CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

200603

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

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

NDA: 200603
Agency receipt date: 12/30/2009
Drug: Lurasidone hydrochloride
Applicant: Sunovion Pharmaceuticals (Originally submitted by Dainippon Sumimoto Pharma America, Inc.)
Indication: schizophrenia

Reviewing Division: Division of Psychiatry Products

Background:
The pharm/tox reviewer and team leader concluded that the nonclinical data support approval of lurasidone for the indication listed above.

Reproductive and Developmental Toxicity:
Reproductive and developmental toxicity studies in rats and rabbits revealed no evidence of teratogenicity or embryofetal toxicity. The high doses in the rat and rabbit embryofetal toxicity studies were 3 and 12 times, respectively, the maximum recommended human dose (80 mg) based on a body surface area comparison.

Carcinogenicity:
Lurasidone was tested in 2 year rat and mouse carcinogenicity studies. These studies were reviewed by the division and the executive carcinogenicity assessment committee. The committee concluded that the studies were adequate and that there was a drug-related increase in mammary carcinomas in female rats at doses of 12 mg/kg and higher and a drug-related increase in mammary carcinomas and adenoacanthomas and pituitary pars distalis adenomas in female mice. The applicant also provided data from various studies showing that lurasidone significantly increases prolactin in several different species including rats and mice.

Conclusions:
I agree with the division pharm/tox conclusion that this application can be approved from a pharm/tox perspective.

The division recommends that lurasidone be labeled with pregnancy category B. This is justified given the lack of effects in the developmental toxicity studies.

The carcinogenicity findings are consistent with other members of this class of drugs. Proposed labeling includes wording noting the potential role of elevated prolactin in these tumor findings and also notes the uncertain relevance to human. This is appropriate.

The division has decided to use the established pharmacologic class of atypical antipsychotic for lurasidone. This is consistent with other members of the class and is a clinically meaningful term.

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/s/

PAUL C BROWN
10/27/2010

SUPERVISORY MEMORANDUM

NDA#	200603
Drug:	Lurasidone
Sponsor:	Dainippon Sumimoto Pharma America, Inc. (DSP)
Indication:	**A**cute treatment of schizophrenia
Division:	Psychiatry Products
Reviewer:	Sonia Tabacova, Ph.D.
Team Leader:	Aisar Atrakchi, Ph.D.
Date:	October 18**,** 2010

Dr. Tabacova based her evaluation and conclusion on the nonclinical data submitted by DSP. She concluded that there are no nonclinical concerns that would affect approval of this drug application. I find her assessment adequate and concur with her conclusion and recommendation to approve this application. This memo is a brief overview of the drug profile highlighting the main drug efficacy and safety issues.

Lurasidone, the drug substance, is an atypical antipsychotic indicated for the acute treatment of schizophrenia at doses 40-120mg/d. The nonclinical efficacy and safety of lurasidone were investigated in standard animal studies. Dr. Tabacova conducted a thorough and comprehensive review and evaluation and made her recommendations based on the submitted data.

Lurasidone's mechanism of action appears to be antagonism through high affinity binding of the dopamine and serotonin receptors specifically D2, 5HT2A and 5HT7 as well as antagonism of alpha2C adrenergic receptors. Lurasidone showed dose dependent responses in standard animal models of schizophrenia such as methamphetamine-induced hyperactivity, apomorphine-induced stereotypy, and conditioned avoidance response in rats. The drug improved cognition and stabilized mood in a number of learning and memory models in rats, and the incidence and severity of extrapyramidal side effects and CNS depression are lower than for other antipsychotic drugs. There are a number of metabolites of lurasidone; two are with pharmacological effects comparable to that of the parent drug.

The potential adverse effects of lurasidone on the CNS, CVS, Respiratory, GI, urinary, and endocrine systems were investigated in a number of in vitro and in vivo safety pharmacology studies in multiple animal species. Many of these effects were extension of the pharmacology of the drug through the dopamine, serotonin and/or adrenergic receptors. Lurasidone caused a significant prolongation of QT_c interval (C_{max} ≈3ug/ml), in conscious dog but not in the guinea pig or the cat; no effect on BP or QRS duration. Lurasidone inhibited the hERG channel current with an estimated IC_{50} of 108nM.

Lurasidone is rapidly absorbed following oral administration (mean T_{max} of 0.7hr but 5hr in the monkey) and its bioavailability is relatively poor (<12% in all species tested). Presence of food seems to improve bioavailability 2-3x. The drug has a large volume of distribution and clearance and $T_{1/2}$ ranged between 1.6-27hrs. Lurasidone is highly bound to plasma proteins with little free circulating drug and it is widely distributed to body tissues/organs including the brain with marked retention in pigmented tissues like the eyes. It crosses the placenta into the fetus. Lurasidone undergoes extensive metabolism mainly by liver CYP3A4 , and all human metabolites including 2 major ones (>10% of dose), have been found and adequately qualified in 1 or more animal test species. The 2 major human metabolites are ID20219 and ID20220 which have been shown not to be pharmacologically active. Main route of excretion of lurasidone is feces with very little detected in urine. There are no or minimal drug-drug interactions and lurasidone did not inhibit or induce CYP enzymes.

The potential toxicity of lurasidone was investigated in single and repeat dose general toxicity studies in rats, monkeys and dogs with oral daily administration up to 6 months in rodent and 1 year in monkeys. Doses tested were up to 100mg/kg/d in the rat, 50mg/kg/d in monkey and, 200mg/kg/d in the dog. The target organs of toxicity include the reproductive system with changes in sex hormones, mammary glands, changes in bone (rat and dog), and increase in prolactin levels in all 3 species. In the 9 month dog study increased incidence of paraventricular contractions was observed in 2/4 200mg/kg/d male group as well as prolongation in the non-corrected QT interval. These dogs had higher plasma levels of lurasidone than other animals in the same dose group. Follow up studies were conducted in the dog up to 2 weeks; no effects of lurasidone were found on PQ, QRS, QT, or cardiac rhythm following 5 or 50mg/kg/d dose.

Lurasidone was negative in the standard battery of genetic toxicity tests i.e. the Ames bacterial gene mutation, in vitro chromosomal aberration using Chinese hamster lung cells and the in vivo bone marrow micronucleous test in the mouse. An impurity in the synthetic pathway of lurasidone with a structural alert for gene mutation tested negative in the Ames gene mutation test.

In 2 year life time bioassay lurasidone caused tumors in mice and rats. In female mice the incidence of malignant mammary gland tumors and pituitary gland adenomas was significantly increased in all doses tested with the lowest dose representing 1.6x the maximum recommended human dose (MRHD), of 120mg/day based on AUC; no increase in any tumor type in male mice was seen up to 20x in humans at the MRHD. In the rat the incidence of mammary gland carcinoma was significantly increased in females dosed 12 and 50/36mg/kg/d; the NOEL of 3mg/kg/d was only 0.5x the MRHD based on AUC exposure. No increase in tumors in male rats was seen up to the highest dose tested. Rodent mammary and pituitary tumors have been considered to be prolactin mediated and their relevance to human cancer risk is unclear at this time.

Lurasidone reduced female rat fertility when administered at daily oral dose of 150mg/kg for 2 weeks premating, during mating, and through gestation day 7. This effect was reversible following 2 week recovery period. The NOEL for decreased fertility is

15mg/kg/d which is 1.2x the MRHD based on body surface area. Abnormalities in estrus cycle were observed at 1.5mg/kg/d dose and above; the 0.1mg/kg/d dose was the NOEL which is only 0.01x the MRHD of 120mg/d based on body surface area. There were no drug effects on male fertility up to the highest dose tested of 150mg/kg/d which is 12x the MRHD of 120mg/d.

Lurasidone was not teratogenic in rats or rabbit when administered during organogenesis at doses up to 25 and 50mg/kg/d respectively. These doses are 2 and 8x the MRHD of 120mg/d based on body surface area. The drug had no effects on fetal/pup development when administered daily to pregnant rats during organogenesis through weaning up to 10mg/kg/d a dose that is equivalent to the MRHD on a body surface area basis.

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/s/

AISAR H ATRAKCHI
10/20/2010

BARRY N ROSLOFF
10/20/2010

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	**200603**
Supporting document/s:	Statistical review of carcinogenicity studies; Exec CAC meeting minutes
Applicant's letter date:	**12/30/2009**
CDER stamp date:	**12/30/2009**
Product:	**Lurasidone hydrochloride**
Indication:	**Acute schizophrenia**
Applicant:	**Dainippon Sumimoto Pharma America, Inc**
Review Division:	**Psychiatric Drug Products**
Reviewer:	**Sonia Tabacova, Ph.D.**
Supervisor/Team Leader:	**Aisar Atrakchi, Ph.D.**
Division Director:	**Thomas Laughren, M.D.**
Project Manager:	**Ann Sohn**

Disclaimer

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Any information or data necessary for approval of NDA 200603 that Dainippon Sumimoto Pharma America does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that [name of applicant] does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 200603.

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability: Approvable

1.1.2 Additional Non Clinical Recommendations: None

1.1.3 Labeling recommendations (current):

(b) (4)

(b) (4)

(b) (4)

(b) (4)

1.2. Brief Discussion of Nonclinical Findings

Pharmacology: Lurasidone has high affinity for human dopamine, serotonin and α-adrenergic receptors. The Ki values of lurasidone for these receptors are as follows: human D2L (0.329 and 0.994 nM depending on experimental system), 5-HT1A (6.38 nM), 5-HT2A (0.357 and 0.470 nM in repeat dose studies), 5-HT7 (0.495 and 2.10 nM depending on experimental system) and α2C receptors (10.8 and 16.2 nM depending on experimental system). *In vitro* functional activity studies conducted with lurasidone and metabolites ID-14283 and ID-14326 suggest that these compounds are partial agonists to the human 5-HT1A receptor but potent antagonists to the human D2L and 5-HT7 receptors. Lurasidone showed little binding affinity (IC50 >1000 nM) for the following receptors: 5-HT3, 5-HT4, noradrenaline β, β1, β2, adenosine A1, A2, benzodiazepine, cholecystokinin CCKA, CCKB, L-type Ca2+ channel, N-type Ca2+ channel, GABAA, glutamate AMPA, Kainate, NMDA, glycine, histamine H1, muscarine M1, M2, nicotine, opiate, sigma, 5-HT uptake sites, and dopamine uptake sites.

In vivo studies showed that lurasidone and few metabolites were effective in animal models of schizophrenia induced by methamphetamine or tryptamine. Improved cognitive effects and mood stabilization in various learning and memory impairment models in rats was also observed.

Evaluation of secondary pharmacodynamic properties of lurasidone, i.e., extrapyramidal syndrome (EPS) and CNS depression showed that the potential for drug-associated EPS and CNS depressive effects such as exaggeration of anesthesia, muscle relaxation, and inhibition of motor coordination, was lower for lurasidone than for other antipsychotic drugs.

Safety pharmacology concerns for lurasidone pertain to the cardiovascular and endocrine systems (increase in prolactin, ACTH, and corticosterone). QTc prolongation was observed in one species (dog) out of the three tested (guinea pigs, cats). Lurasidone oral administration to conscious Beagle dogs at a dose of 300 mg/kg (Cmax 2.78 μg/ml) produced significant prolongation of the QTc, but no marked effects on blood pressure and QRS duration. Lurasidone produced no changes in QT interval and QTc at a dose of 100 mg/kg (Cmax 1.9 μg/ml). Active metabolites ID-14283 and ID-14326 were weaker inhibitors of hERG currents in vitro than the parent compound. In vivo, metabolite ID-14283 at i.v.doses of 10 and 100 μg/kg caused a transient decrease in blood pressure in cats (by 12 and 22 mmHg, respectively) and an increase in heart rate (by 14 and 19 beats/min, respectively); these effects disappeared 2 to 15 min after administration. Blood pressure drop was observed with the parent compound at the same doses. Metabolite ID-11614 induced a greater decrease in blood pressure and heart rate than lurasidone (historical comparison), and unlike lurasidone induced transient respiratory arrest when administered intravenously at low doses (1 μg/kg or higher) to anesthetized cats. Because these effects on blood pressure and heart rate were diminished by pretreatment with a serotonin (5-HT3) receptor antagonist or with bilateral vagotomy and inhibited by pretreatment with a parasympathetic blocker (methylatropine), they were attributed to vagus reflex due to activation of 5-HT3 receptors present on the vagus nerve. ID-11614 had no effect on the ECG in anesthetized dogs.The dose for inducing cardiovascular effects with ID-11614 was lowest for cats, followed by rabbits and dogs in that order. In rats, only a mild hypotensive effect was seen at high doses. No respiration inhibitory effect was seen in animals other than cats. Metabolites ID-15001 and ID-15002 did not exhibit notable effects on the respiratory, cardiovascular, and sympathetic nervous systems or on smooth muscle in rats. These metabolites are present at insignificant levels in humans and therefore are of no safety concern.

PK/ADME: Lurasidone is rapidly absorbed with peak systemic exposure occurring within 0.7 h (in rats and dogs) and 5.3 h of administration (monkeys). The absolute bioavailability is low, <12%, in all species examined (mice, rats, rabbits, dogs and monkeys). Administration with food increased the extent of absorption 2- to 3-fold. Clearance ranged from 17 to 61 ml/min/kg and volume of distribution ranged from 2.4 to 20 l/kg. T1/2 ranged from 1.6 to 27 h.

Lurasidone is bound extensively (>99%) to serum proteins. It is distributed into most tissues including the brain and retained by pigmented tissues including the eye (in pigmented rats, elevated radioactivity levels were still measurable 3 months post-dose). It penetrates the placental barrier and distributes into the fetus.
Lurasidone is extensively metabolized by oxidative *N*-dealkylation, hydroxylation of the norbornane ring or cyclohexane ring, *S*-oxidation, reductive cleavage of the isothiazole ring followed by *S*-methylation, and a combination of two or more of these pathways. The main metabolizing pathways are the hydroxylation of the norbornane skeleton, the S-oxidation of the isothiazolyl ring, and the cleavage of the cyclohexylmethyl-piperazine linkage. The primary metabolizing CYP isoenzyme in humans is CYP3A4.
No human-specific metabolites were identified: all of the primary metabolites detected in humans were also detected in one or more of the nonclinical animal species. Of the metabolites identified, two (ID-20219 and ID-20220) are the major human metabolites present systemically at concentrations >10% of the total radioactivity. These metabolites are pharmacologically inactive. The total systemic exposure (AUC) of these metabolites in mice, rats and dogs following repeated-dose administration of lurasidone was equivalent to or greater than that observed in humans at steady state following administration of the maximum proposed clinical dose of 120 mg. Therefore, the non-clinical toxicology data for these species provide safety qualifications of the metabolites ID-20219 and ID-20220 via the general toxicity, carcinogenicity and reproductive assessments.
Following administration of [14C] lurasidone, the majority of the radioactivity was excreted in feces as parent compound. Approximately 12-48% of the orally administered dose was absorbed. Unchanged parent compound was detected only at trace levels in bile and urine, indicating that the absorbed material was extensively metabolized. Lurasidone is also excreted into milk primarily as unchanged drug at concentrations greater than those in serum.
The potential for protein-based clinical drug-drug interactions appeared to be minimal as no displacement of lurasidone or co-incubated drugs (biperiden, flunitrazepam, haloperidol, or diazepam) from serum proteins was observed in vitro. Similarly, the potential for clinical drug-drug interactions mediated by inhibition or induction of CYP activity by lurasidone is low, since in vitro studies using human tissue preparations suggested that at clinically relevant concentrations lurasidone does not inhibit or induce CYP enzyme activity. In contrast, because lurasidone is primarily and extensively metabolized by CYP3A4, lurasidone PK was significantly altered when co-administered with strong CYP3A4 inhibitors (i.e., ketoconazole).
General toxicology: Single- and repeated-dose (subchronic and chronic) general toxicity studies were performed in rodents, dogs and monkeys. The pivotal GLP-compliant studies performed by oral (gavage) administration of lurasidone are the 6-month study in rats, the 39-week study in dogs and the 52-week study in Cynomolgus monkeys.
The 6-month rat study at doses of 0.03, 1, 10 and 100 mg/kg/day revealed drug-related effects on sex hormones (elevation in serum prolactin in females at all dose levels and in males at ≥ 1 mg/kg/day; and reduction in serum estradiol levels in females at ≥ 10 mg/kg/day) with associated disruption of female estrus cycle, decrease in ovarian corpora lutea, uterine atrophy and mammary gland hyperplasia in males and females at ≥ 1 mg/kg/day. Lurasidone also reduced the density of trabecular bone (femur) and increased fatty infiltration in the bone marrow in females at ≥ 1 mg/kg/day; these changes did not recover after a 3-month discontinuation period and were tentatively attributed (by the sponsor) to lurasidone effects on sex hormones. The issue of the bone density decrease (also seen in the 3-month rat study) was addressed in the Agency's letter dated 2/7/2001 under IND 61 292, which asked the sponsor to further investigate the decreased bone density, and to monitor bone density in a clinical trial. The NOAEL in the rat 6-month oral lurasidone study was 0.03 mg/kg/day because of mammary hyperplasia in both genders, and reduction in trabecular bone density as well as adverse effect on the reproductive system in females at and above the next tested dose of 1 mg/kg/day. There was no safety margin, since lurasidone mean serum levels at the NOAEL in the rat [0.15 and 0.07 ng/ml for M and F, respectively (AUC not determined)] were much lower than the maximal serum concentration in humans (165 ng/ml) following repeated oral administration at the MRHD of 120 mg/day.

The 52-week monkey study at doses of 2, 10, and 50 mg/kg/day resulted, at all dose levels, in subdued behavior, abnormal postures (contortions of the limbs resulting in unusual and sustained posturing) with dose-related incidence, and transient increase in serum prolactin; extrapyramidal side effects i.e., tremors occurred in mid- and high dose groups. Based on these effects, a NOAEL could not be determined (below the lowest tested dose of 2 mg/kg/day). In addition, an MTD could not be established because of lack of a clear dose-limiting toxicity, since there was no body weight change, or other manifestations of general toxicity (except for clinical signs). No significant drug-related changes were found in ECG, hematology, blood biochemistry, urinalysis, bone marrow smears, organ weights and pathology examinations. Myocardial inflammatory infiltrates were found at MD and HD (in 2 of 8 animals in each group), but a re-evaluation of histopathology slides of the heart performed by the sponsor reduced the findings in the high-dose group from 2 animals to 1. Based on this, and on the sponsor's and FDA reviewer's data of high spontaneous incidences of myocardial inflammatory infiltrates in Cynomolgus monkeys, the Division considered that "it is not unreasonable to consider these spontaneous (rather than drug-related) findings in the 1-year monkey study" (see Dr. Lois Freed's P/T Memorandum to IND 61292 of 8/13/2002). This study did not achieve sufficient systemic exposure levels to adequately assess lurasidone safety for humans. Even at the highest tested dose of 50 mg/kg/day, lurasidone systemic exposure values in the monkey after 52 weeks of treatment (Cmax: 86 and 90 ng/ml; AUC: 625 and 558 ng.h/ml for M and F, respectively) were lower than or, at best, similar to the corresponding human exposure values at the MRHD of 120 mg/day (Cmax=165 ng/ml and $AUC_{0\text{-}inf}$ = 687 ng.h/ml).

As this study could not determine a NOAEL, MTD, and did not achieve sufficient systemic exposure levels, the Division recommended that "the sponsor perform lurasidone toxicity studies in another non-rodent species (i.e., dog) in which higher plasma exposures could be achieved to adequately assess lurasidone safety for humans" (Memorandum of Dr. Lois Freed to IND 61292 of 8/13/2002). The sponsor complied with this recommendation and subsequently conducted a 9-month toxicology study in dogs.

The 9-month (39-week) dog study at oral doses of 30, 100 and 200 mg/kg/day caused pharmacologically related clinical signs (decreased activity, somnolence, tremors, miosis) and reduced food consumption at all dose levels, and decreased body weight or weight gain in males at MD and HD. Premature ventricular contractions (PVCs) occurred in HD group (2 of 4 males). Both animals exhibiting PVC had high serum concentrations of lurasidone, and severe (approximately 30%) decrease in body weight relative to control. Non-corrected QT intervals were prolonged in these two HD males, and in one MD male that had a 33% body weight loss (QTc data not provided). Increased total cholesterol and phospholipids were seen in all treated groups except for MDM. Effects potentially related to exaggerated drug pharmacology included increased serum prolactin at all doses throughout treatment, decrease in absolute and relative prostate weights at all doses and decrease in absolute testis weights at MD and HD. Histopathology revealed prostatic atrophy in all treated male groups and, at the two highest dose levels, the testes exhibited multifocal or diffuse seminiferous atrophy; at HD, hypospermia was noted in the epididymis, with cellular debris in the lumen of the tubules and vacuolization of ductal epithelium. In female dogs, uterine atrophy and decreased presence of corpus luteum and secondary ovarian follicles were seen in all treated groups, along with mammary gland "thickening" and microscopic pathology (hydropic appearance of ductal epithelium, diffuse lymphoid cell infiltration and pigmentation). A decrease in trabecular bone in the femur and/or sternum was observed in 1 MD male and 2 HD males in parallel with increased fatty infiltration in bone marrow of the femur and/or sternum. New findings in this study when compared with the chronic toxicity studies in rats and monkeys were the effects on the male genital and cardiovascular systems. MTD: 200 mg/kg/day for females and 100 mg/kg/day for males, due to severe body weight loss (emaciation) and deterioration of general condition in males at the next higher dose of 200 mg/kg/day.

A NOAEL for general toxicity could not be determined in this study because drug-related clinical signs (i.e., tremors), elevation in serum prolactin, and gross- and microscopic pathology changes in the mammary gland, thymus, and male and female reproductive system were present at all tested doses, including the lowest (30 mg/kg/day). The dose of 30 mg/kg was a NOAEL for cardiovascular effects (non-corrected QT interval prolongation). Lurasidone systemic exposure margin at this dose after 39 weeks of treatment was about 15- and 12 times, in males and females respectively (based on AUC in

serum) and 7 times (based on Cmax) the human exposure at MRHD of 120 mg/day ($AUC_{0\text{-}inf}$ = 687 ng.h/ml; Cmax 165 ng/ml).

Special cardiac toxicity studies in dogs employing lurasidone single- and repeated (2 week) oral administration at doses of 5 and 50 mg/kg/day did not show treatment-related changes in PQ, QRS, QT intervals, QTc, or cardiac rhythm (as measured by 24-hour ambulatory ECG on dosing Days 1 through 4, 7 and 14), although treatment-related miosis and decreased activity were present at HD.

Special toxicology studies for drug dependency, antigenicity, phototoxicity did not show notable drug-related effects.

Genetic toxicity: Lurasidone was not mutagenic in the Ames test, and did not exhibit clastogenic potential in the in vitro chromosomal aberrations test, or in the in vivo micronucleus assay. A compound code named (b) (4) (lurasidone starting material and potential impurity with a structural alert), tested negative in the bacterial reverse mutation test. No further genetic toxicity testing of (b) (4) is needed since the specification for this compound is below 0.15% of the drug substance.

Carcinogenicity: Lurasidone carcinogenic potential was investigated in lifetime (2-year) GLP-compliant studies in mice and rats. In mice, oral administration of lurasidone at doses of 30, 100, 300, and 1200/650 mg/kg/day in males for 104 weeks [HD reduced as of Day 410 due to excessive (>20%) weight loss] and at 30, 100, 300, and 650 mg/kg/d in females for 98 weeks (shorter dosing duration in females due to excessive mortality) did not produce neoplastic lesions in the males. In the females, however, drug-related, statistically significant increases in neoplastic lesions (benign pituitary pars distalis adenoma and malignant mammary carcinoma and adenoacanthoma) were induced at all tested dose levels, with highly significant positive trends vs. pooled control groups. The increased incidence of pituitary and mammary tumors in females and the concomitant non-neoplastic findings of estrus cycle disruption and marked elevation of serum prolactin were likely related to the dopamine type 2-receptor antagonistic properties of the drug. Serum prolactin levels in females were 2 to 4 times higher than those in males in all dose groups. Plasma exposure to lurasidone (Cmax and $AUC_{0\text{-}24}$) was higher in females than males across all collection time points. NOEL for neoplasia: Males: 650 mg/kg/day ($AUC_{0\text{-}24}$= 12, 947 ng.hr/ml); Females: A NOEL could not be determined for pituitary adenoma and mammary malignant neoplasia (carcinoma, adenoacanthoma) (below the LD of 30 mg/kg/day, $AUC_{0\text{-}24}$ 1130 ng.hr/ml). Compared to human exposure at the MRHD of 120 mg/day ($AUC_{0\text{-}inf}$= 687 ng.h/ml), the safety margin at the NOEL for males is about 19x; for females, there is no safety margin for humans (based on AUC, the female mice exposure at the lowest tested dose was 1.6x the human exposure at the MRHD).

In rats, oral administration of lurasidone for 104 weeks at doses of 3, 12, 50/36 mg/kg/day (HD reduced to 36 mg /kg /day beginning on Days 403-404 because of excessive decrease in body weight) resulted in increased incidence of mammary carcinomas in female rats at MD and HD. The incidence of all other neoplastic lesions in either gender was not elevated at any of the tested dose levels. Non-neoplastic findings of female estrus cycle disruption occurred at all dose levels in a dose-dependent manner, supported by microscopic findings of increased incidence of absence of ovarian corpora lutea. In males, increased incidence of milk secretion was observed in all dose groups. Serum prolactin was elevated dose-dependently at all dose levels in males and at LD and MD but not at HD in females. Plasma exposure to lurasidone (Cmax and AUC0-24h) was higher in females than in males across all collection time points. According to the conclusion of the Executive CAC, the mammary carcinomas in female rats at mid- and high dose were drug related. NOEL for neoplasia: Females: mammary carcinoma: 3 mg/kg/day ($AUC_{0\text{-}24}$: 365 ng.h/ml). Compared to human exposure at the MRHD of 120 mg/day ($AUC_{0\text{-}inf}$: 687 ng.h/ml), the exposure ratio at the NOEL is 0.53, i.e., there is no safety margin for humans. Males: >50 mg/kg/d ($AUC_{0\text{-}24}$: 5276 ng.h/ml), safety margin for humans greater than 8x.

Reproductive and Developmental Toxicity:

Fertility:

Males: Daily oral (gavage) administration of lurasidone at doses of 6, 30, and 150 mg/kg/day to male rats for 64 consecutive days prior to mating and during the mating period (with untreated females) did not induce adverse effect on male fertility and reproduction. The NOAEL for general toxicity in paternal male rats was 6 mg/kg/day, based on the reduction in body weight and food consumption at the

next tested dose level of 30 mg/kg/day and higher. The NOAEL for reproductive toxicity in paternal males was 150 mg/kg/day since no effects were noted in copulation and fertility indices up to the highest tested dose. On mg/m^2 basis, there is a 12-fold safety margin between the NOAEL for male reproductive toxicity in rats and the MRHD of 120 mg/day.

Females: Daily oral (gavage) administration of lurasidone at doses of 0.1, 1.5, 15 and 150 mg/kg/day for 15 consecutive days prior to mating, during the mating period (with untreated males) and through Day 7 of gestation resulted in decreased maternal body weight gain, prolonged estrus cycle and delayed copulation at all dose levels except the LD. The fertility index and the numbers of corpora lutea, implantations and live fetuses were decreased at 150 mg/kg/day.

NOAEL for reproductive toxicity in females: 0.1 mg/kg/day (based on prolonged estrous cycle and delayed copulation at the next higher tested dose of 1.5 mg/kg/day); NOAEL for general toxicity in female rats: 0.1 mg/kg/day (based on maternal body weight decrease at and above the next higher tested dose of 1.5 mg/kg). On both mg/kg and mg/m^2 basis, the NOAEL for reproductive toxicity in female rats is much lower than the MRHD (120 mg/day). There is no safety margin for female reproductive toxicity in humans.

Embryo-fetal development

Rats: Lurasidone at oral doses of 3, 10 and 25 mg/kg/day administered to pregnant females during the period of fetal organogenesis (Day 6 to Day 17 of gestation) caused a dose-dependent decrease in maternal body weight gain in all dose groups; fetal external malformations were observed in the HD group (cleft palate in 8 fetuses and meningoencephalocele in 1 fetus) versus no external malformations in the control. The frequencies of these findings were beyond the range of the historical control data of the laboratory. However, all fetuses with cleft palate were from the same litter, and we agree with the sponsor that multiple malformation cases confined to a single litter are of a smaller concern than a distribution of affected fetuses in several litters. It is also noteworthy that in the range-finding study in the same species and strain with 12 pregnant rats/group treated with higher doses of lurasidone via the same route from gestation Day 7 to 17 (Study 2784) there were no external malformations up to the highest employed lurasidone dose of 150 mg/kg/day. This supports the sponsor's conclusion that the external malformations observed in the 25 mg/kg (HD) group were spontaneous rather than drug-related.

A NOAEL for general toxicity in maternal rats was not reached (below the lowest tested dose of 3 mg/kg/day). The NOAEL for embryo/fetal developmental toxicity was 25 mg/kg/day. On a mg/m^2 basis, there is a 2-fold safety margin between the NOAEL for developmental toxicity in rats and the MRHD of 120 mg/day.

Rabbits: Lurasidone at oral doses of 2, 10 and 50 mg/kg/day administered to pregnant New Zealand White rabbits during the period of fetal organogenesis (Day 6 to Day 18 of gestation) caused a decrease in maternal body weight gain at all doses but no changes in fetal growth, external, skeletal and visceral observations.

A NOAEL for maternal general toxicity was not reached (<2 mg/kg/day) due to maternal body weight gain decrease at all tested doses; The NOAEL for embryo/fetal developmental toxicity was 50 mg/kg/day. On mg/m^2 basis, there is an 8-fold safety margin between the NOAEL for developmental toxicity in rabbits and the MRHD of 120 mg/day.

Pre- and postnatal development

Oral (gavage) administration of lurasidone at doses of 0.4, 2 and 10 mg/kg/day to pregnant rats from implantation through the end of lactation (Day 6 of gestation through postnatal day 21),did not cause treatment-related effects in the number of pups delivered; sex ratio; viability indices; body weights; physical development; neurobehavioral development (reflex responses, motor activity, learning and memory); sexual development; estrus cyclicity; reproductive capacity; and gross pathological findings.

The NOAEL for general toxicity in maternal (F0) rats was 2 mg/kg/day due to a decrease in maternal body weight and body weight gain at the next tested dose of 10 mg/kg/day. The NOAELs for maternal reproductive function and development of F1 offspring were higher than 10 mg/kg/day, since no changes were seen up to the highest tested dose of 10 mg/kg/day.

In summary, the non-clinical toxicological findings were reasonably anticipated based on the pharmacology of lurasidone and the prior observation of similar effects with marketed atypical antipsychotic drugs. High serum prolactin levels in long-term repeat-dose studies in female rats were associated with effects on bones, adrenal glands, pituitary gland and reproductive system. Mammary gland and/or pituitary gland tumors were observed in the mouse and rat carcinogenicity studies and are most likely due to increased blood prolactin levels. Such findings are common in rodents treated with antipsychotic drugs with dopamine D2 blocking activity.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number: 139563-29-4 (hydrochloride)

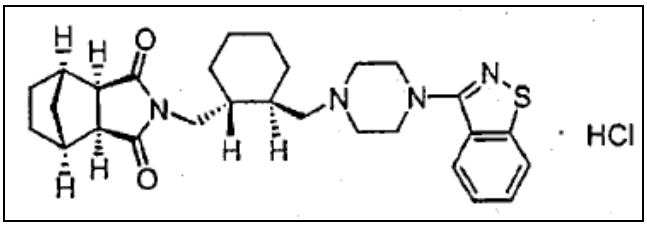
2.1.2 Generic Name: Lurasidone Hydrochloride

2.1.3 Code Name: SM-13496

2.1.4 Chemical Name: *(1R,2S,3R,4S)-N-(1R,2R)-2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinylmethyl)-1-cyclohexylmethyl)]-2,3-bicyclo[2.2.1] heptanedicarboximide hydrochloride*

2.1.5 Molecular Weight: 529.15 (492.69, free)

2.1.6 Structure



2.1.7 Pharmacologic class: D2, 5HT1A, 5HT2A, 5HT7 receptor ligand

2.2 Relevant IND/s, NDA/s, and DMF/s: IND 61 292

2.3 Clinical Formulation:

2.3.1 Drug Formulation: Tablet. Inactive ingredients: (b) (4), (b) (4), croscarmellose sodium, (b) (4) magnesium stearate, (b) (4), carnauba wax, (b) (4)

2.3.2 Comments on Novel Excipients: None

2.3.3 Comments on Impurities/Degradants of Concern:

(b) (4), a starting material and potential impurity of lurasidone (b) (4) (b) (4) belongs to a chemical class with a structural alert for potential genotoxicity. The mutagenic potential of (b) (4) was evaluated and found negative in an *in vitro* bacterial reverse mutation (Ames) test.

2.4 Proposed Clinical Population and Dosing Regimen

Lurasidone is indicated for the acute treatment of adult patients with schizophrenia. The initial and target daily dose of lurasidone is 40 mg or 80 mg. (b) (4) (b) (4) treatment at 40 or 80 mg/day.

2.5 Regulatory Background

The Investigational New Drug Application (IND) 61 292, SN 0000 was originally submitted to the Food and Drug Administration (FDA) on November 15, 2000, for the investigational use of lurasidone in the treatment of patients with schizophrenia, and is currently sponsored by Dainippon Sumitomo Pharma America, Inc (DSPA). Merck and Company conducted an End-of-Phase 2 meeting with the FDA on 9/26, 2006. Merck was the IND sponsor at the time of the meeting; however, DSPA has since reacquired the development rights for lurasidone and is the current IND sponsor.

Specific regulatory issues (relevant to non-clinical studies):

- The issue of bone density decrease in the 6-month rat study that occurred at doses and systemic exposure levels lower than those in humans at MRHD and did not recover after a 3-month discontinuation was addressed in the Agency's letter dated 2/7/2001 under IND 61 292, which asked the sponsor to further investigate the decreased bone density, and to monitor bone density in a clinical trial.
- Since the 1-year monkey study could not determine a NOAEL, MTD, and did not achieve sufficient systemic exposure levels, the Division recommended that the sponsor perform lurasidone toxicity studies in another non-rodent species (i.e., dog) in which higher plasma exposures could be achieved to adequately assess lurasidone clinical safety (Memorandum of Dr. Lois Freed to IND 61292 of 8/13/2002). The sponsor complied with this recommendation and subsequently conducted a 9-month toxicology study in dogs.
- A compound code named (b) (4) (lurasidone starting material and potential impurity with a structural alert) tested negative in the bacterial reverse mutation test. The Division did not require further genetic toxicity testing of (b) (4) since the specification for this compound is below 0.15% of the drug substance.
- Lurasidone metabolites (*R,R*)-ID-20219 and (*R,R*)-ID-20220 are the major metabolites in humans as they meet the threshold of 10% of total exposure or greater in humans (see the sponsor's Response to FDA Comments in Type B Pre-IND meeting (b) (4)), December 23, 2008) and they are not pharmacologically active. Their total systemic exposure (AUC) in rodents and dogs (pivotal toxicity study species) following repeated-dose administration of lurasidone was shown to be equivalent to or greater than that observed in humans at steady state following administration of the maximum proposed clinical dose of 120 mg. Therefore, the current non-clinical toxicity study data provided safety qualifications of these two major metabolites of lurasidone in general toxicity, genotoxicity, carcinogenicity and reproductive/developmental toxicity assessments.

3 Studies Submitted

There are over 160 non-clinical studies that have been completed by DSP and are the primary source of non-clinical data appropriate for inclusion in the NDA. These studies are listed under the relevant chapters in the present review. In addition, there are 8 nonclinical primary pharmacology studies that were conducted by Merck during their tenure as IND sponsor for lurasidone. As per Division's agreement, these eight nonclinical primary pharmacology studies which were preliminary or incomplete (Studies MRPS-01 to 08, as listed in the sponsor's table below) are included in the non-clinical written and tabulated summaries, without submitting the actual study reports (IND 61 292 Pre-NDA face-to-face meeting, 5/22/2009).

Non-clinical studies without submitted study reports
(Primary pharmacology studies sponsored by Merck)

Study	Title
MRPS-01	$5HT_{2A}$ Receptor occupancy of lurasidone in the male fed rat
MRPS-02	Affinity of lurasidone and its metabolites for the rat $5HT_{2A}$ receptor and rat D_2 receptor expressed in HEK cell line
MRPS-03	D_2 Receptor occupancy of lurasidone in the male fed rat
MRPS-04	Dose response curve of L-001710914-000R004 in rotarod and catalepsy
MRPS-05	Dose response curve of L-001710916-000H004 in rotarod and catalepsy
MRPS-06	Profiling of lurasidone in catalepsy and on the rotarod; Study Number: 45003013
MRPS-07	In vitro binding of lurasidone to $5\text{-}HT_{1A}$ receptors in rhesus and human brain
MRPS-08	In vitro human receptor affinity and efficacy profile of lurasidone and metabolites

3.1 Studies Reviewed

All submitted studies except for preliminary dose range-finding studies in various species and studies previously reviewed (see below under 3.2 and 3.3).

3.2 Studies Not Reviewed

Studies not reviewed within this submission are dose range-finding studies in various species.

3.3 Previous Reviews Referenced

The following studies were previously reviewed by Dr. Lois Freed under IND 61292 (original submission) and are cited or reproduced under the relevant study titles in the present review:

General toxicology studies:
- Acute toxicity (rat, cynomolgus monkey);
- Subchronic toxicity:
  - Rat: 2-wk, 3-mo (2 studies)
  - Dog: 2-wk
- Chronic toxicity:
  - Rat: 6-mo

Genetic toxicology studies:
- In vitro bacterial reverse mutation (Ames) test for lurasidone and metabolite (b) (4) (lurasidone starting material and potential impurity with a structural alert)
- Chromosomal aberration test in vitro
- In vivo micronucleus assay in mice

4 Pharmacology

The nonclinical pharmacodynamic properties of lurasidone and several metabolites were determined in a battery of in vitro and in vivo primary and secondary pharmacology studies. Lurasidone had high affinity for human dopamine, serotonin and α-adrenergic receptors. The Ki values of lurasidone for these receptors were as follows: human D2L (0.329 and 0.994 nM depending on experimental system), 5-HT1A (6.38 nM), 5-HT2A (0.357 and 0.470 nM in repeat dose studies), 5-HT7 (0.495 and 2.10 nM depending on experimental system) and α2C receptors (10.8 and 16.2 nM depending on experimental system). *In vitro* functional activity studies conducted with lurasidone and metabolites ID-14283 and ID-14326 suggest that these compounds are partial agonists to the human 5-HT1A receptor but potent antagonists to the human D2L and 5-HT7 receptors. Lurasidone showed little binding affinity (IC50 >1000 nM) for the following receptors: 5-HT3, 5-HT4, noradrenaline β, β1, β2, adenosine A1, A2, benzodiazepine, cholecystokinin CCKA, CCKB, L-type Ca2+ channel, N-type Ca2+ channel, GABAA, glutamate AMPA, Kainate, NMDA, glycine, histamine H1, muscarine M1, M2, nicotine, opiate, sigma, 5-HT uptake sites, and dopamine uptake sites.
In vivo studies showed that lurasidone and some metabolites were effective in animal models of schizophrenia induced by methamphetamine or tryptamine. Improved cognitive effects and mood stabilization in various learning and memory impairment models in rats was also observed.
Secondary pharmacodynamic properties of lurasidone, i.e., extrapyramidal syndrome (EPS) and CNS depression were evaluated and compared to reference drugs. In general, the potential for drug-associated EPS and CNS depressive effects such as anesthesia potentiation, muscle relaxation, and inhibition of motor coordination, was lower for lurasidone than for other antipsychotic drugs.

4.1 Primary Pharmacology

Dopamine, Serotonin, and Noradrenaline Receptor-Mediated Activities
Primary pharmacology studies assessed the *in vitro* binding affinity of lurasidone to dopaminergic, serotonergic, and α-adrenergic receptors, including in vitro functional activity of lurasidone and two active metabolites for human dopamine D2L, human serotonin 5-HT1A and 5-HT7 receptors. Studies on binding activities of lurasidone for other receptor types (such as histamine receptors) and various ion channel types are reviewed in secondary and safety pharmacology. The *in vivo* dopamine antagonistic activity of lurasidone was examined in a battery of behavioral tests in mice and rats i.e., effects of oral lurasidone administration on dopamine agonist-induced behavioral changes including methamphetamine-induced hyperactivity in rats, apomorphine-induced stereotype behavior in rats, apomorphine-induced climbing behavior in mice and conditioned avoidance response. The *in vivo* serotonin antagonistic activity of lurasidone was also examined in a battery of behavioral tests conducted in mice and rats (i.e., effects on serotonin-induced behavioral changes including tryptamine-induced forepaw clonic seizure in rats, p-chloroamphetamine (*p*-CAMP)-induced hyperthermia in rats, 5-hydroxytryptophan (5-HTP)-induced wet dog shake in rats, and 5-methoxytryptamine (5-MT)-induced head twitch in mice).

Dopamine Receptor Effects - In Vitro Studies

Lurasidone
The binding affinities of lurasidone for various dopamine receptor types were evaluated *in vitro* by radioligand binding assays using cultured cells that express these receptors and rat striatal membrane fractions (Studies 7078, 7079, DP1-SM-13496-002).
Lurasidone showed the highest affinity for D2 receptors with a Ki value of 1.68 nM and relatively weak affinity for D1 receptor (Ki 262 nM) (Study 7078) and the highest affinity for D2L receptors (Ki 0.329 and 0.994 nM) (Studies 7079 and DP1-SM-13496-002). Lurasidone affinities for D3 and D4.4 receptors

were weaker than for the D2L receptor, with Ki values of 15.7 and 29.7 nM, respectively (see the following sponsor's table).

Summary of In Vitro Dopamine Binding Data for Lurasidone

RECEPTOR TYPE	SPECIES	TISSUE SOURCE	RADIOACTIVE LIGAND	BINDING AFFINITY MEAN K_i, (NM)	STUDY NO.
D_1	RAT	STRIATUM	^{3}H-SCH23390	262	7078
D_2	RAT	STRIATUM	^{3}H-SPIPERONE	1.68	7078
D_{2L}	HUMAN	CHO-K1 CELL	^{3}H-SPIPERONE	0.329	7079
D_{2L}	HUMAN	SF9 CELL	^{3}H-SPIPERONE	0.994	DP1-SM-13496-002
D_3	HUMAN	CCL1.3 CELL	^{3}H-SPIPERONE	15.7	7079
$D_{4\,4}$	HUMAN	CHO-K1 CELL	^{3}H-SPIPERONE	29.7	7079

Metabolites

In humans, only two metabolites ID-20219 and ID-20220 were considered to be major (as defined by ≥ 10% total drug exposure). As summarized in the following sponsor's table, the affinity of these two major metabolites for the D2 receptor was negligible (IC50 > 1000 nM).

Summary of In Vitro Dopamine Binding Data for Metabolites of Lurasidone

RECEPTOR TYPE	SPECIES	TISSUE SOURCE	RADIOACTIVE LIGAND	COMPOUND ID	BINDING AFFINITY MEAN KI *OR IC50* (NM)	STUDY NUMBER
D2	RAT	STRIATUM	3H-SPIPERONE	ID-14283 ID-20239 ID-20240	3.46 1.46 2.48	7081, 7675
				ID-14326	5.76	7081
				ID-11614, ID-14323, ID-14324, ID-15001, ID-15002, ID-20221, ID-20222	*>1000*	7643
				(*R*,*R*)-ID-20219 (CR-1209) (*R*,*R*)-ID-20220 (CR-1218)	*>1000*	
				ID-11614	590	ISHIBASHI ET AL, 1997
				ID-15001, ID-15002	*>1000*	
D2L	HUMAN	SF9 CELL	3H-SPIPERONE	ID-14283	1.21	DP1-SM-13496-002
				ID-14326	1.62	
				(*R*,*R*)-ID-20219 (*R*,*R*)-ID-20220	*>1000*	

Determination of the affinity of 13 other metabolites of lurasidone for D2 receptor showed that the binding affinities of two metabolites (ID-14283 and ID-14326) for rat D2 receptor (3.46 and 5.76 nM, respectively) and for human D2L receptor (0.99 and 1.21 nM, respectively, were comparable to the

affinity of lurasidone (Ki 1.68 nM for D2 and 1.62 nM for D2L). Metabolites ID-20239 and ID-20240 showed high affinities for D2 receptor (Ki 1.46 and 2.48 nM, respectively); both had similar binding affinities for D2 receptor as ID-14283 and as their parent compound. Nine other metabolites (ID-14323, ID-14324, ID-20221, ID-20222, ID-11614, ID-15001, ID-15002, (*R*,*R*)-ID-20219 (CR-1209), and (*R*,*R*)-ID-20220 (CR-1218) showed no affinity for D2 receptor (IC50 > 1000 nM) (Study 7643). Metabolites (*R*,*R*)-ID-20219 and (*R*,*R*)-ID-20220 had no affinity for human D2L receptor (IC50 > 1000 nM) (Study DP1-SM-13496-002).

In summary, lurasidone and two of its metabolites (ID-14283 and ID-14326, not major metabolites in humans) had high affinity for the rat D2 receptor with Ki values of 1.68, 3.46 and 5.76 nM, respectively, and for the human D2L receptor with Ki values of 0.99, 1.21 and 1.62 nM, respectively. The two major metabolites in humans (ID-20219 and ID-20220) had negligible affinities for both rat D2 and human D2L receptors (IC50 > 1000 nM).

Dopamine Receptor-Mediated Behaviors in Vivo

In vivo dopamine receptor antagonist activity of lurasidone was examined using various behavioral tests in rodents: i.e., inhibitory effects on methamphetamine (MAP)-induced hyperactivity and on apomorphine-induced stereotype behavior in rats; on apomorphine -induced climbing behavior in mice, and on conditioned avoidance response in rats (Study 7031). For comparison, several reference drugs were evaluated separately using the same experimental models (Studies 6993, 7031, 7070, 7592) (see the following sponsor's tables).

Lurasidone and Reference Drugs in In Vivo Dopamine-Mediated Behavioral Studies in Rodents

	Dosage (mg/kg)				
Drugs	MAP-induced Hyperactivity	APO-induced Stereotyped Behaviors	APO-induced Climbing Behavior	Conditioned Avoidance Response	Study Number
Lurasidone (1hr)	0.3, 1, 3, 6	1, 3, 6, 10	1, 3, 6, 10	1, 3, 6, 10, 20	7031
Lurasidone (2hr)	0.3, 1, 3	3, 6, 10, 20	1, 3, 10	1, 3, 10, 20	7031
Risperidone	0.3, 1, 3	3, 10, 20, 30	0.1, 0.3, 1	0.3, 1, 3, 10	7031
Olanzapine	1, 3, 10	1, 3, 10	1, 3, 10	--	6983, 6993
Sertindole	3, 10, 30	10, 30, 100	3, 10, 30	--	6983, 6993
Ziprasidone	0.3, 1, 3, 10	0.3, 1, 3	1, 3, 10	--	7070, 7592
Clozapine	30, 100, 300	100, 200, 400	3, 10, 30	10, 30, 100	7031
Aripriprazole	--	--	1, 3, 10	--	EXA00112
Quetiapine	10, 30, 100	--	10, 30, 100	--	7070
Haloperidol	0.3, 1, 3	1, 2, 3	0.1, 0.3, 1	0.3, 1, 3	7031
Chlorpromazine	3, 10, 30	30, 100, 200, 300	3, 6, 10	1, 3, 10, 30, 100	7031
Thioridazine	30, 60, 100	300, 1000	3, 10, 30	100, 300, 500	7031
Tiapride	100, 200, 300	600, 1000	100, 300, 600	30, 100, 300	7031

MAP = methamphetamine; APO = apomorphine; -- = not tested

Dopamine D2 Blocking Actions of Lurasidone: Comparisons with Other Drugs in Mice and Rats

Inhibition ED_{50} (mg/kg)					
Drugs	MAP-induced Hyperactivity	APO-induced Stereotyped Behaviors	APO-induced Climbing Behavior	Conditioned Avoidance Response	Study Number
Lurasidone (1hr)	2.3	5.0	4.1	6.3	7031
Lurasidone (2hr)	0.87	8.8	5.1	4.6	7031
Risperidone	1.8	11	0.14	1.5	7031
Olanzapine	3.3	5.1	1.1	--	6983, 6993
Sertindole	5.9	42	3.5	--	6983, 6993
Ziprasidone	1.5	1.8	1.4	--	7070, 7592
Clozapine	65	290	9.5	38	7031
Aripriprazole	--	--	6.9	--	EXA00112
Quetiapine	~100	--	29	--	7070
Haloperidol	0.88	1.7	0.44	0.89	7031
Chlorpromazine	16	120	3.8	7.2	7031
Thioridazine	47	>1000	10	170	7031
Tiapride	340	>1000	180	150	7031

Data for reference drugs obtained 1 hour after oral administration.
MAP = methamphetamine; APO = apomorphine; -- = not tested

Effects on Methamphetamine (MAP)-Induced Hyperactivity in Rats

Lurasidone or reference drugs were orally administered to groups of 6 to 13 rats. MAP (1 mg/kg) was administered i.p. at 1 or 2 h after the test drug administration. The locomotor activity was measured using a motility measurement apparatus for 80 minutes starting 10 minutes after MAP administration. The effective dose which inhibited MAP-induced hyperactivity by 50% was calculated. Lurasidone dose-dependently inhibited MAP-induced hyperactivity in rats with ED50 of 2.3 and 0.87 mg/kg at 1 hour and 2 hours after the treatment, respectively (see the sponsor's table above). All the tested antipsychotics inhibited MAP-induced hyperactivity, and the rank order of the potencies of their inhibitory effects at 1 hour post-treatment was as follows: haloperidol >ziprasidone ≥ risperidone ≥ lurasidone > olanzapine > sertindole > chlorpromazine > thioridazine ≥ clozapine > quetiapine > tiapride.

Lurasidone active metabolites ID-14283 and ID-14326 (not major metabolites in humans) also inhibited MAP-induced hyperactivity in a dose-dependent manner, with ED50 values similar to that of the parent compound (Study 7081).

Effects on Apomorphine (APO)-Induced Stereotyped Behavior in Rats

Lurasidone or reference drugs were orally administered to groups of 6 to 12 rats. APO (1.25 mg/kg) was administered i.v. at 1 or 2 hours after the test drug administration. Stereotyped behaviors were scored for

30 minutes immediately after the APO administration, by a 5-point-ranked scale. If the APO-induced stereotyped behavior score (normally 4) was reduced to 2 or less, inhibition of stereotyped behavior was judged as 'positive', and the effective dose which inhibited APO-induced stereotyped behavior in 50% of animals was calculated.

Lurasidone dose-dependently inhibited APO-induced stereotyped behavior in rats with ED50 of 5.0 mg/kg and 8.8 mg/kg at 1 h and 2 h after the treatment, respectively (see the sponsor's table above). The rank order of the potencies of their inhibitory effects at 1 hour post-treatment was as follows: haloperidol ≥ ziprasidone > lurasidone ≥ olanzapine > risperidone > sertindole > chlorpromazine > clozapine. Thioridazine and tiapride inhibited APO-induced stereotyped behavior at doses up to 1000 mg/kg.

Effects on apomorphine-induced stereotyped behavior was also studied after repeated oral administration of lurasidone (5, 10, 20 mg/kg) or haloperidol (3 mg/kg) to rats for two weeks (Study 7031). Lurasidone treatment slightly increased the incidence of apomorphine-induced stereotyped behaviors, but the effect was much weaker than that of haloperidol.

Effects on Apomorphine-Induced Climbing Behavior in Mice

Lurasidone or a reference drug was administered to groups of 5 mice. APO (1 mg/kg) was subcutaneously administered at 1 or 2 hours after the test drug administration. The climbing behavior was observed for 20 minutes beginning 10 minutes after APO administration and was scored by means of 4-point ranked scale. The effective dose which reduced the score of APO-induced climbing behavior by 50% was calculated.

Lurasidone dose-dependently inhibited APO-induced climbing behavior in mice and the ED50 values were 4.1 and 5.1 mg/kg at 1 and 2 hours after the treatment, respectively (see the sponsor's table on the previous page). All the tested antipsychotics inhibited APO-induced climbing behavior in mice, and the rank order of the potencies of their inhibitory effects was as follows: risperidone > haloperidol > olanzapine ≥ ziprasidone > sertindole ≥ chlorpromazine ≥ lurasidone > aripriprazole > clozapine ≥ thioridazine > quetiapine>> tiapride.

Effects on Conditioned Avoidance Response in Rats

Groups of 7 to 16 rats were trained once daily using a two-compartment shuttle-box to learn the conditioned avoidance response. In each trial, rats were given a 5 seconds warning sound and light stimulus (conditioned stimulus, CS), followed by a 5 seconds electroshock (unconditioned stimulus, US). The conditioned avoidance response referred to the movement of the rat to the other compartment during the CS-US interval (5 sec) in order to avoid the electroshock. Escape response referred to the movement of rats to the other compartment after the electroshock was delivered.

For each animal, the number of the conditioned avoidance response was measured in 10 trials (excluding the training trials); only animals that showed a conditioned avoidance response in at least 9 of 10 trials were used in the experiment.

Measurements of conditioned avoidance response were performed over two consecutive days, and the values on the first day were used as controls. On the second day, lurasidone or a reference drug was administered at 1 h or 2 hours before the measurement. The effective dose which inhibited the number of conditioned avoidance response by 50% was then calculated.

Lurasidone dose-dependently inhibited the conditioned avoidance response in rats with ED50 of 6.3 mg/kg and 4.6 mg/kg at 1 hour and 2 hours after the treatment, respectively (see the sponsor's table on the previous page). The inhibitory effect of lurasidone on escape behavior was weaker than on the conditioned avoidance behavior (data not shown). Other antipsychotics also inhibited conditioned avoidance response, and the rank order of the potencies of their inhibitory effects at 1 hour post-dose was as follows: haloperidol ≥ risperidone > lurasidone ≥ chlorpromazine > clozapine > tiapride ≥ thioridazine.

Serotonin Receptor Effects - In Vitro Studies

Lurasidone

The binding affinities of lurasidone for various serotonin receptor subtypes were determined using in vitro radioligand binding assay in Studies 7078, 7397, 7260, DP2-SM-13496-001, DP1-SM-13496-002, and EXA00110.

Lurasidone exhibited high affinity for human 5-HT2A receptor with Ki values of 0.470 and 0.357 nM. The reference drugs aripiprazole, clozapine, olanzapine, and risperidone also exhibited high affinities with Ki values of 2.51, 1.86, 1.43, and 0.187 nM, respectively. Haloperidol and quetiapine showed moderate to weak affinity with Ki values of 36.0 and 128 nM, respectively. Lurasidone also had high affinity for human 5-HT1A receptor with a Ki value of 6.38 nM and for human 5-HT7 receptor with Ki values of 0.495 and 2.10 nM depending on the experimental conditions (see the following sponsor's table).

In Vitro Serotonin Binding Data for Lurasidone and Reference Drugs

Receptor Type	Species	Tissue Source	Radioactive Ligand	Binding Affinity of Lurasidone Mean K_i (nM)	Reference Drug	Binding Affinity Mean K_i or IC_{50} (nM)	Study Number
5-HT_{1A}	Rat	hippocampus	^{3}H-8-OH-DPAT	6.75	--	--	7078
5-HT_{1A}	Human	CHO-K1 cell	^{3}H-8-OH-DPAT	6.38	--	--	DP2-SM-13496-001
5-HT_2	Rat	cerebral cortex	^{3}H-Ketanserin	2.03	--	--	7078
5-HT_{2A}	Human	CHO cell	^{3}H-Ketanserin	0.470	risperidone	0.187	EXA00110
					olanzapine	1.43	
					clozapine	1.86	
					quetiapine	128	
					aripiprazole	2.51	
					haloperidol	36.0	
5-HT_{2A}	Human	CHO-K1 cell	^{3}H-Ketanserin	0.357	--	--	DP1-SM-13496-002
5-HT_{2C}	Pig	choroid plexus	^{3}H-Mesulergine	415	perospirone	4.65	7397
5-HT_7	Human	Sf9 cell	^{3}H-5-CT	0.495	risperidone	2.72	7260
					clozapine	42.2	
					perospirone	2.96	
					haloperidol	*>1000*	
					chlorpromazine	66.3	
					thioridazine	173	
					fluphenazine	11.1	
					trifluoperazine	64.0	
5-HT_7	Human	CHO-K1 cell	^{3}H-SB-269970	2.10	--	--	DP2-SM-13496-001

CHO = Chinese hamster ovary, Sf9 = Spodoptera frugiperda; -- = not determined;
5-CT = 5-carboxamidotryptamine

Metabolites

The affinity of 13 lurasidone metabolites for 5-HT receptor types was determined in a number of studies shown in the following sponsor's table.

In Vitro Serotonin Binding Data for Lurasidone Metabolites

Receptor Type	Species	Tissue Source	Radioactive Ligand	Compound ID	Binding Affinity Mean K_i *or* IC_{50} (nM)	Study Number
5-HT_2	Rat	Cerebral cortex	^{3}H-Ketanserin	ID-14283 ID-20239 ID-20240	1.73 0.947 1.00	7081, 7675
				ID-14326	2.16	7081
				ID-14324	589	7643
				ID-11614	49.4	
				ID-14323, ID-20221, ID-20222, ID-15001, ID-15002	*>1000*	
				(*R*,*R*)-ID-20219 (CR-1209) (*R*,*R*)-ID-20220 (CR-1218)	*>1000*	
5-HT_2	Rat	Cerebral cortex	^{3}H-Ketanserin	ID-11614	30	Ishibashi et al, 1997
				ID-15001, ID-15002	*>1000*	
5-HT_{2A}	Human	CHO-K1 cell	^{3}H-Ketanserin	ID-14283	0.375	DP1-SM-13496-002
				ID-14326	0.337	
				(*R*,*R*)-ID-20219 (CR-1209) (*R*,*R*)-ID-20220 (CR-1218)	*>1000*	
5-HT_{1A}	Human	CHO-K1 cell	^{3}H-8-OH-DPAT	ID-14283	8.36	DP2-SM-13496-001
				ID-14326	2.00	
5-HT_7	Human	CHO-K1 cell	^{3}H-SB-269970	ID-14283	2.06	
				ID-14326	2.16	
5-HT_{1A}	Human	CHO-K1 cell	^{3}H-8-OH-DPAT	(*R*,*R*)-ID-20219 (CR-1209) (*R*,*R*)-ID-20220 (CR-1218)	*>1000*	DP1-SM-13496-001
5-HT_7	Human	CHO-K1 cell	^{3}H-SB-269970	(*R*,*R*)-ID-20219 (CR-1209) (*R*,*R*)-ID-20220 (CR-1218)	*>1000*	

The two major metabolites in humans (ID-20219 and ID-20220) had negligible affinities for the rat 5-HT2, human 5-HT1A, 5-HT2A, and 5-HT7 receptors (IC50 > 1000 nM).

The binding affinities of the active metabolites ID-14283 and ID-14326 for 5-HT2 receptor were 1.73 and 2.16 nM, respectively, comparable to the affinity of lurasidone (Ki 2.03 nM,) (Study 7081). Similarly, the binding affinities of lurasidone, ID-14283 and ID-14326 for human 5-HT2A receptor were similar (0.357, 0.375 and 0.337 nM, respectively) (Study DP1-SM-13496-002). These data suggest that hydroxylation at position 5 or 6 of the norbornane skeleton of lurasidone had little influence on its actions for 5- HT2 receptor. Both ID-14283 and ID-14326 exhibited high affinities for receptor subtypes 5-HT1A (8.36 and 2.00 nM) and 5-HT7 (2.06 and 2.16 nM), similar to the affinity of lurasidone for these receptor subtypes (Study DP2-SM-13496-001).

Metabolites ID-20239 and ID-20240 showed high affinities for 5-HT2 receptor (Ki 0.947 and 1.00 nM, respectively), similar to the binding affinity of ID-14283 (Ki 1.73 nM) (Study 7675). The data suggested that ID-20239 and ID-20240 had similar binding affinities for 5-HT2 receptors as their parent compound.
The rest of the tested metabolites showed either weak/moderate affinity (i.e., ID-14324 and ID-11614), or no affinity for 5-HT2 receptors (i.e., ID-14323, ID-20221, ID-20222, ID-15001, ID-15002, (*R*,*R*)-ID-20219, and (*R*,*R*)-ID-20220.
In summary, lurasidone and two metabolites, ID-14283 and ID-14326, exhibited high affinity for human 5-HT1A, 5-HT2A and 5-HT7 receptor subtypes with Ki values ranging from 0.337 to 8.36 nM. Binding affinities of the two major human metabolites (ID-20219 and ID-20220 for rat
5-HT2, human 5-HT1A, 5-HT2A, and 5-HT7 receptors were negligible (IC50 > 1000 nM).

Serotonin Receptor-Mediated Behaviors in Vivo

Lurasidone
The effect on 5-HT1A and 5-HT2- mediated activity in vivo was examined in behavioral and physiological tests conducted in mice and rats following oral administration of lurasidone (Study 7031) and of several reference drugs (Studies 6993, 7031, 7070, 7592).

Serotonin 5-HT2 Blocking Actions of Lurasidone: Comparisons with Other Drugs

Test Article	Tryptamine-induced clonic forepaw seizure		*p*-CAMP-induced hyperthermia		Study Number
	Dosage (mg/kg)	Inhibition ED_{50} (mg/kg)	Dosage (mg/kg)	Inhibition ED_{50} (mg/kg)	
Lurasidone	3, 6, 10	5.6	1, 3, 10, 30	3.0	7031
Risperidone	0.03, 0.1, 0.3	0.16	0.03, 0.1, 0.3, 1	0.098	7031
Olanzapine	1, 3, 10	1.4	0.3, 1, 3	0.62	6993
Sertindole	1, 3, 6, 10, 30	5.2	3, 10, 30	3.3	6993
Ziprasidone	0.3, 1, 3	1.1	0.3, 1, 3, 10	0.72	7070, 7592
Clozapine	1, 3, 10	5.1	1, 3, 10, 30	5.0	7031
Quetiapine	10, 20, 30	14	--	--	7070
Haloperidol	3, 10, 30	14	1, 3, 10, 30	>30	7031
Chlorpromazine	3, 10, 30	15	1, 3, 10, 30	18	7031
Thioridazine	10, 30, 100	22	3, 10, 30	>30	7031
Tiapride	300, 1000	>1000	30	>30	7031

Data obtained 1 hour after oral administration.
p-CAMP = *p*-chloroamphetamine; -- = not tested

In these studies, lurasidone dose-dependently inhibited the behavioral changes mediated by serotonin 5-HT2 receptors with ED50 values of 3 and 5.6 mg/kg.
In comparison to the reference drugs, the 5-HT2 receptor-blocking actions of lurasidone were at least 2 to 10 times more potent than that of haloperidol, chlorpromazine, thioridazine and quetiapine; weaker than risperidone, ziprasidone, and olanzapine, and comparable to clozapine and sertindole.
The 5-HT2 blocking activity is known to be involved in reduction of EPS of antipsychotics and improvement of refractory and/or negative symptoms of schizophrenia.

Metabolites

The effects of the active metabolites ID-14283 and ID-14326 (0.1, 0.3, 0.6 mg/kg, i.v., for both) on tryptamine-induced clonic seizure of forepaw in rats were evaluated and compared to that of lurasidone at the same i.v. doses (Study 7081). Lurasidone dose-dependently inhibited tryptamine-induced forepaw clonic seizure in rats, with an ED50 value of 0.328 mg/kg. Metabolites ID-11614, ID-14283 and ID-14326 inhibited tryptamine-induced forepaw clonic seizure in a dose-dependent manner, with ED50 values of 0.120, 0.273 and 0.328 mg/kg, respectively. Metabolites ID-15001 and ID-15002 did not inhibit tryptamine-induced forepaw clonic seizure at doses up to 1 mg/kg.

Functional Activity of Lurasidone and Two Metabolites for human D2L, 5-HT1A and 5-HT7 Receptors

The functional activity of lurasidone and two active metabolites (ID-14283 and ID-14326) for human dopamine D2L, serotonin 5-HT1A and 5-HT7 receptors stably expressed on Chinese hamster ovary (CHO) cells, were evaluated in Study DP2-SM-13496-Z001.

Dopamine increased [35S]-GTPγS binding to membranes of D2L-CHO cells in a concentration-dependent fashion with a maximum effect of 240 % of basal binding and an EC50 value of 2.2 μM. Lurasidone, ID-14283 and ID-14326 at 10 μM did not increase the [35S]-GTPγS binding by themselves, but inhibited dopamine-induced [35S]-GTPγS binding with IC50 values of 6.4, 3.1 and 4.0 nM, and KB values of 2.8, 1.3 and 1.8 nM, for lurasidone, ID-14283, and ID-14326, respectively.

5-HT increased [35S]-GTPγS binding to membranes of 5-HT1A-CHO cells in a concentration-dependent fashion with a maximum effect of 170 % of basal binding and an EC50 value of 22 nM. Lurasidone, ID-14283, and ID-14326 increased the [35S]-GTPγS binding in a concentration-dependent fashion with maximum effects of 33, 31 and 51 %, respectively, of 5-HT-induced response.

5-HT increased intracellular cAMP accumulation in 5-HT7-CHO cells in a concentration-dependent fashion from a basal cAMP concentration of 5.6 nM to a maximum cAMP concentration of 170 nM with an EC50 value of 5.0 nM. Lurasidone, ID-14283 and ID-14326 at 300 nM did not increase the intracellular cAMP accumulation by themselves, but inhibited 5-HT-induced cAMP accumulation with IC50 values of 54, 16 and 19 nM, and KB values of 2.6, 0.69 and 0.83 nM, for lurasidone, ID-14283 and ID-14326, respectively.

These results suggested that lurasidone, ID-14283, and ID-14326 are partial agonists of human 5- HT1A receptor, but potent antagonists of human D2L and 5-HT7 receptors.

Adrenergic Receptor Effects - In Vitro Studies

Lurasidone

In Vitro Adrenergic Binding Activity of Lurasidone

Receptor Type	Species	Tissue Source	Radioactive Ligand	Binding Affinity of Lurasidone Mean K_i (nM)	Study Number
α_1	Rat	cerebral cortex	^{3}H-Prazosin	47.9	7078
α_2	Rat	frontal cortex	^{3}H-Rauwolscine	66.7	7078
α_{2A}	Human	Sf9 cell	^{3}H-MK-912	40.7	7078
α_{2C}	Human	Sf9 cell	^{3}H-MK-912	10.8	7078
α_{2C}	Human	CHO-K1 cell	^{3}H-MK-912	16.2	DP2-SM-13496-001

Sf9 = Spodoptera frugiperda

Binding activity of lurasidone for noradrenergic receptor subtypes in vitro showed the highest affinity for α2C receptor and relatively high affinities for α1, α2, α2A adrenaline receptor types (Studies 7078 and DP2-SM-13496-001) (see the following sponsor's table). Lurasidone affinity for α1 receptors (Ki 47.9 nM) was more than 20 times weaker than that for D2 and 5-HT2 receptors (1.68 and 2.03 nM, respectively). This indicated that lurasidone was unlikely to cause side effects induced by α1 receptor blockade such as sedation, hypotension, tachycardia, and orthostatic hypotension.

Metabolites

The two major metabolites in humans (ID-20219 and ID-20220) showed no affinity for human α2C receptor (IC50 > 1000 nM) (Study DP1-SM-13496-0010). The active metabolites ID-14283 and ID-14326 exhibited similar to lurasidone high affinities for α2C receptor with Ki values of 8.31 and 36.5 nM, respectively (Study DP2-SM-13496-001).

In Vitro Adrenergic Binding Data for Lurasidone Metabolites

Receptor Type	Species	Tissue Source	Radioactive Ligand	Compound ID	Binding Affinity Mean K_i *or* IC_{50} (nM)	Study Number
α_{2C}	Human	CHO-K1 cell	^{3}H-MK-912	ID-14283	36.5	DP2-SM-13496-001
				ID-14326	8.31	
				(*R*,*R*)-ID-20219 (CR-1209) (*R*,*R*)-ID-20220 (CR-1218)	*>1000*	DP1-SM-13496-001

Mood Stabilizing Effects

Lurasidone was evaluated by behavioral studies for its mood stabilizing effects [conditioned fear stress-induced freezing behavior test, Vogel's water lick conflict test (Study 7031)], and for its anxiolytic/antidepressive effects [conditioned defensive burying test, and social interaction test (Study 7017)]. Lurasidone inhibited the conditioned fear stress-induced freezing behavior in rats, with a minimal effective dose of 3 mg/kg, suggesting ameliorative effects on mood disorders such as anxiety. Similar effects were found with antidepressants desipramine and imipramine, anxiolytic agent diazepam, antipsychotics clozapine and risperidone, but not with haloperidol and chlorpromazine (see the following sponsor's table).

Inhibitory Effect of Lurasidone on the Conditioned Fear Stress-induced Freezing Behavior: Comparison with Other Drugs

Drugs (Dosage)	Minimum Effective Dose (MED)[a] (mg/kg)	Maximum Suppression (%)
Lurasidone (0.3, 1, 3, 6 mg/kg)	3	93.7
Haloperidol[b]	Tended to increase[c]	(-80.1)
Chlorpromazine[b]	Tended to increase[c]	(-33.3)
Thioridazine[b]	No effects	--
Clozapine[b]	10	46.7
Risperidone[b]	0.1	61.4
Diazepam[b]	1	77.8
Desipramine[b]	Tended to inhibit[d]	52.4
Imipramine[b]	Tended to inhibit[d]	58.8

a) Minimum effective dose to inhibit freezing behavior;
b) Ishida-Tokuda et al, 1996;
c) Behavior tended to worsen with increasing dose;
d) Tendencies for inhibition of behavior with increasing dose; Data obtained 1 hour after oral administration

Lurasidone increased the punished drinking response (number of shocks received) in the Vogel's water lick conflict test, suggesting that it may mediate anti-conflict behaviors in rats. Clozapine produced a similar effect, whereas haloperidol and chlorpromazine did not. Lurasidone significantly increased the social interaction time spent by pairs of naive rats under brightly illuminated conditions in the social interaction test. This effect was similar to that observed for diazepam. Lurasidone selectively suppressed the burying behavior in the conditioned defensive burying test, at doses at which the locomotor activity was not affected. No reference drugs were tested in the experiment.
The findings that lurasidone improved the symptoms in these models of mood disorders suggested that lurasidone could be effective in treating symptoms as fear, anxiety and depression.

Cognitive Effects
The effects of lurasidone on cognition (learning and memory) were tested in rats. Overall, oral administration of lurasidone to rats improved learning and memory impairment induced by MK-801, a NMDA-receptor antagonist (Study EXA00069) and memory impairment induced by a muscarinic-receptor (scopolamine) antagonist (Study EXA00046).

Other in Vivo Studies

Effects on Monoamine Metabolism in Various Brain Regions

Background: Antipsychotic agents increase the contents of dopamine metabolites 3, 4-dihydroxy-phenylacetic acid (DOPAC) and homovanillic acid (HVA) and enhance dopamine turnover in the frontal cortex and striatum. This enhancement of dopamine turnover is considered to be a compensatory response to the blockade of D2 receptors and an alternative approach for evaluating the potency of the D2 receptor blocking activity of various drugs is to compare the changes in DOPAC and HVA contents (DOPAC/DA and HVA/DA ratios) in different regions of the brain.
In Study 7080, three different experiments were performed. In the first experiment, the effects of lurasidone on dopamine turnover in the frontal cortex and striatum were examined in order to compare the potency of its actions on both DA neuron systems. The second experiment evaluated the dose-response relationship of lurasidone and dopamine turnover and compared it with those of haloperidol and clozapine. The third experiment investigated the effects of lurasidone on monoamine contents in eight regions in the rat brain.
Animals were orally administered lurasidone at 10 mg/kg in the first and third experiments (the time-course and site of action experiments). In the second experiment which investigated the dose-response relationship, animals were treated with lurasidone at 0.3, 1, 3, 10, and 30 mg/kg, haloperidol at 0.1, 0.3, 1, 3, and 10 mg/kg, and clozapine at 10, 30, 100, and 300 mg/kg. The ratios of metabolites to its parent were used as indices to assess turnover in these experiments. The metabolites were as follows: DOPAC and HVA for dopamine, 5-hydroxyindole acetic acid (5-HIAA) for serotonin, and 3-methoxy-4-hydroxyphenylglycol (MHPG) for noradrenaline.
a) Effects on Dopamine Turnover in the Frontal Cortex and Striatum – Time Course Evaluation
Lurasidone significantly increased dopamine turnover, *i.e.* the DOPAC/DA and HVA/DA ratios, in the frontal cortex and striatum ($p < .01$) with a maximum at 2 h. after the administration. The maximum DOPAC/DA and HVA/DA ratios were approximately 400 and 450%, respectively, of the control value in the frontal cortex and about 350 and 400%, respectively, of the control value in the striatum. The increase in DA turnover was still statistically significant at 4 hours post-treatment in the frontal cortex ($p < .05$) and at 8 hours post-treatment in the striatum ($p < .01$).
b) Effects on Dopamine Turnover in the Frontal Cortex and Striatum - Dose-Response Relation-ship and Reference Drug Comparison
Lurasidone showed a preferential effect on the frontal cortex (vs. striatum) in increasing DA turnover, as compared with haloperidol. This effect was similar to that of clozapine. Lurasidone increased both the DOPAC/DA and HVA/DA ratios in frontal cortex and striatum to about 400 to 500% of the control

levels. In a dose range of 1 to 10 mg/kg, the ratios increased to a similar extent in the frontal cortex and striatum, and at 30 mg/kg, the increase was significantly ($p < .01$) greater in the frontal cortex. Haloperidol also dose-dependently increased the DOPAC/DA and HVA/DA ratios. Both ratios increased to comparable extents in the frontal cortex and striatum at doses of 0.1 and 0.3 mg/kg, and in a dose range of 1 to 10 mg/kg, the increase was significantly greater in the striatum. Clozapine also dose-dependently increased the DOPAC/DA and HVA/DA ratios. The increases in DOPAC/DA ratio in frontal cortex and striatum were comparable. In the dose range of 10 to 100 mg/kg, the increases in HVA/DA ratio in frontal cortex and striatum were comparable, and at 300 mg/kg, the increase was significantly greater in the frontal cortex.

c) Effects on the Monoamine Contents in Eight Regions in the Brain

This experiment examined the effects on the monoamine contents in 8 regions of the brain - frontal cortex, striatum, hippocampus, thalamus, hypothalamus, mesencephalon, pons/medulla oblongata and cerebellum. Lurasidone significantly increased the DOPAC and HVA contents in the frontal cortex, striatum, hippocampus, thalamus, and cerebellum. In addition, lurasidone significantly decreased the 5-HT contents in the striatum, pons/medulla oblongata and cerebellum, and significantly increased the 5-HIAA contents in the striatum and hypothalamus in comparison to the control group. In contrast, lurasidone did not affect the contents of norepinephrine and MHPG in any of the regions investigated (see the following sponsor's table).

Effects of Lurasidone on Monoamine Metabolism in Rats

Receptor System	Monoamine and Its Metabolites	Quantitative Change (Brain Part) in Comparison with the Control Group
Dopaminergic	Dopamine	No change
	DOPAC	Increase (frontal cortex, striatum, hippocampus, thalamus, cerebellum)
	HVA	Increase (frontal cortex, striatum, hippocampus, thalamus, hypothamlamus, cerebellum)
Serotonergic	5-HT	Slight decrease (striatum, pons/medulla oblongata, cerebellum)
	5-HIAA	Slight increase (striatum, hypothalamus)
Noradrenergic	Noradrenaline	No change
	MHPG	No change

Changes 2 hours after oral administration (10 mg/kg)
DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; 5-HT: serotonin; 5-HIAA = 5-hydroxyindole acetic acid, MHPG = 3-methoxy-4-hydroxyphenylglycol.

Overall, the results from Study 7080 suggested that lurasidone, like clozapine, exerts its effects more selectively on mesolimbic DA neurons, presumably resulting in less EPS than haloperidol, which preferentially acts on nigrostriatal DA neurons.

Effects on the Extracellular Concentration of Dopamine and Serotonin in the Frontal Cortex and the Striatum in Rats

The extracellular levels of dopamine and serotonin in the frontal cortex and the striatum were measured in rats (Studies EXA00086, DP1-SM-13496-003). Lurasidone (3 and 10 mg/kg, po) increased the extracellular dopamine levels in the frontal cortex and striatum to a maximum of 172.3% and 134.2% of baseline, respectively. The 3-h cumulative increase of extracellular dopamine was significant after treatment with lurasidone at 3 and 10 mg/kg in the frontal cortex and at 10 mg/kg in the striatum. At these doses, extracellular serotonin was unchanged in either region. The preferential increase of dopamine in frontal cortex is of therapeutic relevance for treatment of cognitive and/or negative symptoms.

4.2 Secondary Pharmacology

Secondary pharmacodynamic properties of lurasidone that were evaluated and compared to reference drugs include extrapyramidal syndrome (EPS) and CNS depression. In general, the potential for drug-associated EPS and CNS depressive actions such as anesthesia potentiation, muscle relaxation, and inhibition of motor coordination, was lower for lurasidone than for other antipsychotic drugs.

Extrapyramidal Side Effects

The EPS of lurasidone and reference drugs were evaluated by catalepsy tests in rats and mice, the mouse pole-test, and the rat paw-test. Lurasidone at doses of up to 1000 mg/kg did not induce extrapyramidal effects such as catalepsy and bradykinesia, indicating that its extrapyramidal side effect is weak in comparison to other antipsychotic drugs. This can be explained by lurasidone 5-HT2 receptor blocking and 5-HT1A receptor agonistic action, since EPS induced by antipsychotics are known to be reduced by coadministration with 5-HT2 receptor antagonists or 5-HT1A receptor agonists.

Effects on Catalepsy

Cataleptogenic effects of lurasidone in rats and mice were studied in comparison to reference drugs; the effective doses which induced catalepsy in 50% of animals were determined (Studies 7031, 7070, 6994, EXA00112). The results are summarized in the following sponsor's table.

Effects of Lurasidone and Reference Drugs on Catalepsy

Test Article	Dosage in Rats (mg/kg)	Induction ED_{50} (mg/kg)	Dosage in Mice (mg/kg)	Induction ED_{50} (mg/kg)	Study Number
Lurasidone	30, 100, 300, 700, 1000	>1000	30, 100, 300, 700, 1000	>1000	7031
Risperidone	10, 30, 60	20	0.3, 1, 3, 10	0.85	7031
Olanzapine	10, 30, 100	28.3	3, 10, 30 [a]	>10	6994
Sertindole	30, 100, 300, 600, 1000 [b]	>300	30, 100, 300, 1000 [c]	>30	6994
Ziprasidone	10, 30, 100, 300	97	3, 10, 30, 100	63	7070
Clozapine	100, 300	>300	10, 30 [d]	>30	7031
Aripiprazole	--	--	30, 60, 100, 300	55	EXA00112
Haloperidol	5, 10, 20, 30	12	1, 3, 10	2	7031
Chlorpromazine	10, 30, 60, 100	25	3, 10, 20	7.3	7031
Thioridazine	300, 600, 1000	890	10, 30, 60	42	7031
Tiapride	1000	>1000	300, 600, 1000 [e]	>600	7031
Quetiapine	--	--	10, 30, 100, 300	>300	7070

a) Catalepsy induction could not be tested for 3 animals in 30 mg/kg group because of motor deficiency; b) Catalepsy induction could not be tested for 2 animals in 600 mg/kg group and 3 animals in 1000 mg/kg group because of motor deficiency: c) Catalepsy induction could not tested for 2 animals in 100 mg/kg group, 2 animals in 300 mg/kg group, and 7 animals in 1000 mg/kg group because of motor deficiency and for 1 animal in 1000 mg/kg group because of death; d) Catalepsy induction could not be tested at doses of 60 mg/kg or more because of muscle relaxation and motor deficit; e) Catalepsy induction could not be tested at doses of 1000 mg/kg because 4 of 10 mice died.

Lurasidone at doses up to 1000 mg/kg did not induce catalepsy in either rats or mice. Haloperidol, chlorpromazine, thioridazine, risperidone, olanzapine, ziprasidone, and aripiprazole induced catalepsy dose-dependently, and the rank order of the potencies of their cataleptogenic effects were as follows: *Rats*: haloperidol ≥ risperidone ≥ chlorpromazine ≥ olanzapine > ziprasidone >> thioridazine> lurasidone; *Mice*: risperidone > haloperidol > chlorpromazine > thioridazine > aripiprazole > ziprasidone >> lurasidone. Clozapine and tiapride induced no catalepsy in ratsat doses up to 300 and 1000 mg/kg,

respectively, and in mice up to 30 and 600 mg/kg, respectively, and could not be tested at higher doses due to the marked muscle relaxation and motor impairment (clozapine) or death (tiapride).

Effects on Bradykinesia

The bradykinetic properties of lurasidone and reference drugs were examined using the mouse pole-test in which mice were placed head upward on a 45-cm vertical pole 1 h post dose. The time required for mice to turn upside down and the time to descend to the floor were measured, and the minimum effective dose which significantly prolonged the time to descend compared to control was calculated (Studies 7031, 7602). The results are summarized in the following sponsor's table.

Effects on Lurasidone and Reference Drugs on Bradykinesia

Test Article	Dosage in Mice (mg/kg)	Minimum Effective Dose (MED)[a] (mg/kg)	Study Number
Lurasidone	100, 300, 1000	>1000	7031
Risperidone	0.3, 1, 3, 10	3	7031
Olanzapine	3, 10	10	7602
Sertindole	3, 10, 30	10	7602
Ziprasidone	10, 30	30	7602
Clozapine	10, 30[b]	>30	7031
Haloperidol	0.3, 1, 3, 10	1	7031
Chlorpromazine	3, 10, 30	10	7031
Thioridazine	10, 20, 30	30	7031
Tiapride	30, 100, 300, 1000	100	7031

[a] Minimum effective dose to increase the pole-descending time for mice.
[b] Bradykinesia induction could not be tested at doses of 60 mg/kg or more because of muscle relaxation and motor deficit.

Lurasidone was far weaker than other antipsychotic agents in inducing bradykinesia and it did not cause a significant motor impairment (i.e., delay in turning and pole-descending behaviors) even at the highest tested dose of 1000 mg/kg. The rank order of the potencies of the bradykinesia-inducing effects of the tested drugs was as follows: haloperidol > risperidone > chlorpromazine, sertindole, olanzapine > thioridazine, ziprasidone > tiapride >> lurasidone. Clozapine did not prolong the descending time at doses up to 30 mg/kg, but higher doses could not be examined due to the marked muscle relaxation and motor impairment.

Effects on Muscle Rigidity

The propensity of lurasidone or reference drugs to induce motor impairment was examined by the rat paw test (the latency to withdraw forepaws and hind paws from holes used as an index of muscle rigidity) (Studies 7031, 7630, 7114).

Lurasidone and reference drugs thioridazine, sertindole and tiapride did not prolong the forepaw retraction time (FRT) at doses up to 1000 mg/kg. Haloperidol, chlorpromazine, clozapine, risperidone, olanzapine, and ziprasidone all dose-dependently prolonged FRT at lower doses; the minimum effective doses (MED) of these drugs were 1, 30, 300, 30, 30, and 100 mg/kg, respectively.

Lurasidone, thioridazine, tiapride, clozapine, risperidone, olanzapine, sertindole, and ziprasidone prolonged the hind paw retraction time (HRT) at lower doses than for FRT (MED were 300, 1000, 1000, 100, 10, 10, 100, and 10 mg/kg, respectively); haloperidol and chlorpromazine prolonged HRT at the same doses as for FRT (1 and 30 mg/kg, respectively). Thus, for HRT, lurasidone performed better than haloperidol, chlorpromazine, clozapine, risperidone, olanzapine, sertindole, and ziprasidone.

The results are summarized in the following sponsor's table.

Effects on Lurasidone and Reference Drugs on Muscle Rigidity

Test Article	Dosage in Rats (mg/kg)	Minimum Effective Dose (MED)[a] (mg/kg)	Study Number
Lurasidone	30, 100, 300, 1000	>1000	7031
Risperidone	1, 3, 10, 30	30	7031
Olanzapine	3, 10, 30, 100	30	7114
Sertindole	30, 100, 300, 1000	>1000	7630
Ziprasidone	10, 30, 100, 300, 1000	100	7630
Clozapine	10, 30, 100, 300	300	7031
Haloperidol	1, 3, 10, 30	1	7031
Chlorpromazine	3, 10, 30, 100, 200	30	7031
Thioridazine	30, 100, 300, 1000	>1000	7031
Tiapride	30, 100, 300, 1000	>1000	7031

[a] Minimum effective dose to prolong the forepaw retraction time.

Effects on Dystonia

The capacity of oral lurasidone to cause acute dystonia compared to other antipsychotics known to induce EPS in clinical practice was studied in haloperidol-sensitized marmosets (3/sex) with confirmed presence of dystonia-like symptoms elicited by oral administration of haloperidol for 30 weeks (Study L0154). Dosages previously shown to have induced dystonia in clinical practice were used for the comparator drugs. Symptoms were classified as dystonia-like if any of the following were observed: abnormal behavior, abnormal gait, abnormal posture, limbs extension, abnormal limb movement, oral dyskinesia, etc. Other neurological symptoms, i.e., sedation, hyperkinesia, ptosis, tremor, and catalepsy, were also recorded. The results are summarized in the sponsor's table below.

Acute Dystonia-Inducing Effects of Lurasidone in Haloperidol-Sensitized Common Marmosets: Comparison with Other Drugs

Drug	Basic Dose (Clinical Dose) mg/kg/day	No. of Animals in which Dystonia Developed (n=6)				
		0.3[a]	1[a]	3[a]	10[a]	30[a]
Lurasidone	0.96	--[b]	0	--	2	6
Risperidone	0.12	--	3	6	6	6
Clozapine	9.00	--	0	0	0	--
Haloperidol	0.12	--	2	6	6	--
Chlorpromazine	2.00	--	0	5	6	6
Thioridazine	1.80	--	0	0	6	--
Tiapride	2.50	--	0	1	5	6
Bromperidol	0.36	0	6	6	--	--
Sulpiride	12.00	--	0	1	6	--
Diazepam	0.40	--	0	--	0	--
Trihexyphenidyl[c]	0.20	--	6	--	2	--
Solvent[d]	0	--	0	--	--	--

a) Multiples of the basic dose; b) Not conducted; c) Administered 30 minutes before administration of haloperidol at 1.25 mg/kg/day
d) 0.5% methylcellulose solution

Dystonia was induced by haloperidol, bromperidol, and risperidone at approximately the maximum clinical dose. Lurasidone and tiapride induced dystonia at a dose 10-fold the maximum clinical dose level. Clozapine did not induce dystonia even at a dose 10-fold the maximum clinical dose. Administration of non-antipsychotic diazepam or vehicle alone did not induce dystonia in the animal model.
The results of this study suggested that lurasidone was less likely to cause dystonia-like extrapyramidal side effects than risperidone, haloperidol, chlorpromazine, thioridazine, bromperidol and sulpiride.

CNS Depressive Actions
The CNS depressive action of lurasidone and several reference drugs were evaluated in mice by testing drug effects on spontaneous locomotor activity, hexobarbital-induced anesthesia, muscle relaxation, motor coordination, and maximal electric shock (MES)-induced seizures (Studies 7077, 7064, 7016, 7592). Except for the inhibition of spontaneous locomotor activity, the CNS depressive actions of lurasidone (potentiation of anesthesia, muscle relaxation, inhibition of motor coordination, and inhibition of maximum electrical shock convulsion) were considerably weaker than those observed for the other tested antipsychotic agents. The doses and the findings are summarized in the following sponsor's tables.

CNS Depressive Effect of Lurasidone and Reference Drugs in Mice

Drugs	ED_{50} (mg/kg)				
	Inhibition of Spontaneous Activity	Potentiation of Hexobarbital Anesthesia	Muscle Relaxation	Impairment of Motor Coordination	Inhibition of MES Convulsion
Lurasidone	9.9	>1000	>1000	250	>1000
Risperidone	0.15	1.5	11	1.8	>100
Olanzapine	2.1	8.3	11	5.2	>100
Sertindole	4.5	9.7	20	8.7	>1000
Ziprasidone	9.4	14	110	19	>1000
Clozapine	8.5	8.2	32	8.7	>30
Haloperidol	0.73	11	33	2.7	99
Chlorpromazine	5.8	11	23	4.5	150
Thioridazine	17	28	110	43	>100

Data are ED50 (mg/kg) obtained 1 hour after oral administration
MES = maximal electric shock
Data for olanzapine and sertindole were obtained in Study 7064, data for ziprasidone obtained in Study 7016 (spontaneous activity, hexobarbital anesthesia, motor coordination and muscle relaxation) and in Study 7592 (muscle relaxation and MES-induced convulsion); all other data obtained in Study 7077

Lurasidone and Reference Drugs Doses Tested

Drugs	Dosage (mg/kg)				
	Inhibition of Spontaneous Activity	Potentiation of Hexobarbital Anesthesia	Muscle Relaxation	Impairment of Motor Coordination	Inhibition of MES Convulsion
Lurasidone	3, 10, 30	3, 10, 30, 100, 300, 700, 1000	3, 10, 30, 100, 300, 700, 1000	100, 300, 700	3, 10, 30, 100, 300, 600, 1000
Risperidone	0.1, 0.3, 1, 3	0.1, 0.3, 1, 3	3, 10, 30	1, 3, 10, 30	100
Olanzapine	1, 3, 10	3, 10, 30	1, 3, 10, 30	1, 3, 10, 30	3, 10, 30, 100, 300[a], 1000[a]
Sertindole	1, 3, 10, 30	3, 10, 30	3, 10, 30, 100	3, 10, 30, 100	3, 10, 30, 100, 300, 1000
Ziprasidone	1, 3, 10	3, 10, 30	1, 3, 10, 30, 100, 300, 1000	1, 3, 10, 30	10, 30, 100, 300, 1000
Clozapine	3, 10, 30	3, 10, 30	10, 30, 70	3, 10, 30	3, 10, 30
Haloperidol	0.1, 0.3, 1, 3	3, 10, 30	10, 20, 30, 50	1, 3, 10	20, 50, 100, 300[b]
Chlorpromazine	3, 5, 10, 20	3, 10, 30	10, 20, 30	1, 3, 10	10, 20, 30, 100, 300
Thioridazine	3, 10, 30, 100	10, 30, 100	30, 100, 300	3, 10, 30, 100	10, 30, 100

MES = maximal electric shock
a) The test could not be conducted in 8 animals in 300 mg/kg group and 10 animals in 1000 mg/kg groups due to death.
b) The test could not be conducted for 4 animals in 300 mg/kg due to death.

Inhibition of Spontaneous Activity

Lurasidone dose-dependently reduced spontaneous locomotor activity in mice with an ED50 of 9.9 mg/kg. The rank order of the potency of lurasidone inhibitory effect compared to the other tested antipsychotics was as follows: risperidone > haloperidol > olanzapine > sertindole > chlorpromazine ≥ clozapine ≥ ziprasidone ≥ lurasidone ≥ thioridazine.

Potentiation of Hexobarbital-induced Anesthesia

Lurasidone significantly prolonged the duration of hexobarbital-induced anesthesia (the duration of loss of righting reflexes) only at high doses of 700 mg/kg and 1000 mg/kg. The ED50 value was more than 1000 mg/kg, since at the dose of 1000 mg/kg, only 4 of 10 mice showed enhancement of hexobarbital-induced anesthesia. In contrast, other antipsychotics enhanced hexobarbital-induced anesthesia with ED50 values ranging from 1.5 to 28 mg/kg.

Muscle Relaxation

Lurasidone failed to induce muscle relaxation in mice even at a dose of 1000 mg/kg (1 positive out of 10 mice; ED50 value >1000 mg/kg). The other tested antipsychotic drugs induced muscle relaxation dose-dependently. The rank order of their potency was as follows: risperidone = olanzapine > sertindole > chlorpromazine > clozapine ≥ haloperidol > ziprasidone and thioridazine.

Impairment of Motor Coordination

Lurasidone inhibited motor coordination at relatively high doses (100 to 700 mg/kg) with an ED50 value of 250 mg/kg. Other antipsychotics inhibited motor coordination at lower doses with ED50 values ranging from 1.8 to 43 mg/kg.

