M) ↑ HCT (M) ↑ MCV (M) ↓ WBC (M) ↓ LUC count (M)
Organ weight	-	-	↓ Uterus		
Necropsy	-	-	Ovary, ↓ Nodule (F), Pituitary, ↑ Nodule (F), Skin+Subcutis; ↑ Nodule (F) Uterus; ↓ Endometrial cyst (F)		
Histopathology (Neoplastic lesions)	-	-	Mammary gland; ↑ Adenocarcinoma (F) Pituitary gland, pars distalis; ↑ Adenoma (F)		
			Mammary gland; ↑ Adenosquamous carcinoma (F)		
Histopathology (Non-neoplastic lesions)	-	-	Mammary gland; ↑ Lobular hyperplasia and dilatation and ductal/acinar (F)		
			Mammary gland; ↑ squamous metaplasia (F)		
			Uterus; ↑ Atrophy, ↓ Endometrial cystic hyperplasia and ↑ adenomyosis (F)		
			Vagina; ↑ Mucosal atrophy and mucification (F)		
			Ovary; ↓ Cyst (F)		

a): Water for injection; b): 5 w/v% gum arabic solution; c): Additional 11, 50 or 55 animals/sex/group for each group were used as satellite groups for toxicokinetics; M: Male, F: Female; -: No treatment-related effects; ↑: Increase, ↓: Decrease

## 9 Reproductive and Developmental Toxicology

Table 65: The list of reproductive and developmental pivotal studies with brexpiprazole

Study	Species/Strain (Number/Sex/ Group)	Route/ Duration	Dose (mg/kg/day)	Key Results	Report Number
Female Fertility and Early Embryonic Development to Implantation	Rat/Sprague Dawley (20F/group)	Oral (gavage)/ 2 weeks pre mating to Gestation Day 7	0 (vehicle), 0.3, 3, 30	NOAEL: F <sub>0</sub> females 0.3 mg/kg/day for general toxicity and reproductive performance; Embryos 3 mg/kg/day	020004
Male Fertility	Rat/Sprague Dawley (20M/group)	Oral (gavage)/ 93-99 days (63 days pre mating to necropsy)	0 (vehicle), 3, 10, 100	NOAEL: F <sub>0</sub> males 10 mg/kg/day for general toxicity and 100 mg/kg/day for reproductive capacity	020420
Embryo-fetal Development	Rat/Sprague Dawley (18, 19, or 20Fp/group)	Oral (gavage)/ Gestation Days 7 to 17	0 (vehicle), 3, 10, 30	NOAEL: Dams 3 mg/kg/day for general toxicity and 30 mg/kg/day for reproduction; Fetuses 30 mg/kg/day	019640
Embryo-fetal Development	Rabbit/NZW (16, 18, or 20Fp/group)	Oral (gavage)/ Gestation Days 6 to 18	0 (vehicle), 10, 30, 150	NOAEL: Dams 10 mg/kg/day for general toxicity and 30 mg/kg/day for reproduction Fetuses 30 mg/kg/day	020175
Pre- and Postnatal Development, Including Maternal Function	Rat/Sprague Dawley (21 or 22Fp/group)	Oral (gavage)/ Gestation Day 7 to Lactation Day 20	0 (vehicle), 3, 10, 30	NOAEL: Dams 3 mg/kg/day for general toxicity and 10 mg/kg/day for reproduction Offspring 10 mg/kg/day	023985

F0 = initial parent generation; F1 = first filial generation; FP = pregnant female; GD = gestation day, NZW = New Zealand White.

## 9.1 Fertility and Early Embryonic Development

### Female fertility:

**Study title:** OPC-34712: Oral Study of Fertility and Early Embryonic Development to Implantation in Female Rats

Study no.: 025298; Report no.: 020004  
 Study report location: Archives of Tokushima Research Institute,  
 Otsuka Pharmaceutical Co, Ltd  
 Conducting laboratory and location: Tokushima Research Institute Otsuka  
 Pharmaceutical Co., Ltd.  
 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi;  
 Tokushima, JAPAN  
 Date of study initiation: January 31, 2007  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: OPC-34712, Lot # 05J73M, 99.62% purity

### Key Study Findings

- Female rats were dosed orally at 0, 0.3, 3, and 30 mg/kg/d for 2 weeks prior to mating with untreated males, then treatment continued until Day 7 of gestation.
- Findings include: increased body weight (BW) gain before mating (Days 8 – 11 of treatment) by 54 - 94% at HD and by 68 - 99% at MD
- Reduced BW gain during pregnancy (at HD, 53-34%; GD 2–13 and at MD, 20-15%; GD 5–13)
- Decreased fertility at HD and MD
- Greater pre-implantation loss at HD (17%) than in controls (3%)

### Methods

Doses: 0, 0.3, 3, and 30 mg/kg  
 Frequency of dosing: Once a day  
 Dose volume: 5 ml/kg  
 Route of administration: Oral gavage  
 Formulation/Vehicle: Suspension/5% gum Arabic solution  
 Species/Strain: Rats/Sprague-Dawley [CrI: CD (SD)]  
 Number/Sex/Group: 20/sex/group  
 Satellite groups: none  
 Study design: The females were treated with OPC-34712 for 2 weeks prior to mating and throughout the mating period until GD 7. Females were sacrificed on GD 13 and underwent a complete necropsy.

### Observations and Results

## Mortality

No deaths or sacrifice in moribund condition in any group.

## Clinical Signs:

The general condition of all females was observed twice a day; once prior to dosing and approximately four hrs post-dose during the dosing period. After the dosing period, the observations for abortion or deaths were conducted once a day in the morning. Similarly, untreated males used for co-habitation were also observed once a day.

**Results:** Clinical signs were observed at HD of 30 mg/kg only and included hypoactivity and incomplete eyelid closure (20/20), closed eyes (9/20), lacrimation (12/20), and loose stool (2/20) during the treatment period before mating. During the gestational period, hypoactivity and incomplete eyelid closure (19/19), closed eyes (3/19) and lacrimation (6/19) were observed. No findings were noted at LD of 0.3 or MD of 3 mg/kg.

**Reproductive performance:** OPC-34712 caused altered estrous cycle with the incidence of females having prolonged diestrus at MD and HD (0%, 5%, 80%, and 100% at 0, 0.3, 3, and 30 mg/kg, respectively). One HD female failed to copulate.

Decreased fertility was observed at MD and HD as shown in the following Sponsor's table:

Table 66: Summary of reproductive performance in females

Dose (mg/kg)	0	0.3	3	30
Pairs				
Copulated/Paired	20/20	20/20	20/20	19/20
%	100.0	100.0	100.0	95.0
Fertile/Copulated	19/20	18/20	16/20	15/19
%	95.0	90.0	80.0	78.9
Duration of Pairing (Days)				
Mean+SD	2.6+0.8	3.1+2.6	4.1+3.9	6.0+4.4

## Body Weight

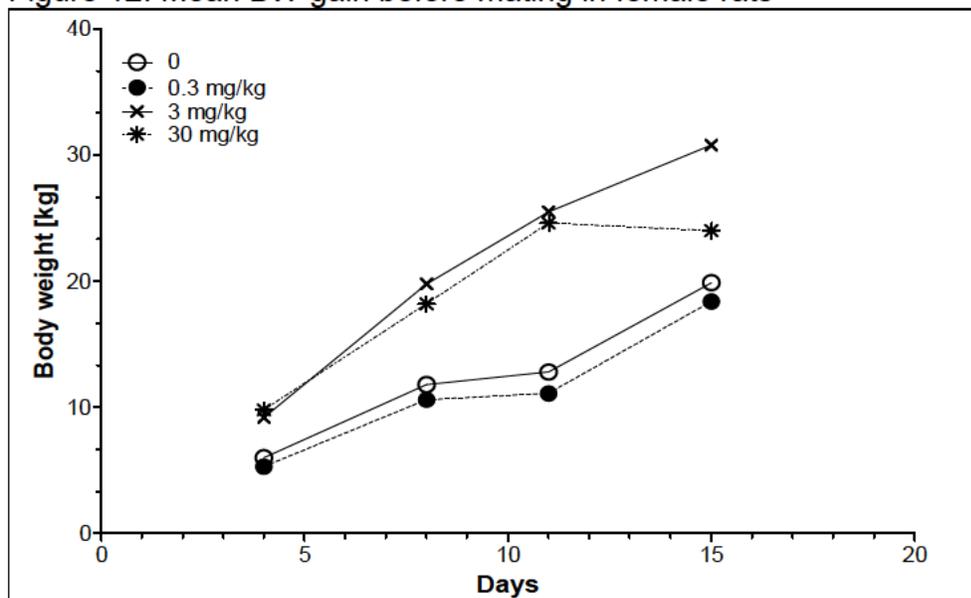
Before mating, the females were weighed twice weekly, than pregnant females were weighed on Days 0–7 and 13 of gestation.

**Results:** Before mating, the BW gain was increased by 68 - 99% and 54 – 92% at MD and HD, respectively on Days 8 -11) compared to controls as it is shown in the following Sponsor's table and this Reviewer's graph:

Table 67: Summary of BW gain (before mating)

Dose (mg/kg) Day	A0	A0.3	A3	A30
4	6.0±4.0 20	5.3±3.7 20	9.2±5.6 20	9.8±8.3 20
8	11.8±4.9 20	10.6±5.0 20	19.8±8.7** 20	18.2±10.1* 20
11	12.8±7.0 20	11.1±7.3 20	25.5±13.0** 20	24.6±13.0** 20
15	19.9±7.4 20	18.4±8.3 20	30.8±12.5** 20	24.0±13.8 20

Figure 12: Mean BW gain before mating in female rats

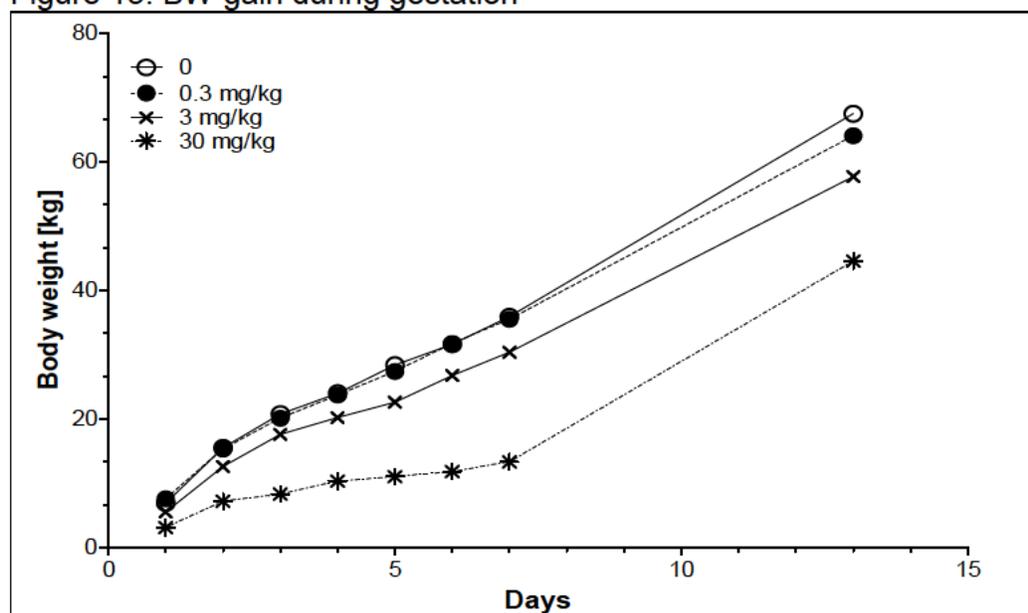


During pregnancy, BW gain of maternal animals was lower between GD 5 and GD 13 at MD (20% - 15%) and between GD 2 and GD 13 at HD (53% - 34%) compared to controls as shown in the following Sponsor's table and this Reviewer's graph:

Table 68: Summary of BW gain during pregnancy

Dose (mg/kg) Day	A0	A0.3	A3	A30
1	6.9±4.1 19	7.6±6.1 18	5.5±3.5 16	3.1±5.7 15
2	15.5±4.5 19	15.3±6.7 18	12.6±4.8 16	7.2±6.4** 15
3	20.8±7.2 19	20.1±7.2 18	17.6±6.0 16	8.3±6.9** 15
4	24.0±5.8 19	23.7±6.7 18	20.2±5.8 16	10.3±7.7** 15
5	28.4±5.7 19	27.4±6.8 18	22.6±7.2* 16	11.0±8.1** 15
6	31.6±7.9 19	31.8±7.8 18	26.8±7.6 16	11.8±7.8** 15
7	35.9±8.4 19	35.5±8.5 18	30.4±7.5 16	13.3±7.9** 15
13	67.5±12.2 19	64.1±12.5 18	57.7±9.3* 16	44.6±11.2** 15

Figure 13: BW gain during gestation



### Feed Consumption

Food consumption was recorded on Day 1 and 8 before mating, and on Days 0–7 and 12 of gestation.

**Results:** OPC-34712 increased food consumptions at MD and HD before mating between Day 1 and Day 7. During pregnancy, the food consumption was decreased at HD (significantly between GD 1 and GD 7) and not changed at MD and LD.

### Toxicokinetics

Not performed

### Dosing Formulation Analysis

OPC-34712 was suspended in 5% gum Arabic solution to make 0.6%, 0.06% and 0.006% suspensions. The Certificate of Analysis (DC060529-2) showed that suspensions were 94.9%, 99.8%, and 102.9%, respectively of the intended concentrations and were confirmed to be acceptable within 90% to 110%.

### Necropsy

Copulated females were sacrificed on GD 13, and examined for pregnancy. Successfully pregnant females were examined for the numbers of corpora lutea, implantations, early deaths (conceptus without embryo or implantation scar), late deaths (embryo limb buds detected), and live embryos were determined.

**Results:** The incidence of pre-implantation loss was greater in the HD group (17%) than in controls (3%). No other changes were observed at HD or at any lower dose of OPC-34712.

**In summary:** Findings of altered estrous cycle, prolonged duration of pairing, decreased fertility, increased pre-implantation losses, increased BW (prior to mating), and increased food consumption are likely to be induced by pharmacologically mediated hyperprolactinemia; similar observations have been made with other antipsychotic drugs in rats. The NOAEL was at 0.3 mg/kg based on BW gain changes and female reproductive performance observed at higher doses of 3 and 30 mg/kg.

