

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*  
**022549Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## SUPERVISORY MEMORANDUM

**NDA#** 22549  
**Drug:** Staccato® Loxapine  
**Sponsor:** Alexza Pharmaceuticals Inc.  
**Indication:** Rapid treatment of agitation associated with schizophrenia or bipolar disorder in adults.  
**Division:** Psychiatry Products  
**Reviewer:** Darren Fegley, Ph.D.  
**Team Leader:** Aisar Atrakchi, Ph.D.  
**Date:** September 14 2010

Dr. Fegley based his evaluation and conclusion on the nonclinical data submitted by Alexza and information found in the literature. He concluded that there are no nonclinical concerns that would affect approval of this drug-device application. I find his assessment adequate and concur with his conclusion.

Loxapine, the drug substance, is a dibenzoxazepine that has been approved for marketing by the FDA since 1975 (caps and tabs) and 1976 (oral concentrate), to treat schizophrenia (NDA# 17-525 and NDA# 17-658). An intramuscular injection was later approved in 1979 (NDA#18-039) but is no longer marketed in the US. Staccato loxapine is a single use drug-device product developed by Alexza and was submitted in August 2005 designed to deliver 5 or 10mg loxapine by oral inhalation. The sponsor submitted the NDA as a 505(b2) application with reference to nonclinical information submitted under the above mentioned marketed loxapine formulations for which Alexza has no right-of-reference. Alexza however conducted some nonclinical studies in support of this drug application, they included single and repeat dose toxicity / TK inhalation studies in rat and dog, cardiovascular and respiratory pharmacology study in the dog, and PK bridging study in dog. Genotoxicity studies were done with the parent drug loxapine, 8-hydroxy loxapine metabolite, and 2 loxapine aerosol impurities ( (b) (4) and (b) (4) )

Alexza proposes that this drug-device product can serve an unmet need for a rapid onset of drug effect through a non-invasive route of administration “oral inhalation”. The oral inhalation triggers heating up to 400C of the drug substance converting it to a vapor which is rapidly condensed into an aerosol particles that penetrates the lung reaching the systemic circulation hence a quick onset of drug effect to alleviate agitation in schizophrenic or depressed patients.

Dr. Fegley made his evaluation and conclusion based on results from nonclinical studies submitted by Alexza as well as information on loxapine from the published literature and SBA. Loxapine’s effects are due to its affinity and antagonism at the D1, D2 and 5HT2A

receptors, its metabolites amoxapine and 7-hydroxy loxapine are also pharmacologically active and contribute to the primary effect. Loxapine in a dose related manner inhibited hERG channel current at  $IC_{50}$  of 1.8 $\mu$ M. In a bridging study, Alexza studied the effects of loxapine in conscious dog following intravenous bolus injection and inhalation of loxapine aerosol and found comparable PK profile between the two routes. There were no persistent drug effects on the cardiovascular system following 1.5mg/kg i.v. dose in the dog and no effects on QT prolongation. Following daily nose-only inhalation for 14 days in the rat loxapine caused dose related CNS signs that were extension of the pharmacology of the drug, these effects (e.g. lethargy), may have indirectly caused the decreases in body weight and weight gain observed at higher doses. Mammary gland hyperplasia observed in both male and female rats and effects on ovaries (follicular cysts) and vagina (mucification) may be related to the dopamine receptor antagonism effect of the drug due to changes in prolactin however, hormone measurement was not done in support of this suggestion. Following 2 week recovery, the changes on mammary gland and vagina were still present though indication towards recovery was seen. Another finding was mild metaplasia of the epithelial cells at the base of the epiglottis observed in all drug treated rats. This finding is thought to be due to impaction of particles in the back of the throat as a result of the method of delivery i.e. inhalation, in animals. A correlation and relevance of this finding to the airway effects observed in COPD patients is unclear. Daily oral inhalation of loxapine aerosol in dogs caused CNS clinical signs likely to be extension of the pharmacology of the drug but no other effects on any of the measured parameters. Loxapine was negative in genetic toxicity assays conducted by Alexza and in the original loxapine NDA. Based on information from the SBA, loxapine did not cause tumors in the rat and was not teratogenic in the rat or rabbit.