Inhibition of Maximal Electric Shock (MES) Convulsion

Lurasidone had a negligible effect on MES-induced seizures (ED50 value >1000 mg/kg). Haloperidol and chlorpromazine inhibited MES-induced seizure with ED50 values of 99 and 150 mg/kg, respectively. Other antipsychotics produced no inhibitory effects at the doses tested.

Other Secondary Pharmacodynamic Effects

The affinity of lurasidone for histamine H1 receptors was investigated in Study 6965. The binding affinities of lurasidone for various types of ion channels were determined in Study 7232 to investigate its capacity to affect the action potential of the cardiac muscle.

In Vitro Histamine Receptor Binding Profile of Lurasidone and Reference Drugs

The affinity of lurasidone for H1 receptors was studied in comparison to other reference drugs (Study 6965). The results are summarized in the following sponsor's table.

In Vitro Histamine Binding Activity Data for Lurasidone and Reference Drugs

Receptor Type	Species	Tissue Source	Radioactive Ligand	Test Article	Binding Affinity Mean K_i (nM)
Histamine 1	Guinea Pig	Whole brain	^{3}H-pyrilamine	lurasidone	>1000 (IC_{50})
				clozapine	2.02
				risperidone	3.46
				chlorpromazine	4.61
				thioridazine	12.0
				fluphenazine	13.2
				trifluoperazine	33.2
				haloperidol	330

Lurasidone negligibly affected the specific binding of [3H]pyrilamine to H1 receptors; the IC50 value was >1000 nM. In comparison, the reference drugs (clozapine, risperidone, chlorpromazine, thioridazine, fluphenazine, trifluoperazine and haloperidol) had much higher affinities for H1 receptors. The data suggest that lurasidone is less likely to induce central effects such as drowsiness and sedation than the other reference drugs tested.

In Vitro Effects on Ion Channels

The binding affinity of lurasidone and chlorpromazine (a typical antipsychotic) to binding sites of ion channels (the L-type Ca2+ channel, the K+ channel, and the Na+ channel) was evaluated in Study 7232, as summarized in the following sponsor's table.

In Vitro Binding Affinity of Lurasidone and Chlorpromazine for Various Ion Channels

Ion Channel Type	Species	Tissue Source	Radioactive Ligand	Lurasidone Binding Affinity Mean K_i, (nM)	Chlorpromazine Binding Affinity Mean K_i, (nM)
L-type Ca^{2+} channel					
dihydropyridine site[a]	Rat	Cerebral cortex	^{3}H-nitrendipine	>1000[b]	--
benzothiazepine site	Rat	Cerebral cortex	^{3}H-diltiazem	>1000[b]	>1000[b]
phenylalkylamine site	Rat	Whole brain	(-)-^{3}H-D-888	641 ± 190	569 ± 110
K^+ channel					
K_A channel	Rat	Cerebral cortex	^{125}I-dendrotoxin	>1000[b]	>1000[b]
K_{ATP} channel	Hamster	Pancreatic β cells	^{3}H-glyburide	>1000[b]	>1000[b]
K_V channel	Rat	Forebrain	^{125}I-charybdotoxin	>1000[b]	>1000[b]
Na^+ channel	Rat	Whole brain[c]	^{3}H-batrachotoxin	398 ± 78	>1000[b]

-- = not tested
[a] Value for L-type Ca^{2+} channel dihydrophyridine site was quoted from Study 7078.
[b] IC_{50} value
[c] except the cerebellum

Lurasidone showed low binding affinities (similar to that of chlorpromazine) to the phenylalkylamine site of the Ca2+ channel with Ki value of 641 nM and to the voltage-gated Na+ channel with Ki value of 398 nM. Lurasidone showed very low affinity to the dihydropyridine site and benzothiazepine site of the L-type Ca2+ channel, the A-type K+ channel, voltage-gated K+ channel and ATP-sensitive K+ channel (IC50>1000 nM).

4.3 Safety Pharmacology

A battery of in vitro and in vivo safety pharmacology studies in multiple species was conducted to investigate the potential undesirable pharmacodynamic effects of lurasidone and its metabolites on physiological functions in relation to exposure. The organ systems evaluated included the CNS, cardiovascular, respiratory, autonomic nervous system, endocrine, urinary, digestive system and smooth muscle. Decreases in spontaneous activity and exploratory behavior in rats were induced at single oral doses of 10 mg/kg and higher and were likely due to the D2 receptor antagonistic activity of lurasidone. Lurasidone decreased the spontaneous EEG activity in rabbits after i.v. administration of 1 mg/kg as a single dose and inhibited emetic response to apomorphine in dogs following oral administration, but exerted no other potent effects on the CNS (i.e., anti-acetylcholine action, anti-hypoxic action, effects on cerebral blood flow, convulsion facilitating action, or anti-adrenergic action).
The safety pharmacology concerns for lurasidone pertain to the cardiovascular and endocrine systems. QTc prolongation was observed in one species (dog) out of the three tested (guinea pigs, cats). Lurasidone oral administration to conscious Beagle dogs at a dose of 300 mg/kg (Cmax 2.78 μg/ml) produced significant prolongation of the QTc, but no marked effects on blood pressure and QRS duration. Lurasidone produced no changes in QT interval and QTc at a dose of 100 mg/kg (Cmax 1.9 μg/ml). Endocrine effects included increases in prolactin, ACTH, and corticosterone; there were no effects on anterior pituitary, thyroid or sex hormones.

Effects on the General Physical Conditions and Behavior

The effects of lurasidone on general physical condition and behavior were studied in male Sprague-Dawley rats (Study G0011). Lurasidone was orally administered at single doses of 1, 10, and 100 mg/kg (0.5% methylcellulose). General physical condition and behavior were observed at 1, 2, 4, 6, and 24 hours post-dose. At 10 mg/kg, a decrease in spontaneous activity and eyelid droop were observed from 2 to 6 hours post-dose (in 2 of 4 rats), and a decrease in exploratory behavior was observed at 2 and 4 hours post-dose (in 1 out of 4 rats). At 100 mg/kg, decreases in spontaneous activity, exploratory behavior, grooming, and touch response were observed from 2 to 6 hours post-dose in at least 1 of 4 rats and eyelid droop was observed in all rats. These symptoms were no longer observed at 24 hours postdose. The NOAEL was 1 mg/kg.
The observed decreases in exploratory behavior and spontaneous activity were likely due to D2 receptor antagonistic activity of lurasidone.

Effects on CNS

Lurasidone slowed the spontaneous EEG activity in rabbits after i.v. administration of 1 mg/kg as a single dose (Study 7082) and inhibited emetic response to apomorphine in dogs following oral administration (Study PS9728), but exerted no other potent effects on the CNS (i.e., anti-acetylcholine action, anti-hypoxic action, effects on cerebral blood flow, convulsion facilitating action, and anti-adrenergic action) (Studies 6932, 7031, 7055, 7241).

The results of the studies are summarized in the following sponsor's table.

Effects of Lurasidone on the Central Nervous System

Test items	Animals	Route	Experimental method	Test results		Study No.
				Lurasidone	Reference drugs	
EEG	Rabbits	IV	Spontaneous EEG	No change up to 0.1 mg/kg Slowed EEG at 1 mg/kg	HAL: Slowed EEG at 0.3 mg/kg	7082[a]
	Rabbits	IV	Arousal response	No effects up to 1 mg/kg	HAL: Increased stimulus threshold at 0.3 mg/kg	7082[a]
Cerebral blood flow	Rats	IV	Laser Doppler (cerebral cortex)	No effects up to 1 mg/kg	n.t.	7055
Convulsive activity	Mice	oral	Electroshock-induced seizure (synergistic)	No effects up to 1000 mg/kg	HAL: No effects up to 30 mg/kg RIS: Increased convulsion at 30 mg/kg	6932
Anti-acetylcholine action	Mice	oral	Inhibition of oxotremorine-induced tremor	ED_{50} > 1000 mg/kg	HAL: ED_{50} = 23 mg/kg	7031[b]
Anti-hypoxia	Mice	oral	Hypobaric hypoxia	No effects up to 100 mg/kg	HAL: No effect up to 3 mg/kg RIS: Increased toxicity ≥ 0.3 mg/kg	7031
	Mice	oral	KCN induced anoxia	No effects up to 100 mg/kg	n.t.	7031
Anti-nor-adrenaline action	Rats	oral	Death by noradenaline	No effects up to 1000 mg/kg	HAL: No effects up to 300 mg/kg RIS: ED_{50} = 1.7 mg/kg	7031, 7241
	Mice	oral	Ptosis	ED_{50} was about 1000 mg/kg	HAL: ED_{50} = 6.3 mg/kg RIS: ED_{50} = 0.68 mg/kg	7031, 7241

HAL = haloperidol; RIS = risperidone; n.t. = not tested
a) HAL data in the report were cited from Ohno-1997; b) HAL data in the report were cited from Hirose-1990.

Effects on EEG in Rabbits

The effects of lurasidone (0.01, 0.1, 1 mg/kg, i.v.) on spontaneous electroencephalogram, EEG arousal response, EEG recruiting response, and hippocampal after-discharge were examined in rabbits (Study 7082). At 1 mg/kg, lurasidone induced a resting EEG pattern (slow waves with high amplitude in the cortex and desynchronization of theta rhythm in the hippocampus) with a peak at 5 to 15 minutes after administration. EEG frequency analysis also showed a tendency of increased delta component in cortical EEG and decreased theta component in hippocampal EEG, after lurasidone administration at 1 mg/kg. There were no abnormal EEG patterns including spikes and seizure waves.

A published study using the same experimental design and methods reported that two reference drugs, haloperidol and chlorpromazine, also induced slow waves in rabbit spontaneous EEG at 0.3 and 0.1 mg/kg, respectively (Ohno-1997, as cited by the sponsor).

Lurasidone slowed the spontaneous EEG at 1 mg/kg, but showed no effect on EEG arousal, EEG recruitment or hippocampal after-discharge response following stimulation. These results suggest that lurasidone has a relatively weak CNS depressant activity.

Effects on Cerebral Blood Flow

The effects of intravenous lurasidone (0.1, 0.3, 1 mg/kg) on blood pressure and cerebral blood flow were evaluated in male Wistar rats (5-6 /group) (Study 7055). Changes in cerebral blood flow between pre- and post-intravenous administration of lurasidone were determined using a laser Doppler probe attached to the parietal cortex of rats. Blood pressure and cerebral blood flow were recorded on a thermal array recorder through a blood pressure transducer and amplifier and through a laser Doppler cerebral blood flow meter,

respectively. Blood pressure and cerebral blood flow were measured at 1 minute before, immediately after, and 1, 3, 5, and 20 minutes after administration of lurasidone.
Lurasidone caused a transient decrease in blood pressure at HD (1 mg/kg, i.v.), but showed no apparent effect on cortical cerebral blood flow at any of the tested doses. Lurasidone also did not affect any of the tested blood gas parameters (PO2, PCO2, and pH).
The transient decrease in blood pressure at the highest dose in this study was likely mediated by α1 receptor antagonism, having in mind that lurasidone had a weak affinity for the adrenergic α1 receptor (Ki 47.9 nM), and exerted a blood pressure-lowering effect in anesthetized cats (Study B000240). However, the blood pressure-lowering effect of lurasidone in the present rat study was mild and transient, without being accompanied by changes in cerebral blood flow.
The results of this study showed that lurasidone (0.1 to 1 mg/kg, i.v.) had no effects on the cerebral blood flow.

Effects on Convulsive Activity

The effect of lurasidone on electrically induced convulsion was examined in male mice (10/group) and compared to reference drugs (Study 6932). Lurasidone or reference drugs were administered as single oral doses. Lurasidone was tested at doses of 3, 10, 30, 100, 300 and 1000 mg/kg. The doses of the reference drugs were selected based on ED50 of anti-dopaminergic activity (as previously determined by inhibition of MAP-induced hyperactivity); the highest doses were 30-fold higher than the respective ED50s. One hour after administration, a sub-threshold electroshock (8 mA, 0.2 sec) was applied to both orbits of each mouse. The occurrence of tonic seizure was recorded immediately after stimulation. The results are summarized in the following sponsor's table:

Lurasidone Effect on Convulsive Activity in Comparison to Reference Drugs

Drugs	Dosage (mg/kg)	Incidence of Electroshock-induced tonic seizure
Lurasidone	3, 10, 30, 100, 300, 1000	10 mg/kg – 1/10 300 mg/kg – 1/10
Risperidone	3, 10, 30, 60	3 mg/kg – 1/10 10 mg/kg – 1/10 30 mg/kg – 4/10[a] 60 mg/kg – 3/10
Olanzapine	3, 10, 30, 100	30 mg/kg – 2/10
Sertindole	10, 30, 100, 300	None
Clozapine	10, 30, 100, 300	100 mg/kg – 3/10 300 mg/kg – 1/2 (8/10 died before electroshock)
Perospirone	3, 10, 30, 100, 300, 1000	100 mg/kg – 1/10
Haloperidol	1, 3, 10, 30	None
Chlorpromazine	0.3, 1, 3, 10, 30, 100, 300, 600	10 mg/kg – 1/10 600 mg/kg – 2/10 died before electroshock

[a] $p < .05$ versus vehicle control group (chi-square test)

Risperidone produced significant enhancement of electrically-induced convulsion at 30 mg/kg compared to control. Clozapine increased electrically-induced seizures in 3 of 10 mice at 100 mg/kg (not significantly different from control) and, at 300 mg/kg, caused mortality due to convulsions without electroshock.
Except for risperidone and clozapine, increase in electrically induced convulsions was not observed in either lurasidone or other reference drug groups. Although tonic seizures occurred in some animals treated with lurasidone, olanzapine, perospirone, and chlorpromazine, there was no dose-dependent effect and no significant differences compared to the control group.

Effects on Emetic Response

The inhibitory effect of lurasidone on apomorphine-induced emesis was evaluated in female Beagle dogs (Study PS9728). Lurasidone was administered orally at single doses of 0.1, 0.3, 1, and 3 mg/kg (suspension in 0.5% methylcellulose). One hour after administration of the test substance, 40 µg/kg of apomorphine was administered intravenously to examine the occurrence and frequency of emesis over a period of 1.5 hours.

Lurasidone, at doses of 0.3 mg/kg and higher, significantly inhibited the apomorphine-induced emetic response.

Effects on Acetylcholine System

Lurasidone showed a negligible inhibitory effect on oxotremorine-induced tremor in mice (Study 7031), an effect mediated by anticholinergic activity. Lurasidone activity was much weaker (ED50 >1000 mg/kg) than that reported for haloperidol, chlorpromazine, and thioridazine (ED50 values of 23, 36, and 19 mg/kg, respectively (Hirose-1990, as cited by the sponsor).

Effects on Hypoxia-Induced Lethality

- Effects on Hypobaric Hypoxia-Induced Lethality

Lurasidone (3, 10, 30, 60, 100 mg/kg), haloperidol (0.1, 0.3, 1, 3 mg/kg), or risperidone (0.1, 0.3, 1, 3 mg/kg) were orally administered to groups of 10 male mice (Study 7031). One hour later, mice were placed in an airtight chamber and the pressure was immediately reduced to 190 mmHg using a vacuum pump. The survival time from the start of pressure reduction to respiratory arrest was measured. Lurasidone and haloperidol, up to the highest tested doses of 100 mg/kg and 3 mg/kg, respectively, showed no effect on the survival time in hypobaric hypoxia-induced lethality in mice. Risperidone significantly decreased the survival time in a dose-dependent manner at doses of 0.3 mg/kg and higher ($p < .01$)

- Effects on KCN (Anoxia)-Induced Lethality

Lurasidone (3, 10, 30, 60, 100 mg/kg) was orally administered to groups of 10 male mice (Study 7031). One hour later, KCN (16 mg/kg) was injected s.c. The survival time from the start of KCN administration to cardiac arrest was measured. Lurasidone at doses up to 100 mg/kg showed no effect on the survival time in KCN (anoxia)-induced lethality in mice.

Other Effects on Adrenergic System

- Induction of Ptosis

Lurasidone (100, 300, 700, 1000 mg/kg) and reference drugs: haloperidol (1, 3, 10 mg/kg), chlorpromazine (3, 10, 20 mg/kg) and risperidone (0.1, 0.3, 1 mg/kg) were orally administered to male mice (10/group) (Studies 7031, 7241). Ptosis was evaluated at 1 hour after administration. Lurasidone dose-dependently induced ptosis, but its activity was weak, with the effect occurring in 5 of 10 animals at 1000 mg/kg. An ED50 value could therefore not be calculated, but was presumed to be about 1000 mg/kg. In contrast, haloperidol, chlorpromazine, and risperidone induced ptosis at low doses with ED50 values of 6.3, 9.2 and 0.68 mg/kg, respectively.

- Effects on Noradrenaline-Induced Lethality

Lurasidone (100, 300, 1000 mg/kg) and reference drugs: haloperidol (50, 100, 300 mg/kg), chlorpromazine (3, 10, 20, 30 mg/kg) and risperidone (0.3, 1 and 3 mg/kg) were orally administered to male Sprague-Dawley rats (5 or 6/group) (Studies 7031, 7241). One hour later, noradrenaline (1.25 mg/kg) was injected i.v. and the survival of animals at 30 minutes after the injection was recorded.

Lurasidone negligibly inhibited noradrenaline-induced lethality at doses up to 1000 mg/kg; at 1000 mg/kg, only one out of six tested animals survived. In contrast, chlorpromazine and risperidone inhibited noradrenaline-induced lethality (ED50 values of 15.5 and 1.7 mg/kg, respectively). Haloperidol had no effect at doses up to 300 mg/kg.

Effects on the Cardiovascular System

A complete battery of cardiovascular safety studies including: in vitro hERG assays, *ex vivo* studies in guinea pig or rat tissues, and in vivo studies in rats, guinea pigs, cats, and dogs were performed to assess the effects of lurasidone on the cardiovascular system.

In Vitro Cardiovascular Safety Studies

The effect of lurasidone on cardiovascular function in vitro was assessed in hERG-transfected HEK293 cells and in fresh tissue preparations isolated from rat aorta, guinea pig atrium or papillary muscles.
Lurasidone inhibited rapidly activating delayed rectifier potassium currents in HEK293 cells with an estimated IC50 value of 108 nM (57 ng/ml).
In isolated rat aorta preparations, lurasidone did not affect resting tension or KCl-induced contraction up to the highest tested concentration of 1000 and 3000 ng/ml, respectively. However, lurasidone (300 ng/ml) produced a competitive antagonistic effect on noradrenaline-induced contractions.
In atrium isolated from guinea pigs, lurasidone had no significant effects on atrial contractile force or heart rate at concentrations of up to 3000 ng/ml.
In papillary muscle of the right ventricle isolated from guinea pig, lurasidone had no effects on any action potential parameters - resting membrane potential (RMP), action potential amplitude (APA), action potential duration at 50% and 90% repolarization (APD50, APD90), or maximum velocity of depolarization (Vmax) at concentrations up to 1 μM (=529 ng/ml). In comparison, chlorpromazine increased RMP at 1 μM (=355 ng/m), and at a concentration of 10 μM (=3,553 ng/ml) further elevated RMP, decreased both Vmax and APA, and extended both APD50 and APD90.

Effects on hERG

The effect of lurasidone on rapidly activating delayed rectifier potassium currents (hERG) in hERG-expressing HEK293 cells was determined in Study SP-0310. Lurasidone inhibited hERG currents in HEK293 cells with an estimated IC50 value of 108 nM.

Effect on Cardiac Smooth Muscle in Rat Aorta

The effects of lurasidone (300, 1000, 3000 ng/ml) on resting tension and on contractions induced by KCl and noradrenaline in smooth muscle tissue from aorta isolated from male rats were examined in Study 7618. Lurasidone did not affect the resting tension or KCl-induced contraction of isolated rat aorta up to the highest tested concentration of 1000 and 3000 ng/ml, respectively. Lurasidone at a concentration of 300 ng/ml exerted a competitive antagonistic effect on noradrenaline-induced contraction that was likely due to its known antagonistic effects on noradrenaline receptors.

Effects on Contractile Force and Heart Rate in Guinea Pig Atrium

The effects of lurasidone (30, 300, 3000 ng/ml) on cardiac contractile force and heart rate were investigated in isolated atrium from guinea pig (Study 7315). The results were compared with those of chlorpromazine (300, 3000, 30 000 ng/ml). Lurasidone had no significant effects on either atrial contractile force or heart rate. In comparison, chlorpromazine showed effects at a concentration of 3000 ng/ml and higher; at the highest chlorpromazine concentration, the left and right atrial contractile forces and the right atrial beating rate were decreased by 82%, 62%, and 49%, respectively.

Effects on Action Potential in Guinea Pig Papillary Muscle

The electrophysiological effects of lurasidone on cardiac action potential parameters were examined in papillary muscle of the right ventricle isolated from guinea pig and compared with chlorpromazine (Study 7245). Lurasidone (0.1, 0.3, 1 μM , equivalent to 52.9, 158.7, 529 ng/ml), chlorpromazine (0.1, 1, 10 μM equivalent to 35.5, 355, 3553 ng/ml) or vehicle (PEG) was applied by perfusion.
Lurasidone showed no effects on action potential in guinea pig right ventricular papillary muscle whereas chlorpromazine elevated the resting membrane potential (RMP) at 1 μM (355 ng/ml) and higher, and at a concentration of 10 μM (3,553 ng/ml) further elevated RMP; decreased both Vmax and action potential amplitude, and extended the action potential duration at 50% and 90% repolarization.

In Vivo Cardiovascular Safety Studies

The studies on the effects of lurasidone on cardiovascular function in vivo are summarized in the following sponsor's table.

Lurasidone Cardiovascular Safety Pharmacology Studies In Vivo

Species	Parameters	Dose (mg/kg) (Route)	Test Results	Study No.
Rat (unanesthetized, unrestrained Spontaneously Hypertensive Rat)	Blood pressure	100, 1000 (po)	• No change in BP seen relative to control group.	B000241
Rat (Wistar)	Blood pressure	0.1, 0.3, 1 (IV)	• At a dose of 1 mg/kg, a significant ↓ in BP was seen with a peak at 3 minutes post-dose ($p < .01$). • This change was transient and returned to the control level < 5 minutes post-dose.	7055
Guinea pig (anesthetized)	Blood pressure, heart rate, ECG, toxicokinetics	0.3, 1 (IV)	• At serum concentrations as high as 761 ng/mL (2 minutes post-dose of 1 mg/kg), no prolongation of the QTc seen. • At 1 mg/kg, ↓ HR (10 min. post dose). • At 0.3 mg/kg, ↓ BP (transient); at 1 mg/kg, further ↓ BP not seen	C0020, C0022
Cat (anesthetized & anesthetized, vagotomized)	Blood pressure, heart rate, blood flow	1, 10, 100 µg/kg (IV)	• Expt 1: At 10 µg/kg, ↓ BP by 21% and 22% in 2 animals; no effect on HR; ↑ BF by 80% in 1 animal. At 100 µg/kg, ↑ BF from 47% to 117% in all 3 animals. • Expt 2: At 10 µg/kg, ↓ BP by 10% to 19%. At 100 µg/, ↓ BP by 32% to 39; no effect on HR	B000240
Dog (anesthetized)	ECG, heart rate	3, 10, 30 µg/kg (IV)	• At doses up to 30 µg/kg, no effects on ECG or HR observed.	7296
Dog (conscious; telemetry)	Blood pressure, heart rate, ECG, toxicokinetics	100, 300 (po)	• No statistically significant effects on systolic, diastolic or mean BP • Statistically significant ↑ HR observed at both doses. ↑ HR < 45 beats per minute. Recovery seen in 8 – 10 hours post-dose. • Statistically significant ↓ in PQ and RR intervals (100 and 300 mg/kg) seen; not dose-dependent. Changes might be due ↑ HR. • No statistically significant changes in QRS. • Statistically significant prolongation of QTc interval, not QT interval, seen (300 mg/kg). QTc intervals calculated using Fridericia's formula. • Sotalol, the positive control, caused ↑ prolongation of the QT and QTc interval.	3815

HR = heart rate; BP = blood pressure; BF = blood flow

Effects on Blood Pressure in Rats

The effects of lurasidone on blood pressure parameters in rats were determined in two *in vivo* studies (Studies B000241 and 7055). No effect on blood pressure was observed following oral administration of lurasidone at single doses of 100 and 1000 mg/kg in unanesthetized, unrestrained spontaneously hypertensive rats (5/group). The effects were compared to those of risperidone (0.3 and 3 mg/kg). In the risperidone 3 mg/kg group, significantly decreased mean blood pressure was noted at 1 to 7 hours after administration compared to control (Study B000241). Intravenous administration of 1 mg/kg to male

Wistar rats resulted in a significant decrease in blood pressure with a peak at about 3 min post dose. This change was transient and returned to the pretreatment level within 5 minutes after administration (Study 7055). Similar transient decreases in blood pressure were also observed following intravenous administration of lurasidone to anesthetized guinea pigs and cats (Studies C0020 and B000240, respectively). These changes in blood pressure were mild and transient; most likely they were related to the relatively weak affinity of lurasidone to noradrenaline α1 receptor (Ki 47.9 nM).

Effects on ECG, Heart Rate and Blood Pressure in Anesthetized Guinea Pigs

Lurasidone effects on ECG and cardiovascular parameters were evaluated after intravenous administration to anesthetized guinea pigs at doses of 0.3 and 1 mg/kg and compared with those of risperidone (0.05, 0.15 mg/kg) and terfenadine (7 mg/kg; a positive control for QTc prolongation) (Study C0020). Serum concentrations of the test articles were determined in blood samples collected 5 min after administration. A follow-up toxicokinetic bridging study was also conducted to determine the serum concentrations of the same test articles at additional time points (2, 5, 15 minutes) (Study C0022).

Significant decrease of heart rate was observed at lurasidone HD (1 mg/kg) at 10 minutes after administration; a slight transient decrease in the mean blood pressure was observed at 0.3 mg/kg, but not at 1 mg/kg. No effects on ECG parameters (PR interval, QRS interval, QTc) were observed at either of the tested lurasidone doses, while the i.v. administration of risperidone (0.15 mg/kg) resulted in a significant decrease of the PR interval and a trend to a decrease in QTc interval. As expected, the positive control terfenadine significantly prolonged the QTc interval. The serum concentrations of lurasidone and risperidone were dose dependent.

Serum Concentrations Following i.v.Administration of Lurasidone, Risperidone or Terfenadine to Anesthetized Guinea Pigs

	Serum Concentration (ng/mL)			
	5 minutes	2 minutes	5 minutes	15 minutes
Lurasidone (0.3 mg/kg)	--	282	130	50.2
Lurasidone (1 mg/kg)	417	761	492	253
Risperidone (0.05 mg/kg)	--	25.2	18.2	14.8
Risperidone (0.15 mg/kg)	55.2	105	88	53.6
Terfenadine (7 mg/kg)	693	1047	641	408

-- = not tested
Each value represents a mean of n=5 in Study C0020; a mean of n=4 in Study C0022

In conclusion, no effects on ECG parameters (PR interval, QRS interval, QTc) were observed in guinea pigs after lurasidone administration at single i.v. doses of up to 1 mg/kg (serum concentrations up to 761 ng/ml).

Effects on ECG and Heart Rate in Anesthetized Beagle Dogs

The effects of single i.v. administration of lurasidone (3, 10, 30 µg/kg) or chlorpromazine (10, 100, 1000 µg/kg, i.v.) on the occurrence of arrhythmia, ECG (PR interval, QRS duration, QT and QTc) and heart rate were examined in anesthetized Beagle dogs (2/sex/group) (Study 7296).

ECG was continuously recorded at 25 mm/sec. Heart rate and ECG parameters (PR, QRS, QT interval and QTc) were recorded and analyzed by ECG analyzer before administration and at 1, 3, 5, 10, 20, and 30 min after administration. The test substances at low, medium and high dose levels in that order, and the negative control (25%-PEG or physiological saline) 0.1 ml/kg were administered through femoral

vein cannula 3 times at 30-minute intervals. The difference between the absolute pre-administration and post-administration values for each animal was calculated.
PEG was used as negative control for lurasidone, and physiological saline as a negative control for chlorpromazine. The effects of 25%-PEG used as vehicle of lurasidone on ECG parameters were evaluated by comparing to the effects of physiological saline.
The effects of lurasidone on each parameter in comparison to the effects of 25%-PEG are shown in the following sponsor's table. Although the PR interval showed significant decrease at 3µg/kg at 20 and 30 minutes after administration, the changes were apparently not drug-related since the PR interval was not affected at the higher doses (10 or 30 µg/kg). Although QT interval showed significant prolongation at 3 µg/kg (at 10 and 20 min after administration), this change was not likely to be drug-related as well, since the QT interval was not prolonged at the higher doses. QT interval was significantly shortened at HD (on a single occurrence 5 min post dosing). No effects on heart rate, QRS duration and QTc were observed. Thus, lurasidone did not appear to affect the ECG or heart rate.

Effects of Lurasidone on PR and QT Intervals in Beagle Dogs

Test Article	Change at Respective Times After Administration (msec)						
	PR interval			QT interval			
	BA	20 min	30 min	BA	5 min	10 min	20 min
Saline (First)	83.7	2.1	2.2	212	0.5	4.2	8.0
25% PEG (First)	100.6	5.7*	7.1	249.4*	4.0	10.4	14.5
Lurasidone 3 µg/kg	108.6	1.5#	1.8#	239.3	4.6	4.0#	4.3#
Saline (Second)	85.8	0.9	-1.3	222.7	3.2	1.7	3.7
25% PEG (Second)	107.7*	1.1	2.7	269.9*	5.2	6.0	10.0
Lurasidone 10 µg/kg	110.4	4.5	4.1	250.5	-1.0	0.7	11.7
Saline (Third)	84.5	3.7	2.0	228.4	4.2	2.7	7.1
25% PEG (Third)	110.3**	2.2	-0.4	282.8*	6.7	1.7	0.7
Lurasidone 30 µg/kg	114.4	-0.7	-0.5	261.0	-0.1#	2.9	3.4

Each value represents the mean of 4 dogs
BA = before administration; Observations at respective minutes after administration
*p < .05, **p < .01, significantly different from saline
#p < .05, significantly different from 25% PEG

Chlorpromazine induced arrhythmia (a single occurrence of ventricular extrasystole) at 100 and 1000 µg/kg (in 1 of 4 animals each) and a trend to increased heart rate at 100 µg/kg. The PR and QT intervals were significantly shortened at 100 µg/kg, at 1 and 3 min and 3 and 5 min after administration, respectively; these changes were associated with the increased heart rate at this dose. Neither QTc nor QRS duration were affected at 100 and 1000 µg/kg. Thus, chlorpromazine induced arrhythmia at 100 and 1000 µg/kg and a trend to increased heart rate with shorter PR and QT intervals at 100 µg/kg.
Overall, in anesthetized dogs, i.v. administration of lurasidone at single escalating doses of up to 30 µg/kg did not induce arrhythmia and had no effect on the ECG parameters or heart rate, while chlorpromazine induced arrhythmia (single occurrence of ventricular extrasystole) at 100 and 1000 µg/kg (in 1 of 4 animals each) and shortening of PR and QT interval at 100 µg/kg associated with a increased heart rate; there were no changes in either QTc or QRS duration.

Effects on ECG, Blood Pressure and Heart Rate in Conscious Beagle Dogs

The effects of lurasidone on blood pressure, heart rate, ECG parameters, and TK were studied in conscious dogs by telemetry (Study 3815). Lurasidone (100 and 300 mg/kg), sotalol (30 mg/kg) as a positive control, or vehicle (0.5% methyl cellulose) were administered orally to 4 female Beagle dogs at 1 or 2-week intervals. On each day of administration, continuous recordings of blood pressure, heart rate and ECG (RR, PQ, QRS, and QT interval) were performed pre-administration and for 24 hours after each administration using a telemetry data collection and computer analysis. The parameters were analyzed before and 0.5, 1, 2, 4, 6, 8, 10, 12, 24 h after each administration. QTc was calculated using the Fridericia's correction [QTc=QT/(RR)1/3]. The results are summarized in the following sponsor's table:

Effects of Lurasidone on Blood Pressure, Heart Rate, ECG and TK Parameters in Conscious Dogs

	Lurasidone (100 mg/kg)	Lurasidone (300 mg/kg)	Solatol (30 mg/kg)
Blood Pressure	No significant differences	No significant differences	Lowering of diastolic blood pressure at 4 hours*
Heart Rate	High values at 4 and 6 hrs** High values at 8 and 12 hrs*	High value at 4 hrs*	No significant differences
Electrocardiogram	PQ interval: low from 0.5 to 2, 6, 8, 12 hours post-dose. QRS duration: no significant differences QT and QTc interval: no significant prolongation	PQ interval: low at 0.5, 1, 6, 8, 10 hours post-dose. QRS duration: no significant differences QT interval: no significant differences QTc interval: prolongation at 4, 6, and 24 hours	QT interval: prolongation at 0.5, 2 to 8, and 12 hours QTc interval: prolongation at 0.5, 4 to 8, and 12 hours
C_{max} (µg/mL) T_{max} (hours) AUC_{0-t} (µg·hr/mL)	1.903 2.5 17.0	2.780 7.5 46.5	22.48 1 173.1

Observations at respective hours after administration
*p < .05; **p < .01

Blood pressure: No differences in systolic, diastolic, and mean blood pressure were observed between lurasidone and vehicle control up to 24 hours after administration. Solatol caused a significant decrease in diastolic blood pressure 4 hours after administration.

Heart rate: The heart rate in lurasidone-treated animals was significantly increased vs. vehicle control at 100 mg/kg (4, 6, 8, and 12 h after administration) and at 300 mg/kg (4 h after administration). Solatol showed no significant differences as compared with the vehicle.

ECG: Significant prolongation in QTc was observed at 300 mg/kg lurasidone vs. vehicle control at 4, 6, and 24 hours after administration. Significant decreases in PQ intervals were observed in 100 mg/kg and 300 mg/kg groups at 0.5 to 2, 6, 8, 10 and 12 h after administration. For QT interval, significantly lower values vs. vehicle control were observed at 100 mg/kg (6 and 8 h after administration), but not at 300 mg/kg. No differences in QRS duration were observed up to 24 hours after administration.

The positive control solatol induced a significant prolongation of QT interval at 0.5, 2 to 8, and 12 hours after administration; in QTc at 0.5, 4 to 8, and 12 h after administration; in PQ interval 0.5 h after administration and in RR interval 0.5 and 4 h after administration.

Lurasidone serum concentration increased gradually after administration; the Tmax was 2.5 h and 7.5 h at 100 and 300 mg/kg, respectively. The Cmax and AUC0-t values at 100 mg/kg were 1.9 µg/ml, and 17 µg·hr/ml, respectively, and at 300 mg/kg - 2.78 µg/ml and 46.5 µg·hr/ml, respectively. The serum concentration of sotalol increased rapidly after administration with a Tmax at 1 h. post dose; Cmax, and AUC0-t were 22.5 µg/ml and 173.1 µg hr/ml, respectively.

In summary, lurasidone oral administration at a dose of 300 mg/kg (Cmax 2.780 µg/ml), produced significant prolongation of the QTc in conscious Beagle dogs, but no marked effects on blood pressure and QRS duration. At doses of 100 and 300 mg/kg, slight increases in heart rate and shortening of PQ and RR intervals were observed. Lurasidone produced no changes in blood pressure, QT interval, QTc and QRS duration at a dose of 100 mg/kg (Cmax 1.903 µg/ml). In contrast, the positive control sotalol at 30 mg/kg caused a marked prolongation of the QT interval and QTc.

Effects on Cardiovascular System in Anesthetized Cats (BP, HR, and blood flow)

The effects of lurasidone (at escalating i.v. doses of 1, 10, 100 µg/kg) on the cardiovascular system were examined in anesthetized cats and in anesthetized *and* vagotomized cats in two separate experiments that also evaluated effects on sympathetic and parasympathetic nervous systems (Study B000240). Cardiac parameters (mean blood pressure, heart rate, and blood flow) were measured and compared with those of risperidone (0.1, 1, 10, 100 µg/kg, i.v.).

The test substances were administered through a femoral vein cannula, starting with the lowest dose and increasing the dose at 20 min intervals.

Both lurasidone and risperidone produced a decrease in the mean blood pressure in anesthetized and in anesthetized *and* vagotomized cats, as well as increase in blood flow in anesthetized cats (see the sponsor's table below).The lowest effective doses for these effects were 10 µg/kg for lurasidone and 0.1 µg/kg for risperidone. Thus, the potency of lurasidone for affecting blood pressure and blood flow was approximately 1/100 that of risperidone. The increased blood flow was thought to be a result of dilation of the peripheral blood vessels due to blockage of the α-receptors. A possible explanation for the lack of increase in blood flow at the HD of risperidone could be the "decreased return blood flow caused by marked depression of mean blood pressure". Both lurasidone and risperidone had no effect on heart rate.

Effects on the Cardiovascular System in Anesthetized Cats

	Experiment 1			Experiment 2	
Test Article	**Mean Blood Pressure**	**Heart Rate**	**Blood flow**	**Mean Blood Pressure**	**Heart Rate**
Lurasidone	10 µg/kg: reduced by 21% and 22% in 2 animals 100 µg/kg: reduced by 24% to 43% in all 3 animals	None	10 µg/kg: increased by 80% in 1 animal 100 µg/kg: increased by 47% to 117% in all 3 animals	10 µg/kg: reduced by 10% to 19% 100 µg/kg: reduced by 32% to 39%	None
Risperidone	0.1 µg/kg :decreased by 11% to 20% 1 µg/kg: decreased by 33% to 41% 10 µg/kg: decreased by 36% to 47% 100 µg/kg: decreased by 41% to 49%	None	0.1 µg/kg: increased by 55% in 1 animal 1 µg/kg: increased by 65% to 197% 10 µg/kg: increased by 49% to 105%	0.1 µg/kg: decreased by 31% and 35% in 2 animals 1 µg/kg: decreased by 33% to 47% 10 µg/kg: decreased by 45% to 54% 100 µg/kg: decreased by 52%	None

Changes are compared to pre-administration values.
Data from Experiment 1 in conjunction with experiment for effects on sympathetic nervous system in anesthetized cats;
Data from Experiment 2 in conjunction with experiment for effects on parasympathetic nervous system in anesthetized and vagotomized cats

Effects on Respiratory System

Effects on Respiratory Function in Rats

The effects of orally administered lurasidone (300 and 1000 mg/kg) on respiratory function were investigated in conscious rats (8/group) using a whole-body plethysmograph under unrestrained conditions (Study G0007). Respiratory waveforms were continuously recorded for at least 6 hours after administration. Respiration rate, tidal volume and minute volume were analyzed before- and at 0.5, 1, 2, 4, and 6 h post-dosing as mean values for 10 minute observation periods.

Significantly higher values were observed in respiratory rate and minute volume in lurasidone low dose group in comparison to control. These changes were not considered to be treatment-related because no similar changes were observed at the high dose of 1000 mg/kg. No significant differences in tidal volume were observed in lurasidone-treated groups compared to control.

Effects on Blood Gas Parameters in Rats

The effects of lurasidone on blood gas parameters (PO2, PCO2, and pH) were tested following i.v. administration of 0.1, 0.3 and 1 mg/kg (in 25% PEG) to male Wistar rats (5-6/group) (Study 7055). No significant changes in any of the measured blood gas parameters were observed.

Effects on Respiratory System in Anesthetized Cats

The effects of lurasidone (1, 10, 100 μg/kg, i.v.) on the respiratory system were examined in anesthetized cats as well as in anesthetized *and* vagotomized cats in two separate experiments and compared with those of risperidone (0.1, 1, 10, 100 μg/kg, i.v.) (Study B000240). Respiration rate was monitored by attaching a thermistor respiration pickup to a cannula inserted into the trachea. The respiratory rate was not affected following lurasidone administration at 1 and 100 μg/kg. At MD (10 μg/kg), an increase of 67% compared to the pre-administration value was observed in 1 of 3 animals, but since this effect was not dose-dependent, it was likely not drug-related.

Overall, the results of this study indicate that lurasidone and risperidone produced no effect on respiratory rate in anesthetized cats.

Effects on Autonomic Nervous System

The effects of lurasidone (1, 10, 100 μg/kg, i.v.) on the autonomic nervous system (sympathetic and parasympathetic) were examined in anesthetized cats in two separate experiments and compared with those of risperidone (0.1, 1, 10, 100 μg/kg, i.v.) (Study B000240). The same study also evaluated effects on cardiovascular and respiratory systems.

The effects on the sympathetic nervous system were tested in anesthetized cats by electrical stimulation-induced contraction response of the nictitating membrane and noradrenaline-induced pressor response. The effects on the parasympathetic nervous system were tested in anesthetized and vagotomized cats by electrical stimulation-induced depressor response, electrical stimulation-induced bradycardiac response, and acetylcholine-induced depressor response.

Lurasidone at 100 μg/kg exerted suppressive effect on the sympathetic nervous system (in 1 of 3 animals). This effect was weaker in comparison to risperidone which, at 100 μg/kg, produced 45% to 65% suppression compared to lurasidone 23% suppression. For the parasympathetic nervous system, lurasidone showed no effect while risperidone suppressed the electrical stimulation- and acetylcholine-induced depressor response (see the following sponsor's table).

Lurasidone Effects on Sympathetic and Parasympathetic Nervous System in Anesthetized Cats

	Sympathetic Nervous System		Parasympathetic Nervous System		
Test Article	**Electric stimulation-induced Contractile Response**	**Noradrenaline-induced pressor response**	**Electrical stimulation induced-Depressor Response**	**Electrical stimulation-induced Bradycardiac Response**	**Acetylcholine-induced Depressor Response**
Lurasidone	100 µg/kg: suppressed by 23% in 1 animal	None	None	None	None
Risperidone	10 µg/kg: suppressed by 15% to 44% 100 µg/kg: suppressed by 45% to 65%	None	10 µg/kg: suppressed by 19% and 29% in 2 animals 100 µg/kg: suppressed by 19% to 67%	None	10 µg/kg: suppressed by 18% to 41% 100 µg/kg: suppressed by 24% to 89%

Changes are compared to pre-administration values.

Effects on the Endocrine System

The effects of lurasidone on the endocrine system were evaluated in two safety pharmacology studies (Studies 6999, 7010). The following sponsor's table summarizes the results of these studies.

Effects of Lurasidone on Endocrine System

Test items	Test results	
	Lurasidone	**Reference drug**
Prolactin	Increased prolactin at 1 mg/kg or more (increasing rate is similar to HAL)	HAL: Elevated prolactin at 3 mg/kg or more Reaction increased with repeated dose (3 days)
ACTH	Increased ACTH at 10 mg/kg	HAL increased ACTH at 3 mg/kg
Corticosterone	Increased corticosterone at 3 mg/kg or more	HAL increased corticosterone at 3 mg/kg
Anterior pituitary hormones: FSH, LH, GH	No effects at 10 mg/kg or less	HAL: No effects at 3 mg/kg
Thyroid hormones: T3, T4	No effects at 10 mg/kg or less	HAL: No effects at 3 mg/kg
Sex hormones: testosterone, progesterone, E2	No effects at 10 mg/kg or less	HAL: No effects at 3 mg/kg

ACTH = adrenocorticotropic hormone; FSH = follicle stimulating hormone; GH = growth hormone;
HAL = haloperidol; LH = luteinizing hormone; T3 = triiodothyronine; T4 = thyroxine
Source: Studies 6999, 7010

Effects on Prolactin

Note: Effects on prolactin were also evaluated in several toxicology studies (Studies No. 2813, 2927, SUP22, 3259, 3879, SM0550, S0260, S0262); the results of these studies are reviewed under General Toxicology. In general, in these studies, lurasidone elevated blood prolactin.

The effects of a single and repeated (3 days) oral administration of lurasidone (10 mg/kg) and haloperidol (3 mg/kg) on serum prolactin were examined in male rats (Study 6999). After a single-dose administration of either lurasidone or haloperidol, serum prolactin was significantly elevated, reaching peak values (2- and 3-fold the initial levels) at 2 and 1 hours after administration, respectively.

The study also evaluated the effects of repeated administration of lurasidone at 10 mg/kg and haloperidol at 3 mg/kg administered once daily for 3 days. Serum prolactin was determined at 0, 1, 2, 4, 8, and 24 hours after the final administration on the 3rd day. In the haloperidol group, the peak serum prolactin level was higher after the repeated vs. single dose administration, while no such effect was found for lurasidone. The dose-response relationship for lurasidone (0.3, 1, 3, 10 mg/kg) and haloperidol (1, 3, 10 mg/kg) at 2 and 1 hour after administration, respectively, were also investigated. Lurasidone increased serum prolactin with a maximum level (approximately 2-fold higher than control) at 1 mg/kg and showed a plateau from 1 to 10 mg/kg. Haloperidol increased prolactin release to a similar extent, with serum prolactin levels of about 1.7-fold to 2.2-fold the control value.
Overall, the study showed that both lurasidone and haloperidol increased serum prolactin levels in rats. The prolactin-increasing effects of both drugs were comparable after a single dose administration, but when administered repeatedly, the effect of lurasidone on serum prolactin level was weaker than that of haloperidol.

Effects on Other Hormones

The effects of single oral doses of lurasidone (1, 3, and 10 mg/kg) and haloperidol (3 mg/kg) on various hormone levels in blood were evaluated in male and female rats at 1 hour after administration (Study 7010). ACTH, prolactin, GH, LH, FSH, corticosterone, testosterone, T3, and T4 were determined in males, and prolactin, LH, FSH, progesterone, E2, and corticosterone were determined in females. Female rats that were in estrus at the time of blood collection were excluded.
Lurasidone significantly increased prolactin in both male and female rats, with maximal values about 2-fold higher than control in males and about 8-fold higher than the control in females. Prolactin increase was also observed after administration of haloperidol. Lurasidone and haloperidol significantly increased the ACTH in males and corticosterone in males and females. Neither lurasidone nor haloperidol showed significant effects on other hormones including anterior pituitary hormones (GH, LH, and FSH), thyroid hormones (T3 and T4), or sex hormones (testosterone, progesterone, and E2).

Effects on the Urinary System

The effects of lurasidone (30, 100 mg/kg) on urinary volume and urinary electrolyte excretion in physiological saline-loaded rats were investigated in Study 6796. At 100 mg/kg, lurasidone significantly increased urinary volume, but did not affect urinary electrolyte excretion. The dose causing the effect of increased urinary volume was 20-fold or more that of principal pharmacological effective dose (0.9 to 4.6 mg/kg) in rats. The dose of 30 mg/kg did not affect urinary volume or the amount of urinary electrolyte excretion compared to control.

Effects on the Digestive System

Lurasidone exhibited no effect on the digestive system in rats and mice at doses up to 100 mg/kg (Study 6762).

Effects on the Smooth Muscle

The effects of lurasidone on smooth muscle were evaluated in two safety pharmacology studies (No. 7604 and 7605). In vitro, lurasidone displayed weak effects on smooth muscles isolated from guinea pig ileum and guinea pig vas deferens.
Lurasidone did not affect the resting tension and contractile responses to acetylcholine and barium chloride of isolated guinea pig ileum at concentrations up to 3000 ng/ml, but produced a slight inhibitory effect in contractile response to histamine at 3000 ng/ml (Study 7604).
Lurasidone did not affect the resting tension of the vas deferens of guinea pigs but produced an inhibitory effect on noradrenaline-induced contraction at concentrations of 30 ng/ml and higher (IC50 = 82 ng/ml).

The latter effect may be due to the α-receptor blocking activity of lurasidone (Study 7605). The results are summarized in the following sponsor's tables:

Effect of Lurasidone on Acetylcholine, Histamine, and BaCl2 Induced Contractions in Isolated Guinea Pig Ileum (Study 7604)

Test Article	% Change		
	Acetylcholine (100 ng/mL)	Histamine (200 ng/mL)	$BaCl_2$ (300 µg/mL)
25% PEG	1.9	-1.7	1.4
Lurasidone (30 ng/mL)	5.5	2.7	0.9
Lurasidone (300 ng/mL)	-3.5	-1.0	-5.2
Lurasidone (3,000 ng/mL)	-19.8	-31.9	-4.3

The value represents the mean of 3 preparations
The percent change from contraction before application of 25% PEG or lurasidone

Effect of Lurasidone on Resting Tonus and Noradrenaline-Induced Contractions in Isolated Guinea Pig Vas Deferens (Study 7605)

Test Article	Change (mm)	% Change
	Resting Tension	Noradrenaline (2 µg/mL)
25% PEG	-1.5	12.2
Lurasidone (3 ng/mL)	-1.3	-3.7
Lurasidone (30 ng/mL)	-1.8	-23.3
Lurasidone (300 ng/mL)	-2.0	-84.1
Lurasidone (3,000 ng/mL)	-1.3	-99.6

The value represents the mean of 3 preparations
The percent change from contraction before application of 25% PEG or lurasidone

Safety Pharmacology Studies of Metabolites

Active metabolites ID-14283 and ID-14326 were tested in vitro for effects on hERG currents in hERG-expressing HEK293 cells. In vivo, several lurasidone metabolites (ID-14283, ID-11614, ID-15001 and ID-15002) were tested for effects on various organ systems after intravenous administration to anesthetized rats, dogs, or cats.

In Vitro Studies

Metabolites ID-14283 and ID-14326

The effects of ID-14283 and ID-14326 on hERG currents were evaluated in hERG-expressing HEK293 cells at concentrations of 0.03, 0.1, 0.3, 1, 3, and 10 µM (Study SP-05001). Compared to the vehicle (DMSO, 0.1% v/v), ID-14326 and ID-14283 showed no statistically significant differences at concentrations of 0.03 and 0.1 µM, but significantly and dose-dependently inhibited rapidly activating delayed rectifier potassium currents at concentrations of 0.3, 1, 3, and 10 µM ($p < .001$). The estimated IC50 of ID-14326 and ID-14283 were 0.676 and 0.821 µM, respectively. These values were approximately 6-8 times higher than those of lurasidone (hERG IC50= 0.108 µM, Study SP-0310), demonstrating that both metabolites are weaker inhibitors of hERG currents than the parent substance.

In Vivo Studies

The effects of intravenous administration of lurasidone metabolites ID-14283, ID-11614, ID-15001 and ID-15002 on various organ systems were tested in anesthetized cats, dogs, and rats. Specifically, the effects of ID-14283 on respiratory and cardiovascular systems were examined in anesthetized cats; the

effects of ID-11614, ID-15001 and ID-15002 on the respiratory, cardiovascular, digestive, autonomic nervous system, and smooth muscle were tested in rats.

Metabolite ID-14283

The effects of metabolite ID-14283 on respiratory and cardiovascular system were studied in anesthetized cats at doses of 1, 10 and 100 μg/kg (i.v.) (Study PS9810).

At 10 and 100 μg/kg, ID-14283 caused a transient decrease in blood pressure (by 12 and 22 mmHg, respectively) and an increase in heart rate (by 14 and 19 beats/min, respectively); these effects disappeared 2 to 15 min after administration. Blood pressure drop was observed with the parent compound at the same doses. ID-14283 had no effect on the amplitude and frequency of respiration at doses up to 100 μg/kg.

Metabolites ID-15001 and ID-15002

Lurasidone metabolites ID-15001 and ID-15002 did not exhibit notable effects on the respiratory, cardiovascular, and sympathetic nervous systems or on smooth muscle in rats (Study 6784).

Metabolite ID-11614

Study 6784 evaluated the effect of ID-11614 on the respiratory, cardiovascular, digestive, autonomic nervous system, and smooth muscle as part of the general pharmacological assessment of the drug. Study B000239 evaluated the effects of ID-11614 (1, 10 μg/kg, i.v.) on ECG in dogs.

Study 6842 evaluated the mechanism of the inhibitory effects of ID-11614 on the respiratory and cardiovascular system in rats, guinea pigs, rabbits, cats, and dogs.

The results of these studies indicated that ID-11614, unlike lurasidone (historical comparison), induced transient respiratory arrest when administered intravenously at low doses (1 μg/kg or higher) to anesthetized cats. ID-11614 induced a greater decrease in blood pressure and heart rate than lurasidone (historical comparison). Because these effects on blood pressure and heart rate were diminished by pretreatment with a serotonin (5-HT3) receptor antagonist (MDL72222) or with bilateral vagotomy and inhibited by pretreatment with a parasympathetic blocker (methylatropine), they were attributed to vagus reflex due to activation of 5-HT3 receptors present on the vagus nerve. No abnormal respiration was observed after subcutaneous administration of 10 μg/kg to unanesthetized cats.

ID-11614 had no effect on PR interval, QRS duration, QT interval, or QTc in the ECG of anesthetized dogs.

In a comparison among the tested animal species, the dose for inducing cardiovascular effects with ID-11614 was lowest for cats, followed by rabbits and dogs in that order. In rats, only a mild hypotensive effect was seen at high doses. No respiration inhibitory effect was seen in animals other than cats.

Drug Interactions

Potential interactions between lurasidone and haloperidol, diazepam, biperiden, imipramine, and carbamazepine were evaluated in rats and mice (Study 7032).

The effects of orally coadministered drugs on the antidopaminergic and antiserotonergic actions of lurasidone were assessed by testing the inhibition of apomorphine-induced climbing behavior (antidopaminergic action) in mice and inhibition of tryptamine-induced forepaw clonic seizure (antiserotonergic action) in rats. As shown in the following sponsor's table, the inhibitory effects of lurasidone on apomorphine-induced climbing behavior were potentiated by haloperidol but not by the other centrally acting drugs tested (diazepam, imipramine, carbamazepine and biperiden), suggesting that the antidopaminergic action of lurasidone is potentiated by antipsychotics with D2-blocking action but not by other anxiolytics, antidepressants, antiepileptics or antiparkisonian drugs. The inhibitory effect of lurasidone on tryptamine-induced forepaw clonic seizure was not significantly affected by any of coadministered centrally acting drugs.

The effects of lurasidone coadministration on the pharmacological actions (e.g. cataleptogenic, anxiolytic, antidepressant, anticonvusant, muscle relaxant, etc) of various centrally acting drugs were also evaluated (as shown in the following sponsor's table). Lurasidone at a dose of 10 mg/kg significantly potentiated the anxiolytic effects of diazepam (Vogel's conflict test), but did not affect its muscle relaxant action

(muscle relaxation test). Lurasidone did not significantly affect the cataleptogenic activity of haloperidol in the catalepsy test and did not affect the antidepressive action of imipramine (forced swimming test) or the anticonvulsive action of carbamazepine (MES-induced seizure), suggesting that lurasidone does not affect the pharmacological actions of these drugs.

Potential Pharmacodynamic Interactions of Lurasidone and Other Drugs (Study 7032)

Reference Drug	Species	Experimental Method	Drug: Doses (mg/kg)	Effect of Lurasidone	Effect of Reference Drug
Haloperidol	Mice	Dopamine blocking action	lurasidone: 1, 3, 10 haloperidol: 0.1, 0.3	N/A	Increased effects at 0.1 and 0.3 mg/kg
	Rats	Serotonin blocking action	lurasidone: 1, 3, 6 haloperidol: 3	N/A	No effects at 3 mg/kg
	Rats	Catalepsy induction action	lurasidone: 10 haloperidol: 3, 10, 30	No effects at 10 mg/kg	N/A
Diazepam	Mice	Dopamine blocking action:	lurasidone 1, 3, 10 diazepam: 10	N/A	No effects at 10 mg/kg
	Rats	Serotonin blocking action	lurasidone: 3, 6, 10 diazepam: 10	N/A	No effects at 10 mg/kg
	Rats	Anti-anxiety action	lurasidone: 0.3, 1, 3, 10, 30 diazepam: 10	Increased effects at 10 mg/kg	N/A
	Mice	Muscle relaxing action	lurasidone: 10 diazepam: 3, 10, 30	No effects at 10 mg/kg	N/A
Biperiden	Mice	Dopamine blocking action	lurasidone: 1, 3, 10 biperiden: 10	N/A	No effects at 10 mg/kg
	Rats	Serotonin blocking action	lurasidone: 3, 6, 10 biperiden: 10	N/A	No effects at 10 mg/kg
Imipramine	Mice	Dopamine blocking action	lurasidone: 1, 3, 10 imipramine: 100	N/A	No effects at 100 mg/kg
	Rats	Serotonin blocking action	lurasidone: 3, 6, 10 imipramine: 100	N/A	No effects at 100 mg/kg
	Rats	Antidepressant action	lurasidone: 1, 3, 10 imipramine: 100	No effects at 1 - 10 mg/kg	N/A
Carbamazepine	Mice	Dopamine blocking action	lurasidone: 1, 3, 10 carbamazepine: 10	N/A	No effects at 10 mg/kg
	Rats	Serotonin blocking action	lurasidone: 3, 6, 10 carbamazepine: 10	N/A	No effects at 10 mg/kg
	Mice	Anti-convulsion action	lurasidone: 10 carbamazepine: 3, 10, 30	No effects at 10 mg/kg	N/A

Dopamine blocking action: Inhibition of apomorphine-induced climbing behavior (1 mg/kg, SQ)
Serotonin blocking action: Inhibition of tryptamine-induced clonic seizure of forepaw (40 mg/kg, IV)
Anti-anxiety action: Vogel's conflict test
Muscle relaxing action: Traction test
Antidepressant action: Forced swimming test
Anti-convulsion action: Inhibition of maximal electroshock convulsion
N/A = not applicable

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Lurasidone is rapidly absorbed with peak systemic exposure occurring within 0.7 h (in rats and dogs) and 5.3 h of administration (monkeys). The absolute bioavailability is low, <12%, in all species examined (mice, rats, rabbits, dogs and monkeys). Administration of lurasidone with food increased the extent of absorption 2- to 3-fold.

Clearance ranged from 17 to 61 ml/min/kg and volume of distribution ranged from 2.4 to 20 l/kg. Terminal elimination half-life ranged from 1.6 to 27 h.

Lurasidone is extensively bound (>99%) to serum proteins including human serum albumin and alpha-glycoprotein. It is distributed into most tissues including the brain and retained by pigmented tissues including the eye (in pigmented rats, elevated radioactivity levels were still observed 3 months post-dose). It penetrates the placental barrier and distributes into the fetus.

Lurasidone is extensively metabolized by oxidative *N*-dealkylation, hydroxylation of the norbornane ring or cyclohexane ring, *S*-oxidation, reductive cleavage of the isothiazole ring followed by *S*-methylation, and a combination of two or more of these pathways. The main metabolizing pathways are the hydroxylation of the norbornane skeleton, the S-oxidation of the isothiazolyl ring, and the cleavage of the cyclohexylmethyl-piperazine linkage. The primary metabolizing CYP isoenzyme in humans is CYP3A4; specific metabolizing isozymes were not identified in nonclinical species.

No human-specific metabolites were identified: all of the primary metabolites detected in humans were also detected in one or more of the nonclinical animal species. Of the metabolites identified, two (ID-20219 and ID-20220) are the major human metabolites present systemically at concentrations >10% of the total radioactivity. The total systemic exposure (AUC) in mice, rats and dogs (pivotal toxicity study species) following repeated-dose administration of lurasidone was shown to be equivalent to or greater than that observed in humans at steady state following administration of the maximum proposed clinical dose of 120 mg. Therefore, the non-clinical toxicology data for these species provide safety qualifications of the metabolites ID-20219 and ID-20220 via the general toxicity, carcinogenicity and reproductive assessments.

Following administration of [14C] lurasidone to the rat, the majority of the radioactivity was excreted in feces as parent compound. Approximately 12-48% of the orally administered dose was absorbed. Unchanged parent compound was detected only at trace levels in bile and urine, indicating that the absorbed material was extensively metabolized. Lurasidone is also excreted into milk primarily as unchanged drug at concentrations greater than those in serum.

The potential for protein-based clinical drug-drug interactions appeared to be minimal as no displacement of lurasidone or co-incubated drugs (biperiden, flunitrazepam, haloperidol, or diazepam) from serum proteins was observed in vitro. Similarly, the potential for clinical drug- drug interactions mediated by inhibition or induction of CYP activity by lurasidone is low, since in vitro studies using human tissue preparations suggested that at clinically relevant concentrations lurasidone does not inhibit or induce CYP enzyme activity. In contrast, because lurasidone is primarily and extensively metabolized by CYP3A4, the pharmacokinetics of lurasidone were significantly altered when co-administered with strong CYP3A4 inhibitors (i.e., ketoconazole).

Absorption

Single-Dose Studies

Following oral administration of lurasidone to rats, dogs and monkeys at single doses comparable to the planned maximum clinical dose (based on body surface area), the PK parameters were similar across the nonclinical species tested and humans (see the following sponsor's table). Lurasidone was rapidly absorbed with a Tmax much shorter in rats and dogs (0.5 – 0.7 h) than in monkeys (3 - 5.3 h). The absolute bioavailability was low (<12%) in all species examined and ranged from <1% to about 7% in fasted Cynomolgus monkeys and Sprague-Dawley rats, respectively. The volume of distribution, clearance and elimination half-life values were variable.

Mean PK Parameters of Lurasidone after Single Oral or Intravenous Administration of Lurasidone to Sprague-Dawley Rats, Beagle Dogs and Cynomolgus Monkeys

Strain/Species (Gender)	Feeding Status	Dose (mg/kg), Route (No. Individuals)	C_{max} (ng/mL)	T_{max} (hr)	AUC (ng•hr/mL) (time period)	$t_{1/2}$ (hr)[a] (time period)	V_{dss} (L/kg)	Cl (mL/min/kg)	F (%)	Study No.
Sprague-Dawley Rat (Male)	Fed	1, IV (3)	--	--	298.1 (0-∞)	2.9 (2-12)	14.7 (V_z)	58.1	--	SMO563
		10, po (3)	72.23	0.5	284.2 (0-∞)	2.6 (0.5-12)	--	--	11.5	
Sprague-Dawley Rat (Male)	Fasted	0.5, IV (4)	--	--	129 (0-∞)	2.4 (2-8)	5.9	61	--	PK001
		10, po (4)	76.4	0.69	176 (0-∞)	--	--	--	6.7	
Beagle Dog (Male)	Fasted	0.5, IV (3)	--	--	525 (0-∞)	27 (6–24)	20	17	--	
		10, po (3)	148	0.50	438 (0-∞)	--	--	--	4.2	
Cynomolgus Monkey (Female)	Fasted	0.5, IV (2)	--	--	269 (0-∞)	1.6 (6–8)	2.4	32	--	
		10, po (2)	11.8	3.0	43.3 (0-∞)	--	--	--	0.8	
Cynomolgus monkey (Male)	NS	1, IV (3)[b]	--	--	689.0 (0-168)	20.0	11.57	26.2	--	SUP24
	Fed	20, po (3)[b]	25.6	4.0	123.6 (0-168)	--	--	--	1.16	
	Fasted	20, po (3)[b]	8.2	5.3	37.8 (0-168)	--	--	--	0.35	
Human (Male)	Fed	120 mg, po	93.4	--	628	--	--	--	--	M1050005 [c]
(Female)			99.6	--	742	--	--	--	--	

[a] half life determined from decline in plasma/serum concentration over this time period;

[b] [isothiazolyl-3-14C]lurasidone administered; lurasidone concentrations determined by HPLC;

[c] Human PK parameters are provided by population PK analysis and are based on a typical non-Asian adult with a body weight of 80 kg (Module 2.7.2.).

-- = Not determined; NS = Not Specified; F = absolute bioavailability

Administration of lurasidone with food increased the extent of absorption. Cynomolgus monkeys administered a single oral 20 mg/kg dose of [isothiazolyl-3-14C] lurasidone (Study SUP24) had higher rate and extent of absorption if they had been fed prior to lurasidone administration. The time to peak exposure was shorter (4 h vs. 5.3 h after fasting) and the peak and total systemic exposures were about 3-fold higher relative to fasted ones (see the sponsor's table on the next page).

In human subjects, administration of lurasidone with a meal resulted in exposure (AUC) 1.7-fold higher compared to subjects who received lurasidone under fasted conditions (based on the overall population PK analysis, Study M1050005, as cited by the sponsor)

Effect of Food on Serum Total Radioactivity, Lurasidone and ID-14283 Concentrations in Male Cynomolgus Monkeys (Study SUP24)

	Feeding Condition					
	Fasted (Mean)			Fed (Mean)		
PK Parameters (at 20 mg/kg)	Total Radioactivity (^{14}C)	Lurasidone	ID-14283	Total Radioactivity (^{14}C)	Lurasidone	ID-14283
C_{max} (ng/mL)	383[a]	8.2	11.2	621 [a]	25.6	18.2
AUC_t (ng·h/mL)	13784[b]	37.8	50.9	19265[b]	123.6	92.1
T_{max} (hours)	5.3	5.3	5.3	4.0	4.0	4.0
F (%)[c]	--	0.35	--	--	1.16	--

Not applicable information denoted as --.
[a] Units in ng equivalents/g
[b] Units in ng equivalents·h/mL
[c] Absolute bioavailability (F) was calculated as (AUC_t oral / AUC iv) × (Dose iv / Dose oral) ×100

These studies indicate that food increases the bioavailability of lurasidone.

Total radioactivity (^{14}C) pharmacokinetic comparison across species

The absorption profile and concentration of labeled lurasidone-derived radioactivity in serum after single oral administration in mouse, rat, rabbit, dog and monkey are presented in the following sponsor's table.

PK Parameters in different animal species following a single oral dose of (^{14}C) lurasidone

Species	Dose (mg/kg)	Radioactive Compound	C_{max} (ng equivalents/g)	T_{max} (hr)	AUC_{0-24hr} (ng equivalents·hr/mL)	Study No.
Mouse (male)	50	[isothiazolyl-3-^{14}C]lurasidone	2070	0.5	7637	6645-184, 8202272
Rat (male)	50	[isothiazolyl-3-^{14}C]lurasidone	2140	2	18314	6645-183
Rabbit (female)	50	[carbonyl-^{14}C]lurasidone	3380	2	27851	6645-187
Rabbit (female)	50	[isothiazolyl-3-^{14}C]lurasidone	2740	2	26273	
Dog (male)	50	[isothiazolyl-3-^{14}C]lurasidone	6510	2	61500	6645-185
Monkey (male)	20	[isothiazolyl-3-^{14}C]lurasidone	1070	8	18223	6645-186

Samples were collected at 0.5, 2, 4, 8, 24 hours postdose
Cmax: mean of n=5 in mice and n=3 in other species

In the mouse, 3.6% of the circulating radioactivity was associated with unchanged parent drug in serum. The terminal elimination half-life (T1/2) of the metabolites in serum ranged from 0.74 to 3.88 h after oral dosing.

In the rat, 3.7% of the circulating radioactivity was associated with unchanged parent drug in serum. T1/2 of the unchanged parent drug in serum was 1.8 h after oral dosing. T1/2 of the metabolites in serum ranged from 1.7 to 4.8 hours after oral dosing.

In the rabbit, AUC or half-life were not determined, as unchanged drug in serum was only detected at 0.5 h after [14C] lurasidone dosing.

In the dog, 3.4% of the circulating radioactivity was associated with unchanged parent drug in serum. T1/2 of the unchanged parent drug in serum was 6 hours after oral dosing. T1/2 of the metabolites in serum ranged from 2.6 to 8.6 hours after oral dosing.
In the monkey, 0.5% of the circulating radioactivity was associated with unchanged parent drug in serum. T1/2 of the unchanged parent drug in serum was 1.6 hours after oral dosing. T1/2 of the metabolites in serum ranged from 2.8 to 9.3 hours after oral dosing.
The results of these studies indicated that lurasidone was extensively and rapidly metabolized in all species examined.

Dose-exposure proportionality
Following a single oral administration of lurasidone (1, 10 and 100 mg/kg) to non-fasted male Sprague-Dawley rats, the total systemic exposure of lurasidone increased more than dose-proportionally up to 10 mg/kg and dose-proportionally from 10 to 100 mg/kg. In contrast, in fasted rats given single oral doses of 2, 10 and 50 mg/kg, the total systemic exposure increased dose-proportionally only at low doses (up to 10 mg/kg) while at higher doses (from 10 to 50 mg/kg) it increased less than dose-proportionally (Study SMO563). In fasted dogs, after single oral doses of 2, 10 and 50 mg/kg, the total systemic exposure of lurasidone increased less than dose-proportionally (Study PK001).
In humans, the pharmacokinetics of lurasidone were linear for both AUC and Cmax within the clinical dose range (40 mg to 120 mg) based on the overall population PK analysis (Study M1050005). However, at doses outside the clinical range (520 mg and higher) there was a trend to a less than dose proportional increase in both AUC and Cmax.

Lurasidone dose-exposure proportionality after single oral administration to rats and dogs
(Studies SMO563 and PK001)

Species	Dose (mg/kg)	Dose Proportionality Factor	C_{max} (ng/mL)	C_{max} Proportionality Factor	$AUC_{0-\infty}$ (ng•hr/mL)	AUC Proportionality Factor
Sprague Dawley rat, fed	1	--	4.87	--	12.7	--
	10	10	72.23	14.8	284.2	22.4
	100	10	485.48	6.7	3280.2	11.5
Sprague-Dawley rat, fasted	2	--	14.2	--	35.3	--
	10	5.0	76.4	5.4	176	5.0
	50	5.0	240	3.1	515	2.9
Beagle dog, fasted	2	--	69.0	--	254	--
	10	5.0	148	2.2	438	1.7
	50	5.0	132	0.9	993	2.3

Not applicable or not determined data reported as --.

Gender Differences in PK Parameters
The PK profile of lurasidone in rats was different in males and females. After administration of the same oral dose (10 mg/kg) to fed male and female Sprague-Dawley rats, the peak and total systemic exposure in males were less than half that of females and the elimination rate was twice that observed in females (see the following sponsor's table). The apparent gender differences were likely due to differences in metabolism since lurasidone is more extensively metabolized in males than in females (see under "Metabolism").

In humans, female subjects had a 1.2-fold higher exposure of lurasidone compared to males based on the overall population PK analysis (Study M1050005, as cited by the sponsor).

Gender differences in Lurasidone PK Parameters in Female and Male Rat Following Administration of a Single Oral Dose (10 mg/kg) (Studies SMO548 and SMO563)

PK PARAMETERS	FEMALE	MALE
CMAX (NG/ML)	171.8	72.23
TMAX (HOURS)	0.5	0.5
AUC_T (NG·HOUR/ML)	634.1	272.2
KEL (HOURS	0.1389	0.2628
T1/2 (HOURS)	5.0	2.6
F (%)		11.5%

Absorption following repeated dose administration

Upon repeated dose administration of [Isothiazolyl-3-^{14}C]lurasidone to rats at a daily dose of 10 mg/kg for 14 days, serum ^{14}C concentration gradually increased and reached steady state on Day 7; on Day 14 the concentration was approximately 4 times higher and the half-life was 1.5 times longer than after single administration (shown in the following sponsor's table).

Effect of Repeated Administration of Lurasidone (10 mg/kg) on PK Parameters in Sprague-Dawley Rats (Study X9308-05)

PK Parameters	Total Radioactivity (^{14}C)	Total Radioactivity (^{14}C)
Day of dosing	Day 1	Day 14
C_{max} (µg equivalents/mL)	0.459	0.679
C_{24h} (µg equivalents/mL)	0.041	0.153
T_{max} (hours)	2	0.5
$t_{1/2}$ (hours) [a]	9.6	14.8
$AUC_{0\text{-}24hr}$ (µg equivalents·hr/mL)	3.38	7.19

Values reported as mean of 5 animals from Day 1 and 4 animals from Day 14.
[a] From 6-24 hours

Tissue Distribution

The tissue distribution of 14C-labeled lurasidone ([isothiazolyl-3-14C] and [carbonyl-14C]) was studied in rats and monkeys. Lurasidone was extensively (>99%) bound to serum proteins and distributed into most tissues, and was retained by pigmented tissues (elevated radioactivity levels in the eyeball of pigmented rats was still observed 3 months post-dose).

Tissue distribution after a single dose administration

In rats (male Sprague-Dawley), tissues which showed high values of radioactivity at 2 hours after administration of [isothiazolyl-3-14C]lurasidone (10 mg/kg) were the stomach, small intestine, liver, adrenal gland, kidney and mesenteric lymph node (study X9308-03). At 24 h post dose, the adrenal gland showed the highest value, followed by the Harderian gland, bladder, mesenteric lymph node, spleen and pituitary gland. At 168 hours post-dose, the adrenal gland still showed the highest level of radioactivity, followed by spleen, mesenteric lymph nodes, thyroid gland and testis (see the following sponsor's table).

Distribution of Total Radioactivity after Administration of a Single Oral Dose of 14C-Lurasidone
(Mean, µg equivalents/g or ml)

Sprague-Dawley Rats (2 h after Administration of 10 mg/kg) (Study X9308-03)

Tissue	[isothiazolyl -3-^{14}C]	[carbonyl -^{14}C]	Tissue	[isothiazolyl -3-^{14}C]	[carbonyl -^{14}C]
Serum	0.458	0.675	Lung	2.16[b]	1.47[a]
Blood	0.430	0.473	Mesenteric lymph	4.17	5.78
Adrenal	6.65	6.90	Muscle	0.627	0.509
Bladder	2.12	1.93	Pancreas	1.60	1.41
Bone marrow	1.41	1.05[a]	Pituitary	1.93	1.38
Cerebellum	0.269	0.139	Prostate	1.32	1.15
Cerebrum	0.304	0.132	Salivary gland	2.98	2.58
Eye ball	0.206	0.190	Skin	0.788	0.607
Fat	0.937	1.57	Small intestine	16.5	22.7
Harderian gland	2.83	2.79	Spinal cord	0.253	0.147
Heart	1.33	1.29	Spleen	1.78	1.25
Kidney cortex	3.19	3.29	Stomach	19.6	21.3
Kidney medulla	4.24	3.23	Testis	0.465	0.304
Large intestine	0.953	1.21	Thymus	0.917	0.732
Liver	8.05	8.58	Thyroid	2.48	2.65

Values reported as mean of five animals, with the following exception:
[a] Mean of four animals; [b] Mean of three animals

Cynomolgus Monkeys (4 h after Administration of 20 mg/kg) (Study SUP24)

Tissue	[isothiazolyl] -3-^{14}C]	[carbonyl -^{14}C]	Tissue	[isothiazolyl -3-^{14}C]	[carbonyl -^{14}C]
Serum	0.293	0.755	Muscles	0.350	1.68
Whole-Blood	0.326	0.535	Pancreas	0.965	1.26
Blood Cells	0.369	0.427	Pituitary	1.02	0.621
Adrenal Glands	0.941	1.22	Prostate	1.05	0.676
Aorta	0.615	0.798	Retina	3.36	2.84
Bile	418	706	Salivary gland	0.699	0.490
Bladder (urinary)	6.06	6.11	Sclera	0.407	0.375
Bone	0.0227	0.0224	Skin (pigmented)	0.521	1.09
Bone marrow	0.588	0.584	Skin (non-pigmented)	0.352	0.434
Brain[a]	0.266	0.119	Spinal cord	0.137	0.198
Cerebrospinal fluid	0.0540	0.0327	Spleen	0.871	0.487
Cerebellum	0.215	0.113	Testis	0.349	0.455
Cerebrum	0.242	0.121	Thymus	0.672	0.635
Choroid	0.489	0.282	Thyroid	0.865	0.612
Eye[b]	0.0363	0.0481	Trachea	0.618	1.12
Fat	0.352	0.846	Vena cava	0.531	0.983
Heart	0.461	0.548	Stomach wall	1.40	7.10
Kidney (cortex)	5.64	8.66	Stomach contents	4.65	216
Kidney (medulla)	4.90	6.64	Small intestine wall	49.8	46.8
Lacrimal Gland	0.415	0.521	Small intestine contents	137	194
Liver	4.90	6.78	Large intestine wall	1.92	105
Lung	0.686	1.07	Large intestine contents	824	548
Lymph nodes	1.03	11.6	--		

[a] The part of the brain remaining after removal of cerebellum and cerebrum.
[b] The part of the eyes remaining after removal of the retina, choroid and sclera.

In monkeys, following a single oral dose (20 mg/kg) of either [isothiazolyl-3-14C]lurasidone or [carbonyl-14C]lurasidone, the highest concentrations of radioactivity in organs and tissues were found at 4 or 24 hours, at levels similar to or greater than those in serum (Study SUP24). Most of the radioactivity was associated with the gastrointestinal tract which accounted for 60-81% and 40-75% of the dose at 4 and 24 hours, respectively. Retina, lymph nodes, and excretory organs (bile, urinary bladder, kidney, liver and the gastrointestinal tract) had radioactivity concentrations significantly greater than those in the serum (see the sponsor's table on the previous page). Detectable concentrations were still present in most tissues up to 168 hours post dose.

Tissue distribution after repeated dose administration

Repeated dose administration of [Isothiazolyl-3-14C]lurasidone (10 mg/kg/day) to male Sprague-Dawley rats for 14 days resulted in accumulation of radioactivity (Study X9308-03). After the last dose on Day 14, the absolute concentrations of radioactivity in the testes, spleen, fat tissue and lung were 3 to 5 times higher than those on Day 1 after a single dose. In other tissues, the total radioactivity concentrations were approximately two-fold higher after the final dose on Day 14 relative to the single dose on Day 1. Radioactivity diminished slowly and was still detected in all tissues (except the spinal cord) 1 month after the final administration.

Distribution of Total Radioactivity Following Repeated Dose Administration of [Isothiazolyl-3-14C]lurasidone (10 mg/kg) to Male Sprague-Dawley Rats for 14 Days (2 h after the last dose) (Study X9308-05)

Tissue	Mean Concentration (µg equivalents/g or mL) (2 Hours after last dose)	Tissue	Mean Concentration (µg equivalents/g or mL) (2 Hours after last dose)
Serum	0.284	Mesenteric lymph node	5.66
Blood	0.394	Muscles	0.470
Adrenal	8.01	Pancreas	1.19
Bladder	3.21	Pituitary	3.26
Bone	0.366	Prostate	1.04
Bone marrow	2.30[a]	Skin	0.576
Cerebellum	0.302	Small intestine	28.4
Cerebrum	0.310	Spinal cord	0.221
Eye ball	0.365	Spleen	5.69
Fat	2.75	Stomach	21.1
Harderian gland	2.18	Salivary gland	2.04
Heart	1.20	Testis	2.27
Kidney (cortex)	3.38	Thymus	0.999
Kidney (medulla)	3.27	Thyroid gland	3.93
Large intestine	1.93	--	--
Liver	9.31	--	--
Lung	6.52[a]	--	--

Each value represents the mean of five animals.
[a] Mean of four animals

Distribution in Pigmented Tissues of Rats

Tissue distribution of lurasidone in pigmented tissues was evaluated in male Long Evans rats following a single and repeated 14-day oral administration of 14C-lurasidone (10 mg/kg) (Studies X9308-03 and X9308-05, respectively). Total radioactivity was measured by whole body autoradiography at 2, 24, 168 h, and at 1 and 3 months following the final administration. In both studies, the highest concentration of ^{14}C was observed in the eyeball; an approximately 20-fold higher level of radioactivity was detected in the eyes of pigmented vs. albino rats, indicating that lurasidone had a preferential distribution and retention in melanin-containing tissues. In the eye, high concentrations were maintained for 3 months following both single and repeated administration; at that time point, the concentrations decreased to less than one fifth of the maximum observed. The following sponsor's table summarizes the distribution of 14C-labeled lurasidone in pigmented rat tissues.

Distribution of Total Radioactivity in Pigmented Tissues after Oral Administration of Single or Repeated Doses of 14C-Lurasidone (10 mg/kg) to Male Long Evans Rats

Dose (Study No.)	Radiolabel Location	Tissue	Concentration (µg equivalents/g or mL)				
			2 hr	24 hr	168 hr	1 month	3 months
Single 10 mg/kg (X9308-03)[a]	Isothiazolyl-3-^{14}C	Serum	0.504	0.047	<0.014	<0.014	<0.014
		Eyeball	3.89	3.91	3.39	1.24	0.666
		Skin (non-pigmented)	1.15	0.178	0.022	<0.014	<0.014
		Skin (pigmented)	2.01	1.17	0.057	0.044	<0.014
	Carbonyl-^{14}C	Serum	0.672	<0.014	<0.014	<0.014	<0.014
		Eyeball	2.33	2.27	1.91	0.957	0.279
		Skin (non-pigmented)	1.05	0.093	<0.014	<0.014	<0.014
		Skin (pigmented)	1.46	0.392	<0.014	<0.014	<0.014
Repeated 10 mg/kg/day for 14 days (X9308-05)	Isothiazolyl-3-^{14}C	Serum	0.383	0.126	0.015	<0.015	<0.019
		Eye	16.9	16.0	12.2	6.58	2.95
		Harderian gland	2.66	1.00	0.163	0.034	0.013
		Skin (non-pigmented)	0.937	0.389	0.160	0.068	0.036
		Skin (pigmented)	1.51	0.781	0.615	0.308	0.157
		Hair (non-pigmented)	2.87	3.28	2.91	2.13	0.389
		Hair (pigmented)	12.2	22.9	20.8	17.7	4.75

[a] Study X9308-03 also measured concentrations 2 weeks following administration.

The results of these two studies indicate that lurasidone had a preferential distribution and retention in melanin-containing tissues.

Protein Binding

Lurasidone was bound extensively (>99.6%) to serum proteins of mice, rats, guinea pigs, dogs, monkeys and humans in a concentration independent (100 – 1000 ng/ml) manner. It also was extensively (99.1% – 99.2%) bound to human serum albumin and alpha-1 acid glycoprotein (99.6% - 99.7% bound). Protein binding of two lurasidone metabolites, ID-14283 and ID-14326, in dog and human serum was also high, >98.8% (see the following sponsor's tables).

Protein Binding (Mean % Bound) of Lurasidone in Serum (Study SMT/01)

Species	Matrix	Lurasidone Concentration (ng/mL)		
		100	300	1000
ICR mouse[a]	Serum	> 99.8	99.9	99.9
Sprague-Dawley rat	Serum	> 99.9	99.9	99.9[b]
Dunkin-Hartley guinea pig	Serum	99.6	99.7	99.7
Beagle dog	Serum	> 99.9	99.8	99.7
Cynomolgus monkey	Serum	> 99.9	99.7	99.6
Human	Serum	>99.9	99.8	99.9
	Human serum albumin	99.1	99.2	99.2
	α-1 acid glycoprotein	99.7	99.7	99.6

[a] Due to instability at 37°C, mouse incubation was performed at room temperature.
[b] Mean of two replicates only, one replicate omitted due to anomalous result.

Protein Binding of Active Metabolites ID-14283 and ID-14326 (Study NA04101)

Metabolite	Matrix	Concentration (ng/mL)		
		100	300	1000
ID-14283	Beagle dog serum	99.1	99.2	99.1
ID-14326		99.3	99.3	99.3
Metabolite	**Matrix**	**Concentration (ng/mL)**		
		10	**30**	**100**
ID-14283	Human serum	98.9	98.8	98.8
ID-14326		98.8	99.0	99.0

Placental Transfer

In both pregnant (dosed on Gestation Day 12) and non-pregnant female rats, the highest concentration of total radioactivity in tissues following a single oral dose (10 mg/kg) of [isothiazolyl-3-14C]lurasidone was observed at 2 and 6 hours post-dose (Study SMO548). High concentrations of radioactivity were found in metabolic and excretory organs (liver, stomach, intestine, kidney, bladder), in brown fat, adrenal gland, Harderian gland, lymph nodes and mammary gland (pregnant only) as well as in ovaries and aorta (non-pregnant only) (see the following sponsor's table). The concentrations of radioactivity in the yolk sac fluid and in whole fetus at 2 hours post-dose were lower than those in the maternal serum. In pregnant females dosed near the end of gestation (on Gestation Day 20), the highest levels of radioactivity were found in fetal kidney and liver; the latter had higher concentrations than the maternal serum (see the following sponsor's table). With the exception of the fetal liver, the concentration of total radioactivity decreased from 2 h to 6 hours after dosing, and was reduced by approximately 50% by 24 hours post dose (see the sponsor's tables on the next page).

Distribution of Total Radioactivity 2 h. after a Single Oral Dose of [Isothiazolyl-3-14C]lurasidone (10 mg/kg) to Non-Pregnant and Pregnant Female Sprague-Dawley Rats (Study SMO548)
(Mean, µg equivalents/g or ml)

Tissue	Non-pregnant	Pregnant (Gestation Day)		Tissue	Non-pregnant	Pregnant (Gestation Day)	
		Day 12	Day 20			Day 12	Day 20
Whole-Blood	0.310	0.362	0.478	Pancreas	1.96	1.96	3.40
Serum	0.350	0.402	0.578	Pituitary	0.628	0.498	1.79
Adrenal Glands	17.9	5.96	8.36	Salivary gland	2.19	2.77	4.20
Aorta	3.58	1.19	1.86	Skin	0.845	0.992	1.25
Bladder (urinary)	3.70	3.12	6.03	Spinal cord	0.247	0.192	0.346
Bone	0.0552	0.0588	0.0781	Spleen	1.62	1.75	2.90
Bone marrow	1.00	0.595	2.15	Thymus	0.932	1.20	2.94
Cerebellum	0.188	0.190	0.291	Thyroid	1.60	0.937	4.00
Cerebrum	0.184	0.234	0.263	Trachea	1.85	0.600	2.64
Brain[a]	0.194	0.234	0.299	Uterus	0.848	0.747	1.03
Eye	0.254	0.168	0.270	Vena cava	0.827	0.664	2.35
Fat (perirenal)	NA	1.22	2.78	Stomach wall	68.6	45.0	132
Fat (white)	1.35	NA	NA	Stomach contents	133	157	328
Fat (brown)	9.89	5.69	11.5	Small intestine wall	70.6	66.5	107
Harderian gland	3.60	3.13	3.95	Small intestine contents	155	213	264
Heart	1.33	1.34	1.96	Large intestine wall	12.8	10.5	28.2
Kidney (cortex)	2.30	2.58	3.75	Large intestine contents	6.72	4.58	9.80
Kidney (medulla)	2.61	2.41	9.51	Yolk sac fluid	NA	0.0437	NA
Liver	7.93	8.36	11.6	Foetus(es) (whole)	NA	0.310	0.347
Lung	1.89	1.91	2.94	Placenta	NA	NA	1.07
Lymph nodes	3.81	2.53	4.39	Amniotic fluid	NA	NA	0.0799
Mammary gland	NA	2.71	4.90	Foetal blood	NA	NA	0.183
Mammary tissue	1.73	NA	NA	Foetal serum	NA	NA	0.164
Muscles	0.535	0.574	0.837	Foetal brain	NA	NA	0.208
Ovaries	3.25	1.33	1.97	Foetal kidney	NA	NA	0.440
--	--	--	--	Foetal liver	NA	NA	0.920

Mean; NA = not applicable.
[a] The part remaining after removal of the cerebellum and cerebrum

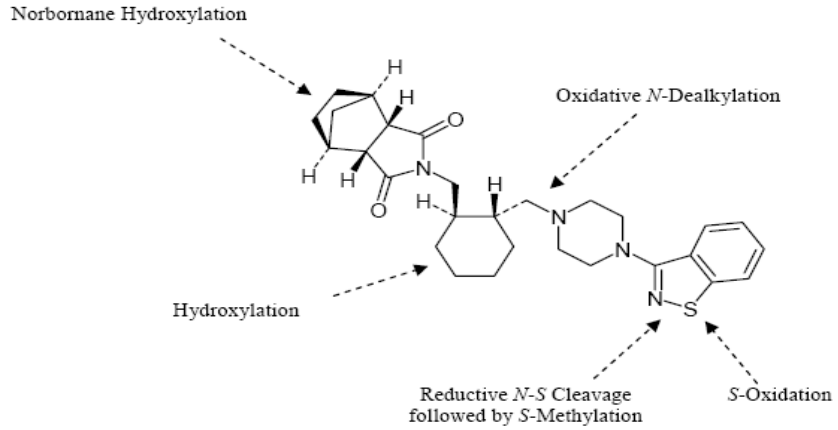
Distribution of Total Radioactivity in the Fetus Following a Single Oral Dose of [Isothiazolyl-3-14C]lurasidone (10 mg/kg) to Pregnant Female Sprague-Dawley Rats (Study SMO548)
(Mean, µg equivalents/g or ml)

Tissue	2 hours	6 hours	24 hours
Yolk sac (Day 12)	0.0437	0.0352	0.0198
Placenta (Day 20)	1.07	1.00	0.490
Amniotic fluid (Day 20)	0.0799	0.0828	0.0988
Fetal blood (Day 20)	0.183	0.165	0.0728
Fetal serum (Day 20)	0.164	0.116	0.0465
Fetal brain (Day 20)	0.208	0.192	0.0907
Fetal kidney (Day 20)	0.440	0.411	0.145
Fetal liver (Day 20)	0.920	1.19	0.513
Whole fetus (Day 12)	0.310	0.227	0.0337
Whole fetus (Day 20)	0.347	0.327	0.185

Metabolism

Lurasidone is extensively metabolized by *N*-dealkylation, hydroxylation of the norbornane ring or cyclohexane ring, *S*-oxidation, reductive cleavage of the isothiazole ring followed by *S*-methylation, and a combination of two or more of these pathways (as shown in the following sponsor's figure).

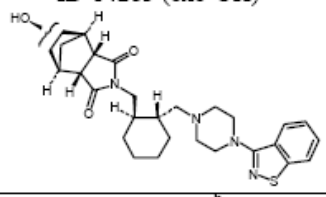
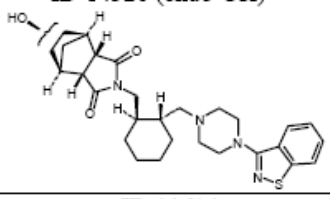
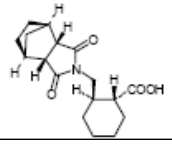
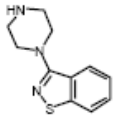
Proposed Primary Metabolic Pathways of Lurasidone



In general, the same metabolites were observed in all species examined, although the proportions varied. In humans, lurasidone is also extensively metabolized; all of the metabolites detected in humans were also detected in one or more of the nonclinical animal species; no human- specific metabolites have been identified. Two of the identified metabolites (ID-20219 and ID-20220) are major human metabolites since they were present systemically at concentrations over 10% of the total radioactivity. The total systemic exposure (AUC) to the two major human metabolites in mice, rats and dogs following repeated-dose administration of lurasidone was equivalent to or greater than that observed in humans at steady state following administration of the maximum proposed clinical dose of 120 mg. Therefore, the non-clinical toxicology assessments of lurasidone general toxicity, carcinogenicity and reproductive toxicity provided safety qualifications of the major human metabolites ID-20219 and ID-20220.

The primary metabolizing CYP isoenzyme in humans is CYP3A4. Specific metabolizing isoenzymes in nonclinical species have not been identified. In vitro studies conducted with human tissue preparations suggest that at clinically relevant concentrations lurasidone does not inhibit or induce CYP enzyme activity.

The chemical structures of lurasidone metabolites are shown in the following sponsor's table.