#### Male fertility:

#### **Study title:** Male Fertility Study of OPC-34712 Administered Orally to Rats

Study no.: 025658; Report no.: 020420  
 Study report location: Archives of Tokushima Research Institute, Otsuka Pharmaceutical Co, Ltd  
 Conducting laboratory and location: Tokushima Research Institute Otsuka Pharmaceutical Co., Ltd.  
 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi; Tokushima, JAPAN  
 Date of study initiation: April 5, 2007  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: OPC-34712, Lot # 05J73M, 99.62% purity

#### **Key Study Findings**

- Male rats were dosed orally at 0, 3, 10 and 100 mg/kg/d for 63 days (9 weeks) before mating with untreated females and throughout the 2 weeks of mating.
- Significant decreases in BW, BW gain, and food consumption were observed only at the high dose of 100 mg/kg/d, from Week 1 through Week 9 of dosing.

#### Methods

Doses: 0, 3, 10, and 100 mg/kg  
 Frequency of dosing: Once a day  
 Dose volume: 5 ml/kg  
 Route of administration: Oral gavage  
 Formulation/Vehicle: Suspension/5% gum Arabic solution  
 Species/Strain: Rats/Sprague-Dawley [CrI: CD (SD)]  
 Number/Sex/Group: 20/sex/group  
 Satellite groups: none  
 Study design: Male rats were dosed for 63 days (9 weeks) then were cohabited with untreated females for 14 days. The copulated females were cesarean-sectioned on GD 13. The treatment of males continued beyond mating until sacrifice after the completion of cesarean-section.

#### **Observations and Results**

##### **Mortality**

No deaths or sacrifice in the moribund condition in any group.

### Clinical Signs:

Observations were made twice (prior to dosing and about 4 h post-dose) daily during the dosing period, except for holidays (observed only prior to dosing). Untreated females used for mating were observed once daily for the occurrence of abortion or death.

**Results:** Hypoactivity, hunchback position, closed eyes, incomplete eyelid closure, and flaccidity and dilatation of scrotum were observed in all HD males (20/20) at about 4 hrs postdose throughout the dosing period (Days 1 – 64). Other observations at HD were more sporadic and included creeping (2/20), tremors (2/20), lacrimation (14/20), reddish stains around the eyes (6/20), stain around the nose (3/20) and loose stool (2/20). No notable changes of the clinical signs were observed at MD and LD.

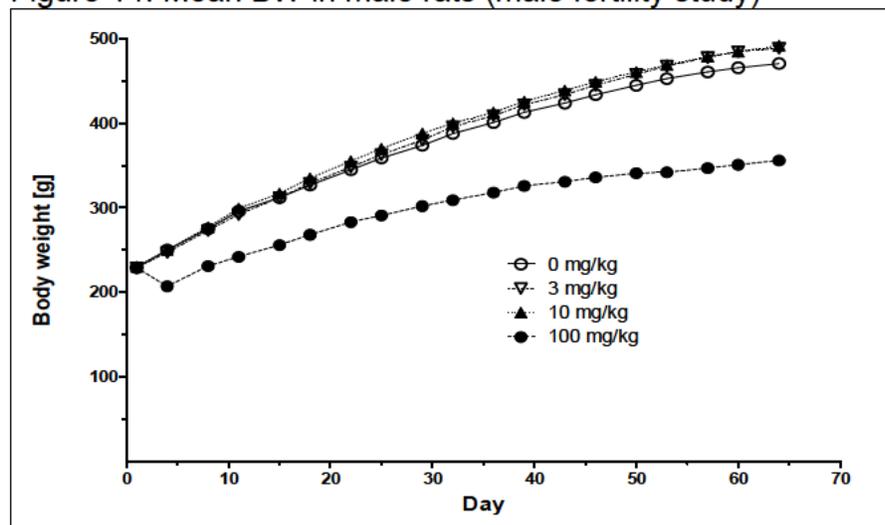
**In the reproductive performance**, evidence of copulation was confirmed in all males during the 14-day mating period, except one pair at LD. The fertility index appeared to decrease dose-dependently; 100%, 90%, and 85% at 3, 10, and 100 mg/kg/d, respectively, however it was not statistically significant vs control value (80%). No difference in pairing duration was also observed among all treatment groups (3.5 - 3.8 days).

### Body Weight

The males before mating were weighed twice weekly. During and after the mating period, the males were weighed once weekly in order to determine the dosing volume and the body weight data were not evaluated.

**Results:** OPC-34712 at HD significantly decreased BW from Day 4 (-17%) up to Day 64 (-24%) of dosing without changes in BW gain at two lower doses as shown on the following graph constructed by this Reviewer based on the mean BW data provided by the Sponsor:

Figure 14: Mean BW in male rats (male fertility study)



### **Feed Consumption**

Food consumption recorded once weekly before mating.

Results: Food consumption was reduced at HD throughout entire dosing period (from 27% on Days 1 – 7 to 14% on Days 57 – 63).

### **Dosing Formulation Analysis**

The suspensions for the initial and final sample preparations were in the range of 100.1 – 104.2% of the intended concentrations.

### **Necropsy**

Cesarean sections of untreated females were performed on GD 13; copulated females were examined for pregnancy and those pregnant were examined for the numbers of corpora lutea, implantations, early deaths (conceptus without embryo or implantation scar), late deaths (embryo limb buds detected), and live embryos (females in which copulation was not determined were sacrificed at the end of mating period).

Males were sacrificed and necropsied after cesarean sections had been performed in females. The external appearance and major internal organs were macroscopically observed.

Results: Cesarean sections revealed no significant differences in the numbers of corpora lutea, implantations, early or late deaths, and live embryos; or the incidences of preimplantation losses, or postimplantation losses between the control group and HD, MD, and LD groups. Necropsy findings in males at HD showed one male with unilateral edematous small testis and small epididymis and 1 male with small prostate; however no abnormalities in the reproductive performance were observed in these males. No other findings potentially related to the treatment were reported.

**Summary:** Treatment related changes in the clinical signs occurred only in males at the HD (100 mg/kg). These findings included hypoactivity, hunchback position, closed eyes, incomplete eyelid closure, and flaccidity and dilatation of scrotum and they were observed throughout dosing period. In addition, some sporadically occurring findings such as creeping, tremors, lacrimation, reddish stains around the eyes, stain around the nose and loose stool were also observed at this HD group. Significant decrease in BW (- 24% versus control) and food consumption also occurred at the HD.

Mating ability and fertility of males were not affected by the treatment with OPC-34712 up to HD of 100 mg/kg. Although, atrophic changes of the reproductive organs (testes, epididymides and prostate) were observed in two HD males, no abnormalities in the reproductive performance of these males were observed. In the 13-Week repeated oral dose toxicity study, similar atrophic changes of the male reproductive organs were also observed at 100 mg/kg/d and higher doses.

The NOAEL for general toxicity was 10 mg/kg/d and for male reproductive capacity was at 100 mg/kg/d.

## 9.2 Embryonic Fetal Development

### Preliminary study in rats:

The purpose of this study (**Study No.: 024309; Report No.: 019146**) was to select doses for the main embryo-fetal development study in rats. OPC-34712 was administered orally to the pregnant rats from GD 7 to 17 at doses of 0 (5% gum Arabic solution), 10, 30 and 100 mg/kg (6 or 7 dams/group). The dams were examined for clinical signs, body weight (BW), food consumption and necropsy. Cesarean section was performed on GD 20 of gestation. The fetuses were examined for their viability, growth and morphology (external, visceral and skeletal examinations).

Results: 2 dams died and 1 dam was sacrificed moribund at 100 mg/kg. Surviving dams showed hypoactivity and incomplete eyelid closure at  $\geq 10$  mg/kg; creeping, closed eyes, lacrimation and stains in the perianal area at  $\geq 30$  mg/kg and hypothermia, hyperreactivity, hunchback position, reddish stain around the eyes, stains around the nose, loose stool, abdominal wetness with urine and vaginal hemorrhage at 100 mg/kg/d. The intensity of observed symptoms was also dose proportional (findings at MD and LD were slight and temporary).

Marked decreases in BW between GD 8 and GD 20 (-29% on GD 20) and food consumption (up to no food consumption) were observed at 100 mg/kg/d. No significant differences were observed at two lower doses on BW but significant decrease in the food consumption was observed at 30 mg/kg/d.

Necropsy findings included, small thymus at  $\geq 30$  mg/kg, and congestion of the lung, pale liver, pale kidneys, dark red content in the intestine, and red and/or black foci in the gastric mucosa at 100 mg/kg/d.

For the embryos and fetuses, total litter losses (all conceptuses resorbed) were observed in three out of four litters at 100 mg/kg, therefore, the incidence of postimplantation losses was increased. These results were considered by the Sponsor to be attributable to the maternal toxicity (e.g., hypothermia and the cessation of the food consumption). Accordingly, the number of live fetuses was so small that the effects on the fetal morphology could not be clearly evaluated at this dose.

In the fetal growth, low fetal weight at 100 mg/kg/d and an increase in the incidences of non-ossified 6<sup>th</sup> sternebra at 30 and 100 mg/kg/d were significantly increased compared with control. In the external, visceral and skeletal morphology, no significant increases in the incidences of the malformations and variations were observed in the 10- and 30-mg/kg/d groups.

Conclusion: Based on the results of this study, a dose of 100 mg/kg/d appeared to be too high for the dams. The Sponsor considered 30 mg/kg/d to be the tolerable toxic dose and would be appropriate as the highest dose in the main embryo-fetal development study in rats.

**Study title: Embryo-Fetal Development Study of OPC-34712  
Administered Orally to Rats**

Study no.: 024869 (Report # 019640)  
 Study report location: Archives of Tokushima Research Institute,  
 Otsuka Pharmaceutical Co., Ltd.  
 Conducting laboratory and Tokushima Research Institute  
 location: Otsuka Pharmaceutical Co., Ltd.; 463-10 Kagasuno,  
 Kawauchi-cho, Tokushima-shi; Tokushima, JAPAN  
 Date of study initiation: October 12, 2006  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: OPC-34712, lot# 05J72M, 99.6% purity

**Key Study Findings**

- Maternal: no deaths or abortions at any dose level (0, 3, 10, and 30 mg/kg/d)
- Hypoactivity, closed eyes, incomplete eyelid closure, reddish stain around the eyes and slight decrease in food consumption at  $\geq 10$  mg/kg/d
- Creeping, lacrimation, loose stool and decreased BW gain (GD 8 – GD 16) by 20% at 30-mg/kg/d
- For the fetuses, no notable changes of the fetal viability or fetal morphology
- NOAEL at 3 mg/kg/d for general toxicology
- NOAEL at 30 mg/kg/d for reproduction in dams and embryo-fetal development

**Methods**

Doses: 0, 3, 10, and 30 mg/kg/d  
 Frequency of dosing: Once a day  
 Dose volume: 5 ml/kg  
 Route of administration: Oral (gavage)  
 Formulation/Vehicle: Suspension/5% gum Arabic solution  
 Species/Strain: Rats/Sprague-Dawley [CrI: CD (SD)]  
 Number of copulated F/Group: 20  
 Satellite groups: None (TK not performed)  
 Study design: Female rats were dosed with OPC-34712  
 formulations for 11 days (GD 7 through GD17).  
 Caesarean section was performed on GD 20.  
 Deviation from study protocol: One control and two HD dams were not pregnant  
 and excluded from evaluation. Accordingly, 19, 20,  
 20, and 18 dams (D) of control, LD, MD, and HD,  
 respectively, were evaluated.

**Observations and Results**

Evaluations included: clinical signs (twice a day), BW and food consumption (daily), gross/histopathology (GD 20), reproductive parameters (corpora lutea, implantations, uterus/placental weight, early/late resorptions, viable fetuses, sex, fetal weight, external malformations/variations, visceral/skeletal malformations/variations).

### Mortality

No deaths or abortions were observed at any dose.

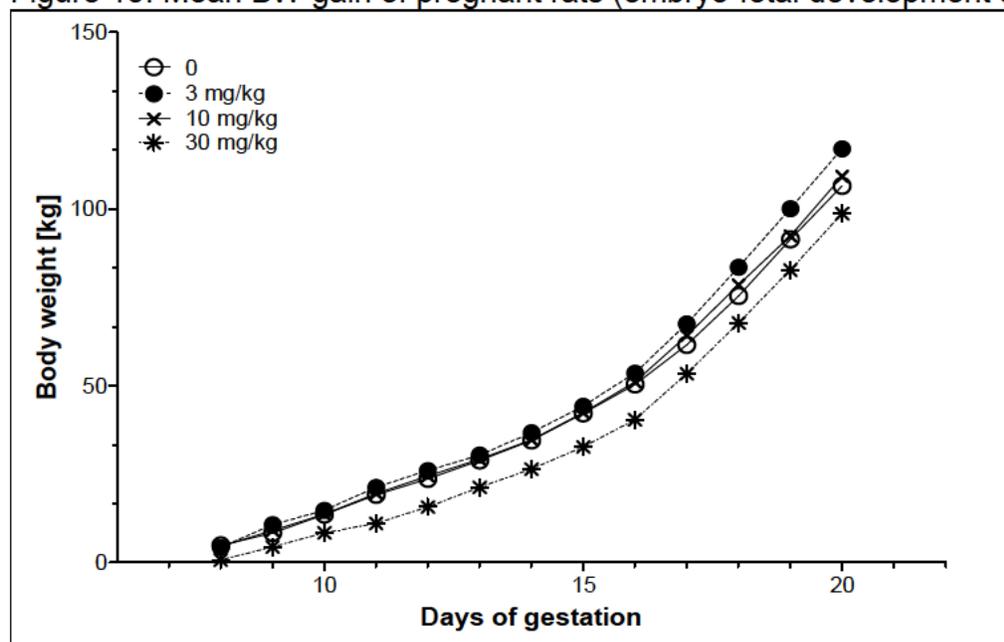
### Clinical Signs

The following findings were observed in HD dams: hypoactivity in all 18/18, creeping in 12/18, closed eyes in 14/18, incomplete eyelid closure in all 18/18, lacrimation in 9/18, reddish stain around eyes in 1/18 and loose stool in 2/18. Findings in MD dams included: hypoactivity in 14/20, closed eyes in 2/20dams, incomplete eyelid closure in 9/20 and reddish stain around the eyes in 1/20. No abnormalities were observed in LD dams.

### Body Weight

BW gain was significantly lower in HD dams on GD 8 through GD 16 (-20% on GD 16) as compared to the corresponding controls. BW gain was not affected in LD dams and MD dams as shown in the following graph constructed by this Reviewer based on provided data:

Figure 15: Mean BW gain of pregnant rats (embryo-fetal development study)



### Feed Consumption

Lower than control food consumption was noted in HD dams on GD 18 and 19 and in MD dams on GD 18.

### Dosing Formulation Analysis

Contents of dosing suspensions were within 95% to 102% of intended concentrations.