Application  
Type/Number

Submission  
Type/Number

Submitter Name

Product Name

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NDA-22549

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ORIG-1

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ALEXZA  
PHARMACEUTICA  
LS INC

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Staccato (loxapine) for Oral  
Inhalation

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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AISAR H ATRAKCHI  
09/16/2010

**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: 022-549  
Supporting document/s: 1/0000/12/11/2009  
Applicant's letter date: 12/11/2009  
CDER stamp date: 12/11/2009  
Product: *Staccato*<sup>®</sup> Loxapine  
Indication: Rapid treatment of agitation associated with  
schizophrenia or bipolar disorder in adults  
Applicant: Alexza Pharmaceuticals Inc., Mountain View,  
CA  
Review Division: Division of Psychiatry Products  
Reviewer: Darren Fegley, Ph.D.  
Supervisor/Team Leader: Aisar Atrakchi, Ph.D.  
Division Director: Thomas Laughren, MD  
Project Manager: Kimberly Updegraff, MS

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## TABLE OF CONTENTS

<b>1</b>	<b>EXECUTIVE SUMMARY .....</b>	<b>3</b>
1.1	RECOMMENDATIONS.....	3
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS .....	3
<b>2</b>	<b>DRUG INFORMATION .....</b>	<b>6</b>
<b>3</b>	<b>STUDIES SUBMITTED.....</b>	<b>7</b>
<b>4</b>	<b>PHARMACOLOGY .....</b>	<b>9</b>
4.1	PRIMARY PHARMACOLOGY .....	9
4.2	SECONDARY PHARMACOLOGY .....	9
4.3	SAFETY PHARMACOLOGY .....	9
<b>5</b>	<b>PHARMACOKINETICS/ADME/TOXICOKINETICS .....</b>	<b>9</b>
5.1	PK/ADME.....	9
<b>6</b>	<b>GENERAL TOXICOLOGY.....</b>	<b>9</b>
6.1	SINGLE-DOSE TOXICITY .....	9
6.2	REPEAT-DOSE TOXICITY .....	10
<b>7</b>	<b>GENETIC TOXICOLOGY .....</b>	<b>16</b>
<b>8</b>	<b>CARCINOGENICITY .....</b>	<b>45</b>
<b>9</b>	<b>REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY .....</b>	<b>45</b>
<b>11</b>	<b>INTEGRATED SUMMARY AND SAFETY EVALUATION.....</b>	<b>45</b>

# 1 Executive Summary

## 1.1 Recommendations

From a Pharmacology/Toxicology perspective, there are no issues that would prevent or delay the approval of this NDA.

### 1.1.1 Approvability

Alexza Pharmaceuticals, Inc. has submitted this NDA under 505(b)(2), using the innovator Lederle labs, data in NDAs# 17-525, 17-658, and 18-039. From a Pharmacology/Toxicology perspective, the non-clinical studies that supported the approval of the innovator product in combination with published literature, and bridging studies submitted by Alexza are considered adequate to support the current submission. The following is a brief review and evaluation of the non-clinical studies that supported the approval of the innovator product and detailed review and evaluation of those studies conducted by Alexza for this application.

### 1.1.2 Additional Non-Clinical Recommendations: none

### 1.1.3 Labeling: Will not be addressed at this time.

## 1.2 Background

Loxapine, a dibenzoxazepine compound, has been marketed in the United States for the treatment of schizophrenia since 1975. The antipsychotic effects of loxapine are thought to be mediated through its antagonist activity at dopamine D<sub>2</sub> receptors. Lederle labs received approval for Loxitane® caps and tabs (NDA# 17-525, 1975) and for Loxitane® C oral concentrate (NDA# 17-658, 1976) for the treatment of schizophrenia at doses up to 250 mg/day (usual daily dose is 60-100 mg). An intramuscular (IM) formulation was approved for the treatment of acutely agitated patients (NDA# 18-039) in 1979, however this formulation is no longer marketed in the US.

Alexza Pharmaceuticals, Inc. has submitted the current NDA for *Staccato*® Loxapine, a single-use, hand-held, drug-device combination product designed to provide rapid systemic delivery by inhalation of loxapine at 5 and 10 mg, for the treatment of agitation associated with schizophrenia or bipolar disorder (5 and 10 mg strengths). The current NDA is submitted as a 505(b)(2) marketing application, referencing the non-clinical information in the approved loxapine NDAs (NDA 17-525 and NDA 18-039) for which Alexza does not have a right of reference, in addition to nonclinical studies conducted by Alexza.