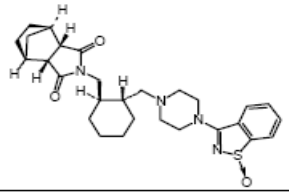
ID-14283 (exo-OH) [a] 	ID-14326 (endo-OH) [a] 
ID-20219 [b] 	ID-11614 

a) Active metabolite; b) Major human metabolite

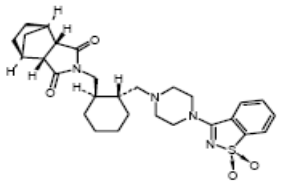
Continued on the next page

Chemical Structures of Lurasidone Metabolites (continued)

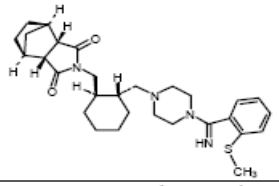
ID-14324



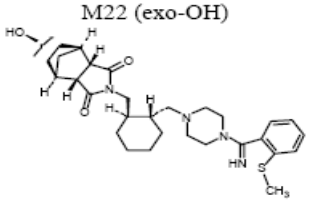
ID-14323



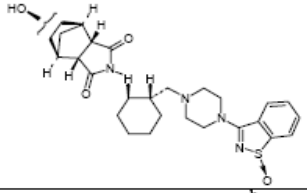
M21



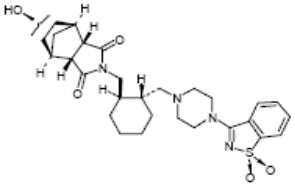
M22 (exo-OH)



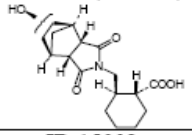
ID-20221 (exo-OH)



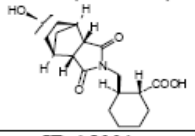
ID-20222 (exo-OH)



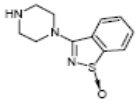
ID-20220 (exo-OH) [b]



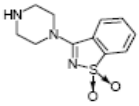
M4 (endo-OH)



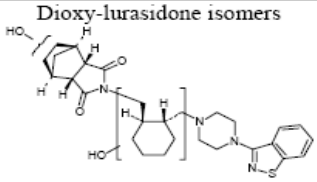
ID-15002



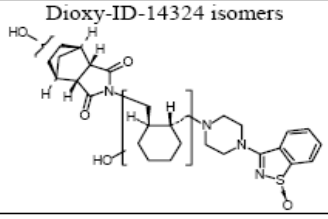
ID-15001



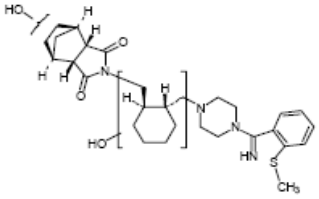
Dioxy-lurasidone isomers



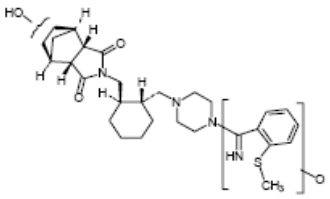
Dioxy-ID-14324 isomers



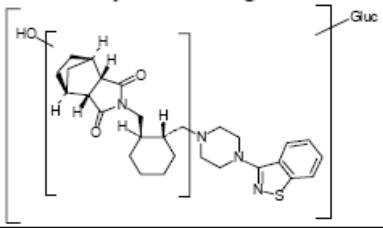
Dioxy-M21 isomers



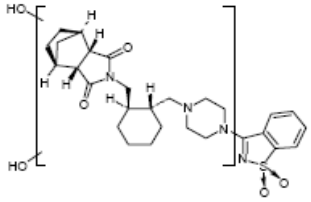
or



Mono-oxy-lurasidone-glucuronide



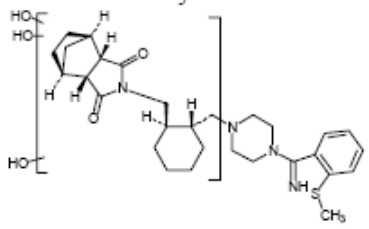
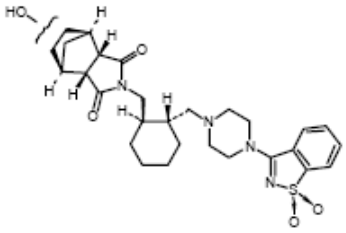
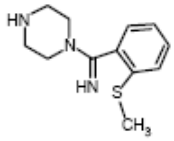
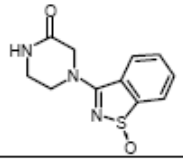
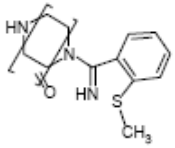
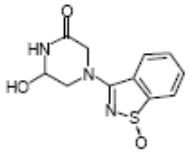
Dioxy-ID-14323 isomers



a) Active metabolite; b) Major human metabolite

Continued on the next page

Chemical Structures of Lurasidone Metabolites (continued)

Trioxy-M21 	ID-20222 isomer 
N-dealkyl-M21 	Keto-ID-15002 
Keto-*N*-dealkyl-M21 	Hydroxy-keto-ID-15002 

In Vitro Metabolite Identification

Lurasidone metabolism was examined in vitro by incubation of mouse, rat, rabbit, dog, monkey and human liver microsomes with [Carbonyl-14C] and [isothiazolyl-3-14C]lurasidone (2 μM) in the presence of NADPH (Study X1K09). Metabolites ID-14283, ID-14326, ID-14324, ID-14323, ID-11614 and ID-20219 and various other were identified, the most abundant being ID-14324 in all species. No human-specific metabolites were detected. Two additional studies (Study X1K10 and X1K11) confirmed that there were no qualitative differences in metabolites identified across species. Another in vitro study (Study PK004) identified lurasidone metabolites in hepatocytes and microsomes from humans and animals (mice, rats, rabbits, dogs, monkeys) incubated with [carbonyl-14C] and [isothiazolyl-3-14C]lurasidone (25 μM). Metabolite identification was performed by liquid chromatography-tandem mass spectrometry with radiometric detection. [14C]lurasidone was metabolized extensively in all species. In mouse, rat, rabbit, dog, monkey and human hepatocytes, an average of 57%, 14%, 20%, 11%, 16% and 24% of the parent compound remained, respectively; in liver microsomes, the percentages were 15%, 34%, 5%, 15%, 4% and 30%, respectively.

Major metabolites in human liver microsomal and hepatocyte incubations were ID-20221 and ID-20222 (arising from a combination of norbornane hydroxylation and S-oxidation), as well as ID-14283 and ID-14324 (arising from hydroxylation of the norbornane ring and S-oxidation, respectively). There was evidence for oxidative *N*-dealkylation occurring in all species, as indicated by the presence of ID-11614 and ID-20219 in both microsomal and hepatocyte incubations. All metabolites generated in incubations with human liver preparations were found in liver preparations from one or more of the nonclinical species examined.

In Vivo Metabolism

Lurasidone metabolic profile determination and quantitation of systemic concentrations of metabolites in mice, rats, rabbits, dogs and monkeys were performed in 13 *in vivo* studies using [isothiazolyl-3-14C] or [carbonyl-14C] lurasidone, as listed in the following sponsor's table. In addition, the systemic exposure to two major human metabolites, ID-20219 and ID- 20220 (as defined by >10% total radioactivity) was determined in 3 TK bridging studies in mice, rats and dogs.

In Vivo Metabolism Studies in Nonclinical Species

Species	Gender / Treatment	Test Compound	Dose (mg/kg)	Matrices	Study No.
Mouse	M / intact	[Isothiazolyl-3-^{14}C]	50	Serum, Urine, Feces	6645-184, 8202272
	M / intact	[Carbonyl-^{14}C]	10	Plasma, Urine, Feces	PK006
Rat	M / intact, BDC	[Isothiazolyl-3-^{14}C]	50	Serum, Urine, Feces, Bile	6645-183
	M, F / intact, BDC	[Carbonyl-^{14}C]	10, 50	Plasma, Urine, Feces, Bile	PK006
	M / intact	[Isothiazolyl-3-^{14}C], [Carbonyl-^{14}C]	10	Serum, Urine, Tissue	X1K08
	F / intact	[Isothiazolyl-3-^{14}C]	10	Serum, Urine, Tissue, Milk	SMO548
	Aged M / intact	[Isothiazolyl-3-^{14}C]	10	Serum, Urine	6645-122
	M /intact	Non-labeled	2, 10, 50	Plasma	PK001
Rabbit	F / intact	[Isothiazolyl-3-^{14}C], [Carbonyl-^{14}C]	50	Serum	6645-187
Dog	M / intact, BDC	[Isothiazolyl-3-^{14}C]	50	Serum, Urine, Feces, Bile	6645-185
	M, F / BDC	[Carbonyl-^{14}C]	10	Plasma, Urine, Feces, Bile	PK006
	M / intact	Non-labeled	2, 10, 50	Plasma	PK001
	F / intact	Non-labeled	100, 300	Serum	3815, NB03083D
Monkey	M / intact, BDC	[Isothiazolyl-3-^{14}C]	20	Serum, Urine, Feces, Bile	6645-186
	M / BDC	[Carbonyl-^{14}C]	50	Plasma, Urine, Feces, Bile	PK006
	F / Intact	Non-labeled	2, 10	Plasma	PK001

M: male, F: female, BDC: bile duct cannulated

Quantification of lurasidone metabolites in nonclinical animal species in vivo

In rats, the primary metabolites detected in serum or plasma following oral administration of [14C]lurasidone to male and female Sprague-Dawley rats, were ID-15001, ID- 15002, ID-11614, dioxy-isomer, ID-14283, ID-14326, ID-20219, ID-20220 and M4 (Studies 6645-83 and PK006).

Determination of PK parameters of lurasidone and 12 metabolites in serum from female Sprague-Dawley rats after administration of a single, 10 mg/kg oral dose of [isothiazolyl-3-14C]lurasidone (Study SMO548) showed that peak concentrations of total radioactivity and all analytes were observed within 30 minutes of drug administration (see the following sponsor's table). At 30 minutes post-dose, lurasidone accounted for 29% of total radioactivity, and lurasidone, ID-14283 and ID-14326 collectively accounted for 52% of total serum radioactivity. During 0.5 to 8 hours after dosing, metabolite ID-14283 was the most abundant radioactive constituent and accounted for 15-20% of serum radioactivity (data not shown); significant proportions of serum radioactivity were also accounted for by ID-14324 (9-16%), ID-20221 (6-14%) and ID-20222 (4-8%). At 24 hours, however, only lurasidone, ID-14283, ID-14326, MX-16 and UK-1 accounted for >1% of serum radioactivity; both metabolites ID-14283, ID-14326 were detected in the serum of a single rat at about 1 ng/ml.

A gender difference was observed in the metabolite profiles between female and male rats. The profiles were qualitatively, but not quantitatively similar. [Carbonyl-14C]lurasidone was metabolized much more extensively in males than in females. The parent compound was a much more abundant radioactive

component in females (26% and 20% of radioactivity at 1 and 4 hours, respectively) than in males (12% at 15 min, 6% at 1 hour and <1% at 4 and 8 hours). Also, the majority of the radioactivity in male rat plasma was comprised of metabolites ID-20220 (27% to 42% of the circulating radioactivity between 0.25 and 8 hours), M4 (~21% to 40%, overlapping with ID-20222) and ID-20219 (15% to 23%). In female rat plasma, metabolite ID-14283 constituted 19% and 21% of radioactivity in plasma at 1 and 4 hours, respectively; ID-14326 and ID-14324 together accounted for 13% and 23% of the radioactivity at 1 and 4 hours, respectively. Metabolite ID-20219 was also an important circulating metabolite (9% and 6% at 1 and 4 hours, respectively), but, M4 and ID-20220 were relatively minor metabolites in females.
In summary, following oral administration of [isothiazolyl-3-14C] or [carbonyl-14C]lurasidone to male and female Sprague-Dawley rats, lurasidone was extensively metabolized via oxidation, *N*dealkylation, *S*-methylation and hydroxylation. The primary metabolites detected in serum or plasma of rats are ID-15001, ID- 15002, ID-11614, dioxy-lurasidone isomer, ID-14283, ID-14326, ID-20219, ID-20220 and M4.

PK Parameters of Lurasidone and metabolites ID-14283 and ID-14326 in Female Sprague-Dawley Rats after a Single Oral Dose of [Isothiazolyl-3-14C]lurasidone (10 mg/kg) (Study SMO548)

Compound	C_{max} [a]	T_{max} (hours)	$t_{1/2}$ (hours)	$AUC_{0-\infty}$ [b]
Total radioactivity	602	0.5	7.6	4860
Lurasidone	171.8	0.5	5.0	653.0
ID-14283	112.0	0.5	3.6	651.3
ID-14326	30.4	0.5	3.9	225.5

[a] C_{max} are expressed as ng equivalents/mL for total radioactivity and ng/mL for other analytes.
[b] $AUC_{0-\infty}$ are expressed as ng equivalents·hr/mL for total radioactivity and ng·hr/mL for other analytes.
For total radioactivity and lurasidone, results are expressed in terms of the hydrochloride salt of lurasidone. For metabolites, results are expressed as the free-base.

PK Parameters of Lurasidone and metabolites in Male Sprague-Dawley Rats after single oral doses of Lurasidone (Study PK001)

Lurasidone Dose	2 mg/kg			10 mg/kg			50 mg/kg		
Analyte	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	M/P AUC	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	M/P AUC	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	M/P AUC
Lurasidone	14.2	35.3	--	76.4	175	--	240	512	--
ID-15001	4.35	16.5	0.47	23.7	74.6	0.43	28.4	92.5	0.18
ID-15002	12.8	33.9	0.96	64.9	179	1.0	89.7	259	0.51
ID-11614	1.78	13.4	0.38	8.42	40.8	0.23	12.0	61.4	0.12
ID-14283	15.5	42.6	1.2	47.6	125	0.71	94.6	261	0.51
ID-14326	5.19	13.7	0.39	16.7	47.2	0.27	33.5	100	0.20
ID-20219	20.2	48.9	1.4	119	260	1.5	281	522	1.0
ID-20220	24.6	57.8	1.6	130	317	1.8	163	415	0.81

--: not applicable

As summarized in the sponsor's table above, determination of PK parameters of lurasidone and 7 metabolites in serum from male Sprague-Dawley rats after administration of single oral doses of lurasidone (2, 10 and 50 mg/kg, Study PK001) showed that the metabolite-to-parent AUC ratios appeared to decrease with increasing dose, ranging from 0.38 (ID-11614) to 1.6 (ID-20220) at LD, from 0.23 (ID-11614) to 1.8 (ID-20220) at MD, and from 0.12 (ID-11614) to 1.0 (ID-20219) at HD.
In dogs, the following primary metabolites were detected in serum or plasma: ID-14324, ID- 14326, ID-20221, ID-20222 and ID-14323(Studies 6645-185 and PK006).

After a single 10 mg/kg oral dose of [carbonyl-14C]lurasidone to male and female Beagle dogs, the major metabolites in plasma were ID-14326 and ID-14324 (19 and 22%, respectively, at 2 hr), ID-20222 (19 and 14%), ID-14323 (11% overlapping with lurasidone) and ID-20221 (6 and 9%), respectively (Study PK006). Cleaved products, ID-20219, M4 and ID-20220, were also present. No gender differences in the metabolic profile were observed.

After a single 50 mg/kg oral dose of [isothiazolyl-3-14C]lurasidone, the proportion of unchanged parent drug was 11.6% of the serum radioactivity at 0.5 hours (Study 6645-185). At 2 hours post-dose, ID-14324 was the major serum metabolite detected (19.1%); ID-20221 (10.2%) and ID-20222 (8.82%) were also detected at this time point. Other serum metabolites identified but present to lesser extent were ID-15001, ID-15002, ID-11614, mono oxy-ID-14324, M22, ID-14283, ID-14326 and ID-14323.

After administration of escalating single oral doses of lurasidone (2, 10 and 50 mg/kg), the metabolite-to-parent AUC ratios ranged from 0.15 (ID-11614) to 0.91 (ID-14283) at LD; from 0.16 (ID-11614) to 0.86 (ID-14283) at MD, and from 0.12 (ID-11614) to 0.68 (ID-14283) at HD, as shown in the following sponsor's table (Studies PK001, 3815 and NB03083D). The metabolite-to-parent ratios appeared to decrease with dose escalation, similar to the rat. After oral lurasidone administration at 100 and 300 mg/kg, the AUC0-24hr values of ID-14283 and ID-14326 were 58% and 32% of the parent compound at the lower dose, while at the higher dose, the proportions decreased to 39% and 20% for ID-14283 and ID-14326, respectively.

PK Parameters of Lurasidone and metabolites in Male Beagle Dogs after single oral doses of Lurasidone (Study PK001)

Lurasidone Dose	2 mg/kg			10 mg/kg			50 mg/kg		
Analyte	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	M/P AUC	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	M/P AUC	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	M/P AUC
Lurasidone	69.0	254	--	148	438	--	132	990	--
ID-15001	16.9	114	0.45	13.9	166	0.38	21.5	251	0.25
ID-15002	18.2	48.7	0.19	18.5	87.3	0.20	19.9	136	0.14
ID-11614	3.75	37.3	0.15	3.75	69.7	0.16	5.92	118	0.12
ID-14283	35.5	230	0.91	42.8	375	0.86	60.0	671	0.68
ID-14326	14.1	108	0.42	16.7	102	0.23	21.8	158	0.16
ID-20219	54.7	167	0.66	48.9	251	0.57	61.7	440	0.44
ID-20220	25.8	87.7	0.35	19.0	191	0.44	25.6	226	0.23

PK Parameters of Lurasidone, ID-14283 and ID-14326 Following Oral Administration of Lurasidone to Female Beagle Dogs (Studies 3815 and NB03083D)

Dose (mg/kg)	Lurasidone			ID-14283			ID-14326		
	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-24hr} (µg·hr/mL)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-24hr} (µg·hr/mL)	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-24hr} (µg·hr/mL)
100	1903 ± 415.4	2.5 ± 1.0	17.0 ± 6.0	708.6 ± 122.6	4.5 ± 2.5	9.9 ± 3.4	379.8 ± 151.0	4.5 ± 2.5	5.4 ± 2.6
300	2780 ± 542.5	7.5 ± 2.5	46.5 ± 6.5	1058 ± 107.8	6.5 ± 1.0	18.0 ± 1.5	567.4 ± 141.8	8.5 ± 1.9	9.4 ± 2.2

Values reported as mean ± standard deviation.

In monkeys, [isothiazolyl-3-14C] lurasidone was extensively metabolized via *S*-oxidation, *N*-dealkylation, *S*-methylation, hydroxylation, hydrogenation and glucuronidation. At 2 hours post-dose, the parent compound contributed only a small proportion (2.76%) of the circulating radioactivity, and ID-15001 and dihydro-lurasidone-glucuronide were the major serum metabolites (7.56% and 4.85%, respectively) (Study 6645-186). Other serum metabolites present to lesser extents were *N*-dealkyl-M21, ID-15002, ID-11614, hydroxyl-dihydro lurasidone-glucuronide, dioxy-lurasidone isomers, ID-20222 and ID-14283. [Carbonyl-14C] lurasidone was extensively metabolized to primarily two products: ID-20219 (8% to 17%) and ID-20220 (4% to 7%), both major metabolites in humans, and to a number of unknown polar products (Study PK006).

After administration of single oral doses of lurasidone (2 and 10 mg/kg) to female monkeys (Study PK001) plasma concentrations of lurasidone and metabolites, except for ID-20219 and ID-20220, were below the lower limit of quantification at 2 mg/kg; at 10 mg/kg, the metabolite-to-parent AUC ratios ranged from 1.2 (for ID-11614 and ID-14283) to 6.7 (for ID-20220), as shown in the following sponsor's table.

PK Parameters of lurasidone and metabolites in plasma of female Cynomolgus monkeys after single oral doses of Lurasidone (Study PK001)

Lurasidone Dose	2 mg/kg			10 mg/kg		
Analyte	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	M/P AUC	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	M/P AUC
Lurasidone	BLQ	--	--	11.8	43.3	--
ID-15001	BLQ	--	--	32.4	124	2.9
ID-15002	BLQ	--	--	24.0	79.8	1.8
ID-11614	BLQ	--	--	7.02	52.2	1.2
ID-14283	BLQ	--	--	12.5	53.9	1.2
ID-14326	BLQ	--	--	BLQ	--	--
ID-20219	3.18	--	--	80.6	611	14
ID-20220	7.17	--	--	90.0	291	6.7

BLQ = below limit of quantitation; "-- " = not determined

Based on the results of these studies, the primary metabolites detected in serum or plasma of Cynomolgus monkeys were ID-20219, ID-20220 (both also major metabolites in humans) and ID-15001.

Inter-species comparisons

In general, metabolite profiles in plasma or serum were qualitatively similar across all species examined. The primary circulating metabolites identified in nonclinical species are summarized in the following sponsor's table.

Primary Circulating Metabolites Identified In Nonclinical Species

Species	Primary Circulating Metabolites Identified
Mice	ID-14283, ID-14324, ID-20219, ID-20220, ID-20222, M4, ID-11614, mono-oxy-lurasidone-glucuronide, hydroxyl-keto-ID-15002, keto-ID-15002
Rats	ID-14283, ID-14326, ID-15001, ID-15002, ID-11614, ID-20219, M4, ID-20220, dioxy-lurasidone isomer
Rabbits	several different dioxy-lurasidone isomers
Dogs	ID-14323, ID-14324, ID-14326, ID-20221, ID-20222
Monkeys	ID-15001, ID-20219, ID-20220

All metabolites identified in human serum (clinical pharmacology studies D1050262 and D1050184) were detected in one or more of the nonclinical animal species, as shown in the following sponsor's tables:

Human Serum Metabolites and their Occurrence in Animals

A. Following Oral Administration of [isothiazolyl-3-14C]lurasidone

	Mouse	Rat	Rabbit	Dog	Monkey	Human
Dose	50 mg/kg	50 mg/kg	50 mg/kg	50 mg/kg	20 mg/kg	40 mg
Study No.	6645-184, 8202272	6645-183, 8202272	6645-187, 8202272	6645-185, 8202272	6645-186, 8202272	D1050262
Metabolite						
N-dealkyl-M21	√	√	√	√	√	√
ID-15001	√	√	√	√	√	√
ID-15002	√	√	√	√	√	√
Keto-*N*-dealkyl-M21	√	√		√	√	√
Hydroxy-keto-ID-15002	√				√	√
Keto-ID-15002	√	√	√	√	√	√
ID-11614	√	√	√	√	√	√
Dioxy-ID-14323	√	√	√	√	√	√
ID-20221	√	√	√	√		√
Trioxy-M21	√		√	√	√	√
ID-20222		√	√	√	√	√
ID-20222 isomer	√	√	√	√	√	√
ID-14283	√	√	√	√	√	√
ID-14324	√	√		√		√
ID-14323		√		√		√

Continued on the next page

B. Following Oral Administration of [carbonyl -14C]lurasidone

	Mouse	Rat	Rabbit	Dog	Monkey	Human
Dose	10 mg/kg	10 mg/kg	50 mg/kg	10 mg/kg	50 mg/kg	40 mg
Study No.	PK006	PK006	6654-187	PK006	PK006	D1050184
Metabolite						
ID-20221	√	√	√	√		√
ID-20220	√	√		√	√	√
ID-20222	√	√	√	√		√
M22	√	√		√		√
ID-14283	√	√	√	√	√	√
ID-14324	√	√		√	√	√
ID-20219	√	√	√	√	√	√

In a clinical pharmacology study using [isothiazolyl-3-14C]lurasidone (Study D1050262), the drug was extensively metabolized and the exposure to unchanged lurasidone was 11.4% of the total drug-related exposure; the most abundant radioactive component in human serum was unchanged lurasidone. In a clinical pharmacology study using [carbonyl-14C]lurasidone (Study D1050184), two metabolites, ID-20219 and ID-20220, were major metabolites in serum in humans as their concentrations exceeded 10% of the total drug-related exposure. The systemic exposure to these metabolites was evaluated in animal toxicology studies (Studies G0020, G0018 and (b) (4)198-117). Animal/human comparisons of systemic exposure (AUC in serum) to metabolites ID-20219 and ID-20220 are summarized in the following sponsor's tables.

Metabolite ID-20219: AUC Comparisons between Animals and Humans
(Studies G0020, G0018 (b) (4)-117 and D1050247)

Species (Study No.)	Dose at TK studies (mg/kg)	Corresponding Toxicology Study	AUC of ID-20219 (ng·hr/mL)			
			Male [a]	Female [a]		Human (120 mg) [b]
Mouse (G0020)	650	Carcinogenicity study	808	2940	>	271
	2000	Micronucleus study	4130 [d]	4120 [d]	>	
Rat (G0018)	36	Carcinogenicity study	568	322	≈ >	
	100	6-month toxicity study	1890	1560	>	
	150	Teratology study [c]	2740	2350	>	
Dog (SBL198-117)	30	9-month toxicity study	1010	1580	>	
	100		1110	2790	>	
	200		2410	1940	>	

a) AUC0-24hr or 8hr following 2-week administration of lurasidone
b) AUC0-tau following 8-day administration of lurasidone from clinical drug-drug interaction study with lithium (Study D1050247)
c) Range-finding preliminary study (Study 2784);
d) AUC0-24hr for a single dose of lurasidone

Metabolite ID-20220: AUC Comparisons between Animals and Humans
(Studies G0020, G0018, (b) (4) 198-117 and D1050247)

Species (Study No.)	Dose at TK studies (mg/kg)	Corresponding Toxicology Study	AUC of ID-20220 (ng·hr/mL)			
			Male [a]	Female [a]		Human (120 mg) [b]
Mouse (G0020)	650	Carcinogenicity study	1050	3950	>	174
	2000	Micronucleus study	2810 [d]	3960 [d]	>	
Rat (G0018)	36	Carcinogenicity study	1540	135	≈ >	
	100	6-month toxicity study	2560	451	>	
	150	Teratology study [c]	2630	618	>	
Dog (SBL198-117)	30	9-month toxicity study	398	697	>	
	100		404	926	>	
	200		664	780	>	

a) AUC0-24hr or 8hr following 2-week administration of lurasidone
b) AUC0-tau following 8-day administration of lurasidone from clinical drug-drug interaction study with lithium (Study D1050247)
c) Range-finding preliminary study (Study 2784)
d) AUC0-24hr for a single dose of lurasidone

The total systemic exposure (AUC) to the two major human circulating metabolites, ID-20219 and ID-20220, in mice, rats and dogs (pivotal toxicity study species) following repeated-dose administration of lurasidone was equivalent to or greater than that observed in humans at steady state following administration of the maximum proposed clinical dose of 120 mg. Therefore, the nonclinical toxicology data provided safety qualifications of the metabolites (ID-20219 and ID-20220) via the general toxicity, carcinogenicity and reproductive assessments.

Enzyme Induction and Inhibition

Enzyme Induction

The potential of lurasidone to induce CYP enzyme activity was tested in rats in vivo and in human hepatocytes in vitro.

The potential for lurasidone to induce enzyme activity in vivo was assessed in male rats following oral administration of either a single (10 mg/kg) or repeated (10 and 100 mg/kg) lurasidone administration for 14 consecutive days (Study 6645-126). Single dose administration of 10 mg/kg lurasidone produced a slight decrease in hepatic microsomal protein yield, but did not produce significant changes in any of the hepatic microsomal parameters. Repeated oral administration produced a moderate nonspecific increase in hepatic microsomal protein (44% and 33% relative to vehicle control at 10 and 100 mg/kg, respectively) and a decrease in hepatic microsomal cytochrome P450 content, but no significant changes in aminopyrine *N*-demethylase, aniline hydroxylase or UDP-glucuronosyltransferase activities.

In vitro treatment of cultured human hepatocytes with lurasidone (0.03, 0.3, 3 and 10 µM) once daily for three consecutive days had little (less than 2-fold) or no effect on CYP1A2, 2B6 and 3A4/5 activities and little or no effect on CYP2B6 mRNA levels (Study XT093011).

Enzyme Inhibition

The potential of lurasidone or several metabolites to inhibit CYP enzyme activity was tested in vitro in human liver microsomes (Studies 6645-128 and PK007). The results indicate that lurasidone and its active metabolite ID-14283 were relatively weak/moderate competitive inhibitors of CYP activity (IC50 values > 5 µM). For lurasidone, the concentration 0f 5 µM corresponds to a 2.6 µg/ml which is >15-fold higher than the predicted steady state Cmax (165 ng/ml; Study D1050160) at the MRHD (120 mg). Therefore, drug-drug interactions mediated by inhibition of CYP activity following oral administration of lurasidone are not likely.

Excretion

The route, extent and metabolic profile of total radioactivity excreted in the form of the 14Clabeled lurasidone or its metabolites in urine, feces and bile was assessed in rodents, rabbits, dogs and monkeys.
Lurasidone was readily excreted after oral dosing, mostly within the first 24 hours in the mouse and rat and within the first 48 hours in rabbits, dogs and monkeys. Following administration of [14C]lurasidone, the majority of the radioactivity (approximately 80% of the dose) was excreted in feces, mostly as the parent compound. Biliary excretion was a major excretion route, as demonstrated in bile-duct cannulated animals. The parent compound was detected only at trace levels in bile and urine, indicating that lurasidone, once absorbed, was subject to extensive metabolism. Many products of the major metabolic pathways were excreted into bile and/or urine Based on the radioactivity excreted in urine and bile after oral administration, approximately 14-48% of the orally administered dose was absorbed.

Route and Extent of Excretion Following Single Oral Administration of Lurasidone to Sprague-Dawley Rats

Location of Radioactivity	Gender (treatment)	Dose (mg/kg)	Sample Collection Time (hr)	Recovery of Radioactivity (% of Dose)				Study No.
				Feces	Urine	Bile	Total	
[Carbonyl-^{14}C]	Male (intact)	50	168	83.3	12.6	--	96.3	6645-183
	Male (BDC)	50	48	42.9	10.4	25.0	79.3	
[Isothiazolyl-3-^{14}C]	Male (intact)	50	168	78.8	13.7	--	93.4	
	Male (BDC)	50	48	41.7	15.0	32.6	92.8	
[Carbonyl-^{14}C]	Male (BDC)	50	72	76.1	6.6	17.3	100	PK006
[Isothiazolyl-3-^{14}C]	Female (intact)	10	168	87.06	7.52	--	95.03	SMO548
[Isothiazolyl-3-^{14}C]	16 months old Male (intact)	10	168	79.7	10.9	--	92.0	6645-122

"--" = not applicable (bile duct intact)
BDC = bile duct cannulated

Cumulative Excretion of Total Radioactivity in Urine and Feces during and after 14-Day Repeated Oral Administration of [Isothiazolyl-3-14C]lurasidone (10 mg/kg/d) **to Male Rats** (Study X9308-05)

Sampling Time	Excretion (% of dose)		
	Feces	Urine	Total
24 hr after 1st dose	54.4	18.6	73.0
24 hr after 14th dose	77.5	17.7	95.3
168 hr after 14th dose	78.0	18.1	96.1
Carcass (168 hr after 14th dose)			0.2
Total recovery			96.3

The excretion profile of metabolites following oral administration of [isothiazolyl-3-14C] lurasidone and [carbonyl-14C] lurasidone was determined in mice, rats, dogs and monkeys.
Major metabolites observed in the urine of mice and rats were ID-15002 and ID-20220; those in dogs and monkeys were ID-15001 and ID-20220. In bile, the metabolite profiles in rats, dogs and monkeys differed. In rats, the main metabolites were dioxy-M21 isomers, ID-14283 and M22; in dogs, dioxy-ID-14324/dioxy-ID-14323, ID-20219 and glucuronide of ID- 20219 were the main metabolites; in monkeys, those were dioxy-ID-14324 isomers and di-, tri- and tetra-oxidized derivatives. In feces, unchanged drug was predominantly excreted and very few metabolites were identified.

Excretion into Milk: Lurasidone was actively secreted into milk of lactating female rats (Study SMO548). During 24 hours following a single oral dose of [14C] lurasidone (10 mg/kg) to lactating rats,

radioactivity concentrations in milk were significantly higher compared with serum concentrations. The mean concentrations of radioactivity in milk reached a maximum of 4.26 µg equivalents/ml at 2 hours after dosing, while in serum, the highest mean radioactivity concentration was 0.448 µg equivalents/ml at 1 h after dosing. At 1 and 2 h post dose, when mean radioactivity concentrations in serum and milk were the highest, the milk/serum ratios of the radioactivity concentrations were about 7:1 and 13:1, respectively. By 24 hours, this ratio declined to about 4:1. A large proportion of radioactivity in the milk was present as lurasidone (60 to 77%), while in the serum, the proportion of radioactivity present as lurasidone was much lower (16 to 22%) at the corresponding time points. The remaining radioactivity in milk was largely present as metabolites ID-14324, ID-14283 and ID-20221 which accounted for 11.2, 7.0 and 5.9%, respectively, of sample radioactivity.

Pharmacokinetic Drug Interactions

In vitro tests were performed to assess lurasidone potential to alter the protein binding or metabolism of several antipsychotic or antianxiety drugs likely to be co-administered in clinical practice (biperiden, flunitrazepam, haloperidol and diazepam), as well as the potential of these drugs to alter the protein binding or metabolism of lurasidone.
The results indicate that the serum protein binding of lurasidone is not affected by the presence of co-administered drugs, and vice versa, the protein binding of the co-administered drugs was not affected by the presence of lurasidone (Study X1K01).

Effect of Co-Incubated Drugs on Protein Binding of Lurasidone (Study X1K01)

Co-incubated Drug	Concentration of Co-incubated Drug (ng/mL)	Lurasidone Protein Binding (% Bound)
None (only Lurasidone)	n/a	99.8
Biperiden	25	99.9
Flunitrazepam	20	99.9
Haloperidol	10	99.9
Diazepam	400	99.8
Each value represents the mean of three experiments; n/a: not applicable		

Effect of Lurasidone on Protein Binding of Co-Incubated Drugs (Study X1K01)

Co-Incubated Drug	Concentration of Co-Incubated Drug	Protein Binding of Co-Incubated Drug (% Bound)	
		In the Absence of Lurasidone	In the Presence of Lurasidone (100 ng/mL)
Biperiden	25 ng/mL	89.4	91.2
Flunitrazepam	20 ng/mL	87.2	86.6
Haloperidol	10 ng/mL	94.2	93.3
Diazepam	400 ng/mL	98.9	98.3
Each value represents the mean of three experiments.			

Inhibition of lurasidone metabolism by coadministered drugs (biperiden, flunitrazepam, haloperidol and diazepam) was investigated in vitro using human liver microsomes. In addition, the effects of ketoconazole (a CYP3A4-selective inhibitor), quinidine (a CYP2D6-selective inhibitor) and cimetidine (a CYP3A4/CYP2D6 inhibitor) on the metabolism of lurasidone were also examined (Study X1K02). The results showed that lurasidone metabolism in vitro was not altered by the co-administered drugs at concentrations up to 1 µg/ml, but was inhibited by biperiden, flunitrazepam, haloperidol and diazepam at concentrations of 100 µg/ml. The metabolism of the coadministered drugs was not affected by lurasidone. The metabolism of lurasidone was markedly reduced by ketoconazole, as compared with quinidine and cimetidine, suggesting that attention should be paid to the interaction of lurasidone with drugs possessing inhibitory effects on CYP3A4.

Effects of Co-administered Drugs on In Vitro Metabolism of Lurasidone by Human Liver Microsomes (Study X1K02)

Concentration of Co-administered Drugs (µg/mL)	Biotransformation of Lurasidone (% of Control)			
	Biperiden	Flunitrazepam	Haloperidol	Diazepam
0.1	105	99	111	100
1	104	92	103	90
10	82	95	70	84
100	43	53	69	22

Effect of Lurasidone on In Vitro Metabolism of Co-administered Drugs by Human Liver Microsomes (Study X1K02)

Concentration of Lurasidone (µg/mL)	Biotransformation of co-administered drugs (% of control)			
	Biperiden (20 ng/mL)	Flunitrazepam (50 ng/mL)	Haloperidol (20 ng/mL)	Diazepam (0.2 µg/mL)
0.1	102	94	80	102
0.5	100	111	97	113
1	94	113	91	112
5	62	103	87	130
10	84	92	91	126

Effect of Ketoconazole, Quinidine, or Cimetidine on In Vitro Metabolism of Lurasidone by Human Liver Microsomes (Study X1K02)

Concentration of Inhibitors (µg/mL)	Biotransformation of Lurasidone (% of control)		
	Ketoconazole	Quinidine	Cimetidine
0.05	65	101	97
0.1	36	98	98
0.5	21	99	92
1	16	98	91
5	12	88	95
10	9	75	95
100	6	32	54

Lurasidone inhibited MDR1-mediated transport of digoxin; ID-20219, the major human metabolite, did not inhibit MDR1-mediated transport of digoxin at test concentrations up to 20 µM (Study GE-0535-G).

P-glycoprotein Inhibition Determination (Study GE-0535-G)

Test Article	Concentration (µM)	% of Control
Lurasidone	0	100.0
	0.01	89.7
	0.1	86.8
	1	56.3
	3	23.6
	10	2.3
Verapamil	30	5.7

In summary, the potential for protein-based clinical drug-drug interactions appears to be minimal as no displacement of lurasidone or co-incubated drugs (biperiden, flunitrazepam, haloperidol, or diazepam) from serum proteins was observed in vitro. Similarly, the potential for clinical drug drug interactions mediated by inhibition or induction of CYP activity by lurasidone is minimal. In contrast, because lurasidone is primarily and extensively metabolized by CYP3A4, the pharmacokinetics of lurasidone was significantly altered when coadministered with a strong CYP3A4 inhibitor, ketoconazole.

5.2 Toxicokinetics

(Not included in toxicity studies)

Single-Dose Toxicokinetic Studies in Rats

A toxicokinetic study of SM-13496 by single dose administration in rats
Study S0260
Serum concentrations of lurasidone and prolactin were evaluated in a non-GLP Study (S0260), after a single oral dose of lurasidone at levels of 0, 10, 50, 150, 500 and 1000 mg/kg in Sprague- Dawley rats (12/sex/group; 6 groups).

Type of Study	Test System	Method of Administration	Doses (mg/kg)	GLP Compliance	Study No.	Module Location
A toxicokinetic study of SM-13496 by single dose administration in rats - Investigation of concentrations of SM-13496 and prolactin in the serum-	Rat/ Crj:CD (SD)	Oral gavage (suspension in aqueous 0.5% methylcellulose)	0, 10, 50, 150, 500 and 1000	No[a]	S0260	4.2.3.1.

The Cmax and AUC0-24hr increased dose-dependently up to 500 mg/kg in males and up to 1000 mg/kg in females. The TK data from Study S0260 are presented in the following sponsor's table.

Toxicokinetic Parameters of Lurasidone Following a Single Oral Dose in Rats (Study S0260)

Dose (mg/kg/day)	Male		Female	
	C_{max} (ng/mL)	$AUC_{0\text{-}24hr}$ (µg·hr/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}24hr}$ (µg·hr/mL)
10	53	NC	104	NC
50	226	NC	599	4.0
150	430	3.8	1185	9.4
500	813	10.2	1334	17.8
1000	802	9.6	1697	25.8

NC = not calculated

Toxicokinetic study of SM-13496 by single oral administration to rats
Study 3340
Serum concentrations of lurasidone were evaluated in a GLP-compliant toxicokinetic study at a single oral dose at 0.03, 1, 10 and 100 mg/kg in male and female Sprague-Dawley rats (12/sex/group, see table below).

Type of Study	Test System	Method of Administration	Doses (mg/kg)	GLP Compliance	Study No.	Module Location
Toxicokinetic study of SM-13496 by single oral administration to rats	Rat/ Crj:CD (SD)	Oral gavage (suspension in aqueous 0.5% methylcellulose)	0.03, 1, 10 and 100	Yes	3340	4.2.3.1.

Within the dose range used in the study, C_{1hr} rose nearly proportionally to dose in both sexes (see the following sponsor's table).

Serum Concentrations of Lurasidone Following a Single Oral Dose in Rats
(Study 3340)

Dose (mg/kg/day)	Male		Female	
	C_{1hr} (ng/mL)	C_{24hr} (ng/mL)	C_{1hr} (ng/mL)	C_{24hr} (ng/mL)
0.03	BLQ	BLQ	BLQ	BLQ
1	2.48	BLQ	2.32	BLQ
10	58.63	0.25	46.33	0.73
100	343.48	6.83	478.63	29.00

BLQ = below the limit of quantitation (< 0.05 ng/ml)

6 General Toxicology

Single-dose and repeated-dose general toxicity studies were performed in mice, rats, dogs and monkeys (see sponsor's table below); the majority was performed in compliance with GLPs.

Lurasidone General Toxicity Studies

Studies	Species	Route	Duration	Dose (mg/kg)
Single-dose Toxicity	Rat	po	acute	1000, 2000
	Monkey	po	acute	10, 50, 250, 1000, 2000
Repeated-dose Toxicity	Mouse	po	3 months	25, 125, 250, 500
	Rat	po	3 months	Male: 0.03, 0.1, 0.3, 3, 30, 150 Female: 0.03, 0.1, 0.3, 3, 30, 300, 1000
		po	6 months	0.03, 1, 10, 100
	Dog	po	1 month	30, 100, 300 (non-GLP)
		po	9 months	30, 100, 200
	Monkey	po	3 months	2, 10, 50
		po	1 year	2, 10, 50

Single dose oral toxicity studies were performed in rats and monkeys at doses of up to 2000 mg/kg. Repeated-dose oral toxicity studies were conducted in mice (for periods of up to 13 weeks), rats (up to 26 weeks); dogs (up to 39 weeks) and monkeys (up to 52 weeks). The repeat-dose studies included:

- Two-week range-finding oral studies in rats, mice and dogs at lurasidone doses of up to 1000 mg/kg and in monkeys at up to 100 mg/kg/day.
- Three-month oral toxicity studies in mice, rats, and monkeys, at doses up to 500, 1000, and 50 mg/kg/day, respectively.
- Pivotal chronic toxicity studies consisted of a 6-month rat study, a 39-week study in dogs, and a 1-year monkey study; lurasidone was administered orally at dose levels of 0.03 to 100 mg/kg/day in rats, 30 to 200 mg/kg/day in dogs and 2 to 50 mg/kg/day in monkeys.

In addition to the above listed studies, there were numerous dose range-finding studies in various species, and special toxicology studies for drug dependency, antigenicity, phototoxicity and cardiovascular toxicity (reviewed under subtitle 10, Special toxicology studies).

The following studies were previously reviewed by Dr. Lois Freed under IND 61292 (original submission) and are cited or reproduced under the relevant study titles in the present review:
- Acute toxicity (rat, cynomolgus monkey);
- Subchronic toxicity:
    - Rat: 2-wk, 3-mo (2 studies)
    - Dog: 2-wk
- Chronic toxicity:
    - Rat: 6-mo

The following studies were not previously reviewed and are reviewed within this submission:
- Subchronic toxicity: Dog: 2-wk (TK), 4-wk; Monkey: 2-wk, 13-wk
- Chronic toxicity: Dog: 39-wk study; Monkey: 52-wk

Studies not reviewed within this submission are dose range-finding studies in various species.

The following sponsor's table lists all general toxicology studies. With the exception of two 2-week i.v. studies (one in rats and another in monkeys) and one 2-week dog study with oral capsule administration, all single- and repeated-dose toxicity studies used administration by oral gavage or gastric intubation of a suspension of lurasidone in 0.5% (w/v) aqueous methylcellulose.

Overview of General Toxicology Studies

Overview of Toxicology Studies					Test Article: lurasidone (SM-13496)			
Type of Study	**Species and Strain**	**Method of Admin.**	**Duration of Dosing**	**Doses (mg/kg)**	**GLP Compliance**	**Testing Facility**	**Study No.**	**Module Location**
Single-Dose Toxicity								
Single-dose oral toxicity study of SM-13496 in rats	Rat/ Crj:CD (SD)	Oral gavage	Single dose	0, 1000 and 2000	Yes	(b) (4)	2737	4.2.3.1.
Single-dose oral toxicity study of SM-13496 in cynomolgus monkeys	Monkey/ Cynomolgus	Intranasal gastric intubation	Single dose	0, 10, 50, 250, 1000 and 2000	Yes		2756	4.2.3.1.
Repeated-Dose Toxicity								
14-Day oral gavage toxicity study with SM-13496 in mice	Mouse/ Crl:CD-1(ICR)BR	Oral gavage	2 weeks	0, 100, 300 and 1000	Yes		6645-135	4.2.3.2.
Two-week oral administration study of SM-13496 in female rats	Rat/ Crj:CD (SD)	Oral gavage	2 weeks	0, 0.04, 0.5, 1, 6, 30 and 150	Yes		2839	4.2.3.2.
Two-week oral toxicity study of SM-13496 in rats	Rat/ Crj:CD (SD)	Oral gavage	2 weeks	0, 10, 50, 150, 500 and 1000	Yes		2759	4.2.3.2.
A 2-week repeated intravenous dose toxicity study of SM-13496 Injection 0.8 mg in rats	Rat/Crl:CD(SD)	Intravenous injection	2 weeks	0(5% glucose), 0(5% glucose + Injection buffer + propylene glycol) and 0.16	Yes		SBL198-092	4.2.3.2.
Two-week oral administration study of SM-13496 in non-pregnant female rabbits	Rabbit/ Kbl:NZW	Oral gavage	2 weeks	0, 50, 100 and 200	Yes		2812	4.2.3.2.
13-week oral gavage preliminary carcinogenicity and toxicokinetic study with SM-13496 in mice	Mouse/ Crl:CD-1(ICR)BR	Oral gavage	13 weeks	0, 25, 125, 250 and 500	Yes		6645-136	4.2.3.2.
Three-month oral toxicity study of SM-13496 in rats	Rat/ Crj:CD (SD)	Oral gavage	3 months	Males: 0, 3, 30 and 150 Females: 0, 3, 30, 300 and 1000	Yes		2813	4.2.3.2.
Three-month oral toxicity study of SM-13496 in rats (NOAEL study)	Rat/ Crj:CD (SD)	Oral gavage	3 months	0, 0.03, 0.1, 0.3 and 3.0	Yes		2927	4.2.3.2.
Six-month oral toxicity study of SM-13496 in rats	Rat/ Crj:CD (SD)	Oral gavage	6 months	0, 0.03, 1, 10 and 100	Yes		3259	4.2.3.2.

Continued on the next page

Overview of General Toxicology Studies (Continued)

Type of Study	Species and Strain	Method of Admin.	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study No.	Module Location
Two-week oral toxicity study of SM-13496 in dogs	Dog/ Beagle	Oral (capsule)	2 weeks	0, 50, 250 and 1000	No[a]	(b) (4)	L0134	4.2.3.2.
Four-week oral toxicity study of SM-13496 in dogs	Dog/ Beagle	Oral gavage	4 weeks	0, 30, 100 and 300	No[b]		L0485	4.2.3.2.
Thirty nine-week oral toxicity study of SM-13496 in dogs	Dog/ Beagle	Oral gavage	39 weeks	0, 30, 100 and 200	Yes		3879	4.2.3.2.
Two-week oral toxicity study of SM-13496 in cynomolgus monkeys	Monkey/ Cynomolgus	Intranasal gastric intubation	2 weeks	0, 4, 20 and 100	Yes[c]		2811	4.2.3.2.
SM-13496 - preliminary study - Repeated oral administration to cynomolgus monkeys for 14 days	Monkey/ Cynomolgus	Oral intubation	2 weeks	4 and 100	No[d]		SUP21	4.2.3.2.
A 2-week repeated intravenous dose toxicity study of SM-13496 Injection 0.8 mg in cynomolgus monkeys	Monkey/ Cynomolgus	Intravenous injection	2 weeks	0(5% glucose), 0(5% glucose + Injection buffer + propylene glycol) and 0.048	Yes		SBL198-090	4.2.3.2.
SM-13496 toxicity to cynomolgus monkeys by repeated oral administration for 13 weeks followed by a 6-week recovery period	Monkey/ Cynomolgus	Oral intubation	3 months	0, 2, 10 and 50	Yes		SUP22	4.2.3.2.
SM-13496 toxicity to cynomolgus monkeys by repeated oral administration for 52 weeks	Monkey/ Cynomolgus	Oral intubation	12 months	0, 2, 10 and 50	Yes		SMO550	4.2.3.2.

Note: In addition to TK evaluations that were performed as part of the toxicology studies listed, two single dose TK studies in rats were performed to determine serum concentrations of prolactin and/or lurasidone (Studies S0260 and 3340), and three repeat-dose TK studies were performed specifically to determine the serum concentration of the primary human metabolites (ID-20219 and ID-20220) in mice, rats and dogs: Studies G0020, G0018 and (b) (4)198-117, respectively. These studies are reviewed under the ADME/TK part of this review.

Single-Dose Toxicity

Single-dose acute toxicity studies were performed in Sprague-Dawley rats and in Cynomolgus monkeys. The studies were reviewed by Dr. Lois Freed under IND 61292. Two additional single-dose studies were performed in Sprague-Dawley rats to determine serum prolactin levels at toxicologic doses and to obtain single-dose toxicokinetic data for lurasidone (reviewed under ADME/TK).

6.1.1. Single-Dose Toxicity Study in Rats
Study Title: Single-dose oral toxicity study of SM-13496 in rats
Study No: 2737
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/18/93
GLP compliance: non-GLP

Key Study findings:

Effects of single oral (gavage) administration of lurasidone were evaluated in Sprague-Dawley rats (5/sex/group) at doses of 1000 or 2000 mg/kg. Animals were observed for 14 days after dosing. No deaths were observed at either of the tested dose levels (the lethal dose was estimated at more than 2000 mg/kg). Decreased spontaneous activity, ptosis and decreased body weight gain and/or body weight loss were noticed at both tested dose levels. Ataxic gait was observed at 2000 mg/kg. Because a uterine horn nodule was observed at autopsy in one female at 2000 mg/kg, Part 2 of the study was conducted on 20 females to confirm whether this finding was treatment-related. No nodules developed and no abnormalities attributable to the administration of the test article were found at autopsy.
The review data below are reproduced from Dr. Lois Freed's review of this study under IND 61292.

Methods:

Dosing
species/strain: Crj: CD(SD) rats (b) (4)
#/sex/group or time point: 5/sex/grp for Test 1, 20 females/grp for Test 2. [There was some discrepancy between the text and summary table in terms of "n".]
age: 5 wks
weight: 151-170 gm for males, 119-153 gm for females
diet/housing: *ad lib* except for 20 hrs prior to and 4 hrs after dosing; 2-3/cage
satellite groups used for toxicokinetics or recovery: no.
dosage groups in administered units: 0, 1000, 2000 mg/kg for Test 1; 0, 2000 mg/kg (females only) for Test 2
route, form, volume, and infusion rate: oral, 10 mL/kg
Dose justification: doses based on results of preliminary study (100, 200, 500, 1000, 2000 mg/kg). There were no unscheduled deaths. Reduced spontaneous motor activity and ptosis were observed at all doses and ataxia was observed at 2000 mg/kg in males.
Drug, lot#, radiolabel, and % purity: SM-13496, lot no. 3CG001M, 100.9%
Formulation/vehicle: suspension/0.5% MC (100, 200 mg/mL)

Observations and times:

Clinical signs: animals were observed at 10 and 30 min, 1, 2, and 4 hrs postdosing, and daily for 2 wks.
Body weights: body wts were recorded prior to dosing and on Days 1, 3, 5, 7, 10, and 14 days postdosing.
Food consumption: no
Ophthalmoscopy: no
EKG: no
Hematology: no
Clinical chemistry: no
Urinalysis: no
Gross pathology: necropsies were performed on all animals.
Organ weights: no
Histopathology: no
Toxicokinetics: no

Results:

Mortality: there were no unscheduled deaths.
Clinical signs: drug-related clinical signs (decreased spontaneous motor activity, ptosis) were evident at both doses in Test 1. Reduced activity was apparent by 2 hrs postdosing; ptosis occurred by 30 min postdosing. Ataxic gait was observed only in HDF (on Day 1). Behavior normalized by Day 2 postdosing in males and by Day 3 in females. In Test 2, decreased spontaneous activity, ataxic gait, and ptosis were observed in treated females. Only decreased activity and ataxia were observed on Day 1 (24-hr postdosing); by Day 2 behavior had normalized.
Body weights: body wt gain was reduced on Day 1 in males at both doses; mean body wt was reduced by 6 and 10% at the LD and HD, respectively. In females, body wt loss was noted at both doses on Day 1. For both males and females, catch-up growth occurred following Day 1. By the end of the 14-day observation period, body wts were fairly similar among grps in Test 1. In Test 2, body wt was still significantly reduced in treated females, but only by <5% compared to CF.
Gross pathology: there were no apparent drug-related findings.

The study results are summarized in the following sponsor's table:

Single-Dose Toxicity				Test Article: lurasidone (SM-13496)			
Species/ Strain	Method of Administration (Vehicle / Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study No.
Rat/ Crj:CD (SD)	Oral gavage (suspension in aqueous 0.5% methylcellulose)	0, 1000 and 2000	5/sex/Gp	2000	> 2000	Part 1: No mortality. Ptosis and decreased spontaneous activity observed at both dose levels. Ataxic gait observed in females at 2000 mg/kg. Decreased body weight gain and/or weight loss due to the treatment were observed. A nodule was observed in the uterine horn of 1 female at 2000 mg/kg.	2737
		0 and 2000	20/females/Gp	2000	> 2000	Part 2: No mortality and no uterine horn nodules.	

6.1.2. Single-Dose Studies in Monkeys

Study Title: Single-dose oral toxicity study of SM-13496 in Cynomolgus monkeys
Study No.: 2756
Conducting laboratory and location:
Date of study initiation: 7/6/93
GLP compliance: Not stated
QA Report: yes

Key study findings:

This single-dose study evaluated the effects of lurasidone in Cynomolgus monkeys (1/sex/group) at doses of 0, **10, 50, 250, 1000 and 2000** mg/kg. No deaths were observed at any dose. Treatment-related clinical signs included decrease of spontaneous activity in all treated groups, tremors accompanied by extrapyramidal symptoms such as persistent abnormal posture and slow movement at and above 50 mg/kg, closed eyelids at 250 mg/kg, and miosis, closed eyelids and vomiting at 2000 mg/kg. Food consumption was reduced at and above 250 mg/kg. At 50 and 2000 mg/kg, elevated alanine aminotransferase (5-fold compared to control) was found in females, but no histopathology abnormalities were observed. At 2000 mg/kg, the liver of one male animal was found to contain brown foci at macroscopic examination; microscopically, "slight" focal hepatocyte atrophy was observed.
The review data below are reproduced from Dr. Lois Freed's review of this study under IND 61292.

Methods

Dosing

Species/strain: Cynomolgus monkey (b) (4), (b) (4)

Number/sex/group: l/sex/group
Age: 71-77 mo. old
Weight: 6.76-7.46 kg in males, 2.65-3.52 kg in females
Diet/housing: 100 g per day, except fasted for 17 hrs pre-dosing

Dosage groups in administered units: 0, 10, 50, 250, 1000, 2000 mg/kg. (Initially the drug was administered at doses of 0, 10 and 50 mg/kg; after a 7-week interval, doses of 250, 1000, and 2000 mg/kg were administered to the same animals used in the initial administration.

Satellite groups used for toxicokinetics or recovery: No

Route, form, volume, and infusion rate: Oral, suspension, 5 or 10 mL/kg.

Drug, lot#, radiolabel, and % purity: SM-13496, lot no. 3CG001M, 100.9%

Formulation/vehicle: suspension/0.5% methylcellulose (MC)

Observations and times

Clinical signs: animals were observed continuously for 2 hrs postdosing, then every 1-2 hrs up to 8 hrs postdosing, then twice daily thereafter (to Day 14).
Body weights: recorded on Days 1, 3, 4, 5, 7, 10, and 14.
Food consumption: food intake measured from Day 5 on.
Ophthalmoscopy: no;
EKG: no

Hematology: blood samples were collected on Days 0, 3, 7, and 14 for analysis of the following parameters: rbc count, hgb, hct, MCH, MCV, MCHC, reticulocyte count, wbc (ct, differential); platelet ct.

Clinical chemistry: blood samples used for assessment of hematology were also used to evaluate the following parameters: total protein, albumin, A/G ratio, glucose, total cholesterol, triglycerides, urea N, uric acid, creatinine, total bilrubin, AST, ALT, GGTP, alkaline phosphatase, LDH, CPK, LAP (leucine aminopeptidase), Na, K, CL, Ca, P.

Urinalysis: no
Gross pathology: necropsies were performed on all animals

Organ weights: the following organs were weighed: brain, pituitary, mandibular glands, thyroid/parathyroid, thymus, lungs, heart, liver/gallbladder, pancreas, spleen, kidney, adrenal, testes, prostate, seminal vesicle, ovaries, uterus.
Histopathology: although a battery of tissues was preserved, only liver sections (from all groups) were examined microscopically (due to macroscopic findings). Liver/gallbladder samples were embedded in paraffin and stained with H & E for analysis.
Toxicokinetics: no

Results
Mortality: there were no unscheduled deaths.
Clinical signs: drug-related clinical signs were evident at all doses. Reduced spontaneous motor activity was apparent at all doses (but not in the male at 10 mg/kg). The sponsor noted that the severity of the effect was dose-related, and by 50 mg/kg, "... seated postures, prone or lateral position, and... slowed motion or continuance of abnormal postures..." were observed. Tremors were observed at 50-2000 mg/kg in males and at 250-2000 mg/kg in females. Vomiting was noted only in females (10, 250, 2000 mg/kg). Drug was vomited in the HDF. Ptosis was evident only in males (250 and 2000 mg/kg). Miosis was observed only in the HDM.
Body weights: body weight was not notably affected.
Food consumption: food intake was reduced for the first few days after dosing. In males, the decrease was dose related from 50 mg/kg on. In females, food intake was reduced at 250-2000 mg/kg, but not in a clearly dose-related manner.
Hematology: the data were too minimal (i.e., 1/sex/gr) to evaluate adequately.
Clinical chemistry: the data were too minimal (i.e., l/sex/gr) to evaluate adequately. However, ALT was elevated in the females receiving 50 and 2000 mg/g (5-fold compared to control F). In the HDF, ALT was higher on Day 14 than on Day 0 (147, 136, 120, and 215 U/L on Days 0, 3, 7, and 14, respectively).
Gross pathology: brown scattered foci were detected in the liver of the HDM.
Organ Weights: data only provided for 3/sex (doses: 250-2000 mg/kg). The data were too minimal to evaluate (l/sex/group).
Histopathology: microscopic analysis of the liver indicated "prominent fat-storing cells" and "focus of atrophic hepatocytes" in the HDM.
The study results are summarized in the following sponsor's table:

Single-dose oral toxicity study of SM-13496 in Cynomolgus monkeys

Single-Dose Toxicity				Test Article: lurasidone (SM-13496)			
Species/ Strain	Method of Administration (Vehicle / Formulation)	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings	Study No.
Monkey/ Cynomolgus	Intranasal gastric intubation (suspension in aqueous 0.5% methylcellulose)	0, 10, 50, 250, 1000 and 2000	1/sex/Gp	2000	>2000	No mortality. Decreased spontaneous activity in all treated groups. Tremors and decrease of spontaneous activity accompanied by extrapyramidal symptoms such as persistent abnormal posture and slow movement noted at 50 mg/kg or higher, along with closed eyelids in males at 250 and 2000 mg/kg. Miosis in a male at 2000 mg/kg. Vomiting was observed in female at 2000 mg/kg. Food consumption was reduced at 250 mg/kg or higher. At 2000 mg/kg one female demonstrated elevated ALT was observed with no histopathologic changes in the liver. At the terminal necropsy, the liver of one male animal from 2000 mg/kg was found to contain brown foci by macroscopy and slight focal hepatocyte atrophy upon histopathologic evaluation.	2756

Repeat-Dose Toxicity

Pivotal Studies:

- Six-month Oral Toxicity Study of SM-13496 in Rats (3259)
- Thirty Nine-Week Oral Toxicity Study of SM-13496 in Dogs (3879)
- Fifty Two-Week SM-13496 Oral Toxicity Study of SM-13496 (SMO550)

Summary tables of these studies are included in studies' reviews.

Non-pivotal Studies: The non-pivotal studies are summarized in the following sponsor's table.

Repeat-Dose Toxicity: Non-pivotal Studies

Repeated-Dose Toxicity		Nonpivotal Studies			Test Article: lurasidone (SM-13496)		
Species/ Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. Per Group	NOAEL (mg/kg)	Noteworthy Findings	Study No.
Mouse/ Crl:CD-1(ICR)BR	Oral gavage (suspension in 0.5% aqueous methylcellulose)	14 days	0, 100, 300 and 1000	6/sex/Gp	100	Hypoactivity and body tremors (male and female) at 300 and 1000 mg/kg/day. Decreased body weight gain and food consumption (male and female) and decreased ovary and uterus weights (females) at 1000 mg/kg/day. Mildly higher BUN and mildly lower absolute and relative lymphocyte counts in high-dose males.	6645-135
Mouse/ Crl:CD-1(ICR)BR	Oral gavage (suspension in 0.5% aqueous methylcellulose)	13 weeks	0, 25, 125, 250 and 500	10/sex/Gp Main 28/sex/Gp TK 10/sex/Gp Prolactin	Not established (less than 25)	Hypoactivity (male and female), increased body weights (female) and prolactin levels (male and female) were observed at all 4 dose levels, along with microscopic findings in female mammary glands and reproductive organs.	6645-136
Female Rat/ Crj:CD (SD)	Oral gavage (suspension in 0.5% aqueous methylcellulose)	2 weeks	0, 0.04, 0.5, 1, 6, 30 and 150	6 non-pregnant females/Gp	Not established (dose range finding)	Decreased body weight and food consumption, with catalepsy and decreased spontaneous activity at 150 mg/kg/day. Prolonged estrous cycle prevalent at 0.5 mg/kg/day or higher. Mammary gland development was noted in an increased number of animals at the dose levels of 6 mg/kg/day or higher.	2839
Rat/ Crl:CD(SD)	Intravenous injection(5% glucose + Injection buffer + propylene glycol)	2 weeks	0(5% glucose), 0(5% glucose + Injection buffer + propylene glycol) and 0.16	10/sex/Gp	Not established (less than 0.16)	In histopathology, prominent mucification of vaginal epithelium was observed in 1 female given 0.16 mg/kg. The incidence of mucification and/or cornification of the vaginal epithelium in females given 0.16 mg/kg group was greater than in the glucose control and vehicle control females.	(b) 98-092
Rat/ Crj:CD (SD)	Oral gavage (suspension in 0.5% aqueous methylcellulose)	2 weeks	0, 10, 50, 150, 500 and 1000	6/sex/Gp	Not established (less than 10)	*	2759

* Decreased spontaneous activity observed at 150 mg/kg/day and above, ptosis at 50 mg/kg/day and above. Decreased body weights observed in males (150 mg/kg/day or more) and females (1000 mg/kg/day). Decreased food consumption from 10 mg/kg/day (males) or 50 mg//kg/day (females) was observed, along with lower water consumption from 150 mg/kg/day (males) and 50 mg/kg/day (females). Miosis was observed from 50 mg/kg/day in males and from 10 mg/kg/day in females. Males exhibited increased erythrocyte count and hemoglobin concentration (from 50 mg/kg/day) and increased hematocrits (from 150 mg/kg/day). Females exhibited decreased mean corpuscular volume (from 150 mg/kg/day) increased neutrophil counts and rations (from 500 mg/kg/day) and decreased mean corpuscular hemoglobin and increased erythrocyte count (1000 mg/kg/day) were observed. Increased total cholesterol in males (from 500 mg/kg/day) and females (1000 mg/kg/day). Triglycerides and phospholipids were increased in females of the 10, 50 and 1000 mg/kg/day group. Ovarian weights tended to or decreased in females (from 50 mg/kg/day) and small ovaries were observed in two females at 1000 mg/kg/day. Females receiving 10 mg/kg/day or more exhibited hyperplasia of mammary gland, decreased number of corpora lutea, increased atretic and cystic follicles (ovarian), endometrial atrophy (uterine) epithelial mucification of the vagina. Increased incidence of foamy cell infiltration in lung was also observed in females of the groups of 500 mg/kg/day or more as well as males of the 1000 mg/kg/day group.

Continued on the next page

Repeat-Dose Toxicity: Non-pivotal Studies (Continued)

Species/ Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. Per Group	NOAEL (mg/kg)	Noteworthy Findings	Study No.
Rat/ Crj:CD (SD)	Oral gavage (suspension in 0.5% aqueous methylcellulose)	3 months	Males: 0, 3, 30 and 150 Females: 0, 3, 30, 300 and 1000	12/sex/Gp Main 6/sex/Gp Recovery 7 or 8/sex/Gp Prolactin 5/sex/Gp TK	Not established	No treatment-related mortality. Major findings consisted of catalepsy at 150 mg/kg/day in males and 1000 mg/kg/day in females and decreased body weights at 150 mg/kg/day in males and 300 and 1000 mg/kg/day in females. Bone mineral loss was seen at 300 and 1000 mg/kg/day. All treated female dose groups exhibited an increase or increasing tendency in the incidence of decreased trabecular bone (femur) and histopathologic changes in mammary gland, uterus, vagina, ovary, femur and sternum, decreased pituitary weights and increased erythrocyte counts. Almost all findings exhibited a tendency to reverse upon withdrawal of treatment.	2813
Rat/ Crj:CD (SD)	Oral gavage (suspension in 0.5% aqueous methylcellulose)	3 months	0, 0.03, 0.1, 0.3 and 3.0	Males: 12/Gp Main 7 or 8/Gp Hormone 5/Gp TK Females: 12/Gp Main 6/Gp Recovery 7 or 8/Gp Hormone 5/Gp TK	Male: 0.3 Female: 0.1	No treatment-related mortality or clinical signs. Tendency for increased body weight gain (0.3 and 3.0 mg/kg/day) and food consumption (3.0 mg/kg/day) in females. Higher values or tendency of serum prolactin (1 hour post final dosing) seem in females (0.3 and 3.0 mg/kg/day) and males (3.0 mg/kg/day) and lower prolactin values/tendency and lower estradiol levels at 24 hours post final dosing, seen in females (0.3 and 3.0 mg/kg/day). Increased incidence of estrus cycle disorder seen at Week 13 (0.3 and 3.0 mg/kg/day). High-dose females tended to exhibit lower relative pituitary weights and development of mammary glands. At 3 mg/kg/day, tubuloalveolar structure of mammary gland was seen in males and acinous hyperplasia or increase of secretion in mammary gland, atrophy of uterus, mucification of epithelium of vaginal mucosa and fatty infiltration in the bone marrow (femur) were observed in females. All above mentioned changes observed in the females reversed or improved during the 6-week recovery period.	2927
Dog/ Beagle	Oral (capsule)	2 weeks	0, 50, 250 and 1000	2/sex/Gp	Not established (dose range finding)	Decreased spontaneous activity, tremors and miosis were observed at all dose levels. Serum concentrations of lurasidone varied widely among animals.	L0134
Dog/ Beagle	Oral gavage (suspension in 0.5% aqueous methylcellulose)	4 weeks	0, 30, 100 and 300	2/sex/Gp	Not established (dose range finding)	Decreased spontaneous activity, tremors, miosis, somnolence were observed in all dosed groups as well as decreased food consumption and body weight (especially in males and females at 300 mg/kg/day), with effects on nipple and mammary glands in females. QT prolongation and hepatic effects were seen in one male at 300 mg/kg/day.	L0485
Monkey/ Cynomolgus	Intranasal gastric intubation (suspension in 0.5% aqueous methylcellulose)	2 weeks	0, 4, 20 and 100 Haloperi-dol: 1 mg/kg/day (Days 1 to 8), 3 mg/kg/day (Days 9 to11) and 5 mg/kg/day (Days 12 to 14).	2/sex/Gp	Not established (dose range finding)	Decreased spontaneous activity was observed at 4 mg/kg/day or above. Tremors (slight or mild), slowed motion and abnormal posture were observed at 20 and 100 mg/kg/day. Similar findings to all of the above were observed with haloperidol administration at 5 mg/kg/day. Decreased food consumption was noted at 20 mg/kg/day or higher. Decreased body weight was noted at 100 mg/kg/day. A low value of thymus weight and mild atrophy in thymus in 1 female at 100 mg/kg/day. Mild development of mammary gland seen in 1 female at 20 mg/kg/day and secretion of milky fluid from mammary glands was observed in 1 female at 100 mg/kg/day of lurasidone. In histopathologic examination, secretion in lumen, dilatation of duct, hyperplasia of glands or epithelial cells in mammary glands were noticed in females of all lurasidone administration groups and haloperidol administration group.	2811

Repeat-Dose Toxicity: Non-pivotal Studies (Continued)

Repeated-Dose Toxicity		Nonpivotal Studies			Test Article: lurasidone (SM-13496)		
Species/ Strain	**Method of Administration (Vehicle / Formulation)**	**Duration of Dosing**	**Doses (mg/kg)**	**Gender and No. Per Group**	**NOAEL (mg/kg)**	**Noteworthy Findings**	**Study No.**
Monkey/ Cynomolgus	Oral intubation (suspension in 0.5% aqueous methylcellulose)	2 weeks	4 and 100	2/sex/Gp	Not established (dose range finding)	A high incidence of subdued behavior and moderate to high incidence of tremors noted at 100 mg/kg/day. Isolated subdued behavior noted for 1 male and 1 female of the 4 mg/kg/day group. Salivation was noted in both dose groups, but more frequently at 100 mg/kg/day. Vomiting noted for 2 animals receiving 100 mg/kg/day. Body weight and food consumption decreases seen mostly at the 100 mg/kg/day level.	SUP21
Monkey/ Cynomolgus	Intravenous injection(5% glucose + Injection buffer + propylene glycol)	2 weeks	0(5% glucose), 0(5% glucose + Injection buffer + propylene glycol) and 0.048	3/sex/Gp	0.048	No treatment-related changes were noted in clinical signs, body weight, food consumption, ophthalmology, electrocardiography, urinalysis, hematology blood chemistry, gross pathology, organ weight or histopathology in any group.	(b) 98-090
Monkey/ Cynomolgus	Oral intubation (suspension in 0.5% aqueous methylcellulose)	13 weeks	0, 2, 10 and 50	3/sex/Gp Main 2/sex/Gp Recovery (control and high dose)	2 mg/kg/day	One male (2 mg/kg/day) killed for humane reasons unrelated to treatment. Subdued behavior seen at 10 mg/kg/day, but mostly at 50 mg/kg/day. Tremors seen in most animals at 50 mg/kg/day during Weeks 1 to 11. Late-onset salivation was observed at 50 mg/kg/day. Body weight and food consumption were decreased at 50 mg/kg/day. Serum prolactin levels were elevated in all treated groups compared to controls at 4 hours after dosing on Day 1 and, to a greater degree, in Week 13. Week 13 prolactin levels tended to be comparable to controls at 24 hours after dosing but were elevated in all treated groups 4 hours post-dose with mean values at of 20.0, 34.5 and 40.5 ng/mL, at doses of 2, 10 and 50 mg/kg/day respectively, relative to a control mean of 1.5 ng/mL. All above findings (50 mg/kg/day) were reversible during 6-week recovery period and without histological correlate.	SUP22
Monkey/ Cynomolgus	NA	NA	NA	NA	NA	A re-evaluation of histopathology slides of the heart from study SUP22 reaffirmed the original diagnosis that focal myocarditis reported in 1 male of the low-dose group was not associated with lurasidone treatment.	3702
Monkey/ Cynomolgus	NA	NA	NA	NA	NA	A re-evaluation of histopathology slides of the heart from study SMO550 reaffirmed the original diagnosis of myocardial inflammatory infiltrate in 2 of 8 animals in the mid-dose group but decreased the findings in the high-dose group, from 2 animals to 1. These are spontaneous lesions without dose-related occurrence and unlikely to be associated with lurasidone treatment.	3703

NA: not applicable, SD: Sprague-Dawley

6.2.1. Repeat-Dose Toxicity: Rat Studies

Study title: **Two-week oral toxicity study of SM-13496 in rats**

Study no.: **2759**

Conducting laboratory and location: (b) (4)

Date of study initiation: 6/16/93

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SM-13496, lot # 3CG001M, 100.9%

Key Study Findings

This 2-week GLP-compliant study was a dose-finding study for the 3-month repeated-dose rat study. Lurasidone administration to Sprague-Dawley rats at daily oral gavage doses of **10, 50, 150, 500 or 1000** mg/kg/day for 2 weeks did not cause mortality. Clinical signs included ptosis (from 50 mg/kg/day) and decreases of spontaneous activity (from 150 mg/kg/day). Lower body weight (males from 150 mg/kg/day and females at 1000 mg/kg/day) and lower food consumption (males from 10 mg/kg/day and females from 50 mg/kg/day) were observed, along with lower water consumption from 150 mg/kg/day (males) and 50 mg/kg/day (females). Miosis occurred in males at and above 50 mg/kg/day. Hematology changes, i.e., increase in erythrocyte count and hemoglobin at and above 50 mg/kg/day, and increases in hematocrit at and above 150 mg/kg/day occurred in males; in females, decreased mean corpuscular volume (from 150 mg/kg/day), increased neutrophil counts and ratios (from 500 mg/kg/day) and decreased mean corpuscular hemoglobin and increased erythrocyte count (at 1000 mg/kg/day) were observed. Total cholesterol values were elevated in males at 500 and 1000 mg/kg/day and in females at 1000 mg/kg/day. Triglycerides and phospholipids were increased in females at 10, 50 and 1000 mg/kg/day. At necropsy, the only clearly drug-related finding was a decrease in ovarian weights, dose-dependent (20% to 40%) in all female groups except LD. Histopathology changes in female reproductive system (i.e., decreased numbers of corpora lutea, increased numbers of atretic and cystic ovarian follicles, endometrial atrophy, vaginal epithelial mucification, and hyperplasia of the mammary gland) were found in females in all dose groups. Pulmonary foamy cell infiltration was found in females at and above 500 mg/kg/day and in males at 1000 mg/kg/day. The mammary and uterine effects were attributed to drug D2 effects on serum prolactin. Ovarian and vaginal effects were attributed to suppression of LH via D2 antagonism. The foamy cell infiltration of lung was considered due to phospholipidosis. Doses of 3, 30 and 150 mg/kg/day for males and 3, 30, 300 and 1000 mg/kg/day for females were chosen for the 3-month study. A NOAEL was not reached (less than the LD of 10 mg/kg/day), due to presence of histopathology changes at all tested doses.

The review data below are reproduced from Dr. Lois Freed's review under IND 61292.

Methods

Dosing

Species/strain: Crj: CD (SD) rat (b) (4)
Number/sex/group: 6/sex/gr; Age: 4 wks
Weight: 148-175 g for males, 113-142 g for females
Diet/housing: ad lib; 2-3/cage
Satellite groups used for toxicokinetics or recovery: no
Dosage groups in administered units: **0, 10, 50, 150, 500, and 1000** mg/kg/day
Route, form, volume, and infusion rate: oral, gavage, 5 mL/kg
Formulation/vehicle: suspension/0.5% MC (stated to be stable for 24 hrs at room temperature and for 14 days refrigerated. Homogeneity was tested and said to be confirmed.

Observations and times

Clinical signs: animals were observed once or twice daily. Catalepsy was defined as maintenance of posture for ~30 sec. Body weights: recorded on Day 1, 4, 8, 11, and 14 of dosing.
Food consumption: Food intake per cage was measured over 48 h on days 1-3 and 8-10.
Ophthalmoscopy: eyes were examined by fundus camera or ophthalmoscope.
ECG: no; Hematology: blood samples were collected from the abdominal aorta at sacrifice from all animals for analysis of the following parameters: rbc ct, hgb, hct, MCV, MCH, MCHC, reticulocyte ct, wbc (ct, differential), platelet ct, PT, APTT, fibrinogen.
Clinical chemistry: blood remaining from samples collected for hematology was used to assess the following parameters: total protein, albumin, A/G, protein, glucose, total cholesterol, TG, PL, total bilirubin, direct bilirubin, urea N, creatinine, AST, ALT, alkaline phosphatase, GGTP, LDH, creatine phosphokinase, leucine aminopeptidase, Na, K, Cl, Ca, P.
Urinalysis: urine samples were collected from animals by "... forced micturition with abdominal compression..." during Wk 2 of dosing for analysis of the following parameters: pH; glucose, protein, occult blood, ketone bodies, bilirbin, urobilinogen.
Gross pathology: complete necropsies were performed on all animals sacrificed at term.
Organ weights: wts of the following organs were recorded: heart, liver, spleen, kidney, adrenal, prostate, testis, ovary, brain, pituitary, lung, thymus, thyroid.
Histopathology: the following tissues were examined microscopically in C and HD animals: heart, liver, spleen, kidney, adrenal, prostate, testis, ovary, stomach, small intestine, large intestine, brain, pituitary, lung, thymus, thyroid/parathyroid, uterus, vagina, mammary gland (females), epididymis, seminal vesicle. In addition, lung, ovary, uterus, vagina, and mammary gland (females) were examined in the lower dose groups.
Toxicokinetics: no

Results

Mortality: there were no unscheduled deaths.
Clinical signs: decreased spontaneous motor activity and ptosis were the clearly drug related findings. These findings were observed at doses > 50 mg/kg in both males and females (all animals at these doses were affected) throughout the dosing period. Ptosis was attributed (by the sponsor) to the (alpha1 antagonist properties of SM-13496.
Body weights: in males, mean body wt was significantly reduced at 150, 500, and 1000 mg/kg (9, 10-15, and 13-17%, respectively) and tended to be lower at 50 mg/kg (4-5; Days 11-14). Body wt loss was noted on Day4 in HDM. Overall body wt gain was reduced by 12, 22, 37, and 42% at 50, 150, 500, and 1000 mg/kg, respectively. In females, mean body wt was significantly elevated at the LD (10 mg/kg, 11 %), but reduced at 150,500, and 1000 mg/kg (8-5, 17-6, and 24-11 %, respectively. The effect was greatest on Day 4. Body wt loss was noted on Day 4 at 500 and 1000 mg/kg. Overall body wt gain was elevated at the LD (36%) and reduced at 500 and 1000 mg/kg (21 and 36%, respectively).

Food consumption: in males, food intake was reduced throughout the dosing period (Day 3, 10) at 150,500, and 1000 mg/kg and on Day 10 at the lower doses. The effect was greatest on Day 3 at the higher doses. In F, food intake was reduced at all but the LD and only on Day 3 (25, 44, 62, and 69% at 50, 150, 500, and 1000 mg/kg, respectively). Food efficiency was decreased at 500 and 1000 mg/kg in M and F.
Water consumption: water intake was reduced in males on Day 3 at 50, 150, 500, and 1000 mg/kg (14, 25, 40, and 53%, respectively) and in females at those same doses (24, 43, 57, and 57%, respectively). On Day 10, water intake was increased at the LD (35%) in females.
Ophthalmoscopy: miosis was observed at all but the LD in males and at all doses in F. This was attributed (by the sponsor) to drug alpha1 antagonist properties.
Hematology: in males, there were increases in rbc parameters (i.e., rbc ct, hgb, hct; 4-9%) at all but the LD (for one or more parameters) however, the magnitude of the effect was not dose-related. A slight increase in reticulocytes (10%) was noted at the HD. In fema1es, rbc ct was slightly (7%) elevated and MCH and MCV were slightly reduced (5-6%) at the HD; Wbc ct was increased only at 500 mg/kg (18%); however, neutrophil ct was elevated at all doses (26, 29, 45, 140, and 130% at 10, 50, 150,500, and 1000 mg/kg, respective1y) and lymphocyte ct was increased only at the HD (12%).
Clinical chemistry: the only reasonably drug-related findings were increases in total cholesterol and phospholipids in HDF (40%). Other findings, primarily in females, (e.g., increased TG, total bilirubin) were not dose-related.
Urinalysis: there were no clear drug-related findings.
Gross pathology: the only gross finding was "small" ovary in 2/6 HDF.
Organ Weights: the only clearly drug-related finding was a decrease in absolute and relative ovary wt at doses over 10 mg/kg (24-30, 32-30, 24-21, and 41-36% at 50, 150, 500, and 1000 mg/kg, respectively).
Histopathology: selected findings are summarized in the table below.

FINDING	TISSUE	MALES						FEMALES					
		0	10	50	150	500	1000	0	10	50	150	500	1000
lung	foamy cell infiltrate												
	minimal	2/6	--	--	2/6	3/6	3/6	0/6	--	--	0/6	5/6	6/6
	slight	0/6			0/6	0/6	1/6	0/6			0/6	0/6	0/6
mammary gland	acinous hyperplasia												
	minimal							0/6	0/6	0/6	2/6	0/6	1/6
	acinous hyperplasia + secretion												
	minimal							0/6	3/6	2/6	1/6	0/6	3/6
	slight							0/6	3/6	3/6	2/6	6/6	1/6
	moderate							0/6	0/6	1/6	1/6	0/6	1/6
	dilatation of ducts												
	minimal							0/6	3/6	5/6	1/6	3/6	0/6
	slight							0/6	0/6	0/6	3/6	1/6	2/6
uterus	endometrial atrophy (mod)							0/6	3/6	4/6	4/6	6/6	4/6
vagina	mucification of epithelium												
	minimal							0/6	0/6	1/6	1/6	0/6	1/6
	slight							0/6	2/6	2/6	2/6	6/6	3/6
ovary	decreased no. corpora lutea												
	minimal							0/6	6/6	3/6	1/6	2/6	1/6
	slight							0/6	0/6	3/6	4/6	4/6	2/6
	moderate							0/6	0/6	0/6	1/6	0/6	3/6
	increased no. atretic follicles												
	minimal							0/6	3/6	3/6	2/6	2/6	3/6
	slight							0/6	0/6	1/6	1/6	4/6	2/6
	increased no. cystic follicles												
	minimal							0/6	3/6	5/6	1/6	3/6	4/6
	slight							0/6	1/6	1/6	3/6	3/6	1/6
	moderate							0/6	0/6	0/6	2/6	0/6	1/6
	loosed interstitium (slight)							0/6	1/6	0/6	1/6	0/6	2/6

The sponsor attributed the mammary and uterine effects to SM-13496's D2 effects on serum prolactin. Ovarian and vaginal effects were attributed to suppression of LH via D2 antagonism. The foamy cell infiltration of lung was considered due to phospholipidosis. The sponsor noted that SM-13496 shares a chemical structure similar to other compounds that induce these changes.

Study title: **Three-month oral toxicity study of SM-13496 in rats**

Study no.: **2813**

Conducting laboratory and location: (b) (4)

Date of study initiation: 10/25/93

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SM-13496, lot no. 3CG001M, purity not stated

Key Study Findings:

A 3-month oral gavage administration of lurasidone to Sprague-Dawley rats at doses of 3, 30 and 150 mg/kg/day for males and 3, 30, 300 and 1000 mg/kg/day for females did not cause mortality; treatment-related clinical signs (ptosis, decreased spontaneous activity, and lacrimation) in both sexes, and miosis in females were observed at and above 30 mg/kg/day; catalepsy was induced at 150 mg/kg/day in males and at 1000 mg/kg/day in females. Body weight, body weight gain and food consumption decreased in males at and above 150 mg/kg/day and in females at and above 300 mg/kg/day, but at 3 and 30 mg/kg/day body weight increased in females, and food intake increased in females at 3 mg/kg/day. Water consumption decreased in females at and above 30 mg/kg/day. Hematological changes were observed in males: increase in erythrocyte count at 3 mg/kg/day or higher, increase in hemoglobin concentration at 30 mg/kg/day or higher, and increase in neutrophil count at 150 mg/kg/day. Changes in serum hormones levels (increase in serum prolactin in both sexes and a tendency toward lower serum estradiol levels in females) were observed at all dose levels, including LD. Increase in the number of females showing disorders of the estrus cycle (persistent diestrus stage) was seen at all dose levels, including LD. Histopathology changes were found in the mammary gland in both sexes and in the ovary, uterus and vagina in females at all dose levels, including LD. Increased fatty infiltration into the bone marrow of femur and sternum, and decreased trabecular bone of the femur were found in females at doses of 3 mg/kg/day or higher. Bone mineral loss was seen at 300 and 1000 mg/kg/day. Decreased pituitary weights were observed in females at doses of 3 mg/kg/day or higher. Almost all findings exhibited a tendency to reverse upon withdrawal of treatment.
Dose-dependent increases of serum lurasidone concentration were observed from 3 to 150 mg/kg/day in males and 3 to 300 mg/kg/day in females. Lurasidone plasma exposure (Cmax and AUC) were higher in females than in males. A NOAEL was not reached (below the lowest tested dose of 3 mg/kg/day) because of adverse effects at all tested doses, i.e., prolactin increase, estrus cycle disorders and histopathology changes in the female reproductive system and in the mammary gland of both genders, as well as trabecular bone decrease and bone marrow fatty infiltration.

The review data below are reproduced from Dr. Lois Freed's review of this study under IND 61292.

Methods

Dosing

Species/strain: Crj:CD(SD) rat (b) (4)

Number/sex/group: 12/sex/grp age: 4 wks)

Weight: 133-154 gm for males, 114-134gm for females

Diet/housing: ad lib12-3 per cage

Satellite groups used for toxicokinetics or recovery: 7-8/sex/gr for serum prolactin study, 5/sex/group (dosed groups only, except 10 F for 3 mg/kg gr) for TK; for 6-wk recovery, 6/gr for males and 6/gr (C, 300, 1000 mg/kg) for females.

Dosage groups in administered units: 0, 3, 30, 150 mg/kg for males, 0, 3, 30, 300, 1000 mg/kg for females.

Route, form, volume, and infusion rate: oral, suspension, 5 mL/kg.

Drug, lot#, and % purity: SM-13496, lot no. 3CG001M, purity not stated.

Formulation/vehicle: 0.5% MC. Suspension stated to be stable at room temperature for 24 hrs or for 14 days if refrigerated. Suspensions were refrigerated and used within 11 days of preparation. Suspensions were within 10% of intended except during Day 3 when concentrations were 12.5 15.3% higher. Homogeneity was stated to be acceptable (CV<5%).

Observations and times

Mortality and clinical signs: animals were observed daily. Catalepsy [defined as maintaing posture (anterior limbs against front of cage) for ~30 sec.] was assessed once a week.

Body weights: animals were weighed 3 times during the first week, then once a week thereafter. In addition, recovery animals were weighed twice during the 6th recovery wk. All animals were weighed just prior to sacrifice. Food/water intake were measured per cage over 48-hr periods once a week throughout the main study and recovery periods. Daily individual consumption was calculated.

Ophthalmoscopy: eyes were examined in 6/sex/group at Wk 12 of main study and Wk 5 of recovery. Examinations were performed before and after induction of mydrasis (Mydrn P) using a fundus camera and an ophthalmoscope or binocular indirect ophthalmoscope.

ECG: no

Hematology: blood samples were collected from the abdominal aorta at sacrifice from main-study and recovery animals for analysis of the following: rbc ct, hgb, hct, MCV, MCH, MCHC, reticulocyte ct and rate, wbc (ct, differential), platelet ct, coagulation parameters: PT, APTT, fibrinogen.

Clinical chemistry: portions of the blood samples collected at sacrifice were used to assess the following parameters: total protein, protein electrophoresis; A/G ratio, glucose, total cholesterol, triglycerides, phospholipids, total bilirubin, direct bilirubin, urea N, creatinine, AST,ALT, alkaline phosphatase, GGTP, LDH, CPK, leucine aminopeptidase, Na, K, CL, Ca, P. Hormones: serum prolactin in blood samples from main study animals at 1 hr after the final dose and from satellite animals at 24 hrs after final dose. Blood samples were stated to have been collected after". . . calming for 1 hr in the necropsy room." Serum prolactin was quantitated using RI assay (rat prolactin). Serum estradiol was quantitated from prolactin satellite females at 1 hr post final dose. Urinalysis: urine samples were collected by "forced micturition with abdominal compression" during Wk 12 or 13 of the main study and during Wk 6 of recovery for analysis of the following parameters: pH, ketone bodies, glucose, bilirubin, protein, urobilnogen, occult blood. In addition, 4-hr samples were collected from the same animals (water provided ad lib) for microscopic analysis of sediment and 24-hr samples were collected (water provided ad lib) for additional analyses (i.e., appearance, total urinary chloride excretion, urine volume, Na/K ratio, total urinary excretion of Na, osmolality, total urinary excretion of K.

Estrus cycle: vaginal smears were collected from satellite (prolactin group) females "since 1 week before the necropsy. . .", stained with Giemsa stain and examined microscopically for the stage of estrus.

Gross pathology: complete necropsy on all animals terminally sacrificed (main and recovery).

Organ weights: heart, liver, spleen, kidney, adrenal, prostate, testis, ovary, brain, pituitary, lung, thymus, thyroid. Adrenal, ovary, pituitary, and thyroid were preserved in 10% neutral buffered formalin prior to weighing.

Histopathology: The following tissues were examined in all C and HD main-study and recovery animals: skin (abdomen), mammary gland, lymph nodes (submandibular, mesenteric), salivary gland (submandibular), bone/bone marrow (sternum, femur), muscle (femoral region), sciatic nerve, thymus, trachea, lung, heart, thyroid/parathyroids, tongue, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum), liver, pancreas, larynx, spleen, kidney, adrenal, urinary bladder, seminal vesicle, prostate, testis, epididymis, ovary, uterus, vagina, brain, pituitary, spinal cord (thoracic), thoracic aorta, eye, Harderian gland, gross lesions, carcass. In addition, mammary glands were examined at the lower doses in males and females; lung, ovary, uterus, femur, sternum, and vagina were examined at the lower doses in females, and mammary gland was examied in the 300-mg/kg recovery group in females. Tissues were stained with H & E (following fixation) for examination.
Additional analyses on bone were conducted (non-GLP) on all females (main study and recovery).

Toxicokinetics: blood samples were collected from satellite-TK, main study (not Controls), and satellite-PRL animals at 1 hr (satellite-PRL animals), 4 hrs (satellite-TK animals), and 24 hrs (main-study animals) following the final dose. SM-13496 was quantitated in serum using HPLC with fluorescence detection. The LLOQ was 4.0 ng/mL for a 1-mL sample and 5.0 ng/mL for <1.0 mL.

Results

Mortality: there was one unscheduled death. One control male died on Day 70 due to gavage error.

Clinical signs: in males, the primary drug-related clinical signs were decreased spontaneous motor activity, ptosis, and muscular flaccidity. Reduced motor activity was noted at the MD and HD primarily during the first 2-3 wks of dosing. Through about Day 34, the daily incidences were fairly dose-related. From Day 35 on, fewer animals were affected and the incidences tended to be greater at the MD. From Day 54 on, ≤ 3/grp (MD, HD) were affected, except for Day 75 on which 16/18 HDM were affected. Ptosis was observed at the MD and HD throughout the dosing period; incidences were not always dose-related. Muscular flaccidity of the scrotum was noted for 2-3 wks starting on Day 6. In general, the incidence decreased with duration and this finding was not observed after Day 64. Lacrimation was observed only sporadically and only in 1-2 animals per group (all doses). Catalepsy was observed only in 1/18 HDM and only after the first dose.
In females, the primary drug-related signs were decreased spontaneous motor activity and ptosis. Reduced activity was observed at all but the LD throughout most of the dosing period, although the incidences were frequently not dose related and tended to decrease with duration of dosing. Ptosis was observed at all but the LD (all animals) throughout the dosing period. Lacrimation was observed in a few females per group at all but the LD; however, the incidences within these groups were not dose-related. Catalepsy was noted only in 5/18 HDF on Day 1 In recovery animals, only ptosis was observed and only in 1-2 animals at 300 and 1000 mg/kg on the first two days of the recovery period.

Body weights: In males, mean body wt was significantly reduced at the HD (9% at final evaluation) throughout the dosing period. Overall bodyweight gain in HDM was reduced by 13% compared to CM. In females, mean bodyweight was increased at 3 and 30 mg/kg (9 and 7%, respectively, at final assessment) and reduced at 300 and 1000 mg/kg (6 and 16%, respectively, at final weighing). Overall body weight gain was increased by 18 and 11% at 3 and 30 mg/kg, respectively, and reduced by 11% and 28% at 300 and 1000mg/kg, respectively.
In recovery animals, there were no differences among groups in males even on Day 1 of the recovery period. (This would suggest that the body wt effect may have been less in this group of animals.) In females, mean body wt remained reduced at 300 and 1000 mg/kg (9 and 10%, respectively, at final assessment) throughout the recovery period. Overall body wt gains during the recovery period were fairly similar among groups for both males and females.
The data were summarized in the following sponsor's figures:

Fig. 1-1. Body weights (Male)
Three-month Oral Toxicity Study of SM-13496 in Rats (Study No. 2813)

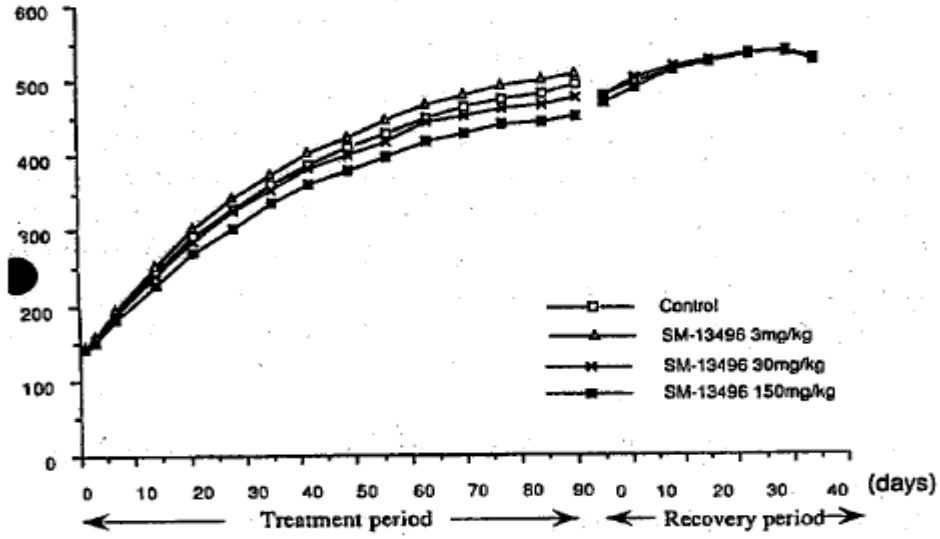
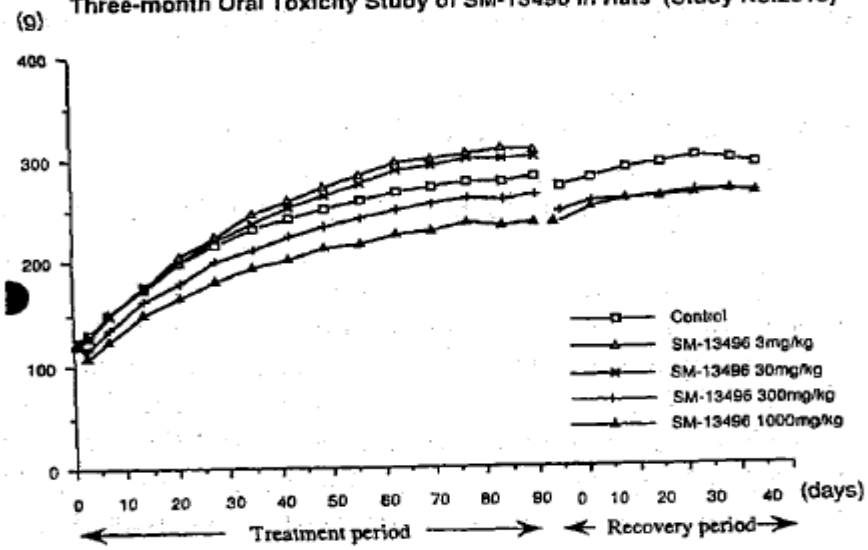


Fig. 1-2. Body weights (Female)
Three-month Oral Toxicity Study of SM-13496 in Rats (Study No.2813)



Food consumption: mean food intake was slightly (<10%), but significantly reduced at the HD in males. In females, food intake was slightly (<20%) and sporadically elevated at the LD, and decreased at 300 and 1000 mg/kg (<25%). The greatest effect was observed on Day 3 on which food intake was reduced by 12, 41, and 65% at 30, 300, and 1000 mg/kg, respectively.