### Necropsy

Enlargement of the adrenals in 1/18 HD dams and red thymus in 1/20 LD dams were the only findings occurred at necropsy. These findings, however, were not considered by the Sponsor to be related to the treatment (only one/group and no findings at MD dams).

**Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):**

No effects of treatment with any dose of OPC-34712 were observed on the numbers of corpora lutea, implants and live fetuses, the incidence of pre- and post-implantation losses, sex ratio, fetal weight, and any placental findings.

**Offspring (Malformations, Variations, etc.)**

In all treated groups, no external anomalies were observed. In the control group, the complicated anomaly of anal atresia and thread-like tail in one fetus was noted.

Visceral examination revealed no significant differences between the control and HD group in the incidence of any type of visceral malformations (the malformations observed were membranous ventricular septum defect in two control fetuses and in one fetus at HD). The only skeletal variation of fetuses showed presence of cervical vertebrae (cervical rib) in 4 fetuses of two litters at HD comparing to none in controls.

**Summary:**

No deaths or abortions were observed in any of the treated dams. Clinical signs revealed hypoactivity, closed eyes, incomplete eyelid closure, reddish stain around the eyes and slight decreased food consumption in the MD and HD groups. In addition, creeping, lacrimation, loose stool and slight decreased body weight gain were observed at the HD. These observations were considered to be related to the pharmacology (CNS depressant action) of the test article. Changes of the fetal viability, fetal growth (weight and ossification) and fetal morphology (external, visceral and skeletal findings) were not remarkable between HD and control groups.

The NOAEL in the present study was 3 mg/kg/d for general toxicological effects in the dams, 30 mg/kg/d for effects on reproduction in the dams, and 30 mg/kg/d for embryo-fetal development.

**Preliminary study in rabbits:**

**Preliminary 13-Days Repeated Oral Dose Study of OPC-34712 in Female Rabbits**  
**Study no. 024440 (report no. 019120)**

This study was conducted to select the appropriate dose for the preliminary embryo-fetal development study of OPC-34712 in rabbits. OPC-34712 was administered orally to the non-pregnant female rabbits (3/group) at doses of 0 (5% gum Arabic solution), 10, 100, 300 and 1000 mg/kg for 13 days. Treated females were examined for the clinical signs, BW, food consumption and necropsy.

**Study results**

Two females died (on Day 3 and Day 9 of treatment) and one was sacrificed moribund on Day 9, at 1000 mg/kg/day. No deaths were observed at lower dose groups.

Findings at 300- and 1000-mg/kg included: sedation, hypoactivity, closed eyes (or incomplete eyelid closure), emaciation, lacrimation, stains around the nose, diarrhea, loose stool, stains in the perianal area, marked decrease in BW and cessation of the food consumption. Several symptoms (e.g. hypoactivity, closed eyes) did not recover at about 24 h after dosing. The necropsy revealed small thymus, red foci in the thymus, congestion of the lung, congestion of the liver, red foci in the gastric mucosa, thickening of the pylorus region of the stomach, retention of gas in the intestinal tract, yellowish white content in the cecum, and pale kidney.

Findings at lower dose of 100 mg/kg included hypoactivity, closed eyes (or incomplete eyelid closure) and loose stool which appeared to be slight and temporary (recovered within 24 h after dosing). In the 10-mg/kg group, no notable changes were observed during the dosing period.

### **Conclusion**

Based on the results of this study, 300 mg/kg was considered to be an excessive dose for the females. It was thought that 100 mg/kg (or intermediate dose between 100- and 300-mg/kg), which was considered to be the tolerable toxic dose, would be appropriate as the highest dose in the preliminary embryo-fetal development study in rabbits.

Therefore, the second dose-selection study entitled: *Preliminary Embryo-Fetal Development Study of OPC-34712 Administered Orally to Rabbits* (Study No.: 024658; Report No.: 019424) was conducted.

In that study, OPC-34712 was administered orally to the pregnant rabbits from GD 6 to 18 at doses of 0 (5% gum Arabic solution), 30, 100 and 150 mg/kg (6, 6, 4 and 7 dams/group were evaluated). The dams were examined for the clinical signs, body weight (BW), food consumption and necropsy. Cesarean section was performed on GD 28. The fetuses were examined for their viability, growth and morphology (external, visceral and skeletal examinations).

### Results:

One dam at HD (150 mg/kg/d) and one at MD (100 mg/kg/d) aborted on GD 23 and GD 28, respectively (hypoactivity, miosis, closed eyes, incomplete eyelid closure and loose stool before abortion).

*Clinical observations* revealed: loose stool at  $\geq 30$  mg/kg/d, hypoactivity, miosis, closed eyes, incomplete eyelid closure at  $\geq 100$  mg/kg/d, and emaciation, lacrimation and abdominal wetness with urine at 150 mg/kg/d.

BW gain was decreased in all treatment groups with the greatest severity at the HD and food consumption was also decreased at MD and HD from the start of dosing.

*Necropsy findings* (surviving dams): Abnormalities were primarily observed at HD and included, pale liver in 3 dams, pale kidney in 3 dams, pale heart in 1 dam, retention of clear fluid in the abdominal, thoracic cavity in 1 dam, red foci in the gastric mucosa in 1 dam, and recessed area in the mucosa of the gallbladder in 1 dam at HD and 1 dam at MD. In addition, pale liver was observed in one dam at LD.

*Effects on the embryos and fetuses:*

The only finding was a decrease in the live fetal weight at 150-mg/kg group (HD) and this finding was considered to be attributed to the aggravation of the maternal condition (e.g., emaciation, hypoactivity and the decrease of the food consumption). No notable changes of the fetal viability and morphology (external, visceral and skeletal examinations) were observed at this dose. No notable changes of the fetal growth, viability and morphology were observed at MD and LD.

In conclusion, the dose of 150 mg/kg/d was selected as the highest dose for the main embryo-fetal development study in rabbits.

**Main Study in Rabbits:**

**Study title: Embryo-Fetal Development Study of OPC-34712  
Administered Orally to Rabbits**

Study no.: 025107 (Report # 020175)  
Study report location: Archives of Tokushima Research Institute,  
Otsuka Pharmaceutical Co., Ltd.  
Conducting laboratory and Tokushima Research Institute  
location: Otsuka Pharmaceutical Co., Ltd.; 463-10 Kagasuno,  
Kawauchi-cho, Tokushima-shi; Tokushima, JAPAN  
Date of study initiation: December 20, 2006  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: OPC-34712, lot# 05J73M, 99.62% purity

**Key Study Findings**

OPC-34712 was administered orally to pregnant rabbits from GD 6 through GD 18 at doses of 0, 10, 30 and 150 mg/kg/d.

Toxicity was observed only at HD of 150 mg/kg/d and included:

- maternal toxicity associated with abortion (4 dams), severe clinical signs of emaciation, hypoactivity, lacrimation, miosis, diarrhea and reduced BW and food consumption; pale liver ( $\geq 30$  mg/kg/d), and thickening of the pyloric region of stomach and black foci in the gastric mucosa
- decreased fetal BW
- retarded ossification
- increased incidence of visceral (increases in the incidence of caudal vena cava left to the descending aorta) and skeletal variations (incidence of 8 lumbar vertebrae)

NOAELs: 10 mg/kg/day for general toxicological effects in dams,

30 mg/kg/day for reproductive effects in dams,  
30 mg/kg/day for embryo-fetal development

#### Methods

Doses: 0, 10, 30, and 150 mg/kg/d  
Frequency of dosing: Once a day  
Dose volume: 5 ml/kg  
Route of administration: Oral (gavage)  
Formulation/Vehicle: Suspension/5% gum Arabic solution  
Species/Strain: Rabbits New Zealand White (Kbl:NZW)  
Number of copulated F/Group: 21/group  
Satellite groups: None (TK performed in another study)  
Study design: Female rats were dosed for 13 days (GD 6 - GD18).  
Caesarean section was performed on GD 28.  
Deviation from study protocol: Several copulated females did not become pregnant, therefore, 18, 20, 18, and 16 dams of control, LD, MD, and HD, respectively were evaluated.

#### Observations and Results

Evaluations included: clinical signs (twice a day), BW and food consumption (daily), gross/histopathology (GD 20), reproductive parameters (corpora lutea, implantations, uterus/placental weight, early/late resorptions, viable fetuses, sex, fetal weight, external malformations/variations, visceral/skeletal malformations/variations).

#### Mortality

One copulated female at HD died 5 days after the final dose administration and was excluded from evaluation as necropsy confirmed it was not pregnant.

On GD 9, one HD dam bit off and swallowed a piece the gastric tube, was sacrificed and excluded from evaluation.

#### Clinical Signs

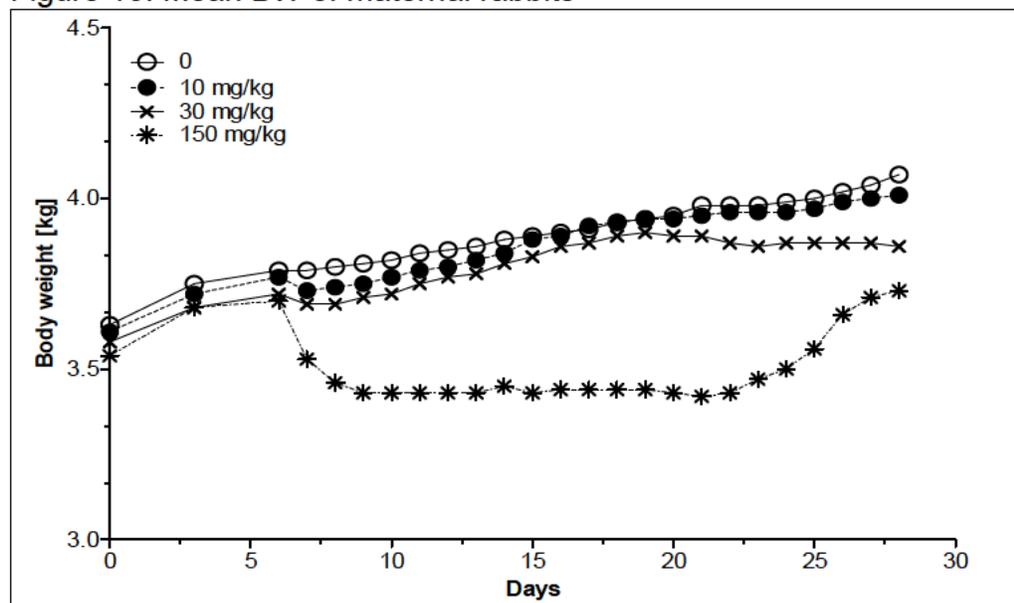
Spontaneous abortions were observed in one control (GD 28), one MD (GD 24), and 4 HD dams (one dam aborted on each of GDs 23, 24, 25, and 26). Clinical signs observed in these dams (HD only) included: emaciation (3/4), hypoactivity 4/4, lacrimation (3/4), miosis (3/4), closed eyes (3/4), incomplete eyelid closure (3/4), diarrhea (2/4), loose stool (4/4) and vaginal hemorrhage (1/4). No abnormal symptoms were observed before abortion of MD and control dams.

Findings in all other HD dams included: emaciation (1/12), hypoactivity (12/12), miosis (3/12), closed eyes (6/12), incomplete eyelid closure (8/12), and loose stool (12/12). At MD diarrhea (1/17) and loose stool (7/17) were also observed.

#### Body Weight

BW and BW gain (compared to GD 6) were significantly lower at the HD as shown on the following graph constructed by this Reviewer based on the mean BW data provided by the Sponsor.

Figure 16: Mean BW of maternal rabbits



### Food consumption

The food consumption was decreased at HD and MD as compared with that of controls. The values were statistically significant on GD 6 through GD 23 for the HD animals and on GD 21 – GD 23 and GD 25 – GD 27 at MD.

### Necropsy

Necropsy findings in 4 aborted dams at HD showed pale heart (2/4), pale liver (4/4), elevated area on the surface of gallbladder (1/4), pale kidney (2/4), thickening of the pyloric region of stomach (3/4), hair ball in the stomach (1/4), decreased rugae in the gastric mucosa (2/4), red foci in the gastric mucosa (1/4) and retention of gas in the intestinal tract (1/4). No anomalies were observed in aborted dam at MD, however in the intrauterine examination of this dam, only two implantation sites were observed in the uterine horns. In addition, for the aborted dam of the control group, red thymus, clear yellow fluid in the thoracic cavity, recessed area in the pyloric region of gastric mucosa, thickening of the pyloric region of stomach and pale liver were observed.

### Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):

The numbers of corpora lutea, implants and live fetuses, sex ratio, the incidence of pre- and post-implantation losses, and placental findings were not affected by the test article administration at any dose. The only finding was decreased weight of live fetuses at HD (statistically not significant) that was reflective of a substantial weight loss by dams at this dose.

### Offspring (Malformations, Variations, etc.)

The incidence of fetal anomaly is summarized in the following Sponsor's table:

Table 69: Summary of fetal anomalies

Dose (mg/kg)		A0	A10	A30	A150
<b>External Anomaly</b>					
Fetuses/Litters Examined	N/N	132/17	150/20	124/17	91/12
Fetuses Affected	N	1	1	0	2
	Mean $\pm$ SD	0.7 $\pm$ 2.7	0.5 $\pm$ 2.0	0.0 $\pm$ 0.0	1.7 $\pm$ 4.0
Litters Affected	N(%)	1( 5.9)	1( 5.0)	0( 0.0)	2( 16.7)
<b>Visceral Variation</b>					
Fetuses/Litters Examined	N/N	132/17	0/0	124/17	91/12
Fetuses Affected	N	56		50	26
	Mean $\pm$ SD	37.6 $\pm$ 23.0		43.2 $\pm$ 20.0	29.8 $\pm$ 19.7
Litters Affected	N(%)	15( 88.2)		17(100.0)	12(100.0)
<b>Visceral Malformation</b>					
Fetuses/Litters Examined	N/N	132/17	0/0	124/17	91/12
Fetuses Affected	N	5		4	5
	Mean $\pm$ SD	3.3 $\pm$ 5.5		2.6 $\pm$ 7.4	5.4 $\pm$ 10.8
Litters Affected	N(%)	5( 29.4)		2( 11.8)	3( 25.0)
<b>Skeletal Variation</b>					
Fetuses/Litters Examined	N/N	132/17	0/0	124/17	91/12
Fetuses Affected	N	86		81	70
	Mean $\pm$ SD	66.1 $\pm$ 22.7		61.7 $\pm$ 29.2	75.7 $\pm$ 27.6
Litters Affected	N(%)	17(100.0)		15( 88.2)	12(100.0)
<b>Skeletal Malformation</b>					
Fetuses/Litters Examined	N/N	132/17	0/0	124/17	91/12
Fetuses Affected	N	2		1	3
	Mean $\pm$ SD	2.0 $\pm$ 8.1		0.7 $\pm$ 2.7	2.8 $\pm$ 6.9
Litters Affected	N(%)	1( 5.9)		1( 5.9)	2( 16.7)

A0 = control, A10 = 10mg/kg, A30 = 30mg/kg, A150 = 150mg/kg

External examination of fetuses showed a club foot in one fetus of each control, LD and HD, a short tail in one fetus at HD and flexion contracture of wrist joint in one HD fetus; these external anomalies are known to occur spontaneously in this strain of rabbits.

Visceral malformations observed included: retrocaval ureter in 3 controls, 4 MD - and 3 HD - fetuses of 3, 2, and 3 litters, respectively, and absence of the gallbladder in 2 fetuses at HD (one litter). The visceral variations observed in the treated groups were the same as observed in control animals with an exception of the incidence of caudal vena cava left to the descending aorta at MD (without statistical significance) and at HD (with statistical significance) was higher than that in the control group.

Skeletal examination showed no significant differences between the control and each treated group in the incidence of any type of skeletal malformations or of the fetuses with skeletal malformations. The malformations observed were deformed cervical vertebral arch in 1 fetus of each, control and HD groups; fused lumbar vertebral bodies in 1 fetus at HD, deformed lumbar vertebral arch in 1 fetus at HD, complex malformation in the sacral and caudal region in 1 fetus at HD (fused sacral and caudal vertebrae, fused caudal vertebral bodies, deformed caudal vertebral body; external examination revealed a short tail), fused ribs in 1 fetus of each of the control and HD groups.

### Dosing Formulation Analysis

Contents of dosing suspensions were within 95% to 102% of intended concentrations.

**Summary:** OPC-34712 administered orally to pregnant rabbits from GD 6 through GD 18 at doses of 0, 10, 30, and 150 mg/kg/day did not cause death at any dose level.

At HD (150 mg/kg/d) four dams aborted. The following clinical signs were observed before abortion: emaciation, hypoactivity, lacrimation, miosis, closed eyes, incomplete eyelid closure, diarrhea, loose stool, vaginal hemorrhage and severely reduced food consumption and decreased BW. Necropsy of aborted dams revealed pale heart, pale liver, elevated area on the surface of gallbladder, pale kidney, thickening of the stomach pyloric region, decreased rugae and red foci in gastric mucosa, and gas retention in the intestinal tract. Clinical observations of surviving dams included loose stool and decreased BW and food consumption, along with emaciation, hypoactivity, miosis, closed eyes and incomplete eyelid closure. Necropsy of surviving dams found thickening of the stomach pyloric region and black foci in the gastric mucosa.

Fetuses showed no notable changes in viability in any treated group. Decreases in live fetal weight and retardation of ossification were observed at 150 mg/kg/d. Fetal morphology did not show any notable external anomalies in all treated groups. No increases in the incidences of visceral and skeletal malformations were observed as compared to controls. However, there was an increased incidence of caudal vena cava left to the descending aorta in visceral variations (in one litter), and the incidence of 8 lumbar vertebrae in skeletal variations in 6 and 7 litters at 30 or 150 mg/kg/d, respectively. These variations occur frequently in rabbits of this strain and were considered minor alterations. At MD (30 mg/kg/day), clinical observations of surviving dams included diarrhea, loose stool, decreased BW, and food consumption. Necropsy of surviving dams noted a hair ball in the stomach, pale liver, and thickening of the pyloric region of stomach. No notable changes in fetal growth were observed in the 10 and 30 mg/kg/day groups.

NOAELs were estimated to be 10 mg/kg/day for general toxicological effects in dams, 30 mg/kg/day for reproductive effects in dams, and 30 mg/kg/day for embryo-fetal development in rabbits.

A separate TK study entitled: Thirteen-day Repeated Oral Dose Toxicokinetics Study of OPC-34712 in Female Rabbits; Study no.:025669 (report no. 020446) was conducted to assess the systemic exposure to OPC-34712. OPC-34712 (lot # 05J73M of 99.62% purity) was administered orally (suspension/5% gum Arabic solution) to the female Kbl:NZW rabbits (3F/group) for 13 days at 10, 30 and 150 mg/kg/d and the plasma concentrations of OPC-34712 were measured after the first and last dosing (Day 1 and Day 13).

The results show the following TK parameters for OPC-34712 in this study:

Table 70: TK parameters in female rabbits

OPC-34712 (mg/kg/d)	C <sub>max</sub> (ng/ml)		t <sub>max</sub> (hours)		AUC <sub>(0-24)</sub> (ng•h/ml)	
	Day 1	Day 13	Day 1	Day 13	Day 1	Day 13
10	220.4	206.4	2	2	2037	1958
30	663.5	842.4	3	4	8129	9202
150	2085	4190	7	4	31170	43680

In summary: The exposure ( $C_{max}$  and  $AUC_{(0-24)}$ ) of OPC-34712 in treated female rabbits has increased dose-dependently on Day 1 and Day 13 (at 150 mg/kg/d, the  $C_{max}$  and  $AUC_{(0-24)}$  on Day 13 were approximately 2 and 1.4 times of those on Day 1, respectively). The  $t_{max}$  on Day 13 was earlier than that on Day 1 in this dose group. In another study entitled: Toxicokinetics Study of OPC-34712 Administered Orally to Pregnant Rabbits (study no.030541; report no. 024787) which used the same doses and dose regimen described above, plasma concentrations of OPC-34712 and its metabolite DM-3411 were determined in pregnant NZW rabbits.

The following Sponsor's table shows the TK parameters of OPC-34712 and its metabolite DM-3411 in pregnant rabbits:

Table 71: TK parameters in pregnant rabbits

OPC-34712 Dose (mg/kg/day)	$C_{max}$ (ng/mL) <sup>a</sup>		$t_{max}$ (hours) <sup>a</sup>		$AUC_{0-24h}$ (ng·h/mL) <sup>a</sup>	
	GD 6	GD 18	GD 6	GD 18	GD 6	GD18
<b>OPC-34712</b>						
10	205.0	1188	2.0	1.3	1987	11870
30	645.9	1720	1.3	4.0	7001	23220
150	2059	3709	4.3	1.3	31640	65350
<b>DM-3411</b>						
10	14.71	42.74	2.0	1.0	88.22	405.0
30	39.89	72.04	1.3	1.3	303.8	716.8
150	155.3	166.6	2.0	9.0	1768	2301

In summary, plasma concentrations of OPC-34712 and DM-3411 were elevated in rabbits in a dose-related manner on GD 6 and 18 at all doses.  $C_{max}$  and  $AUC_{(0-24h)}$  of DM-3411 on GD 18 were increased compared with values on GD 6 at 10 and 30 mg/kg/d but not at 150 mg/kg/d. The exposures of DM-3411 were lower than those of OPC-34712 in each group on GD 6 and GD 18, and the molar ratio of the  $C_{max}$  and  $AUC_{(0-24h)}$  of DM-3411 were less than 7.3% and 5.4% of those of OPC-34712, respectively.

### 9.3 Prenatal and Postnatal Development

#### Study title: An Oral Dose Study for Effects of OPC-331 on Pre- and Postnatal Development, Including Maternal Function, in Rats

Study no.: GH08411 (Otsuka study no.: 028897;  
Report no.: 023985)  
Study report location: EDR  
Conducting laboratory and location: Tokushima Research Institute Otsuka  
Pharmaceutical Co., Ltd., 463-10  
Kagasuno, Kawauchi-cho, Tokushima-shi  
Tokushima, JAPAN  
Date of study initiation: January 15, 2009  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: OPC-331, lot# C07F70M, 99.8% purity

#### Key Study Findings

- OPC-331 was dosed orally to pregnant female rats from implantation to weaning (GD7 to LD20) at 0, 3, 10, and 30 mg/kg/d.
- Maternal generation (F<sub>0</sub>) toxicity:
- Maternal generation (F<sub>0</sub>) toxicity:
  - irregular respiration, incomplete eyelid opening and decrease in movement at  $\geq 10$  mg/kg/d and prone position and piloerection at HD
  - decreased BW and food consumption at HD during the gestation and lactation periods
  - decreased birth index of F<sub>1</sub> generation associated with increased stillbirths and postnatal deaths
  - offspring (F<sub>1</sub> generation) were observed to be scattered in the cage, with subnormal body surface temperature and no milk in the stomach; suggestive of poor nursing of F<sub>0</sub> dams at HD
- F<sub>1</sub> generation toxicity:
  - decreased birth weight and suppressed BW gain in offspring of HD dams
  - decreased viability index on Day 4 of lactation at HD
  - delayed pinna unfolding and decreased number of corpora lutea in the offspring of HD group
  - no treatment-related effects on the weaning index
  - no findings of treatment-related effects on physical development excluding pinna unfolding, early behavior, sensory functions, open field test or conditioned avoidance response
  - no treatment-related effects on mating ability, fertility or gross pathology of offspring (F<sub>1</sub>) or early development of embryos (F<sub>2</sub>)

- The NOAEL for maternal general toxicity was 3 mg/kg/d (based on clinical observation at MD and decreased BW and food consumption at HD) and 10 mg/kg/d for maternal reproductive function (maintenance of pregnancy, delivery and nursing) and for development of the subsequent generation (F<sub>1</sub>).

#### Methods

Doses:	0, 3, 10, and 30 mg/kg/d
Frequency of dosing:	Once a day
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	5% w/v aqueous solution of gum arabic
Species/Strain:	Rats/Crl:CD(SD)
Number/Sex/Group:	F <sub>0</sub> Females, 21 or 22/group
Satellite groups:	None
Study design:	F <sub>0</sub> Females were dosed from gestation day (GD 7) to lactation day (LD) 20 (covering period from implantation to weaning)
Deviation from study protocol:	Not reported

#### Observations and Results (Optional Table)

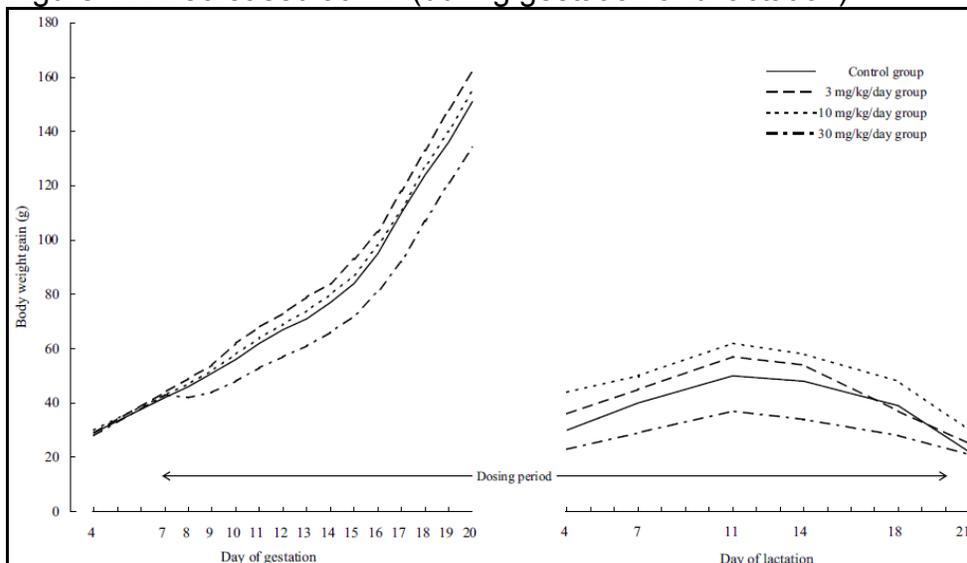
F<sub>0</sub> dams: on LD 21, clinical observations, BW, food consumption, and gross pathology were evaluated. The F<sub>0</sub> dams were also observed for delivery and nursing behavior.

#### F<sub>1</sub> Generation

Status at birth, physical condition, viability index on Day 4 of lactation, weaning index, gross pathology, BW, physical development (pinna unfolding, growth of abdominal hair, eruption of incisors and eyelid opening), early behavior (back righting and negative geotaxis), sensory functions (visual placing response, pupillary reflex, Preyer's reflex and pain response), open field test, conditioned avoidance response (shuttle box test), genital development (preputial separation and vaginal opening), mating ability and fertility. The implantation and viability of embryos (F<sub>2</sub>) was also evaluated.

**Effects on F<sub>0</sub> Dams**

- Survival: No deaths
- Clinical signs: Irregular respiration, incomplete eyelid opening, decreased movement at ≥ 10 mg/kg/d and piloerection (2/22) at 30 mg/kg/d
- Body weight: Figure 17: Decreased at HD (during gestation and lactation)



- Feed consumption: There was lower food consumption at HD (statistically significant decreases on GD 8, 16, and 17 and LD 4, 7, 11, and 14)
- Necropsy observation: Decreased birth index of F<sub>1</sub> generation associated with increased stillbirths and postnatal deaths at MD and HD and decreased BW of newborns at HD as shown in the following Sponsor's table:

	Control	OPC-331 (mg/kg/day)		
		3	10	30
Number of dams	22	21	22	22
Number of dams which delivered	22	21	22	22
Number of dams with a gestation duration of				
21 days	1	3	0	0
22 days	20	16	17	21
23 days	1	2	5	1
Duration of gestation (days, Mean ± S.D.)	22.0 ± 0.3	22.0 ± 0.5	22.2 ± 0.4	22.0 ± 0.2
Number of dams with live newborns	22	21	22	22
Gestation index <sup>a)</sup>	100.0	100.0	100.0	100.0
Number of implantation sites (Mean ± S.D.)	13.4 ± 2.2	14.8 ± 1.9 *	14.2 ± 1.4	14.2 ± 1.3
Number of newborns (Mean ± S.D.)	12.8 ± 2.4	14.1 ± 2.3	13.9 ± 1.4	13.1 ± 1.8
Delivery index (Mean ± S.D.) <sup>b)</sup>	95.2 ± 5.4	95.2 ± 6.9	97.8 ± 3.8	92.6 ± 11.0
Number of live newborns (Mean ± S.D.)	12.7 ± 2.4	13.9 ± 2.2	13.2 ± 1.6	12.0 ± 2.2
Birth index (Mean ± S.D.) <sup>c)</sup>	94.2 ± 5.1	94.1 ± 7.1	93.1 ± 9.0	84.5 ± 14.3 *
Body weight in live male newborns (g, Mean ± S.D.)	6.78 ± 0.50	6.70 ± 0.51	6.69 ± 0.57	6.20 ± 0.37 **
Body weight in live female newborns (g, Mean ± S.D.)	6.38 ± 0.54	6.26 ± 0.44	6.21 ± 0.50	5.89 ± 0.38 **
Male proportion (%; Mean ± S.D.)	48.5 ± 12.4	51.5 ± 13.0	48.4 ± 15.8	46.9 ± 11.7
Number of dead newborns	3	4	15	25
Stillbirths	2	4	9	19
Death <sup>d)</sup>	1	0	6	6
Cannibalism	0	0	0	0
Number of live newborns with external malformations: (%; Mean ± S.D.) <sup>e)</sup>	0 ( 0.0 ± 0.0 )	0 ( 0.0 ± 0.0 )	0 ( 0.0 ± 0.0 )	0 ( 0.0 ± 0.0 )

a) (Number of dams with live newborns / Number of dams) × 100; <sup>b)</sup> (Number of newborns / Number of implantation sites) × 100, litter basis; <sup>c)</sup> (Number of live newborns / Number of implantation sites) × 100, litter basis; <sup>d)</sup> (No. of live newborns with external malformations / No. of live newborns) × 100, litter basis; <sup>e)</sup> Newborns which died immediately after birth. \*: P<0.05, \*\*: P<0.01, significantly different from control

Dosing Formulation Analysis: Contents of dosing suspensions were within 102.5% to 103.9 % of intended concentrations.