Water intake was sporadically reduced (9-17%) in HDM. In females, water intake was reduced at 30, 300, and 1000 mg/kg (<45%); the magnitude of the effect was not consistently dose-related. During the recovery period, food and water intake was similar among groups for both males and females.

Ophthalmoscopy: the only drug-related finding was miosis, which was noted in females at all but the LD. The pupil/eye ratio was reduced by 20, 33, and 29% at 30, 300, and 1000 mg/kg. At the end of the recovery period, miosis was still evident in HDF-R (18% reduction in pupil/eye ratio); but not at 300 mg/kg.

Hematology: in males, the only findings of note were slight increases in rbc ct at all doses (4-5%) and in hgb at 30 and 150mg/kg (3-5%). In females, wbc, neutrophil, and lymphocyte cts were increased at all doses; however, the increases were not dose-related.
In recovery males, hgb and MCV were slightly increased (4%) at the HD. In recovery females, wbc, neutrophil, and lymphocyte cts still tended to be higher, primarily at the HD; fibrinogen was increased at both doses (11 and 17% at 30 and 1000 mg/kg, respectively).

Clinical chemistry: findings were minimal in males. Total protein was slightly (3-5%) reduced at 30 and 150 mg/kg, and creatinine was slightly elevated (13%) at the HD. In females, there were decreases in total protein, albumin, A/G ratio, and PL (<20%); however none of the findings was dose-related. Glucose and AST were reduced (13-14%) at the HD. GGTP was elevated at 300 and 1000 mg/kg. Examination of individual female data indicated slightly increased alkaline phosphatase in 1/12 and 4/12 F at 300 and 1000 mg/kg, respectively. Increases in these females were 6-13% compared to high-C value and 40-49% compared to mean C value. GGTP was elevated (from 2 to 3 U/L) in 2/12 and 10/12 females at 300 and 1000 mg/kg, respectively. There were no findings of note in recovery males. Numerous findings were evident in recovery females; however, none was dose-related.

Urinalysis: in males, decreases were noted in volume (53%), Na excretion (46%), and Na/K ratio (40%) at the HD. Osmolality was increased at the MD and HD (38 and 82%, respectively). Occult blood was higher (+2) in 2/6 HDM compared to CM. In recovery males, osmolality was still elevated at the HD (22%). In females, increases were noted in Na (68 and 120%) and Cl (73 and 170%) excretion at the LD and HD, respectively. The Na/K ratio was increased (43%) at the HD. K excretion was elevated at all doses; however, the increases were not dose-related. Osmolality was reduced slightly (13%) at the HD. In recovery females, Na excretion and the Na/ K ratio were still elevated at the HD (56 and 79%, respectively). Osmolality was increased by 68 62% at 300 and 1000 mg/kg (not dose-related).

Hormones: serum prolactin was elevated in males and females at 1 hr postdosing (but not 24-hrs postdosing). However, the increases were not dose-related in either sex. In males, increases of 6.3, 9.4, and 5.3 fold were obtained at the LD, MD, and HD, respectively. In females, the effect was inversely related to dose (16, 6.3, 2.6, and 2.2 fold at 3, 30, 300, and 1000 mg/kg, respectively).
In females, estradiol was reduced (53-62%) at all doses; however, the differences were not statistically significant.

Estrus cycle: a marked effect was noted on the estrus cycle when evaluated at 1 hr after the last dose. In CF, 4, 3, 1, and 0 animals were in proestrus, estrus, metestrus, and diestrus, respectively. In dosed grous, all females were in diestrus (7/7 in each gr). According to the 24-hr data, 0, 5, 1, and 2 CF were in proestrus, estrus, metestrus, and diestrus, respectively. In the dosed groups, 1/7 females in each group were in metestrus, and the remainder were in diestrus (i.e., 6/7 for each group).

Organ weights: in males, absolute and relative adrenal wt was increased at the HD (11-27%). In females, relative (not absolute) liver wt was slightly elevated at 300 and 1000 mg/g (8-18%), and absolute and relative pituitary wt was increased at all doses (5-26%). For pituitary wt, the increases in absolute wt were dose related, while increases in relative wt were not. In recovery animals, the only finding of note was a decrease in absolute and relative thyroid wt in HDF-R (22-14%).

Gross pathology: findings were as follows: (1) red/dark focus in lung (0/12, 1/12, 2/12, and 3/12 in CM, LDM, MDM, and HDM, respectively; 0/12, 0/12, 0/12, 2/12, and 2/12 at 0, 3, 30, 300, and 1000 mg/kg, respectively, in females) and (2) mammary gland development in 0/12, 10/12, 12/12, 12/12, and 12/12 females at 0, 3, 30, 300, and 1000 mg/kg, respectively.
In recovery animals, mammary gland development was still evident at both doses in females (0/6/, 1/6, and 6/6 at 0, 300, and 1000 mg/kg, respectively).

Histopathology: selected findings for main-study animals are summarized in the table below. In recovery animals, the only findings of note were foamy cell accumulation (slight) in HDF (2/6) and acinous hyperplasia of the mammary gland in the treated groups (300 mg/g: 3/6 slight, 1/6 mild; 1000 mg/kg: 5/6 slight, 0/6 mild).
The sponsor did not consider the lung findings to be drug related.

main-study data

TISSUE	FINDING	MALES 0*	3	30	150	FEMALES 0	3	30	300	1000
lung	bronchiolar cell hyperplasia slight	0/12	--	--	1/12	0/12	3/12	1/12	2/12	0/12
	osseous metaplasia slight	0/12	--	--	2/12	0/12	0/12	0/12	1/12	0/12
	foamy cell accumulation slight	3/12	--	--	5/12	1/12	6/12	1/12	0/12	4/12
	mild	0/12			0/12	0/12	0/12	0/12	0/12	1/12
	foamy cell, diffuse (slight)	0/12	--	--	0/12	0/12	0/12	0/12	0/12	0/12
mammary gland	increased secretion slight	0/12	0/10	0/9	1/11	0/12	1/12	5/12	4/12	1/12
	mild	0/12	0/10	0/9	0/11	0/12	0/12	2/12	2/12	2/12
	tubuloalveolar pattern	0/12	3/10	8/19	11/11	0/12	0/12	0/12	0/12	0/12
	acinous hyperplasia slight	0/12	0/10	0/9	0/11	0/12	8/12	8/12	5/12	7/12
	mild	0/12	0/10	0/9	0/11	0/12	3/12	4/12	7/12	5/12
femur	decrease in trabecular bone slight	0/12	--	--	0/12	0/12	1/12	3/12	4/12	1/12
	increased fatty infiltration slight	0/12	--	--	0/12	0/12	2/12	7/12	5/12	6/12
	mild	0/12			0/12	0/12	0/12	2/12	6/12	5/12
ovary	cyst (slight)					0/12	2/12	2/12	2/12	3/12
	decrease in # of corpora lutea slight					0/12	0/12	1/12	3/12	3/12
	mild					0/12	0/12	3/12	1/12	2/12
	increase in interstitial gland slight					1/12	2/12	3/12	5/12	5/12
	mild					0/12	1/12	2/12	1/12	1/12
sternum	increased fatty infiltration slight					0/12	2/12	1/12	6/12	8/12
uterus	atrophy slight					0/12	3/12	4/12	5/12	5/12
	mild					0/12	4/12	2/12	3/12	4/12
	moderate					0/12	0/12	2/12	0/12	1/12
vagina	mucification of epithelium slight					0/12	0/12	2/12	4/12	3/12
	mild					0/12	2/12	4/12	2/12	4/12
	moderate					0/12	1/12	1/12	2/12	2/12

* doses given in mg/kg

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Bone mineral analyses: The sponsor did not appear to provide a protocol for the bone mineral analyses performed. According to the report provided, "bone mineral contents", "area", and "bone mineral density" were evaluated in 11 females per group. Bone mineral content was significantly increased in LDF (8%), but decreased at 300 and 1000 mg/kg (7 and 16%, respectively). Area was increased in LDF (7%) and decreased in HDF (7%). Bone mineral density was reduced at 300 and 1000 mg/kg (8 and 10%, respectively). In recovery females, all three parameters had nearly normalized. There was a tendency for bone mineral content and density to be lower in HDF-R (6-7%); however, differences were not significant. Bone (and bone marrow) effects were attributed to decreases in estradiol.

Toxicokinetics: summary data are provided in the following table:

DOSE (mg/kg)	MALES T_{max} (hr)	C_{max} (ng/mL)	AUC (µg•hr/mL)	FEMALES T_{max} (hr)	C_{max} (ng/mL)	AUC (µg•hr/mL)
3	1	18.2 ± 3.09	--	1	29.4 ± 7.08	--
30	1	200 ± 59.8	1.61	1	326 ± 74.0	4.33
150	4	465 ± 89.2	6.61			
300				4	1290 ± 166	17.9
1000				4	1270 ± 77.1	18.5

Dose-dependent increases of serum lurasidone concentration were observed from 3 to 150 mg/kg/day in males and 3 to 300 mg/kg/day in females. Peak serum levels and the areas under the concentration-time curve were higher in females than in males.

Study title: Three-month oral toxicity study of SM-13496 in rats (NOAEL study)

Study no.: **2927**

Conducting laboratory and location: (b) (4)

Date of study initiation: 7/28/94

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SM-13496, lot no. 3CG001M, 100.9%

Key Study Findings:

This 3-month repeated-dose GLP-compliant toxicity study was designed to confirm the results of the preceding 3-month study performed at higher dose levels (Study# 2813), as well as to evaluate recovery of some findings, and to determine a NOAEL. Lurasidone was administered to rats via oral gavage at 0.03, 0.1, 0.3 and 3.0 mg/kg/day for 3 months. Main study groups consisted of 12 rats/sex, recovery groups consisted of 6 female rats in the vehicle control, 0.3 and 3 mg/kg/day, hormone evaluation groups consisted of 7 or 8 rats/sex/group and satellite groups for toxicokinetic evaluation of 5 rats/sex/group.
No treatment-related mortality or clinical signs were noted in this study. Most of the changes observed were also observed in Study 2813. In females, serum prolactin was increased, serum estradiol was decreased and number of animals with estrus cycle disorders was increased at all dose levels. At HD, females tended to have lower relative pituitary weights and grossly exhibited development of the mammary gland; microscopically, acinous hyperplasia and/or increased secretion of mammary glands, atrophy of uterus, mucification of vaginal mucosa, and fatty infiltration in the bone marrow (femur) were observed (bone mineral density analyses were not performed). In HD males, increases in serum prolactin and mammary histopathology changes (tubuloalveolar structure) were observed. The observed effects are attributable to the anti-dopaminergic action of the drug. All changes in female rats either partially or completely recovered following the recovery period. Bone marrow changes did not recover fully during the 6-week recovery period. Serum lurasidone concentrations increased dose-dependently in both sexes. Both peak serum levels and AUC tended to be slightly higher in females than in males.
The NOAEL was 0.3 mg/kg/day for males and 0.1 mg/kg/day for females because changes in mammary gland were found at ≥ 3 mg/kg/day in males and hormonal changes and disturbance of estrous cycles were found at ≥ 0.3 mg/kg/day in females.
The review data below are reproduced from Dr. Lois Freed's review of this study under IND 61292.

Methods

Dosing

Species/strain: Cij:CD(SD) rats (b) (4)
Number/sex/group: 12/sex/group
Age: 3 wks
Weight: 115-155 gm for males, 102- 1 26 gm for females
Diet/housing: ad lib, 2/cage
Satellite groups used for toxicokinetics or recovery: 7-8/sex/gr for prolactin and estradiol assays, 5/sex/gr for TK (no C groups for TK)

Dosage groups in administered units: 0, 0.03, 0.1, 0.3, and 3.0 mg/kg

Route, form, volume, and infusion rate: oral, gavage, 5 mL/kg.

Drug, lot#, and % purity: SM-13496, lot no. 3CG001M, 100.9%

Formulation/vehicle: suspension/0.5% MC. Stability and homogeneity of suspensions were tested and stated to be acceptable.

Observations and times

Clinical signs: daily. Satellite animals were checked daily only for mortality. Body weights: recorded weekly, except for twice weekly during Wks 1 and 13 and Wk 6 of recovery. Also, animals were weighed just prior to necropsy. Food consumption: food intake over 48-hr periods was recorded once weekly during the dosing and recovery periods. Daily individual consumption was calculated.

Ophthalmoscopy: no; ECG: no

Hematology: blood samples were collected at necropsy from all main-study and recovery animals for the following parameters: rbc ct, hgb, hct, MCV, MCH, MCHC, reticulocyte ct.

Clinical chemistry: plasma remaining from blood samples collected for hematology was analyzed for: total cholesterol, triglycerides and phospholipids. Hormones: blood samples were collected at 1 and 24 hrs following the final dose for analysis of serum prolactin (from main study and satellite animals). Serum prolactin was quantitated using RI assay (rat prolactin). Serum estradiol was quantitated in blood samples from main-study, recovery, and satellite females. Urinalysis: no

Estrus cycle: vaginal smears were prepared from females used for analysis of serum estradiol for one week prior to sacrifice. Smears were stained with Giemsa stain for determination of estrus stages.

Gross pathology: a complete necropsy was performed on all animals sacrificed at term. Organ weights: pituitary; Histopathology: the following tissues were examined microscopically in all main-study and recovery animals: femur and sternum (including bone marrow), ovary, vagina, pituitary, uterus, mammary gland in females, and mammary gland in males. Tissues were stained with H & E for examination. In this study, the sponsor apparently did not perform bone mineral analyses.

Toxicokinetics: blood samples were collected from 5/sex/gr (main-study, recovery, and satellite animals) at 1 hr (hormone satellite), 4 hr (satellite-TK), and 24 hrs (main study) following the final dose. SM 13496 was quantitated in serum using LC-MS/MS (ESI ionization). The LLOQ = 0.05 ng/mL for 1 mL of serum and 0.06 and 0.07 ng/mL for 0.8 and 0.7 mL of serum, respectively.

Deviations from protocol (selected): 5 M in the 0.3 mg/kg group received 1/3 of the intended dose on Day 82. However, a second dose was apparently administered to this group on the same day providing the remainder (i.e., 2/3) of the intended dose.

Results

Mortality: there was one unscheduled death. One control F-R died during the recovery period. At necropsy, liver and spleen were found to be enlarged and malignant lymphoma was diagnosed upon microscopic examination

Clinical signs: there were no drug-related clinical signs.

Body weights: there was no drug-related effect on body wt in males. In females, mean body wt was increased at the HD (about 15 %) throughout most of the dosing period. (Mean body wt tended to be higher at 0.3 mg/kg as well (10%); however, this effect was not statistically significant.) The body wt effect was evident (to a similar degree) in HDF-R throughout the recovery period. Overall, mean body wt gain was increased by 20% in HDF by the end of the main study, but was reduced at both doses assessed by the end of the recovery period (21± 15.1 g for C vs. 5±13.7 g for the 0.3 and 3.0 mg/kg groups).

Food consumption: food intake was not affected in either males or females.

Hematology and Clinical chemistry: no drug-related effects were noted.

Hormones: serum prolactin was elevated in HDM (2.4 fold) at 1 hr post dosing, but was similar among groups by 24 hrs post dosing. In females, serum prolactin was elevated at 0.3 and 3.0 mg/kg (5.7 and 20-fold, respectively) at 1 hr post dosing, but reduced (compared to CF) at these doses at 24 hrs post dosing (56 and 92%). Serum estradiol levels in females were reduced at 0.3 and 3.0 mg/kg (53 and 70%). In HDF-R, estradiol levels were slightly higher than in CF (39%); this difference was not statistically significant.

Gross pathology: the only notable gross finding was mammary gland development in 3/12 HDF (no incidence in other groups or in males). No mammary gland findings were detected in recovery females.

Estrus cycle: assessment of estrus cycles indicated a shift toward diestrus at 0.3 and 3.0 mg/kg in animals examined at 1 and 24 hrs after the last dose. The data relating serum prolactin levels and estrus cycles in satellite animals are summarized in the following sponsor's tables:

Table 7-3. Analysis of hormones and estrus cycle :
Serum prolactin concentration and stage of estrus cycle in female
- group mean values
Three-month Oral Toxicity Study of SM-13496 in Rats (NOEL Study, Study No.2927)

Group and dose	1hr[a] Proestrus	Oestrus	Metestrus	Diestrus
Control	9.9 ± 10.01 (3)	32.4 ± - (1)	13.7 ± - (1)	< 38.0 [b] ± - [c] (3)
SM-13496 0.03mg/kg	4.5 ± - (1)	61.7 ± 12.30 (2)	20.1 ± - (1)	14.6 ± 10.90 (3)
SM-13496 0.1mg/kg	37.9 ± - (1)	40.8 ± 39.77 (3)	64.8 ± 84.29 (2)	1.7 ± - (1)
SM-13496 0.3mg/kg	- ± - (-)	118.3 ± 52.89 (2)	308.9 ± - (1)	< 99.1 [b] ± - [c] (4)
SM-13496 3mg/kg	- ± - (-)	- ± - (-)	668.3 ±228.61 (2)	407.7 ±178.04 (5)

Mean ± SD, ng/ml
Figures in parentheses represent the number of animals.
a): Time after the final dosing
b): Mean value is calculated from individual data including the lower limit of detection (0.7ng/ml).
c): Standard deviation not calculated.

Table 7-3. Analysis of hormones and estrus cycle :
Serum prolactin concentration and stage of estrus cycle in female
- group mean values (continued)
Three-month Oral Toxicity Study of SM-13496 in Rats (NOEL Study, Study No.2927)

Group and dose	24hr[a] Proestrus	Oestrus	Metestrus	Diestrus
Control	16.3 ± 12.98 (4)	15.2 ± - (1)	22.4 ± 28.07 (2)	35.5 ± - (1)
SM-13496 0.03mg/kg	19.5 ± 4.45 (2)	12.6 ± 1.41 (2)	14.0 ± - (1)	7.6 ± 1.56 (2)
SM-13496 0.1mg/kg	< 10.4 [b] ± - [c] (2)	19.7 ± 26.41 (3)	23.8 ± - (1)	- ± - (-)
SM-13496 0.3mg/kg	4.0 ± - (1)	1.3 ± 0.14 (2)	17.8 ± 19.34 (3)	2.1 ± - (1)
SM-13496 3mg/kg	- ± - (-)	4.3 ± - (1)	< 0.7 [b] ± - [c] (1)	< 1.7 [b] ± - [c] (5)

Mean ± SD, ng/ml
Figures in parentheses represent the number of animals.
a): Time after the final dosing
b): Mean value is calculated from individual data including the lower limit of detection (0.7ng/ml).
c): Standard deviation not calculated.

The sponsor also noted that the incidence of "abnormal estrus cycles" was increased at 0.3 and 3.0 mg/kg (1/20, 2/19, 2/19, 5/19, and 19/19 for 0, 0.03, 0.1, 0.3, and 3.0 mg/kg, respectively). There were no apparent drug-related changes in recovery females.

Organ Weights: pituitary gland wt was not significantly affected by drug.

Histopathology: selected findings are summarized in the following table:

TISSUE	FINDING	MALES 0	0.03	0.3	0.3	3.0	FEMALES 0	0.03	0.3	0.3	3.0
MAIN STUDY											
mammary gland	tubuloalveolar pattern	0/12	0/12	0/12	0/12	4/12	0/12	0/12	0/12	0/12	0/12
	acinous hyperplasia (slight)	0/12	0/12	0/12	0/12	0/12	1/12	0/12	0/12	0/12	7/12
	increased secretion (slight)	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	4/12
femur	inc. fatty infiltration (slight)						0/12	1/12	0/12	1/12	4/12
sterum	inc. fatty infiltration (slight)						0/12	0/12	0/12	0/12	1/12
uterus	atrophy										
	slight						1/12	0/12	0/12	0/12	5/12
	mild						0/12	0/12	0/12	0/12	3/12
vagina	mucification of epithelium										
	slight						1/12	0/12	0/12	0/12	3/12
	mild						0/12	0/12	0/12	0/12	3/12
RECOVERY											
femur	inc. fatty infiltration (slight)						1/5	--	--	0/6	2/6

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Toxicokinetics: the data are summarized in the following sponsor's table:

Table 13 Pharmacokinetic parameters of SM-13496 after repeated oral administration to rats for 3 months

Dose (mg/kg)	Male Tmax (hr)	Male Cmax[a)] (ng/ml)	Male AUC (ng·hr/ml)	Female Tmax (hr)	Female Cmax[a)] (ng/ml)	Female AUC (ng·hr/ml)
0.03	1	0.07 ± 0.018	-	1	0.16 ± 0.035	-
0.1	1	0.30 ± 0.035	-	1	0.34 ± 0.079	-
0.3	1	1.20 ± 0.285	-	1	1.49 ± 0.389	-
3	1	22.38 ± 5.457	144.5	1	32.62 ± 11.068	159.0

a) Each value represents the mean ± SE of 5 rats.
-: not calculated

Dose-dependent increases of serum lurasidone concentration were observed from 0.03 to 3 mg/kg/day in both sexes. Both peak serum levels and area under the concentration-time curves tended to be slightly higher in females than in males.

Comments: Increased body weight gain at relatively low doses in females, changes in mammary glands in both sexes and changes in organs of reproductive system in females are attributable to the anti-dopaminergic action of the drug; these changes are commonly seen in studies of other drugs of this class. In this study, the sponsor apparently did not perform bone mineral analyses. Bone mineral content was significantly decreased at higher dose levels used in the preceding 3-month rat study (300 and 1000

mg/kg, 7 and 16% decrease, respectively); bone mineral density was also reduced at the same doses (8 and 10%, respectively).
Decrease in trabecular bone in the femur and increase in fatty infiltration into bone marrow were also reported with other dopamine D2 receptor blockers, such as haloperidol and chlorpromazine that also induced changes in the endocrine and reproductive systems (Tsuji, 1997, as cited by the sponsor)[*]. Decreased trabecular bone in the femur is seen in ovariectomized animals (Isobe, 1996, as cited by the sponsor)[*] and post-menopausal women whose serum estrogen concentrations are reduced. Therefore, it is likely that the bone lesions described above might be caused by alterations in sex hormones due to treatment with compounds possessing dopamine D2 receptor blocking potential.

[*] Tsuji R, Yamaguchi T, Miyata K, Inoue T, Yoshioka K, Kamita Y, et al. A Study for Effects of Haloperidol and Chlorpromazine Hydrochloride on Bone Remodeling in Female Rats. Clinical report. 1997;31(2):359-91
[*] (b)(4)

Study title: **Six-month oral toxicity study of SM013496 in rats**

Study no.: **3259**

Conducting laboratory and location: (b) (4)

Date of study initiation: 5/21/97

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SM-13496, lot no. 3CG001M, 100.9%

Key Study Findings

Six-month administration of lurasidone at oral (gavage) doses of 0.03, 1, 10 and 100 mg/kg/day to Sprague-Dawley rats (followed by a 3-month recovery) did not cause drug-related mortality. Clinical signs that were considered to be extension of the pharmacological effect of the drug were observed at ≥ 10 mg/kg/day and included decrease in spontaneous activity, ptosis in both genders and miosis in females); at 100 mg/kg/day, catalepsy developed in a few animals from both genders. Effects on sex hormones (prolactin, estradiol) occurred at lower doses than in the prior 3-month rat studies: elevation in serum prolactin was seen in females at all dose levels and in males at ≥ 1 mg/kg/day; reduction in serum estradiol was found in females at ≥ 10 mg/kg/day. Disruption of the estrus cycle in females with increased incidence of diestrus was observed at ≥ 1 mg/kg/day. Gross and microscopic pathology examination showed mammary gland changes (tubulo-alveolar pattern in males and acinous hyperplasia in females) at ≥ 1 mg/kg/day along with a decreased number and increased size of ovarial corpora lutea in females, increase in the frequency and extent of vaginal epithelium mucification and uterine atrophy. Reduction in the density of trabecular bone (femur) and increased fatty infiltration into the bone marrow was found in females at ≥ 1 mg/kg/day. These effects were noted in the 3-month rat study as well, and were considered attributable to the dopamine blocking action of lurasidone and its effects on sex hormones (prolactin and estrogen). An increase in the incidence and extent of thickened zona glomerulosa in adrenal gland was noted in females at ≥ 1 mg/kg/day (a finding newly noted in the 6-month study). Adrenal changes have also been reported with haloperidol and chlorpromazine administration to female rats for 6 months (lit. data); it is likely that they are mediated through elevation in prolactin levels. Recovery or a trend toward recovery was noted after a 3-month discontinuation period, except for the following changes: reduction in density of trabecular bone, increase in fatty infiltration in the bone marrow of the femur, and increase in absolute and relative ovary weights. The NOAEL in males was 0.03 mg/kg/day because of elevated serum prolactin levels and histopathology changes in the mammary gland noted at ≥ 1 mg/kg/day. For females the NOAEL was 0.03 mg/kg/day as well because of adverse effects on reproductive parameters (i.e., disruption of estrus cycle, mammary hyperplasia, decreased corpora lutea, uterine atrophy, reduction in trabecular bone density and increased fatty infiltration in bone marrow) seen at ≥ 1 mg/kg/day; it is noted that elevation or a trend towards elevation in serum prolactin was recorded at ≥ 0.03 mg/kg/day. There was no safety margin, since lurasidone rat serum levels at the NOAEL (0.15 and 0.07 ng/ml for M and F, respectively) were much lower than the maximal serum concentrations measured in humans after repeated oral administration at the MRHD of 120 mg/day (165 ng/ml).

The review data below are reproduced from Dr. Lois Freed's review of this study under IND 61292.

Methods

Dosing

Species/strain: Crj:CD(SD) rats (b) (4)

Number/sex/group: 12/sex/gr

Age: 4 wks

Weight: 162-188 g for males, 123-160 g for females

Diet/housing: ad lib/2 per cage

Satellite groups used for toxicokinetics or recovery: 3-mo recovery: 6 males for 0 and 100 mg/kg groups and 6 females for 0, 1, 10, and 100 mg/kg groups; an additional 6/sex/gr were used for "various tests"

Dosage groups in administered units: **0, 0.03, 1, 10, and 100** mg/kg

Route, form, volume: oral, gavage, 5 mL/kg

Drug, lot#, and % purity: SM-13496, lot no. 3CG001M, 100.9%

Formulation/vehicle: suspension/0.5% methyl cellulose; dosing suspensions were analyzed for concentration and homogeneity. Dosing concentrations were within 10% of intended. Homogeneity was documented.

Observations and times

Clinical signs: all main-study and recovery animals were observed daily. Animals were tested for catalepsy once a week. Satellite animals were checked daily only for mortality.

Body weights: all animals were weighed weekly through Wk 13 and monthly from Wk 14 on during the dosing period. Recovery animals were weighed weekly during the recovery period. In addition, animals were weighed just prior to sacrifice.

Food consumption: food consumption (per cage) was quantitated for 48-hr periods around days on which animals were weighed. Individual food intakes were calculated.

Ophthalmoscopy: examinations were performed during Wk 25 of the dosing period in 6/sex/grp from the main study and in all recovery animals during Wk 12 of recovery. Examinations involved assessment of"... cornea, iris, conjunctiva, lens, vitreous body, and ocular fundus..." of both eyes. The right eye was dilated (Midrin-P) for examination. In addition, ocular diameter and pupil diameter were assessed at 2 hrs postdosing during Wks 25 and 26 of dosing and during Wk 12 of the recovery period.

ECG: no

Hematology: blood samples were collected (abdominal aorta) just prior to sacrifice from main-study and recovery animals for analysis of the following parameters: rbc ct, hgb, hct, MCV, MCH; MCHC, wbc (ct, differential), platelet ct, reticulocyte ct, PT, APTT, fibrinogen.

Clinical chemistry: blood samples collected just prior to sacrifice were also analyzed for the following: total protein, A/G ratio, protein electrophoresis, glucose, total cholesterol, TG, PK, total bilirubin, direct bilirubin, urea N, creatinine, P, AST, ALT, alkaline phosphatase, GGTP, LDH, CPK,LAP, cholinesterase, Na, K, CL, and Ca.

Urinalysis: the following parameters were assessed in urine samples collected by "forcible urination" during Wks 25 and 26 of the recovery period and in Wks 12- 13 of recovery: pH, glucose, protein, occult blood, ketone bodies, bilirubin, urobilinogen. Microscopic examination of sediment was assessed in 4-hr urine samples collected in metabolism cages. The following parameters were assessed in 18-hr urine samples collected in metabolism cages: urine volume, osmolarity, Na and K concentration, Na/K ratio, Cl concentration, appearance.

Hormones: prolactin levels were quantitated in blood samples (serum) collected just prior to sacrifice (6/sex/group from main study, all recovery animals). Blood samples were collected from main-study animals 24 hrs after the last dose. Serum estradiol levels were quantitated in terminal blood samples from all main study and recovery females.

Bone analyses: blood samples collected at sacrifice were analyzed for serum osteocalcin levels (females; main-study, recovery). Deoxpyridinoline was quantitated in 4-hr urine samples from females. Bone density was determined using femoral samples from all main-study and recovery females. The following parameters were assessed (at 2 and 10 mm from the distal epiphysial plate): total bone density, trabecular bone density, cortical bone density.

Gross pathology: complete necropsy on all main-study and recovery animals.

Organ weights recorded: liver, kidneys, spleen, heart, lungs, brain, thymus, adrenals, pituitary, thyroid/parathyroid, testes, prostate, ovaries, and uterus.

Histopathology: the following tissues were examined microscopically in all Control and HD animals at the end of the dosing and recovery periods: liver, kidneys, spleen, heart, lungs, brain, thymus, prostate, testes, pituitary, thyroid/parathyroid, adrenals, ovaries, eyeballs, harderian glands, salivary glands (submandibular, "large" sublingual), submandibular lymph nodes, trachea, esophagus, larynx, tongue, stomach, small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum), pancreas, mesenteric lymph nodes, aorta (thoracic), spinal cord (cervical, thoracic, lumbar), sciatic nerve, bone and bone marrow (sternum, right femur), muscle (femoral), urinary bladder, skin (abdominal), epididymis, seminal vesicles, .uterus; vagina, mammary glands, gross lesions. Mammary gland, pituitary, and adrenals were also examined in both males and females at the lower doses (main study). Femoral bones, ovaries, uterus, and vagina were examined in females at the lower doses (main study). Mammary gland, adrenals, femoral bone, and ovaries were examined at the lower doses in recovery females. Tissues were stained with H & E for examination.

Estrus cycle: vaginal smears were collected from all females once daily during the last 7 days of the dosing period (included the day of sacrifice) and stained with Giemsa stain for assessment of stage of estrus.

Toxicokinetics: blood samples were collected from main-study dose groups (6/sex/gr) and from all satellite animals (except Cs) for analysis of serum levels of SM-13496.
Samples were collected at 1 hr following the final dose in satellite animals and at 24 hrs following the final dose in main-study animals.

Results

Mortality: there were 3 unscheduled deaths, all in LDM and all found to be due to dosing error. Foamy fluid was found in the lungs at necropsy and microscopic examination indicated "...pulmonary edema and congestion and hemorrhage".

Clinical signs: catalepsy was noted in a few HD animals (1/18 HDM, 2-3/18 HDF) on Days 1 -3 of dosing. Decreased spontaneous motor activity was observed at the two highest doses (10 and 100 mg/kg) in both males and females from Wk 1 through Wk 11 or 15 at 10 mg/kg and through Wk 17-18 at the HD. All animals at these doses appeared affected at some time during this period. Ptosis was observed at 10 and 100 mg/kg in both males and females. At 10 mg/kg, ptosis was observed in 1 to 6/12 animals during any one week from Wk 1 through Wk 2 (M) or Wk 6 (F). At 100 mg/kg, ptosis was observed in the majority of animals from Wk 1 through Wk 6-7, then in fewer animals through Wk 10-13. Muscular flaccidity of the scrotum was noted in HDM during the first 4 wks of dosing.

Body weights: In males, the mean body wt was reduced at HD throughout the dosing period. The effect increased with duration; by the end of the dosing period there was a 17% decrease in mean body wt in HDM compared to CM. There was a tendency for mean body wt to be lower at 1 and 10 mg/kg (3 and 5%) and higher at the LD (0.03 mg/kg) (7%) compared to CM; however, none of these differences was statistically significant. Overall body wt gain was 24% lower in HDM compared to CM.
In females, mean body wt was increased at 1 and 10 mg/kg, and decreased at 100 mg/kg. At the HD (100 mg/kg), mean body wt was 14% lower compared to CF by the end of the dosing period. At 1 mg/kg, mean body wt was increased (5- 8%) from Wk 29 on. At 10 mg/kg, mean body wt was transiently elevated (5-8%) from Wks 8 through 92. At the end of the dosing period, body wt was similar to CF at 0.03 and 10 mg/kg, elevated at 1 mg/kg (8%) and reduced at 100 mg/kg (14%). Overall body wt gain was

similar to CF at 0.03 and 10 mg/kg, but 14% higher and 24% lower at 1 and 100 mg/kg, respectively, compared to CF.

In recovery animals, mean body wt was still reduced in HDM-R throughout the recovery period; however, some catch-up growth was noted. On Day 3 of recovery, body wt in HDM-R was 15% lower than in CM. At the end of the recovery period, body wt in HDM-R was 11% lower than in CM. Overall body wt gain during the recovery period was 38% higher in HDM-R compared to CMR; however, this difference was not statistically significant. Mean body wt was also reduced in HDF-R throughout the recovery period; minimal catch-up growth was noted. Body wt was 22% reduced in HDF-R on Day 3 of recovery and by 19% at the end of the recovery period. At 1 mg/kg, body wt was reduced (although not significantly) throughout the recovery period. By the end, mean body wt was 11% lower in the 1 mg/kg group than in CF-R. Overall body wt gain during .the recovery period was not significantly different at 10 and 100 mg/kg compared to CF-R; however, body wt loss was noted at 1 mg/kg.

Food consumption was reduced (up to 20%) in HDM and (up to 26%) in HDF during the dosing period. Food consumption was fairly similar in CM-R and HDM-R during the recovery period, although intake tended to be lower in HDM-R (no significant difference). There were no significant differences among recovery groups in females, although food intake tended to be lower in treated groups.

Ophthalmoscopy: no findings were reported. Miosis was not observed. Mydriasis was, however, noted in LDF. Pupil-to-eye size was increased significantly (26%) in this group. No findings were noted in recovery animals.

Hematology: in males, hemoglobin was slightly elevated (3-4%) and decreases were noted in absolute lymphocyte (15, 8, and 22% at 1, 10, and 100 mg/kg, respectively), monocyte (36, 27, and 36% at 1, 10, and 100 mg/kg, respectively), eosinophil (38-40%), and basophil (50%) counts at all but the LD. In females, the only findings were: decreases in APTT at 10 and 100 mg/kg (6%) and increases in fibrinogen at all but the LD (7, 6, and 9% at 1, 10, and 100 mg/kg, respectively).

No findings were noted in recovery males. In females, however, the following were noted: (1) slight increases in RBC parameters (ct, hgb, hct; 5-7%) in all treated groups followed, (2) increased neutrophil count at the HD (120%), (3) increased fibrinogen at 10 and 100 mg/kg (6 and 11%, respectively). Only the rbc effects were statistically significant.

Clinical chemistry: <u>in males</u>, the following were noted: (1) slight increases in total protein at all but the LD (3-5%), (2) slight increases in albumin at 10 and 100 mg/kg (5-6%), (3) increased gamma-globulin at 10 and 100 mg/kg (14 and 27%), (4) increased creatine at the HD (12%); levels were increased in 4/12 HDM (0.9-1.1 vs. 0.7- 0.8 in CM), (5) increases in ChE at 10 and 100 mg/kg (38 and 58%, respectively), (6) decrease in K at the HD (10%), (7) small increases in serum Ca at 0.03 and 1 mg/kg (2-3%), and (8) increases in AST and ALT in individual HDM (#55: 120-170% increase compared to mean CM, 44-87% compared to high CM value; #63: 150-130% increase compared to mean CM, 63-57% compared to high CM value).

<u>In females,</u> the following were noted: (1) small decreases in total protein (4-6%) and albumin (7-9%) at all but the LD, (2) increased total cholesterol at the LD (17%), (3) increases in PL at the LD (15%) and decreases at 10 and 100 mg/kg (5 and 17%, respectively), (4) increases in LAP at 10 and 100 mg/kg (10 and 17%, respectively; no individual animal markedly affected), (5) small decrease in Ca at 1 and 10 mg/kg (2-3%), (6) increases in AST, ALT, alkaline phosphatase, GGTP, and LDH in individual animals. Findings in these animals are summarized in the following table. Data (all increases) are expressed as % of mean C value (% of high C value) except for GGPT which is expressed in U/L (compared to C mean of 0, range of 0-2).

DOSE (mg/kg)	ANIMAL #	AST	ALT	ALK PHOS	γ-GPT	LDH
0.03	519	--	--	120% [34%]	--	56% [5%]
	520	--	--	130% [45%]	--	--
1	531	--	--	330% [168%]	--	--
	532	200% [100%]	220% [120%]	--	3	140% [60%]
	534	--	--	--	--	92% [29%]
	535	--	--	140% [50%]	--	--
	540	--	--	--	3	--
	542	--	--	160% [63%]	--	--
10	549	--	--	120% [40%]	--	--
	550	--	--	140% [46%]	--	--
	551	--	--	130% [43%]	--	--
	552	--	--	180% [73%]	--	--
	553	--	--	82% [13%]	--	--
	557	--	--	100% [32%]	--	--
	560	--	--	100% [36%]	--	--
100	567	800% [530%]	800% [500%]	--	4	380% [220%]
	568	--	--	100% [34%]	--	--
	569	260% [160%]	330% [200%]	--	3	130% [57%]
	571	--	--	130% [41%]	--	--
	575	--	--	120% [40%]	--	--
	576	--	--	200% [94%]	--	--
	578	130% [63%]	300% [170%]	--	--	--

Urinalysis: Na and CL excretion and Na/ ratio were increased at 10 and 100 mg/kg in both males and females. Differences are summarized in the following table:

DOSE (mg/kg)	MALES			FEMALES		
	Na EXCRETION	Cl EXCRETION	Na/K	Na EXCRETION	Cl EXCRETION	Na/K
0.03	--	--	--	--	--	--
1	--	--	--	--	57%	--
10	60% ↑	90% ↑	48% ↑	130% ↑	63% ↑	52% ↑
100	74% ↑	120% ↑	57% ↑	120% ↑	72% ↑	63% ↑

Urine volume was increased at 1 and 100 mg/kg (52 and 36%, respectively). The degree of urobilnogen tended to be increased at 10 and 100 mg/kg in males.

In recovery animals, Na and Cl excretion, and Na/K ratio were elevated in HDMR (46, 28, and 50%, respectively); only the Na/K difference was statistically significant. In females, urine volume was increased (not significantly) at 10 and 100 mg/kg, although the effect was not dose-related (67 and 28%, respectively).

Na excretion and Na/ ratio were elevated in HDF-R (68 and 110%, respectively); however, only the Na/K effect was statistically significant. (CL excretion was elevated in treated groups (F; 16-30%); however, the effect was not dose-related or statistically significant at any dose.)

Hormones: serum prolactin levels were elevated at 1 hr after the final dose in both males and females. However, in neither sex were the increases dose-related (M: 272, 400, and 200% at 1, 10, and 100 mg/kg, respectively; F: 2500, 1100, 450% at 1, 10, and 100 mg/kg, respectively). At 24 hrs after the final dose, serum prolactin was similar among groups (and to C) in males; in females, however, serum prolactin was reduced at 1 and 10 mg/kg (95 and 86% at 1 and 10 mg/kg, respectively).

In recovery animals, serum prolactin levels were not significantly affected in HDM-R, but were reduced at all doses (followed through recovery) in females (88, 71, and 66% at 1, 10, and 100 mg/kg, respectively).

Estradiol was increased at the LD (82%) and decreased at 10 and 100 mg/kg (40 and 56%, respectively); however, only the LD effect was statistically significant.

In recovery females, estradiol was not significantly affected; however, levels were lower in dosed groups (36, 43, and 48% at 1, 10, and 100 mg/kg, respectively).

Bone parameters: osteocalcin was increased in females at all but the LD (24, 11, and 20% at 1, 10, and 100 mg/kg, respectively.) This effect was not observed in recovery females. Deoxypyridinoline was not affected during the dosing period. In recovery females, deoxypyrdinoline was increased at all doses (110- 160%); however, this effect was probably due to a low C value. Increases were not dose-related.

Estrus cycle: estrus cycles were abnormal in all females at 1, 10, and 100 mg/kg, with the majority of treated females in diestrus (7/18, 10/18, 15/18, 16/18, 18/18 for 0, 0.03, 1, 10, and 100 mg/kg, respectively). In recovery animals, there were no increases in abnormal estrus in treated females; although 2/6 HDF were noted to be in diestrus.

Gross pathology: gross findings at necropsy were as follows: (1) retention of white substance in the urinary bladder was increased in HDM (0/12, 1/9, 1/12, 2/12, and 5/12 at 0, 0.03, 1, 10, and 100 mg/kg, respectively), (2) mammary gland development was noted at all but the LD (0/12, 0/12, 1/12, 10/12, and 12/12 at 0, 0.03, 1, 10, and 100 mg/kg, respectively).
In recovery animals, mammary gland development was still noted in females (0/6, 0/6, 3/6, and 6/6 at 0, 1, 10, and 100 mg/kg, respectively). Retention of white substance in the urinary bladder was detected in 2/6 and 3/6 recovery males at 0 and 100 mg/kg, respectively.

Histopathology: selected findings are summarized in the following table.

Main study

TISSUE	FINDING	MALES					FEMALES				
		0	0.03	1	10	100	0	0.03	1	10	100
adrenal	thickened z. glomerulosa										
	+/-	1/12	2/12	3/12	3/12	2/12	2/12	3/12	5/12	8/12	6/12
	+	1/12	1/12	0/12	1/12	3/12	0/12	0/12	2/12	2/12	5/12
	2+	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	1/12
b6ne/bone marrow, femur	fatty infiltration										
	+/-	0/12	0/12	0/12	0/12	0/12	1/12	2/12	2/12	3/12	5/12
	+	0/12	0/12	0/12	0/12	0/12	0/12	0/12	3/12	3/12	6/12
bone/bone marrow, sternum	fatty infiltration										
	+/-	0/12	0/12	0/12	0/12	0/12	1/12	0/12	0/12	0/12	2/12
mammary gland	tubuloalveolar pattern	0/12	0/9	4/12	9/12	11/11	0/12	0/12	0/12	0/12	0/12
	acinous hyperplasia										
	+/-	0/12	0/12	0/12	0/12	0/12	0/12	0/12	1/12	6/12	4/12
	+	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	3/12	8/12
	increased secretion (+)						0/12	0/12	0/12	3/12	6/12
ovary	dec. no. corpora lutea						0/12	0/12	3/12	9/12	7/12
	develop follicles/# corp lutea						3/12	3/12	0/12	0/12	0/12
	enlarged corpora lutea										
	+/-						1/12	2/12	3/12	4/12	4/12
	+						0/12	0/12	1/12	7/12	7/12
uterus	atrophy										
	+/-						0/12	0/12	1/12	4/12	2/12
	+						0/12	0/12	3/12	2/12	2/12
	2+						0/12	0/12	0/12	1/12	3/12
	dilatation										
	+/-						0/12	1/12	1/12	2/12	1/12
	+						0/12	4/12	2/12	0/12	0/12
	2+						3/12	1/12	0/12	0/12	0/12
vagina	mucification of epithelium										
	+/-						0/12	0/12	1/12	1/12	6/12
	+						1/12	0/12	6/12	4/12	2/12
	2+						0/12	0/12	0/12	0/12	1/12

Continued on the next page

Recovery

TISSUE	FINDING	MALES		FEMALES			
		0	100	0	1	10	100
adrenal	focal cortical hypertrophy (+)	0/6	2/6	0/6	0/6	0/6	0/6
	thickened z glomerulosa +/-	0/6	0/6	0/6	1/6	2/6	3/6
	+	0/6	0/6	0/6	1/6	2/6	2/6
bone/bone marrow, femur	dec in trabecular bone (+/-)	0/6	0/6	0/6	1/6	3/6	3/6
	increase in fatty infiltration (+/-)	0/6	0/6	0/6	1/6	6/6	3/6
mammary gland	tubuloalveolar pattern	0/6	2/6	0/6	0/6	0/6	0/6
	acinous hyperplasia (+/-)	0/6	0/6	0/6	0/6	3/6	3/6

Mammary gland changes (tubuloalveolar pattern in males and acinous hyperplasia in females) were seen at ≥ 1 mg/kg/day along with a decrease in number of corpora lutea in the ovaries, increase in the frequency and extent of vaginal epithelium mucification, and uterine atrophy in females. Reduction in the density of trabecular bone in the femur and increased fatty infiltration into the bone marrow of the femur was seen in females at ≥ 1 mg/kg/day. These effects were noted in the 3-month administration study as well, and were considered attributable to the dopamine blocking action of lurasidone or its effects on sex hormones (prolactin and estrogen). An increase in the incidence and extent of thickened zona glomerulosa in adrenal gland was noted in females at ≥ 0.03 mg/kg/day.

Organ Weights: absolute and relative adrenal wt was increased in HDM (20-45%) and decreased in females at 1, 10, and 100 mg/kg (18-27, 20-20, and 27-19%, respectively). Absolute and relative pituitary wt was increased in HDM (14- 41 %), and uterine wt was decreased at 1, 10, and 100 mg/kg (36-48, 49-48, and 55-41 %, respectively).

In recovery animals, pituitary wt (absolute and relative) was still elevated in HDM (21-33%). In females, absolute and relative wts of ovary (90-100, 74-77, and 33-67% at 1, 10, and 100 mg/kg, respectively) were increased and uterine wt (absolute and relative) were decreased (31-25, 38-40, and 41-29% at 1, 10, and 100 mg/kg, respectively). Absolute adrenal wt was reduced (15, 16, and 20% at 1, 10, and 100 mg/kg, respectively); however, relative wts were similar among groups.

Toxicokinetics: the data are summarized in the following sponsor's table:

Serum concentrations of SM-13496 after repeated oral administration of SM-13496 to rats for 6 months - group mean values

	Dose(mg/kg)	Concentration (ng/ml)[a] 1hr[b]	24hr[b]
Male	0.03	0.15 ± 0.047	<0.05 [c]
	1	8.53 ± 3.769	0.64 ± 0.451
	10	88.24 ± 21.630	9.10 ± 4.651
	100	528.43 ± 279.7	100.10 ± 71.339
Female	0.03	0.07 ± 0.054	<0.05
	1	9.34 ± 4.254	0.89 ± 0.583
	10	166.68 ± 133.422	14.67 ± 6.646
	100	608.11 ± 82.850	171.36 ± 37.035

a) : Values are the mean ± S.D. of six animals.
b) : Time after final administration
c) : n = 4

Serum drug concentrations increased in a dose-dependent fashion, and were higher in females at doses of 1 mg/kg day and above. C_{1hr} mean value at the dose 1 mg/kg/day (the lowest dose at which adverse effects were observed in both sexes) was 8.5 and 9.3 ng/mL in males and females, respectively.

Comments:
Effects on sex hormones (prolactin, estradiol) were found in the 6-month study at lower doses than in the prior 3-month rat studies: elevation in serum prolactin was seen in females at all dose levels and in males at ≥ 1 mg/kg/day; reduction in serum estradiol levels was found in females at ≥ 10 mg/kg/day. Disruption of the estrus cycle in females with increased incidence of diestrus was observed at ≥ 1 mg/kg/day. Gross and microscopic pathology examination showed mammary gland hyperplasia in females at ≥ 1 mg/kg/day along with a decrease in number of corpora lutea in the ovaries, increase in the frequency and extent of vaginal epithelium mucification, and uterine atrophy. Reduction in the density of trabecular bone (femur) and increased fatty infiltration of bone marrow of the femur was seen in females at ≥ 1 mg/kg/day. These effects were noted in the 3-month administration study as well, and were considered attributable to the dopamine blocking action of lurasidone or its effects on sex hormones (prolactin and estrogen). Thickening of the adrenal zona glomerulosa (dose-dependent in females) was newly noted at ≥ 0.03 mg/kg/day in the 6-month study. As suggested by the sponsor, it is likely that "this change is a result of lurasidone-induced elevation in prolactin levels, which may have affected corpora lutea in the ovary, potentially producing a low estrogen state with increased progesterone production, which in turn elicited an increase in production of aldosterone in the adrenal glands. Elevated aldosterone levels have been reported when progesterone was administered to ovariectomized rats (Braley, 1996[*]), and changes in the adrenal have also been found when haloperidol and chlorpromazine were administered to female rats for 6 months (Inoue, 1998[*])."
The following discussion is reproduced from the Study on the Effects of Dopamine D2 Receptor Blockers on the Adrenal Gland in Female Rats (Inoue, 1998[**]).

> It has been well known that dopamine D_2 receptor blockers cause the condition of high prolactin levels by stimulating the secretion of prolactin from the pituitary[3)]. Prolactin serves as a luteotropin and causes the corpus luteum to accelerate the progesterone secretion, which is a phenomenon unique to rodents[4)]. A high level of prolactin, an increase or a tendency toward an increase in ovary weight, and large corpus luteum were actually observed in the animals treated with HP and CP in the study titled "A Study for Effects of Haloperidol and Chlorpromazine Hydrochloride on Bone Remodeling in Female rats (Study No. S0353)[2)], as in animals treated with SM-13496[1)]. In addition, it has been reported that an increase in progesterone leads to an increase in blood aldosterone concentration[5)]. There are some reports that the secretion of aldosterone is stimulated by a direct effect of prolactin on the zona glomerulosa in the adrenal gland[6, 7, 8)] and by the administration of dopamine D_2 receptor blockers such as metoclopramide[9)], haloperidol, sulpiride and domperidone[10)]. In the six month oral toxicity study of SM-13496 in rats[1)], a change in the adrenal gland was observed in females. Therefore, there is a strong possibility that an increase in progesterone may be associated with the change in the adrenal gland. A detailed mechanism for this association is not yet understood because aldosterone was not determined in the present study. However, the thickened zona glomerulosa in the adrenal gland could be considered a finding which is associated with the stimulation of aldosterone secretion by prolactin or progesterone following administration of the test article. The finding in the adrenal gland observed in the study titled "Six month oral toxicity study of SM-13496 in rats"[1)] is considered due to anti-D_2 activity, which is common with the dopamine D_2 receptor blockers.

Recovery or a trend toward recovery was noted after a 3-month discontinuation period except for the following: reduction in density of trabecular bone, increase in fatty infiltration in the bone marrow of the femur, and increase in absolute and relative ovary weights. The sponsor suggested that "recovery of the

[*] Braley LM, Menachery AI, Yao T, Mortensen RM, Williams GH. Effect of progesterone on aldosterone secretion in rats. Endocrinology. 1996; 137:4773-8 (as cited by the sponsor)

(b

bone could not be observed because osteogenesis was decreased in the aging animals, which were aged seven months and whose growth period was already over at the start of recovery".

The issue of the bone density decrease seen consistently in the subchronic and chronic rat studies was addressed in the Agency's letter dated 2/7/2001 under IND 61 292, which asked the sponsor to further investigate the decreased bone density observed in the 3-mo rat study, and to monitor bone density in the proposed clinical trial:

> Clinical Comment #3: "The decreased bone densities observed in the 3-month rat studies need further exploration. We recommend that you monitor patients' bone density throughout and after this study for some appropriate time."

The sponsor responded in Amendment N-007 to IND 61 292, as reviewed by Dr. Lois Freed:

> The sponsor agreed that the bone density findings in rat "would be problematic" and indicated that "Review of the 3 and 6-month animal data suggest that those that received higher doses did indeed have accelerated bone loss". This finding was tentatively attributed to hyperprolactinemia (by the sponsor) although the sponsor acknowledged that a direct drug-related effect on bone was possible. No nonclinical studies were proposed by the sponsor. The sponsor discussed a monitoring plan for humans, and this information will be reviewed by the medical officer (see Dr.Freed's P/T Memorandum to IND 61 292 of 8/13/2002) .

Conclusion:

The NOAEL in the 6-month oral study of lurasidone in rats is 0.03 mg/kg/day for males because of elevated serum prolactin levels and histopathology changes in the mammary gland noted at and above the next tested dose of 1 mg/kg/day. For females the NOAEL is 0.03 mg/kg/day as well because adverse effects (i.e., disruption of estrus cycle, mammary hyperplasia, reduced number of ovarian corpora lutea, uterine atrophy, reduced trabecular bone density and increased fatty infiltration in bone marrow) occurred at and above the next tested dose of 1 mg/kg/day. Serum prolactin levels were elevated at $\geq$ 0.03 mg/kg/day. There was no safety margin, since lurasidone rat serum levels at the NOAEL (0.15 and 0.07 ng/ml for M and F, respectively) were much lower than the maximal serum concentrations in humans following repeated oral administration at the MRHD of 120 mg/day (165 ng/ml).

6.2.2. Studies in monkeys

Study title: **SM 13496 Toxicity to Cynomolgus monkeys by repeated oral administration for 13 weeks followed by a 6-week recovery period**

Study no.: **SUP22**

Conducting laboratory and location: (b) (4)

Date of study initiation: 29 March, 1994

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SM 13496, lot # 3CG001M, 100.9%

Key Study Findings

A 3- month administration of lurasidone (suspension of 0.5% aqueous methylcellulose) to Cynomolgus monkeys (3/sex/group) by oral intubation at doses of 0 (vehicle) **2, 10 and 50 mg/kg/day**, followed by a 6-week recovery period did not cause drug-related mortality. Subdued behavior was observed at MD (10 mg/kg) and mostly at HD (50 mg/kg/day). Tremors, salivation and decreased body weight and food consumption were observed at HD. Serum prolactin was elevated in all treated groups vs. control on Day 1 and, to a greater degree, in Week 13 when prolactin levels were elevated in all treated groups with mean values of 20, 34.5 and 40.5 ng/ml, at LD, MD and HD respectively, vs. the control mean of 1.5 ng/mL; at 24 h post dosing, prolactin tended to be comparable to controls. Prolactin changes had no histological correlates and disappeared after a 6-week recovery period. There were no treatment-related changes in hematology, blood biochemistry, urinalysis, body temperature, ophthalmology, ECG, organ weights, bone marrow, gross pathology or histopathology (focal myocarditis found in 1 low-dose male was assessed as non-treatment-related). Lurasidone systemic exposure levels (Week 13) were dose-dependent with mean AUC_{0-t} serum values of 58 and 73 ng.hr/mL (M and F, respectively) at 10 mg/kg and 401 and 959 ng.hr/mL (M and F, respectively) at 50 mg/kg/day (the AUC_{0-t} values at the LD of 2 mg/kg/day could not be calculated). The mean Cmax serum concentration was 4.7 and < 2.0 ng/mL (males and females, respectively at Week 13) at a dose of 2 mg/kg/day, a dose that elevated serum prolactin levels. The NOAEL in this study is 2 mg/kg/day, because the next higher tested dose of 10 mg/kg/day caused subdued behavior, while 2 mg/kg/day yielded no toxic findings associated with the elevation of prolactin levels. The mean Cmax concentration at this dose after 13 wks was 4.7 and < 2.0 ng/ml (M and F, respectively); the AUC_{0-t} values could not be calculated. This is a much lower exposure than that in humans at the MRHD of 120 mg/day (Cmax: 165 ng/ml)

Methods

Dose selection: The dose selection was based on a preliminary 14-day repeated-dose oral toxicity study (Study No.SUP21, non-GLP) conducted at lurasidone doses of 4 and 100 mg/kg/day in Cynomolgus monkeys (2/sex/group) at the same lab facilities in which the 13-week study was conducted. Subdued behavior and salivation were observed at both doses; tremors, vomiting and decreases in body weight and food consumption were observed at 100 mg/kg/day. A NOAEL was not reached (less than 4 mg/kg/day). Based upon these results, dosage levels of 2, 10 and 50 mg/kg/day were selected for the 13-week study.

Doses:	2, 10 and 50 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral intubation
Dose volume:	4 ml/kg
Formulation/Vehicle:	suspension in 0.5% aqueous methylcellulose
Species/Strain:	Monkey/ Cynomolgus
Number/Sex/Group:	3/sex/group
Age:	Range: 2.5 – 4.5 years
Weight:	Range: 2.2 – 5.2 kg
Satellite groups:	No [6-month recovery animals (2/sex/group) were selected from HD and Control groups]
Unique study design:	None
Deviation from study protocol:	Did not affect the study results and conclusions

Observations

Mortality and clinical signs: Daily, at 2-hour intervals
Rectal temperature: Recorded in weeks 4, 8 and 13, pre-dose, and 2 and 6 hrs after dosing.
Food consumption: Daily
Body weight: Weekly, starting 4 weeks before dosing
Ophthalmoscopy: In all animals, before commencement of treatment and again on one day during Week 13
Hematology/Biochemistry: Routine, at the following time points:

Week -4
Day 1 (Prolactin assay)
Week 4
Week 8
Week 13
Week 13 (Prolactin assay)
Recovery Week 6
Recovery Week 6 (Prolactin assay)

Urinanalysis: Routine, at the following time points:

Week -4
Week 4
Week 8
Week 13
Recovery Week 6

Prolactin: Determined by RIA in blood samples from all animals collected At 4 and 24 h. after dosing on Day 1 and on one day during Week 13, and on one day during the 6-week recovery period, at times corresponding to 4 and 24 h. after dosing
ECG: (as reproduced from the study report)

Electrocardiograms (ECGs) were recorded once before dosing commenced and again on one day during Weeks 6 and 13 of dosing and on one occasion in the final week of the recovery period. During the dosing period, recordings were made before the daily dose was given.

Recordings were made on a three channel ECG machine using the standard limb leads I, II and III, augmented limb leads aVR, aVL and aVF. A standardisation of 10 mm = 1 mV was used and a paper speed of 25 mm/second. An additional recording of lead II was taken at 50 mm/second. The recordings were examined visually for any abnormalities of the electrical complexes, and heart rate was recorded.

Organ weights were determined for the following organs:

adrenals	lungs	thymus
brain	pancreas	thyroids
heart	pituitary	uterus or prostate (with seminal vesicles)
kidneys	salivary gland (sub-mandibular)	testes (with epididymides) or ovaries
liver	spleen	

Bone marrow examination: A bone marrow sample was obtained from all animals prior to necropsy; the smears were stained using a modified Wright's stain and examined.
Histopathology: The following tissues were examined by light microscopy:

adrenals	gall bladder	skeletal muscle (quadriceps femoris)
alimentary tract (oesophagus, stomach (body and antrum), duodenum, jejunum, ileum, caecum, colon rectum)	heart	skin
aorta (arch and abdominal)	kidneys	spinal cord (cervical, thoracic and lumbar levels)
brain (cerebral cortex, thalamic nuclei, mid-brain, medulla and cerebellum)	liver	spleen
eyes (with optic nerve)	lungs (with bronchi)	sternum
femur (with joint) - left leg	lymph nodes (cervical and mesenteric)	tattoo*
femur (whole) - right leg*	mammary gland	testes (with epididymides)
	ovaries	thymus
	pancreas	thyroids (and parathyroids)
	pituitary	tongue
	prostate	trachea
	salivary gland (submandibular)	urinary bladder
	sciatic nerve	uterus
	seminal vesicles	vagina

* Preserved only

TK: In all animals on Days 1, 14 and on one day during Wk 13 at 1, 2, 4, 6 and 24 hrs after dosing.

Results

Mortality: One low-dose animal was humanely sacrificed for reasons unrelated to test article treatment.
Clinical signs: Subdued behavior was observed at MD (10 mg/kg) and mostly at HD (50 mg/kg/day). Tremors and late-onset salivation were observed at HD.
Body weight: During Week 1, bodyweight losses were noted in some animals from all groups but were more marked and numerous in the HD animals. Thereafter (including the recovery period), bodyweight losses and/or variable weight gains were seen in all groups, with no obvious treatment-related trends.

Bodyweights - group mean values (g)

Week	Dosage mg/kg/day							
	Control		2		10		50	
	♂	♀	♂#	♀	♂	♀	♂	♀
-4	3674	2816	3216	2671	3595	2799	3564	2795
-3	3726	2821	3374	2682	3626	2824	3618	2797
-2	3731	2820	3365	2677	3540	2798	3536	2789
-1	3780	2838	3233	2760	3595	2823	3580	2792
0	3826	2921	3436	2823	3684	2872	3676	2865
1	3841	2832	3345	2736	3661	2804	3519	2712
2	3860	2889	3452	2802	3847	2871	3571	2746
3	3835	2919	3488	2797	3807	2871	3668	2764
4	3904	2911	3426	2825	3898	2914	3668	2812
5	3920	2885	3455	2859	3965	2864	3709	2895
6	3883	2881	3406	2842	3975	2917	3750	2845
7	3905	2886	3598	2798	3975	2922	3742	2990
8	3937	2895	3509	2828	3964	2912	3755	2958
9	3959	2889	3495	2866	3985	2891	3781	3010
10	4026	2896	3572	2867	4108	2912	3819	3041
11	4141	2961	3659	2926	4173	2943	3902	3053
12	4098	2919	3654	2914	4181	2926	3868	3041
13	4046	2935	3582	2919	4202	2931	3760	2966
Change 0 to 1 ♂ + ♀	15	-89 -37	-91	-88 -89	-23	-68 -46	* -157	* -153 -155
1 to 13 ♂ + ♀	205	103 154	237	184 205	541	127 334	241	253 247
0 to 13 ♂ + ♀	220	14 117	146	96 116	518	59 289	84	100 92
13	3700						3413	
R1	3646						3375	
R2	3701						3328	
R3	3778						3411	
R4	3839						3585	
R5	3981						3699	
R6	3839						3525	
Change 13 to R6 ♂ + ♀	140						112	

* P <0.05
\# Mean of two animals
R Recovery

Food consumption was decreased at HD.

Food consumption - group mean values (g)

Week	Dosage mg/kg/day							
	Control		2		10		50	
	♂	♀	♂#	♀	♂	♀	♂	♀
-4	990	1144	995	1007	1033	1087	1010	1046
-3	1012	1116	1015	1010	963	1073	990	1016
-2	1012	1166	970	1003	983	1070	1024	1024
-1	1006	1104	980	1023	993	1040	988	1064
Mean -4 to -1	1005	1133	990	1011	993	1067	1003	1038
1	1020	1088	990	1010	967	990	876	694
2	1010	1080	1020	1043	990	993	876	674
3	996	1086	1020	1013	953	997	888	686
4	996	1094	1055	1003	983	1017	958	750
5	1004	1136	1015	1043	1013	1037	968	892
6	1020	1108	970	1000	997	1030	986	706
7	958	1008	940	977	963	960	970	732
8	978	1114	960	937	953	967	974	746
9	978	1128	985	983	960	993	958	882
10	982	1114	1000	1040	990	1047	958	804
11	956	1102	945	1050	963	920	966	744
12	1004	1138	960	1030	963	967	938	812
13	928	1060	975	983	907	947	906	794
Mean 1 to 13	987	1097	987	1009	969	989	* 940	** 763
♂ + ♀	1042		1000		980		** 852	
R1	1018						923	
R2	985						943	
R3	1013						995	
R4	1023						1020	
R5	980						1028	
R6	940						940	
Mean R1 to R6 ♂ + ♀	993						975	

* P <0.05; ** P <0.01
\# Mean of two animals
R Recovery

Ophthalmology: no treatment-related changes
ECG: no treatment-related changes
Hematology and blood biochemistry: no treatment-related changes
Hormones: Serum **prolactin** levels were elevated in <u>all</u> treated groups compared to controls at 4 hours after dosing on Day 1 and, to a greater degree, in Week 13. Week 13 prolactin levels were elevated in all treated groups 4 hours post-dose with mean values at of 20.0, 34.5 and 40.5 ng/mL, at doses of 2, 10 and 50 mg/kg/day respectively, vs. control mean of 1.5 ng/mL; at 24 h post dosing, prolactin tended to be comparable to controls. These changes had no histological correlates and disappeared after a 6-week recovery period.

Prolactin (ng/ml) - group median values

Males

Dosage mg/kg/day	Hours after dosing	
	4	24
Week 1		
Control	0.50	3.00
2	(12.50)	(2.75)
10	* 13.00	3.00
50	** 14.00	4.00
Week 13		
Control	1.50	9.00
2	(17.50)	(4.75)
10	* 31.00	1.50
50	** 24.00	** 1.50

* P <0.05; ** P <0.01 (Distribution free Williams' test)
( ) Median of two animals
Non-parametric analysis performed due to large number of values below the level of detection, therefore table of medians presented

Females

Dosage mg/kg/day	Hours after dosing	
	4	24
Week 1		
Control	2.00	7.00
2	13.00	6.00
10	* 26.00	0.50
50	** 39.00	14.00
Week 13		
Control	1.50	8.00
2	20.00	5.00
10	* 39.00	1.50
50	** 60.00	4.00

* P <0.05; ** P <0.01 (Distribution free Williams' test)
Non-parametric analysis performed due to large number of values below the level of detection and one value greater than 200, therefore table of medians presented

(Prolactin (ng/ml) - continued)

Males and females combined

Dosage mg/kg/day	Hours after dosing#	
	4	24
Week 1		
Control	2.00	6.00
2	13.00 *	5.00
10	18.50 **	1.75
50	24.50 **	6.00
Week 13		
Control	1.50	8.50
2	20.00	5.00
10	34.50 **	1.50
50	40.50 **	1.50 **
Recovery Week 6		
Control	0.75	1.50
50	3.00	3.00

* P <0.05; ** P <0.01 (Distribution free Williams' test)
\# Or equivalent times during recovery period
Non-parametric analysis performed due to large number of values below the level of detection and one value greater than 200, therefore table of medians presented

Urinalysis: no treatment-related changes
Gross pathology: no treatment-related changes
Organ weights: no treatment-related changes
Histopathology: Focal myocarditis was found in 1 LD male animal; however, a re-evaluation of histopathology slides of the heart (study 3702) reaffirmed that focal myocarditis reported in 1 male of the low-dose group was not associated with lurasidone treatment.

Summary: The study results are summarized in the following sponsor's table:

Species/ Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. Per Group	NOAEL (mg/kg)	Noteworthy Findings	Study No.
Monkey/ Cynomolgus	Oral intubation (suspension in 0.5% aqueous methylcellulose)	13 weeks	0, 2, 10 and 50	3/sex/Gp Main 2/sex/Gp Recovery (control and high dose)	2 mg/kg/day	One male (2 mg/kg/day) killed for humane reasons unrelated to treatment. Subdued behavior seen at 10 mg/kg/day, but mostly at 50 mg/kg/day. Tremors seen in most animals at 50 mg/kg/day during Weeks 1 to 11. Late-onset salivation was observed at 50 mg/kg/day. Body weight and food consumption were decreased at 50 mg/kg/day. Serum prolactin levels were elevated in all treated groups compared to controls at 4 hours after dosing on Day 1 and, to a greater degree, in Week 13. Week 13 prolactin levels tended to be comparable to controls at 24 hours after dosing but were elevated in all treated groups 4 hours post-dose with mean values at of 20.0, 34.5 and 40.5 ng/mL, at doses of 2, 10 and 50 mg/kg/day respectively, relative to a control mean of 1.5 ng/mL. All above findings (50 mg/kg/day) were reversible during 6-week recovery period and without histological correlate.	SUP22

Toxicokinetics:
Measurements of serum lurasidone levels showed that systemic exposure levels were dose-dependent with mean AUC0-t values of 57.6 and 73.0 ng.hr/ml (males and females, respectively) at a dose of 10 mg/kg/day and 401.1 and 959.5 ng.hr/ml (males and females, respectively) at a dose of 50 mg/kg/day (Week 13). The AUC0-t values at 2 mg/kg/day could not be calculated. The mean Cmax serum concentration was 4.7 and < 2.0 ng/ml (males and females, respectively at Week 13) at a dose of 2 mg/kg/day, a dose that elevated serum prolactin levels.

Toxicokinetic Parameters of Lurasidone on Days 1, 14 and after 13 Weeks of Oral Gavage Dosing in Monkeys (Study SUP22)

Dose Levels (mg/kg/day)	C_{max} (ng/mL) Males			C_{max} (ng/mL) Females		
	Day 1	Day 14	Week 13	Day 1	Day 14	Week 13
2	5.7	7.4	4.7	3.1	3.4	<2.0
10	13.1	13.5	14.8	12.7	18.1	19.1
50	29.6	59.4	49.3	29.9	49.2	72.0
Dose Levels (mg/kg/day)	**AUC_{0-t} (ng·hr/mL) Males**			**AUC_{0-t} (ng·hr/mL) Females**		
	Day 1	Day 14	Week 13	Day 1	Day 14	Week 13
2	NC	NC	NC	NC	NC	NC
10	51.6	86.1	57.6	46.7	59.5	73.0
50	285.8	557.4	401.1	370.4	380.6	959.5

Group mean data provided from groups of 3 or 5/sex/group.
NC = not calculated

Stability and Homogeneity: Lurasidone 0.1 to 200 mg/ml (0.5% MC suspension) was stable for 24 h at room temperature or for 14 days in refrigerator in the dark.

Conclusion: The NOAEL in this study is 2 mg/kg/day, because the next higher tested dose of 10 mg/kg/day caused subdued behavior, while 2 mg/kg/day yielded no toxic findings associated with the elevation of prolactin levels. The mean Cmax serum concentration at this dose after 13 wks of dosing was 4.7 and < 2.0 ng/ml in M and F, respectively; the AUC_{0-t} values could not be calculated. This is a much lower exposure than that in humans at the MRHD of 120 mg/day (Cmax humans: 165 ng/ml)

Study title: **SM-13496 Toxicity to Cynomolgus Monkeys by Repeated Oral Administration for 52 Weeks** Study no.: **SMO550**

Conducting laboratory and location: (b) (4)

Date of study initiation: December 17, 1996

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SM-13496, Lot # 3CG001M, 100.9%

Key Study Findings

Lurasidone oral administration (as suspension in 0.5% aqueous methylcellulose) to Cynomolgus monkeys (4/sex/group) by oral intubation at doses of 0 (vehicle) **2, 10 and 50 mg/kg/day** for 1 year (52 weeks) did not cause drug-related mortality. Clinical signs attributable to the pharmacological effects of lurasidone, i.e., subdued behavior, quietness, slow/unsteady movement, inactivity and/or hunched posture, salivation (at all dose levels) and tremors (at MD and HD) were noted from the first week of the study with a dose-related incidence. A reduction in food consumption, possibly related to treatment-related subdued behavior, particularly in the HD group, was not of sufficient severity to affect body weight gain.
Serum prolactin was increased dose-dependently in all treated groups in weeks 13, 26, 39 and 52 at 4 hours after dosing, but was highly variable at 24 hours after dosing without a clear trend. Increase in serum prolactin is a known effect of the dopamine antagonism associated with neuroleptic agents. No significant drug-related changes were found in ophthalmoscopy, electroretinography, ECG, hematology, blood biochemistry, urinalysis, bone marrow smears, organ weights and gross pathology examinations. Histopathology changes in the pituitary (enlarged pale staining cells in pars distalis) were observed in both genders at HD. Myocardial inflammatory infiltrates were found at MD and HD (in 2 of 8 animals in each group). A re-evaluation of histopathology slides of the heart (study 3703) reaffirmed the original diagnosis of myocardial inflammatory infiltrates in 2 of 8 animals in the mid-dose group but decreased the findings in the high-dose group from 2 animals to 1. It is likely that these changes were spontaneous rather than drug-related. Serum concentration of lurasidone and its metabolite, ID-14283 (not a major metabolite in humans), showed that their systemic exposure levels were dose-dependent. Peak serum concentrations of lurasidone were generally achieved between 1 and 6 hours after administration. Serum exposure parameters in males increased over the length of the study, compared to Day 1, indicating accumulation. A NOAEL was not reached in this study (< 2 mg/kg/day) due to presence of adverse neurological clinical signs and prolactin increase at all tested dose levels, including the LD of 2 mg/kg/day. This study did not achieve sufficient systemic exposure levels to adequately assess lurasidone safety for humans. Even at the highest tested dose of 50 mg/kg/day, lurasidone systemic exposure values in the monkey after 52 weeks of treatment (Cmax: 86 and 90 ng/ml; AUC: 625 and 558 ng.h/ml for M and F, respectively) were lower than or, at best, similar to the corresponding human exposure values at the MRHD of 120 mg/day (Cmax=164.7 ng/ml and $AUC_{0\text{-}inf}$ = 686.6 ng.h/ml).

METHODS

DOSES:	2, 10 AND 50 MG/KG/DAY
ROUTE OF ADMINISTRATION:	ORAL INTUBATION

DOSE VOLUME:	3 ML/KG
FORMULATION/VEHICLE:	SUSPENSION IN 0.5% AQUEOUS METHYLCELLULOSE
SPECIES/STRAIN:	MONKEY/CYNOMOLGUS
NUMBER/SEX/GROUP:	4
AGE:	37 TO 41 MONTHS (INITIAL)
WEIGHT:	2.1 – 4.1 KG
SATELLITE GROUPS:	NO
UNIQUE STUDY DESIGN:	NONE
DEVIATION FROM STUDY PROTOCOL:	DID NOT AFFECT SCIENTIFIC INTEGRITY OF THE STUDY

Observations

Clinical signs: Daily, throughout the day ; Body weight: Weekly; Food consumption: Daily

Ophthalmoscopy:
Pre-dose
Week 13
Week 26
Week 52

Electroretinography:
Pre-dose
Week 24/25
Week 50/51

Electrocardiography: (as reproduced from the study report)

Electrocardiograms (ECGs) were recorded before dosing commenced and again during Weeks 13, 26, 39 and 52. During the dosing period, recordings were made prior to administration of the daily dose.

Recordings were made on a multi-channel electrocardiograph and consisted of standard limb leads, I II and III and augmented limb leads aVR, aVL and aVF. A standardisation of 10 mm = 1 mV was used, and a paper speed of 25 mm/second. An additional recording of Lead II was taken at 50 mm/second.

The recordings were examined visually for any abnormalities of the electrical complexes and heart rate was calculated.

Hematology and biochemistry (routine)
Pre-dose
Week 13
Week 26
Week 39
Week 52

In addition, in the same weeks as other laboratory investigations, serum prolactin was assessed. Samples of blood (1 ml into tubes containing no anticoagulant) were obtained by femoral venipuncture at times corresponding to predose (24 hours after dosing) and 4 hours after dosing.

Urinanalysis (routine)
Pre-dose
Week 13
Week 26
Week 39
Week 52

Bone marrow examination: A bone marrow sample was obtained from all animals prior to necropsy; the smears were stained using a modified Wright's stain and examined.

Organ weights:

adrenals	ovaries	spleen
brain	pancreas	testes (with epididymides)
heart	pituitary	thymus
kidneys	prostate (with seminal vesicles)	thyroids (with parathyroids)
liver	salivary glands (submandibular)	uterus
lungs		

Histopathology: The following tissues were examined by light microscopy:

adrenals	lungs (with bronchi)	spinal cord (cervical, thoracic and lumbar regions)
alimentary tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum)	lymph nodes (cervical and mesenteric)	spleen
aorta (arch and abdominal)	mammary gland	sternum
brain (cerebrum, thalamic nuclei, midbrain, medulla and cerebellum)	ovaries	testes (with epididymides)
eyes (with optic nerve)	pancreas	thymus
femur (with joint)	pharynx	thyroids (and parathyroids)
gall bladder	pituitary	tongue
heart	prostate	trachea
kidneys	salivary glands (submandibular and parotid)	urinary bladder
lachrymal gland	sciatic nerve	uterus
larynx	seminal vesicles	vagina
liver	skeletal muscle (quadriceps femoris)	
	skin	
	spinal column (lumbar vertebrae*)	

* slides prepared, but not examined

TK: Blood samples for determination of SM-13496 and metabolite ID-14283 were drawn at 1, 2, 4, 6 and 24 h. post-dose on the following days:

Day 1
Day 30
Week 13
Week 26
Week 52

Results

Mortality: There were no unscheduled deaths during the study.

Clinical signs: Clinical manifestations were noted from the first week of the study with a dose-related incidence. Clinical signs attributable to the pharmacological effects of lurasidone, i.e., subdued behavior, quietness, slow/unsteady movement, inactivity and/or hunched posture, salivation, occurred at all dose levels; tremors were observed at MD (isolated occurrences) and mainly at HD starting from the 1st week, 2 to 3 h after dosing, accompanying subdued behavior. Fixed posture (no movement seen for several minutes), often accompanied by unusual postures (dystonic behavior, expressed as contortions of the extremities resulting in unusual and sustained posturing, particularly of the limbs) were noted with a dose-related incidence, i.e., while at LD seen only in a single monkey from Week 27, at HD they were noted from the 1st week, 3 to 5 h after dosing, and occasionally persisted overnight.

Food consumption: A reduction in food consumption was seen at HD, possibly as a result of treatment-related subdued behavior.

Body weight: There were no significant changes in body weight or weight gain.

Ophthalmology, electroretinography: no drug-related changes

ECG: no drug-related changes

Hematology: no drug-related changes

Blood biochemistry: Mean serum prolactin concentrations were increased dose-dependently in all treated groups at all time points (Weeks 13, 26, 39 and 52), at 4 hours after dosing, but were highly variable at 24 hours after dosing and did not present a clear trend. The transient post-dose increase in serum prolactin was attributable to the drug effect of dopamine antagonism.

Urinalysis: no drug-related changes

Bone marrow smears: no drug-related changes

Organ weights: no drug-related changes

Gross pathology: no drug-related changes

Histopathology: Enlarged pale staining cells in pituitary pars distalis were observed in both genders at HD (3/4 M and 3/4 F). Myocardial inflammatory infiltrates were found in 2 of 8 animals in the mid-dose group and in 2 of 8 animals in the high dose group. A re-evaluation of histopathology slides of the heart from the 52-week study (study 3703) reaffirmed the original diagnosis of myocardial inflammatory infiltrate in 2 of 8 animals in the mid-dose group but decreased the findings in the high-dose group from 2 animals to 1. The severity of these changes was "minimal". The sponsor contends that "these are spontaneous lesions without dose-related occurrence and unlikely to be associated with lurasidone treatment." In previous communications (Amendment 007 of 10/25/2001, reviewed by Dr. Lois Freed on 8/13/2002 under IND 61292), the sponsor concluded that the finding was incidental due to the "similarity to previously reported spontaneous lesions, no dose-related occurrence and individual difference in the location of lesions" and cited spontaneous rates of myocardial inflammatory infiltrates in wild-caught Cynomolgus monkeys up to 44% in males and 41% in females [Ito et al, Exp Anim 41(4):455-469, 1992 and Shimoi et al, J Toxicol Pathol 11 :85-94, 1998]. Dr. Freed's comments on this issue were as follows:

> "The sponsor's re-analysis of the heart sections from the l-yr monkey study indicates that the incidence of myocardial inflammatory infiltrates are not dose-related, are "slight" in severity, and are not associated with other signs of toxicity (e.g., degeneration). [Only 1 LD animal was affected in the 13-wk study.] The published reports provided by the sponsor as an estimate of spontaneous incidence are of limited usefulness since the incidences of focal myocarditis or mononuclear cell infiltration were based on data from control and drug-treated animals. However, the fairly high incidences reported would suggest that these may, to some extent, be spontaneous findings in wild-bred monkey. In addition, examination of control data in a study using cynomolgus monkeys (3/sex/grp; (b) (4) indicated frequencies of 30-60% for mononuclear cell infitration. Therefore, it is not unreasonable to consider these spontaneous findings in the 1-yr monkey study". *(End citation)*

This reviewer agrees with Dr. Freed's conclusion.

The study results are summarized in the following sponsor's table:

Study Title: SM-13496 Toxicity to Cynomolgus Monkeys by Repeated Oral Administration for 52 Weeks							Test Article: lurasidone (SM-13496)	
Species/Strain: Monkey/ Cynomolgus		Duration of Dosing: 12 months					Study No. SMO550	
Daily Dose (mg/kg)	**0 (Control)**		**2**		**10**		**50**	
No. of Animals	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Noteworthy Findings:								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body Weight (%)								
Week 0	3186 g	2673 g	-6	-1	-3	+6	-4	0
Week 3	3115 g	2628 g	-4	+1	-2	+8	-2	0
Week 52	4227 g	3149 g	0	+2	-3	+6	+1	-8
Food Consumption (%)								
Weeks -4 to -1	955 g/week	798 g/week	-5	+6	+4	+9	+11	+20
Weeks 1 to 3	730 g/week	487 g/week	-7	+47	-9	+19	-27	+4
Weeks 4 to 52	1103 g/week	1058 g/week	+8	+10	+8	+5	+4	-14
Clinical Observations (No. of animals)								
Subdued behavior[a]	1	2	4	4	4	4	4	4
Fixed/unusual postures	0	0	1	0	2	3	4	4
Tremors	0	0	0	0	3	2	4	4
Salivation[b]	0	0	0	0	0	0	2	2
Ophthalmoscopy	--	--	--	--	--	--	--	--
Electrocardiography	--	--	--	--	--	--	--	--
Hematology	--	--	--	--	--	--	--	--
Serum Prolactin (ng/mL)								
Week 13 (4, 24hr)	4.6, 3.9	6.1, 8.6	9.9*, 4.3	14.7, 3.3*	32.1**, 2.4	48.4**, 3.5*	34.2**, <1.0	45.2**, 3.1*
Week 26 (4, 24hr)	<1.0, 1.5	2.6, 7.1	8.9, 1.7	7.5, 2.1	25.0*, <1.0	37.5*, 1.5	31.0**, <1.0	43.3*, 2.9
Week 39 (4, 24hr)	1.7, 1.6	2.2, 2.4	6.0, 2.1	6.2, 1.8	9.1, <1.0	25.7*, 1.6	36.8**, <1.0	32.8*, 1.5
Week 52 (4, 24hr)	7.0, 7.9	9.4, 3.6	22.5, 4.8	21.8, 9.2	33.2**, 4.2	33.5, 3.2	58.3**, 2.5*	94.8**, 14.8*
Serum Chemistry	--	--	--	--	--	--	--	--
Urinalysis	--	--	--	--	--	--	--	--
Organ Weights (%)	--	--	--	--	--	--	--	--
Gross Pathology	--	--	--	--	--	--	--	--
Histopathology (No. of animals)								
No. Examined	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Enlarged pale-stained cells in *pars distalis* of pituitary	0	0	0	0	0	0	3	3
Additional Examinations								
Bone marrow smears	--	--	--	--	--	--	--	--

a Incidence of subdued behavior (quietness, slow/unsteady movement, inactive/reluctant to move, huddled/hunched posture).
b Incidence of late post-dose salivation (typically noted 1.5 to 6 hours after dosing).
*P < .05; **P < .01 (Williams test); N.E. Not examined; -- No abnormality

Toxicokinetics

TK parameters were obtained for the parent drug (lurasidone) and for metabolite ID-14283 on Days 1 and 30 and during Weeks 13, 26 and 52 of the study. ID-14283 is not a major metabolite in humans. The following sponsor's table shows lurasidone TK parameters on Day 1 and 30 and Weeks 13, 26 and 52.

Serum concentrations of lurasidone increased with dose increments. Peak serum concentrations were variable and generally achieved between 1 and 6 hours after administration. The mean Cmax and AUC0-24hr values increased dose-dependently. Lurasidone peak serum levels and 24-h exposure levels were

generally lower in females than in males (but not statistically significant). Systemic exposure generally increased over the length of the study compared to values on Day 1, indicating accumulation. The mean TK data for metabolite ID-14283 are shown in the following sponsor's table. ID-14283 systemic exposure levels were dose-dependent, although values were lower than dose-proportional.

TK Parameters of Lurasidone and metabolite ID-14283 Following 1 and 30 Days, and 13, 26 and 52 Weeks of Oral Dosing in Monkeys (Study SMO550)

Daily Dose (mg/kg)	0 (Control)		2		10		50	
No. of Animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Toxicokinetics: mean Cmax (ng/mL)								
Day 1								
Lurasidone (parent)	N.E.	N.E.	2.16	4.19	37.51	11.48	43.11	26.52
ID-14283 (metabolite)	N.E.	N.E.	2.70	3.37	14.08	8.37	20.65	16.72
Day 30								
Lurasidone (parent)	N.E.	N.E.	6.86	1.79	22.19	9.51	57.70	39.96
ID-14283 (metabolite)	N.E.	N.E.	4.41	2.46	15.79	8.78	32.89	24.28
Week 13								
Lurasidone (parent)	N.E.	N.E.	3.96	1.60	21.38	13.09	48.87	49.10
ID-14283 (metabolite)	N.E.	N.E.	3.73	2.35	13.27	11.32	22.83	24.64
Week 26								
Lurasidone (parent)	N.E.	N.E.	6.38	2.54	20.66	19.60	72.46	85.39
ID-14283 (metabolite)	N.E.	N.E.	4.34	3.70	10.83	12.62	27.28	39.34
Week 52								
Lurasidone (parent)	N.E.	N.E.	5.83	2.77	9.52	22.14	85.84	89.70
ID-14283 (metabolite)	N.E.	N.E.	4.15	2.57	7.89	9.62	34.61	43.68
Toxicokinetics: mean $AUC_{0\text{-}24hr}$ (ng·hr/mL)								
Day 1								
Lurasidone (parent)	N.E.	N.E.	12.8	17.2	143.2	89.1	504.4	282.6
ID-14283 (metabolite)	N.E.	N.E.	35.4	38.5	94.9	56.7	239.2	170.1
Day 30								
Lurasidone (parent)	N.E.	N.E.	24.7	8.0	175.4	90.3	592.5	431.8
ID-14283 (metabolite)	N.E.	N.E.	26.2	20.2	149.2	85.0	382.7	305.0
Week 13								
Lurasidone (parent)	N.E.	N.E.	12.5	6.7	167.4	105.8	619.7	560.9
ID-14283 (metabolite)	N.E.	N.E.	16.7	11.3	125.8	93.8	309.0	312.8
Week 26								
Lurasidone (parent)	N.E.	N.E.	15.1	10.7	196.8	180.5	710.3	927.1
ID-14283 (metabolite)	N.E.	N.E.	18.5	17.4	123.7	123.0	315.1	527.3
Week 52								
Lurasidone (parent)	N.E.	N.E.	23.8	13.7	110.1	154.4	625.5	558.1
ID-14283 (metabolite)	N.E.	N.E.	21.3	14.8	84.4	84.4	322.2	360.1

Even at the highest tested dose of 50 mg/kg/day, lurasidone systemic exposure values in the monkey after 52 weeks of treatment (Cmax: 86 and 90 ng/ml; AUC: 625 and 558 ng.h/ml for M and F, respectively) were lower than the corresponding human exposure values at the MRHD of 120 mg/day (Cmax=164.7 ng/ml and $AUC_{0\text{-}inf}$ = 686.6 ng.h/ml). Human pharmacokinetic data for lurasidone and its metabolites upon multiple oral administration of lurasidone are reproduced in the following sponsor's tables.

Pharmacoklnetic parameters of healthy male Caucasian with multiple oral doses of SM-13496
(Study D1050002)

	80 mg once-daily administration		
	Day 1 (n=5)	Day 5 (n=4)	Day 9 (n=1)
SM-13496			
T_{max} [hr]	1.40 ± 0.418	-	2.0
C_{max} [ng/ml]	110 ± 35.3	106 ± 59.0[a]	168
C_{min} [ng/ml]	2.16 ± 1.05	3.97 ± 2.59	9.24
$AUC_{0-\infty}$ [ng·hr/ml]	451 ± 155	-	-
AUC_{0-t} [ng·hr/ml]	381 ± 127	-	677
$t_{1/2}$ [hr]	21.0 ± 6.81	-	37.0
ID-14283			
T_{max} [hr]	2.10 ±□0.894	-	2.0
C_{max} [ng/ml]	32.9 ± 7.01	24.5 ± 10.5[a]	38.7
C_{min} [ng/ml]	0.688 ± 0.176	0.82 ± 0.505	1.92
$AUC_{0-\infty}$ [ng·hr/ml]	177 ± 52.7	-	-
AUC_{0-t} [ng·hr/ml]	165 ± 50.8	-	217
$t_{1/2}$ [hr]	11.6 ± 3.37	-	20.7
ID-14326			
T_{max} [hr]	3.10 ± 1.02	-	3.0
C_{max} [ng/ml]	1.28 ± 0.227	0.79 ± 0.212[a]	1.13
C_{min} [ng/ml]	0.0880 ± 0.0268	0.11 ± 0.053	0.140
$AUC_{0-\infty}$ [ng·hr/ml]	11.8 ± 2.34	-	-
AUC_{0-t} [ng·hr/ml]	10.3 ± 2.19	-	10.9
$t_{1/2}$ [hr]	12.9 ± 3.47	-	12.2
ID-11614			
T_{max} [hr]	1.70 ± 0.447	-	2.0
C_{max} [ng/ml]	3.08 ± 0.611	2.38 ± 0.664[a]	3.69
C_{min} [ng/ml]	0.140 ± 0.0283	0.13 ± 0.060	0.230
$AUC_{0-\infty}$ [ng·hr/ml]	19.0 ± 3.47	-	-
AUC_{0-t} [ng·hr/ml]	17.3 ± 3.22	-	21.3
$t_{1/2}$ [hr]	8.08 ± 0.972	-	-

PK parameters in patients with multiple oral dose administration*
(A MTD study of SM- 13496 in patients with schizophrenia, 2002 , (b) (4) Prot. No. DI050160-P03)

	SM-13496 AUC (0-inf) (ng h/mL)					
	120 mg		140 mg		160 mg	
	Mean	Range	Mean	Range	Mean	Range
Period I[1] Assay results	481.3	260-1057	728.6	471-1450	902.4	424-1614
Period I DN	4.011	2.171-8.808	5.204	3.366-10.35	5.640	2.649-10.09
Period II[2] Assay results	686.6	419-1685	793.3	454-1188	899.3	518-1418
Period II DN	5.72	3.489-14.04	5.666	3.243-8.487	5.621	3.235-8.862

	SM-13496 C_{max} (ng/mL)					
	120 mg		140 mg		160 mg	
	Mean	Range	Mean	Range	Mean	Range
Period I[1] Assay Results	125.3	45.2–208	170.4	102–246	196.5	72.9–470
Period I DN	1.044	0.376–1.736	1.217	0.730–1.756	1.228	0.455–2.935
Period II[2] Assay Results	164.7	75.0–357	194.0	119–235	233.4	156–411
Period II DN	1.372	0.625–2.978	1.386	0.853–1.682	1.459	0.977–2.569

DN: Dose-normalized
1 Period I serial PK samples were collected over the 72 hours beginning on Day 1
2 Period II serial PK samples were collected over 24 hours beginning on Day 8
* (8 subjects at each dose level, repeat-dose administration on days 1 and 4 through 8)

Stability and Homogeneity: Lurasidone 0.1 – 200 mg/ml (0.5% MC suspension) stable for 24 h at room temperature or for 14 days in refrigerator in the dark.