F <sub>1</sub> Generation	
Survival:	The numbers of stillbirths and postnatal deaths were increased at HD group and a statistically significant decrease was noted in the birth index in this group as compared to the control group as shown in the Sponsor's table above. Viability index (Day 4 of lactation) was significantly decreased at HD (84%) as compared to the control (100%).
Clinical signs:	Death was observed in some offspring from 1, 1, 8 and 13 dams of control, LD, MD and HD groups, respectively. Subnormal body surface temp and no milk in the stomach in offspring from 7 and 8 dams at HD, respectively were also observed.
Body weight:	Decreases in BW were noted in male and female offspring at HD (0, 4, 7, 11, 14, 18, and 21 days of age) as compared to the control group. Decreased BW was still observed in HD male offspring after weaning, up to 70 days of age.
Feed consumption:	Not performed
Physical development:	Statistically significant decreases in the incidence of pinna unfolding were noted at HD in male offspring at 2 days of age and in female offspring at 2 and 3 days of as compared to the control group, indicating delayed development.
Neurological assessment:	The sensory functions: pupillary reflexes, Preyer's reflexes and pain responses were 100% in all groups. No treatment-related effects were noted in the open field test and in conditioned avoidance response.
Reproduction:	There were no differences in the incidence of preputial separation or vaginal opening between the control and any treated group. Mating and fertility index were not different between any treatment group and control during the first or second mating period.
Other:	No clinical signs or BW differences were observed between treatment groups and control during gestation.

#### Reproductive observations of dams (F<sub>1</sub>) – Sponsor's table:

	Control	OPC-331 (mg/kg/day)		
		3	10	30
Number of dams	21	20	21	22
Number of corpora lutea (Mean ± S.D.)	15.9 ± 2.0	15.1 ± 1.6	15.9 ± 1.3	14.4 ± 1.7 **
Number of implantations (Mean ± S.D.)	15.0 ± 1.4	14.5 ± 2.1	14.6 ± 2.3	13.4 ± 2.6 *
Pre-implantation loss (%; Mean ± S.D.) <sup>a)</sup>	5.1 ± 7.4	4.4 ± 9.1	8.2 ± 12.5	6.9 ± 13.2
Number of live embryos (Mean ± S.D.)	14.2 ± 1.7	13.6 ± 2.6	13.7 ± 2.7	12.6 ± 2.5
Post-implantation loss: (%; Mean ± S.D.) <sup>b)</sup>	17 ( 5.4 ± 6.3 )	18 ( 6.4 ± 11.3 )	19 ( 7.0 ± 8.0 )	17 ( 5.7 ± 6.2 )
Early	0 ( 0.0 ± 0.0 )	0 ( 0.0 ± 0.0 )	0 ( 0.0 ± 0.0 )	0 ( 0.0 ± 0.0 )
Resorb	16 ( 5.1 ± 6.0 )	18 ( 6.4 ± 11.3 )	19 ( 7.0 ± 8.0 )	17 ( 5.7 ± 6.2 )
Dead	1 ( 0.3 ± 1.5 )	0 ( 0.0 ± 0.0 )	0 ( 0.0 ± 0.0 )	0 ( 0.0 ± 0.0 )

a)  $[(\text{Number of corpora lutea} - \text{Number of implantations}) / \text{Number of corpora lutea}] \times 100$ , litter basis

b)  $(\text{Post-implantation loss} / \text{Number of implantations}) \times 100$ , litter basis; \* P<0.05, \*\* P<0.01

In summary: OPC-331 was administered to pregnant female rats from implantation to weaning (GD7 to LD20) at oral doses of 0, 3, 10, and 30 mg/kg/d. No deaths occurred in any dam (F<sub>0</sub>). Toxicities in the maternal generation (F<sub>0</sub>) included irregular respiration, incomplete eyelid opening and decrease in movement at ≥ 10 mg/kg/d and prone position and piloerection at HD. Decreased BW and food consumption, lower birth index and scattering of all offspring in the cage and subnormal body surface temp and no milk in the stomach in offspring suggestive of poor nursing were also observed at HD.

F<sub>1</sub> generation showed low birth weight, decreased viability index on Day 4 of lactation, suppressed BW gain, delayed pinna unfolding and decreased number of corpora lutea were noted in the offspring of HD group. No treatment-related effects were noted on the weaning index, physical development excluding pinna unfolding, early behavior, sensory functions, open field test, conditioned avoidance response, mating ability, fertility or gross pathology of offspring (F<sub>1</sub>) or early development of embryos (F<sub>2</sub>).

The NOAEL for maternal general toxicity was LD of 3 mg/kg/d (based on clinical observation at MD and decreased BW and food consumption at HD) and MD of 10 mg/kg/d for maternal reproductive function (maintenance of pregnancy, delivery and nursing) and for development of the subsequent generation (F<sub>1</sub>).

### **Summary of reproductive toxicities**

Effects of brexpiprazole (OPC-331 or OPC-34712) on fertility and early embryonic development were assessed in rats with males and females treated in separate studies. In females dosed at 0, 0.3, 3, and 30 mg/kg/d for two weeks, altered estrous cycle (prolonged diestrus) and decreased fertility occurred at doses ≥ 3 mg/kg/d, and pre-implantation losses were increased significantly at 30 mg/kg/d. The NOAELs for brexpiprazole in this study were 0.3 mg/kg/d for general toxicological effect in females (clinical signs of hypoactivity, incomplete eyelid closure, and lacrimation at higher doses), 0.3 mg/kg/d for effects on female reproductive performance, and 3 mg/kg/d for early embryonic development. Slightly impaired fertility at MD and HD, including increased preimplantation losses, was considered to be related to prolonged diestrus caused by hyperprolactinemia due to an effect of brexpiprazole at the D2 receptor (partial agonist).

Treatment of male rats with brexpiprazole (at doses of 0, 3, 10 and 100 mg/kg/d for 63 days before mating with untreated females) had no effect on mating, copulation, fertility index and cesarean section parameters. The NOAELs for brexpiprazole in this study were 10 mg/kg/d for general toxicity (clinical signs and decreased BW gain) and 100 mg/kg/d for effects on male reproductive capacity.

In embryo-fetal development studies, brexpiprazole was not teratogenic in rats at doses up to 30 mg/kg/d or in rabbits, up to 150 mg/kg/d. In rats dosed orally for 11 days (GD 7 – GD 17) at 0, 3, 10, and 30 mg/kg/d, no deaths or abortions occurred in any dam. The MTD was achieved based on observed clinical signs of hypoactivity, incomplete eyelid closure, creeping, lacrimation and significant reduction in BW gain at HD. No notable reproductive effects in dams or effects on embryo-fetal development were observed at

any dose level. The NOAEL for general toxicity was at 3 mg/kg/d and for reproduction and embryo-fetal development at 30 mg/kg/d.

Pregnant rabbits were dosed orally for 13 days (GD 6 – GD18) at 0, 10, 30, and 150 mg/kg/d. The MTD was achieved; 4 dams with clinical signs, severely reduced food consumption and BW aborted at 150 mg/kg/d. Necropsy of aborted dams revealed findings in the heart, liver, gastrointestinal tract and kidney. Similar findings were noted in dams with regular pregnancy at HD. In fetuses, decreased BW and retardation of ossification and slightly increased incidences of skeletal and visceral variations were observed at 150 mg/kg/d. The NOAELs were at 10 mg/kg/d for general toxicity in dams and 30 mg/kg/d for reproductive effects, and for embryo-fetal development in rabbits.

In the pre- and postnatal study in rats, brexpiprazole was dosed orally to pregnant females from implantation to weaning (GD 7 to LD 20) at 0, 3, 10, and 30 mg/kg/d. No deaths occurred in any dam (F<sub>0</sub>). Toxicities in the maternal generation (F<sub>0</sub>) included irregular respiration, incomplete eyelid opening and decrease in movement at ≥ 10 mg/kg/d and prone position and piloerection at HD. In dams treated with brexpiprazole at HD, decreased BW and food consumption, increased stillbirths and postnatal deaths associated with decreased birth index of subsequent F<sub>1</sub> generation were also observed. Other findings included offspring scattered in the cage, subnormal body surface temperature, and no milk in the stomach of F<sub>1</sub> generation suggesting poor nursing by the F<sub>0</sub> dams at HD. F<sub>1</sub> generation showed low birth weight, decreased viability index on Day 4 of lactation, suppressed BW gain, delayed pinna unfolding and decreased number of corpora lutea in the offspring of HD group. No treatment-related effects were noted on the weaning index, physical development excluding pinna unfolding, early behavior, sensory functions, open field test, conditioned avoidance response, mating ability, fertility or gross pathology of offspring (F<sub>1</sub>) or early development of embryos (F<sub>2</sub>).

The NOAEL for maternal general toxicity was at 3 mg/kg/d (based on clinical observation at 10 mg/kg/d and decreased BW and food consumption at 30 mg/kg/d) and the NOAEL at 10 mg/kg/d for maternal reproductive function (maintenance of pregnancy, delivery and nursing) and for development of the subsequent generation (F<sub>1</sub>).

## **10 Special Toxicology Studies**

### **Mechanistic studies**

#### **1) Influence of Alleviation of Hypothermia on Brain and Testes Toxicity in Male Rats Treated with OPC-34712**

Study No. 025190; Report No. 020031

Study initiation date: 15 Feb 2007; GLP compliant

#### **Background:**

In the previously conducted repeat-dose toxicity studies of OPC-34712 in rats, the neuropathological changes and testicular toxicities were observed and were considered to be associated with extreme low body temperature. To investigate the pathogenesis of these changes current study was conducted to evaluate a preventive effect of hypothermia-alleviation on OPC-34712 induced brain and testis toxicities in rats.

Two groups of male rats (20 animals each) were housed under different conditions:

- 1) individually in stainless-steel bracket cages with a wire bottom floor without any bedding material
- 2) 5 animals were housed collectively in each polycarbonate cage with paper pulp bedding.

All rats were treated with OPC-34712 at 1000 mg/kg/day by gavage for 7 days. Mortality, general conditions, body weights, body temperature, plasma concentrations of OPC-34712 and histopathological examinations of the brains, testes and epididymides were evaluated.

### Results:

Four animals died and two animals were sacrificed due to deteriorating conditions in the individually housed group, and four animals died and one animal was sacrificed in the collectively housed group, indicating similar results between the two groups. Clinical signs observed in this study were consistent with those in previously conducted repeat dose studies, including hypoactivity, abnormal postures (hunchback position or creeping), closed eyes or incomplete eyelid closure, and scrotal flaccidity and dilatation were observed in all animals of both groups. In contrast, hypothermia (cold to touch at body surface) was observed in most individually housed animals but not in the collectively housed group suggesting that collective housing prevents the OPC-34712 induced hypothermia.

Low body temperatures were recorded throughout the dosing period, particularly, the mean body temperatures were reduced drastically at the beginning of dosing (Day 1 to Day 3) and then they became somewhat milder on Day 4 and thereafter. In contrast, no animals revealed extremely low body temperature throughout the dosing period in the collectively housed group as it is shown in the following Sponsor's table:

Table 72: Body temperature of individually or collectively housed rats treated with OPC-34712

Time Point	1000 mg/kg Individually Housed	1000 mg/kg Collectively Housed
Day 1, Pre-Dose <sup>a</sup>	37.04 ± 0.36	36.85 ± 0.26
Day 1	30.30 ± 0.94	35.90 ± 0.96**
Day 2	26.72 ± 1.15	36.40 ± 0.47**
Day 3	29.33 ± 3.04	35.13 ± 0.76**
Day 4	32.22 ± 2.69	36.43 ± 0.85**
Day 7	32.44 ± 1.58	35.42 ± 0.56**

<sup>a</sup> All values are for 6 hours postdose except for Day 1 pre-dose.

### Histopathology;

Histopathological findings in the brain and/or testes in individually and collectively housed rats (dosed with OPC-34712 at 1000 mg/kg for 7 days) are shown in the following Sponsor's table:

Table 73: Brain and testicular histopathology in individually and collectively housed rats

Findings <sup>a</sup>	Stage	During Dosing <sup>b</sup>		End of Dosing <sup>c</sup>		Total	
	Housing Condition	Individual	Collective	Individual	Collective	Individual	Collective
	Number of Rats	6	5	14	15	20	20
<b>Brain</b>							
Necrosis, Granule Cells, Olfactory Bulb		4	0	10	0	14	0
Necrosis, Oligodendrocytes		3	0	1	0	4	0
Vacuolation, Cerebrum		2	0	4	0	6	0
Demyelination		0	0	4	0	4	0
Necrosis, Purkinje Cells		3	0	7	0	10	0
Fluoro-Jade C-positive Purkinje Cells		1	0	7	0	8	0
Vacuolation, Cerebellum		2	0	5	0	7	0
Vacuolation, Medulla Oblongata		1	0	3	0	4	0
Autolysis		1	4	0	0	1	4
<b>Testes</b>							
Nuclear Vacuolation, Rounded Spermatids		4	0	8	0	12	0
Degeneration/Necrosis, Germ Cells, Stage VII		0	0	1	7	1	7
Degeneration/Necrosis, Germ Cells, Stage XIV		3	0	5	0	8	0
Multinucleated Giant Cell Formation, Seminiferous Tubules		2	0	7	0	9	0
Decrease, Germ Cells		2	0	11	0	13	0
Retention, Step 19 Spermatids		2	0	8	2	10	2
Autolysis		1	3	0	0	1	3

<sup>b</sup> Data are from animals that died or were euthanized in moribund condition prior to the end of dosing.

<sup>c</sup> Data are from animals sacrificed as scheduled on Day 8, after the end of dosing.

The neurotoxicity and testicular lesions were observed frequently in the individually housed group, but no drug-related neurotoxic lesions were observed in the collectively housed group. The degeneration/necrosis of germ cells at stage VII was observed mainly in the collectively housed group. The sponsor offered the explanation that the lesion might be caused by the nutrient starvation or the disturbance of hormones and surfaced under the condition as without depressed spermatogenesis at stage XIV. In the epididymides, the changes reflecting depressed spermatogenesis in the testes as the decrease of sperms and exfoliated germ cells in epididymal duct were found in the

individually housed group. Exfoliated germ cells were also observed in the collectively housed group, however the grade of findings was limited to very slight in this group and it was considered that the finding would be a within normal limit of the histological variations.

#### Toxicokinetics:

$C_{max}$  and  $AUC_{24h}$  of OPC-34712 on Days 1 and 7 of the collectively housed group were slightly lower than those of the individually housed group, but the differences between the results of two groups were less than 2-fold as it is shown in the following Sponsor's table:

Table 74: TK parameters of OPC-34712 in individually and collectively housed rats

Parameter	Day	1000 mg/kg <sup>a</sup>	
		Individually Housed	Collectively Housed
$C_{max}$ (µg/mL)	1	4.41	3.63
$C_{max}$ (µg/mL)	7	10.87	7.95
$AUC_{0-24h}$ (µg·h/mL)	1	89.9	62.9
$AUC_{0-24h}$ (µg·h/mL)	7	197	108

#### **Summary and conclusion:**

Based on the results of this study, pharmacologically-induced hypothermia, rather than a direct toxicity of OPC-34712, was considered to be the cause of histopathologic lesions in the brain and testes of rats (individually housed) in the repeat-dose toxicity studies. Thermogenesis is under the direct control of the CNS, particularly by the medial preoptic/anterior hypothalamic region. In the temperature regulation, it is imperative that the animal body can transfer internal heat out away from the body and into the external environment, thus, the housing condition is an important factor regarding the thermogenesis. From the result of this study, hypothermia in individually housed rats was considered to be related to the pharmacology of OPC-34712 which caused CNS depression. Moreover, in rats housed collectively in the cage containing paper pulp bedding, OPC-34712 did not cause body temperature to decrease, no brain lesions and testicular abnormalities were developed.

#### **2) Serum Prolactin Level in Mice and Rats**

These studies were conducted in order to confirm if brexpiprazole increases serum prolactin level, as was suggested in the carcinogenicity study in mice and rats and the repeated dose toxicity studies and female fertility study in rats.

In mice, brexpiprazole was orally administered by gavage to groups of 6 males and 6 females each at doses of 0 (control) and 5 mg/kg, and serum prolactin levels were determined. Results are shown in the following Sponsor's table (*Report No. 028842*):

Table 75: Serum concentrations of prolactin in mice given a single oral dose of brexpiprazole (OPC-34712)

Sex	Dose (mg/kg)	Number of Animals	Serum Prolactin Concentration (ng/mL)	
			1 h After Administration	4 h After Administration
Male	0 <sup>a</sup>	6	<1.56	<1.56
	5	6	4.47*	2.04
Female	0 <sup>a</sup>	6	<1.56	<1.56
	5	6	90.15**	23.06**

<1.56 ng/mL = below the lower limit of quantification; <sup>a</sup> Animals in the control group were administered the vehicle (5% gum arabic solution); \*\*P ≤ 0.05, \*\*P ≤ 0.01 vs the control group.

Brexpiprazole at the oral dose of 5 mg/kg increased serum prolactin level at 1 h after administration in males and 1 and 4 h after administration in females and the increase was higher in females than in males.

In rats, brexpiprazole was orally administered by gavage to groups of 6 males and 6 females each at doses of 0 (control) and 100 mg/kg for males and 0 (control) and 30 mg/kg for females, and serum prolactin levels were determined. The results of this study demonstrated that serum prolactin was increased at 1 and 4 h after brexpiprazole administration as it is shown in the following Sponsor's table (*Report No. 020467*):

Table 76: Serum concentrations of prolactin in rats given a single oral dose of brexpiprazole

Sex	Dose (mg/kg)	Number of Animals	Serum Prolactin Concentration (ng/mL)	
			1 h After Administration	4 h After Administration
Male	0 <sup>a</sup>	6	6.19	7.16
	100	6	42.05**	28.33**
Female	0 <sup>a</sup>	6	4.29	1.49
	30	6	424**	140**

<sup>a</sup> Animals in the control group were administered the vehicle (5% gum arabic solution); \*\*P ≤ 0.01 vs the control group

### 3) Drug-dependence Studies

The Controlled Substance Staff will review the abuse potential for brexpiprazole.

### 4) Immunotoxicity Studies

#### Four-week Repeated-dose Oral Immunotoxicity Study in Rats (*Report No.025948*)

The immunotoxic potential of brexpiprazole was evaluated by determining the effects on humoral immune response to sheep red blood cells (SRBC) in Sprague-Dawley rats. Brexpiprazole at doses of 0 (control), 3, 10, or 30 mg/kg/d was administered orally (gavage) groups of 10 males and 10 females for 4 weeks. At 4 days before scheduled sacrifice, animals were immunized with an SRBC suspension by single IV injection. On the day of sacrifice, the spleen was weighed and processed to prepare a single-cell suspension, which was reacted with the antigen to evaluate antigen-specific antibody (IgM) response by plaque-forming cell assay.

Treatment-related findings in males or females at 30 mg/kg/d included CNS-related findings such as hypoactivity and incomplete eyelid closure and decreased BW and food consumption. Increased BW and food consumption were noted in females at 10 mg/kg/day. No changes in spleen weight or SRBC-specific antibody response were observed in brexpiprazole-dosed animals compared with the control group, suggesting that brexpiprazole has no effect on the rat humoral immune system, therefore was not considered to be immunogenic.

## 5) Photosafety studies

### In Vitro Phototoxicity Test Using BALB/3T3 Cells (Report No. 023818)

The potential phototoxicity of brexpiprazole was examined using BALB/3T3 clone A31 cells. The cells were treated with brexpiprazole for 1 h and then exposed to 5 J/cm<sup>2</sup> of UVA light using a solar simulator or kept in the dark for 50 minutes. On the following day, cell viability was measured by neutral red uptake assay. Cells were treated with brexpiprazole at 0 (vehicle control) or 0.0632-200 µg/mL in the dose-finding test and 0 (vehicle control) or 0.6-10 µg/ml in the main test.

In the preliminary test, brexpiprazole showed cytotoxic effect at a precipitating dose of 20 µg/ml without irradiation, and IC<sub>50</sub> was calculated to be 17.53 µg/ml. With irradiation, cytotoxicity was noted at lower non-precipitating doses and IC<sub>50</sub> was calculated to be 3.285 µg/ml. In the definitive test, without irradiation, cell viability was 99.6% even at the highest dose of 10 µg/ml and therefore IC<sub>50</sub> could not be calculated. With irradiation, brexpiprazole showed cytotoxicity, with an IC<sub>50</sub> of 2.021 µg/ml.

Brexpiprazole was considered to be phototoxic based on established criteria (the mean photo effect of 0.506, exceeding the phototoxicity criteria of 0.15).

Brexpiprazole was not phototoxic *in vivo* in female albino (CD-1) mice (Report No. 024516) or pigmented (B6D2F1) mice (Report No. 024515) given up to 20 mg/kg brexpiprazole ±Irr once or for 3 consecutive days, respectively.

## 6) Impurities and Intermediates

Several impurities have been identified in the drug substance and those were tested for potential mutagenicity in the Bacteria Reverse Mutation assay; *S typhimurium* (TA100, TA1535, TA98, and TA1537) and *Escherichia coli* (*E coli*; WP2uvrA/pKM101 or WP2uvrA) were used as test systems. The substances were dissolved in DMSO, and tested with and without S9 up to a max dose of 5000 µg/plate or to a cytotoxic dose. The results of these studies are shown in the following Sponsor's table:

Table 77: Mutagenicity results for OPC-34712 production intermediates and impurities

Substance	Result				Report Number
	<i>S. typhimurium</i>		<i>E. coli</i>		
	+S9	-S9	+S9	-S9	
(b) (4)				(b) (4)	029244
					025670
					029245
					029246
					029247
					029248
					029249

(b) (4) When a positive mutagenic response occurred, the strain that exhibited the response is indicated.

Except for (b) (4), all of the intermediates were not mutagenic in the bacterial reverse mutation assay with or without S9. (b) (4) was identified as an impurity in the drug substance for which in silico modeling, showed structural alert for mutagenicity. The results of the Bacteria Reverse Mutation assay showed that (b) (4) is mutagenic. There was an increase in numbers of revertant colonies equal to or higher than twice the mean number of the negative control group in *S. typhimurium* TA1535 and TA100 strains with S9 as well as in TA1535 strain without S9. In this study (*Report No. 029246*) the numbers of revertant colonies in the negative and positive controls were within the acceptable variation range of the controls in the testing center, therefore, this test was judged to be valid. A specification of  $\leq$  (b) (4) ppm was established for this impurity. Based on a MHRD of 4 mg/day, the estimated dose of this impurity was calculated to be (b) (4)  $\mu\text{g}/\text{day}$ ; a dose below the threshold of toxicological concern ((b) (4)  $\mu\text{g}/\text{day}$ ).

(b) (4) was also identified as an impurity in the drug substance. The specification of this impurity has been established at  $<$  (b) (4) %, and is considered qualified. The levels of this impurity in the batches used for the pivotal toxicology studies were about (b) (4) % and (b) (4) %. At the highest doses used in the 26-week and 39-week toxicity studies with rats and monkeys, respectively, the estimated dose of (b) (4) administered to animals was 90  $\mu\text{g}/\text{kg}$  for rats and 27  $\mu\text{g}/\text{kg}$  for monkeys. At these estimated doses for (b) (4), the margin of safety (body surface area basis) to the MRHD (4 mg/day), corrected for the maximum (b) (4) % specification, was approximately 103- and 61-fold for the rat and monkey, respectively. Furthermore, brexpiprazole is not genotoxic at calculated doses of the impurity of (b) (4)  $\mu\text{g}/\text{kg}$  and higher. Therefore, additional testing with this impurity was not undertaken.

During the review cycle, the CMC reviewer identified another impurity (b) (4) and requested additional information from the Sponsor.

(b) (4). In addition to being a process impurity and a possible degradation product, (b) (4) is a minor metabolite of brexpiprazole in animals and humans. In order to assess the potential genotoxicity of (b) (4), a DEREK *in-silico* analysis for mutagenicity was conducted prior the NDA submission and the data showed that it was not mutagenic. Furthermore, no (b) (4) was detected in any batches of the drug substance used in the developmental stage (including nonclinical batches) or formal stability studies (limit of detection: < (b) (4) %). The amounts of (b) (4) in the drug substance were < (b) (4) % which was below the reporting threshold according to ICH Q3A (R2) indicating it is well controlled in the brexpiprazole drug substance.

To supplement DEREK *in-silico* analysis for mutagenicity with a statistical *in-silico* method in accordance with the ICH M7 guideline, a second *in silico* analysis was conducted with (b) (4). The Leadscope Genetox Expert Alerts and two Leadscope statistical-based QSAR models for 'Microbial in vitro - (b) (4)' and 'Microbial in vitro - (b) (4) mutation' were used for the assessment. The specific functionality in Leadscope called 'ICH M7 settings' was applied when running the predictions.

The obtained data allowed the overall conclusion that (b) (4) would be classified as a non-mutagenic substance.

## 11 Integrated Summary and Safety Evaluation

**Pharmacology:** Brexpiprazole is a partial agonist at serotonergic 5-HT<sub>1A</sub> (K<sub>i</sub>=0.09/0.12nM; rat/human, respectively) and at dopaminergic D<sub>2</sub> (K<sub>i</sub>=0.35/0.30nM; rat/human) receptors and antagonist at serotonergic 5-HT<sub>2A</sub> (K<sub>i</sub>=3.8/0.47nM; rat/human) as well as antagonist at noradrenergic α<sub>1/2</sub> receptors, having a broad spectrum of binding affinities and actions on several other central monoaminergic receptor subtypes. Overall, the broad spectrum of brexpiprazole receptor binding profile shows that it has high affinity for multiple monoaminergic receptors including serotonin 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5HT<sub>7</sub>, dopamine D<sub>2</sub>, D<sub>3</sub>, and noradrenergic α<sub>1A</sub>, α<sub>1B</sub>, α<sub>1D</sub>, and α<sub>2C</sub> receptors. Brexpiprazole acts as a partial agonist at the 5-HT<sub>1A</sub>, D<sub>2</sub>, and D<sub>3</sub> receptors and as an antagonist at 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5HT<sub>7</sub>, α<sub>1A</sub>, α<sub>1B</sub>, α<sub>1D</sub>, and α<sub>2C</sub> receptors. Additionally, brexpiprazole showed some inhibitory effect and *ex vivo* binding occupancy of 5-HT transporters in rats, but has lower affinity for the human 5-HT transporter. This assessed receptor profile of brexpiprazole was reflected in the results of microdialysis studies in rats (nucleus accumbens, mPFC, and dorsal hippocampus). The slight reduction in extracellular DA in nucleus accumbens, combined with a small increase in DA metabolite levels, is consistent with the D<sub>2</sub> receptor partial agonist activity at DA autoreceptors. However, no significant changes were seen in mPFC and dorsal hippocampus compared to vehicle-treated rats, except that histamine levels were increased in mPFC. None of the 5-HT, NE, or ACh levels were also affected. According to the Sponsor, this unexpected observation could be due to potential additional target effects (e.g., α<sub>1</sub>-adrenergic antagonism) which may counteract and, accordingly, mask any expected increases such as in 5-HT in mPFC.

The brexpiprazole main metabolite DM-3411 occurs in significant concentrations in plasma from rodents and humans (> 10% compared with total drug-related exposure) and has a pharmacological profile similar to brexpiprazole, but is generally less potent (i.e., binding affinity to hD<sub>2L</sub>, 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> is 16-, 70-, and 5.5-fold, respectively, lower than brexpiprazole). However, DM-3411 was not detected in brains of rats treated with a 1000 mg/kg dose of brexpiprazole, suggesting poor brain penetration. Thus, it is unlikely this metabolite will influence the CNS-related effects of brexpiprazole.