Comments:

Issues of toxicology and safety concern in the 1-year monkey study were the findings of myocardial infiltrates in the mid- and high-dose groups, and the insufficient systemic exposure levels achieved for lurasidone in the monkey serum that, even at the highest tested dose level, did not reach the levels of human exposure at MRHD.
These issues were addressed in the Memorandum of Dr. Lois Freed to IND 61292 of 8/13/2002 [Re: sponsor's submissions, Amendments N-007 (10/25/01), N-011 (5/7/02), N-015 (7/11/02)].
Relevant parts of this document are cited below:

> "The sponsor was limited to clinical trials of 6 wks in duration due to a finding of myocardial infiltrate in the 1-yr monkey study. In addition, in the Agency letter dated 2/7/01, it was noted that serum drug concentrations achieved in the l-yr (52-wk) monkey study did not reach the levels anticipated in humans. The sponsor responded to the issues in Amendments N-007, N-011, and N-015.
> Amendment N-007
> In the Agency's letter (2/7/01), the sponsor was informed that:
> **"Clinical Comment #2:** The myocardial infiltrate in the 1-year monkey study is a finding that is not easily monitored and needs further investigation before human studies proceed beyond this 6-week time frame. Serum concentrations of SM-13496 in the 1-year monkey study did not reach the levels to which humans are exposed. These (sic) studies are unfortunately of little value unless much lower doses than those currently proposed are to be used in humans.
> Monkey: In Amendment N-007, the sponsor stated that the monkey was selected as a nonclinical species because metabolism of SM- 13496 is similar to human. The HD (50 mg/kg) used in the 1-yr study was considered to be an MTD based on clinical signs (subdued behavior). According to the sponsor, doses > 50 mg/kg "prevents the monkeys from maintaining adequate self-care". At Wk 52, Cmax and AUC for SM-13496 were 86 ng/mL and 626 ng hr/mL, respectively. The Cmax and AUC in humans following a 6-wk dosing period at 120 mg/day were 173 ng/ml and 663 ng.hr/mL. The lower Cmax in monkeys was attributed to the later T max in that species compared to humans (i.e., 4-5 hrs vs. 1.5 hrs in humans).
> Regarding the myocardial infiltrates detected in the l-yr study, the sponsor noted that these findings "were of minimal extent even after one year of treatment...and were unaccompanied by related clinical findings..." The sponsor considered the finding incidental, but requested input from the Division as to appropriate follow-up studies.
> Amendment N-011
> The sponsor re-examined heart sections from the 13 + 6-wk recovery and 1-yr cynomolgus monkey studies. These data were provided in this amendment.
> Review of sections of the heart from SM-14396: Toxicity to cynomolgus monkeys by repeated oral administration for 52 weeks (Study No. 3703, study completion date: 2/27/02, conducting laboratory: sponsor, Japan)
> The data were summarized in the following sponsor's Text Table (severity graded: within normal limits, slight, mild, moderate, severe):

Text table. Summary of findings

Group	Animal No./ Sex	Focal cell infiltration			Myocardial inflammatory infiltrate a)
		Infiltrated cells	Location	Severity	
1	606 / M	Lymphocytes, Histiocytes	Left ventricle	Very slight	Not detected
3	622 / F	Lymphocytes, Plasma cells, Histiocytes	Left ventricular papillary muscle	Slight	Minimal
3	623 / M	Lymphocytes, Plasma cells	Left auricle	Slight	Minimal
3	624 / F	Lymphocytes	Left ventricular papillary muscle	Very slight	Not detected
4	628 / F	Lymphocytes, Plasma cells, Histiocytes	Left ventricular papillary muscle	Slight	Minimal
4	630 / F	Not detected	Not detected	Not detected	Minimal
4	632 / F	Lymphocytes	Epicardium, periartery	Slight	Not detected
		Lymphocytes	Left ventricular papillary muscle	Very slight	

a) Original diagnosis (Ref. 2)

The sponsor noted that the results of the re-examination differed from the original report in that "myocardial inflammatory infiltrate" was detected in 0/8, 0/8, 2/8, and 1/8 C, LD, MD, and HD animals, respectively. (These incidences presume that "very slight" = "within normal limits".) In the original report, 2 HD animals were reported to be affected. The sponsor concluded that the finding was incidental due to the "similarity to previously reported spontaneous lesions, no dose-related occurrence and the individual difference in the location of lesion [sic]". The sponsor cited the spontaneous rates reported by Ito et al (1992) and Shimoi et al (1998). Copies of these published articles were requested by the Division, and provided by the sponsor in Amendment N-015.

Amendment N-015

Ito T et al. ExpAnim 41(4):455-469, 1992: Ito et al. (1992) reported on spontaneous histopathology findings in wild-caught cynomolgus monkeys (22I/sex) used in toxicology studies. These studies were performed by (b) (4). during the years 1979- 1990. Mononuclear cell infiltration was detected in 97/221 (44%) males and in 91/221 (44%) females. However, it appears that the frequencies reported for "spontaneous" lesions were based on data from both dosed and control monkeys. The authors determined that certain histopathology findings were drug-related (these were listed in Table 1 of the publication), and that "Findings other than the lesions shown in Table I, which were considered to be treatment-related, were assumed to be spontaneous". According to Table 1, "mononuclear cell infitration" in the heart was considered a spontaneous finding.

Shimoi A et al. J Toxicol Pathol 11:85-94, 1998: Shimoi et al. (1998) compared spontaneous lesions in wild-caught cynomolgus monkeys (89 M, 72 F) and laboratory-bred rhesus monkeys (78 M, 88 F) used in toxicology studies performed by (b) (4) No description was given as to the treatment of these monkeys in the studies, i.e., control or drug-treated. The study years were not specified. The incidences of focal myocarditis" were as follows: (a) 18/89 (20%) and 17/72 (24%) in male and female cynomolgus monkeys, respectively and (b) 18/78 (24%) and 15/88 (18%) in male and female rhesus monkeys, respectively. The severity of the focal myocarditis was specified as "slight".

Reviewer *(Dr Freed's)* comments

The sponsor's re-analysis of the heart sections from the l-yr monkey study indicates that the incidence of myocardial inflammatory infiltrates are not dose-related are "slight" in severity, and are not associated with other signs of toxicity [e.g., degeneration]. [Only 1 LD animal was affected in the 13-wk study.] The published reports provided by the sponsor as an estimate of spontaneous incidence are of limited usefulness since the incidences of focal myocarditis or mononuclear cell infiltration were based on data from control and drug-treated animals. However, the fairly high incidences reported would suggest that these may, to some extent, be spontaneous findings in wild-bred monkey. In addition, examination of control data in a study using cynomolgus monkeys (3/sex/grp; (b) (4) indicated frequencies of 30-60% for

mononuclear cell infitration. Therefore, it is not unreasonable to consider these spontaneous findings in the 1-yr monkey study.

Regarding the lack of a safety margin for exposure (as noted in Clinical Comment #2), the sponsor noted that the Cmax and AUC at the high-dose in the 52-wk monkey are "0.5 and 1 times the Cmax and AUC, respectively, in humans at 120 mg/day. According to the sponsor, an MTD was achieved in the 52-wk study based on clinical signs. According to the study report, body wt was not significantly affected in either males or females, although final body wt in HD females was slightly lower (8%) compared to CF. It was noted that body wt loss was noted in all grps, including controls, during the first 3 wks of the study, but that some HD animals were particularly affected. However, when access to food was extended, "the bodyweight performance of the majority of animals improved". Food consumption was more persistently affected in some HD animals, an effect thought to be due to "treatment-related subdued behavior". However, since body wt was not affected, the effect on food consumption was not considered to be "of toxicological importance". There were no drug-related effects on clinical pathology, ECG, ophthalmology, gross pathology, or organ wts. Upon microscopic examination, the only drug-related finding was "Minimal enlarged pale staining cells in pars distalis" in 3/4 M and 3/4 F at the HD.

…….

From the findings observed, it is not entirely clear that higher doses could not have been used in the l-yr study. Although the clinical signs were notable, they were not sufficient to adversely affect food consumption or body wt [transient decreases were observed in all grps, including C].

The sponsor indicated that the monkey was selected as the nonrodent toxicity species since metabolism in monkey and human are similar; however, no data were provided to support this position. Considering the lack of clear dose-limiting toxicity, additional data should be provided to support the position that higher exposures cannot be tolerated in monkeys and, if not, why monkey is a better animal model for human than, for example, dog. In vitro metabolism data would suggest that SM-13496 may be metabolized via CYP2D6 and/or CYP3/4 in humans.

Therefore, there is a possibility that plasma exposures to parent and metabolites may vary over a considerable range when SM-13496 is administered in vivo. This possibility needs to be taken into account when assessing safety margins.

The l-yr monkey study was adequately conducted (except for the apparent lack of sufficiently high doses) and provides some data on the potential toxicity of SM 13496 at plasma exposures (of SM 13496) similar to the highest proposed clinical dose. Therefore, there is no objection to the sponsor initiating studies or extending ongoing studies beyond 6 wks in duration. However, the sponsor should be asked to provide additional data to document the adequacy of the l-yr monkey study to support long-term clinical trials.

(Note: Paragraphs underlined by this reviewer (S.T.)

Recommendation: there is no objection to the sponsor conducting trials of greater than 6 wks in duration. However, the sponsor should provide the following in a timely manner:

(a) data in monkey to document that doses higher than 50 mg/kg (the high-dose in the l-yr study) could not have been achieved. The clinical signs observed in the l-yr study were not clearly dose-limiting since body wt and food consumption were not affected over the course of the study.

(b) if it is determined that higher doses can be achieved in monkey, a chronic (39-wk) toxicity study in monkey using higher doses should be initiated as soon as possible.

(c) if higher doses and, therefore, higher plasma exposures cannot be achieved in monkey, please provide data to document that higher plasma exposures cannot be achieved in another nonrodent species (e.g., dog) or that monkey is the most appropriate nonrodent model for assessment of human risk."

(End citation)

The present reviewer agrees with Dr. Freed's conclusion that, based on the provided additional information from the sponsor, it is not unreasonable to consider the myocardial inflammatory infiltrates seen in dosed groups as spontaneous findings in the 1-yr monkey study, and that the 1-year monkey study was adequately conducted (except for the apparent lack of sufficiently high doses) and provides some data on the potential toxicity of SM 13496 at plasma exposures (of SM 13496) similar to the highest proposed clinical dose. Complying with Dr. Freed's recommendations, the sponsor achieved higher plasma exposures in another non-rodent species (dog) in the subsequent general toxicology studies reviewed herewith.

Conclusion:
Lurasidone oral administration to Cynomolgus monkeys at doses of 2, 10, and 50 mg/kg/day for 1 year resulted in treatment-related findings at all dose levels. Some of these were not associated with toxicity, i.e., subdued behavior was associated with the intended pharmacological action of the test substance, and the transient increase in serum prolactin was attributable to the known effect of the dopamine antagonism of neuroleptic agents. There were, however, clinical signs indicative of extrapyramidal syndrome, i.e., tremors at mid- and high dose and abnormal postures (dystonic behavior, expressed as contortions of the extremities resulting in unusual and sustained posturing, particularly of the limbs) noted at all dose levels with a dose-related incidence. Based on this neurobehavioral effect, a NOAEL was not established (below the lowest tested dose of 2 mg/kg/day). However, there was a lack of clear dose-limiting toxicity, since there was no body weight change, or other manifestations of general toxicity. No significant drug-related changes were found in ECG, hematology, blood biochemistry, urinalysis, bone marrow smears, organ weights and pathology examinations. Myocardial inflammatory infiltrates were found at MD and HD (in 2 of 8 animals in each group), but a re-evaluation of histopathology slides of the heart (study 3703) reduced the findings in the high-dose group from 2 animals to 1. Based on this, and on literature data and Division's review experience of high spontaneous incidences of myocardial inflammatory infiltrates in Cynomolgus monkeys, it is likely that these changes were spontaneous rather than drug-related.
The main issue in this study is the insufficient systemic exposure level achieved for lurasidone in the monkey serum that, even at the highest tested dose level, did not reach the levels of human exposure at MRHD of 120 mg/day. As this study did not achieve sufficient systemic exposure levels to adequately assess lurasidone safety for humans, the Division recommended that the sponsor achieve higher plasma exposures in another non-rodent species (dog). The sponsor complied with this recommendation in subsequent general toxicology studies in dogs, reviewed herewith.

6.2.3. Dog Studies

Study title: **Two-week oral toxicity study of SM-13496 in dogs**

Study no.: **L0134**

Conducting laboratory and location: (b) (4)

Date of study initiation: 11/5/93

GLP compliance: Non-GLP

Drug, lot #, and % purity: SM-13496, lot # 3CG001M, purity = 100.9%

Key Study Findings

A 2-week repeated-dose non-GLP study was performed to evaluate oral toxicity of lurasidone administered orally (capsules) to Beagle dogs (2 animals/sex/group) at dose levels of 0, 50, 250 and 1000 mg/kg/day. Clinical signs related to the administration of the test compound included decrease of spontaneous activity, tremors and miosis in all dose groups. Decreased food consumption was noted at all three dose levels. No significant abnormalities were determined by ophthalmology, ECG, hematology or urinalysis. Gross pathology findings of note included: enlarged uterus (2/2 MDF, 1/2 HDF) and mammary gland development (2/2 MDF). Microscopic examination revealed hypertrophy of the uterine endometrium and tunica muscularis found in MD and HD, and mammary gland changes (hyperplasia, secretion, hemorrhage in interstitium, and dilation of duct) in MDF (these findings were not detected in HDF). Since peak serum levels and exposure levels were highly variable, it was decided to administer lurasidone in an aqueous suspension by gavage tube (instead of capsules) in subsequent dog studies.
A NOAEL was not reached (due to clinical signs of tremors, miosis and decreased food intake at all tested dose levels, including the lowest tested dose of 50 mg/kg/day).

The review data below are reproduced from Dr. Lois Freed's review of this study under IND 61292.

Methods: this study was conducted on Beagle dogs (2/sex/group), age 8-9 mo and weight 9.4-11.9 kg (M) and 6.7-12.2 kg (F) at study initiation. SM-13496 was administered orally (gelatin capsules) at doses of **0, 50, 250, and 1000** mg/kg/day for 2 wks. Doses were based on the results of an acute study (report appended to study report) (10, 50, 250, 1000, 2000 mg/kg). In the preliminary study, doses ≥250 mg/kg were associated with tremors, persisting for 6 days post dosing at the HD. Miosis and "slight" decrease in motor activity were observed at the two highest doses. Lower food intake was noted at HD.

Observations: clinical signs, body wt, food consumption, ophthalmology, ECG (Wk 0 and Wk 2; limb lead II; conscious), hematology (Wks 0, 1 and 2; rbc ct, hgb, hct, MCV, MCH, MCHC, reticulocyte ct, wbc (ct, differential), platelet, PT, APTT), clinical chemistry (Wks 0, 1 and 2; total protein, protein electrophoresis, A/G ratio, total cholesterol, TG, PL, urea N, uric acid, creatinine, total bilirubin, direct bilirubin, AST; ALT, LDH, alkaline phosphatase, GGTP, LAP, CPK, ChE, Na, K, CL, Ca, P), urinalysis (Wks 0 and 2; 6.5 hr collections: pH, glucose, protein, blood, ketone bodies, bilirubin, urobilinogen, microscopic analysis of sediment; overnight collections: urine volume, specific gravity, osmotic pressure, GGTP, NAG, ALP, LDH, creatinine, Na, K, CL), terminal studies [gross pathology, organ weights (brain,

pituitary, lungs, liver/gallbladder, spleen, kidneys, testes, prostate, ovaries, uterus); histopathology (brain, pituitary, lungs, liver/gallbladder, spleen, kidneys, testes, prostate, ovaries, uterus, mammary gland, epididymis, vagina; H & E; incomplete battery of tissues)], TK (Days 1 and 14 at 0.5, 1, 2, 6, and 24 hrs post dosing).

Results: There were no unscheduled deaths. Clinical signs were observed at all doses (i.e., decreased spontaneous motor activity, tremor, miosis). Body wt loss was noted in 1/2 MDF and in all HD animals. Food intake was sporadically affected in all dosed groups. No clear drug-related findings were detected during ophthalmology examination or on ECG, hematology, or clinical chemistry parameters. There was, however, a tendency for total cholesterol to increase with duration of dosing in treated females). Of urinalysis parameters, the following were of note: bilirubin was detected in urine of 1/2 HDM and NAG was increased in 1/2 MDM and 2/2 HDM.
Kidney wt (absolute-relative) was increased in 1/2 MDM (75%) and 2/2 HDM (28-29%). Uterus wt (absolute-relative) was increased in 2/2 MDF (3-6 fold; 200-500%) and 1/2 HDF (3-4 fold; 200-300%). Gross pathology findings of note included: (1) large uterus (2/2 MDF, 1/2 HDF); (2) mammary gland development (2/2 MDF), and (3) "sparse white foci" on lung (2/2 MDF).
Upon microscopic examination, the following findings were of note: (1) slight basophilic "uriniferous" tubules in the MDM with increased NAG and kidney wt, (2) hypertrophy of the uterine endometrium and tunica muscularis in 2/2 MDF (mild and moderate severity) and 1/2 HDF (mild severity), and (3) mammary gland changes (including hyperplasia (high), secretion (slight, moderate), hemorrhage in interstitium (mild), and dilation of duct (with fluid-filled cyst; moderate) in 2/2 MDF. (These findings were not detected in either HDF).
The TK data are summarized in the following sponsor's table (data are expressed as individual values):

Toxicokinetic Parameters of Lurasidone Following 2 Weeks of Oral (Capsule) Dosing in Dogs (Study L0134)

Dose (mg/kg/day)	Male		Female	
	C_{max} (ng/mL)	$AUC_{0\text{-}24hr}$ (ng·hr/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}24hr}$ (ng·hr/mL)
50	14 244	191 2946	888 389	4227 2542
250	391 259	5435 3053	481 237	3006 3562
1000	2047 1605	15750 12820	2944 863	39395 5502

Individual data provided since there were 2 dogs/sex/group.

Time to peak serum levels (Tmax) following 2 weeks of dosing ranged from 1 to 6 hours. As shown in the sponsor's table, lurasidone was absorbed following oral administration of gelatin capsules but peak serum levels and exposure levels were highly variable. For this reason, the sponsor decided to administer lurasidone in an aqueous suspension by gavage tube in subsequent dog studies.

Conclusion: A NOAEL was not reached (due to clinical signs of tremors, miosis and decreased food intake at all tested dose levels, including the lowest tested dose of 50 mg/kg/day).
Lurasidone plasma exposure levels were highly variable; for this reason in the subsequent dog studies, lurasidone was administered in an aqueous suspension by gavage (instead of capsules).

Study title: **Two-Week Repeated Oral Dose Toxicokinetic Study of SM-13496 in Beagles**

Study no. (b) (4) **198-117**

Conducting laboratory and location: (b) (4)

Date of study initiation: 6/19/2009

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity: SM-13496, Lot # 3CG001M, Purity 100.9%

Key Study Findings

Lurasidone at oral dose levels of 30, 100, and 200 mg/kg (suspension in aqueous methylcellulose), was administered by gavage to 3 male and 3 female beagle dogs per group once daily for 2 weeks, to evaluate systemic exposure to the parent compound (SM-13496) and its main human metabolites, ID-20219 and ID-20220. Dose selection was based on the previously performed 39-week oral toxicity study of SM-13496 in dogs (Study No. 3879) which employed the same dose levels. The parent compound, SM-13496, showed the highest serum exposure levels in males and females, followed by ID-20219 (exposure values ≥ 10% of the parent at LD and MD) and ID-20220 (exposure values less than 10% of the parent in all dosed groups). For the parent compound, the Cmax and AUC_{0-t} tended to increase with dose; for metabolite ID-20219, Cmax and AUC_{0-t} were higher at 100 and 200 mg/kg/day than at 30 mg/kg/day, but showed no obvious difference between 100 and 200 mg/kg/day, and for metabolite ID- 20220, Cmax and AUC_{0-t} did not show obvious differences between doses tested. With repeated dosing, no constant tendency was noted in C_{max} or AUC_{0-t} of the parent compound, while for metabolites ID-20219 and ID-20219, a tendency to decrease was noted in C_{max} and AUC_{0-t}. No obvious gender differences were noted in Cmax or AUC_{0-t} for any of the tested compounds. The study showed that lurasidone major metabolites in humans (ID-20219 and ID-20220) are also major metabolites in dogs.

Methods

Dosing

Species/strain: Dog, Beagle

Number/sex/group: 3/sex/group

Age: 8 to 10 months (at the initiation of acclimation)

Weight: Males: 7.0 to 9.4 kg, females: 6.4 to 8.1 kg

Dosage groups in administered units: 30, 100, and 200 mg/kg/day [Dose selection was based on the previously performed 39-week oral toxicity study of SM-13496 in dogs (Study No. 3879) which employed the same dose levels]

Route, form, volume: oral, gavage, 3 mL/kg

Drug, lot#, and % purity: SM-13496, lot no. 3CG001M, 100.9%

Formulation/vehicle: suspension in aqueous 0.5% methylcellulose

Observations: Parameters evaluated included clinical signs, body weight, food consumption and concentration of lurasidone, ID-20219 and ID-20220 in serum (sampling points: 30 minutes, and 1, 2, 3, 4, 6, 8, and 24 hours after dosing on the 1st and the final day of dosing). Analysis was conducted by LC/MS/MS.

Results:
There were no deaths. Clinical signs (miosis, a decrease in spontaneous activity, somnolence and tremor) were observed in all treated groups, and ataxic gait was observed in the 100 and 30 mg/kg/day groups throughout the treatment period. Vomiting, decreased body weight and food consumption were noted in all treated groups; the latter tended to recover by Week 2 in all groups.
The toxicokinetic data from the study are presented in the following sponsor's table.

TK parameters of SM-13496 and its metabolites ID-20219 and ID-20220
Following 2 Weeks of Oral Gavage Dosing in Dogs (Study (b)(4)198-117)

SM-13496

Sex	Dose (mg/kg)	C_{max} (ng/mL)		t_{max} (h)		AUC_{0-t} (ng·h/mL)	
		First day	Final day	First day	Final day	First day	Final day
Male	30	679	979	1.5	1.0	3890	4660
	100	2400	1710	5.7	1.3	31000	10600
	200	2680	3450	3.3	2.0	28300	35500
Female	30	512	920	0.7	0.8	3380	4540
	100	2040	2100	3.0	0.8	25200	12500
	200	3420	3730	12.0	1.7	62400	24800

ID-20219

Sex	Dose (mg/kg)	C_{max} (ng/mL)		t_{max} (h)		AUC_{0-t} (ng·h/mL)	
		First day	Final day	First day	Final day	First day	Final day
Male	30	141	109	1.0	2.7	1250	1010
	100	253	132	6.7	2.3	3760	1110
	200	287	249	2.7	2.3	3960	2410
Female	30	181	176	1.0	0.8	2320	1580
	100	374	307	2.2	1.0	5020	2790
	200	292	222	2.7	1.7	5030	1940

ID-20220

Sex	Dose (mg/kg)	C_{max} (ng/mL)		t_{max} (h)		AUC_{0-t} (ng·h/mL)	
		First day	Final day	First day	Final day	First day	Final day
Male	30	49.1	25.7	4.3	1.5	617	398
	100	50.7	28.0	10.7	1.5	874	404
	200	65.3	40.3	3.0	9.7	926	664
Female	30	60.7	56.1	3.3	4.0	895	697
	100	74.4	60.9	2.0	1.7	1150	926
	200	72.9	48.0	2.0	1.7	1080	780

The parent compound, SM-13496, showed the highest serum exposure levels in males and females, followed by ID-20219 (exposure values ≥ 10% of the parent at LD and MD) and ID-20220 (exposure values less than 10% of the parent in all dosed groups). For the parent compound, the Cmax and AUC_{0-t} tended to increase with dose; for metabolite ID-20219, Cmax and AUC_{0-t} were higher at 100 and 200 mg/kg/day than at 30 mg/kg/day, but showed no obvious difference between 100 and 200 mg/kg/day, and for metabolite ID- 20220, Cmax and AUC_{0-t} did not show obvious differences between doses tested. With repeated dosing, no constant tendency was noted in C_{max} or AUC_{0-t} of the parent compound, while for metabolites ID-20219 and ID-20219, a tendency to decrease was noted in C_{max} and AUC_{0-t}. No obvious gender differences were noted in Cmax or AUC_{0-t} for any of the tested compounds.

Study title: **Four-Week Repeated-Dose Oral Toxicity Study in Dogs**

Study no.: **L0485**

Conducting laboratory and location: (b) (4)

Date of study initiation: 3/11/2003

GLP compliance: GLP-Noncompliant

QA statement: No

Drug, lot #, and % purity: SM-13496, lot# E-278, purity 100.3%

Key Study Findings

Four weeks of daily oral (gavage) administration of lurasidone (suspension in methylcellulose) to beagle dogs at doses of **0, 30, 100 and 300** mg/kg/day resulted in pharmacologically related clinical signs (miosis, tremor, somnolence and decreased spontaneous activity), as well as in decreased food consumption and body weight, with nipple- and mammary gland hyperplasia in females in all dose groups. Loss of body weight was pronounced in the high dose group (300 mg/kg/day). QT prolongation and hepatic effects were seen in one male at 300 mg/kg/day. Thymic atrophy or involution was seen in all dose groups, and changes in the adrenal glands in high-dose group. A NOAEL was not reached in this dose range-finding study. The maximum tolerated dose was less than 300 mg/kg/day because of deteriorated general condition (severe clinical signs and emaciation) at this dose.

Methods

Doses:	**0, 30, 100 and 300** mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	3 ml/kg
Formulation/Vehicle:	Suspension in 0.5% aqueous methylcellulose
Species/Strain:	Dog/Beagle
Number/Sex/Group:	3/sex/group
Age:	13 months
Weight:	8.7-11.5 kg (males); 8.3-12.7 kg (females)
Satellite groups:	No

Justification for the formulation and dose selection (as stated by the sponsor):

Doses of up to 1000 mg/kg were given in the previously conducted two-week oral dosing study of SM-13496 on dogs (Study No. L0134), but pronounced variation in the serum concentration of the test substance was observed as a result of administration with capsules. There tended to be less variation in the serum concentration of the test substance in the study where SM-13496 was given to dogs in the form of a suspension (Study No. CH124, entitled "Pharmacological Study of Safety of SM-13496: Preliminary Study of TK in Dogs," dose: 300 mg/kg) compared to when given in the form of capsules. Administration in the form of a suspension was thus selected in order to minimize variation in the serum concentration of the test substance. The serum concentration (Cmax: about 2000 ng/mL) of the test substance in the above study of SM-13496 was given in the form of a suspension to dogs was well over the serum concentration of the test substance in humans (168 ng/mL) at the high dose (80 mg/man) in the (Phase I) clinical study.

A dose of 300 mg/kg was thus established for the high dose group in this study, and the 100 and 30 mg/kg doses were established as the intermediate and low doses, respectively by reducing the above dose at a common ratio of about 3.

Observations and results

Mortality: There was no mortality observed in the study.

Clinical signs related to the administration of the test compound included decrease of spontaneous activity, tremors, miosis and somnolence at all dose levels. “Thickened” mammary glands were observed from Week 2 or 3 through the end of the study, in females of all treated groups, along with lactation at MD and HD (one female in each of the 100 and 300 mg/kg/day groups).

Body weights were decreased throughout the study in all dosed groups, especially in HD males. Two HD animals (a male and a female) were emaciated from Week 2 onwards.

Food consumption was decreased in all treated groups throughout the study, but less so during Weeks 3 and 4.

Electrocardiography (in non-anesthetized animals, 4 and 24 hrs post dosing in weeks 0 and 4, standard limb leads: I, II, III, and augmented vectors aVR, aVL, aVF) revealed prolongation of the QT interval in one HD male at 4 and 24 hours after dosing in Week 4. The body weight of the affected animal was decreased 36% from the mean of the vehicle control group and the animal was described as showing emaciation. The QT interval data were not corrected for heart rate (see the sponsor’s table below).

Sex	Group	Animal Number	QRS Duration (msec)				Q-T Interval (msec)				Heart Rate (bpm)			
			0		4(Week)		0		4(Week)		0		4(Week)	
			4	24(hour)	4	24(hour)	4	24(hour)	4	24(hour)	4	24(hour)	4	24(hour)
Male	Control	501	46	55	58	48	166	176	183	186	132	110	127	98
	(0.5%MC)	502	63	68	60	73	190	200	191	193	99	90	118	121
	SM-13496	511	56	53	55	58	186	178	188	176	135	121	111	139
	30mg/kg	512	60	48	53	53	183	186	188	175	136	122	138	126
	SM-13496	521	50	60	61	60	156	190	198	220	151	113	100	70
	100mg/kg	522	45	41	48	46	208	213	211	198	96	100	88	123
	SM-13496	531	58	55	56	58	193	185	311	326	105	97	117	105
	300mg/kg	532	51	48	43	53	186	201	158	210	110	99	133	86
Female	Control	905	38	46	43	56	188	171	168	175	108	126	110	124
	(0.5%MC)	906	46	50	38	60	185	191	188	195	145	120	118	138
	SM-13496	915	51	55	51	55	180	193	181	205	108	87	106	95
	30mg/kg	916	50	50	46	55	203	206	190	220	104	98	122	74
	SM-13496	925	51	50	51	51	173	200	188	193	130	98	125	102
	100mg/kg	926	50	43	45	50	218	240	190	205	75	60	127	100
	SM-13496	935	51	50	56	53	183	173	195	201	110	129	118	90
	300mg/kg	936	35	35	31	28	168	163	216	221	155	132	91	77

Hematology: elevated leukocyte count, neutrophils, monocytes and fibrinogen, as well as prolonged prothrombin time and activated partial thromboplastin time were found in the emaciated HD male, but these changes returned to normal in Week 4. Reduced erythrocyte count, hemoglobin and hematocrit, and elevated platelet counts and fibrinogen were found in one MD female in Week 4.

Clinical chemistry In the emaciated HD male, elevated alpha-2 globulin and gamma globulin, with reduced alpha-1-globulin and albumin to globulin (A/G) ratio were observed in Week 2, as well as elevated alkaline phosphatase (ALP) in Weeks 2 and 4 and elevated AST and ALT in Week 4. Elevated ALP was also noted in Week 4 in one MD female.

Hormone analysis (testosterone and luteinizing hormone, 4 and 24 hrs post dose in wks 0, 2, and 4): no abnormalities

Ophthalmoscopy: no abnormalities.

Urinalysis: "Mild" occult blood in Week 2 in the emaciated HD male and in a HD female, and "mild" glucose in Week 4 in another HD male. Elevated N-acetyl-β-glucosaminidase (NAG) excretion was also noted in Weeks 2 or 4 in two HD males. One MD female showed increased NAG excretion and urine volume, and lower osmotic pressure in Week 4.

Organ weight: Lower absolute and relative weight of the thymus and higher relative weight of the adrenal glands was observed in 2 HD animals (the emaciated male and one female).

Gross- and microscopic pathology: "Thickened" mammary glands, and enlarged nipples were observed in females of all dosed groups, with acinar proliferation in mammary glands of females of all dosed groups, and ductal dilation and increased secretion at and above 100 mg/kg/day. Thymic atrophy or involution was found in males and females in all treated groups and decreased cytoplasmic vacuoles in the adrenal cortex were noted in HDM and HDF.

TK: Peak serum (Cmax) levels were reached within one to two hours; 24 h after dosing the serum concentrations were much lower. Cmax and AUC0-t values increased with increasing dose, but they tended to be saturated in the 300 mg/kg/day group.

Multiple dosing did not result in drug accumulation. Although there were considerable individual variations, there were no pronounced gender differences.

The ranges of Cmax values at doses of 30, 100 and 300 mg/kg/day were 198 to 525 ng/ml; 144 to 4732 ng/ml; and 1259 to 2615 ng/ml, respectively. Compared with the maximum serum concentration of unchanged compound (165 ng/ml) in human Phase 1 studies (MRHD of 120 mg/person following multiple dosing), these levels, at the lower values of the exposure range at LD and MD were close to human Cmax at the maximum therapeutic dose level. The high dose, 300 mg/kg/day, resulted in Cmax in dogs well over the maximum serum concentration of unchanged compound (165 ng/mL) in humans at the maximum targeted therapeutic dose level.

TK Parameters of Lurasidone Following 4 Weeks of Oral Gavage Dosing in Dogs (Study L0485)

Dose (mg/kg/day)	Male		Female	
	C_{max} (ng/mL)	AUC_{0-t} (µg·hr/mL)	C_{max} (ng/mL)	AUC_{0-t} (µg·hr/mL)
30	198 525	1.11 2.47	466 1943	1.28 6.34
100	182 144	1.74 1.00	4732 1217	23.63 11.39
300	2615 1259	26.15 10.23	1279 1378	8.11 7.71

Individual data provided since there were 2 dogs/sex/group.

Comments: The sponsor provided the following valid comments on the study findings:

SM-13496 is known to have dopamine (D_2) receptor-blocking action in animal experiments. Among the dopaminergic neuron pathways in the brain, the blockade of D_2 receptors in the midbrain-limbic system is thought to bring central depressant action such as sedation and tranquilization (the pharmacological action of neuroleptics), while blockade of D_2 receptors in the midbrain-cerebral cortex system is thought to aggravate negative symptoms, and blockade of D_2 receptors in the nigro-striatal system is thought to induce the onset of extrapyramidal syndrome such as tremors. Blockade of D_2 receptors in the tubero-infundibular system is known to result in prolactin oversecretion, and prolactin is known to have action in mammary gland development and lactation.[2)] That is, it would seem that the decrease of spontaneous activity and somnolence observed in this study were caused by central depressant action, and that the tremors originated from extrapyramidal symptoms. The effects on the mammary glands and nipple were considered secondary changes caused by prolactin oversecretion. In vitro studies of SM-13496 revealed weak α_1 receptor-blocking action of adrenergic nerves,[3)] and the miosis was thus concluded to be related to such action. As such changes were similarly found in two-week oral dosing studies of SM-13496 on rats, cynomolgus monkeys, and dogs,[4,5,6)] all these changes were attributed to the effects of administration of the test substance.

(End citation)

Summary and conclusion: Four weeks of daily oral (gavage) administration of lurasidone (suspension in methylcellulose) to beagle dogs at doses of 0, 30, 100 and 300 mg/kg/day resulted in pharmacologically related clinical signs (miosis, tremor, somnolence and decreased spontaneous activity), as well as in decreased food consumption and body weight, with nipple- and mammary gland hyperplasia in females in all dose groups. Loss of body weight was pronounced in the high dose group (300 mg/kg/day), with prolonged QT interval and hepatic effects in an emaciated male. Thymic atrophy or involution was seen in all dose groups, and changes in the adrenal glands in high-dose group. A NOAEL was not reached in this dose range-finding study; the maximum tolerated dose was > 100 mg/kg/day, but less than 300 mg/kg/day because of deteriorated general condition at this dose.

Study title: **39-week Oral Toxicity Study of SM-13496 in Dogs** Study no.: **3879**

Conducting laboratory and location: (b) (4)

Date of study initiation: November 8, 2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SM-13496 (Lot E-278, 100.3%)

Key Study Findings

A 9-month (39-week) oral gavage administration of lurasidone (suspension in 0.5% aqueous methylcellulose) to Beagle dogs (4/sex/group) at daily doses of **30, 100 and 200 mg/kg/day** caused pharmacologically related clinical signs (decreased spontaneous activity, somnolence, tremors, miosis) at all dose levels. Reduced food consumption was observed in all treated groups and decreased body weight or weight gain - in males at MD and HD. Ophthalmoscopy and electroretinography did not reveal any treatment-related effects. Premature ventricular contractions (PVCs) occurred at HD (2 of 4 males) from Wk 13 to 22. Both animals exhibiting PVC had high serum concentrations of lurasidone, and severe (approximately 30%) decrease in body weight relative to control. Non-corrected QT intervals were prolonged in these two HD males, and in one MD male that had a 33% body weight loss. Increased total cholesterol and phospholipids were seen in all treated groups except for MDM. Serum prolactin was elevated at all doses throughout treatment. Absolute and relative prostate weights were lower at all doses and absolute testis weights were lower at MD and HD. Grossly, prostates, ovaries and/or uteri appeared small in all treated groups. Histopathology examination revealed prostatic atrophy in all treated male groups. At the two highest dose levels, the testes exhibited multifocal or diffuse seminiferous atrophy; at HD, hypospermia was noted in the epididymis, with cellular debris in the lumen of the tubules and vacuolization of ductal epithelium. Uterine atrophy and decreased presence of corpus luteum and secondary ovarian follicles were seen in all female treated groups, along with mammary gland "thickening" and microscopic pathology (hydropic appearance of ductal epithelium, diffuse lymphoid cell infiltration and pigmentation). A decrease in trabecular bone in the femur and/or sternum was observed in 1 MD male and 2 HD males in parallel with increased fatty infiltration in bone marrow of the femur and/or sternum. All the three males exhibited severe body weight loss (20 to 30% by the end of the treatment period); two of these males were listed as "emaciated". It is likely that the bone loss was secondary to the severe body weights loss rather than a prolactin effect in this species. Thymic atrophy was seen in all dose groups, and decreased numbers of cytoplasmic vacuoles were observed in the adrenal cortex of animals of both sexes at the two highest dose levels, compared to controls. New findings in this study when compared with the chronic toxicity studies in rats and monkeys were the effects on the male genital system and premature ventricular contractions. A NOAEL for general toxicity was not reached in this study since drug-related clinical signs (i.e., tremors), elevation in serum prolactin, cholesterol and triglycerides, and gross- and microscopic pathology changes in the mammary gland, thymus, and male and female reproductive system (i.e., prostate and uterine atrophy) were present at all tested doses, including the lowest (30 mg/kg/day). The dose of 30 mg/kg was a NOAEL specifically for cardiovascular effects (non-corrected QT interval prolongation). Lurasidone systemic exposure margin at this dose after

39 weeks of treatment was about 15- and 12 times, in males and females respectively (based on AUC in serum) and 7 times (based on Cmax) the human exposure at MRHD of 120 mg/day ($AUC_{0\text{-}inf}$ = 687 ng.h/ml). The MTD was 200 mg/kg/day for females and 100 mg/kg/day for males, due to severe body weight loss (emaciation) and deterioration of general condition in males at the next higher tested dose of 200 mg/kg/day.

Justification of dog studies:

The following justification of lurasidone dog studies (as a third species used in general toxicology studies in addition to rodents and monkeys) was provided by the sponsor:

> "A one-year oral toxicity study of SM-13496 in cynomolgus monkeys was previously conducted as part of the safety evaluation of the test substance in non-rodents. The maximum serum concentration (Cmax) of the test substance following administration of the highest dose (50 mg/kg) in the study in cynomolgus monkeys was 85.84 ng/mL in males and 89.70 ng/mL in females. These values did not exceed the maximum serum concentration (168 ng/mL) of the test substance obtained in humans following repeated oral administration of the high dose (80 mg/man) in the phase I clinical study. Therefore, it was concluded that it is necessary to perform a safety evaluation of SM-13496 in non-rodents at a higher exposure in order to ensure the safety of long-term use in humans. However, since long-term administration of higher doses to cynomolgus monkeys was expected to result in emaciation and deaths due to severe decrease of spontaneous activity, it was decided that nine-month oral toxicity study should be performed in beagle dogs instead of cynomolgus monkeys."

Methods

Doses:	SM-13496: 0, 30, 100 and 200 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	3 ml/kg
Formulation/Vehicle:	0.5% aqueous methylcellulose
Species/Strain:	Dog, Beagle
Number/Sex/Group:	4/sex/group
Age:	8 months
Weight:	9.6 – 12.4 kg (M); 7.8 – 11.7 kg (F)
Satellite groups:	No
Unique study design:	Prolactin, Bone density measurements
Deviation from study protocol:	Did not affect the reliability of the study

Observations:

Clinical signs: daily, prior to dosing and at 1- to 3-hourly intervals post-dose; Food consumption: weekly; Body weight: once weekly (prior to feeding) from 2 weeks prior to the initiation of treatment and during the treatment period; if the rate of change in body weight exceeded 5 % when compared with the previous week, re-measurements were performed immediately to confirm reproducibility.

Ophthalmoscopy: at Weeks 0, 11, and 37 of the treatment, macroscopically or by retinal camera (GENESIS K9L22, Kowa Co., Ltd.). Electroretinography: at Weeks 0, 11, and 37; the a- and b-waves, and oscillatory potentials were recorded using an evoked potential system; Electrocadiogram (ECG): 2 and 24 hrs after dosing (or at the equivalent time at Week 0) at Weeks 0, 4 and 13, and 2, 6 and 24 hours after dosing at Weeks 17, 22, 26, 30, 35, 39 of treatment. ECG was recorded in conscious animals in a right lateral position using an electrocardiograph (standard lead I, II, III; augmented unipolar extremity lead aVR, aVL, aVF for animals (Labo System ZM-5012, Fukuda M-E Kogyo Co., Ltd.). Using a standard lead II, the following parameters were measured simultaneously with heart rate: amplitude of the P and R waves, PR interval, QRS duration, and QT interval.

Hematology: at Weeks 0, 4, 13, 26 and 39 of treatment: RBC, Hb, Ht, MCV, MCH, MCHC, Plt, WBC, Neut, Lymph, Mono, Eos, Baso, Ret, PT, APTT, Fbg.

Blood Biochemistry: at Weeks 0, 4, 13, 26 and 39 of treatment: TP, Alb, alpha-1, -2, -3-Glob, beta-Glob, gamma-Glob, Alb/Glob ratio, total cholesterol, phospholipids, triglycerides, BUN, creatinine, uric acid, T-bilirubin, AST, ALT, ALP, gamma-GTP, LDH, creatine kinase, Na, K, Ca, P, Cl.
Hormone Analysis (RIA): 2 and 24 hours after dosing at Weeks 0, 4, 13, 26 and 39: Prolactin, Testosterone, Luteinizing hormone (testosterone and luteinizing hormone were measured only in the sample drawn from males 24 hours after dosing).
Urinalysis: at Weeks 0, 4, 13, 26 and 39: Appearance, pH, Glucose, Protein, Occult blood, Ketone bodies, Bilirubin, Urobilinogen, Erythrocytes, Leukocytes, Epithelium, Small round cells, Cast; the following parameters were measured using pooled urine collected for 24 hrs: Urine volume, Osmolarity, Creatinine excretion, N -acetyl-ß-D-glucosaminidase excretion, Gamma-glutamyl transpeptidase excretion, Lactate dehydrogenase excretion, Na-, K-, Cl-excretion.
Pathology: Gross observations of the external appearance, subcutaneous tissue, thoracic and abdominal cavity, and all organs. Organ weights (absolute and relative): Brain, Pituitary, Submandibular gland, Thyriod (including parathyroid), Thymus, Lung, Heart, Pancreas, Liver (including gall bladder), Spleen, Adrenal, Kidney, Testis, Prostate, Ovary, Uterus
Histopathology: The above listed organs as well as the following organs and tissues were fixed and preserved in 10% neutral buffered formalin: Eyeball (including optic nerve), Submandibular lymph nodes, Parotid gland, Tongue, Larynx, Trachea, Aorta, Urinary bladder (including ureter), Esophagus, Stomach, Small intestine (duodenum, jejunum, ileum), Large intestine (cecum, colon, rectum), Mesenteric lymph nodes, Spinal cord, Sciatic nerve, Femoral skeletal muscle, Abdominal skin, Mammary gland, Sternum (bone/bone marrow), Femur (bone/bone marrow), Epididymis, Vagina, Eyelids (meibomian glands), Nictitating membrane, Lacrimal gland and Gross lesions . The eyeball (including optic nerve) was fixed with Davidson's solution, and the testis was fixed with Formal Sucrose Acetic acid (FSA) solution, respectively, prior to being preserved in 10% neutral buffered formalin.
All organs and tissues were dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H/E) and subjected to light microscopic examination.
Toxicokinetics: Blood samples were collected via the cephalic vein 0.5, 2, 6 and 24 h post-dose from all animals on Day 1 and at Weeks 4, 13, 26, and 39 and 2, 6, 24 h post-dose from all males at Week 19.

Results:

Mortality: All animals survived to the end of the study.
Clinical signs: Decreased spontaneous activity, somnolence, miosis, tremors and dry muzzle were observed in all treated animals, except for LDF where tremors were seen in 3 of 4, and somnolence in 2 of 4 females. “Thickened mammary glands” and lactation were observed in 50% of females at all dose levels, without dose-dependence. The decrease of spontaneous activity and somnolence observed in all treated groups were attributed to the intended pharmacological action of the test substance and tremors were indicative of an extrapyramidal syndrome associated with neuroleptic agents. These signs were noted especially during the early stage of treatment. Although these signs subsided in most of the animals during the course of treatment, some animals in the high dose group were persistently affected. TK results indicated that high Cmax was noted in HD males, but there was no clear relationship between Cmax and expression of signs in females.
Food consumption was reduced dose-dependently in all treated groups, statistically significant at 100 and 200 mg/kg/day, mostly during the first 4 weeks, but HDM being affected throughout the treatment period.
Body weight was decreased in males at 100 or 200 mg/kg/day (-23% at MD and -14% at HD vs. control by Week 39. In females body weight was decreased in MD (-9% vs. control by Wk 39), but increased at HD (+19% by Wk 39). Body weight decrease may be partially related to the reduction of food intake as a result of treatment-related clinical signs. Body weights in most of treated animals were affected during Week 1 and, in some animals, until Week 20.
Ophthalmoscopy and **electroretinography** did not show any treatment-related effects.

ECG: Premature ventricular contractions (PVCs) were observed in 2 HD males, from Week 13 to 22. Both animals exhibiting PVC had high serum concentrations of lurasidone, and approximately a 30% decrease in body weight relative to the control (described as emaciation). In one of these animals, a single incidence of PVC was reported at Week 17; in the other animal, multiple incidences of ECG events were reported beginning at Week 13 and described as a "ventricular bigeminal rhythm pattern with regularly coupled PVCs arising from a single focus." The sponsor suggested that an "alternative explanation for the ECG events in this animal is premature atrial contractions with aberrant conduction in the ventricle"; this alternative interpretation was "supported by one ECG in which P waves apparently preceded the wide complex beat." The sponsor contends that "both of these rhythms (ventricular bigeminy or atrial bigeminy with aberrant conduction) are generally not considered to be malignant rhythms and could be attributed to the poor physical condition of the animal (e.g., low body weight with emaciation and poor food consumption) rather than a direct drug effect".

Non-corrected QT intervals prolongation was found in 1 MD male and 2 "emaciated" HD males from Week 13 onwards. All these three animals showed body weight loss (26%-33% decrease relative to the control group mean; the MD male had 33% body weight loss), and they also were found to have high blood concentrations of the test substance.

Hematology and clinical chemistry analysis showed some decrease in leukocyte and neutrophil counts in HD males from Week 13 onward (statistically significant only at the Wk 13 time point), and a time-dependent increase in total cholesterol and phospholipids in all treated groups except for MDM.

Hormones: Serum **prolactin** levels were markedly elevated throughout the entire treatment period (most pronounced at the Wk 4 time point) in all treated groups and animals with no apparent dose dependence. Block of D2 receptors at tubero-infundibular system has been reported in the literature to increase prolactin release from the pituitary. No treatment-related changes were seen in testosterone and LDH.

Urinalysis showed a decrease in pH levels at MD and HD after Week 13.

Organ weights: Absolute and relative prostate weights were lower in all treated male groups with no apparent dose dependence. Absolute testis weights were decreased relative to control at MD and HD.

Gross pathology: Prostates, ovaries and/or uteri appeared small in all treated groups. **Histopathology examination** revealed prostatic atrophy in all treated male groups. At the two highest dose levels, the testes exhibited multifocal or diffuse seminiferous atrophy, and hypospermia was noted in the epididymis, with cellular debris in the lumen of the tubule and vacuolization of ductal epithelium at HD. Uterine atrophy and decreased presence of corpus luteum and secondary ovarian follicles were found in all female treated groups. Mammary glands of all treated female groups exhibited hydropic appearance of ductal epithelium, diffuse lymphoid cell infiltration and pigmentation. At HD, cholesterol granuloma and dilated duct containing cholesterol crystals were observed in the mammary gland of 1 HDF. These changes were likely due to prolactin increase; it is well known that prolactin develops the mammary gland and milk secretion, and prolonged hyperprolactinemia affects genital system due to hormonal disbalance.

A decrease in trabecular bone in the femur and/or sternum was observed in one MD male and 2 HD males; the latter also exhibited increased fatty infiltration in bone marrow of the femur and/or sternum. All the three males exhibited severe body weight loss (20 to 30% by the end of the treatment period) and two of these males were listed as emaciated in the clinical observations. It is likely that the bone loss was secondary to the severe body weights loss in these animals rather than a prolactin effect in this species, since prolactin increase took place in all tested dose groups including animals that did not display trabecular bone decrease. The sponsor speculated that the stress caused by the deterioration of the general condition could have increased the secretion of adrenal cortical hormone, which is known to cause reduction of bone mineral density. This seems to be supported by the histopathologic finding of decreased cytoplasmic vacuoles in the adrenal cortex of these 3 males along with thymic atrophy, which suggested enhancement of secretion of adrenal cortical hormone. Decreased numbers of cytoplasmic vacuoles were also observed in the adrenal cortex of animals without trabecular bone decrease, i.e., in both sexes at the two highest dose levels, and thymic atrophy was noted in all male dose groups and in females at MD and HD.

Mid- and high-dose males exhibited foamy cell infiltration in the mesenteric lymph nodes and a tendency towards increased severity of foamy cell accumulation in the lungs, with cholesterol crystals or inflammatory cell infiltration.
The results are summarized in the following sponsor's table:

Study Title: Thirty Nine-Week Oral Toxicity Study of SM-13496 in Dogs		Test Article: lurasidone (SM-13496)	
Species/Strain: Dog/ Beagle	Duration of Dosing: 39 weeks	Study No. 3879	
Initial Age: 8 months	Duration of Postdose: None	Module Location: 4.2.3.2.	
Date of First Dose: 25/Nov/2004	Method of Administration: Oral gavage	GLP Compliance: Yes	
Vehicle/Formulation: Suspension in aqueous 0.5% methylcellulose			
Special Features: None			

Daily Dose (mg/kg)	0 (Control)		30		100		200	
No. of Animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Noteworthy Findings:								
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Body Weight (%)								
Week 1	11.1 kg	9.7 kg	-7	-7	-6	-13	-3	-6
Week 13	12.2 kg	10.4 kg	-5	-3	-13	-8	-21	+4
Week 39	12.9 kg	11.3 kg	+5	-2	-23	-9	-14	+19
Food Consumption (%)								
Week 1	2100 g	1990 g	-39	-25	-54*	-47**	-56*	-64**
Week 2	2100 g	2040 g	-6	-4	-26*	-23*	-31*	-44**
Week 3	2100 g	2060 g	-9	-1	-18*	-9	-20*	-30**
Week 4	2100 g	2020 g	-7	+1	-15	-11	-16*	-28*
Week 5	2100 g	2040 g	-4	+2	-12	-3	-11	-19*
Clinical Observations (No. of animals)								
Decreased spontaneous activity	0	0	4	4	4	4	4	4
Somnolence	0	0	4	2	4	4	4	4
Tremors	0	0	4	3	4	4	4	4
Dry muzzle	0	0	4	2	4	4	4	4
Miosis	0	0	4	4	4	4	4	4
Thickened mammary gland	0	0	0	2	0	2	0	2
Lactation	0	0	0	2	0	2	0	2
Ophthalmoscopy	--	--	--	--	--	--	--	--
Electroretinography	--	--	--	--	--	--	--	--
Electrocardiography (No. of animals)								
Premature ventricular contraction								
Week 13 (2, 24 hr)	--, --	--, --	--, --	--, --	--, --	--, --	+, +	--, --
Week 17 (2, 6, 24 hr)	--, --, --	--, --, --	--, --, --	--, --, --	--, --, --	--, --, --	--, +, +	--, --, --
Week 22 (2, 6, 24 hr)	--, --, --	--, --, --	--, --, --	--, --, --	--, --, --	--, --, --	--, --, +	--, --, --
QT interval (Mean value, msec)								
Week 13 (2, 24 hr)	176, 183	188, 194	170, 171	180, 183	179, 195	180, 184	178, 207↑	174, 171
Week 17 (2, 6, 24 hr)	175, 182, 181	183, 180, 185	169, 162, 177	182, 178, 185	163, 172, 182	184, 190, 189	183↑, 187↑, 198↑	170, 177, 181
Week 30 (2, 6, 24 hr)	179, 176, 180	180, 181, 186	179, 170, 177	177, 178, 186	183, 171, 199↑	187, 189, 190	183, 173, 189	184, 179, 182
Week 35 (2, 6, 24 hr)	173, 176, 179	189, 184, 194	179, 170, 178	187, 181, 184	181, 178, 197	186, 185, 183	193, 206↑, 213↑	177, 181, 186
Week 39 (2, 6, 24 hr)	191, 182, 186	187, 186, 189	173, 176, 176	185, 183, 196	177, 177, 182	191, 187, 202	214↑, 196, 215↑	184, 183, 183

39-week Oral Toxicity Study in Dogs (continued)

Daily Dose (mg/kg)	0 (Control)		30		100		200	
No. of Animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Hematology								
Leukocyte count (Mean value, $x10^3/\mu L$)								
Week 13	10.89	9.93	9.81	8.82	8.00	8.50	6.70*↓	9.47
Week 26	11.06	9.95	11.14	8.74	8.38	8.83	7.16↓	11.00
Week 39	9.13	11.61	9.36	8.50	10.32	9.15	7.11↓	11.29
Neutrophils (Mean value, $x10^3/\mu L$)								
Week 13	7.00	5.83	6.02	5.16	4.80	4.90	4.15*↓	5.24
Week 26	7.27	5.78	7.32	5.47	5.17	5.54	4.32	6.70
Week 39	5.82	7.48	5.73	5.48	7.09	6.07	4.64	6.97
Blood biochemistry								
Total Cholesterol (Mean value, mg/dL)								
Week 4	152	147	143	188↑	137	183↑	161	180↑
Week 13	153	148	186↑	191↑	158	203↑	164↑	221*↑
Week 26	144	219	184↑	204	149	202	176↑	248↑
Week 39	141	193	196↑	241↑	149	221↑	192↑	244↑
Phospholipids (Mean value, mg/dL)								
Week 4	332	310	319	380↑	311	385↑	339	381↑
Week 13	339	306	384↑	391↑	339	413↑	329	433↑
Week 26	315	406	381↑	413↑	328	413↑	363↑	477↑
Week 39	313	375	406↑	457↑	329	443↑	388↑	495↑
ALT (Mean value, U/L)								
Week 13	32	33	40	37	182↑	30	219↑	32
AST (Mean value, U/L)								
Week 13	34	29	27	27	34	31	62↑	30
Hormone analysis								
Serum Prolactin (Mean value, ng/mL)								
Week 4 (2, 24hr)	5.38, 4.53	5.14, 4.76	33.30**↑, 22.86↑	40.52↑, 23.71↑	30.16**↑, 21.70↑	37.58↑, 28.51↑	30.40**↑, 26.32↑	37.03↑, 33.06↑
Week 13 (2, 24hr)	4.65, 5.95	4.48, 4.04	31.49↑, 29.77↑	32.10↑, 21.65↑	28.55↑, 28.24↑	34.95↑, 39.15↑	23.84↑, 28.62↑	40.10↑, 38.00↑
Week 26 (2, 24hr)	3.31, 3.47	2.91, 2.63	23.99↑, 21.40↑	35.99↑, 14.79↑	12.39↑, 12.26↑	40.42↑, 22.11↑	25.17↑, 23.26↑	30.77↑, 36.69↑
Week 39 (2, 24hr)	1.86, 1.41	1.94, 2.84	29.76↑, 14.62↑	27.78↑, 11.93↑	10.34↑, 6.07↑	19.65↑, 9.43↑	23.04↑, 12.23↑	12.08↑, 11.79↑
Urinalysis								
pH (range of the individual values)								
Week 13	8.5 - ≥9.0	>9.0	7.5 - ≥9.0	8.0 – 8.5	6.0↓ - 8.5	7.5 – 8.5	5.5↓ - ≥9.0	6.5↓ - ≥9.0
Week 26	≥9.0	≥9.0	7.5 - ≥9.0	8.5 - ≥9.0	7.5 - ≥9.0	6.5↓ - ≥9.0	5.5↓ - ≥9.0	6.0↓ - ≥9.0
Week 39	8.5 - ≥9.0	8.0 - ≥9.0	7.0 - ≥9.0	8.0 - ≥9.0	6.5↓ - ≥9.0	6.5↓ - ≥9.0	5.5↓ - ≥9.0	7.0 – 8.5
Organ Weights								
absolute prostate (g)	9.9	N.E.	3.1**↓	N.E.	4.0*↓	N.E.	3.5**↓	N.E.
relative prostate (% to bw)	0.076	N.E.	0.024**↓	N.E.	0.041↓	N.E.	0.031*↓	N.E.
Absolute testis (g)	16.0	N.E.	16.7	N.E.	10.8↓	N.E.	13.0↓	N.E.

(continued on the next page)

39-week Oral Toxicity Study in Dogs (continued)

Daily Dose (mg/kg)	0 (Control)		30		100		200	
No. of Animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Gross Pathology (No. of animals)								
Prostate (small)	0	N.E.	4	N.E.	2	N.E.	1	N.E.
Ovary (small)	N.E.	0	N.E.	2	N.E.	2	N.E.	2
Uterus (small)	N.E.	0	N.E.	2	N.E.	0	N.E.	1
Lung (yellow to white foci)	0	0	0	1	2	1	3	0
Histopathology (No. of animals)								
No. Examined	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Prostate/ atrophy	0	N.E.	4	N.E.	2	N.E.	3	N.E.
Testis/ exfoliated cells or giant cells	0	N.E.	0	N.E.	2	N.E.	3	N.E.
Testis/ accumulation of spermatozoa in the lumen (seminiferous tubule)	0	N.E.	0	N.E.	1	N.E.	1	N.E.
Testis/ multifocal or diffuse seminiferous atrophy	0	N.E.	0	N.E.	2	N.E.	2	N.E.
Epididymis/ hypospermia	0	N.E.	0	N.E.	2	N.E.	3	N.E.
Epididymis/ cellular debris (lumen of the tubule)	0	N.E.	0	N.E.	0	N.E.	2	N.E.
Epididymis/ vacuolation (ductal epithelium)	0	N.E.	0	N.E.	0	N.E.	1	N.E.
Ovary/ decrease in secondary follicle	N.E.	0	N.E.	3	N.E.	2	N.E.	3
Uterus/ atrophy	N.E.	0	N.E.	2	N.E.	0	N.E.	1
Mammary gland/ hydropic appearance (ductal epithelium)	0	0	0	2	0	3	0	2
Mammary gland/ lymphoid cell	0	0	0	1	0	1	0	1
Mammary gland/ cholesterin granuloma	0	0	0	0	0	0	0	1
Mammary gland/ dilated duct containing cholesterin crystals	0	0	0	0	0	0	0	1
Bone/ decrease in trabecular bone(sternum or femur)	0	0	0	0	1	0	2	0
Bone marrow/ increase in fatty infiltration (sternum or femur)	0	0	0	0	0	0	3	0
Urinary bladder/ increase in cytoplasmic eosinophilic granule (epithelium)	0	0	4	4	3	4	2	4
Lung/ increase in foamy cell accumulation	1	2	1	2	2	3	3	1
Lung/ cholesterin crystals or inflammatory cell infiltrations	0	0	1	0	2	0	2	0
Mesenteric lymph node/ foamy cell infiltration (sinus)	0	0	0	0	1	0	2	0
Adrenal/ decreased cytoplasmic vacuole (cortex)	0	0	0	0	1	2	1	1
Thymus/ atrophy or involution	0	0	1	0	3	2	2	1

Bartlett's, *t* and Steel's or Dunnett's statistical tests; * Significantly different from control group ($p < .05$); ** ($p < .01$);
+ Observed; ↓ Decrease or low value; ↑ Increase or high value
N.E. Not examined; -- No abnormality

Toxicokinetics

Toxicokinetic parameters were obtained for the parent drug (lurasidone) and for metabolites ID-14283 and ID-14326. These data are presented in the following sponsor's tables.

Peak serum concentrations of lurasidone were generally achieved between 0.5 and 6 hours after administration. The concentration gradually decreased from Cmax with the mean half-life of about 3 to 10 hours. The mean Cmax and AUC_{0-t} increased dose-dependently. The time course of serum concentrations of the two metabolites showed almost similar trend to that of the unchanged test substance. Among the analytes (SM-13496, ID-14283 and ID-14326), the unchanged test substance exhibited the highest concentration at every sampling point. There were no marked sex differences in the serum concentration of analytes. The difference in Cmax between male and female dogs (male to female ratio) generally fell between 0.7 and 2.0. However, apparent individual differences were observed for each of the analytes. Some male dogs in the 100 and 200 mg/g groups showed higher Cmax than other dogs, and there was a link between abnormal ECG or QT prolongation and serum drug concentration. The Cmax and AUC_{0-t} of lurasidone and its metabolites remained almost at the same level throughout the study, indicating that there was no accumulation. Since the two metabolites are not major metabolites in humans, they are not discussed further here. For TK data of the main human metabolites in dogs, see the study entitled "Two-week repeated oral dose TK study of SM-13496 in Beagles"(Study No. (b) (4)198-117) reviewed herewith.

Toxicokinetic Parameters of Lurasidone metabolites ID-14283 and ID 14326 Following 4 and 39 Weeks of Oral Gavage Dosing in Dogs

Toxicokinetics: mean AUC_{0-t} (µg·hr/mL)								
Daily Dose (mg/kg)	0 (Control)		30		100		200	
No. of Animals	M:4	F:4	M:4	F:4	M:4	F:4	M:4	F:4
Lurasidone (parent)								
Week 4	N.E.	N.E.	10.2	8.7	12.1	12.3	32.1	15.6
Week 39	N.E.	N.E.	10.1	8.3	20.1	10.5	67.0	16.7
ID-14283 (metabolite)								
Week 4	N.E.	N.E.	2.9	4.8	5.9	4.8	8.0	6.9
Week 39	N.E.	N.E.	4.9	3.9	6.7	4.6	9.4	5.9
ID-14326 (metabolite)								
Week 4	N.E.	N.E.	1.2	2.3	3.2	1.6	4.1	4.0
Week 39	N.E.	N.E.	3.2	2.3	3.3	1.6	4.8	3.6
Toxicokinetics: mean C_{max} (µg/mL)								
Lurasidone (parent)								
Week 4	N.E.	N.E.	0.91	1.28	1.50	1.45	2.68	1.99
Week 39	N.E.	N.E.	1.15	1.15	2.08	1.21	5.86	1.77
ID-14283 (metabolite)								
Week 4	N.E.	N.E.	0.21	0.42	0.40	0.37	0.49	0.52
Week 39	N.E.	N.E.	0.38	0.36	0.43	0.36	0.62	0.41
ID-14326 (metabolite)								
Week 4	N.E.	N.E.	0.10	0.22	0.23	0.13	0.27	0.32
Week 39	N.E.	N.E.	0.26	0.22	0.23	0.12	0.35	0.27

Toxicokinetic Parameters of Lurasidone Following 4, 13 and 39 Weeks of Oral Gavage Dosing in Dogs (Study 3879)

Dose (mg/kg/day)	Male		Female	
	C_{max} (ng/mL)	AUC_{0-t} (µg·hr/mL)	C_{max} (ng/mL)	AUC_{0-t} (µg·hr/mL)
Week 4				
30	910	10.2	1280	8.7
100	1500	12.1	1450	12.3
200	2680	32.1	1990	15.6
Week 13				
30	1240	14.4	910	5.8
100	3160	35.2	1400	11.3
200	3900	54.0	1760	13.9
Week 39				
30	1150	10.1	1150	8.3
100	2080	20.1	1210	10.5
200	5860	67.0	1770	16.7

Group mean data provided from groups of 4 dogs/sex/group.

Compared to the human systemic exposure at MRHD (120 mg/day) after repeated oral administration (Cmax=164.7 ng/ml and AUC_{0-inf} = 686.6 ng.h/ml) (see the sponsor's table below), the corresponding exposures in dogs at the lowest tested dose of 30 mg/kg/day at the end of the study were 7 x (Cmax) and 12 to 15 x (AUC for F and M, respectively) higher.

TK parameters in humans after repeat-dose oral Lurasidone administration*

	SM-13496 AUC (0-inf) (ng h/mL)					
	120 mg		140 mg		160 mg	
	Mean	Range	Mean	Range	Mean	Range
Period I[1] Assay results	481.3	260-1057	728.6	471-1450	902.4	424-1614
Period I DN	4.011	2.171-8.808	5.204	3.366-10.35	5.640	2.649-10.09
Period II[2] Assay results	686.6	419-1685	793.3	454-1188	899.3	518-1418
Period II DN	5.72	3.489-14.04	5.666	3.243-8.487	5.621	3.235-8.862

	SM-13496 C_{max} (ng/mL)					
	120 mg		140 mg		160 mg	
	Mean	Range	Mean	Range	Mean	Range
Period I[1] Assay Results	125.3	45.2–208	170.4	102–246	196.5	72.9–470
Period I DN	1.044	0.376–1.736	1.217	0.730–1.756	1.228	0.455–2.935
Period II[2] Assay Results	164.7	75.0–357	194.0	119–235	233.4	156–411
Period II DN	1.372	0.625–2.978	1.386	0.853–1.682	1.459	0.977–2.569

DN: Dose-normalized

1 Period I serial PK samples were collected over the 72 hours beginning on Day 1

2 Period II serial PK samples were collected over 24 hours beginning on Day 8

***Source:** A maximum tolerated dose study of SM- 13496 in patients with schizophrenia, 2002 (b) (4) Prot. No. DI050160-P03) (8 subjects at each dose level, repeat-dose administration on days 1 and 4 through 8)

Stability and homogeneity: Although the uniformity of the dosing suspensions was confirmed in the same study as the stability of the dosing suspensions described in the protocol, the measurement of the uniformity was not done in that study. The uniformity was confirmed in another study using a different lot. Because the analysis data of the uniformity were within the normal range and all concentrations of dosing suspensions were within the normal range, it was considered that this would not affect the reliability of the study.

Conclusion: A NOAEL for general toxicity could not be determined in lurasidone 39-week chronic oral toxicity study in dogs at doses of 30, 100 and 200 mg/kg/day since drug-related clinical signs (i.e., tremors), elevation in serum prolactin, cholesterol and triglycerides, and gross- and microscopic pathology changes in the mammary gland, thymus, and male and female reproductive system (i.e., prostate and uterine atrophy) were present at all tested doses, including the lowest (30 mg/kg/day). The dose of 30 mg/kg was a NOAEL specifically for cardiovascular effects (non-corrected QT interval prolongation). Lurasidone systemic exposure margin at this dose after 39 weeks of treatment was about 15- and 12 times, in males and females respectively (based on AUC in serum) and 7 times (based on Cmax) the human exposure at MRHD of 120 mg/day ($AUC_{0\text{-}inf}$ = 687 ng.h/ml). The MTD in the 39-week dog study was 200 mg/kg/day for females and 100 mg/kg/day for males, due to severe body weight loss (emaciation) and deterioration of general condition in males at the next higher tested dose of 200 mg/kg/day.

7 Genetic Toxicology

Genotoxicity Studies of Lurasidone

The potential of lurasidone to induce genetic toxicity was investigated in vitro [in bacterial reverse mutation test (Ames test, Study 2773) and mammalian cell systems (chromosomal aberration test using Chinese hamster lung cells, Study 2749)] and in an in vivo micronucleus assay in mice (Study 2767).

STUDIES	SPECIES	ROUTE	DURATION	DOSE (MG/PLATE, MG/ML, MG/KG)
Genotoxicity				
Ames assay	Bacteria	In vitro	48 hours	Up to 5000 µg/plate
Chromosomal Aberration Test	CHL/IU cells	In vitro	24, 48 hours	Up to 5000 µg/mL
Micronucleus Test	Mouse	po	1 dose	500, 1000, 2000

Other genotoxicity studies

A lurasidone metabolite (ID-11614) and a compound code named (b) (4) (lurasidone starting material and potential impurity with a structural alert), were evaluated in bacterial reverse mutation (Ames) tests (Studies No. 3117 and A02097, respectively) (see under 7.4 "Other Genotoxicity Studies").

All of the above studies, except one (Study A02097) were previously reviewed by Dr. Lois Freed (under IND 61 292 N 000, original submission), and the data of her review are cited and reproduced in the next pages under the corresponding studies. Study A02097 (b) (4) bacterial reverse mutation test) has not been reviewed before.

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

Study title: **Reverse mutation test of SM-13496 in bacterial systems** Study no.: **2773**

Conducting laboratory and location: (b) (4)

Date of study initiation: 7/19/93

GLP compliance: Yes (Japanese testing guidelines)

QA statement: Yes

Drug, lot #, and % purity: SM-13496, lot no. 3CG001M, purity 100.9%

Key Study Findings

The mutagenic potential of lurasidone (SM-13496) was tested in *S. typhimurium* strains, TA100, TA98, TA1535, and TA1537, and *E. coli* strain WP2uvrA, without and with metabolic activation. In the dose-ranging study (15-5000 µg/plate), precipitation was detected at doses ≥500 µg/plate, both with and without S9. No cytotoxicity was observed. Based on these results, concentrations of 156, 313, 625, 1250, 2500, and 5000 µg/plate were selected for the definitive study. In the definitive study, precipitation was

detected at concentrations ≥625 µg/plate both with and without S9. There was no evidence of cytotoxicity or increases in revertants with any tester strain, with or without S9. Positive controls produced increases in revertants consistent with a positive response. In conclusion, lurasidone was not mutagenic under the test conditions.

Methods

Strains:	S. typhimurium strains, TA100, TA98, TA1535, and TA1537, and E. coli.strain WP2uvrA without and with metabolic activation (male Sprague-Dawley rat liver S9, □henobarbital and 5.6-benzoflavone inducers). Tester strains were obtained from the (b) (4) (b) (4). It was stated that phenotypic characteristics of each strain were tested and confirmed.
Concentrations in definitive study:	156, 313, 625, 1250, 2500, and 5000 µg/plate
Basis of concentration selection:	Dose-ranging study (15-5000 µg/plate)
Negative control:	DMSO
Positive control:	-S9: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2uvrA), sodium azide (TA1535) 9-aminoacridine (TA1535) +S9: 2-aminoanthracene (all tester strains)
Formulation/Vehicle:	Lurasidone was suspended in DMSO (it was not soluble in DMSO at a concentration of 50 mg/mL; ultrasonication was required for form suspension).
Incubation & sampling time:	The preincubation method was used for evaluation of mutagenicity. Prior to plating, the bacterial suspension was incubated with drug for 20 min at 37° C. Plates were incubated for 48 h at 37°C.

Study Validity: This is a valid study. Concentrations (and negative and positive controls) were tested in triplicate in both a dose-ranging study and the definitive study. The criterion for a positive response was a "dose dependent increase in the number of revertant colonies to at least twice as many as that of the solvent control". Statistical evaluation was not performed.

Results

In the dose-ranging study (15-5000 µg/plate), precipitation was detected at doses ≥500 µg/plate both with and without S9. No cytotoxicity was observed. Based on these results, concentrations of 156, 313, 625, 1250, 2500, and 5000 µg/plate were selected for the definitive study. There was no clear evidence of cytotoxicity, although the number of revertants was slightly decreased with TA100 in the absence of S9. There were no increases in revertants with any tester strain, either with or without S9. Positive controls produced increases in revertants consistent with a positive response.

In the definitive study, precipitation was detected at concentrations ≥625 µg/plate both with and without S9. There was no evidence of cytotoxicity or increases in revertants with any tester strain, with or without S9. Positive controls produced increases in revertants consistent with a positive response.

The results are summarized in the following sponsor's table:

Study Title: Reverse mutation test of SM-13496 in bacterial systems			**Test Article: lurasidone (SM-13496)**
Test for Induction of: Bacterial reverse mutation		**No. of Independent Assays: 2**	**Study No. 2773**
Strains: *Salmonella typhimurium* and *Escherichia coli*		**No. of Replicate Cultures: 3**	**Module Location 4.2.3.3.1.**
Metabolizing System: S9 liver fraction from Sprague-Dawley rat		**No. of Cells Analyzed/Culture: -**	**GLP Compliance: Yes**
Vehicles:	**For Test Article: Dimethyl sulfoxide (DMSO)**		
	For Positive Controls: Sterile purified water or DMSO		
Treatment: 20-minute pre-incubation + 48-hour treatment			**Date of Treatment: July, 1993**
Cytotoxic Effects: None			
Genotoxic Effects: None			

Metabolic Activation	Test Article	Dose Level (µg/plate)	Mean of revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2*uvrA*
Dose-finding Assay							
Without Activation	Vehicle control	DMSO	30	108	11	6	23
	Positive control[a]	[a]	354	541	324	751	184
	Lurasidone	15	32	104	12	7	14
		50	30	99	14	11	18
		150	30	105	10	9	20
		500[b]	29	103	7	9	16
		1500[b]	22	105	8	5	16
		5000[b]	32	82	8	6	21
With Activation	Vehicle control	DMSO	36	91	8	10	23
	Positive control[c]	[c]	292	549	120	170	695
	Lurasidone	15	31	101	11	12	30
		50	42	113	9	13	26
		150	52	117	10	11	29
		500[b]	34	92	10	9	21
		1500[b]	35	97	10	10	19
		5000[b]	33	96	8	7	20

Continued on the next page

Reverse mutation test of SM-13496 in bacterial systems (continued)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Mean of revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2*uvrA*
Main assay							
Without Activation	Vehicle control	DMSO	26	88	8	6	13
	Positive control[a]	[a]	276	654	337	770	174
	Lurasidone	156	28	119	6	4	13
		313	24	122	9	7	18
		625[b]	24	99	7	7	19
		1250[b]	27	100	8	10	15
		2500[b]	29	106	8	8	17
		5000[b]	27	102	10	9	14
With Activation	Vehicle control	DMSO	36	87	10	7	28
	Positive control[c]	[c]	266	496	144	120	681
	Lurasidone	156	42	108	7	8	25
		313	46	101	9	7	26
		625[b]	49	112	10	8	21
		1250[b]	32	102	7	5	24
		2500[b]	39	107	9	7	27
		5000[b]	33	102	8	8	28

[a] TA98 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide 0.1 µg/plate
TA100 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide 0.01 µg/plate
TA1535 sodium azide 0.5 µg/plate
TA1537 9-aminoacridine 80 µg/plate
WP2*uvrA* 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide 0.01 µg/plate

[b] Precipitates of the test compound were observed.

[c] TA98 2-aminoanthracene 0.5 µg/plate
TA100 2-aminoanthracene 1.0 µg/plate
TA1535 2-aminoanthracene 2.0 µg/plate
TA1537 2-aminoanthracene 2.0 µg/plate
WP2 *uvrA* 2-aminoanthracene 10 µg/plate

7.2. *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study no.: **2749**

Conducting laboratory and location: (b) (4)

Date of study initiation: 5/26/93

GLP compliance: Yes (Japanese testing guidelines)

QA statement: Yes

Drug, lot #, and % purity: SM-13496, lot no. 3CG001M, purity = 100.9%

Key Study Findings

The clastogenic potential of lurasidone (SM-13496) was tested using Chinese hamster lung cells in the absence and presence of metabolic activation (-S9 or +S9). Based on the results of a cytotoxicity assay (20-5000 μg/mL), a definitive study was conducted using doses of 0, 50, 100, 200, and 400 μg/mL (in the direct method) and 0, 625, 1250, 2500, and 5000 μg/mL (in the metabolic activation method). In the definitive study, there were no concentration-related increases in structural aberrations, although the number of cells with structural aberrations was increased at certain concentrations. The percent of cells with polyploidy was increased at 5000 μg/mL under one condition (6 hr, -S9); however, the value did not exceed that at lower concentrations under other conditions. The incidence of cells with structural chromosomal aberrations clearly increased in the positive control groups [mitomycin C (direct method) and cyclophosphamide (metabolic activation method)]. In conclusion, lurasidone did not exhibit potential to induce chromosomal aberrations in Chinese hamster lung cells under the conditions of this test.

Methods

The clastogenic potential of SM-13496 was tested using Chinese hamster lung cells in the absence and presence of metabolic activation (male Sprague-Dawley rat liver S9); inducers: phenobarbital, 5, 6-benzoflavone. SM-13496 was suspended in 1 % CMC (stated to be insoluble in physiological saline or DMSO) and 1 % CMC was used as the negative control. Positive controls were Mitomycin C (direct) and cyclophosphamide (indirect). Cells were incubated with drug for 24 and 48 hrs in the absence of S9 and for 6 hrs in the presence of S9 (and absence of S9); concentrations were tested in duplicate. Separate cultures (in duplicate) were used for assessment of cytotoxicity and for clastogenicity. One hundred metaphases per slide were scored for structural and numerical chromosomal aberrations. The criteria for a positive response were as follows: (1) a ≥10% increase in aberrations compared to control, (2) increase should demonstrate "reproducibility or dose dependency". A 5-10% increase in aberrations would be considered a "marginal" response. Statistical analyses were not performed.

Cell line:	Chinese hamster lung cells
Concentrations in definitive study:	0, 50, 100,200, and 400 μg/mL in the absence of metabolic activation (-S9) and 0, 625, 1250, 2500, and 5000 μg/mL in the presence of metabolic activation (+S9).
Basis of concentration selection:	Cytotoxicity assay (20-5000 μg/mL): growth rate was inibited by "50% or less" at concentrations of ≥625 μg/mL in the -S9, 24 hr condition, and at concentrations of ≥313 μg/mL in the -S9, 48 hr.
Negative control:	1 % carboxymethyl cellulose (CMC)
Positive control:	Mitomycin C (direct) and cyclophosphamide (in-direct)
Formulation/Vehicle:	SM-13496 suspended in 1 % CMC (SM-13496 was stated to be insoluble in physiological saline or DMSO)
Incubation & sampling time:	Cells were incubated with drug for 24 and 48 hrs in the absence of S9 and for 6 hrs in the presence of S9 (and absence of S9); concentrations were tested in duplicate. Separate cultures (in duplicate) were used for assessment of cytotoxicity and for clastogenicity. One hundred metaphases per slide were scored for structural and numerical chromosomal aberrations.

Study Validity

This is a valid study. The criteria for a positive response were as follows: (1) a ≥10% increase in aberrations compared to control, (2) increase should demonstrate "reproducibility or dose dependency". A 5-10% increase in aberrations would be considered a "marginal" response.

Results

In the cytotoxicity assay (20-5000 μg/mL), the high concentration was not scored due to methodological problems. The growth rate was inhibited by >50% at concentrations of ≥625 μg/mL in the -S9, 24 hr condition, and at concentrations of ≥313 μg/mL in the -S9, 48 hr condition (according to the summary table, not included here). No cytotoxicity was observed in the presence of S9. Based on these results, the definitive study was conducted using doses of 0, 50, 100,200, and 400 μg/mL (-S9) and 0, 625, 1250, 2500, and 5000 μg/mL (+S9).

In the definitive study, there appeared to be some problems with drug suspension. It was noted that "The test chemical was almost evenly suspended" at concentrations of 200 and 400 μg/mL (-S9, 24 and 48 hr incubation) and at all concentrations tested in the 6-hr incubation assays (±S9). [(In the cytotoxicity assay, it was noted that concentrations of 625, 1250, 2500, and 5000 μg/mL were "evenly suspended in the medium", whereas at 313 μg/mL was adequately suspended for the -S9 (24, 48-hr) assays, but not for the+S9 (6-hr) assay].

There were no concentration-related increases in structural aberrations, although the number of cells with structural aberrations was increased at certain concentrations. The percent of cells with polyploidy was increased at 5000 μg/mL under one condition (6 hr, -S9); however, the value did not exceed that at lower concentrations under other conditions. No cytoxicity data were provided for the definitive tests. Statistical analyses were not performed. The data were summarized in the following sponsor's table:

Chromosomal Aberration Test

Study Title: In vitro chromosomal aberration test of SM-13496 in Chinese hamster lung cells (CHL/IU)			**Test Article: lurasidone (SM-13496)**
Test for Induction of: Chromosomal aberration		**No. of Independent Assays: 1**	**Study No. 2749**
Strains: Chinese hamster lung cells (CHL/IU)		**No. of Replicate Cultures: Two**	**Module Location: 4.2.3.3.1.**
Metabolizing System: S9 mixture from Sprague-Dawley rat		**No. of Cells Analyzed/Culture: 100**	**GLP Compliance: Yes**
Vehicles:	**For Test Article: 1% Carboxymethylcellulose sodium solution (CMC-Na)**	**For Positive Controls: Physiological saline**	
Treatment: Direct method, 24 and 48 hours continuous treatment **Metabolic activation method, 6 hours treatment followed by 18 hour-expression time**			**Date of Treatment: May, 1993**

Treatment group	Concentration (μg/mL)	No. of observed cells	Numbers of structural aberrations						Cells with structural aberrations (%)		Judge (+/-)	Polyploid cells (%)	Judge (+/-)
			gap	ctb	cte	csb	cse	frg	+g	-g			
Direct method (24 hours continuous treatment)													
Solvent Control[a]	--	200	0	0	0	0	0	0	0.0	0.0	-	0.0	-
Positive Control[b]	0.03	200	5	44	51	0	0	0	43.0	41.0	+	0.0	-
Lurasidone	50	200	1	1	1	0	0	0	1.5	1.0	-	0.0	-
	100	200	0	1	0	0	0	0	0.5	0.5	-	0.0	-
	200[c]	200	1	0	1	0	0	0	1.0	0.5	-	0.5	-
	400[c]	200	0	0	0	0	0	0	0.0	0.0	-	0.5	-
Direct method (48 hours continuous treatment)													
Solvent Control[a]	--	200	0	1	0	0	1	0	1.0	1.0	-	0.0	-
Positive Control[b]	0.03	200	4	57	93	0	2	0	61.5	61.0	+	0.0	-
Lurasidone	50	200	0	0	0	0	0	0	0.0	0.0	-	0.0	-
	100	200	0	1	0	0	0	0	0.5	0.5	-	0.0	-
	200[c]	200	0	1	1	0	1	0	1.5	1.5	-	0.0	-
	400[c]	200	0	1	0	0	0	0	0.5	0.5	-	0.0	-
Metabolic activation method (with S9mix)													
Solvent Control[a]	--	200	0	0	0	0	0	0	0.0	0.0	-	0.0	-
Positive Control[d]	15	200	2	83	139	0	0	0	78.0	77.5	+	0.0	-
Lurasidone	625[c]	200	0	0	0	0	0	0	0.0	0.0	-	1.0	-
	1250[c]	200	1	1	0	0	0	0	1.0	0.5	-	0.5	-
	2500[c]	200	0	0	0	0	0	0	0.0	0.0	-	1.0	-
	5000[c]	200	0	1	1	0	0	0	1.0	1.0	-	0.5	-
Metabolic activation method (without S9mix)													
Solvent Control[a]	--	200	1	2	0	0	0	0	1.5	1.0	-	0.5	-
Positive Control[d]	15	200	0	2	1	0	0	0	1.5	1.5	-	0.0	-
Lurasidone	625[c]	200	0	2	1	0	0	0	1.5	1.5	-	0.0	-
	1250[c]	200	0	0	0	0	0	0	0.0	0.0	-	0.0	-
	2500[c]	200	2	3	1	0	0	0	3.0	2.0	-	0.5	-
	5000[c]	200	0	2	1	0	0	0	1.5	1.5	-	1.0	-

a 1% CMC-Na; b Mitomycin C; c The test chemical was almost evenly suspended; d Cyclophosphamide
A statistic method was not used for the evaluation of the results of this test.
frg = fragmentations, +g = aberrations including gaps, -g = aberrations excluding gaps, gap = chromatid gaps and chromosome gaps,
ctb = chromatid breaks, cte = chromatid exchanges, csb = chromosome breaks, cse = chromosome exchanges

Conclusion: No genotoxic effect was observed under the conditions of this study. There was cytotoxic effect in the direct method (the dose level at which the growth rate was suppressed >50% vs. the solvent control was 625 μg/mL and higher, and 313 μg/mL and higher in the 48-h-treatment group); in the metabolic activation method the drug did not show cytotoxicity at any dose level either in the presence or in the absence of S9).

7.3 *In Vivo* Clastogenicity Assay in Rodents (Micronucleus Assay)

Study title: **Micronucleus test of SM-13496 in mice** **Study no.: 2767**

Conducting laboratory and location: (b) (4)

Date of study initiation: 7/8/93

GLP compliance: Yes (Japanese testing guidelines)

QA statement: Yes

Drug, lot #, and % purity: SM-13496, Lot 3CGOOIM, purity = 100.9%

Key Study Findings

The study was conducted in male CD-1 mice at SM-13496 single oral doses of 500, 1000, and 2000 mg/kg (selected on the basis of a dose-ranging study). No deaths were reported; therefore, doses of 0, 0, 500, 1000, and 2000 mg/kg were selected for the definitive study. Cyclophosphamide (80 mg/kg) was used as the positive control. In the time-course part of the study, bone marrow smears were prepared 24, 48, and 72 h. after a single oral administration of the HD to examine the incidence of micronucleated polychromatic erythrocytes (MNPCEs). The incidence of MNPCEs tended to increase in the 48 h. treatment group (statistically not significant). Based on this finding, in the definitive dose-response study the specimens were prepared 48 h. after drug administration in all dosed groups. Clinical signs (ptosis and reduced spontaneous motor activity) were observed at all doses. There was no evidence of cytotoxicity at any of the doses tested. No significant increase in the incidence of micronuclei was found in any dose group compared to the solvent control, and no significant difference was found in the ratio of polychromatic erythrocytes (PCEs). Cyclophosphamide produced 1.7- fold increases in the % micronucleated cells (per total PCEs examined). Based on these findings, SM-13496 has no potential to induce micronuclei in murine bone marrow under the tested conditions.

Methods

SM-13496 was administered orally to male CD-1 mice (8 wks old (b) (4)). Food and water were provided ad lib. Cyclophosphamide (80 mg/kg) was used as the positive control. A dose-ranging study was conducted using acute oral doses of 500, 1000, and 2000 mg/kg. Clinical signs and body wt were recorded on Days 0 (untreated), 0 (solvent), 1, 2, 3, 5, and 7 postdosing. No deaths were reported in this study; therefore, doses of 0, 0, 500, 1000, and 2000 mg/kg were selected for the definitive study. In the time-course portion of the study, animals (5/group) received an acute 0, 0, or 2000 mg/kg dose and were sacrificed at 24, 48, and 72 hrs postdosing; the treated negative control and the positive control groups were sacrificed at 24 hrs postdosing. In the dose-response portion of the study, animals (5/group) were dosed at single doses of 0, 0, 500, 1000, and 2000 mg/kg and sacrificed at 48 hrs postdosing. The solvent control group was sacrificed at 48 hrs postdosing, and the positive control was sacrificed at 24 hrs postdosing. At sacrifice, bone marrow cells were collected from the femur. One thousand PCEs were examined for micronuclei in each animal (1/2 of the recommended cells according

to the OECD guidelines); the PCE:NCE ratio was assessed in 1000 eryhrocytes per animaL. The criteria for a positive response were a statistically significant, reproducible or dose-dependent increase in micronucleated PCEs.

Doses in definitive study: 0, 0, 500, 1000, and 2000 mg/kg

Frequency of dosing: Single

Route of administration: Oral

Formulation/Vehicle: Solvent: 0.5% MC

Species/Strain: CD-1 mice

Number/Sex/Group: 5/group (males only)

Satellite groups: No

Basis of dose selection: Dose-ranging study at 500, 1000, and 2000 mg/kg

Negative control: Vehicle: 0.5% MC

Positive control: Cyclophosphamide (80 mg/kg)

Study Validity: Valid criteria for a positive response were used: a statistically significant, reproducible or dose-dependent increase in micronucleated PCEs. One thousand PCEs were examined for micronuclei in each animal (1/2 of the recommended cells according to the OECD guidelines).

Results

In the dose-ranging study using acute oral doses of 500, 1000, and 2000 mg/kg no deaths were reported; therefore, doses of 0, 0, 500, 1000, and 2000 mg/kg were selected for the definitive study. In the time-course part of the study, bone marrow smears were prepared 24, 48, and 72 h. after a single oral administration of the HD (2000 mg/kg) to examine the incidence of micronucleated polychromatic erythrocytes (MNPCEs). The incidence of MNPCEs tended to increase in the 48 h. treatment group (0.44% vs. 0.26% in the solvent control, statistically not significant). Based on this finding, in the definitive dose-response study the specimens were prepared 48 h. after a single oral administration of SM-13496 at doses of 500, 1000, and 2000 mg/kg. No significant increase in the incidence of micronuclei was found in any dose group compared to the solvent control, and no significant difference was found in the ratio of polychromatic erythrocytes (PCEs).
Clinical signs were observed at all doses. Ptosis and reduced spontaneous motor activity were noted at 30 min to 2 hrs postdosing at all doses. There were no effects on body weight. There was no evidence of cytotoxicity at any of the doses tested. There was no increase in micronuclei (% of total PCEs) at any dose in either the time-course or dose-dependency study.
Cyclophosphamide produced 1.7- fold increases in the % micronucleated cells (per total PCEs examined).

The results are summarized in the following sponsor's tables:

Study Title: Micronucleus Test of SM-13496 in Mice		Test Article: lurasidone (SM-13496)
Test for Induction of: Micronucleus	Treatment Schedule: Single dose	Study No. 2767
Species/Strain: Male Mouse/ CD-1 (ICR)	Sampling Time: 24, 48 and 72 hours	Module Location: 4.2.3.3.2.
Age: 8-week old	Method of Administration: Oral gavage	GLP Compliance: Yes
Cells Evaluated: Polychromatic erythrocytes (PCE)	Vehicle/Formulation: Suspension in aqueous 0.5% methylcellulose	
No. of Cells Analyzed/Animal: 1000 PCE		Date of Dosing: July, 1993
Special Features: None		
Toxic/Cytotoxic Effects: All groups treated with lurasidone showed toxic signs of ptosis and decreased spontaneous activities.		
Genotoxic Effects: None		
Evidence of Exposure: None		

Treatment group	Dose (mg/kg)	Sampling time (hr)	No. of Animals	%PCE in PCE+NCE (SD)	% MNPCE in PCE (SD)
Time-course study					
Untreated Control	--	--	5	54.3 (10.68)	0.26 (0.182)
Vehicle control (0.5% Methylcellulose solution)	--	24	5	53.5 (10.58)	0.26 (0.167)
Positive control (Cyclophosphamide)	80	24	5	44.4 (12.17)	4.50 (1.806)**
Lurasidone	2000	24	5	59.2 (5.01)	0.08 (0.084)*
	2000	48	5	55.0 (4.18)	0.44 (0.297)
	2000	72	5	59.7 (8.04)	0.20 (0.187)
Dose-response study					
Untreated Control	--	--	5	41.9 (3.67)*	0.28 (0.205)
Vehicle control (0.5% Methylcellulose solution)	--	48	5	51.6 (7.68)	0.14 (0.055)
Positive control (Cyclophosphamide)	80	24	5	35.1 (11.14)*	4.88 (1.599)**
Lurasidone	500	48	5	45.6 (9.01)	0.16 (0.055)
	1000	48	5	47.1 (4.71)	0.20 (0.071)
	2000	48	5	51.2 (5.47)	0.22 (0.110)

PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes; MNPCE = Micronucleated polychromatic erythrocytes
The chemical was administered at a rate of 10 mL/kg.
The incidence of MNPCE was statistically analyzed by the Kastenbaum and Bowman's method (significance levels: $p < .05$ and $p < .01$) and t-test was employed to analyze the ratio of PCE
(significance levels: $p < .05$ and $p < .01$, two-tailed analysis). *: $p < .05$, **: $p < .01$ (Compared with the solvent control group)

Conclusion: Based on the study findings, SM-13496 has no potential to induce micronuclei in murine bone marrow under the tested conditions.

7.4 Other Genetic Toxicity Studies

7.4.1. Lurasidone Metabolite ID-11614

Lurasidone is extensively metabolized in several animal species and in humans (See ADME). Since there are no unique human metabolites, toxicity and mutagenicity studies for lurasidone metabolites were not conducted. However, as stated by the sponsor "one of the lurasidone metabolites, ID-11614, was evaluated in an acute toxicity study and in a mutagenicity study (Ames test) during the course of development for another investigational drug". The relevant data are summarized below.