In predictive animal models for antipsychotic-like efficacy, brexpiprazole inhibited apomorphine-induced hyperlocomotion and stereotyped behavior in rats as well as conditioned avoidance response in rats. Furthermore, despite the lower intrinsic activity for D<sub>2</sub> receptor and higher antipsychotic potency, the liability for catalepsy of brexpiprazole was comparable with that of aripiprazole, but less than that for haloperidol, olanzapine, and risperidone, suggesting brexpiprazole has a low potential to induce EPS. In rodent models of antidepressant-like activity (forced swim test and chronic mild stress) brexpiprazole did not show any antidepressant effect, but markedly potentiated the effects of antidepressants (e.g., SSRIs or SNRIs).

**Safety pharmacology:** Brexpiprazole had an effect on the CNS that was related to its exaggerated pharmacology; it decreased body temperature in rats, a finding also observed in repeat-dose toxicity studies, with the hypothermic response related to an

effect of the drug at the D<sub>2</sub> receptor. Moreover, the severe hypothermic response observed in the repeat-dose studies in rats may explain the brain lesion at ≥300 mg/kg/d (730-fold MRHD on mg/m<sup>2</sup> basis) in males and ≥100 mg/kg/d in females (243-fold MRHD of 4 mg; see discussion below).

Decreased blood pressure (BP) and prolonged QT interval and QTc were noted in the conscious dog model, and on Day 1 of the 13-week repeat-dose toxicity study with monkeys. The effect of brexpiprazole on decreased BP was suggested to be due to a blockade of α<sub>1</sub>-adrenoceptors in peripheral blood vessels, which is consistent with its pharmacological profile. The observed QT prolongation in these studies appeared to correlate with the decrease in body temperature, and QT prolongation has been associated with a decrease in body temperature. Therefore, the QT effect observed in the monkey may be indirectly associated with the pharmacologically-mediated decrease in body temperature, although brexpiprazole inhibited the hERG current; the IC<sub>50</sub> was at 0.117 μM.

**Pharmacokinetics:** The PK of brexpiprazole were investigated in a number of *in vitro* and *in vivo* studies conducted in mice, rats, rabbits, dogs, and monkeys, species that were utilized for toxicological assessment. An approximately linear pharmacokinetic profile was observed in rats from 1 to 30 mg/kg and in monkeys from 0.1 to 3 mg/kg. Brexpiprazole showed low bioavailability in rats (13.6%) and monkeys (31%); however, this did not preclude achieving relevant systemic exposures to brexpiprazole in pivotal nonclinical toxicity studies. There was no appreciable sex difference in tissue distribution pattern in rats.

The biotransformation profiles of brexpiprazole were qualitatively similar across species. CYP3A4 was the primary enzyme responsible for the metabolism of brexpiprazole in human liver microsomes. In addition, CYP2D6 and FMO3 were considered partially involved in the metabolism of brexpiprazole. Fecal excretion was the main route of elimination of brexpiprazole in rats and monkeys. There was minimal potential for pharmacokinetic drug interaction by brexpiprazole and the major metabolite DM-3411.

The nonclinical studies demonstrated that animal species tested were acceptable species for the safety evaluation of brexpiprazole and its major metabolites.

**General Toxicology:** In the single-dose oral toxicity studies, the minimum lethal dose in male rats was > 2000 mg/kg and between 800 mg/kg and 2000 mg/kg in female rats. In male and female monkeys, the minimum lethal dose was > 100 mg/kg, the highest dose tested. Clinical signs observed in these studies were related to the pharmacological effects of brexpiprazole on the CNS and included hypoactivity, closed eyes, and abnormal postures, and hypothermia. Based on these data, brexpiprazole has low acute toxicity in the rat and monkey.

Repeat-dose toxicity studies were conducted in rats at doses up to 100 mg/kg/day for up to 26 weeks and in cynomolgus monkeys at doses up to 30 mg/kg/day for the

duration up to 39 weeks. The clinical signs noted in both species, and in the mouse in a 13-week study, were similar to those observed with single dose administration.

In rats, main toxicological findings across all general toxicity studies include decreased body weight and food consumption, and decrease in body temperature which occurred in male rats at  $\geq 10$  mg/kg/d and females at  $\geq 30$  mg/kg/d with an extreme decrease in body temperature, e.g., to approximately 30 °C or less, in males at  $\geq 300$  mg/kg/d and in females at  $\geq 100$  mg/kg/d. The declines in temperature noted at doses lower than 100 mg/kg/d in the rat were generally minimal and resolved as dosing continued or with recovery. Observations of flaccidity and dilatation of the scrotum in male rats was a common observation in the repeat dose toxicity studies with the incidence persisting for 26 weeks in rats given  $\geq 30$  mg/kg/d. Convulsions were observed at 100 mg/kg/d (243-fold of MRHD on mg/m<sup>2</sup> bases) in 3 males and 2 females (26-week study); they developed later during the treatment period and were not observed in the 13-week toxicity study with the same dose.

Hematology changes included increased RBC parameters and decreased platelet count in males at  $\geq 30$  mg/kg which were also present at the end of recovery (HD, except decreases in platelets count). Increased WBC, mainly due to an increase in lymphocytes were observed in females at  $\geq 10$  mg/kg. Clinical chemistry changes included decreased triglyceride, glucose and potassium; increased AST, inorganic phosphorus, and gamma-globulin. In addition, increased CPK and ALP and lower albumin were noted in females at  $\geq 30$  mg/kg. There were no toxicologically significant changes observed at the end of the recovery period.

Histopathological examination in the 13-week study (doses up to 300 mg/kg/d in males and 100 mg/kg/d in females) revealed brain lesions (2/6) and depressed spermatogenesis that included degeneration/necrosis of germ cells (4/6) in surviving males at 300 mg/kg/d. In females, hypertrophy of corpus luteum and atrophy and mucification of cervical part of the uterus that were accompanied by increased ovary weights and decreased weights of uteri were observed. In addition, an increased incidence of lobular hyperplasia with secretion of milk in the mammary gland indicating the pseudopregnancy in females, and feminization of mammary gland in males; all were the consequences of drug-related pharmacologically mediated hyperprolactinemia similar to those changes observed with other antipsychotics.

Brain lesions were observed in rats in several studies at  $\geq 300$  mg/kg/day in males and  $\geq 100$  mg/kg/day in females which included necrosis of oligodendrocytes, demyelination, and vacuolation of white matter or gray matter; chromatolysis of neurons; necrosis of granule cells in olfactory bulb; and necrosis of Purkinje cells. The incidence of these lesions was low and these lesions were observed primarily in rats which had severe hypothermia, that died or were moribund (i.e., a time-dependent change could not be demonstrated). These lesions were not observed in the 26-week study with rats at doses up to 100 mg/kg/d. It has been demonstrated by the Sponsor that brain and testis toxicities caused by brexpiprazole were related to a substantial body temperature decrease in individually housed rats (stainless-steel wire-mesh cages

without any bedding material) versus rats housed collectively (5 rats per polycarbonate cage with paper pulp bedding) in which no brain lesions or testicular abnormalities were developed (see review of the Study No. 025190 under “Special Toxicology Studies”)

Granules of pigment depositions were observed in many organs of males and females at  $\geq 30$  mg/kg. Depending on the special staining procedures and EM examination, these pigment granules were described by the Sponsor as same material to lipofuscin. Although the toxicological meaning of these findings could not be clarified, the Sponsor has offered the following explanations: “1) that lipofuscin pigment gradually accumulated with aging or 2) due to continuous slight cytotoxic effects of the compound as a result of incomplete phagocytic removal of the intracellular components (e.g. lipids, membrane protein, DNA) degenerated by superoxides or hydroxy radicals”. The NOAEL was estimated to be 3 mg/kg/day (males) and 10 mg/kg/day (females).

In cynomolgus monkeys, dosed orally with brexpiprazole (0, 1, 3, and 30 mg/kg/d), treatment-related clinical signs of slight tremors (limited to extremities), decreased movement, prone, crouching, or lateral position, partially closed eyes, and/or drowsiness were observed in males and females at all doses. All findings except tremors of the extremities were related to pharmacologically mediated CNS depression, including decreased food consumption, and slight and transient decreases in BW (at 1 mg/kg signs were predominantly observed during first two weeks of treatment).

In the 4-week and 13-week studies, brexpiprazole at 10 mg/kg/d and 30 mg/kg/d, respectively, prolonged QT interval or QTc on Day 1 in both studies, although QT prolongation was not readily observed at or near the end of the studies (except one female monkey dosed at 30 mg/kg/d in the 4-week study had prolonged QT interval on Day 24). Prolongation of QT was not observed in the 39-week toxicity study. The observed QT prolongation in these studies appeared to correlate with the decrease in body temperature, and QT prolongation has been associated with a decrease in body temperature. Therefore, the QT effect observed in the monkey may be indirectly associated with the pharmacologically-mediated decrease in body temperature although an effect due to hERG affinity ( $IC_{50}$  of 0.117  $\mu$ M) cannot be excluded. Thus, QT prolongation associated with brexpiprazole administration may be considered to represent a potential risk to patients particularly at dose initiation. However, in clinical trials with brexpiprazole, no QT prolongation has been observed.

In addition to the effect on QT, decreases in blood pressure also were observed in the monkey in the 4-, 13- and 39-week toxicity studies. The decrease was most prominent at Day 1 in each study at 30 mg/kg/d and tended to resolve as dosing continued. The mechanism of the hypotensive effect of brexpiprazole was investigated using isolated rat aorta and in beagle dogs. Brexpiprazole at  $\geq 30$   $\mu$ M decreased the tension of isolated aorta induced by the  $\alpha_1$  receptor agonist phenylephrine. Also brexpiprazole at  $\geq 0.3$  mg/kg inhibited the phenylephrine-induced increase in blood pressure. Thus, brexpiprazole may induce depressor responses by blockade of  $\alpha_1$  receptors in peripheral blood vessels.

The histopathological findings in monkeys at 10 mg/kg/d or 30 mg/kg/d included atrophy of the thymus, adrenals, spleen; these findings were considered to be associated with a stress response. The toxicological findings noted during the dosing phase of the studies were generally reversible or showed a tendency towards reversibility.

**Genotoxicity:** An adequate battery of genotoxicity studies were conducted which included the bacterial reverse mutation test, the mammalian cell forward mutation test, the mammalian cell chromosome aberration test, the rat micronucleus test, and the rat UDS test.

Brexpiprazole was not genotoxic in the bacterial reverse mutation test, the rat bone marrow micronucleus test, or the rat UDS test. Brexpiprazole was mutagenic in the *in vitro* forward mutation test in mouse lymphoma cells after 3-h exposure (+S9) and was not mutagenic after 3- or 20-h exposures (–S9). Brexpiprazole was also clastogenic in the *in vitro* chromosome aberration test in CHO cells after 3-h treatment (–S9), but results (+S9) were not reproducible and considered equivocal. Brexpiprazole was not clastogenic in CHO cells after 20-h incubation (–S9). The positive responses observed *in vitro* were generally slight and occurred only at high concentrations that were also moderately cytotoxic. These results are considered to have no clinical relevance.

**Carcinogenicity:** In the 2-year carcinogenicity study in mice, brexpiprazole was administered by oral gavage at doses of 0, 0, 0.75, 2, and 5 mg/kg/d. These dose levels are 0.9- to 6-fold the oral MRHD (4 mg) on a body surface area basis. In this study, lobular hyperplasia, adenocarcinoma and adenosquamous carcinoma in mammary gland, and pars distalis adenoma in the pituitary gland were observed in female mice. Non-neoplastic changes were also observed in females in the mammary gland, ovary, uterus, and vagina. These changes, likely mediated by dopamine D<sub>2</sub> antagonist effects or hormonal alterations as a result of increased prolactin, were consistent with the pharmacology of brexpiprazole.

No drug-related increases in neoplastic or non-neoplastic lesions were observed in males. Exposure (based on C<sub>max</sub> and AUC<sub>0-24h</sub>) to brexpiprazole in Week 26 at the highest dose (5 mg/kg/day) was 1076 and 412 ng/ml and 12430 and 4321 ng•h/ml in males and females, respectively.

In the 2-year carcinogenicity study in rats dosed at: males - 0, 0, 1, 3, 10 mg/kg/d and female - 0, 0, 3, 10, 30 mg/kg/d which are 2.3- to 73-fold the MRHD (4 mg) on a body surface area basis, no biologically relevant, drug related increases in incidence of neoplasms at any dose level were observed in either male or female rats. Exposure (based on C<sub>max</sub> and AUC<sub>0-24h</sub>) to brexpiprazole in Week 26 at the MTD was 418.5 and 1433 ng/ml and 3912 and 17400 ng•h/ml in males and females, respectively.

**Reproductive toxicology:** Effects of brexpiprazole on fertility and early embryonic development were assessed in rats with males and females treated in separate studies. In females dosed at 0, 0.3, 3, and 30 mg/kg/d for two weeks, altered estrous cycle (prolonged diestrus) and decreased fertility occurred at doses ≥ 3 mg/kg/d, and pre-

implantation losses were increased significantly at 30 mg/kg/d. The NOAELs for brexpiprazole in this study were 0.3 mg/kg/d for general toxicological effect in females (clinical signs of hypoactivity, incomplete eyelid closure, and lacrimation at higher doses), 0.3 mg/kg/d for effects on female reproductive performance, and 3 mg/kg/d for early embryonic development. Slightly impaired fertility at MD and HD, including increased preimplantation losses, was considered to be related to prolonged diestrus caused by hyperprolactinemia due to an effect of brexpiprazole at the D<sub>2</sub> receptor (partial agonist). Treatment of male rats with brexpiprazole (at doses of 0, 3, 10 and 100 mg/kg/d for 63 days before mating with untreated females) had no effect on mating, copulation, fertility index or cesarean section parameters. The NOAELs for brexpiprazole in this study were 10 mg/kg/d for general toxicity (clinical signs and decreased BW gain) and 100 mg/kg/d for effects on male reproductive capacity.

In embryo-fetal development studies, brexpiprazole was not teratogenic in rats at doses up to 30 mg/kg/d (73-fold MRHD of 4 mg on mg/m<sup>2</sup>) or in rabbits, up to 150 mg/kg/d (143-fold MRHD on mg/m<sup>2</sup>). In rats dosed orally for 11 days (GD 7 – GD 17) at 0, 3, 10, and 30 mg/kg/d, no deaths or abortions occurred in any dam. The MTD was achieved based on observed clinical signs of hypoactivity, incomplete eyelid closure, creeping, lacrimation and significant reduction in BW gain at HD. No notable reproductive effects in dams or effects on embryo-fetal development were observed at any dose level. The NOAEL for general toxicity was at 3 mg/kg/d and for reproduction and embryo-fetal development at 30 mg/kg/d.