Study title: **Reverse mutation test of ID-11614 in bacterial systems** Study no **3117**

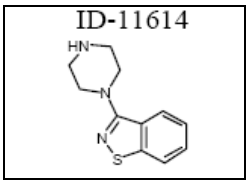
Conducting laboratory and location: (b) (4)

Date of study initiation: 1/31/96

GLP compliance: Yes (Japanese testing guidelines)

QA statement: Yes

Drug, lot #, and % purity: ID-11614 (lot no. 5CG001, purity = 99.9%)



Key Study Findings

The mutagenic potential of metabolite ID-11614 (a product of oxidative *N*-dealkylation of lurasidone and one of the primary circulating metabolites) was evaluated in vitro using a reverse mutation test (Ames assay) with four strains of *Salmonella typhimurium* (TA100, TA98, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2*uvrA*), with and without a rat liver metabolic activation system (S9 mix). In the preliminary assay, cytotoxicity was observed at ≧ 1250 μg/plate for TA100, TA1535 and TA1537 without S9 mix, at ≧ 313 μg/plate for TA98 without S9 mix, at 5000 μg/plate for WP2*uvrA* without S9 mix, at 5000 μg/plate for TA100, TA98, TA1537 and WP2*uvrA* with S9 mix, and at ≧ 1250 μg/plate for TA1535 with S9 mix. Based on the results of the preliminary assay, the main assays were conducted twice at dose levels ranging from 19.5 to 5000 μg/plate. Precipitation of the test substance was observed at ≧ 2500 μg/plate with S9 mix. The test drug did not induce a 2-fold or greater increase in revertants relative to solvent control in any of the tester strains up to the maximum tested concentration of 5000 μg/plate. The positive control chemicals produced the expected increase in revertants, indicating that the microbial mutagenesis assay was performed properly. In conclusion, ID-11614 was not mutagenic under the test conditions.

Methods

The mutagenic potential of ID-11614 was tested using S. typhimurium strains, TA100, TA98, TA1535, and TA1537, and E. coli strain, WP2uvrA. ID-11614 was dissolved in DMSO, which was used as the negative control. ID-11614 was stated to be stable in DMSO (no data provided). The phenotypic characteristics of the tester strains were stated to have been confirmed. The positive controls used were as follows: (a) 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide [TA100, TA98, WP2uvrA], sodium azide [TA1535], 9-aminoacridine [TA1537] in the absence of metabolic activation and (b) 2-aminoanthracene for all tester strains in the presence of metabolic activation. Metabolic activation was accomplished by addition of male Sprague-Dawley rat liver S9 (phenobarbital- and 5, 6-benzoflavone-induced). The pre-incubation method was used. ID-11614 was incubated with bacterial suspensions for 20 mi at 37° C prior to plating. Plates were incubated for 48 hrs at 37° C. Revertant colonies were quantitated using automatic counting methods. In the definitive study (two separate assays, I and II), concentrations were tested in duplicate. The criterion for a positive response was a "... dose-dependent increase in the number of revertant colonies to at least twice as many as that of the solvent control."

Strains:	*S. typhimurium* strains, TA100, TA98, TA1535, and TA1537, and *E. coli* strain WP2uvrA without and with metabolic activation (male Sprague-Dawley rat liver S9, phenobarbital and 5.6-benzoflavone inducers). It was stated that phenotypic characteristics of each strain were tested and confirmed.
Concentrations in definitive study:	**- S9:** 39.1, 78.1, 156, 313, 625, and 1250 µg/plate for TA100, TA1535, and TA1537. 19.5, 39.1, 78.1, 156, 313, and 625 µg/plate for TA98. 156, 313, 625, 1250, 2500, and 5000 µg/plate for WP2uvrA. **+S9**: 156, 313, 625, 1250, 2500, and 5000 µg/plate for TA100, TA98, TA1537, and WP2uvrA. 78.1, 156, 313, 625, 1250, and 2500 µg//plate for TA1535.
Basis of concentration selection:	Preliminary study at levels up to 5000 µg/plate
Negative control:	DMSO
Positive control:	-S9: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2uvrA), sodium azide (TA1535) 9-aminoacridine (TA1535) +S9: 2-aminoanthracene (all tester strains)
Formulation/Vehicle:	ID-11614 was dissolved in DMSO, which was used as negative control. (ID-11614 was stated to be stable in DMSO required for form suspension).
Incubation & sampling time:	ID-11614 was incubated with bacterial suspensions for 20 min at 37° C prior to plating. Plates were incubated for 48 hrs at 37° C.

Study Validity: This is a valid study. In the definitive study (two separate assays), concentrations were tested in duplicate. The criterion for a positive response was a “dose- dependent increase in the number of revertant colonies to at least twice as many as that of the solvent control”. Statistical evaluation was not performed. The positive control chemicals produced the expected increase in revertants, indicating that the microbial mutagenesis assay was performed properly.

Results

The results are summarized in the following sponsor's table:

Study Title: Reverse Mmutation Test of ID-11614 in Bacterial Systems						**Test Article: ID-11614 (metabolite of lurasidone)**	
Test for Induction of: Bacterial reverse mutation		**No. of Independent Assays: 2**				**Study No. 3117**	
Strains: *Salmonella typhimurium* and *Escherichia coli*		**No. of Replicate Cultures: 2**				**Module Location: 4.2.3.7.5.**	
Metabolizing System: S9 liver fraction from Sprague-Dawley rat		**No. of Cells Analyzed/Culture: -**				**GLP Compliance: Yes**	
Vehicles:	**For Test Article: Dimethyl sulfoxide (DMSO)**						
	For Positive Controls: Sterile purified water or DMSO						
Treatment: 20-minute pre-incubation + 48-hour treatment					**Date of Treatment: February, 1996**		
Cytotoxic Effects: Yes							
Genotoxic Effects: None							

Metabolic Activation	**Test Article**	**Dose Level (µg/plate)**	**Mean of revertants per Plate**				
			TA98	**TA100**	**TA1535**	**TA1537**	**WP2*uvrA***
Main assay I							
Without Activation	Vehicle control	DMSO	14	136	14	10	22
	Positive control[a]	[a]	389	623	315	535	166
	ID-11614	19.5	21	NT	NT	NT	NT
		39.1	24	153	11	11	NT
		78.1	15	159	9	5	NT
		156	19	163	11	10	26
		313	28	150	9	16	27
		625	11[b]	173	12	15	27
		1250	NT	1108[b]	[b]	11[b]	20
		2500	NT	NT	NT	NT	17[b]
		5000	NT	NT	NT	NT	21[b]
With Activation	Vehicle control	DMSO	42	139	13	12	26
	Positive control[c]	[c]	242	654	216	142	550
	ID-11614	78.1	NT	NT	10	NT	NT
		156	36	195	12	13	30
		313	32	210	10	13	21

Continued on the next page

In the preliminary study, precipitation was detected at 5000 µg/plate in the presence of S9. Cytotoxicity (decrease in revertants) was observed with all tester strains (+/- S9) at concentrations of 1250 and/or 5000 µg/plate. There were slight increases in revertants with TA100, without S9 (20% at 313 µg/plate) and with S9 (32% at 1250 µg/plate) (these concentrations were just below the cytotoxic concentrations). Based on the results of the preliminary assay, the main assays were conducted twice at dose levels ranging from 19.5 to 5000 µg/plate. In the definitive study (Main assay I), cytotoxicity (decrease in revertants) was observed at 625 µg/plate (-S9) for TA98 and at 1250 µg/plate for the other tester strains (-S9), and at 2500 µg/plate for all tester strains in the presence of S9. With TA98, there was an about 2-fold increase in revertants at the highest non-cytotoxic concentration (i.e., 313 µg/plate) in the absence of S9 (27 and 28 vs 13 and 14 revertants for the negative control). In Main assay II, cytotoxicity was observed

at similar concentrations as in Main assay I. There were no notable increases in revertants with any tester strain, either in the absence or presence of S9.
In both assays, the positive controls produced increases in revertants consistent with a positive response.

Reverse mutation test of ID-11614 in bacterial systems (continued)

Metabolic Activation	Test Article	Dose Level (µg/plate)	Mean of revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2*uvrA*
With Activation	ID-11614	625	41	217	8	11	23
		1250	30	202	6	17	30
		2500[d]	39	163[b]	6[b]	14[b]	33[b]
		5000[d]	0[b]	92[b]	NT	7[b]	23[b]
Main assay II							
Without Activation	Vehicle control	DMSO	18	128	8	7	22
	Positive control[a]	[a]	366	560	309	470	154
	ID-11614	19.5	21	NT	NT	NT	NT
		39.1	16	147	12	12	NT
		78.1	16	156	9	5	NT
		156	17	140	6	4	22
		313	16	138	9	8	25
		625	20[b]	134	12	13	28
		1250	NT	128[b]	13[b]	10[b]	22
		2500	NT	NT	NT	NT	15[b]
		5000	NT	NT	NT	NT	17[b]
With Activation	Vehicle control	DMSO	46	136	11	10	26
	Positive control[c]	[c]	253	611	205	131	511
	ID-11614	78.1	NT	NT	14	NT	NT
		156	31	194	14	13	27
		313	27	189	12	18	21
		625	39	202	16	14	27
		1250	39	240	10	15	25
		2500[d]	30[b]	165[b]	12[b]	12[b]	28[b]
		5000[d]	0[b]	0[b]	NT	7[b]	17[b]

a TA98 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide 0.1 µg/plate; TA100 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide 0.01 µg/plate; TA1535 sodium azide 0.5 µg/plate; TA1537 9-aminoacridine 80 µg/plate; WP2*uvrA* 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide 0.01 µg/plate
b Toxic effect was observed.
c TA98 2-aminoanthracene 0.5 µg/plate ; TA100 2-aminoanthracene 1.0 µg/plate ; TA1535 2-aminoanthracene 2.0 µg/plate; TA1537 2-aminoanthracene 2.0 µg/plate; WP2 *uvrA* 2-aminoanthracene 10 µg/plate
d Precipitates of the test compound were observed.
NT: Not tested

Conclusion: Based on these results, ID-11614 was not mutagenic under the test conditions.

7.4.2. Lurasidone Impurities

7.4.2.1. (b) (4)

(b) (4), a starting material and potential impurity of lurasidone [chemical name (b) (4) (b) (4) belongs to a chemical class with a structural alert for potential genotoxicity. The mutagenic potential of (b) (4) was evaluated in an in vitro bacterial reverse mutation (Ames) test.

(b) (4)

Study title: **Reverse mutation test of** (b) (4) **in bacterial systems** Study no.: **A02097**

Conducting laboratory and location: (b) (4)

Date of study initiation: 2/19/2003

GLP compliance: Yes (Japanese testing guidelines)

QA statement: Yes

Drug, lot #, and % purity: (b) (4) (lot M02A-051-01A, purity = 99.1%)

Key Study Findings

The mutagenic potential of a compound code named (b) (4), starting material and potential impurity of lurasidone) was evaluated *in vitro* using a reverse mutation test (Ames assay) with four strains of *Salmonella typhimurium* (TA100, TA98, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2*uvrA*), with and without metabolic activation. In the dose-finding assay, the highest dose was 5000 µg/plate (the maximum dose recommended in the guidelines), and the assay was conducted with 7 doses. The main assay was conducted at dose levels ranging from 313 to 5000 µg/plate, with 5 doses. In both dose-finding and main assays, no bacterial growth inhibition was observed either with or without metabolic activation. Precipitation of the test compound was not observed up to the highest tested concentration. Throughout the assays, the test compound did not show any dose-dependent increase in the number of revertant colonies to at least twice as many as that of the vehicle control in any of the strains with or without metabolic activation. The positive control chemicals induced more than 2-fold increase in revertant colonies in each strain as compared to the vehicle control, which indicated that the assays were performed properly. Based on these results, (b) (4) was not mutagenic under the test conditions.

Methods

Ames test was conducted in four strains of *Salmonella typhimurium* (TA100, TA98, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2*uvrA*) (Study A02097) (b) (4) was tested with and without a rat liver metabolic activation system (S9 mix). In the dose-finding assay, the highest dose of the test chemical was 5000 µg/plate (the maximum dose recommended in the guidelines), and the assay was conducted with 7 doses at a dilution factor of 4 for each strain. The main assay was conducted at dose levels ranging from 313 to 5000 µg/plate, with 5 doses at a dilution factor of 2 for each strain.

Strains: *S. typhimurium* strains, TA100, TA98, TA1535, and TA1537, and *E. coli* strain WP2uvrA without and with metabolic activation (male Sprague-Dawley rat liver S9, phenobarbital and 5.6-benzoflavone inducers). It was stated that phenotypic characteristics of each strain were tested and confirmed

Concentrations in definitive study: 313, 625, 1250, 2500, and 5000 µg/plate.

Basis of concentration selection: A dose-finding study at concentrations of up to 5000 µg/plate

Negative control: DMSO

Positive controls:

Bacterial strain	Without S9 Mix (µg/plate)		With S9 Mix (µg/plate)	
TA100	AF-2	(0.01)	2AA	(1.0)
TA1535	NaN_3	(0.5)	2AA	(2.0)
WP2 *uvrA*	AF-2	(0.01)	2AA	(10.0)
TA98	AF-2	(0.1)	2AA	(0.5)
TA1537	ICR-191	(1.0)	2AA	(2.0)

*** AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide**
*** NaN3: Sodium azide**
*** ICR-191: 6-Chloro-9-[3-(2-chloroethylamino)-propylamino]-2-methoxyacridine dihydrochloride**
*** 2AA: 2-Aminoanthracene**

Formulation/Vehicle: (b) (4) was dissolved in DMSO, which was used as negative control.

Incubation & sampling time: (b) (4) was incubated with bacterial suspensions for 20 min at 37° C prior to plating. Plates were incubated for 48 hrs at 37° C.

Study Validity:

This is a valid study. The assays were carried out in duplicate plating for each dose level except for the vehicle control. The vehicle control groups were treated in triplicate. The criterion for a positive response was a "dose-dependent increase in the number of revertant colonies to at least twice as many as that of the solvent control". Statistical evaluation was not performed. The positive control chemicals produced the expected increase in revertants, indicating that the microbial mutagenesis assay was performed properly.

Results

The results are summarized in the following sponsor's tables:

(b) (4) **Ames Test: Dose-finding assay**

Name of the test chemical: (b) (4)

Test dates: From February 19 to February 21, 2003

With (+) or Without(−) S9 Mix	Dose level (μg/plate)	Number of revertant colonies/plate									
		Base-pair substitution type						Frameshift type			
		TA100		TA1535		WP2 *uvrA*		TA98		TA1537	
S9 Mix (−)	Vehicle control	96	81	14	16	26	19	20	21	13	12
		104 (	94)	12 (	14)	22 (	22)	17 (	19)	7 (	11)
	1.2	96		13		20		8		7	
		111 (	104)	8 (	11)	20 (	20)	15 (	12)	8 (	8)
	4.9	99		19		22		17		14	
		100 (	100)	14 (	17)	31 (	27)	24 (	21)	8 (	11)
	20	109		13		33		16		6	
		93 (	101)	15 (	14)	20 (	27)	18 (	17)	8 (	7)
	78	91		15		22		23		7	
		96 (	94)	16 (	16)	22 (	22)	19 (	21)	8 (	8)
	313	88		10		30		16		4	
		93 (	91)	9 (	10)	21 (	26)	26 (	21)	4 (	4)
	1250	125		14		20		18		7	
		104 (	115)	20 (	17)	26 (	23)	23 (	21)	9 (	8)
	5000	150		19		19		14		8	
		146 (	148)	13 (	16)	27 (	23)	14 (	14)	5 (	7)
S9 Mix (+)	Vehicle control	108	105	13	13	26	21	26	26	15	16
		118 (	110)	12 (	13)	30 (	26)	30 (	27)	17 (	16)
	1.2	92		15		27		34		16	
		89 (	91)	8 (	12)	28 (	28)	25 (	30)	15 (	16)
	4.9	112		11		34		30		17	
		132 (	122)	15 (	13)	36 (	35)	21 (	26)	14 (	16)
	20	108		17		27		21		13	
		96 (	102)	14 (	16)	20 (	24)	20 (	21)	17 (	15)
	78	122		11		23		27		10	
		102 (	112)	18 (	15)	30 (	27)	32 (	30)	15 (	13)
	313	108		8		28		33		14	
		115 (	112)	9 (	9)	27 (	28)	27 (	30)	14 (	14)
	1250	149		10		25		26		16	
		120 (	135)	13 (	12)	28 (	27)	27 (	27)	17 (	17)
	5000	165		10		25		24		8	
		143 (	154)	12 (	11)	37 (	31)	27 (	26)	13 (	11)
Positive control without S9 Mix	Name	AF-2		NaN_3		AF-2		AF-2		ICR-191	
	Dose level (μg/plate)	0.01		0.5		0.01		0.1		1.0	
	Number of colonies/plate	668		409		194		616		3608	
		642 (	655)	422 (	416)	184 (	189)	664 (	640)	3421 (	3515)
Positive control with S9 Mix	Name	2AA		2AA		2AA		2AA		2AA	
	Dose level (μg/plate)	1.0		2.0		10.0		0.5		2.0	
	Number of colonies/plate	1230		270		1261		551		211	
		1058 (	1144)	280 (	275)	1196 (	1229)	556 (	554)	208 (	210)

Notes

( ): mean revertant colonies/plate

Positive controls

AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

NaN_3: Sodium azide

ICR-191: 6-Chloro-9-[3-(2-chloroethylamino)-propylamino]-2-methoxyacridine dihydrochloride

2AA: 2-Aminoanthracene

(b) (4) **Ames Test - Main assay**

Name of the test chemical:		(b) (4)								
Test dates:		From February 26 to February 28, 2003								
With (+) or Without(−) S9 Mix	Dose level (μg/plate)	Number of revertant colonies/plate								
		Base-pair substitution type						Frameshift type		
		TA100		TA1535		WP2 *uvrA*		TA98	TA1537	
S9 Mix (−)	Vehicle control	117	109	6	8	35	20	14 24	6	8
		90 (	105)	11 (	8)	19 (	25)	18 (19)	10 (	8)
	313	110		11		30		17	10	
		97 (	104)	6 (	9)	26 (	28)	19 (18)	8 (	9)
	625	108		8		30		23	6	
		111 (	110)	10 (	9)	22 (	26)	17 (20)	6 (	6)
	1250	133		8		19		16	4	
		122 (	128)	7 (	8)	21 (	20)	27 (22)	9 (	7)
	2500	146		7		16		21	5	
		140 (	143)	11 (	9)	19 (	18)	15 (18)	4 (	5)
	5000	144		17		19		20	7	
		125 (	135)	11 (	14)	25 (	22)	18 (19)	10 (	9)
S9 Mix (+)	Vehicle control	96	118	7	11	28	29	32 23	13	8
		115 (	110)	14 (	11)	19 (	25)	24 (26)	16 (	12)
	313	119		14		20		34	12	
		130 (	125)	10 (	12)	20 (	20)	22 (28)	7 (	10)
	625	139		15		31		29	14	
		131 (	135)	9 (	12)	30 (	31)	29 (29)	21 (	18)
	1250	120		8		31		20	19	
		142 (	131)	9 (	9)	30 (	31)	27 (24)	16 (	18)
	2500	130		8		35		30	10	
		145 (	138)	11 (	10)	36 (	36)	21 (26)	8 (	9)
	5000	137		6		41		15	16	
		144 (	141)	7 (	7)	30 (	36)	26 (21)	12 (	14)
Positive control without S9 Mix	Name	AF-2		NaN_3		AF-2		AF-2	ICR-191	
	Dose level (μg/plate)	0.01		0.5		0.01		0.1	1.0	
	Number of colonies/plate	634		341		188		696	5389	
		581 (	608)	395 (	368)	201 (	195)	666 (681)	5483 (	5436)
Positive control with S9 Mix	Name	2AA		2AA		2AA		2AA	2AA	
	Dose level (μg/plate)	1.0		2.0		10.0		0.5	2.0	
	Number of colonies/plate	1201		268		901		560	218	
		1254 (	1228)	221 (	245)	881 (	891)	533 (547)	206 (	212)

Notes

( ) ; mean revertant colonies/plate

Positive controls

AF-2 : 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

NaN_3 : Sodium azide

ICR-191 : 6-Chloro-9-[3-(2-chloroethylamino)-propylamino]-2-methoxyacridine dihydrochloride

2AA : 2-Aminoanthracene

As shown in the sponsor's tables, (b) (4) at concentration levels ranging from 313 to 5000 μg/plate, did not induce a 2-fold or greater increase in revertants relative to solvent control in any of the tester strains evaluated up to the maximum tested concentration, with and without a rat liver metabolic activation system (S9 mix). The test chemical did not produce cytotoxicity in the bacterial tester strains. Precipitation of the test compound was not observed up to the highest concentration. The positive control chemicals produced the expected increase in revertants, indicating that the microbial mutagenesis assay was performed properly.

Historical control data

		Vehicle control (the number of revertant colonies/plate)			Positive control (the number of revertant colonies/plate)		
Strain	S9	Mean ±	SD	Number of data	Mean ±	SD	Number of data
TA100	–	112 ±	11.6	316	537 ±	60.1	316
TA1535	–	9 ±	2.4	243	402 ±	58.3	243
WP2 *uvrA*	–	33 ±	6.8	207	169 ±	14.3	207
TA98	–	24 ±	4.9	331	621 ±	61.1	331
TA1537	–	10 ±	3.1	236	3580 ±	807.0	236
TA100	+	117 ±	12.5	303	1367 ±	97.2	303
TA1535	+	9 ±	2.2	234	276 ±	37.4	234
WP2 *uvrA*	+	37 ±	7.7	202	1266 ±	187.4	202
TA98	+	29 ±	4.8	323	589 ±	85.1	323
TA1537	+	18 ±	4.9	228	247 ±	54.2	227

The above are pooled data from January, 2002 to December, 2002 including screening data.

Positive control chemicals

Test strain	Chemical	Dose (μg/plate)
Without S9 mix		
TA100	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide	0.01
TA1535	Sodium azide	0.5
WP2 *uvrA*	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide	0.01
TA98	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide	0.1
TA1537	6-Chloro-9-[3-(2-chloroethylamino)-propylamino]-2-methoxyacridine dihydrochloride	1.0
With S9 mix		
TA100	2-Aminoanthracene	1.0
TA1535	2-Aminoanthracene	2.0
WP2 *uvrA*	2-Aminoanthracene	10.0
TA98	2-Aminoanthracene	0.5
TA1537	2-Aminoanthracene	2.0

In conclusion, (b) (4) was not mutagenic in the Ames test under the test conditions.

Comments: Regarding the further qualification of (b) (4), as stated by the Division at the pre-NDA meeting (May 22, 2009), "if the specification for this compound is below 0.15% of drug substance, no further (genotoxicity) testing is required in view of the negative Ames test." According to the reviewer chemist, Dr. Shastri Bhamidipati, (e-mail communication of 2/16/2010), the sponsor has tested the lurasidone free base (obtained prior to its conversion to the salt form) for (b) (4) by GC-MS method and has shown tha (b) (4) levels are below (b) (4) in a total of 25 batches (including batches used in nonclinical, clinical, and commercial scale); since the levels were below (b) (4) in the batches used in Phase III studies, the drug product at the MRHD (120 mg) will have no more than (b) (4) of the drug substance). Therefore, no further genetic toxicity testing of (b) (4) is needed.

7.4.2.2. (b) (4)

Another potentially genotoxic impurity, (b) (4), (b) (4) (structure shown below), was addressed in the following CMC communication and response from the sponsor (reproduced below).

(b) (4)

(b) (4)

(

Content of (b) (4) in Lurasidone HCl and (b) (4)

ID-11594		Lurasidone HCl		
Batch No.	Content of (b) (4) (ppm)	Batch No.[a]	Corresponding (b) (4) Batch No.	Content of (b) (4) (ppm)
N81102	(b) (4)	090307[c]	N81102 N81103	(b) (4)
N81103		090308[c]	N81103 N81201	
N81201		090309[c]		
		090310[c]		
N81202		100501[d]	N00201	
N00201		100502[d]	N00201 N00202	
N00202		100503[d]		
N00203		100504[d]	N00201 N00202 N81202	
		100505[d]	N00202 N00203	
		100506[d]		

(b) (4)

According to the reviewer chemist, Dr. Shastri Bhamidipati, "the sponsor has effectively shown that the levels of the (b) (4) impurity in the starting material (b) (4) and the drug substance were well controlled (b) (4)). Hence from CMC perspective we do not have further concerns about this impurity."

8 Carcinogenicity

Study title: **104-week oral gavage carcinogenicity and TK study with SM-13496 in mice**

Study no.:	6645-138
Conducting laboratory and location:	(b) (4), (b) (4)
Date of study initiation:	02/23/2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SM -13496, Lot E-278, 100.3%
CAC concurrence:	Yes

Key Study Findings

Neoplastic findings: Oral administration of Lurasidone HCl in 0.5% methylcellulose to Crl:CD-1®(ICR)BR mice (60/sex/dose) at doses of **0, 0, 30, 100, 300, and 1200/650** mg/kg/day **in males for 104 weeks** [HD reduced as of Day 410 due to excessive (>20%) weight loss] and at **0, 0, 30, 100, 300, and 650** mg/kg/d **in females for 98 weeks** (shorter dosing duration in females due to excessive mortality) did not produce neoplastic lesions in the males. In the females, however, statistically significant increases in neoplastic lesions [benign pituitary pars distalis adenoma and malignant mammary tumors (carcinoma, adenoacanthoma)] were induced at all tested dose levels, with highly significant positive trends vs. pooled control groups. In particular, the tests of overall trend and pairwise comparisons between the highest dose group and pooled control in females were statistically significant for mammary carcinoma, as were the tests of pooled mammary tumors (adenomas, carcinomas, carcinosarcomas, and adenoacanthomas). The pairwise comparisons of mammary carcinoma and adenoacanthoma at mid-high and middle dose to control were statistically significant; for the low dose group the difference for mammary carcinoma was close to the adjusted statistical significance ($p \approx 0.01$). The pairwise comparisons of pituitary pars distalis adenoma to the pooled controls were statistically significant in all dose groups. Some other neoplasms were increased in single dose groups without dose-dependence. Thus, the pooled cancers of the ovary were statistically significantly higher vs. pooled control in the middle dose group only and adrenal pheochromocytoma and islet cell adenoma of the pancreas were statistically significant in the mid-high dose group, but not in the highest dose group. No other tests achieved statistical significance.

Non-neoplastic findings: Dose-related significant increases in mortality occurred in females at 300 and 650 mg/kg/day. The proportion of females with a lack of estrus cycling was increased at 100 mg/kg/day and higher, supported by histopathology findings in the female reproductive system indicative of estrus cycle disruption, i.e., ovarian, uterine, cervical and vaginal atrophy. The estrus cycle disruption along with the marked elevation of serum prolactin and the increased incidence of tumors in the pituitary and mammary gland is likely related to the dopamine type 2-receptor antagonistic properties of the drug. Serum prolactin (measured during Week 52) was markedly and significantly elevated at all dose levels in comparison to control, and prolactin levels in females were 2 to 4 times higher than those in males in all dose groups. Plasma exposure to lurasidone increased with dose, less than dose-proportionally, in both males and females. Females had higher systemic exposure values (Cmax and AUC_{0-24}) than males across all collection time points, as well as higher systemic exposure after multiple dosing.

NOEL for neoplasia: M: 650 mg/kg/day (AUC_{0-24} 12 947 ng.hr/ml); F: NOEL not reached for pituitary adenoma and mammary malignant neoplasia (below LD of 30 mg/kg/day, AUC_{0-24} 1130 ng.hr/ml).

Adequacy of Carcinogenicity Study: Adequate; **Appropriateness of Test Models:** Yes

Evaluation of tumor findings: Mammary carcinomas and adenoacanthomas and benign pituitary pars distalis adenomas were drug related in females only, at all dose groups.

Note: All tables and figures are reproduced from the sponsor, unless otherwise indicated

Methods

Doses:	Males: **0, 30, 100, 300, 1200/650 mg/kg/d** for 104 weeks (HD reduced as of Day 410 due to weight loss) Females: **0, 30, 100, 300, 650 mg/kg/day** for 98 weeks (dosing duration reduced because of a rapid decline in survival)
Frequency of dosing:	Daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose in reverse osmosis water
Basis of dose selection:	Toxicological findings in a 14-Day Oral Gavage Toxicity Study at dose levels of 0, 100, 300, and 1000 mg/kg/day and 13-Week Oral Gavage Preliminary Carcinogenicity and TK study at dose levels of 0, 25, 125, 250, and 500 mg/kg/day. In the 14-day study, decreases in body weight gain were observed in males (-57%) and females (-24%) at 1000 mg/kg/day (not statistically significant). No deaths or remarkable changes were observed in other parameters. In the 13-week study, there were no drug-related mortalities. Increases in body weights and body weight gains were noted for females in all treated groups; in males, a decrease in mean body weight gain (-16%, not statistically significant) was noted at HD (500 mg/kg/d). No notable effects were registered in clinical pathology, except for markedly increased prolactin levels in both genders at all dose levels (not dose-dependent). Remarkable microscopic findings were observed in the uterus, cervix, vagina, and mammary glands of females at all doses (potentially induced by dopamine receptor antagonism or dysregulation of prolactin and estrogen levels). Plasma exposure to SM-13496 (C_{max}, AUC_{0-24}) at Week 13 in females was approximately 2-fold higher than in males. Based upon this information and taking a 2-year treatment into consideration, the high doses for the 2-year carcinogenicity study were set at 1200 and 650 mg/kg/day in males and females, respectively. The low, mid-low, and mid-high dose for both genders were set at 30, 100, and 300 mg/kg/day, respectively.
Species/Strain:	Mouse, Crl:CD-1.(ICR)BR
Number/Sex/Group:	60 (main study)
Age:	38-44 days old (males); 41-47 days old (females)
Animal housing:	Male and female mice were housed individually in stainless steel cages (except during acclimation, animals were group-housed) and offered Certified Rodent Diet #8728C (Harlan Teklad) and water ad libitum. Environmental controls for the animal room were set to maintain 18 to 26°C, a relative humidity of 30 to 70%, a minimum of 10 room air changes/hour, and a 12-hour light/12-hour dark cycle. The light/dark cycle was interrupted for study-related activities. Any variations to these conditions are maintained in the raw data and had no effect on the outcome of the study. *(from the Methods section in sponsor's submission)*
Paradigm for dietary restriction:	No
Dual control	Yes
Scheduled sacrifice:	Week 105 (males); Weeks 99-100 (females, due to a rapid decline in survival)

Interim sacrifice: Toxicokinetic and Prolactin Animals: Week 52

Satellite groups: For TK (39/sex/group) and Prolactin measurements (10 sex/group)
Animals/Group allocation is shown in the following sponsor's table:

Group	No. of Animals Male	No. of Animals Female	Dose Level[a] (mg/kg/day)	Dose Concentration[a] (mg/mL)
Carcinogenicity Animals				
1 (Control)[b]	60	60	0	0
2 (Control)[b]	60	60	0	0
3 (Low)	60	60	30	3
4 (Mid-Low)	60	60	100	10
5 (Mid-High)	60	60	300	30
6 (High)	60	60	1200/650[d,e]	120/65
Toxicokinetic Animals[c]				
7 (Low)	39	39	30	3
8 (Mid-Low)	39	39	100	10
9 (Mid-High)	39	39	300	30
10 (High)	39	39	1200/650[d,e]	120/65
Prolactin Analysis Animals				
11 (Control)[b]	10	10	0	0
12 (Low)	10	10	30	3
13 (Mid-Low)	10	10	100	10
14 (Mid-High)	10	10	300	30
15 (High)	10	10	1200/650[d]	120/65

a Animals were given daily doses of either SM-13496 or control article by oral gavage at a dose volume of 10 mL/kg.
b The animals in Groups 1, 2, and 11 received the control article only.
c Three animals/sex/group served as extras to be used as needed based on mortality.
d Males were dosed at 1200 mg/kg/day and females were dosed at 650 mg/kg/day.
e Beginning on Day 410, males in Groups 6 and 10 were dosed at 650 mg/kg/day.

Deviation from study protocol: None of the deviations affected the integrity or interpretability of the results.

Observations: mortality; clinical signs; body weight and food consumption; hematology, clinical chemistry; anatomic pathology; TK; estrus cycle (females only), prolactin (see the sponsor's table below).

Procedure	Frequency/Comment
Inlife Procedures	
Clinical Observations (Groups 1 through 6)[a]	Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Findings were recorded as observed. Detailed observations were done once prior to treatment (for randomization), weekly thereafter, and on the day of scheduled sacrifice.
Mass Palpations/Observations (Groups 1 through 6)[a]	The following information on each grossly visible or palpable mass was recorded. time of onset location size (small or large) appearance progression Additional findings were recorded as observed.
Clinical Observations - Toxicokinetic and Prolactin Animals	Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Additional findings were recorded as observed. No other observations were performed.

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Procedure	Frequency/Comment
Inlife Procedures	
Estrus Cycle Determinations (Groups 11 through 15)	Vaginal smears were done daily for all females during Weeks 51 and 52.
Body Weights	Taken at least once prior to treatment, weekly for Weeks 1 through 14, once every 4 weeks thereafter, and at scheduled termination
Food Consumption (Groups 1 through 6)[a]	Measured weekly for Weeks 1 to 14 and every 4 weeks thereafter
Clinical Pathology[a]	Samples were taken for hematology and clinical chemistry at scheduled sacrifice for all surviving carcinogenicity animals in Groups 1 through 6. The anticoagulant for the hematology tests was potassium EDTA. Samples for clinical chemistry were collected without anticoagulant. At unscheduled and scheduled sacrifices, blood smears were prepared from carcinogenicity animals. Blood smears were examined for specific animals when needed for a manual differential count.
Hematology Tests	red blood cell (erythrocyte) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell (leukocyte) count, differential blood cell count, and blood smear
Clinical Chemistry Tests	glucose, urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, triglycerides, total bilirubin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, calcium, potassium, inorganic phosphorus, sodium, and chloride
Toxicokinetic Analyses[a]	Samples were taken from three toxicokinetic animals/sex/group/time point on Day 1 and during Week 52 approximately 0.5, 2, 6, and 24 hours postdose. Additional samples were taken from three toxicokinetic animals/sex/group/time point during Weeks 26 and 100 approximately 2 and 24 hours postdose. Serum analysis of toxicokinetic samples was performed by (b) (4) Toxicokinetic analysis (e.g., calculation of TK parameters such as AUC) of samples collected during Weeks 1 and 52 were done; samples collected during Weeks 26 and 104 were analyzed and serum concentrations were reported, but toxicokinetic analysis was not done because of the limited number of time points at these intervals.
Prolactin Analysis[a]	During Week 52, blood was collected over several days for prolactin analysis animals only (Groups 11 through 15). Blood was collected from all males on the first scheduled day. For females, blood was collected only from females that were not in the proestrous phase of the estrus cycle. Blood was collected at the approximate time to peak SM-13496 concentration (T_{max}) of 0.5 hours postdose, as determined from the results of Day 1 toxicokinetics in (b) (4) 6645-136. Serum analysis was done by (b) (4)
Disposition of Animals	
Unscheduled Deaths[a]	A necropsy was done on only carcinogenicity animals that died or were sacrificed at an unscheduled interval.
Scheduled Sacrifice[a]	In females, a terminal necropsy was performed on all surviving females during Weeks 99 and 100 after 98 weeks of dosing because of a rapid decline in survival. A terminal necropsy was performed on all surviving males during Week 105 after 104 weeks of dosing.

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Unscheduled Deaths - Toxicokinetic and Prolactin Animals	Toxicokinetic and prolactin analysis animals that died or were sacrificed at an unscheduled intervals were euthanatized with carbon dioxide and discarded.
Scheduled Sacrifice - Toxicokinetic and Prolactin Animals	Toxicokinetic and prolactin analysis animals were sacrificed with carbon dioxide following blood collection and discarded without necropsy. Toxicokinetic animals that survived past the final toxicokinetic blood collection were sacrificed with carbon dioxide inhalation and discarded without necropsy.
Bone Marrow Smears	Prepared from the femur of animals sacrificed at scheduled and unscheduled intervals. Bone marrow smears were not evaluated.
Tissue Preservation[a]	The following tissues (when present) were preserved in 10% neutral-buffered formalin. adrenal (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, eye (2), femur with bone marrow (articular surface of the distal end), gallbladder, Harderian gland, heart, ileum, jejunum, kidney (2), larynx, lesions, liver, lung with mainstem bronchi, lymph node (mesenteric), mammary gland (males and females), nasal cavity, optic nerve (2), ovary (2), pancreas, pituitary gland, preputial/clitoral gland, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle (2), skeletal muscle, skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis (2), thymus, thyroid (2) with parathyroid, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal's gland
Histopathology[a]	All tissues from each carcinogenicity animal (Groups 1 through 6) were processed and examined microscopically at (b) (4) (b) (4) Slides were returned to (b) (4) for record retention prior to report finalization. A pathology peer review of histopathology findings was conducted at (b) (4) by pathologists from Merck & Co.

Results

Mortality: In males, there were no significant increases in mortality at any dose level. Survival in males at 30, 100, and 300 mg/kg/day was comparable to the control groups through approximately Week 95 and then appeared to decline at a higher rate than the control groups. At HD (1200 mg/kg/day), a more rapid decline in survival as compared to controls started approximately at Week 40 (high dose reduced to 650 mg/kg/day as of week 59). By the end of Week 104, the number of surviving males was 27, 29, 24, 22, 24, and 20 at 0, 0, 30, 100, 300, and 1200/650 mg/kg/day, respectively. The decline in survival in dosed groups was not statistically significant, as based on the sponsor's statistical evaluation.

In females, dose-related significant increases in mortality occurred at 300 and 650 mg/kg/day: at these doses, the survival was comparable to control through approximately Week 55, but after this point it declined at a faster rate compared to controls; the increase in mortality was dose-dependent and statistically significant. At the end Week 98, the number of surviving females at 0, 0, 30, 100, 300, and 650 mg/kg/day was 25, 24, 24, 20, 14, and 13, respectively. Due to the excessive mortality at 300 and 650 mg/kg/day, the dosing for all female groups was discontinued at the end of week 98 and the groups were terminated during weeks 99 and 100. The survival of females at 30 or 100 mg/kg/day was comparable to one or both of the control groups throughout the study.

The mean results are presented in the following sponsor's graphs and tables.

Adjusted Survival Data (%) - Males

GROUP AND DOSE LEVEL (MG/KG/DAY)	WEEK:	72	73	74	75	76	77	78	79	80	81	82	83
						MALE							
1													
0		54/60	54/60	54/60	54/60	54/60	54/60	54/60	51/60	51/60	51/60	51/60	50/60
		90%	90%	90%	90%	90%	90%	90%	85%	85%	85%	85%	83%
2													
0		46/60	46/60	46/60	46/60	46/60	45/60	44/60	42/60	42/60	40/60	39/60	38/60
		77%	77%	77%	77%	77%	75%	73%	70%	70%	67%	65%	63%
3													
30		49/60	48/60	47/60	47/60	46/60	46/60	45/60	45/60	44/60	44/60	44/60	44/60
		82%	80%	78%	78%	77%	77%	75%	75%	73%	73%	73%	73%
4													
100		48/58	48/58	47/58	47/58	46/58	45/58	45/58	45/58	44/58	43/58	43/58	42/58
		83%	83%	81%	81%	79%	78%	78%	78%	76%	74%	74%	72%
5													
300		47/58	45/58	44/58	44/58	44/58	43/58	43/58	42/58	42/58	42/58	42/58	42/58
		81%	78%	76%	76%	76%	74%	74%	72%	72%	72%	72%	72%
6													
1200/650		41/53	40/53	40/53	40/53	40/53	40/53	40/53	39/53	39/53	39/53	39/53	39/53
		77%	75%	75%	75%	75%	75%	75%	74%	74%	74%	74%	74%

GROUP AND DOSE LEVEL (MG/KG/DAY)	WEEK:	84	85	86	87	88	89	90	91	92	93	94	95
						MALE							
1													
0		48/60	48/60	47/60	45/60	44/60	42/60	41/60	39/60	38/60	38/60	38/60	36/60
		80%	80%	78%	75%	73%	70%	68%	65%	63%	63%	63%	60%
2													
0		37/59	37/59	37/59	37/59	37/59	37/59	37/59	36/59	36/59	35/59	34/59	32/59
		63%	63%	63%	63%	63%	63%	63%	61%	61%	59%	58%	54%
3													
30		44/60	43/60	43/60	42/60	41/60	40/60	38/60	37/60	37/60	35/60	34/60	34/60
		73%	72%	72%	70%	68%	67%	63%	62%	62%	58%	57%	57%
4													
100		40/58	38/58	37/58	37/58	37/58	36/58	36/58	36/58	36/58	34/58	32/58	31/58
		69%	66%	64%	64%	64%	62%	62%	62%	62%	59%	55%	53%
5													
300		41/58	38/58	37/58	37/58	37/58	36/58	35/58	33/58	33/58	33/58	33/58	33/58
		71%	66%	64%	64%	64%	62%	60%	57%	57%	57%	57%	57%
6													
1200/650		39/53	38/53	37/53	36/53	33/52	31/52	30/52	28/52	27/52	27/52	27/52	27/52
		74%	72%	70%	68%	63%	60%	58%	54%	52%	52%	52%	52%

GROUP AND DOSE LEVEL (MG/KG/DAY)	WEEK:	96	97	98	99	100	101	102	103	104
					MALE					
1										
0		36/60	34/60	33/60	32/60	31/60	30/60	29/60	28/60	27/60
		60%	57%	55%	53%	52%	50%	48%	47%	45%
2										
0		32/59	32/59	32/59	32/59	31/59	31/59	31/59	29/59	29/59
		54%	54%	54%	54%	53%	53%	53%	49%	49%
3										
30		33/60	32/60	32/60	29/60	27/60	27/60	27/60	25/60	24/60
		55%	53%	53%	48%	45%	45%	45%	42%	40%
4										
100		29/58	29/58	27/58	26/58	25/58	25/58	24/58	23/58	22/58
		50%	50%	47%	45%	43%	43%	41%	40%	38%
5										
300		33/58	32/58	32/58	30/58	28/58	27/58	26/58	26/58	24/58
		57%	55%	55%	52%	48%	47%	45%	45%	41%
6										
1200/650		26/52	26/52	24/52	24/52	23/52	22/52	20/52	20/52	20/52
		50%	50%	46%	46%	44%	42%	38%	38%	38%

Adjusted Survival Data (%) – Females

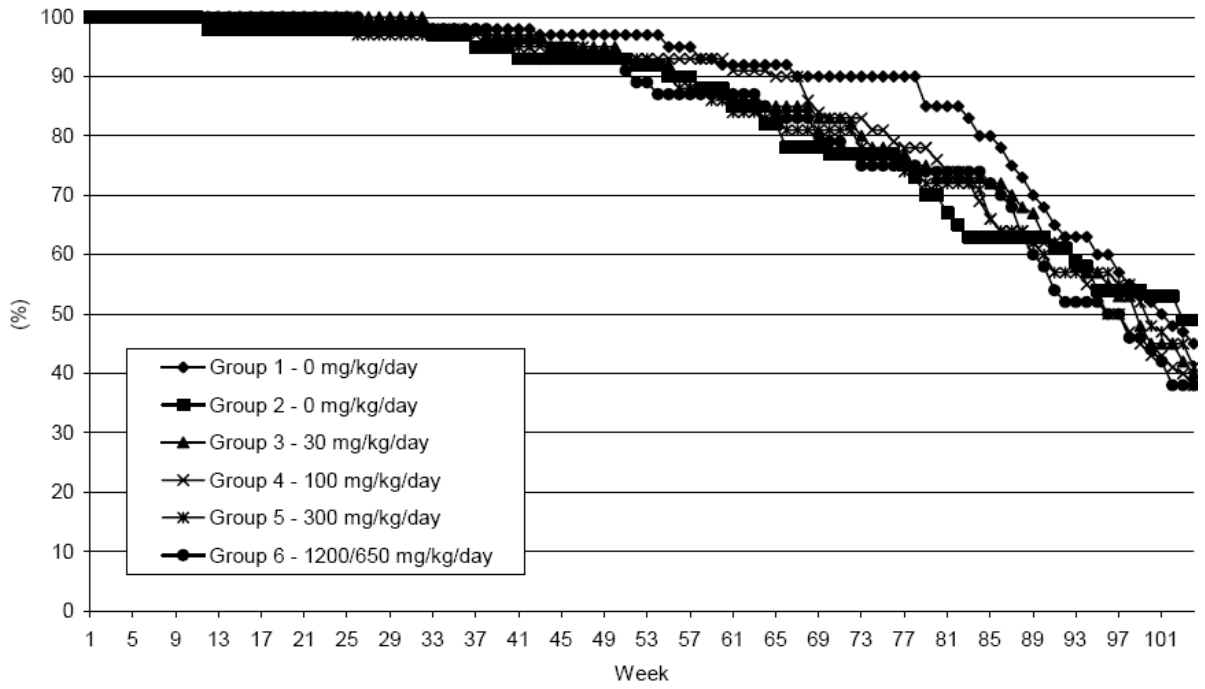
GROUP AND DOSE LEVEL (MG/KG/DAY)	WEEK:	60	61	62	63	64	65	66	67	68	69	70	71
						FEMALE							
1													
0		54/59	54/59	54/59	53/59	53/59	53/59	52/59	51/59	51/59	50/59	50/59	50/59
		92%	92%	92%	90%	90%	90%	88%	86%	86%	85%	85%	85%
2													
0		56/60	56/60	56/60	56/60	56/60	56/60	55/60	55/60	52/60	52/60	52/60	51/60
		93%	93%	93%	93%	93%	93%	92%	92%	87%	87%	87%	85%
3													
30		50/59	50/59	49/59	49/59	49/59	49/59	48/59	48/59	46/59	45/59	45/59	45/59
		85%	85%	83%	83%	83%	83%	81%	81%	78%	76%	76%	76%
4													
100		54/60	54/60	53/60	53/60	52/60	52/60	52/60	52/60	52/60	51/60	51/60	51/60
		90%	90%	88%	88%	87%	87%	87%	87%	87%	85%	85%	85%
5													
300		51/59	51/59	51/59	51/59	49/58	49/58	48/58	48/58	47/58	47/58	47/58	47/58
		86%	86%	86%	86%	84%	84%	83%	83%	81%	81%	81%	81%
6													
650		49/59	48/59	48/59	47/59	47/59	47/59	47/59	47/59	44/59	42/59	41/59	40/59
		83%	81%	81%	80%	80%	80%	80%	80%	75%	71%	69%	68%

GROUP AND DOSE LEVEL (MG/KG/DAY)	WEEK:	72	73	74	75	76	77	78	79	80	81	82	83
						FEMALE							
1													
0		50/59	49/59	48/59	47/59	47/59	47/59	45/59	44/59	44/59	43/59	42/59	42/59
		85%	83%	81%	80%	80%	80%	76%	75%	75%	73%	71%	71%
2													
0		50/60	50/60	50/60	49/60	48/60	46/60	46/60	45/60	45/60	44/60	44/60	43/60
		83%	83%	83%	82%	80%	77%	77%	75%	75%	73%	73%	72%
3													
30		43/59	42/59	41/59	41/59	39/59	38/59	37/59	35/59	35/59	35/59	35/59	34/59
		73%	71%	69%	69%	66%	64%	63%	59%	59%	59%	59%	58%
4													
100		51/60	50/60	47/60	47/60	47/60	47/60	46/60	45/60	44/60	44/60	42/60	42/60
		85%	83%	78%	78%	78%	78%	77%	75%	73%	73%	70%	70%
5													
300		47/58	46/58	45/58	43/58	43/58	43/58	39/58	38/58	38/58	36/58	35/58	34/58
		81%	79%	78%	74%	74%	74%	67%	66%	66%	62%	60%	59%
6													
650		40/59	40/59	39/59	39/59	38/59	36/59	34/59	33/59	33/59	32/59	30/59	29/59
		68%	68%	66%	66%	64%	61%	58%	56%	56%	54%	51%	49%

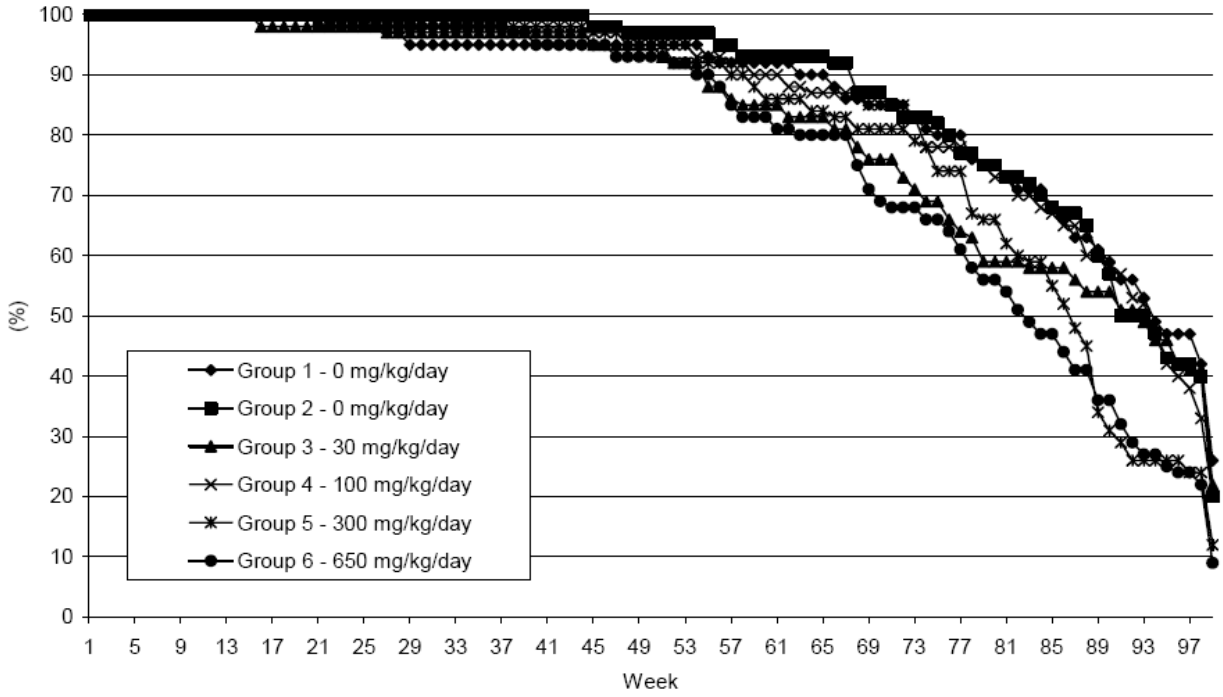
GROUP AND DOSE LEVEL (MG/KG/DAY)	WEEK:	84	85	86	87	88	89	90	91	92	93	94	95
						FEMALE							
1													
0		42/59	40/59	39/59	37/59	37/59	36/59	35/59	33/59	33/59	31/59	29/59	28/59
		71%	68%	66%	63%	63%	61%	59%	56%	56%	53%	49%	47%
2													
0		42/60	41/60	40/60	40/60	39/60	36/60	34/60	30/60	30/60	30/60	28/60	26/60
		70%	68%	67%	67%	65%	60%	57%	50%	50%	50%	47%	43%
3													
30		34/59	34/59	34/59	33/59	32/59	32/59	32/59	30/59	30/59	29/59	27/59	27/59
		58%	58%	58%	56%	54%	54%	54%	51%	51%	49%	46%	46%
4													
100		41/60	40/60	39/60	39/60	36/60	36/60	35/60	34/60	32/60	31/60	28/60	25/60
		68%	67%	65%	65%	60%	60%	58%	57%	53%	52%	47%	42%
5													
300		34/58	32/58	30/58	28/58	26/58	20/58	18/58	17/58	15/58	15/58	15/58	15/58
		59%	55%	52%	48%	45%	34%	31%	29%	26%	26%	26%	26%
6													
650		28/59	28/59	26/59	24/59	24/59	21/59	21/59	19/59	17/59	16/59	16/59	15/59
		47%	47%	44%	41%	41%	36%	36%	32%	29%	27%	27%	25%

GROUP AND DOSE LEVEL (MG/KG/DAY)	WEEK:	96	97	98	99
			FEMALE		
1					
0		28/59	28/59	25/59	12/46
		47%	47%	42%	26%
2					
0		25/60	25/60	24/60	10/50
		42%	42%	40%	20%
3					
30		25/59	24/59	24/59	10/45
		42%	41%	41%	22%
4					
100		24/60	23/60	20/60	10/51
		40%	38%	33%	20%
5					
300		15/58	14/58	14/58	6/50
		26%	24%	24%	12%
6					
650		14/59	14/59	13/59	5/54
		24%	24%	22%	9%

Figure **Adjusted Survival Data (%) – Males**



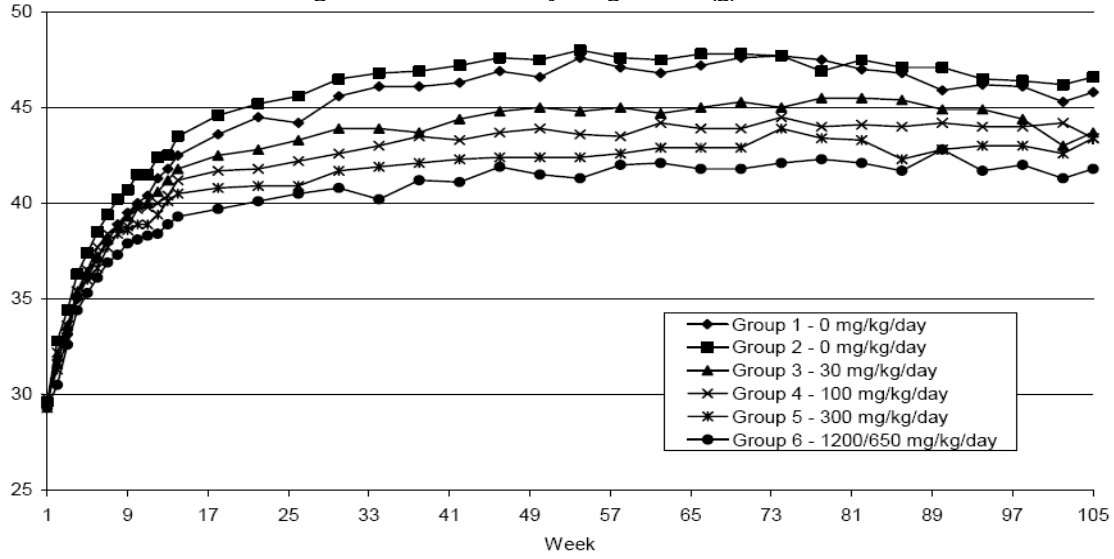
Adjusted Survival Data (%) – Females



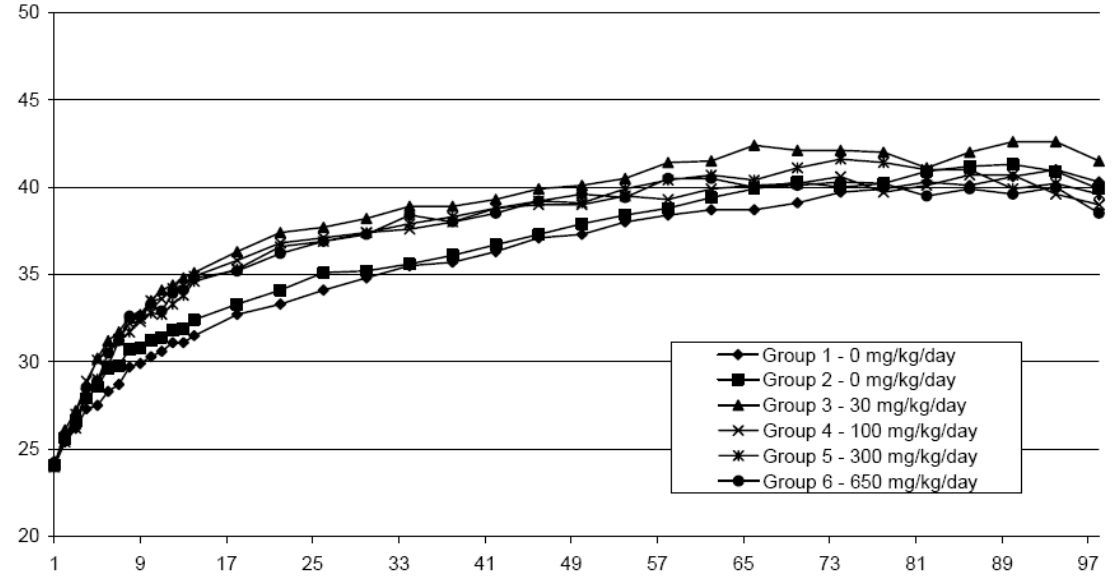
Clinical Signs: The clinical observations were comparable among the groups across the time intervals. Animals in all groups developed palpable masses at locations typical for mice of this strain and duration of study. As subjectively assessed by the sponsor, the females given SM-13496 appeared to have a higher incidence of palpable masses in various locations.

Body Weights: In males, a dose- and time-dependent reduction in body weight occurred in all dose groups in comparison to control. At Week 58 (a week before the high dose level was reduced from 1200 to 650 mg/kg/day), the percentage difference of mean body weight versus control group 1 was +1, -5, -8, -10, and -11% for control group 2 and the dosed groups of 30, 100, 300, and 1200 mg/kg/day, respectively. After Week 58, the group differences in body weight remained stable on absolute and percentage basis. The body weight gain changes were biphasic: lower at all dose levels in comparison to one or both control groups during Weeks 1 through 54, and higher than control from Week 54 through 78. In females, SM-13496 was generally associated with a dose- dependent *increase* in mean body weight in all dose groups versus controls that occurred mostly between Weeks 4 and 74. For females at all dose levels, the body weight gain values for Week 1 through 14 were significantly *higher* than those for control females. During Weeks 14 through 54, the body weight gain values at the two high dose groups (100 and 650 mg/kg/day) were *lower* in comparison to control. The group body weight or body weight gain differences associated with drug administration in either gender were not of toxicological concern. The mean results are presented in the following sponsor's graphs and tables.

Fig. Mean body weight data (g) – Males



Mean body weight data (g) – Females



Summary of body weight data (g) – Males

Test Article		Control		SM-13496			
Dose Unit		mg/kg/day		mg/kg/day			
Group		1	2	3	4	5	6
Dose Level [Male]		0	0	30	100	300	1200/650
[Female]		0	0	30	100	300	650

Week		Group 1M	Group 2M	Group 3M	Group 4M	Group 5M	Group 6M
46	Mean	46.9	47.6	44.8 A,B	43.7 A,B	42.4 A,B	41.9 A,B
	SD	5.11	4.40	4.11	4.69	4.17	3.45
	N	58	56	57	56	55	52
50	Mean	46.6	47.5	45.0 B	43.9 A,B	42.4 A,B	41.5 A,B
	SD	4.99	4.72	4.12	4.77	3.94	3.38
	N	58	56	57	55	55	51
54	Mean	47.6	48.0	44.8 A,B	43.6 A,B	42.4 A,B	41.3 A,B
	SD	5.41	4.61	4.25	4.65	3.68	3.41
	N	58	55	55	54	55	48
58	Mean	47.1	47.6	45.0 A,B	43.5 A,B	42.6 A,B	42.0 A,B
	SD	5.59	4.59	4.48	5.06	4.14	3.24
	N	57	54	53	54	52	47
62	Mean	46.8	47.5	44.7 A,B	44.2 A,B	42.9 A,B	42.1 A,B
	SD	4.89	4.22	4.02	5.13	3.73	3.33
	N	55	51	52	53	49	47
66	Mean	47.2	47.8	45.0 A,B	43.9 A,B	42.9 A,B	41.8 A,B
	SD	5.40	4.76	4.05	4.84	4.20	3.40
	N	55	49	51	52	48	45
70	Mean	47.6	47.8	45.3 A,B	43.9 A,B	42.9 A,B	41.8 A,B
	SD	5.38	4.80	3.94	3.78	3.97	3.70
	N	54	47	50	49	47	43
74	Mean	47.7	47.7	45.0 A,B	44.5 A,B	43.9 A,B	42.1 A,B
	SD	5.46	4.71	3.99	4.43	3.96	3.17
	N	54	46	48	48	45	40
78	Mean	47.5	46.9	45.5	44.0 A,B	43.4 A,B	42.3 A,B
	SD	5.53	4.88	4.08	4.16	4.27	3.37
	N	54	45	46	45	43	40
82	Mean	47.0	47.5	45.5 B	44.1 A,B	43.3 A,B	42.1 A,B
	SD	5.47	4.55	4.20	4.37	4.10	3.63
	N	51	40	44	43	42	39
86	Mean	46.8	47.1	45.4	44.0 A,B	42.3 A,B	41.7 A,B
	SD	5.21	4.44	4.31	4.70	4.00	4.37
	N	48	37	43	38	38	38
90	Mean	45.9	47.1	44.9 B	44.2 B	42.8 A,B	42.8 A,B
	SD	5.13	4.46	4.00	5.01	3.93	3.62
	N	42	37	38	36	36	31
94	Mean	46.2	46.5	44.9	44.0 A,B	43.0 A,B	41.7 A,B
	SD	5.01	5.00	3.70	4.49	3.81	4.13
	N	38	35	35	34	33	27
98	Mean	46.1	46.4	44.4	44.0 A,B	43.0 A,B	42.0 A,B
	SD	5.42	4.64	3.96	3.80	3.76	3.43
	N	34	32	32	29	32	26
102	Mean	45.3	46.2	43.0 B	44.2	42.6 A,B	41.3 A,B
	SD	5.83	4.52	4.27	4.06	4.40	3.39
	N	30	31	27	25	26	22
105	Mean	45.8	46.6	43.7 B	43.4 B	43.4 B	41.8 A,B
	SD	5.74	4.90	3.37	3.55	4.40	3.17
	N	27	29	24	22	24	20

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.

Feed Consumption

In males, a dose-dependent slight (usually less than 10%) reduction in food intake occurred in all dose groups; statistical differences were mostly found in the early part of the study and rarely after Week 33.

In females, SM-13496 was not associated with any consistent changes in group mean food consumption values.

Estrus cycling The percentages of females with evidence of estrus cycling (assessed during Weeks 51 and 52) were 90, 90, 60, 56, and 50% for dose levels of 0, 30, 100, 300, and 650 mg/kg/day SM-13496, respectively, showing that SM-13496 affected the estrus cycle at dose levels of 100 mg/kg/day and higher.

Hematology and Clinical Chemistry There were no drug-related effects on hematology parameters, except for some minor hematology findings at Week 100 (slightly lower hematocrit and higher platelet count for females at ≥100 mg/kg/day). Mildly higher urea nitrogen and triglycerides and lower alkaline phosphatase activity was found in all groups of treated females (see the following sponsor's table). Because of the mild and not-dose-dependent changes, the toxicological significance of these findings is unclear.

Summary of clinical pathology changes

Test Article		Control		SM-13496			
Dose Unit		mg/kg/day		mg/kg/day			
Group		1	2	3	4	5	6
Dose Level	[Male]	0	0	30	100	300	1200/650
	[Female]	0	0	30	100	300	650

Group		RBC E6/UL Week 100	HGB G/DL Week 100	HCT % Week 100	MCV FL Week 100	MCH PG Week 100	MCHC G/DL Week 100	PLT E3/UL Week 100
1F	Mean	7.42	11.7	38.3	51.9	15.8	30.5	1091
	SD	1.185	1.84	5.03	4.39	1.18	1.34	345.7
	N	23	23	23	23	23	23	23
2F	Mean	7.68	12.2	39.5	51.6	15.9	30.9	1068
	SD	0.852	1.20	4.24	2.78	0.82	0.91	379.4
	N	19	19	19	19	19	19	19
3F	Mean	7.35	11.6	37.1	51.0	15.8	31.1	1262
	SD	1.210	1.75	5.44	5.40	1.10	1.52	429.9
	N	22	22	22	22	22	22	22
4F	Mean	6.71	10.7	34.6 AB	51.7	16.0	31.0	1332
	SD	0.806	1.31	3.72	2.84	0.72	1.12	442.5
	N	18	18	18	18	18	18	18
5F	Mean	7.30	11.3	35.9 B	49.2	15.4	31.3	1348
	SD	0.583	0.90	2.65	1.78	0.88	1.00	479.0
	N	12	12	12	12	12	12	12
6F	Mean	6.99	10.8	35.2 B	50.5	15.5	30.7	1376
	SD	0.987	1.46	3.95	2.52	0.52	1.43	564.2
	N	10	10	10	10	10	10	10

Group		TRIG MG/DL Week 100	CHOL MG/DL Week 100	ALK PHOS IU/L Week 100	UN MG/DL Week 100
1F	Mean	47	91	91	32
	SD	27.2	23.1	47.5	16.3
	N	24	24	24	24
2F	Mean	47	116	105	30
	SD	40.6	62.8	51.1	7.1
	N	19	19	19	19
3F	Mean	80 AB	131	60 AB	40 B
	SD	43.2	79.2	29.9	21.5
	N	23	22	23	22
4F	Mean	75 AB	115	41 AB	41 B
	SD	41.2	67.8	24.8	14.7
	N	19	19	18	18
5F	Mean	88 AB	123	37 AB	44 AB
	SD	37.4	43.1	21.6	12.5
	N	14	14	14	14
6F	Mean	90 AB	114	35 AB	45 AB
	SD	39.7	31.7	17.3	13.6
	N	8	8	8	8

A Statistically significant from Group 1 at $p \leq 0.05$
B Statistically significant from Group 2 at $p \leq 0.05$

Prolactin: The only clearly manifested drug-related clinical pathology effect of SM-13496 was a markedly higher prolactin in all treated animals. Serum prolactin (measured during Week 52) was elevated at all dose levels (although not dose-dependently), in both males and females (see the following sponsor's table).

Serum Prolactin Concentrations during Week 52

	Serum Prolactin (ng/mL)				
	SM-13496 (mg/kg)				
	0	30	100	300	1200/650[a]
Male					
Mean	5.1	78.4[d]	94.4[d]	126.2[d]	103.1[d]
SD[b]	1.22	29.68	47.79	53.92	36.1
N[c]	10	10	9	9	9
Female					
Mean	21.3	346.6[d]	316.5[d]	268[d]	315.5[d]
SD[b]	32.36	22.79	53.54	44.68	38.34
N[c]	10	10	10	9	8

a 1200 mg/kg/day for males, 650 mg/kg/day for females.
b Standard deviation.
c Number of animals.
d Considered as test article response (statistical evaluation not required by protocol).

Gross Pathology

No test article-related macroscopic findings were noted in males. In females, however, drug-related increase in the incidences of masses in the pituitary and mammary glands were found at all dose levels compared to either control group (see the following sponsor's table). In contrast, the incidence of masses in the uterus, cervix, and ovaries was decreased in females at all dose levels compared with either control group, in agreement with the microscopic findings of atrophy in these organs.

Macroscopic findings

ORGAN AND KEYWORD(S)	SEX:	FEMALE					
	GROUP:	-1-	-2-	-3-	-4-	-5-	-6-
	NUMBER:	25	20	24	19	14	10
PITUITARY (PI)	NUMBER EXAMINED:	25	20	24	19	14	10
	NOT REMARKABLE:	23	17	19	8	4	4
DARK FOCUS(I)/AREA(S)		0	0	0	1	2	1
LARGE		0	0	1	1	2	3
MASS(ES)		1	0	3	7	6	3
RED FOCUS(I)/AREA(S)		1	2	1	3	1	1
DIFFUSELY RED		0	0	0	0	0	1
MISSING/NOT IDENTIFIED		0	1	0	0	0	0
MAMMARY, FEMALE (MF)	NUMBER EXAMINED:	25	20	24	19	14	10
	NOT REMARKABLE:	25	20	19	16	8	7
MASS(ES)		0	0	5	3	5	2
MASS #2		0	0	0	0	2	0
THICKENED		0	0	0	0	1	1
OVARY (OV)	NUMBER EXAMINED:	25	20	24	19	14	10
	NOT REMARKABLE:	4	1	6	7	7	4
CYST(S)		21	19	18	12	7	6
MASS(ES)		0	2	0	0	0	0
UTERUS (UT)	NUMBER EXAMINED:	25	20	24	19	14	10
	NOT REMARKABLE:	0	1	12	8	8	5
CYST(S)		22	15	10	11	5	5
LARGE, DIFFUSE		1	0	0	0	0	0
MASS(ES)		6	6	3	1	1	0
THICKENED WALL		0	1	1	0	0	0

Histopathology

Neoplastic

Sponsor's assessment

In males, administration of SM-13496 did not induce increased incidence of neoplastic lesions. It should be noted, however, that the number of males examined for mammary histopathology (3, 4, 8, 8, 6, 5 at 0, 0, 30, 100, 300 and 650 mg/kg/d, respectively) was much smaller than those of females examined for mammary histopathology, or for males examined for other organs' histopathology (not explained by the sponsor).

In females, SM-13496 caused a dose-dependent increase in the incidence of tumors and hyperplasia in the pituitary and in the mammary gland of females at all tested dose levels.

In the pituitary, the findings included pars distalis adenoma and diffuse hyperplasia that resulted in an overall enlargement of the gland. Ventral compression of the hypothalamic area of the brain due to the presence of pituitary tumors was noted in 5, 9, 17, and 17 females at 30, 100, 300, and 650 mg/kg/d, respectively, as compared to 0 cases in either control. The following table shows the incidence of test article-related findings in the pituitary of females.

Incidence of Pituitary Microscopic Findings in Female Mice

Dose Level (mg/kg/day):	0	0	30	100	300	650
No. Examined:	58	58	59	60	60	60
Pituitary						
Adenoma, Pars Distalis	3	4	11	17	27	29
Hyperplasia, Pars Distalis	2	4	7	11	14	15

The incidence of mammary hyperplasia and neoplasia (mammary gland carcinoma and malignant adenoacanthoma) was also markedly and dose-dependently increased in all female dosed groups

compared with either of the two control groups. The following sponsor's table summarizes the incidence of mammary gland neoplasia and hyperplasia in females.

Incidence of Mammary Neoplasia and Hyperplasia in Female Mice

Dose Level (mg/kg/day):	0	0	30	100	300	650
No. Examined:	58	57	55	60	60	57
Mammary Gland						
Carcinoma	2	1	7	12	18	13
Adenoacanthoma	1	0	7	6	7	5
Carcinosarcoma	0	0	0	1	2	2
Hyperplasia	3	6	23	36	39	34
Galactocele/Increased Secretion	1	4	8	27	26	32

Drug-related neoplastic lesions that showed statistically significant increases in females were benign pituitary (pars distalis) adenoma and malignant mammary carcinoma and adenoacanthoma (independently and in combination). In both cases, highly significant positive trends were noted in the dosed groups versus both control groups.

FDAs Statistical Reviewer's assessment

No increase in neoplastic lesions was seen in the males. In the females, statistically significant increases in neoplastic lesions [benign pituitary pars distalis adenoma and malignant mammary tumors (carcinoma, adenoacanthoma)] were induced at all tested dose levels, with highly significant positive trends vs. pooled control groups (see table on the next page). In particular, the tests of overall trend and pairwise comparison between the highest dose group and pooled control in mammary carcinoma in females were statistically significant, as were the tests of pooled tumors (adenomas, carcinomas, carcinosarcomas, and adenoacanthomas) for trend and pairwise comparisons. Similarly the tests of overall trend and pairwise comparison between the highest dose group and control in pituitary pars distalis adenoma in females were highly statistically significant. The pairwise comparisons of mammary carcinoma in the mid-high dose and middle dose groups to control were statistically significant, while for the low dose group the difference was close to adjusted statistical significance ($p \approx 0.01$). The pairwise comparisons for these groups were also statistically significant for mammary adenoacanthoma. Similarly the pairwise comparisons of the mid-high dose, middle, and low dose groups to the pooled controls for pituitary pars distalis adenoma were statistically significant In pooled mammary tumors all these comparisons were also statistically significant.

Some other neoplasms were increased in single dose groups without dose-dependence. Thus, the pooled cancers of the ovary were statistically significantly higher in the middle dose group vs. pooled vehicle; and adrenal pheochromocytoma and islet cell adenoma of the pancreas were statistically significant in the mid-high dose group vs. pooled vehicle, but not in the highest dose group. No other tests achieved statistical significance.

Selected Neoplasms in Mice (All Female)*

	Veh 1	Veh 2	Low	Mid-Med	Hi	High Hi	Trend	High vs Veh	Med-Hi vs Veh	Medium vs Veh	Low vs Veh
N	60	60	60	60	60	60					
ADRENAL, MEDULLA											
B-PHEOCHROMOCYTOMA	0	0	0	0	3	0	0.3776	.	0.0261	.	.
HARDERIAN GLAND											
Adenoma/Carcinona	5	3	7	3	5	8	0.0354	0.0489	0.3406	0.5383	0.1383
MAMMARY, FEMALE											
Adenoma/Carc./-sarcoma/-canth.	2	1	13	19	26	20	0.0000	0.0000	0.0000	0.0000	0.0000
M-ADENOACANTHOMA	1	0	7	6	7	5	0.1028	0.0080	0.0011	0.0061	0.0014
M-CARCINOMA	2	1	7	12	18	13	0.0011	0.0000	0.0000	0.0002	0.0113
M-CARCINOSARCOMA	0	0	0	1	2	2	0.0259	0.0800	0.0898	0.3333	.
OVARY											
Cystad./Gran./Thecal/Tubul.	1	0	2	4	1	2	0.2543	0.1961	0.5073	0.0446	0.2307
PANCREAS											
B-ISLET CELL ADENOMA	0	0	0	1	3	1	0.1546	0.2857	0.0261	0.3333	.
PITUITARY											
B-ADENOMA, PARS DISTALIS	3	4	11	17	27	29	0.0000	0.0000	0.0000	0.0001	0.0068

* FDA statistical analysis (statistical reviewer: Steve Thomson)

Non Neoplastic

A number of findings in the female reproductive system indicated a disruption in the estrus cycle. These findings included ovarian, uterine, cervical and vaginal atrophy, a decrease in the normal aging lesion of cystic endometrial hyperplasia in the uterus, and an increase in the incidence of adenomyosis in the uterus (see the sponsor's table on the next page).

Microscopic findings in other organs:

The incidence of amyloidosis was slightly and not dose-dependently elevated in the testis in males at 300 mg/kg/day, but the overall incidence of this systemic condition was comparable in other tissues. A slight elevation of mononuclear cell infiltrates was noted in the lungs of females at 300 mg/kg/day (but not in males at any dose). These infiltrates were "minimal" to "slight" and absent at the higher dose level and apparently not related to the test article. The findings were not dose-dependent and considered incidental and not drug-related.

Microscopic findings occurring spontaneously in aging mice were noted during the study. None of these findings appeared to be test article-related.

The sponsor's tables on the next page list the incidences of the findings in the reproductive and other organs.

Incidence of Microscopic Findings in Mouse Female Reproductive system

Dose Level (mg/kg/day)	0	0	30	100	300	650
No. Examined	60	60	59	60	59	59
Ovary						
Atrophy	5	4	10	16	25	37
No. Examined	60	60	60	60	60	60
Uterus						
Hyperplasia, Cystic Endometrial	54	46	21	29	15	17
Adenomyosis	3	2	30	22	25	15
Atrophy	0	2	11	16	25	30
No. Examined	57	57	58	56	59	60
Cervix						
Atrophy	2	2	10	12	18	28
No. Examined	60	58	58	58	58	59
Vagina						
Atrophy	3	2	8	8	13	24
Mucification, Epithelium	2	2	8	16	28	26

Microscopic findings in other mouse organs

SEX: ----------MALE----------

ORGAN AND FINDING DESCRIPTION		GROUP: -1-	-2-	-3-	-4-	-5-	-6-
	NUMBER:	27	29	24	22	23	20
KIDNEY (KD)	NUMBER EXAMINED:	27	29	24	22	23	20
	NOT REMARKABLE:	0	0	0	0	0	0
--AMYLOIDOSIS		2	2	2	1	4	2
JEJUNUM (JE)	NUMBER EXAMINED:	27	29	24	22	23	20
	NOT REMARKABLE:	26	25	23	20	18	16
--AMYLOIDOSIS		1	3	1	2	5	3
TESTIS (TE)	NUMBER EXAMINED:	27	29	24	22	23	20
	NOT REMARKABLE:	17	16	16	13	13	13
--AMYLOIDOSIS		0	0	1	0	4	2
AUDITORY SEB GL (AS)	NUMBER EXAMINED:	26	28	24	22	22	19
	NOT REMARKABLE:	11	19	11	11	12	13
--AMYLOIDOSIS		2	2	2	0	4	3

SEX: ----------FEMALE----------

ORGAN AND FINDING DESCRIPTION		GROUP: -1-	-2-	-3-	-4-	-5-	-6-
	NUMBER:	25	20	24	19	14	10
LUNG (LU)	NUMBER EXAMINED:	25	20	24	19	14	10
	NOT REMARKABLE:	15	9	12	10	10	3
--INFILTRATE, MONONUCLEAR CELL		0	0	1	0	2	0

Continued on the next page

ESOPHAGUS (ES)	NUMBER EXAMINED:	25	20	24	19	14	10
	NOT REMARKABLE:	25	20	24	19	13	10
--INFILTRATE, MONONUCLEAR CELL		0	0	0	0	1	0
LARYNX (LA)	NUMBER EXAMINED:	24	18	22	18	12	8
	NOT REMARKABLE:	23	17	20	16	11	8
--INFILTRATE, MONONUCLEAR CELL		0	1	0	0	1	0
UTERUS (UT)	NUMBER EXAMINED:	25	20	24	19	14	10
	NOT REMARKABLE:	0	1	3	1	3	1
--AMYLOIDOSIS		0	1	1	0	0	1

Peer Review: A pathology peer review of histopathology findings was performed by pathologists from Merck & Co.

Toxicokinetics

The mean TK data are summarized in the sponsor's tables below.
Exposure to SM-13496 increased with dose in both males and females. Females had higher exposure (Cmax and $AUC_{0\text{-}24hr}$ values) than males across all blood collection time points. Tmax values ranged from 0.5 to 2 hours. Elimination half-life (t1/2) was not determined.

After multiple dosing (week 52), serum exposures in females (Cmax and AUC0-24hr) were generally higher than on Day 1; a markedly (>2-fold) higher Cmax value was observed in females at the 30-mg/kg/day dose level. The increases in Cmax and AUC0-24hr were generally less-than-dose-proportional, with the exception of AUC0-24hr at 30 and 100 mg/kg/day, where the increases were roughly dose-proportional. In males, there was some increase in Cmax, but not in AUC0-24hr after multiple dosing.

TK parameters of SM-13496 in serum of male and female mice

Dose Group	Dose Level (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-24hr} (ng·hr/mL)
Day 1						
7	30	M	123	0.500	310	428
		F	193	0.500	401	714
8	100	M	266	0.500	957	1397
		F	495	0.500	1282	2126
9	300	M	383	2.00	3949	3949
		F	731	0.500	2344	5131
10	1200	M	870	2.00	11958	11958
	650	F	665	2.00	6909	6909
			Week 52			
7	30	M	138	0.500	283	418
		F	420	0.500	1130	1130
8	100	M	403	0.500	1142	1142
		F	558	0.500	4217	4217
9	300	M	518	0.500	2844	2844
		F	748	0.500	9927	9927
10	1200	M	1124	0.500	12947	12947
	650	F	956	2.00	13599	13599

Dose Proportionality ratios

Interval	Proportional Dose Level Increase	Males		Females	
		C_{max}	AUC_{0-24hr}	C_{max}	AUC_{0-24hr}
Day 1	1.0:3.3:10:40/22-fold	1.0:2.2:3.1:7.1-fold	1.0:3.3:9.2:28-fold	1.0:2.6:3.8:3.4-fold	1.0:3.0:7.2:9.7-fold
Week 52	1.0:3.3:10:40/22-fold	1.0:2.9:3.8:8.2-fold	1.0:2.7:6.8:31-fold	1.0:1.3:1.8:2.3-fold	1.0:3.7:8.8:12-fold

Stability and Homogeneity

Homogeneity[a]	Samples (1.0 mL each) were taken from the low and high dose levels mixed for Week 1. Duplicate samples from the top, middle, and bottom of the dose preparations were collected and analyzed for test article content. All samples were stored, protected from light, at room temperature until analysis (Week 1 samples) or in a refrigerator, set to maintain 2 to 8°C (Day 729 to Day 732 samples). Homogeneity analysis was repeated at the low and high dose levels when the batch size changed by more than 30%.
Stability	Dose preparations at concentrations of 0.1 and 100 mg/mL were shown to be stable (when protected from light) after 31 days of refrigerated storage followed by at least 24 hours of room temperature storage (b) (4)5645-142).

(As cited from the sponsor)

Overall, the dose analysis data showed that the provided dose concentrations were accurate, homogenous, and stable for the conditions of use in this study.

Conclusion: Mammary carcinomas and adenoacanthomas and benign pituitary pars distalis adenomas were drug related in females only, at all dose groups.
NOEL for neoplasia: Males: 650 mg/kg/day (AUC_{0-24}= 12, 947 ng.hr/ml); Females: An NOEL was not reached for pituitary adenoma and mammary malignant neoplasia (carcinoma, adenoacanthoma) (below the LD of 30 mg/kg/day, AUC_{0-24} 1130 ng.hr/ml). Compared to human exposure at the MRHD of 120 mg/day (AUC_{0-inf}= 687 ng.h/ml), the safety margin at the NOEL for males is about 19x; for females, there is no safety margin for humans as an NOEL was not reached (based on AUC, the female mice exposure at the lowest tested dose was 1.6x the human exposure at the MRHD).

Study title: **104-Week Oral Gavage Carcinogenicity and TK Study with SM-13496 in Rats**

Study no.:	6645-139
Conducting laboratory and location:	(b) (4), (b) (4)
Date of study initiation:	02/23/2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SM -13496, Lot E-278, 100.3%
CAC concurrence:	Yes
Date of study initiation:	14 November 2003

Key Study Findings: Oral (gavage) administration of lurasidone in 0.5% methylcellulose to Crl:CD(SD)®IGS BR rats (65/sex/dose) for 104 weeks at doses of 0, 0, 3, 12, 50/36 mg/kg/day (HD reduced to 36 mg /kg of body weight/day beginning on Days 404 and 403 for males and females, respectively) resulted in the following neoplastic findings: Males: Skin fibroma/fibrosarcoma was significantly increased over the pooled vehicle control only at mid-dose, but not at high dose. No other significant effects, either in terms of positive trend or significant increase over the controls, were noted. Females: Increased incidence of mammary carcinomas was found at mid- and high-dose; the test of trend was statistically significant, as was the test comparing the HD and MD to the pooled vehicle; at the mid-dose, the incidence of mammary adenomas was also significantly increased over pooled controls, but there was no increase in this tumor incidence at the high dose. The incidence of other mammary tumors was similar for control and treated females. No other tests of trend or comparisons between the high dose and controls achieved significance. Non-neoplastic findings: No increase in mortality in either gender. Body weight reduction: in females at MD and HD and in males at all doses; due to excessive reduction in mean body weight (>20% for both genders) at the initial HD of 50 mg/kg/day, it was reduced to 36 mg/kg/day beginning on Days 404 and 403 for M and F, respectively. The first statistically significant change in body weight occurred at weeks 46, 13, and 3 for the LD, MD and HD, respectively. In F, the changes in body weight were biphasic across the dose range: at LD, a significant increase vs. control was registered from Week 2 through 66. Food consumption was decreased at HD (both genders), at MD (females) and increased at LD (females only). Female estrus cycle disruption occurred at all dose levels in a dose-dependent manner, supported by microscopic findings of increased incidence of absence of corpora lutea in the ovary and increased vaginal cornification at the terminal sacrifice of females at all dose levels. In males, increased incidence of milk secretion was observed at all dose groups. Serum prolactin was elevated dose-dependently vs. controls in the males at all dose levels (reaching a plateau between MD and HD); in the females, prolactin was increased at LD and MD but not at HD. Plasma exposure to lurasidone increased greater-than-dose-proportionally; Cmax and AUC0-24h values were higher in females than in males across all collection time points. A marked (>2-fold) increases in Cmax and AUC0-24h values were determined in all dose groups after multiple dosing, indicative of drug accumulation. MTD: 36 mg/kg/day, due to excessive (>20%) body weight decrease versus control at the next tested dose of 50 mg/kg/day in both genders. In summary, lurasidone resulted in increased incidence of mammary carcinomas in female rats at MD and HD (12 and 50/36 mg/kg/d, respectively), and increased incidence of milk secretion in males at all dose levels. The incidence of all other neoplastic lesions in either gender was not elevated at any of the tested dose levels. NOEL for neoplasia: Females: mammary carcinoma: 3 mg/kg/day (AUC_{0-24}: 365 ng.h/ml); Males: 36 mg/kg/day (AUC_{0-24}: 5276 ng.h/ml). Adequacy of Carcinogenicity Study: Adequate; Appropriateness of Test Model: Yes; Evaluation of tumor findings: The mammary

carcinomas in female rats at mid- and high dose lurasidone (12 and 50/36 mg/kg/day, respectively) were drug related.

Note: All tables and figures are reproduced from the sponsor, unless otherwise indicated

Methods

Doses: 0, 0, 3, 12, 50/36 mg/kg/day

Frequency of dosing: Daily

Dose volume: 5 ml/kg

Route of administration: Oral gavage

Formulation/Vehicle: Methylcellulose (b) (4) Lot # 063K0060);
The control article was 0.5% (w/v) medium viscosity (1500 cps) methylcellulose in reverse osmosis water

Basis of dose selection: Six-month oral gavage toxicity study with SM-13496 in rats (b) (4) (b) (4) Study No. 3259) at dose levels of 0, 0.03, 1, 10, and 100 mg/kg/day. Significantly reduced mean body weight gain (-23.5% for both genders) was observed at HD. Based on the body weight decrease at 100 mg/kg/day, the high dose level for the present study was selected at 50 mg/kg/day. The HD was reduced from 50 to 36 mg/kg/d at study week 58 [Day 404 (M) and 403 (F)] due to excessive decrease in body weight. Mid- and low dose levels were 12 and 3 mg/kg/day, respectively.

Species/Strain: Rats/Crl:CD (SD)®IGS BR

Number/Sex/Group: 65/sex/group

Age: Approximately 6 weeks old at initiation of treatment (initial body weights from 167 to 241 g for M and 144 to 199 g for F).

Animal housing: The animals were housed individually in stainless steel cages and given Certified rodent diet (#8728C, Harlan Teklad) and water *ad libitum*. Environmental controls for the animal room were set to maintain19 to 26°C, a relative humidity of 30% to 70%, a minimum of 10 room air changes/hour, and a 12-hour light/dark cycle.

Paradigm for dietary restriction: No

Dual control employed: Yes

Interim sacrifice: Toxicokinetic and Prolactin Animals: Sacrificed following Week 53 or 54

Satellite groups: TK and prolactin analysis animals (15 /sex/ group)

	No. of Animals		Dose Level	Dose Concentration
Group	Male	Female	(mg/kg/day)[a]	(mg/mL)[a]
Carcinogenicity Animals				
1 (Control)[b]	65	65	0	0
2 (Control)[b]	65	65	0	0
3 (Low)	65	65	3	0.6
4 (Mid)	65	65	12	2.4
5 (High)	65	65	50/36[e]	10/7.2[e]
Toxicokinetic and Prolactin Analysis Animals[c]				
6 (Control)[b,d]	15	15	0	0
7 (Low)	15	15	3	0.6
8 (Mid)	15	15	12	2.4
9 (High)	15	15	50	10

Note: A total of 770 animals (385 males and 385 females) were assigned to this study.
a The dose volume was 5 ml/kg.
b The animals in Groups 1, 2, and 6 received the control article only.
c Up to twelve animals/sex/group (Groups 7, 8, and 9) were used for toxicokinetic analysis; up to 12 animals/sex/group (Groups 6, 7, 8, and 9) were used for prolactin analysis. Remaining animals were used as extra animals for blood collection.
d Animals in Group 6 (control) were used for prolactin analysis only.

e The dose level was reduced to 36 mg of test article/kg of body weight/day (mg/kg/day) (dose concentration of 7.2 mg/ml) beginning on Days 404 and 403 for male and female, respectively

Deviation from study protocol: Study deviations did not affect the overall interpretation of results or compromised its integrity.

Observations: Mortality; clinical signs, ophthalmology, body weight and food consumption, hematology, clinical chemistry, estrous cycle (females), prolactin evaluations, TK and anatomic pathology (see the following sponsor's table).

Inlife Procedures	
Husbandry[a]	Male and female rats were housed individually in stainless steel cages and offered Certified rodent diet (#8728C, Harlan Teklad) and water was provided ad libitum. Environmental controls for the animal room were set to maintain 19 to 26°C, a relative humidity of 30% to 70%, a minimum of 10 room air changes/hour, and a 12-hour light/12-hour dark cycle. Any variations to these conditions are maintained in the raw data and had no effect on the outcome of the study.
Dose Administration[a]	Administered by oral gavage daily for at least 104 weeks at a dose volume of 5 mL/kg. Treatment continued through the day prior to scheduled sacrifice.
Clinical Observations (Groups 1 through 5)[a]	Animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Additional findings were recorded as observed. Detailed observations were made weekly and on the day of scheduled sacrifice.
Mass Palpations/Observations (Groups 1 through 5)	The following information on each grossly visible or palpable mass was recorded. time of onset location size (small or large) appearance progression Additional findings were recorded as observed.
Estrous Cycle Determinations (Groups 6 through 9)	Vaginal smears were done daily for all females during Weeks 52, 53, and 54.
Ophthalmic Examinations (Groups 1 through 5)	Examined once prior to treatment and during the predose phase and during Weeks 52 and 102 and 103 for 10 carcinogenicity animals/sex/group by a board certified veterinary ophthalmologist using an indirect ophthalmoscope and slit lamp. The eyes were dilated with a mydriatic agent prior to examination.
Body Weights	Taken at least once prior to treatment, weekly for Weeks 1 through 14, once every 4 weeks thereafter, and at Week 105
Food Consumption (Groups 1 through 5)[a]	Measured weekly for Weeks 1 to 14 and every 4 weeks thereafter
Clinical Pathology[a]	Samples were taken during Week 53 from toxicokinetic/prolactin analysis animals (Groups 6 through 9) and at scheduled sacrifice for carcinogenicity animals (Groups 1 through 5). Blood smears were examined for specific animals when requested by the pathologist to aid in the diagnosis of hematopoietic disorders. Animals were bled for hematology and clinical chemistry during Week 53 (all surviving animals in Groups 6 through 9) and at scheduled sacrifice (all surviving animals in Groups 1 through 5). Animals were fasted overnight for scheduled collections. Blood was collected via a jugular vein. The anticoagulant was potassium EDTA for the hematology tests. Samples collected for clinical chemistry were collected without anticoagulant.
Hematology Tests[a]	Red blood cell (erythrocyte) count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell (leukocyte) count, differential blood cell count, and blood smear
Clinical Chemistry Tests[a]	Glucose, urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, triglycerides, total bilirubin, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, aspartate aminotransferase, calcium, inorganic phosphorus, sodium, potassium, and chloride

Procedure	Frequency/Comment
Toxicokinetic Analyses[a]	Samples were taken on Day 1 and during Weeks 26 and 52; three toxicokinetic animals/sex/group/time point from Groups 7, 8, and 9 were bled approximately 0.5, 2, 6, and 24 hours postdose. During Week 104, three carcinogenicity animals/sex/group/time point were bled approximately 2 and 24 hours postdose. Serum analysis was done by (b) (4) Toxicokinetic analysis for samples collected during Weeks 1, 26, and 52 was also done by (b) (4) Toxicokinetic samples were not analyzed for Week 104.
Prolactin Analysis[a]	Samples were taken during Week 53 or 54 at least three days following toxicokinetic sampling from up to 12 females in Groups 6, 7, 8, and 9. Serum analysis for prolactin levels was done by (b) (4) The remaining samples will be disposed at study finalization.
Disposition of Animals	
Toxicokinetic and Prolactin Animals	Following blood collection for prolactin analysis during Week 53 or 54, animals were sacrificed with carbon dioxide and discarded without necropsy. The extra animals assigned to these groups were sacrificed with carbon dioxide and discarded following completion of the Week 54 blood collection.
Unscheduled Deaths	A necropsy was done on animals in Groups 1 through 5 that died or were sacrificed at an unscheduled interval.
Scheduled Sacrifice[a]	After at least 104 weeks of treatment, all surviving carcinogenicity animals were sacrificed and necropsied. Terminal body weights were recorded.
Necropsy	Necropsy included an examination of the external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.
Bone Marrow Smears[a]	Prepared from the femur of animals sacrificed at scheduled and unscheduled intervals. Bone marrow smears were evaluated when deemed necessary by the study pathologist and study director (added by amendment).
Tissue Preservation	Adrenal (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, eye (2), femur with bone marrow (articular surface of the distal end), Harderian gland, heart, ileum jejunum, kidney (2), larynx, lesions, liver, lung with mainstem bronchi, lymph node (mesenteric), mammary gland (males and females), nasal cavity, ovary (2), optic nerve (2), pancreas, pituitary gland, preputial/clitoral gland, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle (2), skeletal muscle, skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis (2), thymus, thyroid (2) with parathyroid, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal gland
Histopathology[a]	All tissues from animals in Groups 1 through 5 and animals that died or were sacrificed at an unscheduled interval were processed and examined microscopically. Protocol specified tissues were processed and examined microscopically.

Results:

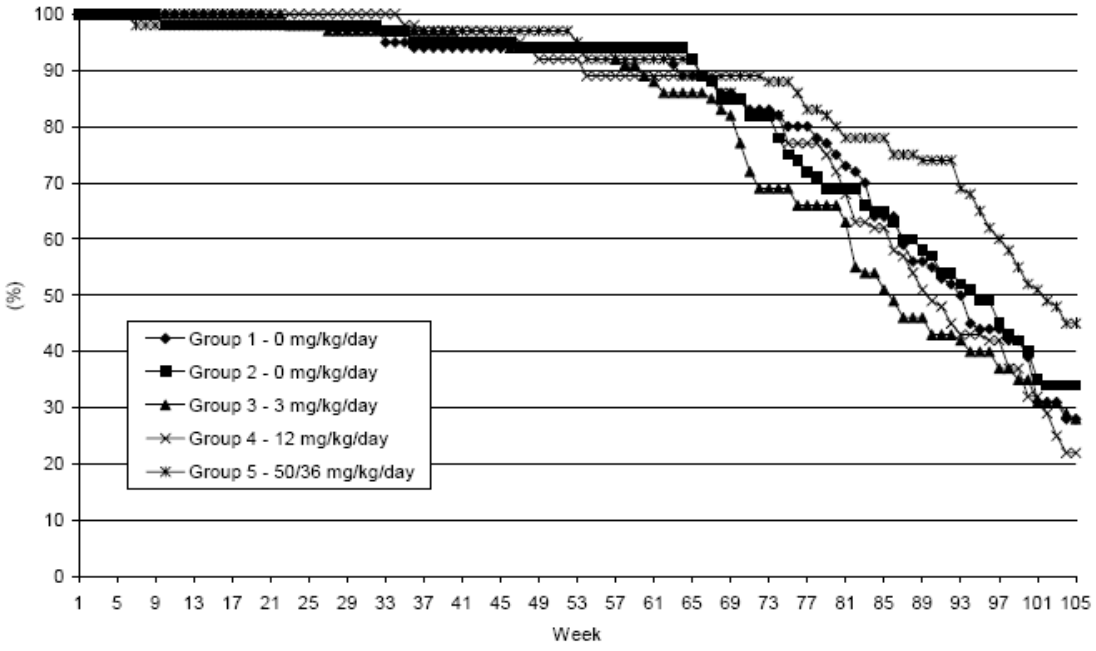
Mortality: There was no increase in mortality at any dose in either males or females.

In males, survival in the two control groups was comparable throughout the study, and the survival for LD and MD groups was comparable to controls. At HD, the survival was comparable to controls until approximately Week 60; from Weeks 60 to 90 it declined at a slower rate than controls or other treated groups and at comparable rates thereafter. The number of males that survived until the end of Week 105 was 18, 22, 18, 14, and 29 for the dose groups of 0, 0, 3, 12, and 50/36 mg/kg/day, respectively. Treatment with the test article resulted in dose-dependently reduced mortality, significant in the high-dose males.

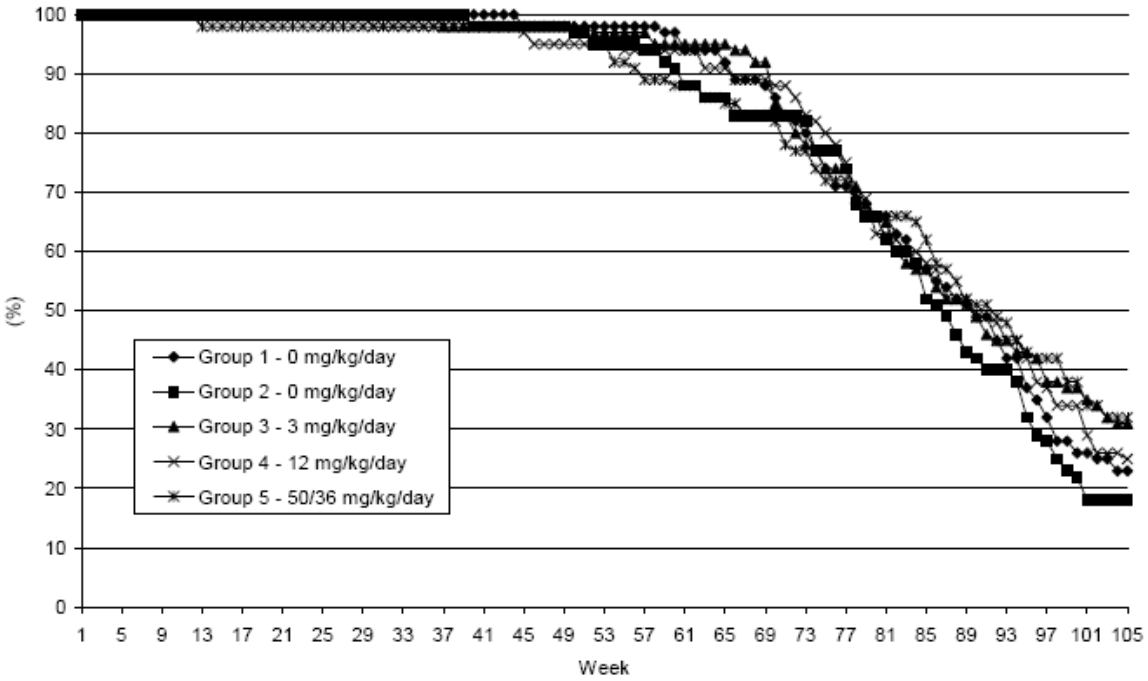
In females, survival in the two control groups was comparable throughout the study and the survival for all dosed groups was comparable to controls until approximately Week 80; between Week 80 and 102, the survival declined faster in the two control groups than in the dosed groups. The number of females surviving until the end of Week 105 was 15, 12, 20, 16, and 21 for the dose groups of 0, 0, 3, 12, and 50/36 mg/kg/day, respectively. Although not statistically significant, there were reduced mortalities in all treated groups compared with either control (see the following sponsor's figures).

Figure

Adjusted Survival Data (%) – Males



Adjusted Survival Data (%) – Females



Clinical Signs

Clinical observations (means for Days 1 through 183, 184 through 365, 366 through 547, and 548 through 736) were comparable among the groups across these intervals. Palpable masses were registered in all groups, including controls, at locations typical for rats of this strain and duration of study.
Ophthalmic observations [recorded during Weeks 52 and 102 (F) or 103 (M)] did not show any drug-related changes.

Body Weights

Mean body weight values for specific time periods (excerpts from sponsor's tables) are shown on the next page.
In both males and females, the mean body weight values of the two control groups were similar across the entire study.
In males, a dose- and time-dependent reduction in body weight in comparison to control occurred at all dose levels. A statistically significant change in body weight in comparison to both control groups was first registered at 3, 13, and 46 weeks for the HD, MD and LD, respectively. At Week 58 (when the high dose level was reduced from 50 to 36 mg/kg), the percentage difference of mean body weight versus control was -7, -10, and -20% for LD, MD and HD groups, respectively. This large difference for the HD group was a rationale for lowering the dose level to 36 mg/kg/day. The peak body weight for the control groups occurred at Week 78; at this time, the percentage difference of mean body weight in treated groups in comparison to control was -8, -13, and -22% for LD, MD and HD, respectively. The similarity in percentage differences across time indicates that the lowering of the dose from 50 to 36 mg/kg/day resulted in maintaining the existing weight status but it did not reverse the reduction of body weight.
In females, biphasic and time-dependent changes in body weight were observed. At LD, statistically significant increase in comparison to both control groups were registered from Week 2 through 66; at MD, the mean body weight was higher than controls during Weeks 2 through 18 and lower than controls from Week 46 onwards; at HD, a significant decrease vs. controls was detected from Week 22 onwards. At Week 58, when the high dose level was reduced from 50 to 36 mg/kg, the percentage difference of mean body weight versus control was +12, -8, and -19% for LD, MD and HD, respectively. This large difference for the HD group was the reason for lowering the dose level to 36 mg/kg/day. At Week 90, when the mean body weight of control groups was at its peak, the percentage differences of the mean body weight at LD, MD, and HD in comparison to control were +3, -15, and -25%, respectively.

Body weight change

Males: The body weight change values were similar for the two control groups; for the treated dose groups the body weight gain was generally lower than controls, except for LD fromWeek 1 to 14. For Weeks 78 through 105, the mean body weight change values were negative for all dose groups, although the differences were not statistically significant.
Females: The body weight change values were similar for the two control groups, except for the period from Week 1 to 14 for which a statistical difference was detected (Group 2 higher than Group 1). At LD and MD, the mean body weight change values were significantly higher compared to each of the control groups during weeks 1 to 14. At MD, the body weight change values were significantly lower than those of the control groups during Weeks 14 to 54 and Weeks 54 to 78. At HD, the body weight change values were significantly lower than the control groups for Weeks 14 to 54 and Weeks 54 to 78. For Weeks 78 through 105, no statistical differences were registered.

Summary of Body Weight Means (g)

Test Article		Control		SM-13496		
Group		1	2	3	4	5
Dose Level (mg/kg/day)		0	0	3	12	50/36

Males

Week		Group 1M	Group 2M	Group 3M	Group 4M	Group 5M
1	Mean	206	207	206	206	206
	SD	17.7	17.0	16.3	16.7	17.3
	N	65	65	65	65	65
3	Mean	322	320	320	319	309 A,B
	SD	22.9	25.1	23.7	22.9	25.5
	N	65	65	65	65	65
13	Mean	567	579	566	547 A,B	512 A,B
	SD	51.7	56.7	49.5	44.1	47.8
	N	65	64	65	65	64
46	Mean	755	768	716 A,B	683 A,B	609 A,B
	SD	93.0	104.9	74.7	70.0	68.1
	N	61	62	62	62	63
58	Mean	792	803	739 A,B	712 A,B	633 A,B
	SD	104.5	117.1	79.8	77.7	76.3
	N	61	61	60	58	60
78	Mean	837	842	773 A,B	730 A,B	653 A,B
	SD	119.6	122.1	86.5	83.4	78.3
	N	51	47	43	50	54
105	Mean	806	762	735 A	680 A,B	608 A,B
	SD	93.4	99.2	91.1	89.1	76.5
	N	18	22	19	14	29

Females

1	Mean	173	171	173	172	171
	SD	12.3	12.4	12.1	12.3	12.6
	N	65	65	64	65	65
2	Mean	194	195	207 A,B	205 A,B	190
	SD	14.0	14.4	15.9	16.4	15.9
	N	65	65	64	65	65
18	Mean	330	338	389 A,B	350 A,B	318 B
	SD	33.9	37.0	42.9	34.1	32.8
	N	65	65	65	65	64
22	Mean	343	353	405 A,B	357 A	327 A,B
	SD	37.1	38.5	48.2	36.2	34.1
	N	65	65	65	65	64
58	Mean	446	454	500 A,B	411 A,B	363 A,B
	SD	75.0	72.8	90.4	64.7	50.5
	N	64	61	63	61	58
66	Mean	462	486	519 A,B	422 A,B	367 A,B
	SD	81.6	84.2	93.6	71.5	53.3
	N	60	56	62	59	55
90	Mean	524	530	542	446 A,B	394 A,B
	SD	119.8	111.1	106.4	87.4	62.3
	N	33	28	34	34	33
105	Mean	530	600	544	418 A,B	400 A,B
	SD	136.4	101.7	102.8	95.2	68.9
	N	15	12	20	17	21

A Statistically significant from Group 1 at $p < 0.05$.
B Statistically significant from Group 2 at $p < 0.05$.

Summary of Body Weight Change Data (g)

Test Article	Control	SM-13496		
Group	1 2	3	4	5
Dose Level (mg/kg/day)	0 0	3	12	50/36

Males

Week		Group 1M	Group 2M	Group 3M	Group 4M	Group 5M
1 to 14	Mean	374	383	369	350 A,B	317 A,B
	SD	50.4	51.4	43.5	40.2	41.7
	N	65	64	65	65	64
14 to 54	Mean	200	203	156 A,B	132 A,B	103 A,B
	SD	60.8	64.9	45.6	52.6	39.4
	N	61	61	61	60	62
54 to 78	Mean	57	64	41 A,B	37 A,B	32 A,B
	SD	55.2	36.3	31.9	45.2	34.6
	N	51	47	43	50	54
78 to 105	Mean	-7	-31	-41	-32	-33
	SD	56.1	83.7	71.2	37.3	47.0
	N	18	22	19	14	29

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p < 0.05$.

Females

Week		Group 1F	Group 2F	Group 3F	Group 4F	Group 5F
1 to 14	Mean	141	151 A	196 A,B	164 A,B	136 B
	SD	25.4	26.4	30.6	29.6	24.4
	N	65	65	64	65	64
14 to 54	Mean	125	127	123	69 A,B	51 A,B
	SD	52.3	47.8	53.1	37.9	29.7
	N	64	62	63	62	62
54 to 78	Mean	65	65	55	35 A,B	25 A,B
	SD	44.1	64.0	46.4	34.1	30.6
	N	46	48	48	49	47
78 to 105	Mean	16	50	15	-1	17
	SD	74.4	41.4	68.5	50.7	16.8
	N	15	12	20	17	21

A Statistically significant from Group 1 at $p \leq 0.05$.
B Statistically significant from Group 2 at $p \leq 0.05$.

Feed Consumption

Males: There were no consistent effects on food consumption at LD and MD; At HD, food consumption was reduced in comparison to each control group for most of the measured time intervals.

Females: At LD, food consumption was increased in comparison to both control groups through Week 33; after this time there were only occasional differences. At MD, food consumption was generally decreased beginning at Week 29; and at HD, food consumption was reduced in comparison to both control groups for most of the measured intervals beginning in Week 1.

Overall, in LD females, food consumption was increased in comparison to controls. Conversely, in MD females, and in HD males and females, food consumption was reduced in comparison to controls. At HD, the lower food consumption values were consistent with the lower body weights and body weight gains for both genders.

Food Consumption Summary (g)

Test Article		Control	SM-13496		
Group		1 2	3 4	5	
Dose Level (mg/kg/day)		0 0	3 12	50/36	

Males

Week		Group 1M	Group 2M	Group 3M	Group 4M	Group 5M
8	Mean	241	247	250 A	238 B	228 A,B
	SD	21.4	22.5	21.6	19.7	20.0
	N	65	65	65	65	64
13	Mean	234	240	236	231 B	222 A,B
	SD	23.0	23.8	24.6	20.2	21.5
	N	65	64	64	65	63
25	Mean	226	229	236 A	218 A,B	202 A,B
	SD	24.8	24.4	22.0	20.7	20.9
	N	64	64	64	65	64
37	Mean	215	219	222	218	191 A,B
	SD	21.0	24.2	21.3	25.5	24.3
	N	60	62	63	63	63
45	Mean	230	237	225 B	227 B	207 A,B
	SD	24.8	29.0	31.2	26.7	20.9
	N	61	62	62	62	63
57	Mean	232	240	224 B	219 A,B	198 A,B
	SD	26.7	31.4	28.8	24.1	22.7
	N	61	61	60	58	59
73	Mean	225	222	215	214	195 A,B
	SD	36.3	41.9	36.5	29.7	34.8
	N	54	52	45	53	57
85	Mean	220	219	209	218	193 A,B
	SD	32.9	30.4	45.0	33.6	33.5
	N	41	42	33	40	51
101	Mean	220	219	220	203	193 A,B
	SD	35.6	24.6	19.7	20.6	37.8
	N	19	22	20	21	33

A Statistically significant from Group 1 at $p < 0.05$.
B Statistically significant from Group 2 at $p < 0.05$.

Females

Week		Group 1M	Group 2M	Group 3M	Group 4M	Group 5M
1	Mean	146	143	146	148 B	117 A,B
	SD	11.9	13.7	14.5	12.7	17.1
	N	65	64	64	65	65
2	Mean	157	157	174 A,B	161	150 A,B
	SD	12.9	16.5	21.8	18.2	25.1
	N	65	65	64	65	64

Continued on the next page

Food consumption Females (Continued)

Week		Group 1M	Group 2M	Group 3M	Group 4M	Group 5M
8	Mean	161	166	180 A,B	159 B	151 A,B
	SD	16.4	18.1	21.7	18.5	18.6
	N	65	65	65	65	65
17	Mean	152	159 A	179 A,B	156	138 A,B
	SD	16.1	16.2	23.3	17.0	18.2
	N	64	65	65	65	64
25	Mean	157	161	176 A,B	152 B	139 A,B
	SD	18.8	17.1	23.4	18.4	17.6
	N	65	65	64	65	64
29	Mean	158	167 A	170 A	148 A,B	140 A,B
	SD	20.1	20.5	26.7	17.8	19.5
	N	65	65	65	65	64
33	Mean	163	164	173 A,B	151 A,B	134 A,B
	SD	21.4	20.3	23.5	19.2	18.4
	N	64	65	65	64	64
45	Mean	169	169	171	154 A,B	137 A,B
	SD	19.9	23.9	25.0	21.1	16.5
	N	64	64	64	63	64
57	Mean	174	178	181	158 A,B	142 A,B
	SD	28.0	27.7	32.5	20.9	29.3
	N	64	61	63	61	58
65	Mean	172	175	175	153 A,B	139 A,B
	SD	28.2	34.5	29.5	21.1	19.5
	N	59	56	62	59	55
89	Mean	156	159	156	134 A,B	128 A,B
	SD	34.1	38.2	26.8	25.8	23.6
	N	33	28	34	34	33
101	Mean	157	171	161	139 A,B	131 A,B
	SD	34.9	20.3	26.8	29.2	20.6
	N	17	12	23	19	22

A Statistically significant from Group 1 at $p < 0.05$.
B Statistically significant from Group 2 at $p < 0.05$.

Estrus Evaluations

Estrus cycle evaluations (performed during Weeks 52 and 53) showed a dose-dependent disruption in estrus cycle at all tested dose levels. The proportion of females entering estrus during this period were 86, 53, 40, and 27% in control, LD, MD, and HD, respectively.

Prolactin Determinations

Prolactin levels were determined in serum from animals that received 0, 3, 12 and 50 mg/kg/d of SM-13496 via oral gavage for 53 weeks (females) or 54 weeks (males). Serum samples (12 animals/ gender/ group) collected during Week 53 or 54 from animals in the toxicokinetic and prolactin analysis study were used for prolactin analysis. In females, blood was only collected from animals that were not in the proestrus phase of the estrus cycle. Blood was collected at the approximate time of Cmax (2 hours post dose).

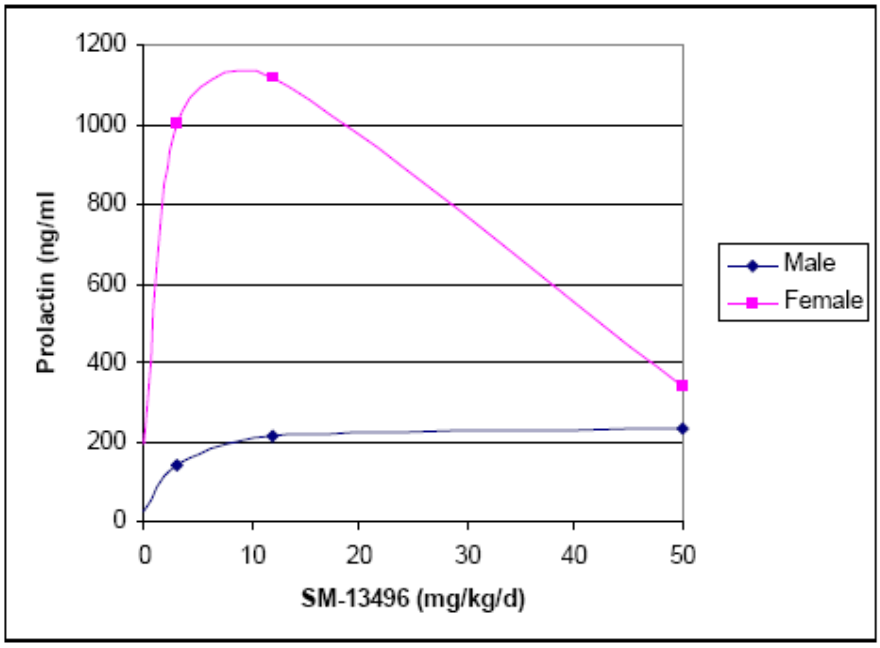
In <u>males</u>, SM-13496 produced a significant increase in serum prolactin levels at all doses tested and appeared to reach a plateau after 12 mg/kg/d. The average values of prolactin were 29.6, 140.5, 216.9 and 233.7 ng/ml at 0, 3, 12 and 50 mg/kg/d of SM-13496, respectively. In <u>females,</u> SM-13496 significantly increased serum prolactin at 3 and 12 mg/kg/d, <u>but not</u> at 50 mg/kg/d (see the graph and tables in the following 2 pages). The average values of prolactin were 199.3, 1004.2, 1116.7 and 340.6 ng/ml at 0, 3, 12 and 50 mg/kg/d of SM-13496, respectively.

Serum Prolactin in SM-13496-treated rats (Mean, ng/ml)

	SM-13496 (mg/kg)			
	0	3	12	50
	Male			
Mean	29.7	141[c]	217[c]	234[c]
SD[a]	18.47	50.3	96.2	79.5
N[b]	12	12	12	12
	Female			
Mean	199	1004[c]	1117[c]	341
SD[a]	137.3	516.9	648.5	198.5
N[b]	12	12	12	12

[a] standard deviation (SD); [b] number (N); [c] $p<0.001$ in comparison to vehicle group of same sex (ANOVA test).

Figure **Serum Prolactin Levels in SM-13496-treated male and female rats**



Serum Prolactin Individual Values in SM-13496-treated rats

CONTROL (VEHICLE)		SM-13496					
		3 MG/KG/D		12 MG/KG/D		50 MG/KG/D	
ANIMAL NUMBER	PROLACTIN NG/ML	ANIMAL NUMBER	PROLACTIN NG/ML	ANIMAL NUMBER	PROLACTIN NG/ML	ANIMAL NUMBER	PROLACTIN NG/ML
MALES							
(b) (4)							
MALES, MEAN (SD)							
12	**29.65**	12	**140.54**	12	**216.87**	12	**233.71**
	(18.47)		(50.32)		(96.16)		(79.46)

Prolactin Individual Values in SM-13496-Treated rats (continued)

Control (Vehicle)		SM-13496					
		3 mg/kg/d		12 mg/kg/d		50 mg/kg/d	
Animal Number	**Prolactin** ng/ml	Animal Number	**Prolactin** ng/ml	Animal Number	**Prolactin** ng/ml	Animal Number	**Prolactin** ng/ml
Females							
(b) (4)							
Females, Mean (SD)							
12	**199.31**	12	**1004.21**	12	**1116.69**	12	**340.59**
	(137.33)		(516.92)		(648.53)		(198.50)

** The value of the diluted sample was below the limit of quantification for the assay.

Hematology

There were no notable changes in hematology parameters at the 2 sampling time points (weeks 56 and 106), except for an increase in neutrophil counts and decrease in lymphocytes at week 106 (at all doses, both genders), and a slight decrease in eosinophils (at all doses, females) at week 106, as reproduced from the sponsor's table below.

Hematology

MALES

Group		LYMPH AI E3/UL Week 10	NEUT % % Week 10	LYMPH % % Week 10
1M	Mea	4.52	33.3	59.1
	S	1.165	9.89	10.38
		17	17	17
2M	Mea	4.29	38.3	54.8
	S	1.139	15.87	15.74
		21	21	21
3M	Mea	3.80	43.5 A	49.0 A
	S	1.204	12.35	12.10
		17	17	17
4M	Mea	3.07 J	49.1 A	43.5 A
	S	1.111	11.08	10.03
		14	14	14
5M	Mea	2.96 J	49.9 A	43.3 A
	S	1.399	10.73	10.78
		28	28	28

FEMALES

Group		LYMPH % % Week 1(	NEUT % % Week 10	EOSIN % % Week 1(
1F	M	49.7	42.7	1.9
		12.73	12.75	1.29
		15	15	15
2F	M	49.9	42.1	1.7
		16.03	16.17	0.71
		12	12	12
3F	M	36.2 J	56.9 A	1.2 J
		13.73	14.23	0.60
		19	19	19
4F	M	36.4 J	56.7 A	1.0 J
		13.65	15.24	0.72
		16	16	16
5F	M	43.4	50.2	1.3 J
		15.75	16.10	0.97
		21	21	21

Test Article	Control		SM-13496		
Group	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	3	12	50/36

A: Statistically significant from control (1) at $p < 0.05$.
B: Statistically significant from control (2) at $p < 0.05$

Clinical Chemistry

Week 53: Lower glucose and triglycerides at all doses (males and females), mildly higher aspartate aminotransferase at MD and HD (males), and slightly lower potassium in males at all doses and in females at MD and HD.
Week 106: lower glucose at all doses (males and females), and lower triglycerides (at all doses in females and at MD and HD males) higher total protein (primarily due to higher globulin) at LD (males), MD (males) and HD (males and females), and minimally lower alkaline phosphatase and potassium at LD and MD (males). Lower glucose and triglycerides were the only findings consistent at both time intervals and in both sexes. Statistically significant findings for other clinical pathology parameter were considered incidental, as they were usually very small, inconsistent between genders, and lacked a dose-related response.

Clinical Chemistry Data

Week 53

		Males				Females	
Group		GLU MG/DL Week 53	TRIG MG/DL Week 53	AST/SGOT IU/L Week 53	K MMOL/L Week 53	GLU MG/DL Week 53	AST/SGOT IU/L Week 53
6M	Mean	112	155	108	6.4	98	126
	SD	7.8	91.6	20.3	0.47	9.7	35.3
	N	13	13	13	13	13	13
7M	Mean	102 A	131	119	5.7 A	90 A	135
	SD	7.1	61.5	28.5	0.43	7.5	39.7
	N	13	13	13	13	15	15
8M	Mean	98 A	122	146 A	5.7 A	82 A	117
	SD	7.5	48.7	35.0	0.31	8.1	20.2
	N	15	15	15	15	15	15
9M	Mean	97 A	131	134 A	6.0 A	86 A	130
	SD	10.8	51.0	33.2	0.57	8.5	37.5
	N	15	15	15	15	15	15

A: Statistically significant from control at $p < 0.05$.

Week 106

		Males					Females		
Group		GLU MG/DL Week 106	TRIG MG/DL Week 106	T PRO G/DL Week 106	ALK PHOS IU/L Week 106	K MMOL/L Week 106	GLU MG/DL Week 106	TRIG MG/DL Week 106	T PRO G/DL Week 106
1M	Mean	81	146	7.0	52	5.8	78	118	7.2
	SD	15.8	65.4	0.47	19.0	0.64	23.5	143.0	0.54
	N	18	18	18	18	18	15	15	15
2M	Mean	80	119	7.0	57	6.0	90	139 A	7.1
	SD	20.3	55.7	0.38	13.4	0.60	14.1	69.0	0.75
	N	21	21	21	21	21	12	12	12
3M	Mean	69 A	113	7.2	55	5.9	67 B	84 B	7.5
	SD	14.1	45.8	0.68	40.4	0.63	17.4	56.6	0.86
	N	18	18	18	18	18	19	19	19
4M	Mean	62 AB	102 A	7.3 B	40 B	5.6 B	74 B	75 B	7.2
	SD	20.6	56.3	0.43	14.0	0.54	21.7	55.2	0.92
	N	14	14	14	14	14	16	16	16
5M	Mean	63 AB	71 AB	7.6 AB	35 AB	5.5 B	73 B	79 B	7.7 AB
	SD	18.9	27.4	0.74	15.7	0.69	13.3	50.4	0.47
	N	29	29	29	29	29	21	21	21

Test Article	Control		SM-13496		
Group	1	2	3	4	5
Dose Level (mg/kg/day)	0	0	3	12	50/36

A: Statistically significant from control (1) at $p < 0.05$.
B: Statistically significant from control (2) at $p < 0.05$

Gross Pathology

Males (N examined: 18, 22, 18, 14, and 29 for control 1, control 2, LD, MD, and HD, respectively): Gross pathology changes in males were registered almost exclusively at the high dose, i.e., increased incidence of mammary cysts (2, 0, 3, 2, and 7 for control 1, control 2, LD, MD, and HD, respectively); larger size and/or mottled appearance of adrenal cortex (2, 2, 2, 2, and 8 for control 1, control 2, LD, MD, and HD, respectively); pituitary masses (4, 1, 4, 2 and 9 for control 1, control 2, LD, MD, and HD, respectively), enlarged pituitary (0, 1, 0, 1 and 4 for control 1, control 2, LD, MD, and HD, respectively); red focal areas in liver (3, 2, 4, 1, and 11 for control 1, control 2, LD, MD, and HD, respectively). The only gross finding in MD males was that of pancreatic masses, non-dose-dependent (1, 1, 2, 5, and 1 for control 1, control 2, LD, MD, and HD, respectively)..
Females (N examined: 15, 12, 20, 16 and 21 for control 1, control 2, LD, MD, and HD, respectively): Mammary masses in all dosed groups (11, 6, 14, 13, and 15 for control 1, control 2, LD, MD, and HD, respectively); larger size and/or mottled appearance of adrenal cortex in all dosed groups, non-dose-dependent (3, 1, 7, 3, and 4 for control 1, control 2, LD, MD, and HD, respectively).

Histopathology

Sponsor's assessment
Males:
- Neoplastic Lesions
In males, as shown in the next sponsor's table, the mid-dose (12 mg/kg/d) group was significantly increased over Control 2 for skin/muscle, skeletal fibrosarcoma, and fibroma/fibrosarcoma combined. No other significant effects, either in terms of positive trend or significant increase over the controls, were noted.

Neoplastic Lesions - Males

Skin/Muscle, Skeletal, Fibrosarcoma-M (P)	2/65	0/65	2/65	5/65	0/65
1 vs 2 p-value (one-sided)		0.2489 -			
1 vs 3-5 p-value (one-sided)	0.3299 -		NA	0.2054 +	0.2331 -
2 vs 3-5 p-value (one-sided)		0.4143 +	0.2296 +	0.0269 + *	NA

* = Significant at 0.05 level.
NA = Not Analyzed

"The apparent significant mid-dose group increase over Control 2 for fibrosarcoma and fibroma/fibrosarcoma combined in the skin/muscle, skeletal is considered background noise because of the lack of such effect versus Control 1 as well as the lack of trend versus both controls. No other significances were noted in males."

- Non-neoplastic lesions
Increased milk secretion was observed in males at all dose levels in a dose-dependent manner:

Test Article-Related Microscopic Findings – Males

Dose Level (mg/kg/day):	0	0	3	12	50/36
No. Examined:	41	40	45	49	54
Mammary Gland Increased Secretion					
Minimal	7	7	6	6	14
Slight	0	2	7	9	15
Moderate	4	0	7	12	8

Females:

- Neoplastic lesions

Increased incidence of mammary carcinomas was found in mid- and high-dose females (statistically significant); significant positive trends versus Control 2 were observed, with high-dose significantly increased over Control 2 ($p = 0.0025$), and the mid-dose significantly increased over both controls ($p = 0.0277$ and $p = 0.0026$, respectively) for mammary carcinoma. The mid-dose group also showed significant increase over both controls ($p = 0.0492$ and $p = 0.0021$, respectively) in mammary adenoma. The incidence of other mammary tumors was similar for control and treated females. When the adenoma, carcinoma, and fibroadenoma incidences were combined, the significant trend disappeared, but the mid-dose group significant increase against the two controls remained ($p = 0.0286$ and $p = 0.0367$, respectively). "In other words, the combined mammary tumor incidences did not show any biologically meaningful detrimental effect". No other increased tumor incidences were observed in the females.

Neoplastic Lesions - Females

Dose Level (mg/kg/day):	0	0	3	12	50/36
No. Examined:	65	65	65	65	65
Mammary Gland Carcinoma					
Present	19	14	21	30	32

"Significant negative trends for pituitary adenoma and adenoma/carcinoma combined versus both controls were observed, with high-dose significant decrease over Control 2 ($p = 0.0026$ for adenoma and $p = 0.0081$ for pituitary adenoma/carcinoma combined). Statistically significant decreases in the mid-dose group of pituitary adenoma and adenoma/carcinoma combined versus both controls were also noted. The decreased tumor incidences, such as in the female pituitary, showed inconsistent patterns and were probably of biological variation".

- Non-neoplastic lesions

A dose-related incidence of absent corpora lutea in the ovary and increased cornification of the vagina was noted at all dose levels (see the following sponsor's table). This finding was indicative of an interruption of the estrous cycle in the treated females.

Ovarian and Vaginal Microscopic Findings
Terminal Sacrifice - Females

Dose Level (mg/kg/day):	0	0	3	12	50/36
No. Examined:	15	12	20	16	21
Ovary Corpora Lutea					
Absent	7	4	16	14	20
Vagina Cornification					
Present	6	3	13	11	14

The most common causes of death were pituitary adenomas for males and females and mammary fibroadenomas and carcinomas for controls and treated animals.

Peer Review: The following statement was provided by the sponsor (as cited from the study report):

A microscopic peer review was performed at (b) (4), April 17-26, 2006. Diagnostic terminology was discussed and agreement was reached between the Study Pathologist and the Reviewing Pathologists.

(End citation)

FDA's statistical reviewer's assessment of the neoplastic findings in rats:

Males: Skin fibroma/fibrosarcoma was significantly increased over the pooled vehicle control only at mid-dose, but not at high dose (see table below). No other significant effects, either in terms of positive trend or significant increase over the pooled controls, were noted.
Females: Increased incidence of mammary carcinomas was found at mid- and high-dose; the test of trend was statistically significant, as was the test comparing the HD and MD to the pooled vehicle control; at the mid-dose, the incidence of mammary adenomas was also significantly increased over pooled controls, but there was no increase in this tumor incidence at the high dose. The incidence of other mammary tumors was similar for control and treated females. No other tests of trend or comparisons between the high dose and controls achieved the multiplicity adjusted significance levels.

Selected Neoplasms in Rats*

	Incidence					Significance Levels			
	Veh 1	Veh 2	Low	Med	High	Trend	High vs Veh	Medium vs Veh	Low vs Veh
N	65	65	65	65	65				
Male Rats									
HEMATO NEOPLASIA									
M-LYMPHOMA	0	1	4	1	2	0.4121	0.3047	0.5432	0.0396
MAMMARY, MALE									
Adenona/Carc./Fibro.	0	1	1	4	1	0.5059	0.6019	0.0372	0.5284
SKIN									
Fibrona/Fibrosarcoma	2	0	2	7	1	0.6429	0.2982	0.0064	0.3789
M-FIBROSARCOMA	1	0	2	5	0	0.8026	0.3630	0.0156	0.2364
Female Rats									
MAMMARY, FEMALE									
Adenona/Carc./Fibro./mixed	38	38	41	50	42	0.1771	0.1966	0.0121	0.3523
B-ADENOMA	11	5	10	20	12	0.2538	0.2477	0.0044	0.4292
M-CARCINOMA	19	14	21	30	32	0.0008	0.0009	0.0027	0.2483
THYROID									
Adenona/Carc. C cell	4	4	2	4	8	0.0398	0.1423	0.3807	0.7363

* FDA statistical analysis (statistical reviewer: Steve Thomson)

Toxicokinetics

Exposure to SM-13496 increased with the dose from 3 to 50 mg/kg/day; the increases in Cmax and AUC0-24hr were generally greater than dose-proportional. The mean concentrations of SM-13496, as well as Cmax and AUC0-24hr values were generally higher after multiple dosing [i.e, the serum levels at Week 104 at the protocol nominal times of 2 and 24 hours postdose were markedly (>2-fold) higher than those on Day 1 even at the high-dose group]. Tmax values ranged from 0.5 to 2 h across all collections. Elimination half-life ($t_{1/2}$) was not determined. Females had higher Cmax and AUC_{0-24hr} values than males across all collections. The data are summarized in the following sponsor's tables and figures.

Dose Proportionality Ratios for SM-13496 in Male and Female Rat Serum

	Proportional Dose	Males		Females	
Interval	Level Increase	C_{max}	AUC_{0-24hr}	C_{max}	AUC_{0-24hr}
Day 1	1:4:17-fold	1.0:5.0:16-fold	1.0:12:28-fold	1.0:5.4:23-fold	1.0:9.3:58-fold
Week 26	1:4:17-fold	1.0:3.3:6.7-fold	1.0:4.5:22-fold	1.0:3.6:20-fold	1.0:4.3:29-fold
Week 52	1:4:17-fold	1.0:3.2:6.6-fold	1.0:6.8:30-fold	1.0:6.0:26-fold	1.0:5.0:30-fold

Toxicokinetic Parameters for SM-13496 in Male and Female Rat Serum

Dose Group	Dose Level (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-24hr} (ng·hr/mL)
Day 1						
7	3	M	14.8	0.500	53.9	53.9
		F	22.4	2.00	79.2	107
8	12	M	74.0	0.500	668	668
		F	121	2.00	994	994
9	50	M	239	2.00	1531	1531
		F	513	2.00	6133	6133
Week 26						
7	3	M	39.7	0.500	169	169
		F	58.9	0.500	299	299
8	12	M	130	2.00	755	755
		F	213	0.500	1277	1277
9	50	M	266	2.00	3724	3724
		F	1181	2.00	8542	8542
Week 52						
7	3	M	66.5	0.500	178	178
		F	67.6	0.500	365	365
8	12	M	210	2.00	1216	1216
		F	408	0.500	1826	1826
9	50	M	440	0.500	5276	5276
		F	1743	2.00	10881	10881

SM-13496 AUC0-24hr Dose Relationships in Male Rat Serum

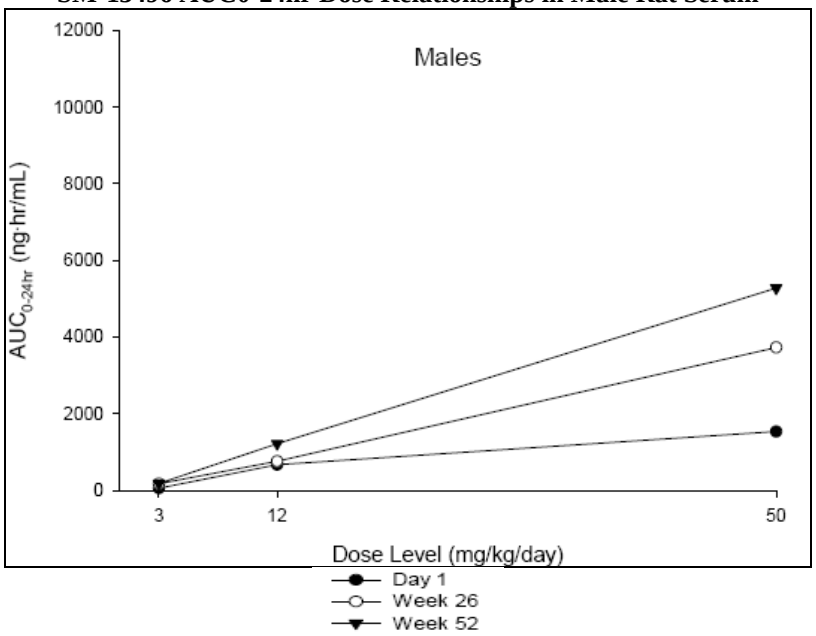
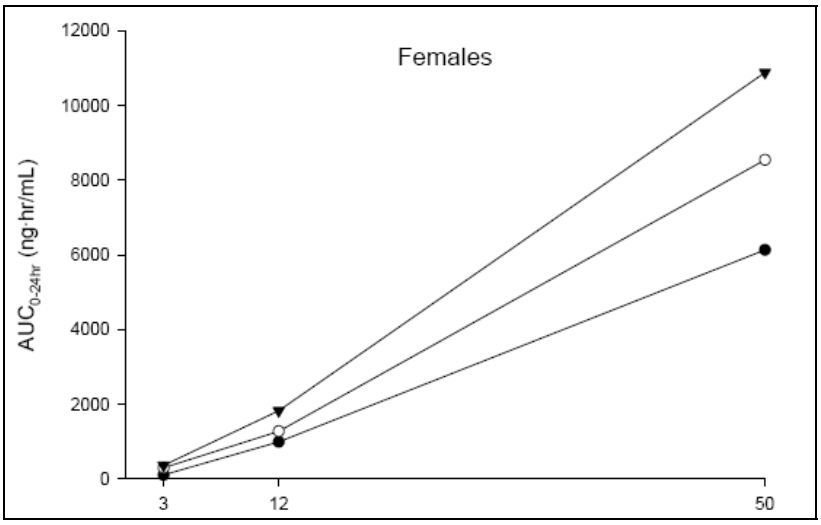


Figure for females on the next page

SM-13496 AUC0-24hr Dose Relationships in Female Rat Serum



Stability and Homogeneity

Stability: "Dose preparations at concentrations of 0.1 and 100 mg/ml (bracketing the concentrations used in this study) were shown to be stable, protected from light, after 31 days of refrigerated storage followed by at least 24 hours of room temperature storage."
Homogeneity: "Samples (1.0 ml each) were taken from the low and high dose levels mixed for use during Week 1. Duplicate samples from the top, middle, and bottom of the dose preparations were collected and analyzed for test article content. All samples were stored protected from light in a refrigerator, set to maintain 2 to 8°C. Homogeneity analysis was repeated at low and high dose levels when the batch size changed by more than 30%."
Overall, the dose analysis data showed that the provided dose concentrations were accurate, homogenous, and stable for the conditions of use in this study.
The dose analysis data showed that the provided dose concentrations were "accurate, homogenous, and stable for the conditions of use in this study".

Conclusion: Lurasidone resulted in increased incidence of mammary carcinomas in female rats at MD and HD (12 and 50/36 mg/kg/d, respectively). The incidence of all other neoplastic lesions in either gender was not elevated at any of the tested dose levels. According to the conclusion of the Executive CAC, the mammary carcinomas in female rats at mid- and high dose lurasidone (12 and 50/36 mg/kg/day, respectively) were drug related. NOEL for neoplasia: Females: mammary carcinoma: 3 mg/kg/day (AUC_{0-24}: 365 ng.h/ml). Compared to the human exposure at the MRHD of 120 mg/day (AUC_{0-inf}: 687 ng.h/ml), the exposure ratio at the NOEL is 0.53, i.e., there is no safety margin for humans.

9 Reproductive and Developmental Toxicology

The reproductive and developmental toxicity studies performed with lurasidone included:
- Fertility and early embryonic development studies in male and female rats,
- Embryo-fetal development studies in rats and rabbits (range-finding and main), and
- Pre- and postnatal development study in rats (dose range-finding and main).

All studies were GLP-compliant (Japanese guidelines), unless otherwise stated.

Fertility and Early Embryonic Development

The effects of lurasidone on reproductive performance in the rat were examined in males and females separately, in studies with lurasidone administration prior to and in early stage of pregnancy.

Study title: Study by oral administration of SM 13496 prior to and in the early stages of pregnancy in rats – Effect on Males
Study no.: Study 2771
Conducting laboratory and location: (b) (4)

Date of study initiation: July 26, 1993
GLP compliance: Yes (Japanese guidelines)
QA statement: Yes
Drug, lot #, and % purity: SM-13496, Lot #3CG001M, purity 100.9%

Key Study Findings

This study evaluated the effects of lurasidone on the reproductive performance of male rats and on the embryo/fetal development of their generation. Male Sprague-Dawley rats (22/group) were administered lurasidone at daily oral gavage doses of 6, 30, and 150 mg/kg/day for 64 consecutive days prior to mating and during the mating period (with untreated females) until copulation. Caesarean sections were performed on the pregnant females at term (Day 20 of gestation) and fetuses were examined externally, viscerally and skeletally.

Decreased paternal body weight gain and food consumption, and clinical signs attributable to the pharmacological effect of the drug occurred at MD and HD (decrease in spontaneous activity at 30 mg/kg/day and above; catalepsy and ptosis at HD of 150 mg/kg/day). There were no effects on copulation or fertility indices of males. No signs of embryo/fetal toxicity were found, except for a non-significant increase in post-implantation embryonic loss in all dosed groups that did not result in a reduction of the number of fetuses at term. External, visceral and skeletal examinations of fetuses showed no drug-related abnormalities.

The NOAEL for male reproductive toxicity and paternally-mediated developmental toxicity was 150 mg/kg/day. The NOAEL for general toxicity in paternal male rats was 6 mg/kg/day, based on the significant decrease in body weight gain and food intake at 30 mg/kg/day.

Methods

Doses: 6, 30, and 150 mg/kg/day (selected on the basis of a preceding 2-week oral study in male rats at doses up to 1000 mg/kg/d, showing that doses of 150 mg/kg and greater resulted in reduced body weight and food consumption).

Frequency of dosing: Daily for 64 consecutive days prior to mating and during the mating period (mated with untreated females) until copulation

Dose volume: 5 ml/kg body weight

Route of administration: Oral

Formulation/Vehicle: Suspension in 0.5% aqueous solution of methylcellulose

Species/Strain: Rat, Sprague-Dawley

Number/Sex/Group: 22/group

Satellite groups:

Study design: Male Sprague-Dawley rats (22/group) were administered daily oral gavage doses of lurasidone at levels of 6, 30, and 150 mg/kg/day, and vehicle control suspension for 64 consecutive days prior to mating, during the mating period (mated with untreated females) until copulation. Cesarean sections were performed on females at term (Day 20 of gestation) and fetuses were examined externally, viscerally and skeletally.

Deviation from study protocol: Not reported

Observations and Results

Males: Mortality and general signs (twice daily); body weight and food consumption (weekly); reproductive performance (days to copulation, indices of copulation and fertility); necropsy (on the day of copulation or at the end of mating period); gross examination of organs/tissues; testes, seminal vesicles and abnormal organs/tissues were preserved in 10% formalin solution (microscopic examination not performed)

Females (Untreated): Mortality and general signs (once daily); body weight (on day 0 and 20 of gestation); gross necropsy and caesarean section of pregnant females on gestation day 20; number of fetuses, implantation sites, corpora lutea; live fetuses weighed, sexed and processed/examined for external, visceral and skeletal abnormalities; embryo/fetal death rate and sex ratio).

The results are summarized in the following sponsor's table:

Study Title: Study by Oral Administration of SM-13496 Prior to and in the Early Stages of Pregnancy in Rats- Effects on Males		Test Article: lurasidone (SM-13496)
Design similar to ICH 4.1.1: Yes	Duration of Dosing: 64 days pre-mating, through mating, until copulation (males only)	Study No. 2771
Species/Strain: Rat/ Crj:CD (SD)	Day of Mating: Day 0 of gestation	Module Location: 4.2.3.5.1.
Initial Age: 6 weeks	Day of C-Section: Day 20 of Gestation	GLP Compliance: Yes
Date of First Dose: 4/Aug/ 93	Method of Administration: Oral gavage	
Special Features: None	Vehicle/Formulation: Suspension in aqueous 0.5% methylcellulose	

Continued on the next page

Study by oral administration of SM 13496 prior to and in the early stages of pregnancy in rats – Effect on Males
(continued)

Daily Dose (mg/kg/day)	0 (Control)	6	30	150
Males:				
No. Evaluated	22	22	22	22
No. Died or Sacrificed Moribund	1 (Wound)	0	0	0
Clinical Observations				
Decrease in Spontaneous Activity	-- [a]	-- [a]	+ [b]	++ [c]
Catalepsy	-- [a]	-- [a]	-- [a]	+ [c]
Ptosis	-- [a]	-- [a]	-- [a]	+ [c]
Necropsy Observations [a]	--	--	--	--
Body Weight (%)[d]	516 ± 37.6 g	- 0.6	- 11.4**	- 16.9**
Food Consumption (%)[d]	27 ± 3.1 g/day	- 7.4	- 14.8**	- 22.2**
No. of Males that Mated	21	22	22	21
Copulation Index (%)	100	100	100	95.5
No. of Fertile Males	21	22	22	20
Fertility Index (%)	100	100	100	95.2
Females[e]:				
No. Evaluated	21	22	22	22
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations [a]	--	--	--	--
Necropsy Observations [a]	--	--	--	--
Gestation Body Weight (%)[f]	428 ± 22.7 g	+ 2.1	+ 1.9	+ 0.7
No. of Pregnant Females	21	22	22	20
No. Corpora Lutea	16.3 ± 1.49	16.6 ± 1.65	16.3 ± 1.70	16.7 ± 1.59
No. Implantations	15.3 ± 2.95	16.0 ± 1.59	15.9 ± 1.46	15.7 ± 1.93
Preimplantation Loss (%)	6.1	3.3	2.8	6.3
No. Live Fetuses	14.9 ± 2.8	15.4 ± 1.44	15.2 ± 1.76	14.9 ± 1.84
No. of Embryonic or Fetal Deaths	9	14	15	16
Postimplantation Loss (%)	2.8	4.0	4.3	5.1
Fetal Body Weight (g)	Male: 3.74 ± 0.169 Female: 3.57 ± 0.157	Male: 3.78 ± 0.160 Female: 3.57 ± 0.142	Male: 3.75 ± 0.277 Female: 3.59 ± 0.242	Male: 3.78 ± 0.180 Female: 3.57 ± 0.164
Fetal Sex Ratios (%, Males)	54	48	53	54
Fetal Anomalies:				
Gross External [a]	--	--	--	--
Visceral Anomalies [a]	--	--	--	--
Skeletal Anomalies [a]	--	--	--	--
Skeletal Ossification [a]	--	--	--	--

Mean ± SD
a Evaluations were done, but no significant findings were observed.
b Decrease in spontaneous activity was observed from Day 22 through Day 28 of treatment.
c Decrease in spontaneous activity was observed from Day 2 through Day 31 of treatment; catalepsy was observed sporadically from Day 1 through Day 17 of treatment; ptosis was observed in 1 animal on Day 1 of treatment.
d After 64 days of dosing. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
e Only males dosed with the test article. Untreated females were used for mating.
f At end of gestation (Day 20 of gestation). For controls, group means are shown. For treated groups, % differences from controls are shown.
** Statistically different from control, $p < .01$ (Body weight: Student's t-test; where there was a difference in distribution ratio, the Aspin-Welch's test was applied. Food consumption: One way analysis of variance disclosed significance, LSD test was applied.)

Mortality and Clinical Signs: No deaths occurred during the study period. Decrease of spontaneous activity in treated males at and above 30 mg/kg/day; catalepsy and ptosis at 150 mg/kg/day (sporadically observed 1 to 3 h after treatment)

Body Weight and Feed Consumption:

A dose-dependent and statistically significant reduction in male body weight and feed consumption was noted at 30 and 150 mg/kg/day during the administration period. For females that copulated, body weight and body weight gain during gestation did not differ between dosed and control groups.

Toxicokinetics: Not performed

Necropsy: No notable changes in the females. One male in the 30 mg/kg group showed "small and soft testes, light purple color of epididymis, and unrecognizable seminal vesicles". These changes were not dose-dependent and were likely spontaneous and not drug related.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

In the 14-day mating period, copulation was confirmed in all animals except for 1 pair in the HD (150 mg/kg/d) group. Of these copulating pairs, 1 female mated with a HD male was not pregnant. There was no difference in copulation and fertility indices between the treated and control groups. There was a dose-related, but not statistically significant increase in post-implantation losses in all dosed groups vs. control (see sponsor's table below). No differences in the number of corpora lutea, number of implantations, implantation rate, number of live fetuses at term, sex ratio, or body weight of live fetuses were noted between the dosed and control groups.

Study by Oral Administration of SM-13496 Prior to and in the Early Stages of Pregnancy in Rats
—Effects on Males—

Dose Group	Number of Animals	Number of Corpora Lutea[a)]	Number of Implantations[a)]	Implantation Rate[b)]	Number of Embryonic or Fetal Death[c)]	Number of Live Fetuses[a)]	Sex Ratio[d)]	Mean Live Fetal Body Weight (g)[e)]	
								Male	Female
Control (0.5%MC)	21	342 16.3 ± 1.49	321 15.3 ± 2.95	93.9	9 (2.8)	312 14.9 ± 2.80	54	3.74 ± 0.169	3.57 ± 0.157
SM-13496 6mg/kg	22	365 16.6 ± 1.65	353 16.0 ± 1.59	96.7	14 (4.0)	339 15.4 ± 1.44	48	3.78 ± 0.160	3.57 ± 0.142
SM-13496 30mg/kg	22	359 16.3 ± 1.70	349 15.9 ± 1.46	97.2	15 (4.3)	334 15.2 ± 1.76	53	3.75 ± 0.277	3.59 ± 0.242
SM-13496 150mg/kg	20	334 16.7 ± 1.59	313 15.7 ± 1.93	93.7	16 (5.1)	297 14.9 ± 1.84	54	3.78 ± 0.180	3.57 ± 0.164

a): Total, mean ± S.D.
b): (Number of implantations/Number of corpora lutea) × 100
c): Parenthesized figures represent mortalities (%), or (Number of embryonic or fetal death/Number of implantations) × 100
d): (Number of male fetuses/Total number of live fetuses) × 100
e): Mean ± S.D.

Fetal observations:
No external abnormalities were noted in any of the groups. Skeletal abnormalities (variations) were limited to cervical rib in 1 fetus in the HD group, and a supernumerary (14^{th}) rib and splitting of ossification centers of thoracic vertebral bodies in all treated and control groups. The incidences of these abnormalities did not differ or show consistent trends between control and treated groups. Visceral malformations noted were: ventricular septal defect in 1 animal each in the 30 and 150 mg/kg/d groups, and diaphragmatic hernia in 1 fetus in the LD group. As minor anomalies, abnormal bifurcation of pulmonary artery was found in control and LD groups (2 fetuses each), MD (1 fetus) and HD group (4 fetuses). As variations, thymic remnant in the neck, convoluted ureter, and ureteral dilatation were noted in all groups, including control. There was no significant difference between control and dosed groups in the incidences of any of these abnormalities.

Conclusion:
Daily oral administration of lurasidone at doses of 6, 30, and 150 mg/kg/day to male rats for 64 consecutive days prior to mating and during the mating period (with untreated females) did not induce adverse effect on male fertility and reproduction. The NOAEL for general toxicity in paternal male rats was 6 mg/kg/day, based on the findings of reduction in body weight and food consumption at the next tested dose level of 30 mg/kg/day and higher. The NOAEL for reproductive toxicity in paternal males was 150 mg/kg/day since no effects were noted in copulation and fertility indices up to the highest tested dose. The NOAEL for embryo/fetal toxicity in the progeny of the treated males and untreated females was 150 mg/kg/day, based on the lack of changes in fetal weight and morphological abnormalities at any of the tested dose levels.

Study title: Fertility and Early Embryonic Development Study – Effect on Females
Study no.: Study 2865
Conducting laboratory and location: (b) (4)

Date of study initiation: Jan. 31, 1994
GLP compliance: Yes (Japanese testing guidelines)
QA statement: Yes
Drug, lot #, and % purity: SM-13496, Lot 3CG001M, purity=100.9%

Key Study Findings

Female Sprague-Dawley rats (22/group) were administered daily oral gavage doses of lurasidone at 0.1, 1.5, 15, or 150 mg/kg/day for 15 consecutive days prior to mating, during the mating period (mated with untreated males) and through Day 7 of gestation. Recovery groups of 11 females each were administered lurasidone at HD level for 28 days, withdrawn for 14 days and then mated with untreated males. Upon Cesarean sections at term (Day 20 of gestation), fetuses were examined externally, viscerally and skeletally. There were no mortalities or adverse clinical signs related to lurasidone administration. Prior to pregnancy, body weight gain and food consumption were decreased at a dose of 150 mg/kg/day and increased at 1.5 and 15 mg/kg/day. During pregnancy, decreased maternal body weight gain was seen at all dose levels except the LD, with lower values of food consumption at 15 and 150 mg/kg/day. Prolonged estrous cycle and delayed copulation were observed at levels of 1.5 mg/kg/day and above. The copulation and fertility indices and the numbers of corpora lutea, implantations and live fetuses were decreased at 150 mg/kg/day (attributable to the low number of corpora lutea). Low fetal body weight and delayed ossification, indicative of retarded fetal development, were found at 150 mg/kg/day; no teratogenicity was found. The effects on maternal body weight, food consumption and reproductive performance including the effects on estrous cycle, corpora lutea count and intrauterine fetal development noted in the high-dose group were recovered after the two-week withdrawal.

NOAEL for reproductive function in females: 0.1 mg/kg/day (based on prolonged estrous cycle and delayed copulation at the next higher tested dose of 1.5 mg/kg/day); NOAEL for general toxicity in female rats: 0.1 mg/kg/day (based on maternal body weight decrease at and above the next higher tested dose of 1.5 mg/kg); NOAEL for embryo/fetal toxicity (upon prenatal exposure before organogenesis): 15 mg/kg/day (based on low fetal body weight and delayed ossification at the next higher tested dose of 150 mg/kg/day).

Methods

Doses:	0.1, 1.5, 15, or 150 mg/kg/day (based on a preceding 2-week toxicology study at the same doses, the selected HD was expected to produce clear signs of toxicity, i.e., body weight gain decrease); the LD was expected to be a NOAEL
Frequency of dosing:	Daily for 15 consecutive days prior to mating, during the mating period (mated with untreated males) and through Day 7 of gestation.
Dose volume:	5 ml/kg body weight
Route of administration:	Oral
Formulation/Vehicle:	0.5% aqueous methylcellulose
Species/Strain:	Rat, Sprague-Dawley
Number/Sex/Group:	22/group
Satellite groups:	Recovery groups (11/group)
Study design:	Female Sprague-Dawley rats (22/group) were administered daily oral gavage doses of lurasidone at levels of 0.1, 1.5, 15, or 150 mg/kg/day, and vehicle control suspension for 15 consecutive days prior to mating, during the mating period (mated with untreated males) and through Day 7 of gestation. Recovery groups of 11 females each were administered vehicle alone or lurasidone at a level of 150 mg/kg/day, for 28 days, withdrawn for 14 days and then mated with untreated males. Cesarean sections were performed at term (Day 20 of gestation) and fetuses were examined externally, viscerally and skeletally.
Deviation from study protocol:	Did not affect the study integrity and conclusions

Observations:

Mortality and clinical signs (daily for both females and males); female body weights (days 1, 3, 5, 7, 11, 15, 22 and 29 of administration and, for pregnant females, on gestation days 0, 7, 12, 17 and 20); female food consumption (daily); estrous cycle (daily during the administration and withdrawal periods, and through mating period); reproductive performance examination [from Day 15 of administration, the females were mated with males on a 1:1 basis, for 14 days; presence of sperm in the vaginal smears was indicative of copulation; females that failed to copulate in the 14-day mating period (7 in the HD group) were again mated after a 14 day drug withdrawal to assess reproductive performance]; necropsy of males, non-pregnant and pregnant females on gestation day 20 (gross examination of major thoracic and abdominal organs and mammary glands; ovaries, uteri and vaginas of non-pregnant females, and specimens of organs with any abnormal findings were stored in 10% neutral formalin); Caesarean section in pregnant females (corpora lutea counts, number of implantations, live and dead fetuses); fetal examinations (gender, weight, external, skeletal and visceral abnormalities).

Results

There were no mortalities or adverse clinical signs related to lurasidone administration. Body weight gain and food consumption were decreased prior to pregnancy at a dose of 150 mg/kg/day and increased at 1.5 and 15 mg/kg/day. During pregnancy, suppressed body weight gain was detected in all except the lowest dose group, with lower values of food consumption at 15 and 150 mg/kg/day. Prolonged estrous cycle and delayed copulation were observed at 1.5 mg/kg/day and above; the copulation and fertility indices and the numbers of corpora lutea, implantations and live fetuses were decreased at 150 mg/kg/day. The low number of live fetuses was attributable to the low number of corpora lutea. At maternal necropsy, there were no drug-related adverse findings. Lower fetal body weight and delayed ossification, indicative of retarded fetal development, were found at 150 mg/kg/day; there were no morphological anomalies or variations related to lurasidone. The effects on maternal body weight, food consumption and reproductive performance including the effects on estrous cycle, corpora lutea count and intrauterine fetal development in the high-dose group were recovered after the two-week recovery period upon discontinuation of dosing.
The results are summarized in the following sponsor's table:

Study Title: Study by Oral Administration of SM-13496 Prior to and in the Early Stages of Pregnancy in Rats-Effects on Females		**Test Article: lurasidone (SM-13496)**
Design similar to ICH 4.1.1: Yes	**Duration of Dosing: 15 days pre-mating, during mating, through Day 7 of gestation. Animals in recovery group were dosed for 28 days then put on 14-Day withdraw prior to mating. (females only)**	**Study No. 2865**
Species/Strain: Rat/ Crj:CD (SD)	**Day of Mating: Day 0 of gestation**	**Module Location: 4.2.3.5.1.**
Initial Age: 10 weeks	**Day of C-Section: Day 20 of gestation**	**GLP Compliance: Yes**
Date of First Dose: 16/FEB/94	**Method of Administration: Oral gavage**	
Special Features: None	**Vehicle/Formulation: Suspension in aqueous 0.5% methylcellulose**	
No Observed Adverse Effect Level: F_0 **Females: 0.1 mg/kg/day for general toxicological effects, 0.1 mg/kg/day for reproductive effects, 15 mg/kg/day for developmental effects**		

Daily Dose (mg/kg/day)	**0 (Control)**	**0.1**	**1.5**	**15**	**150**	**0 (R)[a]**	**150 (R)[a]**
Females:							
No. Evaluated	22	22	22	22	22	11	11 + 7[b]
No. Died or Sacrificed Moribund	0	0	0	0	0	0	0
Clinical Observations[c]	--[d]	--	--	--	--[d]	--	--
Necropsy Observations[c]	--	--	--	--	--	--	--
Premating Body Weight (%)[e]	256 ± 14.9 g[d]	- 0.4	+ 8.6**	+ 4.7*	- 2.3[d]	280 ± 20.8 g	- 5.0
Gestation Body Weight (%)[e]	296 ± 18.4 g	+ 2.0	+ 4.1*	- 1.0	- 12.5**	325 ± 30.5 g	- 7.1
Gestation Body Weight Gain (g)[f]	33 ± 8.1	36 ± 10.1	27 ± 8.7*	15 ± 6.5**	3 ± 6.1**	31 ± 6.7	24 ± 9.8
Premating Food Consumption (%)[e]	17 ± 2.6 g/day[d]	0.0	+ 11.8**	0.0	- 5.9[d]	18 ± 2.1 g/day	0.0
Gestation Food Consumption (%)[e]	24 ± 2.6 g/day	+ 4.2	- 4.2	- 16.7**	- 33.3**	23 ± 8.1 g/day	+ 4.3

Continued on the next page

Daily Dose (mg/kg/day)	0 (Control)	0.1	1.5	15	150	0 (R)[a]	150 (R)[a]
No. Females with Normal Estrous Cycles	31[d]	22	0	0	0[d]	8[g]	0[g]
No. Females with Irregular Estrous Cycles	1[d]	0	0	0	0[d]	0[g]	0[g]
No. Females with Prolonged Estrous Cycles (estrous cycle of >5 days)	1[d]	0	22**	22**	33**[d]	2[g]	3[g]
No. Females that Recovered the Cyclicity (showing at least one estrous cycle)	0[d]	--	8	2	0[d]	1[g]	15[g]
No. of Females that Mated (Total)	22	22	22	22	15	11	18
Mated on Day 1-7 of copulation	21	22	16	8	9	10	18
Mated on Day 8-14 of copulation	1	0	6	14	6	1	0
Copulation Index (%)	100	100	100	100	68.2*	100	100
No. of Pregnant Females	18	20	20	18	9	10	14
Fertility Index (%)	81.8	90.9	90.9	81.8	60.0	90.9	77.8
No. Corpora Lutea	18.5 ± 3.85	19.2 ± 2.95	19.9 ± 3.37	18.0 ± 4.55	16.0± 1.73*	20.2 ± 3.01	18.4 ± 3.59
No. Implantations	15.6 ± 2.28	16.7 ± 1.59	16.0 ± 2.67	14.3 ± 4.47	13.8 ± 1.86*	14.6 ± 2.22	16.2 ± 1.89
Preimplantation Loss (%)	15.6	13.0	19.8	20.4	13.9	27.7	11.7 **
No. Live Fetuses	14.9 ± 2.48	15.5 ± 1.70	15.0 ± 2.87	13.7 ± 4.28	12.7 ± 2.06*	13.8 ± 1.87	15.6 ± 1.82*
No. of Embryonic or Fetal Death	12	24	20	11	10	8	8
Postimplantation Loss (%)	4.3	7.2	6.3	4.3	8.1	5.5	3.5
Fetal Body Weight (g)	Male: 3.38 ± 0.197 Female: 3.18 ± 0.176	Male: 3.36 ± 0.228 Female: 3.18 ± 0.238	Male: 3.51 ± 0.271 Female: 3.37 ± 0.242*	Male: 3.50 ± 0.266 Female: 3.41 ± 0.227**	Male: 3.12 ± 0.511 Female: 2.97 ± 0.443	Male: 3.16 ± 0.207 Female: 3.00 ± 0.210	Male:3.44 ± 0.241** Female: 3.26 ± 0.236*
Fetal Sex Ratios (%, Males)	48	44	51	45	59	43	53
Fetal Anomalies:							
Gross External[c]	--	--	--	--	--	--	--
Visceral Anomalies[c]	--	--	--	--	--	--	--
Skeletal Anomalies[c]	--	--	--	--	--	--	--
Skeletal Ossification	--[c]	--[c]	--[c]	--[c]	Delayed	--[c]	--[c]

Mean ± SD
a Recovery groups of 11 animals, dosed for 28 days with a 14-day withdrawal prior to mating.
b This assessment included 7 animals that did not mate from the main study group.
c Evaluations were done, but no significant findings were observed.
d This pre-mating assessment included animals assigned to the recovery group as well as the main study group.
e At end of pre-mating (Main groups, Day 15 of treatment; Recovery groups, Day 15 of withdrawal) or Day 7 of gestation. For controls, group means are shown.
For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
f The body weight gain on Day 7 of gestation from the Day 0 of gestation. Group means are shown.
g This assessment was done during the withdrawal and mating periods.
* Significantly different from control, $p < .05$; ** Significantly different from control, $p < .01$ (Body weight, Body weight gain, No. of corpora lutea, No. of implantations, No. of live fetuses, Fetal body weight: Student's t-test; where there was a difference in distribution ratio, the Aspin-Welch's test was applied.
Food consumption: One way analysis of variance disclosed significance, LSD test was applied. No. of females with abnormal estrous cycles, Copulation index: $\chi 2$ test was applied. Preimplantaion loss: Mann-Whitney's U-test was applied.)

Conclusion: NOAEL for reproductive function in females: 0.1 mg/kg/day (based on prolonged estrous cycle and delayed copulation at the next higher tested dose of 1.5 mg/kg/day); NOAEL for general toxicity in female rats: 0.1 mg/kg/day (based on maternal body weight decrease at and above the next higher tested dose of 1.5 mg/kg); NOAEL for embryo/fetal toxicity (upon prenatal exposure before organogenesis): 15 mg/kg/day (based on low fetal body weight and delayed ossification at the next higher tested dose of 150 mg/kg/day).

Embryo/Fetal Development

Embryo-fetal Development Study in Rats

Study title: Study for Effects on Embryo-Fetal Development of SM-13496 Administered Orally to Rats
Study no: 3850
Conducting laboratory and location: (b) (4)

Date of study initiation: June 24, 2003
GLP compliance: Yes (Japanese testing guidelines)
QA statement: Yes
Drug, lot #, and % purity: SM-13496, Lot E-278, purity=100.3%

Key Study Findings

Lurasidone developmental toxicity in rats (Spraue-Dawley) was assessed at doses of 3, 10 and 25 mg/kg/day administered by oral gavage to pregnant females during the period of fetal organogenesis (Day 6 to Day 17 of gestation). The high dose level was selected based on a preceding range-finding study in which the dose of 25 mg/kg/day suppressed maternal body weigh gain upon administration from Day 7 to Day 17 of gestation (Study 2784).
In the present study, there was no drug-related maternal mortality or adverse clinical signs. Maternal body weight gain was suppressed significantly and dose-dependently in all 3 dose groups; maternal food consumption was decreased at 10 and 25 mg/kg/day. Fetal external malformations were observed in the 25 mg/kg (HD) group (cleft palate in 8 fetuses and meningoencephalocele in 1 fetus) versus no external malformations in the control; there were no external malformations at MD. The frequencies of these findings were beyond the range of the historical control data of the laboratory. However, all fetuses with cleft palate were from the same litter, and we agree with the sponsor that multiple malformation cases confined to a single litter are of a smaller concern than a distribution of affected fetuses in several litters. It is also noteworthy that in the range-finding study in the same species and strain with 12 pregnant rats/group treated with higher doses of lurasidone via the same route from gestation Day 7 to 17 (Study 2784) there were no external malformations up to the highest employed lurasidone dose of 150 mg/kg/day. This supports the sponsor's conclusion that the external malformations observed in the 25 mg/kg (HD) group were spontaneous rather than drug-related. A NOAEL for general toxicity in maternal rats was not reached (below the lowest tested dose of 3 mg/kg/day). The NOAEL for embryo/fetal developmental toxicity was 25 mg/kg/day.

Methods

Doses: 3, 10 and 25 mg/kg/day
The dose levels were selected based on a range-finding study in pregnant rats (11 or 12 per group) at dose levels of 25, 50, 100 and 150 mg/kg/day from Day 7 to Day 17 of gestation (Study 2784) that caused a dose-dependent decrease in maternal body weight gain at all dose levels, reduction in food consumption at 100 and 150 mg/kg/day, clinical signs (lacrimation, decrease of spontaneous activity) at 150 mg/kg/day, and retarded embryo/fetal development (low fetal body weight and delayed

	ossification) at 150 mg/kg/day. Based of these findings, the dose of 25 mg/kg/day, which suppressed maternal body weigh gain, was selected as the high dose for this study.
Frequency of dosing:	Daily, Day 6 through Day 17 of gestation
Dose volume:	5 ml/kg body weight
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension in aqueous 0.5% methylcellulose
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	20 pregnant females
Satellite groups:	None (TK was not performed)
Study design:	Daily doses of 3, 10 and 25 mg/kg/day or vehicle control suspension were administered to pregnant rats during the period of fetal organogenesis (Day 6 to Day 17 of gestation), by oral gavage. Cesarean sections were performed at term (Day 20 of gestation) and fetuses were examined externally, viscerally and skeletally.

Observations: Mortality and clinical signs (daily); female body weights and food consumption (on gestation days 0, 3, 6, 9, 12, 15, 17 and 20); necropsy (gross pathology only); caesarean section in pregnant females (corpora lutea counts, number of implantations, live and dead fetuses); fetal examinations (gender, weight, external, skeletal and visceral abnormalities). TK was not performed.

Results: There were no drug-related maternal mortalities or adverse clinical signs. Maternal body weight gain was suppressed dose-dependently and statistically significantly in all treatment groups (from 10% at LD up to 18% at HD); maternal food consumption was decreased at MD and HD (by 30% and 40%, respectively, on gestation days 15 through 20), but not at LD. There were no other treatment-related maternal findings. There were no treatment-related changes in embryo/fetal lethality (post-implantation losses), or fetal weight at term.

Fetal Observations:

The following external, skeletal and visceral findings were reported (skeletal and visceral examinations were performed only in the HD and control groups; only external examinations were performed in all dosed groups).

- External abnormalities (see sponsor's table on the next page): cleft palate in 8 fetuses (all from one and the same litter) and meningoencephalocele in 1 fetus from another litter. In the LD group, external abnormalities were seen in 3 fetuses (anencephaly in 1 case; and 2 cases of omphalocele in combination with either anophthalmia, or anal atresia and tail hypoplasia). No external malformations were reported in the MD and control groups.
- Skeletal abnormalities (examined in control and HD group only): No skeletal malformations were found in either HD or control group. Skeletal variations (presacral vertebra 25, supernumerary (14^{th}) rib, shortening of the 13^{th} rib, bipartite ossification of sternebrae or thoracic vertebrae) were found in a similar number of fetuses from the control and HD group with no differences in the frequency of occurrence (see the sponsor's table on the next page).
- Visceral abnormalities (examined in control and HD group only): There was no drug-related increase in visceral abnormalities in the HD group. Minor visceral abnormalities were found in both control and HD group (i.e., abnormal branch of pulmonary artery in 9 control and 4 HD fetuses; remnant of the right azygous vein (in 1 HD fetus), absent renal papilla (in 1 control fetus). Variations (i.e., "remnant of thymus in the neck" and dilated or convoluted ureter) were observed in both control and HD group, without significant difference in incidence.

Developmental toxicity study in rats: Fetal external malformations

Study for effects on embryo-fetal development of SM-13496 administered orally to rats

Dose groups	No. of fetuses examined a)	No. of fetuses with malformations (%)	No. of fetuses with each malformation (%) anophthalmia	omphalocele	thread-like tail	anal atresia
Control (0.5%MC)	308 (159/149)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SM-13496 3 mg/kg	310 (149/161)	3 (1.0)	1 (0.3)	2 (0.6)	1 (0.3)	1 (0.3)
SM-13496 10 mg/kg	293 (137/156)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SM-13496 25 mg/kg	315 (174/141)	9 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

a):Total, (Male/Female)

Dose groups	No. of fetuses examined a)	No. of fetuses with each malformation (%) meningoencephalocele	cleft palate	acephaly
Control (0.5%MC)	308 (159/149)	0 (0.0)	0 (0.0)	0 (0.0)
SM-13496 3 mg/kg	310 (149/161)	0 (0.0)	0 (0.0)	1 (0.3)
SM-13496 10 mg/kg	293 (137/156)	0 (0.0)	0 (0.0)	0 (0.0)
SM-13496 25 mg/kg	315 (174/141)	1 (0.3)	8 (2.5)	0 (0.0)

a):Total, (Male/Female)

Developmental toxicity study in rats: Fetal skeletal variations

Dose groups	No. of fetuses examined a)	No. of fetus with variatio	No. of fetuses with each variation (%) Vertebrae: presacral vertebra 25	Cervical vertebra: cervical rib	Thoracic vertebral body: bipartite ossification	Rib: 14th rib	Rib: short 13th rib	Sternebra: bipartite ossif
Control (0.5%MC)	154 (79/ 75)	10 (6.9	1 (0.6)	1 (0.6)	5 (3.2)	2 (1.3)	3 (1.9)	1 (0.
SM-13496 25 mg/kg	152 (85/ 67)	9 (5.9	2 (1.3)	0 (0.0)	2 (1.3)	3 (2.0)	3 (2.0)	1 (0.

The overall results are summarized in the following sponsor's table:

Study Title: Study for Effects on Embryo-Fetal Development of SM-13496 Administered Orally to Rats		Test Article: lurasidone (SM-13496)
Design similar to ICH 4.1.3: Yes	Duration of Dosing: Day 6 through Day 17 of gestation Day of Mating: Day 0 of gestation Day of C-Section: Day 20 of gestation	Study No. 3850
Species/Strain: Rat/ Crj:CD (SD)	Method of Administration: Oral gavage	Module Location: 4.2.3.5.2.
Initial Age: 11-12 weeks	Vehicle/Formulation: Suspension in aqueous 0.5% methylcellulose	GLP Compliance: Yes
Date of First Dose: 14/Jul/03		
Special Features: None		

Daily Dose (mg/kg/day)	0 (Control)	3	10	25
Dams:				
No. Pregnant	20	20	20	20
No. Died or Sacrificed Moribund	0	0	0	0
No. Aborted or with Total Resorption of Litter	0	0	0	0
Clinical Observations [a]	--	--	--	--
Necropsy Observations [a]	--	--	--	--
Body Weight (%)[b]	345 ± 18.7 g	- 2.6	- 3.5	- 4.3*
Body Weight Gain During Gestation (g)[c]	77 ± 9.2	70 ± 9.1*	65 ± 11.5**	63 ± 8.9**
Food Consumption (%)[b]	26 ± 2.4 g/day	- 3.8	- 7.7**	- 11.5**
No. Corpora Lutea	17.8 ± 1.94	18.0 ± 2.42	18.0 ± 2.37	17.0 ± 1.85
No. Implantations	16.1 ± 1.73	16.1 ± 1.48	15.3 ± 3.08	16.5 ± 1.64
Preimplantation Loss (%)	9.8	10.3	14.8	2.7*
Litters:				
No. Litters Evaluated	20	20	20	20
No. Live Fetuses	308	310	293	315
No. Live Fetuses	15.4 ± 1.93	15.5 ± 1.57	14.7 ± 3.12	15.8 ± 2.12
No. of Embryonic or Fetal Deaths	13	12	13	15
Postimplantation Loss (%)	4.0	3.7	4.2	4.5
Fetal Body Weight (g)	Male: 3.46 ± 0.249 Female: 3.28 ± 0.209	Male: 3.39 ± 0.231 Female: 3.27 ± 0.199	Male: 3.39 ± 0.271 Female: 3.21 ± 0.283	Male: 3.29 ± 0.231 Female: 3.15 ± 0.233
Fetal Sex Ratios (%, Males)	52	48	47	55
Fetal Anomalies:				
Gross External [a]	--	--	--	--
Visceral Anomalies [a]	--	N.E.	N.E.	--
Skeletal Anomalies [a]	--	N.E.	N.E.	--
Skeletal Ossification [a]	--	N.E.	N.E.	--

Mean ± SD, N.E.: Not examined
a Evaluations were done, but no significant findings were observed.
b At end of dosing (Body weight, Day 17 of gestation; Food consumption, Days 15 to 17 of gestation). For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).
c The body weight gain on Day 17 of gestation from the Day 6 of gestation. Group means are shown.
* Significantly different from control, $p < .05$; ** Significantly different from control, $p < .01$ (Body weight, Body weight gain, Food consumption: Dunnett's multiple comparison test; where there was a difference in distribution ratio, Steel's multiple comparison test was applied. Preimplantaion loss: Steel's multiple comparison test was applied.)

Comments: The incidence of external malformations was higher in the 25 mg/kg (HD) group (cleft palate in 8 fetuses and meningoencephalocele in 1 fetus) versus no external malformations in the control. The frequencies of these findings were beyond the range of the historical control data of the laboratory, as stated by the sponsor. However, all fetuses with cleft palate were from the same litter, and we agree with

the sponsor that multiple malformation cases confined to a single litter are of a smaller concern than a distribution of affected fetuses in several litters[1]. It is also noteworthy that in the range-finding study in the same species and strain with 12 pregnant rats/group treated with higher doses of lurasidone via the same route from gestation Day 7 to 17 (Study 2784), there were no external malformations up to the highest dose of 150 mg/kg/day. This supports the sponsor's conclusion that the external malformations observed in the 25 mg/kg (HD) group in the present study were spontaneous rather than drug-related. In the LD group (3 mg/kg/day), the external malformations (found in 3 fetuses) were also likely spontaneous because there were no similar findings in the next higher dose group. No skeletal or visceral abnormalities were found in any of the dosed groups. Thus, it appears that the external malformations observed at the HD of 25 mg/kg/day and LD of 3 mg/kg/day were not treatment related.

Conclusion: A NOAEL for general toxicity in maternal rats was not reached (less than the lowest tested dose of 3 mg/kg/day) in view of the significant dose-dependent decrease in the maternal weight gain at all tested dose levels. The NOAEL for developmental embryo/fetal toxicity was the HD of 25 mg/kg/day.

[1] Health Effects Division Standard Evaluation Procedure (Developmental Toxicity Studies), U.S. EPA, 44 (1993) *(as cited by the sponsor)*

Embryo-fetal Development Study in Rabbits

Study title: Study by Oral Administration of SM-13496 During the Period of Fetal Organogenesis in Rabbits
Study no: 2874

Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 16, 1994
GLP compliance:	Yes (Japanese guidelines)
QA statement:	Yes
Drug, lot #, and % purity:	SM-13496, Lot # 3CG0011M, Purity=100.9%

Key Study Findings

The potential effects of lurasidone on embryo-fetal development in rabbits was studied at daily doses of 2, 10 and 50 mg/kg/day (vehicle control suspension 0.5% aqueous methylcellulose) administered to pregnant New Zealand White rabbits during the period of fetal organogenesis (Day 6 to Day 18 of gestation), by oral gavage. Cesarean sections were performed at term (Day 28 of gestation) and fetuses were examined externally, viscerally and skeletally. There were no drug-related mortalities, abortions, premature deliveries or adverse maternal clinical signs. Maternal body weight gain was suppressed at all doses (at and above 2 mg/kg/day) while there was no drug effect on food consumption. There were no notable necropsy (gross pathology) and Cesarean section findings (corpora lutea number, implantation rate, intrauterine embryo/fetal loss, number of live fetuses, sex ratio and fetal weight). There were no treatment-related changes in fetal growth, external, skeletal and visceral observations.
NOAEL for maternal general toxicity: Not reached (<2 mg/kg/day) due to maternal body weight gain decrease at all tested doses; NOAEL for embryo/fetal developmental toxicity: 50 mg/kg/day.

Methods

Doses:	2, 10 and 50 mg/kg/day Dose selection was based on a previous two-week oral study of SM-13496 in non-pregnant female rabbits (Study No. 2812) at doses of 0, 50, 100, and 200 mg/kg that showed a reduction or tendency toward reduction in body weight and weight gain in all treated groups, and decreased food consumption.
Frequency of dosing:	Daily (Day 6 to Day 18 of gestation)
Dose volume:	5 ml/kg body weight
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension in 0.5% aqueous methylcellulose
Species/Strain:	Rabbit / New Zealand White
Number/Sex/Group:	Pregnant F: n=14 (Control & LD); 15 (MD); 13 (HD)
Satellite groups:	No

Study design: Pregnant rabbits (artificially inseminated with sperm from untreated males) were dosed during the period of fetal organogenesis [Gestation Days 6 – 18 (insemination day considered as Gestation Day 0)]. Cesarean sections were performed at term (Gestation Day 28) and the fetuses were examined externally, viscerally and skeletally.

Observations: Maternal mortalities, clinical signs (twice daily), abortions, premature deliveries, maternal weight and food consumption (days 0, 6, 9, 12, 15, 18, 22, 25, and 28 of gestation), body weight gain [on each weighing day compared to body weight on the first dosing day (day 6 of gestation)]; necropsy (gross pathology) and Cesarean section (corpora lutea count, implantation number and rate, intrauterine embryo/fetal loss, number of live fetuses), fetal weight, sex ratio, external, skeletal and visceral observations.

Results:

Mortality:

No maternal deaths, abortions or premature deliveries occurred during the study period.

Summary of Disposition: Pregnancy, Mortality, Exclusion from Study

Dose Group	Number of Animals Subjected to Artificial Insemination	Number of Excluded Animals	Number of Non-pregnant Animals	Number of Pregnant Animals	Number of Dead or Moribund Sacrificed Animals	Number of Animals Aborted or Prematurely Delivered	Number of Animals with Live Fetuses
Control (0.5%MC)	15	0	1	14	0	0	14
SH-13496 2mg/kg	15	0	1	14	0	0	14
SH-13496 10mg/kg	15	0	0	15	0	0	15
SH-13496 50mg/kg	16	1[a)]	2	13	0	0	13

a) : Excluded from the study since it showed decreased food consumption on day 6 of gestation (i.e., at the initiation of dosing)

Clinical Signs:

No maternal clinical signs were observed during the study period

Body Weight:

There were no statistically significant changes in maternal body weight, but a dose-dependent trend to a decrease in body weight was seen at MD and HD throughout the treatment period and at LD from Gestation day 15 through day 22.

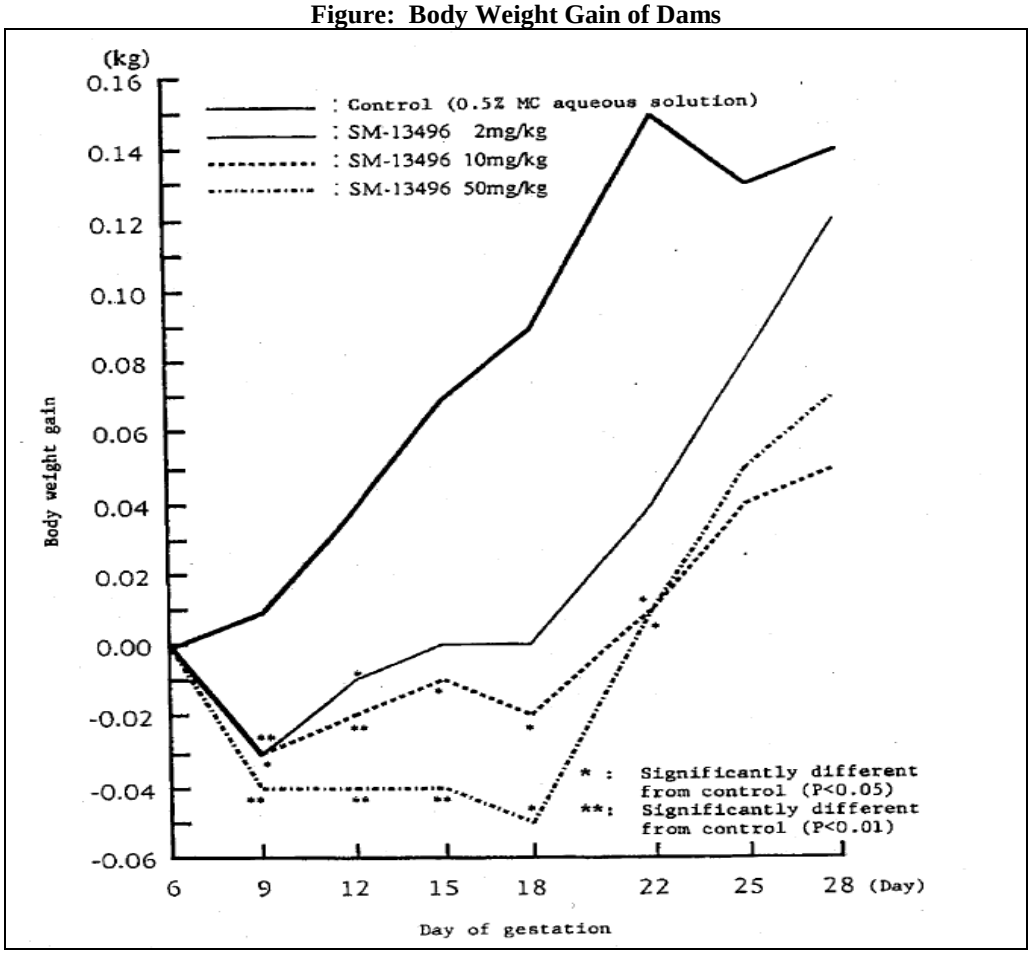
Dose-dependent suppression of **body weight gain** was noted in all groups (at and above 2 mg/kg). Significantly lower values were noted from Gestation day 9 onwards in all dosed groups (see sponsor's table and figure on the next page).

Maternal body weight gain

Dose Group	Mean Body Weight Gain (kg) on Day of Gestation							
	6	9	12	15	18	22	25	28
Control (0.5%MC)	0.00 ±0.000 (14)	0.01 ±0.035 (14)	0.04 ±0.050 (14)	0.07 ±0.108 (14)	0.09 ±0.147 (14)	0.15 ±0.115 (14)	0.13 ±0.119 (14)	0.14 ±0.149 (14)
SM-13496 2mg/kg	0.00 ±0.000 (14)	-0.03** ±0.040 (14)	-0.01* ±0.054 (14)	0.00 ±0.089 (14)	0.00 ±0.131 (14)	0.04 ±0.172 (14)	0.08 ±0.170 (14)	0.12 ±0.187 (14)
SM-13496 10mg/kg	0.00 ±0.000 (15)	-0.03* ±0.067 (15)	-0.02** ±0.063 (15)	-0.01* ±0.081 (15)	-0.02* ±0.129 (15)	0.01* ±0.198 (15)	0.04 ±0.252 (15)	0.05 ±0.301 (15)
SM-13496 50mg/kg	0.00 ±0.000 (13)	-0.04** ±0.032 (13)	-0.04** ±0.046 (13)	-0.04** ±0.089 (13)	-0.05* ±0.133 (13)	0.01* ±0.174 (13)	0.05 ±0.186 (13)	0.07 ±0.208 (13)

( ): Number of animals examined
* : Significantly different from control (P<0.05)
** : Significantly different from control (P<0.01)

Figure: Body Weight Gain of Dams



Feed Consumption:

No significant differences were noted between any of the dosing groups and the control group, but a trend to a decreased food consumption was seen at HD from Gestation day 9 through 22, and at MD and LD on Gestation days 12, 18, and 22.

Toxicokinetics: Not performed

Stability and Homogeneity

SM-13496 suspensions at concentrations of 0.001 % and 20% were confirmed to be stable for 14 days under refrigeration) and the suspensions in this study were used with 8 days of preparation.

Necropsy: No gross maternal pathology changes (microscopic examination not performed)

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

Cesarean section on day 28 of gestation revealed no significant drug-related differences between any of the dosing groups and the control group in the number of corpora lutea, number of implantations, implantation rate, embryonic/fetal death rate, number of live fetuses, sex ratio, or body weight of live fetuses (see sponsor's summary table at the end).

Offspring (Malformations, Variations, etc.)

No external abnormalities were found.
Skeletal observations (see sponsor's tables on the next page) showed no malformations at HD and LD. At MD, malformations of the cranium and sternebrae were found in 2 fetuses (cranial bone anomaly in one fetus with fusion of frontal bone and paretal bone, and thoracic vertebral anomaly in another fetus with fused thoracic vertebral bodies and absent thoracic vertebral arch). Since these malformations were noted in only 1 fetus each and were not dose-related, they were considered to be incidental. Minor skeletal anomalies (incomplete ossification of nasal bone in 1 fetus in the LD group, incomplete ossification of frontal bone in 1 fetus each in the control and MD group, incomplete ossification of parietal bone in 1, 8, and 1 fetus in the LD, MD and HD groups, respectively, and fused sternebrae in 2 fetuses in the control group and 1 fetus in the HD group) and variations were observed in both dosed and control groups, showed no dose-dependence, and were not attributable to the test substance. Nearly all indicators of ossification degree (i.e., numbers of ossified sacrococcygeal vertebrae, unossification rates of the sternebrae, middle or proximal phalanges, and the talus) showed no effect from administration of the test substance.
Visceral malformations (see sponsor's tables on the next page): diaphragmatic hernia occurred in a single animal in the LD group, and was considered to be incidental. Minor anomalies and variations in all cases showed no specific trend in the incidences, and were not treatment-related.

In conclusion, SM-13496 prenatal exposure to rabbits during the period of organogenesis through maternal oral administration of doses up to 50 mg/kg/day on gestation days 6 to 17 did not cause increase in embryonic/fetal mortality, teratogenic effects, or effects on the intrauterine development of fetuses. The NOAEL for embryo/fetal developmental toxicity was 50 mg/kg/day.