Pregnant rabbits were dosed orally for 13 days (GD 6 – GD18) at 0, 10, 30, and 150 mg/kg/d. The MTD was achieved; 4 dams with clinical signs, severely reduced food consumption and BW aborted at 150 mg/kg/d. Necropsy of aborted dams revealed findings in the heart, liver, gastrointestinal tract and kidney. Similar findings were noted in dams with regular pregnancy at HD. In fetuses, decreased BW and retardation of ossification and slightly increased incidences of skeletal and visceral variations were observed at 150 mg/kg/d. The NOAELs were at 10 mg/kg/d for general toxicity in dams and 30 mg/kg/d for reproductive effects, and for embryo-fetal development in rabbits.

In the pre- and postnatal study in rats, brexpiprazole was dosed orally to pregnant females from implantation to weaning (GD 7 to LD 20) at 0, 3, 10, and 30 mg/kg/d. No deaths occurred in any dam (F<sub>0</sub>). Toxicities in the maternal generation (F<sub>0</sub>) included irregular respiration, incomplete eyelid opening and decrease in movement at ≥ 10 mg/kg/d and prone position and piloerection at HD. In dams treated with brexpiprazole at HD, decreased BW and food consumption, increased stillbirths and postnatal deaths associated with decreased birth index of subsequent F<sub>1</sub> generation were also observed. Other findings included offspring scattered in the cage, subnormal body surface temperature, and no milk in the stomach of F<sub>1</sub> generation suggesting poor nursing by the F<sub>0</sub> dams at HD. F<sub>1</sub> generation showed low birth weight, decreased viability index on Day 4 of lactation, suppressed BW gain, delayed pinna unfolding and decreased number of corpora lutea in the offspring of HD group. No treatment-related effects were noted on the weaning index, physical development excluding pinna unfolding, early behavior, sensory functions, open-field test, conditioned avoidance response, mating

ability, fertility or gross pathology of offspring (F1) or early development of embryos (F2).

The NOAEL for maternal general toxicity was at 3 mg/kg/d (based on clinical observation at 10 mg/kg/d and decreased BW and food consumption at 30 mg/kg/d) and the NOAEL at 10 mg/kg/d for maternal reproductive function (maintenance of pregnancy, delivery and nursing) and for development of the subsequent generation (F<sub>1</sub>).

### Overall Safety Evaluation

Brexpiprazole was adequately tested in standard non-clinical studies.

An approximately linear pharmacokinetic (PK) profile was observed in animals (rats and monkeys) used in pivotal studies of non-clinical evaluations. In contrast to human bioavailability (95%), brexpiprazole has low bioavailability in rats (13.6%) and monkeys (31%). The biotransformation profiles of brexpiprazole were qualitatively similar across species. CYP3A4 was the primary enzyme responsible for metabolism of brexpiprazole in human liver microsomes.

Toxicities observed in mice, rats and monkeys were related to the exaggerated pharmacological activity of brexpiprazole. Clinical signs included hypoactivity, closed eyes, abnormal postures, tremors (monkey), flaccidity and dilatation of the scrotum (male rats) and hypothermia. Decreased blood pressure (BP) and prolonged QT/QTc interval were noted at 30 mg/kg (243-fold the MRHD of 4 mg on mg/m<sup>2</sup> basis) in conscious dogs (safety pharmacology study) and in monkeys at 146 times the MRHD on Day 1 of the 4- and 13-week repeated-dose toxicity study. Prolongation of QT/QTc was not observed at later evaluation times in the 4- and 13-week studies or in the 39-week study in monkeys. The observed QT prolongation after the initial dose in these studies appeared to correlate with the decrease in body temperature (QT prolongation has been associated with a decrease in body temperature). However, brexpiprazole did not inhibit the hERG channel current with IC<sub>50</sub> of 0.117 μM. There was no significant QTc prolongation effect observed in a thorough QT study in humans at doses up to 12 mg. The effect of brexpiprazole to decrease BP in dogs and monkeys was suggested to be due to a blockade of α<sub>1</sub>-adrenoceptors in peripheral blood vessels, which is consistent with the pharmacological profile for brexpiprazole.

Lobular hyperplasia with secretion of milk in the mammary gland and changes/toxicities in reproductive tissues related to pseudopregnancy were observed in female rats at all dose levels. A feminization of the mammary glands in males, atrophy of seminiferous tubules, prostate, and seminal vesicles were observed in males at ≥ 30 mg/kg/d (73 times the MRHD). These effects on male and female reproductive tissues may have been a consequence of pharmacologically-mediated effects on prolactin and/or low food consumption and decreased body temperature. In general, histopathological findings in male and female reproductive organs were attributed to pharmacologically mediated (D<sub>2</sub> antagonism) increases in serum prolactin. The mechanism of prolactin-mediated luteal

function in rodents is well established and considered by many to be rodent-specific with uncertain toxicological significance.

In the 2-year carcinogenicity study in mice, lobular hyperplasia, the incidence of adenocarcinoma in mammary gland increased in females in all dose levels (0.9 to 6.1 times the MRHD) and the incidence of adenosquamous carcinoma was increased in females at 2.4 and 6.1 times the MRHD. Elevated prolactin levels in mice were observed in a 2-week study of brexpiprazole administered at 6.1 times the MRHD. Increases in prolactin level are known to cause mammary tumors in rodents. No increase in the incidence of tumors was observed in male mice. No drug-related increases in neoplastic or non-neoplastic changes were observed in a 2-year study in rats. Overall, brexpiprazole is considered to represent a low carcinogenic risk to patients.

Slightly impaired fertility in female rats at 7.3 and 73 times the MRHD including increased preimplantation losses was considered to be related to prolonged diestrus caused by hyperprolactinemia due to an effect of brexpiprazole at the D2 receptor (partial agonist). Brexpiprazole was not teratogenic in rats at doses up to 73 times the MRHD, or rabbits at doses up to 730 times the MRHD, although in embryo-fetal development study in rabbits, decreased body weight, retarded ossification, and increased incidences of visceral and skeletal variations were observed in fetuses at 150 mg/kg/d, a dose that induced maternal toxicity (abortions, severely reduced food consumption and body weight loss). In the pre- and postnatal study in rats, increased stillbirths and postnatal deaths associated with decreased birth index of subsequent generation at 73 times MRHD was observed. At this dose level the offspring toxicity expressed as delayed growth and physical development and impaired viability related to impaired nursing behavior (attributable to pharmacologically-mediated effects on the CNS) were observed.

The nonclinical studies showed that many of the findings were associated with an exaggerated pharmacological effect of brexpiprazole e.g. hypoactivity, hypothermia or hyperprolactinemia. Although a clearly identified NOAEL was established in the pivotal nonclinical studies for observed toxicities, the effects observed at doses above the NOAEL may have clinical relevance, e.g., prolonged QT, decreased blood pressure or effects on clinical pathology parameters. Therefore, a comparison of the systemic exposure across species at the NOAEL and at the highest dose from repeat-dose toxicity studies to exposure in humans at MRHD (4 mg/d) for brexpiprazole and the major metabolite, DM-3411, have been provided by the Sponsor and is included in the Appendix/Attachments of this review.

## 12 Appendix/Attachments

**Appendix 1: Table 78: Histopathology Inventory for NDA 205422**

Study	26-week	39-week	104-week	104-week
Species	Rat	Monkey	Rat	Mouse
Adrenals	X*	X*	X*	X*
Aorta - thoracic	X	X	X (thoracic)	X (thoracic)
Bone Marrow - sternum	X	X	X (sternum)	X (sternum)
Bone (femur)	X	X	X (knee joints)	X (knee joints)
Femoral muscles		X	X	X
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Clitoral glands			X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Ears			X	X
Epididymis	X*	X*	X	X
Esophagus	X	X	X	X
Eyes	X*		X	X
Gall bladder	X	X*	X	X
Gross lesions	X		X	X
Heart	X*	X*	X*	X*
Harderian Glands			X	X
Ileum	X	X	X (+Peyer's patch)	X (+Peyer's patch)
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Liver	X*	X*	X*	X*
Lung	X*	X*	X* (+bronchus)	X* (+bronchus)
Lymph nodes		X	X	X
Lymph nodes mandibular	X	X*	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X (females)
Optic nerves	X	X	X	X
Ovaries	X*	X*	X*	X
Pancreas	X	X*	X	
Parathyroid	X*	X	X	X
Penis			X	
Pituitary	X*	X*	X*	X
Preputial glands			X	X
Prostate	X*	X*	X*	X*
Rectum		X	X	
Salivary glands	X	X	X* (+sublingual & submandibular)	X* (+sublingual & submandibular)
Sciatic nerve	X	X	X	X
Seminal vesicles	X*	X*	X*	X*

Study	26-week	39-week	104-week	104-week
Species	Rat	Monkey	Rat	Mouse
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X*	X*	X*	X*
Stomach	X	X	X	X
Tail			X	
Testis	X*	X*	X*	X*
Thymus	X*	X*	X	X
Thyroid	X*	X* (+parathyroid)	X*(+parathyroid)	X*(+parathyroid)
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X*	X*
Vagina	X	X	X	X
Zymbal's Glands			X (preserved)	X (preserved)

X, histopathology performed  
 \*, organ weight obtained;

Table 79: Comparison of PK parameters for brexpiprazole (OPC-34712) and DM-3411 across nonclinical species at the NOAEL and human subjects

Species	Rat		Monkey		Rat (Pregnant)	Rabbit (Pregnant)	Rat		Rat		Mouse		Human
Study Duration	26 Weeks		39 Weeks		EFD	EFD	Fertility		Carcinogenicity				
Gender	Male	Female	Male	Female	Female	Female	Male	Female	Male	Female	Male	Female	-
NOAEL (mg/kg/day)	3	10	1	1	30	30	100	0.3 <sup>b</sup>	10 <sup>c</sup>	30 <sup>c</sup>	5 <sup>c</sup>	<0.75 <sup>c</sup>	-
<b>C<sub>max</sub> (ng/mL)</b>													
OPC-34712	91.3	551.3	156.2	165.6	1476 <sup>d</sup>	1720	3425 <sup>d</sup>	161 <sup>d</sup>	418.5	1433	1076	<65.3	199
Margin of Exposure <sup>a</sup>	0.5	2.8	0.8	0.8	7.4	8.6	17.2	0.8	2.1	7.2	5.4	<0.3	-
DM-3411	16.9	87.4	78.1	91.6	303.2 <sup>d</sup>	72.0	580.3 <sup>d</sup>	32 <sup>d</sup>	104.3	259.7	19.0	<6.2	64.3
Margin of Exposure <sup>a</sup>	0.3	1.4	1.2	1.4	4.7	1.1	9.0	0.5	1.6	4.0	0.3	<0.1	-
<b>AUC<sub>0-24h</sub> (ng·h/mL)</b>													
OPC-34712	591	3285	1680	1773	16390 <sup>d</sup>	23220	45930 <sup>d</sup>	1140 <sup>d</sup>	3912	17400	12430	<773	3950
Margin of Exposure <sup>a</sup>	0.1	0.8	0.4	0.4	4.1	5.9	11.6	0.3	1.0	4.4	3.1	<0.2	-
DM-3411	103	440.3	640.7	751.9	2123 <sup>d</sup>	716.8	4647 <sup>d</sup>	195 <sup>d</sup>	861.4	2081	246.8	<58.6	1280
Margin of Exposure <sup>a</sup>	0.1	0.3	0.5	0.6	1.7	0.6	3.6	0.2	0.7	1.6	0.2	<0.05	-

EFD = embryo-fetal development; <sup>a</sup> the margin of exposure, determined as the ratio of exposure (AUC or C<sub>max</sub> of brexpiprazole or DM-3411) in animals to the exposure in humans; <sup>b</sup> NOAEL for reproduction; <sup>c</sup> NOEL for carcinogenicity

Table 80: AUC<sub>0-24</sub> and C<sub>max</sub> values for brexpiprazole (OPC-34712) and DM-3411 at the highest dose tested in nonclinical studies compared to human PK values at the MRHD

Species	Rat		Monkey		Rat (Pregnant)	Rabbit (Pregnant)	Rat		Rat		Mouse		Human
Study Duration	26 Weeks		39 Weeks		EFD	EFD	Fertility		Carcinogenicity				
Gender	Male	Female	Male	Female	Female	Female	Male	Female	Male	Female	Male	Female	-
High dose (mg/kg/day)	100	100	30	30	30	150	100	30	10	30	5	5	-
<b>C<sub>max</sub> (ng/mL)</b>													
OPC-34712	2859	2651	537.3	412.9	1476	3709	3425	1476	418.5	1433	1076	412	199
Margin of Exposure <sup>a</sup>	14.4	13.3	2.7	2.1	7.4	18.6	17.2	7.4	2.1	7.2	5.4	2.1	-
DM-3411	605	491.3	320.9	330.9	303.2	166.6	580.3	303.2	104.3	259.7	19.0	45.2	64.3
Margin of Exposure <sup>a</sup>	9.4	7.6	5.0	5.1	4.7	2.6	9.0	4.7	1.6	4.0	0.3	0.7	-
<b>AUC<sub>0-24h</sub> (ng·h/mL)</b>													
OPC-34712	44230	34420	7193	5235	16390	65350	45930	16390	3912	17400	12430	4321	3950
Margin of Exposure <sup>a</sup>	11.2	8.7	1.8	1.3	4.1	16.5	11.6	4.1	1.0	4.4	3.1	1.1	-
DM-3411	6830	3982	3818	3587	2123	2301	4647	2123	861.4	2081	246.8	465.5	1280
Margin of Exposure <sup>a</sup>	5.3	3.1	3.0	2.8	1.7	1.8	3.6	1.7	0.7	1.6	0.2	0.4	-

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/s/  
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VIOLETTA M KLIMEK  
03/19/2015

LINDA H FOSSOM  
03/19/2015

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

**NDA Number: 205422**

**Applicant: Otsuka  
Pharmaceutical Co., Ltd.**

**Stamp Date: July 11, 2014**

**Drug Name: Brexpiprazole**

**NDA Type: 505(b)(1) - Standard**

On **initial** overview of the NDA 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?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
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)?	√		
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).			The route of administration for the formulation to be marketed is not different from the formulation used in the toxicology studies
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 <u>submitted</u> a rationale to justify the alternative route?	√		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
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?	√		Yes on face, but it is a subject of review
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		
11	Has the applicant addressed any abuse potential issues in the submission?	√		CSS has been consulted.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

**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.

N/A

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

None at this time

Violetta Klimek, Ph.D.

\_\_\_\_\_  
Reviewing Pharmacologist

\_\_\_\_\_  
Date

Linda Fossom, Ph.D.

\_\_\_\_\_  
Team Leader/Supervisor

\_\_\_\_\_  
Date

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09/03/2014

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