Developmental toxicity study in rabbits: Fetal skeletal abnormalities

Skeletal Malformations

Dose Group	Number of Litters	Number of Fetuses Examined (Male/ Female)	Number of Fetuses with Malformations	Skeletal Malformation (Number of fetuses by the type of malformation; parenthesized figures represent incidences of the malformation.) Cranial Bone Anomaly[a)]	Thoracic Vertebral Anomaly[b)]
Control (0.5%MC)	14	113 (51/62)	0	0 (0.0)	0 (0.0)
SM-13496 2mg/kg	14	126 (59/67)	0	0 (0.0)	0 (0.0)
SM-13496 10mg/kg	15	104 (58/46)	2	1 (1.0)	1 (1.0)
SM-13496 50mg/kg	13	97 (53/44)	0	0 (0.0)	0 (0.0)

a): Complex of the fusion and separation of frontal bone, the fusion and incomplete ossification of parietal bone, and incomplete ossification of nasal bone

b): Complex of the fusion of 8th and 9th thoracic vertebral bodies, absence of 7th thoracic vertebral arch, and deformation of 7th thoracic vertebral body

Dose Group	Number of Litters	Number of Fetuses Examined (Male/ Female)	Number of Fetuses with Minor Anomalies	Minor Anomaly (Number of fetuses by the type of minor anomaly; parenthesized figures represent incidences of the minor anomaly.) Nasal Bone, Incomplete Ossification	Frontal Bone, Incomplete Ossification	Parietal Bone, Incomplete Ossification	Parietal Bone, Separation	Sternebra, Fusion
Control (0.5%MC)	14	113 (51/62)	3	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	2 (1.8)
SM-13496 2mg/kg	14	126 (59/67)	2	1 (0.8)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)
SM-13496 10mg/kg	15	104 (58/46)	11	0 (0.0)	1 (1.0)	8 (7.7)	1 (1.0)	1 (1.0)
SM-13496 50mg/kg	13	97 (53/44)	1	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)

Skeletal variations

Dose Group	Number of Litters	Number of Fetuses Examined (Male/ Female)	Number of Fetuses with Variations	Skeletal Variation (Number of fetuses by the type of variation; parenthesized figures represent incidences of the variation.) Hyoid Bone Body, Separation	Hyoid Bone Body, Unossification	Hyoid Bone Arch, Flexure	Cervical Vertebra, Cervical Rib	Vertebra, 27 Presacral Vertebrae
Control (0.5%MC)	14	113 (51/62)	83	29 (25.7)	11 (9.7)	0 (0.0)	0 (0.0)	18 (15.9)
SM-13496 2mg/kg	14	126 (59/67)	104	39 (31.0)	11 (8.7)	1 (0.8)	2 (1.6)	17 (13.5)
SM-13496 10mg/kg	15	104 (58/46)	71	25 (24.0)	11 (10.6)	0 (0.0)	1 (1.0)	4 (3.8)*
SM-13496 50mg/kg	13	97 (53/44)	70	20 (20.6)	9 (9.3)	1 (1.0)	1 (1.0)	14 (14.4)

*: Significantly different from control ($P<0.05$)

Developmental toxicity study in rabbits: Fetal visceral abnormalities

Dose Group	Number of Litters	Number of Fetuses Examined (Male/ Female)	Number of Fetuses with Malformations	Visceral Malformation (Number of fetuses by the type of malformation; parenthesized figures represent incidences of the malformation.) Diaphragm, Diaphragmatic Hernia
Control (0.5%MC)	14	113 (51/62)	0	0 (0.0)
SM-13496 2mg/kg	14	126 (59/67)	1	1 (0.8)
SM-13496 10mg/kg	15	104 (58/46)	0	0 (0.0)
SM-13496 50mg/kg	13	97 (53/44)	0	0 (0.0)

Dose Group	Number of Litters	Number of Fetuses Examined (Male/ Female)	Number of Fetuses with Minor Anomalies	Visceral Minor Anomaly (Number of fetuses by the type of minor anomaly; parenthesized figures represent incidences of the minor anomaly.) Lung, Hypoplasia of Left Lobe and Intermediate Lobe	Cecum, Bifurcation of the Appendix	Cecum, Duplication of the Appendix
Control (0.5%MC)	14	113 (51/62)	3	0 (0.0)	0 (0.0)	1 (0.9)
SM-13496 2mg/kg	14	126 (59/67)	1	1 (0.8)	0 (0.0)	0 (0.0)
SM-13496 10mg/kg	15	104 (58/46)	1	0 (0.0)	1 (1.0)	0 (0.0)
SM-13496 50mg/kg	13	97 (53/44)	0	0 (0.0)	0 (0.0)	0 (0.0)

Dose Group	Number of Litters	Number of Fetuses Examined (Male/ Female)	Number of Fetuses with Variations	Visceral Variation (Number of fetuses by the type of variation; parenthesized figures represent incidences of the variation.) Heart, Supernumerary Coronary Orifice	Artery, Abnormal Course of Right Subclavian Artery	Vein, Abnormal Course of Caudal Vena Cava
Control (0.5%MC)	14	113 (51/62)	20	16 (14.2)	0 (0.0)	5 (4.4)
SM-13496 2mg/kg	14	126 (59/67)	23	17 (13.5)	0 (0.0)	8 (6.3)
SM-13496 10mg/kg	15	104 (58/46)	10	6 (5.8)	0 (0.0)	6 (5.8)
SM-13496 50mg/kg	13	97 (53/44)	19	11 (11.3)	1 (1.0)	8 (8.2)

The overall results of the study are summarized in the following sponsor's table:

Study Title: Study by Oral Administration of SM-13496 During the Period of Fetal Organogenesis in Rabbits		Test Article: lurasidone (SM-13496)
Design similar to ICH 4.1.3: Yes	Duration of Dosing: Days 6 through 18 of gestation Day of Mating: Day of Artificial Insemination: Day 0 of gestation Day of C-Section: Day 28 of gestation	Study No. 2874
Species/Strain: Rabbit/ Kbl:NZW	Method of Administration: Oral gavage	Module Location: 4.2.3.5.2.
Initial Age: 21 weeks	Vehicle/Formulation: Suspension in aqueous 0.5% methylcellulose	GLP Compliance: Yes
Date of First Dose: 22/Mar/94		
Special Features: None		

Daily Dose (mg/kg/day)		0 (Control)	2	10	50
Dams:					
No. Pregnant		14	14	15	13
No. Died or Sacrificed Moribund		0	0	0	0
No. Aborted or with Total Resorption of Litter		0	0	0	0
Clinical Observations [a]		--	--	--	--
Necropsy Observations [a]		--	--	--	--
Body Weight (%)[b]		4.15 ± 0.351 kg	- 1.4	- 2.7	- 3.6
Body Weight Gain During Gestation (kg) [c]	Day 9 of Gestation	0.01 ± 0.035	- 0.03 ± 0.040**	- 0.03 ± 0.067*	- 0.04 ± 0.032**
	Day 12 of Gestation	0.04 ± 0.050	- 0.01 ± 0.054*	- 0.02 ± 0.063**	- 0.04 ± 0.046**
	Day 18 of Gestation	0.09 ± 0.147	0.00 ± 0.131	- 0.02 ± 0.129*	- 0.05 ± 0.133*
Food Consumption (%)[b]		141 ± 57.9 g/day	- 11.3	- 17.0	- 29.1
No. Corpora Lutea		10.8 ± 1.67	12.2 ± 2.08	11.7 ± 2.22	11.5 ± 1.66
No. Implantations		8.6 ± 2.14	9.9 ± 2.60	7.7 ± 3.41	8.2 ± 3.24
Preimplantation Loss (%)		20.5	19.3	34.1	29.3
Litters:					
No. Litters Evaluated		14	14	15	13
No. Live Fetuses		113	126	104	97
No. Live Fetuses		8.1 ± 2.16	9.0 ± 2.60	6.9 ± 2.87	7.5 ± 3.41
No. of Embryonic or Fetal Deaths		7	12	12	9
Postimplantation Loss (%)		5.8	8.7	10.3	8.5
Fetal Body Weight (g)		Male: 36.6 ± 5.91 Female: 35.8 ± 5.21	Male: 35.3 ± 4.51 Female: 34.2 ± 4.07	Male: 36.7 ± 7.04 Female: 35.6 ± 8.02	Male: 36.4 ± 4.98 Female: 36.1 ± 6.99
Fetal Sex Ratios (%, Males)		45	47	56	55
Fetal Anomalies:					
Gross External [a]		--	--	--	--
Visceral Anomalies [a]		--	--	--	--
Skeletal Anomalies [a]		--	--	--	--
Skeletal Ossification [a]		--	--	--	--

Mean ± SD
a Evaluations were done, but no significant findings were observed.
b At end of dosing (Day 18 of gestation). For controls, group means are shown. For treated groups, percent differences from controls are shown.
c The body weight gain on Days 9, 12 and 18 of gestation from the Day 6 of gestation. Group means are shown.
* Significantly different from control, $p < .05$; ** Significantly different from control, $p < .01$ (Student's t-test; where there was a difference in distribution ratio, the Aspin-Welch's test was applied.)

Conclusion: Lurasidone administration at daily oral (gavage) doses of 2, 10 and 50 mg/kg/day to pregnant rabbits during the period of fetal organogenesis (Day 6 to Day 18 of gestation), caused significant dose-dependent decrease in maternal body weight gain at all doses, but no effect on embryo/fetal development. NOAEL for maternal general toxicity: Not reached (<2 mg/kg/day) due to maternal body weight gain decrease at all tested doses; NOAEL for embryo/fetal developmental toxicity: 50 mg/kg/day.

Prenatal and Postnatal Development

Study title: Study for effects of SM-13496 on pre- and postnatal development, including maternal fuction in rats by oral administration
Study no: (b) (4) 08006

Conducting laboratory and location: (b) (4)

Date of study initiation: May 26, 2008
GLP compliance: Yes (Japanese guidelines)
QA statement: Yes
Drug, lot #, and % purity: SM-13496, Lot #3CG001M, Purity =100.9%

Key Study Findings

Lurasidone effect on pregnant/lactating female rats, on development of the conceptus and offspring, and on reproductive performance of the offspring, was evaluated upon oral (gavage) administration of lurasidone (suspension in 0.5% aqueous methylcellulose) at doses of **0.4, 2 and 10 mg/kg/day** to pregnant Sprague-Dawley rats from implantation through the end of lactation (Day 6 of gestation through postnatal day 21). The dose selection was based on a range-finding study (Study D0254) in pregnant rats of the same strain at oral doses of 0.3, 1, 3 and 10 mg/kg/day from implantation to the end of lactation, resulting in decreased maternal body weight gain at and above 1 mg/kg/day, and a tendency toward increased offspring mortality rate at parturition at 10 mg/kg/day which was chosen as the HD for the definitive study. The dams were allowed to deliver spontaneously and nurse their offspring. One male and 1 female offspring per litter were selected at weaning for evaluation of growth, development, physical and neurobehavioral development, and reproductive performance of F1 generation. Maternal body weights were significantly lower than controls by end gestation in the 10 mg/kg/day group and maternal body weight gain was suppressed in the same dose group during the gestation period. No treatment-related effects were found in gross pathological findings, maintenance of pregnancy, parturition and nursing status. Among the offspring, no treatment-related effects were found in the number of pups delivered; sex ratio; viability indices; body weights; physical development; neurobehavioral development (reflex responses, motor activity, learning and memory); sexual development; estrous cyclicity; reproductive capacity; and gross pathological findings.
The NOAEL for general toxicity in maternal (F0) rats was 2 mg/kg/day due to a decrease in body weight and body weight gain at the next tested dose of 10 mg/kg/day. The NOAELs for maternal reproductive function and development of F1 offspring were 10 mg/kg/day.

Methods

Doses:	**0.4, 2 and 10 mg/kg/day** The dose selection was based on a range-finding study (Study D0254) in pregnant rats of the same strain at oral doses of 0.3, 1, 3 and 10 mg/kg/day from implantation to the end of lactation, resulting in maternal body weight gain decrease at and above 1 mg/kg/day, and a tendency toward increased offspring mortality rate at parturition at 10 mg/kg/day which was chosen as the HD for the definitive study.
Frequency of dosing:	Daily, Day 6 of gestation through Day 21 of lactation
Dose volume:	5 ml/kg body weight
Route of administration:	Oral gavage
Formulation/Vehicle:	Suspension in 0.5% aqueous methylcellulose
Species/Strain:	Rat, Sprague-Dawley
Number/Sex/Group:	22 mated females/group
Satellite groups:	None
Study design:	Lurasidone oral doses of 0.4, 2 and 10 mg/kg/day were administered to pregnant Sprague-Dawley rats from implantation through the end of lactation (Day 6 of gestation through postnatal Day 21). The F0 dams were allowed to deliver spontaneously and nurse their offspring (F1); pup viability, growth (weight), physical development (pinna detachment, incisor eruption) and reflex responses, were assessed weekly. On postnatal Day 4, the litters were standardized by culling to 4 pups/sex/litter. At weaning (postnatal day 21), one male and one female offspring per litter were selected for evaluation of growth, neurobehavioral development (motor activity, learning and memory); sexual maturation (testes descent, vaginal opening); estrous cyclicity; and reproductive performance by male/female (non-sibling) mating within dose groups to produce the next (F2) generation.

Observations and Results

F_0 Dams

Survival:	In F0 maternal rats, there were no deaths related to administration of the test substance in any of the treated groups.
Clinical signs:	No drug-related clinical signs
Body weight:	In HD (10 mg/kg) group, maternal body weights were significantly lower at late pregnancy and parturition (on gestation days, 19 and 20, and on lactation day 0). Body weight gains in this group were below the control values throughout the gestation period (statistically significant). These changes were attributable to the test substance. On the other hand, maternal body weight gains during the lactation period were significantly higher in this group, indicating that decreases in the body weights in the late gestation and on lactation day 0 were recovered. No significant differences were found in the body weights, body weight gains or food consumption in LD and MD groups throughout the gestation and lactation as compared with the control group (see sponsor's tables on the next page).

Body weights in the gestation period in maternal (F0) rats

Generation	Group	Number of animals		Dosing period: Body weight (g) on gestation day 0	3	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
F0	Control	22	Mean	244.8	270.0	289.6	293.2	300.2	303.8	310.6	317.9	324.8	330.3	336.0	343.2	354.1	366.1	381.3	399.0	415.5
			S.D.	11.3	12.3	13.6	13.9	14.9	13.3	14.6	15.1	16.2	16.4	17.4	17.2	18.2	20.2	21.3	22.6	25.8
	SM-13496 0.4 mg/kg	22	Mean	245.0	269.5	288.9	294.0	299.7	304.6	311.8	320.3	325.3	331.5	336.8	344.4	354.5	368.0	382.4	400.8	416.4
			S.D.	13.0	13.5	16.1	13.5	16.1	15.4	16.4	16.9	18.3	17.8	18.3	18.2	19.7	19.9	19.1	20.4	21.4
	SM-13496 2 mg/kg	22	Mean	244.6	271.2	289.4	293.7	300.2	305.1	309.7	316.7	324.0	329.7	336.5	344.5	354.4	368.4	383.0	399.3	416.1
			S.D.	11.2	11.9	12.6	12.5	12.2	13.8	14.4	13.0	14.7	14.5	14.7	15.7	16.5	17.1	17.3	18.8	18.6
	SM-13496 10 mg/kg	22	Mean	244.8	266.5	284.0	286.1	292.9	296.3	301.1	307.1	313.4	319.7	325.3	332.7	342.3	354.3	367.0 *	383.1 *	398.0 *
			S.D.	11.5	10.9	12.7	12.3	13.6	12.5	14.8	15.2	14.9	15.8	15.6	16.0	17.6	18.8	20.1	20.2	22.7

Body weight gains in the gestation period in maternal (F0) rats

Generation	Group	Number of animals		Dosing period: Body weight gain (g) on gestation days 0-6	6-7	6-8	6-9	6-10	6-11	6-12	6-13	6-14	6-15	6-16	6-17	6-18	6-19	6-20
F0	Control	22	Mean	44.8	3.6	10.6	14.1	21.0	28.2	35.1	40.6	46.3	53.6	64.5	76.5	91.6	109.3	125.9
			S.D.	8.5	3.5	3.8	3.8	5.4	5.2	6.6	6.7	7.7	8.0	9.2	10.9	11.5	13.0	16.4
	SM-13496 0.4 mg/kg	22	Mean	44.0	5.0	10.8	15.7	22.9	31.4	36.4	42.6	47.9	55.5	65.5	79.0	93.5	111.9	127.5
			S.D.	8.4	4.1	4.0	4.8	5.3	5.0	6.6	6.9	7.5	8.0	9.1	10.1	10.6	12.9	15.8
	SM-13496 2 mg/kg	22	Mean	44.8	4.4	10.8	15.7	20.3	27.4	34.7	40.4	47.1	55.1	65.0	79.0	93.6	110.0	126.7
			S.D.	6.8	4.0	3.3	5.1	6.4	6.2	6.6	5.7	7.4	8.6	8.3	9.3	9.5	11.0	12.8
	SM-13496 10 mg/kg	22	Mean	39.2 *	2.1	8.8	12.2	17.0	23.1 *	29.3 *	35.7 *	41.3	48.6	58.2 *	70.3	82.9 *	99.1 *	114.0 *
			S.D.	5.3	4.4	4.5	5.0	6.3	6.2	6.1	6.5	6.4	6.4	8.0	9.3	11.7	11.5	16.1

*: Significantly different from the control at $p \leq 0.05$ by Dunnett's test.

Body weights in the lactation period in maternal (F0) rats

Generation	Group	Number of animals		Dosing period: Body weight (g) on lactation day 0	4	7	11	14	18	21	22[a]
F0	Control	22	Mean	317.0	336.2	342.6	347.7	350.1	334.6	320.4	317.3
			S.D.	24.1	20.2	18.1	17.7	15.1	16.2	15.7	16.5
	SM-13496 0.4 mg/kg	22	Mean	317.7	337.6	340.9	351.0	345.6	329.1	319.7	318.9
			S.D.	26.6	21.4	21.1	19.5	16.8	15.2	18.8	16.3
	SM-13496 2 mg/kg	22	Mean	320.7	339.0	341.6	351.5	344.6	328.5	319.8	318.7
			S.D.	22.4	16.2	14.5	15.5	12.1	14.3	13.4	13.6
	SM-13496 10 mg/kg	22	Mean	298.1 *	331.0	331.8	344.0	344.1	327.7	311.0	307.6
			S.D.	17.1	16.8	16.6	19.2	17.1	20.9	16.1	17.2

a: Autopsy day.
*: Significantly different from the control at $p \leq 0.05$ by Dunnett's test.

Body weight gains in the lactation period in maternal (F0)rats

Generation	Group	Number of animals		Dosing period: Body weight gain (g) on lactation days 0-4	0-7	0-11	0-14	0-18	0-21	0-22[a]
F0	Control	22	Mean	19.1	25.5	30.6	33.0	17.5	3.3	0.3
			S.D.	18.8	14.2	15.4	16.4	17.9	17.2	16.3
	SM-13496 0.4 mg/kg	22	Mean	19.9	23.2	33.3	27.9	11.4	2.0	1.2
			S.D.	16.6	14.0	18.3	16.5	23.3	21.8	22.1
	SM-13496 2 mg/kg	22	Mean	18.3	20.9	30.8	23.9	7.7	-1.0	-2.0
			S.D.	15.6	16.1	14.5	14.7	14.7	15.7	14.3
	SM-13496 10 mg/kg	22	Mean	32.9 *	33.7	45.8 **	46.0 *	29.5	12.8	9.5
			S.D.	12.1	10.1	10.9	10.3	12.9	11.9	13.6

Feed consumption At HD, significantly lower food consumption was noted on lactation days 4-7 (this change was likely incidental because it was transient and because body weight gains in the lactation period were higher in HD than in the control group). No significant differences were found in the food consumption in LD and MD groups throughout the gestation and lactation as compared with the control group.

Uterine content: No significant difference was observed in the gestation index, delivery index, live birth index, gestation length or number of implantations in any of the SM-13496-treated groups as compared with the control group (see the sponsor's table below). No abnormality was observed in delivery or nursing behavior in any of the SM -13496-treated groups.

Reproductive findings in maternal (F0) rats

Gener-ation	Group	Number of animals	Gestation index Fraction (incidence, %)		Gestation length (days)	Number of implanta-tions	Delivery index (%)[a]	Live birth index (%)[b]	Number of pups delivered	Sex ratio[c]	Viability index (%)[d] on postnatal day : 0	4	22
										F1 pup data			
F0	Control	22	22/22	Mean	22.0	15.9	92.5	91.2	14.7	0.494	98.4	98.8	100.0
			(100)	S.D.	0.5	1.7	11.4	13.4	2.4	0.107	6.2	2.7	0.0
	SM-13496 0.4 mg/kg	22	22/22	Mean	21.9	15.3	95.1	95.1	14.5	0.459	100.0	99.0	100.0
			(100)	S.D.	0.3	1.3	6.4	6.4	1.4	0.089	0.0	2.5	0.0
	SM-13496 2 mg/kg	22	22/22	Mean	21.8	15.0	94.0	93.7	14.1	0.506	99.7	99.0	100.0
			(100)	S.D.	0.5	1.1	5.8	6.0	1.3	0.147	1.6	2.6	0.0
	SM-13496 10 mg/kg	22	22/22	Mean	22.0	15.0	95.8	95.0	14.3	0.511	99.1	98.2	99.4
			(100)	S.D.	0.0	1.3	5.9	6.2	1.4	0.103	2.3	3.7	2.7

Gestation index (%) = (number of females that delivered live pups/number of pregnant females) x 100.
Delivery index (%) = (number of pups delivered/number of implantations) x 100.
Live birth index (%) = (number of live pups on postnatal day O/number of implantations) x 100.
Sex ratio = number of male live pups/number of live pups on postnatal day o.
Viability index on postnatal day 0 (%) = (number of live pups on postnatal day O/number of pups delivered) x 100.
Viability index on postnatal day 4 (%) = (number of live pups on postnatal day 4/no. of live pups on postnatal day 0) x 100.
Viability index on postnatal day 22 (%) = (number of live pups on postnatal day 22/number of live pups selected for use on postnatal day 4) x 100.
a, b, c and d: The litter is the unit evaluated.

Necropsy observation: The gross pathological examination of F0 maternal rats on lactation day 22 revealed no drug-related abnormalities in any of the treated groups. One HD animal showed a cyst on the left uterine horn (the finding was considered not drug-related since it was observed in only one animal and not observed at the same or higher dose levels (up to 100 mg/kg/day) in the six-month oral toxicity study of SM-13496 in rats (Study No. 3259, dose levels: 0, 0.03, 1, 10 and 100 mg/kg/day).

Toxicokinetics: Not performed

Stability and homogeneity: Stability of the test substance was confirmed by the analytical results of the same lot supplied by the study sponsor; homogeneity and stability of the dosing suspensions were determined for the concentrations of 0.01, 0.1 and 200 mg/ml. The assays showed homogeneity of the test substance in 0.5% methylcellulose aqueous solution and its stability for 3 hours at room temperature and for 14 days under cold conditions. Accordingly, preparation of dosing suspensions was performed 5 times at intervals of 5 to 13 days during the dosing period.

F_1 Generation

Survival: During the lactation period there was no significant difference between the control group and any of the SM-13496-treated groups in the number of pups delivered, sex ratio or viability indices of pups on postnatal days 0, 4 and 22.

Clinical findings in F1 rat pups during postnatal days 0-4 following oral administration of SM-13496 to the dams

		Postnatal day 0								Postnatal days 1-4							
		Male				Female				Male				Female			
		SM-13496 (mg/kg)				SM-13496 (mg/kg)				SM-13496 (mg/kg)				SM-13496 (mg/kg)			
Gener-ation	Item	Control	0.4	2	10	Control	0.4	2	10	Control	0.4	2	10	Control	0.4	2	10
F1	Number of pups examined	161	146	158	162	163	174	153	153	159	146	158	160	160	174	152	152
	Number of pups with abnormal findings	3	0	0	2	3	0	1	1	3	2	2	1	2	1	1	5
	Findings[a]																
	Found dead/lost	2	0	0	2	3	0	1	1	2	2	2	1	2	1	1	5
	Forelimb/tail; Loss/scab	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

a: Values represent the number of pups that showed abnormal findings.

In the clinical observations of F1 offspring from birth through weaning to adulthood, there were no abnormalities related to administration of the test substance in any of the SM-13496-treated groups

Body weight: There were no significant differences between the control group and any of the SM-13496-treated groups in the body weights of male and female pups from birth to weaning. After weaning, the body weights and food consumption of F1 males in LD group were significantly increased. This finding was likely not drug related but rather due to an unintentional "biased selection of high body weight weanlings on postnatal day 22 in this group because there were no significant differences in their body weights on postnatal day 21 (the day before selection of weanlings)". No significant differences were observed in the post-weaning body weights of F1 females in any of the dosed groups.

Feed consumption: After weaning, significant increases in the food consumption were sporadically found in the MD group in F1 males. However, these changes were not toxicologically significant because they were transient, and no significant difference was observed in body weights. The food consumption of F1 females was significantly increased in the LD group during postnatal days 28-35 and in the HD group during postnatal days 22-28 and 28-35. However, these increases were not toxicologically significant because they were slight, transient, and not associated with significant differences in body weights.

Physical development: No significant changes were found in any of the pre-weaning landmarks of physical development (pinna detachment, incisor eruption, eye opening, testes descent) in F1 pups in any of the treated groups (see the following sponsor's table). Post-weaning, there were no treatment-related differences between dosed and control groups in growth (body weight, weight gain) and sexual maturation (as indicated by preputial separation for males and vaginal opening for females).

Physical development in Fl rat pups following oral administration of SM-13496 to the dams*

Gener-ation	Group	Number of litters		Pinna detachment Completion rate (%) Male	Pinna detachment Completion rate (%) Female	Incisor eruption Completion rate (%) Male	Incisor eruption Completion rate (%) Female	Eye opening Completion rate (%) Male	Eye opening Completion rate (%) Female	Descending testes Completion rate (%) Male
F1	Control	22	Mean	88.0	88.8	97.7	97.4	77.3	79.5	95.5
			S.D.	31.0	28.8	10.7	8.7	36.9	37.5	21.3
	SM-13496 0.4 mg/kg	22	Mean	94.3	93.2	94.3	90.9	76.9	89.8	95.5
			S.D.	21.7	22.3	13.2	19.7	33.6	22.7	9.9
	SM-13496 2 mg/kg	22	Mean	86.0	87.5	96.6	98.9	56.1	65.2	97.7
			S.D.	30.5	30.6	8.8	5.3	39.0	41.2	10.7
	SM-13496 10 mg/kg	22	Mean	98.5	97.2	94.3	96.6	78.4	76.1	95.5
			S.D.	7.1	10.0	13.2	8.8	24.8	26.1	9.9

***Pinna detachment, incisor eruption, eye opening and descending testes were observed on postnatal day 3, 11, 14 and 20, respectively**

Neurological assessment: Reflex responses assessed in F1 pups during pre-weaning - the first 3 weeks of life (surface righting reflex on postnatal day 5; air righting reflex on postnatal day 18; corneal reflex, pain response, auditory startle response, grasping reflex – all on p.n. day 20) showed no differences in treated groups vs. control in both genders and indicated a normal sensory/motor development.

Neurobehavioral assessments during the post-weaning period, i.e., activity (open field test) and learning ability (T-maze) did not show treatment-related differences in performance.

In the open field test, no significant differences from the control group were found in ambulation (horizontal motor activity) or the numbers of rearing, defecation, urination or grooming in any of the treated groups except for a significant decrease in ambulation on the third day of testing in the LD group. In the learning test, no significant difference was found in the time required to reach the goal or the number of errors for maze trials in any of the treated groups except the time required to reach the goal on the second day of the trials, which was significantly longer in the LD females as compared to control. These findings were most likely unrelated to administration of the test substance because no significant difference was observed in either MD or HD groups.

Gross pathology: In the gross pathological examination of F1 pups found dead on postnatal days 0-5 and euthanized on postnatal day 4 or 22, there were a few findings such as: dilatation of the renal pelvis (in 2 and 5 male pups in LD and MD groups, respectively, and in 2, 1 and 2 female pups in LD, MD and HD groups, respectively); small size of the testis (in 1 male pup each in the LD, MD and HD groups); diverticulum of the ileum (in 1 female pup each in LD and MD groups). These findings were most likely spontaneous because they occurred at a low incidence and were not dose-dependent.

Gross pathological findings in F1 generation following oral administration ofSM-13496 to the dams

F1 males

Gener-ation	Item	Control				SM-13496 (mg/kg) 0.4				2				10			
		A	B	C	T	A	B	C	T	A	B	C	T	A	B	C	T
F1	Number of animals examined	20	1	1	22	21	1	0	22	20	2	0	22	20	2	0	22
	Number of animals with abnormal findings	2	0	1	3	3	0	-	3	4	0	-	4	5	1	-	6
	Findings[a]																
	Incisor: Crushing and Malocclusion	0	0	1	1	0	0	-	0	0	0	-	0	0	0	-	0
	Thymus: Atrophy	0	0	1	1	0	0	-	0	0	0	-	0	0	0	-	0
	Ileum: Diverticulum	0	0	0	0	1	0	-	1	0	0	-	0	0	0	-	0
	Kidney: Dilatation, renal pelvis	2	0	0	2	0	0	-	0	3	0	-	3	4	1	-	5
	Testis: Atrophy	1	0	0	1	2	0	-	2	0	0	-	0	1	0	-	1
	Hypertrophy	0	0	0	0	0	0	-	0	0	0	-	0	1	0	-	1
	Epididymis: Atrophy	1	0	0	1	2	0	-	2	0	0	-	0	1	0	-	1
	Seminal vesicle: Atrophy	0	0	0	0	0	0	-	0	1	0	-	1	0	0	-	0

Fate: A, animals that impregnated a female; B, animals that unsuccessfully mated or did not impregnate a female; C, animals that euthanized during the study; T, total (A+B+C).
a: Values represent the number of animals that showed abnormal findings.
-: Not applicable.

F1 females

Gener-ation	Item	Control			SM-13496 (mg/kg) 0.4			2			10		
		A	B	T	A	B	T	A	B	T	A	B	T
F1	Number of animals examined	22	0	22	21	1	22	21	1	22	20	2	22
	Number of animals with abnormal findings	1	-	1	0	0	0	0	0	0	0	0	0
	Findings[a]												
	Ileum: Diverticulum	1	-	1	0	0	0	0	0	0	0	0	0

Fate: A, animals that were pregnant; B, animals that unsuccessfully mated or were not pregnant; T, total (A+B).
a: Values represent the number of animals that showed abnormal findings.
-: Not applicable.

Reproduction: Mating of F1 animals to produce F2 generation was initiated at 11 weeks of age; each female was continuously housed with a non-sibling male from the same dose group for a period of 3 weeks. Successful copulation was confirmed by the presence of vaginal plug and/or sperm in vaginal smears (gestation day 0). The following indices were calculated: - Male copulation index (%) = (number of males with successful copulation/ number of males paired) x 100
- Female copulation index (%) = (number of females with successful copulation! number of females paired) xl 00
- Male fertility index (%) = (number of males that impregnated a female/ number of males with successful copulation) x100
- Female fertility index (%) = (number of pregnant females/ number offemales with successful copulation) x100

Estrous cycle examination in F1 females during 2 weeks before cohabitation showed abnormal estrous cycle (persistent diestrus) in 2 females each in the LD and HD groups (not statistically significant). The average length of the estrous cycle was significantly shorter only in the HD group vs. control (see sponsor's table on the next page). However, this change was not considered toxicologically significant because all the values of the HD group (i.e., 4 days) were within the normal range for rats of this strain.[2]

[2] Ohta, T., Izumi, H., Kimura, E., Shimazu, S., Kato, H., Yoshinaga, K., Kitazato, M. and Takechi, M. (2001): Comparison of reproductive parameters between Crj:CD (SD) and Crj:CD (SD) IGS rats. Biological Reference Data on CD(SD) IGS Rats-2001, 115-11S.; Ohta, T., Izumi,

Successful copulation was not achieved within the 3-week mating period in 1 and 2 mating pairs in the control and LD groups, respectively. The females were subsequently housed with different "proven" males from the same test group; successful copulation then occurred in 1 of the 2 LD females within the 1-week additional mating period. In the mean number of days until copulation, no significant difference was noted between the control group and any of the SM-13496-treated groups.
Except for 1 LD and 2 HD females, all the females that successfully mated were proven to be pregnant. Three males (1LD and 2 HD) did not impregnate females. However, in copulation or fertility index of male or female rats, no significant difference was found between the control group and any of the SM-13496-treated groups.

Vaginal estrous cycles of Fl female rats following oral administration of SM-13496 to the dam

Gener-ation	Group	Number of animals		Estrous cyclicity: Normality Incidence (%)[a]	Estrous cyclicity: Length (days)
F1	Control	22	Mean	22/22	4.23
			S.D.	(100)	0.40
	SM-13496 0.4 mg/kg	22	Mean	22/22	4.17
			S.D.	(100)	0.39
					(20)
	SM-13496 2 mg/kg	22	Mean	20/22	4.40
			S.D.	(90.9)	0.50
					(20)
	SM-13496 10 mg/kg	22	Mean	20/22	4.00 $
			S.D.	(90.9)	0.00

a: Incidence of females with the normal estrous cycle (%) = (no. of females cycling normally/number of females examined) x 100.
The normal estrous cycle is defined as having a mean cycle length between 4.0 and 6.0 days.
Values in parentheses in the column of the length of estrous cycles are the number of animals examined.
$: Significantly different from control at $p \leq 0.05$ by Steel's test.

In summary, in the examination of F1 fertility, no significant difference was found in the incidence of females with the normal estrous cycle in any of the SM-13496-treated groups. The average length of the estrous cycle was significantly shorter in the 10 mg/kg group than that in the control group. This change was not considered toxicologically significant because all the values of the 10 mg/kg group were 4.0 days that is within the normal range for rats of this strain. No significant differences were noted in the number of days until copulation in females, or copulation or fertilty index of both sexes.

F_2 Generation: Pregnant F1 females were autopsied on gestation day 15, and the ovaries and uterine contents were examined. The numbers of corpora lutea, implantations, and live and dead or resorbed F2 fetuses were recorded. The following parameters were calculated:

- Implantation index (%) = (number of implantations/number of corpora lutea) x 100
- Viability index of embryos (%) = (number of live embryos/number of implantations) x l00
- Incidence of dead or resorbed embryos (%) = (n. dead or resorbed embryos/ n. implants)x100

In the intrauterine examination of F1 pregnant females performed on gestation day 15, no significant differences were observed in the numbers of corpora lutea, implantations, implantation index; live F2 embryos; viability

H., Kimura, E., Shimazu, S., Kato, H., Yoshinaga, K., Kitazato, M. and Takechi, M. (200212003): Comparison of reproductive parameters between Crj:CD (SD) and Crj:CD (SD) IGS rats (ll). Biological Reference Data on CD(SD) IGS Rats-2002/2003, 135-140 (as cited by the sponsor)

index of embryos; or incidence of dead or resorbed embryos in any of the SM-13496-treated groups as compared with the control group (see the following sponsor's table).

Reproductive pedormance in F1 male and female rats and intrauterne findings in Fl female rats on gestation day 15 following oral administrtion of SM-13496 to the dams

Gener-ation	Group	Reproductive performance: Copulation index (%) Male	Copulation index (%) Female	Fertility index (%) Male	Fertility index (%) Female		Days until copu-lation	Number of corpora lutea	Number of implan-tations	Implan-tation index (%)[a]	Dead or resorbed embryos: Total	Inci-dence (%)[b]	Implan-tation sites	Placental remnants	Embryo resorption	Number of live embryos	Viability index of embryos (%)[c]
F1	Control	20/21	22/22	20/20	22/22	Mean	3.3	15.7	15.4	97.8	1.3	7.9	0.0	1.2	0.0	14.1	92.1
		(95.2)	(100)	(100)	(100)	S.D.	2.6	1.6	1.9	4.6	1.4	8.7	0.0	1.3	0.2	1.9	8.7
	SM-13496 0.4 mg/kg	22/22	22/22	21/22	21/22	Mean	2.6	15.7	15.2	96.6	1.0	6.4	0.0	1.0	0.0	14.2	93.6
		(100)	(100)	(95.5)	(95.5)	S.D.	1.6	1.6	1.7	4.3	1.3	8.3	0.0	1.3	0.0	2.1	8.3
	SM-13496 2 mg/kg	20/22	21/22	20/20	21/21	Mean	3.6	15.9	14.3	89.5	1.2	8.4	0.0	1.2	0.0	13.1	91.6
		(90.9)	(95.5)	(100)	(100)	S.D.	2.0	1.8	3.4	17.1	1.0	7.3	0.0	1.0	0.0	3.4	7.3
	SM-13496 10 mg/kg	22/22	22/22	20/22	20/22	Mean	3.5	15.3	14.1	91.2	0.8	5.4	0.0	0.8	0.0	13.3	94.6
		(100)	(100)	(90.9)	(90.9)	S.D.	3.4	1.6	3.5	19.5	1.2	8.0	0.0	1.2	0.0	3.4	8.0

Copulation index = (number of animals with successful copulation/number of animals paired) x 100.
Fertility index = (number of animals that impregnated a female or were pregnant/number of animals with successful copulation) x 100.
Implantation index = (number of implantations/number of corpora 1utea) x 100.
Incidence of dead or resorbed embryos = (number of dead or resorbed embryos/number of implantations) x 100.
Viability index of embryos = (number of live embryos/number of implantations) x 100.
a, b and c: The litter is the unit evaluated.

Conclusion: In a pre-/postnatal developmental toxicity study with lurasidone continuous administration to pregnant rats from implantation (Gestation Day 6) through pregnancy, parturition and lactation to weaning of progeny (postnatal Day 21) at oral doses of 0.4, 2 and 10 mg/kg/day, the NOAEL for general toxicity in maternal rats was 2 mg/kg/day due to decreases in body weights and body weight gains during gestation at the next tested dose level of 10 mg/kg/day. For maternal reproductive function and pre-/postnatal development of offspring, the NOAEL was 10 mg/kg/day because no adverse effects related to lurasidone administration were found in any parameters up to the highest tested dose of 10 mg/kg/day. (The average length of the estrous cycle of the female generation was significantly shorter in the 10 mg/kg/day group vs. control, but this change is not considered toxicologically significant because all the values of the HD group were within the normal range for rats of this strain.)

10 Special Toxicology Studies

10. 1. Cardiac Toxicity Studies

Dogs

Study title: **Oral Single Dose Cardiac Toxicity Study in Beagles**

Study no. (b) (4) **97-4241**

Conducting laboratory and location: (b) (4)

Date of study initiation: December 4, 1997

GLP compliance: Yes (Japanese guidelines)

QA statement: Yes

Drug, lot #, and % purity: SM-13496 (Lot # 3CG001M, 100.9%)

Key Study Findings

Assessment of potential cardiac effects of oral (capsule) administration of lurasidone at single doses of 5, 15 and 50 mg/kg/day and chlorpromazine (as a positive control, 120 mg/kg/day, equivalent by potency to 15 mg/kg/day of lurasidone) to Beagle dogs showed that lurasidone did not cause changes in ambulatory electrocardiogram, but produced clinical signs in males (tremors and a decrease in movements) at 50 mg/kg/day. Lurasidone had no effect on hematology, blood biochemistry, or necropsy parameters. Chlorpromazine resulted in clinical signs of vomiting, decrease in movement, staggering gait, miosis, ptosis and dry nose, persisting to the evening of the next day. Blood biochemical examination showed increases in the levels of creatine kinase ALT, AST, alkaline phosphatase, glucose and phospholipids. Ambulatory ECG showed increase in heart rate in males and females, prolongation of QTc in males and shortening of PQ interval in females, with statistically significant differences from control at many time points. In conclusion, lurasidone single oral administration to Beagle dogs at doses of up to 50 mg/kg/day caused clinical signs (tremors and a decrease in movements) at the high dose, but did not affect cardiac parameters as measured by 24-hour ambulatory ECG on Days 0 to 1, 3 to 4 and 13 to 14 post dose.

Methods

Doses: Lurasidone was administered at single doses of 5, 15 and 50 mg/kg/day and the positive control chlorpromazine was administered at a dose of 120 mg/kg/day. The dose of chlorpromazine was selected to be "equivalent to 15 mg/kg/day of lurasidone, in consideration of the potency ratio (1:8) to lurasidone in mice and rats". The 50 mg/kg/day dose of lurasidone was selected "as the dose equivalent to 10 times or more a clinical dose of 10 mg/person, for comparison between the maximum serum concentration in a

previous single dose Phase 1 study and the maximum serum concentration in the previous 2-week administration study in dogs", as explained by the sponsor below:

> In a previous oral 2-week study in dogs [1], animals of the 50 mg/kg group showed no abnormal clinical signs on day 1, but had a decrease in locomotor activity, tremor, and miosis from day 2 of administration. Serum concentrations (C_{max}) of SM-13496 for 2 males and 2 females of the 50 mg/kg group were 56, 819, 266, and 214 ng/ml on day 1 of administration. In the phase I clinical trial of SM-13496 [2], on the other hand, serum concentrations (C_{max}) of SM-13496 were 12.5±5.5 ng/ml (n=6) and 43.5±20.6 ng/ml (n=6) for the 10 and 30 mg single administration, respectively. From the above results, the high dose for the present study was determined to be 50 mg/kg that would result in C_{max} nearly 10-fold or higher than that expected clinically in humans given 10 mg/man.

Frequency of dosing: Single
Route of administration: Oral
Formulation/Vehicle: Gelatin capsules
Species/Strain: Dogs/Beagle
N animals/sex/group: 3

Observations: During the 14-day observation period, animals were observed for clinical signs and subjected to measurements of body weight, food consumption, ambulatory ECG, hematological and blood biochemistry examinations:

ECG**:** 24-hour ambulatory ECGs (Holter's electrocardiograph) were obtained on Days -1 (before administration), Days 0 (after administration) to 1, Days 3 to 4 and Days 13 to 14;

> electrocardiograms by M-X lead (episternum: -, xiphosternum: +) and R-L lead (right thorax: -, left thorax: +) were recorded in a cassett tape. ECG traces were printed out with an ambulatory ECG analyzer (Quick Scan QS-2200, Fukuda M-E Ind., Lit.). Heart rate (mean of heart rates for 30 seconds at neighboring 3 points) and wave intervals (PQ, QRS, QT: mean of 3 waves in succession) were measured at 1-hour interval by using ECG taken on the day of administration until 1 day after administration or at 2-hour interval for the other ECGs. R-R interval was calculated from the heart rates, and QT_c was calculated using the following formula. Arrhythmia was analyzed for the entire course of time for all ECGs.
>
> $QT_c = QT / \sqrt{RR}$

Hematology examination was performed on Days -1, 1, 3, and 13 after administration (RBC, WBC, Hb, Ht, MCH, MCV, MCHC, Ret, Pl, differential leukocyte count)
Blood biochemistry examinations were performed on the same days (GOT, GTP, LDH, gamma-GTP, CK, Glu, T. Cholesterol, Triglycerides, Phospholipids, T. Protein, Alb., A/G ratio, BUN, Creatinine, T. Bilirubin, Na, K, Ca, Pi, Mg, Creatine kinase fractions, LDH fractions, Protein fractions)
Pathology**:** Gross examination
At necropsy, heart specimens from each animal were collected, fixed and stored for light microscopy. Papillary muscle of the left ventricle, parts of the left ventricle and interventricular septum were collected and processed for electron microscopy. (Microscopic examination was not performed)

Results:
Lurasidone: At HD (50 mg/kg/day), males showed drug-related clinical signs (decrease in movement and tremor) at 3 to 4 hours post-dose and recovered to normal at 6 to 7 hours after administration. There were no changes in hematology, blood biochemistry, or necropsy parameters.
No treatment-related changes were found in ambulatory ECG.
Chlorpromazine: Chlorpromazine dose of 120 mg/kg/day (equivalent to 15 mg/kg/day of lurasidone), resulted in clinical signs of vomiting, decrease in movement, staggering gait, miosis, ptosis and dry nose,

persisting to the evening of the next day. Blood biochemical examination showed increases in total creatine kinase (CK) activity, ALT, AST, alkaline phosphatase, glucose and phospholipids, and changes in CK fractions. Ambulatory ECG revealed an increase in heart rate in males and females, prolongation of QTc in males and shortening of PQ interval in females, with statistically significant differences from control at many time points.
The following sponsor's text adequately describes the study findings:

> For the SM-13496 groups, decrease in movement and tremor were found in males of the 50 mg/kg groups at 3 - 4 hours after administration, but the changes were transient and disappeared at 6 - 7 hours after administration. Ambulatory electrocardiography revealed an increased incidence of upper ventricular complementary beats in 1 male of the 5 mg/kg group on the day of administration until 1 day after administration and on 3 - 4 days after administration. Complementary beats are a physiological phenomenon to compensate the delay of the fundamental cardiac rhythm, and the clinical attention is directed to bradycardiac arrhythmia that induces complementary beats. In the above-mentioned animal, this change appeared in 27 beats/day on the day of administration until 1 day after administration and 66 beats/day on 3 - 4 days after administration. The incidences were very low compared with the 24-hour total heart rates (120,000 - 140,000 beats) and continual bradycardiac arrhythmia was not found. Complementary beats were also found in 1 male littermate of the 50 mg/kg group before and after administration with similar incidences on both occasions, and the change appeared, in both dogs, before feeding of the day or from midnight to early morning when heart rates decline physiologically. Above-mentioned upper ventricular complementary beats in the 5 mg/kg group, therefore, was considered to be changes within physiological variations and unrelated to treatment. In addition, there were no changes in CK and LDH levels in the blood, which are the escape enzymes of the cardiac muscle, or in the heart at necropsy. In consideration of these facts altogether, SM-13496 is considered to have no effects on the heart at dose levels of 50 mg/kg or lower.
>
> For the chlorpromazine group, animals had vomiting from immediately after administration to 4 hours after administration and also showed signs consisting of decrease in movement, ataxic gate, miosis, ptosis, and dry nose. These signs disappeared by the evening of the next day, but the effects on animals were severer than those found in animals of the 50 mg/kg SM-13496 group. Ambulatory electrocardiography revealed an increase in heart rates in males and females, prolongation of QT_c in males, and shortening of PQ interval in females slightly on the day of administration until 1 day after administration, suggesting the functional effects of chlorpromazine on the heart. Transient bradycardia accompanying complementary beats found from immediately after administration to 1 hour after administration was considered to be changes related to vomiting in consideration of the type and time of their occurrences. Changes in blood biochemistry in 1 female consisted of increases in CK, GOT, and GPT with an increase in fraction CK-MB. These changes may suggest some disorders of the cardiac muscle. As shown in the above, chlorpromazine at 120 mg/kg caused an increase in heart rate, prolongation of QT_c, shortening of PQ interval, and probably pathological changes of the cardiac muscle, which clearly indicate the effects on the heart. Effects on the liver were also suggested as evidenced by the increased ALP, Glu., and PL levels in the blood.

Conclusion: Lurasidone single-dose oral (capsule) administration to Beagle dogs at doses of 5, 15 and 50 mg/kg/day caused clinical signs (tremors and a decrease in movement) at the high dose, but did not affect cardiac parameters as measured by 24-hour ambulatory ECG on Days 0 to 1, 3 to 4 and 13 to 14.

Study title: **Oral Repeated Dose Cardiac Toxicity Study in Beagles**

Study no.: (b) (4) **98-4306**

Conducting laboratory and location: (b) (4)

Date of study initiation: March 3, 1998

GLP compliance: Yes (Japanese guidelines)

QA statement: Yes

Drug, lot #, and % purity: SM-13496 (Lot # 3CG001M, 100.9%)

Key Study Findings

Repeated oral administration of lurasidone (in gelatin capsules) at doses of 0, 5 and 50 mg/kg/day for 14 consecutive days to male Beagle dogs produced treatment-related miosis and a decrease in movement at HD. ECG (as measured by 24-hour ambulatory ECG on dosing Days 1 to 4, 7 and 14) revealed lack of nighttime physiological drop in heart rate in one HD animal on Days 1 to 2 that gradually resolved with repeated administration. There were no treatment-related changes in PQ, QRS, QT intervals, QTc, or cardiac rhythm.

Methods:
Doses: 0, 5 and 50 mg/kg/day
Frequency of dosing: Daily for 14 consecutive days
Route of administration: Oral
Formulation/Vehicle: Gelatin capsules
Species/Strain: Dogs/Beagle
N animals/sex/group: 3 males

Observations:
Hematology examination was performed on the acclimation day and weeks 1 and 2 of administration (RBC, WBC, Hb, Ht, MCH, MCV, MCHC, Ret, Pl, differential leukocyte count)
Blood biochemistry examinations were performed on the same time points (GOT, GTP, LDH, gamma-GTP, CK, Glu, T. Cholesterol, Triglycerides, Phospholipids, T. Protein, Alb., A/G ratio, BUN, Creatinine, T. Bilirubin, Na, K, Ca, Pi, Mg, Creatine kinase fractions, LDH fractions, Protein fractions)
Pathology**:** Gross examination
At necropsy, heart specimens from each animal were collected, fixed and stored for light microscopy. Papillary muscle of the left ventricle, parts of the left ventricle and interventricular septum were collected and processed for electron microscopy. (Microscopic examination was not performed)

ECG: Continuous 24-hour ambulatory electrocardiograms (Holter electrocardiograph) were performed twice in the acclimation period (18 - 17 days and 5 - 4 days before start of administration), days 1 - 2, 3 - 4, 7 - 8, and 13 - 14 of administration. A specially designed jacket for ECG was put on each animal on the previous day of examination. By using Holter's electrocardiograph (QR1300 Fukuda M-E Ind., Ltd.), electrocardiograms by M-X lead (episternum: -, xiphosternum: +) and R-L lead (right thorax: -, left thorax: +) were recorded in a cassett tape. ECG traces were printed out with an ambulatory ECG analyzer (Quick Scan QS-2200, Fukuda M-E Ind., Lit.). Heart rate (mean of heart rates for 30 seconds at neighboring 3 points) and wave intervals (PQ, QRS, QT: mean of 3 waves in succession) were measured at 1-hour interval by using an ECG taken at the 2nd examination in the acclimation period and all ECGs taken during the administration period. R-R interval was calculated from the heart rates, and QT_c was calculated using the following formula. Arrhythmia was analyzed for the entire course of time for all ECGs.

$$QT_c = QT / \sqrt{RR}$$

Results:

Treatment-related miosis and decreased movement appearing on Day 2 were observed in all animals at 50 mg/kg; the incidence decreased after the 1st week of treatment.

There were no changes in body weights, body weight gain, and food consumption. Hematology and blood biochemistry showed no treatment-related changes. Differences from control were found in some hematological parameters (MCHC and Pl) and chemical parameters (Glu, Ca, and LDH fraction) in LD and HD groups, but there were no clear differences from the corresponding pretest values.

ECG revealed lack of nighttime physiological drop in heart rate in one HD animal on Days 1 to 2 that gradually resolved with repeated administration, as described by the sponsor below:

> For the 50 mg/kg group, heart rates on days 1 - 2 began to decrease from approximately 12 hours after administration in degrees lower than those in the control group and were significantly higher than the control at 10 and 11 hours after administration. When individually assessed, 1 animal (No.132) had a lack of physiological decrease in heart rate at night, but other 2 animals showed a similar pattern of change as seen in the control group. Therefore, the delay of physiological decrease in heart rate as shown in the group mean was because of the delay in 1 animal mentioned above. This animal still showed a similar pattern of change on days 3 - 4, but the pattern became similar to that of the control group thereafter. The group mean on days 3 - 4 was significantly lower at 4 hours after administration, but the change was accidental because of a lack of change in sequence.
>
> For the 5 mg/kg group, there were no differences from the control throughout the administration period.

No treatment related changes were found in PQ, QRS, QT intervals, QTc, or rhythm.

Conclusion: Repeated oral administration of lurasidone (by capsules) at doses of 5 or 50 mg/kg/day for 14 consecutive days to male Beagle dogs (3/group) caused drug-related clinical signs at HD (miosis, decrease in movements) but did not induce changes in PQ, QRS, QT intervals, QTc, or cardiac rhythm.

Cardiac Toxicity Studies in Monkeys

Study title: **Oral Single-Dose Cardiac Toxicity Study in Monkeys**

Study no.: (b) (4) **97-6242**

Conducting laboratory and location: (b) (4)

Date of study initiation: December 11, 1997

GLP compliance: Yes (Japanese guidelines)

QA statement: Yes

Drug, lot #, and % purity: SM-13496 (Lot # 3CG001M, 100.9%)

Key Study Findings

Single-dose oral administration of lurasidone (10, 50 and 250 mg/kg) to Cynomolgus monkeys produced clinical signs described as "crouching position and listlessness" in 1 HD male on the 2nd day after administration. Ambulatory 24-hr. ECG (obtained on Days 1, 3 and 14) showed an increase in heart rate at HD (starting from 1 h. after administration) and tachycardia (over 200 bpm) at 3 to 6 h. after administration. Heart rate declined at 7 h. post-dose, but increased subsequently and remained slightly higher (140-180 bpm) until the next morning. On days 3 to 4 post-dose, heart rates were similar to control at daytime, but were slightly higher at night. No treatment-related changes in PQ, QRS, QT intervals, or QTc were found. There were no treatment-related arrhythmias or other changes except for multiple electromyogram patterns in HDS animals, probably due to tremor. No treatment-related changes were found in body weight, food consumption, hematology, and at necropsy.

Methods:
Doses: 0, 10, 50 and 250mg/kg/day
Justification of dose selection: Based on Cmax values in a previous 3-month study in monkeys and in Phase 1 clinical trial, the dose of 10 mg/kg/day was selected as a dose equivalent to a 10 mg clinical dose and 50 mg/kg/day as "slightly lower" lower than a 30 mg clinical dose, and 250 mg/kg/day as a dose 5 times 50 mg/kg/day.
Frequency of dosing: Single dose
Route of administration: Oral (intubation)
Formulation/Vehicle: Suspension/0.5% Methylcellulose
Dosing volume: 2 ml/kg
Species/Strain: Monkey/Cynomolgus
N animals/sex/group: 3 males

Observations:
Clinical signs: 2-3 times daily
Body weight and food consumption: Once weekly during the acclimation period, on the day of administration, and 1, 2, 3, 4, 7, 11 and 14 days after administration.

ECG: Ambulatory 24-hr. ECG data were obtained on Days -1 to 0 (before administration), Days 0 to 1 (after administration), Days 3 to 4 and Days 13 to 14. The method was described by the sponsor as follows:

Ambulatory electrocardiography (approximately for 24 hours continuous) was performed twice in the acclimation period, on the previous day of administration until the day of administration, on the day of administration until 1 day after administration, on 3 - 4 days after administration, and on 13 - 14 days after administration. A specially designed jacket for ECG was put on each animal on the previous day of examination. By using Holter's electrocardiograph (QR1300 Fukuda M-E Ind., Ltd.), electrocardiograms by M-X lead (episternum: -, xiphosternum: +) and R-L lead (right thorax: -, left thorax: +) were recorded in a cassett tape. At the time of the jacketing and at the start and end of the recording, animals were sedated with an intramuscular injection of ketamine hydrochloride (Ketalal® 50 for animal, Sankyo Co., Ltd.). In preparation for the ECG practice, animals had been trained to accustom to the putting on the jacket and Holter's electrocardiograph in the acclimation period. ECG traces were printed out with an ambulatory ECG analyzer (Quick Scan QS-2200, Fukuda M-E Ind., Lit.). Heart rate (mean of heart rates for 30 seconds at neighboring 3 points) and wave length (PQ, QRS, QT: mean of 3 waves in succession) were measured at 1-hour interval by using ECG taken on the day of administration until 1 day after administration or at 2-hour interval for the other ECGs. R-R interval was calculated from the heart rates, and QT_c was calculated using the following formula. Arrhythmia was analyzed for the entire course of time for all ECGs.

$$QT_c = QT / \sqrt{RR}$$

Hematology: Pre-dose and on Days 1, 3, and 13 (RBC, WBC, Hb, Ht, MCH, MCV, MCHC, Ret, Pl, differential leukocyte count)

Blood biochemistry examinations were performed on the same time points (GOT, GTP, LDH, gamma-GTP, CK, Glu, T. Cholesterol, Triglycerides, Phospholipids, T. Protein, Alb., A/G ratio, BUN, Creatinine, T. Bilirubin, Na, K, Ca, Pi, Mg, Creatine kinase fractions, LDH fractions, Protein fractions)

Pathology (Gross examination) At necropsy, heart specimens from each animal were collected, fixed and stored for light microscopy. Papillary muscle of the left ventricle, parts of the left ventricle and interventricular septum were collected and processed for electron microscopy. (Microscopic examination was not performed).

Results:

Clinical signs: General observation revealed crouching position and listlessness in one animal (250 mg/kg group) on Day 2 after drug administration. These signs were not present on Day 3.

In a previous oral single-dose study in monkeys performed by the sponsor, a decrease in locomotor activity and tremor was observed in males at doses of 50 mg/kg and higher, and in females at doses of 10 mg/kg and higher and 250 mg/kg, respectively. These results are different from this study. According to the sponsor, one of the possible reasons is that the present study focused on assessing ECG parameters, with observations of clinical signs restricted to the minimum by limiting the time of observation.

ECG:

Heart rate: No treatment-related changes in HR were found at LD and MD (10 and 50 mg/kg). Increased heart rate was found in the HD (250 mg/kg) group; the increase was slight at 1 hour post dose (approximately 170 bpm), and marked at 3 through 6 hours after administration (over 200 bpm). Heart rates declined beginning 7 hours after administration, but increased subsequently and remained slightly higher (140 to 180 bpm) until the next morning. Statistically significant differences from control were registered at 3, 5, 6, 7 and 9 hrs post dose. On Days 3 and 4 after administration, the heart rates were

similar to controls during the daytime, but were slightly higher (130-140 bpm) at night (mostly due to one animal). There were no differences in HR on Days 13 and 14 post dose.
A decrease in QT interval was found at HD in comparison to control, beginning at 1 h post dose until the next morning. The decrease was most pronounced (below 150 msec) at 4-6 h post dose, and statistically significant at 5 hrs. post dose. The QT decrease was due to changes in heart rate, since no changes in QTc were found in this group. No treatment-related changes in PQ and QRS intervals were found in any group.
Rhythm: Upper ventricular complementary beats (41 beats/day) were registered in 1 HD animal on the day of administration and 1 day post dose; this incidence was low as compared with the 24-hour total heart rates, and was considered a spontaneous variation, having in mind that one control animal also showed complementary beats of up to 25 beats/day.
Multiple electromyogram patterns, probably due to tremor, were noted in HD animals.
Blood biochemistry did not show changes in LDH or CK, which reflect pathological changes in the heart, and no gross changes in the heart were found at necropsy.
Conclusion: A single oral dose administration of lurasidone (10, 50 and 250 mg/kg) to Cynomolgus monkeys induced clinical signs (crouching position and listlessness) and increase in heart rate at HD (1 male) up to 4 days post-dose. No treatment-related changes in PQ, QRS, QT intervals, or QTc were found.

The cardiac toxicity studies in dogs and monkeys are summarized in the sponsor's table below.

Cardiac toxicity studies

Objective	Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No.
Single-dose cardiac toxicity	Dog/ Beagle	Oral (capsule) (lurasidone)	Single dose	0, 5, 15 and 50 Chlorpromazine (120 mg/kg positive control)	3/sex/ group	Males at 50 mg/kg exhibited transient tremors and decreased movement at 3 to 4 hours post-administration. ECGs, hematology and clinical chemistry parameters were unaffected. Positive control suggested numerous functional and pathological cardiac effects.	(b) 97-4241
Repeated-dose cardiac toxicity	Dog/ Beagle	Oral (capsule) (lurasidone)	2 weeks	0, 5 and 50	3 males/ group	Decreased movement was observed at 50 mg/kg/day. Treated animals exhibited miosis daily during the first week and less frequently thereafter. The only ECG finding from treatment was a lack of physiological decrease in nocturnal heart rate on the first day or 2 of dosing.	(b) 98-4306
Single-dose cardiac toxicity	Monkey/ Cynomolgus	Intranasal gastric intubation (suspension in aqueous 0.5% methylcellulose	Single dose	0, 10, 50 and 250	3 males/ group	At 250 mg/kg one animal exhibited listlessness and crouching position. No indication of cardiac disorder or arrhythmia, but tachycardia was observed at 3 to 6 hours after 250 mg/kg dose.	(b) 97-6242

Review of Heart Sections from 13-Week Repeated-Dose Oral Toxicity Study in Monkeys (Study 3702)
A re-evaluation of histopathology slides of the heart from the 13-week oral toxicity study in monkeys (Study No. SUP22) reaffirmed the original diagnosis that focal myocarditis reported in 1 male of the low-dose group was not associated with lurasidone treatment.

Review of Heart Sections from 52-Week Repeated-Dose Oral Toxicity Study in Monkeys Study (Study 3703)
A re-evaluation of histopathology slides of the heart from the 52-week oral toxicity study in monkeys (Study No. SMO550) reaffirmed the original diagnosis of myocardial inflammatory infiltrate in 2 of 8 animals in the mid-dose group but decreased the findings in the high-dose group, from 2 animals to 1 and

concluded that these were "spontaneous lesions without dose-related occurrence and unlikely to be associated with lurasidone treatment ".

10. 2. Antigenicity (Study No. 2968)

Antigenicity of oral and subcutaneous doses of lurasidone was studied in guinea pigs (5 males/group) sensitized with lurasidone in three groups: low oral dose (0.35 mg/animal) and high oral dose (3.5 mg/animal), 3 times a week for 3 weeks; and subcutaneous high dose (3.5 mg/animal) with Freund's complete adjuvant (FCA; an immunopotentiator) once a week for 3 weeks. Antigenicity was assessed in active systemic anaphylactic reaction assays, passive cutaneous anaphylactic reaction assays, gel precipitation reaction and intradermal tests (see sponsor's table below).

Objective	Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No.
Antigenicity	Male Guinea pig/ Hartley	Sensitization: oral and subcutaneous	Sensitization: 3 weeks, 3 times/week (po), weekly(SQ)	Sensitization: 0.35 and 3.5 mg/animal (po) or 3.5 mg/animal (SQ)	5 males/ group	Delayed-type hypersensitivity was observed in the group receiving lurasidone along with Freund's complete adjuvant, subcutaneously, but not in the group receiving lurasidone orally. Active systemic anaphylactic reaction assays, passive cutaneous anaphylactic reaction assays and gel precipitation reaction assays were negative.	2968
		Challenge: Intradermal and intravenous	Challenge: Single dose	Challenge: 50 µg/site (i.d.) 200 and 500 µg/animal (IV)			

Lurasidone caused delayed-type allergic reactions upon subcutaneous administration at a dose of 3.5 mg/animal, along with FCA. However, since lurasidone did not show antigenicity when orally administered, it is unlikely that it will show antigenicity when orally administered to humans.

10.3. Phototoxicity (Study No. L-08-029)

The potential phototoxicity of lurasidone was studied following a single oral administration of lurasidone (100, 300 and 1000 mg/kg), vehicle (negative control) or positive control (8-methoxypsoralen, 10 mg/kg) to Sprague-Dawley rats (5 males/group). An additional group received 1000 mg/kg lurasidone served as a non-irradiated control group. All groups except the non-irradiated control group then were irradiated with ultraviolet A (UVA) radiation at a dose of 10 J/cm2.
The results are summarized in the following sponsor's table.

Objective	Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No.
Phototoxicity Study	Rat/ Crl:CD (SD)	Oral Gavage Irradiation: (UVA) at a dose of 10 J/cm^2	Single dose	0, 100, 300 and 1000 (irradiated) 1000 (non-irradiated) 8-Methoxypsoralen 10 mg/kg (positive control)	5 males/ group	No remarkable skin reaction or increase in ear thickness observed in lurasidone-treated or vehicle control groups.	L-08-029

No remarkable skin reaction or increase in ear thickness was observed in lurasidone-treated or vehicle control groups. No skin reaction was detected in the non-irradiated control group. The validity of the experiment was confirmed by the presence of erythema and edema in the ears and dorsal skin, at 48 and 72 hours post-irradiation in the positive control group, as well as an increase in ear thickness. Lurasidone had no phototoxic effect on the skin under the conditions of the study.

10.4. Drug Dependence (Study No. (b)(4)-89A)

The potential of lurasidone to induce drug dependence, as measured by acute central nervous system effects, was studied in experiments in rhesus monkeys and rats: psychic dependence liability in rhesus monkeys through an intragastric self-administration experiment; cross-physical dependence liability in rhesus monkeys through a barbital withdrawal sign suppression experiment; and physical dependence liability in rats through a drug-admixed food intake experiment, as summarized in the following sponsor's table.

Objective	Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No.
Psychic dependence (reinforcing effect)	Monkey/ Rhesus	Oral (intragastric continuous self-administration)	14 or 21 days	1, 4, 16 and 64/infusion	2 or 4	No frequent or persistent spontaneous intake was observed with any unit dose in any monkeys.[a]	(b)(4)-89A
Cross-physical dependence (suppression of barbital-withdrawal signs)	Monkey/ Rhesus	Intranasal gastric intubation (suspension in aqueous 0.5% methylcellulose)	Single dose	4, 16, 64, 128 and 256	1 to 6	Mild or marked suppression was seen at 64 mg/kg or higher, which was considered to result from general suppression, as in the case of chlorpromazine that has no physical dependence ability.[a]	(b)(4)-89A
Physical dependence (withdrawal signs)	Rat/ Jcl:SD	Oral (drug-admixed food)	4 weeks	Low dose, 19 to 90; High dose, 72 to 316	10 males	No withdrawal signs were evident.[a]	(b)(4)-89A

[a] Minimal dosage required for CNS effects was 4 mg/kg in monkeys and 16 mg/kg in rats

Lurasidone appeared to be free of cross-physical dependence with barbital, and free of potential for physical dependence formation. No reinforcing effect was noted with lurasidone, suggesting that this compound has no potential to induce psychic dependence.

11 Integrated Summary and Safety Evaluation

General toxicology

Single- and repeated-dose (subchronic and chronic) general toxicity studies were performed in rodents, dogs and monkeys. The pivotal GLP-compliant studies in these species, performed by oral (gavage) administration of lurasidone (suspension in 0.5% aqueous methylcellulose) are as follows:

- Six-month Oral Toxicity Study of SM-13496 in Rats (Study # 3259)
- Thirty Nine-Week Oral Toxicity Study of SM-13496 in Dogs (Study # 3879)
- SM-13496 Toxicity to Cynomolgus Monkeys by Repeated Oral Administration for 52 Weeks (Study # SMO550)

Rats: A 6-month rat study (followed by a 3-month recovery) was performed at oral gavage doses of 0.03, 1, 10 and 100 mg/kg/day. Effects on sex hormones included elevation in serum prolactin in females at all dose levels and in males at ≥ 1 mg/kg/day; and reduction in serum estradiol levels in females at ≥ 10 mg/kg/day. These hormonal changes correlated with the disruption of the estrus cycle in females with increased incidence of diestrus (at ≥ 1 mg/kg/day) and with gross and microscopic pathology of mammary gland hyperplasia in females at ≥ 1 mg/kg/day along with a decrease in number of corpora lutea in the ovaries, increased frequency and extent of vaginal epithelium mucification, and uterine atrophy. Lurasidone also reduced the density of trabecular bone (femur) and increased fatty infiltration in the bone marrow observed in females at ≥ 1 mg/kg/day; similar effects were also noted in the 3-month rat study, and were tentatively attributed (by the sponsor) to lurasidone effects on sex hormones (prolactin and estrogen). Thickening of the adrenal zona glomerulosa (dose-dependent in females) was a new finding noted at ≥ 0.03 mg/kg/day in the 6-month study. It was speculated that "this change is a result of lurasidone-induced elevation in prolactin levels which may have affected corpora lutea in the ovary, potentially producing a low estrogen state with increased progesterone production, which in turn elicited an increase in production of aldosterone in the adrenal glands". However, plasma aldosterone levels were not analyzed to confirm this proposal. Recovery or a trend toward recovery was noted after a 3-month discontinuation period, except for the following changes: reduction in density of trabecular bone, fatty infiltration in the bone marrow, and increase in absolute and relative ovary weights. The issue of the bone density decrease (seen consistently in the subchronic and chronic rat studies) was addressed in the Agency's letter dated 2/7/2001 under IND 61 292, which asked the sponsor to further investigate the decreased bone density, and to monitor bone density in a clinical trial. The NOAEL in the rat 6-month oral lurasidone study was 0.03 mg/kg/day for males because of the elevated serum prolactin levels and the corresponding histopathology changes in the mammary gland noted at this and higher doses. In females, the NOAEL was also 0.03 mg/kg/day although serum prolactin was also increased at ≥ 0.03 mg/kg/day however, additional adverse effects i.e., disruption of estrus cycle, mammary hyperplasia, decreased number of corpora lutea, uterine atrophy, reduction in trabecular bone density and increased fatty infiltration in bone marrow occurred at and above the next tested dose of 1 mg/kg/day. There was no safety margin, since lurasidone mean serum levels at the NOAEL in the rat [0.15 and 0.07 ng/ml for M and F, respectively (AUC not determined)] were much lower than the maximal serum concentration in humans (165 ng/ml) following repeated oral administration at the MRHD of 120 mg/day.

Monkeys: Lurasidone oral administration to Cynomolgus monkeys at doses of 2, 10, and 50 mg/kg/day for 1 year (52 weeks) resulted in treatment-related findings at all dose levels. Some of these were considered extension of the drug pharmacology i.e., subdued behavior and transient increase in serum prolactin. Lurasidone also induced extrapyramidal side effects i.e., tremors (in mid- and high dose groups) and abnormal postures (dystonic behavior, expressed as contortions of the extremities resulting in unusual and sustained posturing, particularly of the limbs) at all dose levels with a dose-related incidence. Based on these effects, a NOAEL could not be determined (below the lowest tested dose of 2 mg/kg/day). In addition, an MTD was not established because of lack of a clear dose-limiting toxicity, since there was no body weight change, or other manifestations of general toxicity (except for clinical signs). No significant

drug-related changes were found in ECG, hematology, blood biochemistry, urinalysis, bone marrow smears, organ weights and pathology examinations. Myocardial inflammatory infiltrates were found at MD and HD (in 2 of 8 animals in each group), but a re-evaluation of histopathology slides of the heart performed by the sponsor (study 3703) reduced the findings in the high-dose group from 2 animals to 1. Based on this, and on the sponsor's literature data and FDA reviewer's data of high spontaneous incidences of myocardial inflammatory infiltrates in Cynomolgus monkeys, the Division considered that "it is not unreasonable to consider these spontaneous (*rather than drug-related*) findings in the 1-year monkey study" (see Dr. Lois Freed's P/T Memorandum to IND 61292 of 8/13/2002). This study did not achieve sufficient systemic exposure levels to adequately assess lurasidone safety for humans. Even at the highest tested dose of 50 mg/kg/day, lurasidone systemic exposure values in the monkey after 52 weeks of treatment (Cmax: 86 and 90 ng/ml; AUC: 625 and 558 ng.h/ml for M and F, respectively) were lower than or, at best, similar to the corresponding human exposure values at the MRHD of 120 mg/day (Cmax=164.7 ng/ml and $AUC_{0\text{-}inf}$ = 686.6 ng.h/ml).

The concerns over the findings of myocardial infiltrates in mid- and high-dose groups and the lack of safety margin based on systemic exposure levels achieved for lurasidone that were below those measured in the clinic rendered this monkey study inadequate. These concerns were addressed in the Memorandum of Dr. Lois Freed to IND 61292 of 8/13/2002. As this study did not achieve sufficient systemic exposure levels to adequately assess lurasidone safety for humans, the Division recommended that the sponsor achieve higher plasma exposures in another non-rodent species (dog). The sponsor complied with this recommendation and subsequently conducted general toxicology studies in dogs, reviewed herewith.

Dogs: A 9-month (39-week) oral gavage administration of lurasidone to Beagle dogs at doses of 30, 100 and 200 mg/kg/day caused pharmacologically related clinical signs (decreased spontaneous activity, somnolence, tremors, miosis), reduced food consumption at all dose levels, and decreased body weight or weight gain in males at MD and HD. Premature ventricular contractions (PVCs) occurred at HD (2 of 4 males) from Week 13 to 22. Both animals exhibiting PVC had high serum concentrations of lurasidone, and severe (approximately 30%) decrease in body weight relative to control. Non-corrected QT intervals were prolonged in these two HD males, and in one MD male that had a 33% body weight loss. Increased total cholesterol and phospholipids were seen in all treated groups except for MDM. Effects potentially related to exaggerated drug pharmacology included increased serum prolactin at all doses throughout treatment decrease in absolute and relative prostate weights at all doses and decrease in absolute testis weights at MD and HD. These decreases were evident grossly, where the prostates, ovaries and/or uteri appeared small in all treated groups. Histopathology revealed prostatic atrophy in all treated male groups and, at the two highest dose levels, the testes exhibited multifocal or diffuse seminiferous atrophy; at HD, hypospermia was noted in the epididymis, with cellular debris in the lumen of the tubules and vacuolization of ductal epithelium. In female dogs, uterine atrophy and decreased presence of corpus luteum and secondary ovarian follicles were seen in all treated groups, along with mammary gland "thickening" and microscopic pathology (hydropic appearance of ductal epithelium, diffuse lymphoid cell infiltration and pigmentation). A decrease in trabecular bone in the femur and/or sternum was observed in 1 MD male and 2 HD males in parallel with increased fatty infiltration in bone marrow of the femur and/or sternum. All three males exhibited severe body weight loss (20 to 30% by the end of the treatment period); two of these males were listed as "emaciated". Thymic atrophy was seen in all dose groups, and decreased numbers of cytoplasmic vacuoles were observed in the adrenal cortex of animals of both sexes at the two highest dose levels, compared to controls. New findings in this study when compared with the chronic toxicity studies in rats and monkeys were the effects on the male genital system and premature ventricular contractions. The MTD was 200 mg/kg/day for females and 100 mg/kg/day for males, due to severe body weight loss (emaciation) and deterioration of general condition in males at the next higher tested dose of 200 mg/kg/day.

A NOAEL for general toxicity could not be determined in this study because drug-related clinical signs (i.e., tremors), elevation in serum prolactin, and gross- and microscopic pathology changes in the mammary gland, thymus, and male and female reproductive system (i.e., prostate and uterine atrophy) were present at all tested doses, including the lowest (30 mg/kg/day). The dose of 30 mg/kg was a

NOAEL for cardiovascular effects (non-corrected QT interval prolongation). Lurasidone systemic exposure margin at this dose after 39 weeks of treatment was about 15- and 12 times, in males and females respectively (based on AUC in serum) and 7 times (based on Cmax) the human exposure at MRHD of 120 mg/day ($AUC_{0\text{-}inf}$ = 687 ng.h/ml).

Genetic toxicity

The potential of lurasidone to induce genetic toxicity was investigated *in vitro* in the bacterial reverse mutation test (Study 2773), in mammalian cell chromosomal aberration test using Chinese hamster lung cells (Study 2749) and in an *in vivo* micronucleus assay in mice (Study 2767). A lurasidone metabolite (ID-11614) and a compound code named (b) (4) (lurasidone starting material and potential impurity with a structural alert), were evaluated in bacterial reverse gene mutation (Ames) tests (Studies No. 3117 and A02097, respectively)

Under the test conditions, lurasidone was not mutagenic in the Ames test, and did not exhibit clastogenic potential in the in vitro chromosomal aberrations test, or in the in vivo micronucleus assay. Both compounds code named ID-11614 and (b) (4) tested negative in the bacterial reverse mutation test. Regarding the further qualification of (b) (4) (the potential impurity with a structural alert), "if the specification for this compound is below 0.15% of drug substance, no further (genotoxicity) testing is required in view of the negative Ames test", as stated by the Division at the pre-NDA meeting (May 22, 2009). According to the reviewer chemist, Dr. Shastri Bhamidipati, (e-mail communication of 2/16/2010), the sponsor has tested the lurasidone free base (obtained prior to its conversion to the salt form) for (b) (4) by GC-MS method and has shown that (b) (4) levels are below (b) (4) in a total of 25 batches (including batches used in nonclinical, clinical, and commercial scale); since the levels were below (b) (4) in the batches used in Phase III studies, the drug product at the MRHD (120 mg) will have no more than (b) (4) (below 0.15% of the drug substance). Therefore, no further genetic toxicity testing of (b) (4) is needed.

Carcinogenicity

Lurasidone carcinogenic potential was investigated in lifetime (2-year) GLP-compliant studies in mice and rats (Studies No. 6645-138 and No. 6645-139, respectively).

Mice: Oral administration of Lurasidone (suspension in 0.5% methylcellulose) to mice (60/sex/dose) at doses of 0, 0, 30, 100, 300, and 1200/650 mg/kg/day in males for 104 weeks [HD reduced as of Day 410 due to excessive (>20%) weight loss] and at 0, 0, 30, 100, 300, and 650 mg/kg/d in females for 98 weeks (shorter dosing duration in females due to excessive mortality) did not produce neoplastic lesions in the males. In the females, however, statistically significant increases in neoplastic lesions (benign pituitary pars distalis adenoma and malignant mammary carcinoma and adenoacanthoma) were induced at all tested dose levels, with highly significant positive trends vs. pooled control groups. In particular, the tests of overall trend and pair-wise comparisons between the highest dose group and pooled control in females were statistically significant for mammary carcinoma, as were the tests of pooled mammary tumors (adenomas, carcinomas, carcinosarcomas, and adenoacanthomas). The pairwise comparisons of mammary carcinoma and adenoacanthoma at mid-high and middle doses to control were statistically significant; for the low dose group the difference for mammary carcinoma was close to the adjusted statistical significance ($p \approx 0.01$). The pairwise comparisons of pituitary pars distalis adenoma to the pooled controls were statistically significant in all dose groups. Some other neoplasms were increased in single dose groups without dose-dependence. Thus, the pooled cancers of the ovary were statistically significantly higher vs. pooled control in the middle dose group only and adrenal pheochromocytoma and islet cell adenoma of the pancreas were statistically significant in the mid-high dose group, but not in the highest dose group. No other tests achieved statistical significance.

Non-neoplastic findings: Dose-related significant increases in mortality occurred in females at 300 and 650 mg/kg/day. The proportion of females with a lack of estrus cycling was increased at 100 mg/kg/day and higher, supported by histopathology findings in the female reproductive system indicative of estrus cycle disruption, i.e., ovarian, uterine, cervical and vaginal atrophy. The estrus cycle disruption along

with the marked elevation of serum prolactin and the increased incidence of tumors in the pituitary and mammary gland is likely related to the dopamine type 2-receptor antagonistic properties of the drug. Serum prolactin (measured during Week 52) was markedly and significantly elevated at all dose levels in comparison to control, and prolactin levels in females were 2 to 4 times higher than those in males in all dose groups. Plasma exposure to lurasidone increased with dose, less than dose-proportionally, in both males and females. Females had higher systemic exposure values (Cmax and AUC_{0-24}) than males across all collection time points, as well as higher systemic exposure after multiple dosing.
According to the conclusion of the Executive CAC, mammary carcinomas and adenoacanthomas and benign pituitary pars distalis adenomas were drug related in females only, at all dose groups. NOEL for neoplasia: Males: 650 mg/kg/day (AUC_{0-24}= 12, 947 ng.hr/ml); Females: A NOEL could not be determined for pituitary adenoma and mammary malignant neoplasia (carcinoma, adenoacanthoma) (below the LD of 30 mg/kg/day, AUC_{0-24} 1130 ng.hr/ml). Compared to human exposure at the MRHD of 120 mg/day (AUC_{0-inf}= 687 ng.h/ml), the safety margin at the NOEL for males is about 19x; for females, there is no safety margin for humans since a NOEL could not be determined (based on AUC, the female mice exposure at the lowest tested dose was 1.6x the human exposure at the MRHD).

Rats: Oral (gavage) administration of lurasidone (suspension in 0.5% methylcellulose) to rats (65/sex/dose) for 104 weeks at doses of 0, 0, 3, 12, 50/36 mg/kg/day (HD reduced to 36 mg /kg /day beginning on Days 404 and 403 for males and females, respectively, because of excessive decrease in body weight) resulted in the following neoplastic findings: Males: Skin fibroma/fibrosarcoma incidence was significantly increased over the pooled vehicle control only at mid-dose, but not at high dose. No other significant effects, either in terms of positive trend or significant increase over the controls, were noted. Females: Increased incidence of mammary carcinomas was found at mid- and high-dose; the test of trend was statistically significant, as was the test comparing the HD and MD to the pooled vehicle; at the mid-dose, the incidence of mammary adenomas was also significantly increased over pooled controls, but there was no increase in this tumor incidence at the high dose. The incidence of other mammary tumors was similar for control and treated females. No other tests of trend or comparisons between the high dose and controls achieved significance.
Non-neoplastic findings: Mortality was not increased in either gender relative to the incidence in corresponding controls. Body weight reduction: in females at MD and HD and in males at all doses; due to excessive reduction in mean body weight (>20% for both genders) at the initial HD of 50 mg/kg/day, the dose was reduced to 36 mg/kg/day beginning on Days 404 and 403 for M and F, respectively. The first statistically significant change in body weight occurred on weeks 46, 13, and 3 for the LD, MD and HD, respectively. In females, the changes in body weight were biphasic across the dose range: at LD, a significant increase vs. control was registered from Week 2 through 66. Food consumption was decreased at HD (both genders), at MD (females) and increased at LD (females only). Female estrus cycle disruption occurred at all dose levels in a dose-dependent manner, supported by microscopic findings of increased incidence of absence of corpora lutea in the ovary and increased vaginal cornification in females at all dose levels. In males, increased incidence of milk secretion was observed in all dose groups. Serum prolactin was elevated dose-dependently vs. controls in the males at all dose levels (reaching a plateau between MD and HD); in the females, prolactin was increased at LD and MD but not at HD. Plasma exposure to lurasidone increased greater-than-dose-proportionally; Cmax and AUC0-24h values were higher in females than in males across all collection time points. A marked (>2-fold) increases in Cmax and AUC0-24h values were determined in all dose groups after multiple dosing, indicative of drug accumulation. MTD: 36 mg/kg/day, due to excessive (>20%) body weight decrease versus control at the next tested dose of 50 mg/kg/day in both genders. In summary, lurasidone resulted in increased incidence of mammary carcinomas in female rats at MD and HD (12 and 50/36 mg/kg/d, respectively). The incidence of all other neoplastic lesions in either gender was not elevated at any of the tested dose levels. According to the conclusion of the Executive CAC, the mammary carcinomas in female rats at mid- and high dose lurasidone (12 and 50/36 mg/kg/day, respectively) were drug related. NOEL for neoplasia: Females: mammary carcinoma: 3 mg/kg/day (AUC_{0-24}: 365 ng.h/ml). Compared to human exposure at the

MRHD of 120 mg/day (AUC_{0-inf}: 687 ng.h/ml), the exposure ratio at the NOEL is 0.53, i.e., there is no safety margin for humans. Males: >50 mg/kg/d (AUC_{0-24}: 5276 ng.h/ml), safety margin for humans greater than 8x.

Reproductive and Developmental Toxicology

The reproductive and developmental toxicity studies performed with lurasidone included:
- Fertility and early embryonic development studies in male and female rats,
- Embryo-fetal development studies in rats and rabbits, and
- Pre- and postnatal development study in rats.

All studies were GLP-compliant. TK measurements were not performed.

Fertility and early embryonic development:

Effect on Males (Study 2771)

This study evaluated the effects of lurasidone on the reproductive performance of male rats and on the embryo/fetal development of their generation. Daily oral (gavage) administration of lurasidone at doses of 6, 30, and 150 mg/kg/day to male rats for 64 consecutive days prior to mating and during the mating period (with untreated females) did not induce adverse effect on male fertility and reproduction. There are no changes in fetal weight and morphological abnormalities at any of the tested dose levels. The NOAEL for general toxicity in paternal male rats was 6 mg/kg/day, based on the reduction in body weight and food consumption at the next tested dose level of 30 mg/kg/day and higher. The NOAEL for reproductive toxicity in paternal males was 150 mg/kg/day since no effects were noted in copulation and fertility indices up to the highest tested dose. On a mg/m^2 basis, there is a 12-fold safety margin between the NOAEL for male reproductive toxicity in rats and the MRHD of 120 mg/day.

Effect on Females (Study 2865)

Female Sprague-Dawley rats were administered daily oral (gavage) doses of lurasidone at 0.1, 1.5, 15, or 150 mg/kg/day for 15 consecutive days prior to mating, during the mating period (mated with untreated males) and through Day 7 of gestation. Upon Cesarean sections at term (Day 20 of gestation), fetuses were examined externally, viscerally and skeletally. There were no mortalities or adverse clinical signs related to lurasidone administration. During pregnancy, decreased maternal body weight gain was seen at all dose levels except the LD, with lower values of food consumption at 15 and 150 mg/kg/day. Prolonged estrus cycle and delayed copulation were observed at levels of 1.5 mg/kg/day and above. The copulation and fertility indices and the numbers of corpora lutea, implantations and live fetuses were decreased at 150 mg/kg/day (attributable to the low number of corpora lutea). Low fetal body weight and delayed ossification, indicative of retarded fetal development, were found at 150 mg/kg/day; no teratogenicity was found. The effects on maternal body weight, food consumption and reproductive performance including the effects on estrus cycle, corpora lutea count and intrauterine fetal development noted in the high-dose group were recovered after a two-week withdrawal period.

NOAEL for reproductive toxicity in females: 0.1 mg/kg/day (based on prolonged estrous cycle and delayed copulation at the next higher tested dose of 1.5 mg/kg/day); NOAEL for general toxicity in female rats: 0.1 mg/kg/day (based on maternal body weight decrease at and above the next higher tested dose of 1.5 mg/kg). On both mg/kg and a mg/m^2 basis, the NOAEL for reproductive toxicity in female rats is much lower than the MRHD (120 mg/day or 74 mg/m2) There is no safety margin for female reproductive toxicity in humans.

Embryo-fetal development

Rats

Lurasidone developmental toxicity in rats was assessed at doses of 3, 10 and 25 mg/kg/day administered by oral gavage to pregnant females during the period of fetal organogenesis (Day 6 to Day 17 of gestation). The high dose level was selected based on a preceding range-finding study in which the dose of 25 mg/kg/day suppressed maternal body weigh gain upon administration from Day 7 to Day 17 of gestation (Study 2784).

There was no drug-related maternal mortality or adverse clinical signs. Maternal body weight gain was suppressed significantly and dose-dependently in all 3 dose groups; maternal food consumption was decreased at 10 and 25 mg/kg/day. Fetal external malformations were observed in the 25 mg/kg (HD) group (cleft palate in 8 fetuses and meningoencephalocele in 1 fetus) versus no external malformations in the control; there were no external malformations at MD. The frequencies of these findings were beyond the range of the historical control data of the laboratory. However, all fetuses with cleft palate were from the same litter, and we agree with the sponsor that multiple malformation cases confined to a single litter are of a smaller concern than a distribution of affected fetuses in several litters. It is also noteworthy that in the range-finding study in the same species and strain with 12 pregnant rats/group treated with higher doses of lurasidone via the same route from gestation Day 7 to 17 (Study 2784) there were no external malformations up to the highest employed lurasidone dose of 150 mg/kg/day. This supports the sponsor's conclusion that the external malformations observed in the 25 mg/kg (HD) group were spontaneous rather than drug-related.

A NOAEL for general toxicity in maternal rats was not reached (below the lowest tested dose of 3 mg/kg/day). The NOAEL for embryo/fetal developmental toxicity was 25 mg/kg/day. On a mg/m^2 basis, there is a 2-fold safety margin between the NOAEL for developmental toxicity in rats and the MRHD of 120 mg/day.

Rabbits

Lurasidone developmental toxicity in rabbits was studied at daily doses of 2, 10 and 50 mg/kg/day administered to pregnant New Zealand White rabbits during the period of fetal organogenesis (Day 6 to Day 18 of gestation), by oral gavage. Cesarean sections were performed at term (Day 28 of gestation) and fetuses were examined externally, viscerally and skeletally. There were no drug-related mortalities, abortions, premature deliveries or adverse maternal clinical signs. Maternal body weight gain was suppressed at all doses (at and above 2 mg/kg/day) while there was no drug effect on food consumption. There were no notable necropsy (gross pathology) and Cesarean section findings (corpora lutea number, implantation rate, intrauterine embryo/fetal loss, number of live fetuses, sex ratio and fetal weight). There were no treatment-related changes in fetal growth, external, skeletal and visceral observations.

NOAEL for maternal general toxicity: Not reached (<2 mg/kg/day) due to maternal body weight gain decrease at all tested doses; NOAEL for embryo/fetal developmental toxicity: 50 mg/kg/day. On a mg/m^2 basis, there is an 8-fold safety margin between the NOAEL for developmental toxicity in rabbits and the MRHD of 120 mg/day.

Pre- and postnatal development

Lurasidone effect on pregnant/lactating female rats, on development of the conceptus and offspring, and on reproductive performance of the offspring, was evaluated following oral (gavage) administration of lurasidone at doses of 0.4, 2 and 10 mg/kg/day to pregnant rats from implantation through the end of lactation (Day 6 of gestation through postnatal day 21). The dams were allowed to deliver spontaneously and nurse their offspring. One male and 1 female offspring per litter were selected at weaning for evaluation of growth, development, physical and neurobehavioral development, and reproductive performance of F1 generation. Maternal body weights were significantly lower than controls by end of gestation in the 10 mg/kg/day group and maternal body weight gain was suppressed in the same dose group during the gestation period. No treatment-related effects were found in gross pathological findings, maintenance of pregnancy, parturition and nursing. Among the offspring, no treatment-related effects were found in the number of pups delivered; sex ratio; viability indices; body weights; physical development; neurobehavioral development (reflex responses, motor activity, learning and memory); sexual development; estrus cyclicity; reproductive capacity; and gross pathological findings.

The NOAEL for general toxicity in maternal (F0) rats was 2 mg/kg/day due to a decrease in body weight and body weight gain at the next tested dose of 10 mg/kg/day. The NOAELs for maternal reproductive function and development of F1 offspring were higher than 10 mg/kg/day, since no changes were seen up to the highest tested dose of 10 mg/kg/day.

Safety evaluation

Toxicity	Species	NOAEL (mg/kg/d) M/F	Safety Margin Based on AUC*
General	Rat (6 mo)	0.03/0.03	none
	Dog (9 mo)	Not reached (less than 30 mg/kg/d)	Not determined
	Monkey (1 yr)	Not reached (less than 2 mg/kg/d)	Not determined
Carcinogenicity	Mouse	650 (M)/ less than 30 (F)	19x (M) Not determined (F)
	Rat	50 (M)/ 3 (F)	7.7x (M)/ 0.53 (F)
Reproductive & Developmental			
Fertility	Rat	150 (M)/ 0.1 (F)	24x (M)/ none (F)
Embryo/fetal development	Rat	25	2x**
	Rabbit	50	8x**
Pre- and postnatal development	Rat	> 10 (HD) (No effect at maternally toxic doses)	Not determined

*AUC in human: 686.6 ng hr/ml at 120 mg/day.
**On a mg/m2 basis (TK data not available)

12 Appendix/Attachments

12.1. Statistical Review and Evaluation of Carcinogencity Studies

12.2. Executive CAC Meeting Minutes

ATTACHMENT 1

STATISTICAL REVIEW AND EVALUATION OF CARCINOGENICITY STUDIES

Attachment 1 is a 37 page Duplicate Review with a Submitted date of December 2009, that can be found in the Statistical Review Section of this Approved NDA. Please refer to this section for this Review.

ATTACHMENT 2

EXECUTIVE CAC MEETING MINUTES

Attachment 2 is 4 pages of the Duplicate Executive CAC Meeting Minutes dated 7/13/10 that can be found in the Other Reviews Section of this Approved NDA. Please refer to this section for these Meeting Minutes.

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

/s/

SONIA A TABACOVA
10/19/2010

AISAR H ATRAKCHI
10/19/2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 200603 **Applicant: Sumimoto** **Stamp Date: 12/30/2009**

Drug Name: (b) (4) **(lurasidone HCl)** **NDA/BLA Type: NDA**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
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).			N.A.: The administration route 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 submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		During the pre-submission discussions regarding the further qualification of the process impurity (b) (4) (b) (4) a starting material for

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
				which there is a structural alert) the Division indicated (pre-NDA meeting of May 2009) that according to ICH Q3A guidance, "if the specification for this compound is below 0.15%, no further genetic toxicity testing is required in view of the negative Ames test". In communication with the CMC reviewer, Dr. Shastri Bhamdipati, this reviewer obtained the information that (b) (4) levels are below 0.15% (specifically, at or below (b) (4) in both drug substance and drug product – therefore no further genetic toxicity testing is required.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		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.)	X		See comment to point 8 above
11	Has the applicant addressed any abuse potential issues in the submission?	X		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			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.

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

None at this time

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

Sonia Tabacova, Ph.D.	March 1, 2010
Reviewing Pharmacologist	Date
Aisar Atrakchi, Ph.D.	
Team Leader/Supervisor	Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-200603	ORIG-1	DAINIPPON SUMITOMO PHARMA AMERICA INC	Lurasidone HCl

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

/s/

SONIA A TABACOVA
03/01/2010

AISAR H ATRAKCHI
03/02/2010