Alexza Pharmaceutical, Inc. proposes that *Staccato*<sup>®</sup> Loxapine addresses the unmet need for rapid onset of effects, while affording patients and clinicians the benefits of reliable, non-invasive administration. Oral inhalation through the product initiates the controlled rapid heating (up to 400°C) of a thin film of excipient-free loxapine, resulting in a thermally generated drug vapor, which rapidly condenses into aerosol particles. These particles are of appropriate size for delivery to the deep lung where the drug is rapidly absorbed. The temperature of the mouthpiece of the inhaler remains relatively cool ( (b) (4) ) and, therefore, safe for the patient. The device is designed as a single-use unit and can not be reused once activated.

Non-clinical studies conducted by Alexza to support the safety of inhalation delivery of loxapine include single and repeat dose inhalation toxicology and toxicokinetic studies in rats and dogs, a cardiovascular and respiratory safety pharmacology study in dogs, pharmacokinetic studies in rats and dogs and *in vitro* metabolism studies. In addition, *in vitro* genotoxicity studies were carried out with loxapine, a loxapine metabolite (8-OH-loxapine), and two loxapine aerosol impurities ( (b) (4) ) and ( (b) (4) ).

### 1.3 Brief Discussion of Non-Clinical Findings

Alexza Pharmaceuticals, Inc. has submitted this NDA under 505(b)(2), referencing the innovator (Lederle labs, NDA# 17-525, 17-658, and 18-039). From a Pharmacology/Toxicology perspective, the non-clinical studies that supported the approval of the innovator product in combination with published literature, and bridging studies submitted by Alexza are considered adequate to support the current submission. The following is a review and evaluation of the non-clinical studies submitted by Alexza and a brief overview as appropriate, of the nonclinical information found in the SBA (summary basis of approval) of the innovator product.

The *in vivo* pharmacological activity of loxapine is primarily related to its affinity and antagonist activity at dopamine D<sub>1</sub> (K<sub>i</sub> = 18 nM) and D<sub>2</sub> (K<sub>i</sub> = 9.8 nM) receptors and the serotonin 5HT<sub>2a</sub> receptors (K<sub>i</sub> = 2 nM). The primary metabolites of loxapine in rat, dog, and human are amoxapine, 7-OH-loxapine, 8-OH-loxapine and loxapine n-oxide. Amoxapine is a pharmacologically active tricyclic antidepressant. 7-OH-loxapine is pharmacologically active at the D<sub>2</sub> receptor. 8-OH-loxapine and loxapine n-oxide are pharmacologically inactive at dopamine and serotonin receptors.

Loxapine dose-dependently blocked the hERG channel current with an IC<sub>50</sub> value of 1.8 μM. In a cardiovascular study in support of NDA# 17-525, IV doses up to 4 mg/kg in dogs, caused: transient hypotension, decreased arterial blood flow, increased cardiac contractility, and increased cardiac output. Alexza conducted an additional cardiovascular and respiratory safety study in conscious telemetered beagle dogs to evaluate the rapid delivery of loxapine following IV exposure. Bridging studies conducted by Alexza demonstrated that an IV bolus dose and inhalation exposure resulted in comparable PK profiles supporting the IV route in safety studies. IV doses of

0.15 and 0.5 mg/kg did not induce cardiovascular changes. The high dose of 1.5 mg/kg resulted in a transient decrease in blood pressure followed by a transient increase. No effect on ECG intervals attributable to loxapine, no QT or QTc prolongation, and no effect on respiratory parameters were observed

The acute toxicity of loxapine was investigated in the mouse, rat, guinea pig, and dog following oral, parenteral, and inhalation administration. Clinical signs included decreased motor activity, ataxia, rigidity (including catalepsy), altered sleep, inhibition of righting reflex, hypothermia, sedation, prostration, ptosis, relative lack of responsiveness to painful stimuli, tremors, clonic convulsions, opisthotonus, tetanus, and respiratory depression. Convulsions appeared 10-30 minutes post administration at doses of 60-80 mg/kg in mouse, 200-300 mg/kg in rat, 100-200 mg/kg in guinea pig, 25-50 mg/kg in rabbit and 50 mg/kg in cat and dog. Death due to respiratory paralysis frequently followed convulsions.

Repeated dose toxicity studies were conducted in mice, rats, and dogs. The primary clinical signs observed consisted of sedation, decreased motor activity, and catalepsy in all species, and seizure in rats and dogs. Decreased bodyweight and food consumption was observed across all species.

Repeat dose toxicity studies conducted by Alexza include a 14-day inhalation study in rats (for detail see section 6.2 of this review), and 5-day and 28-day inhalation studies in beagle dog conducted previously

Briefly, the administration of loxapine by daily nose-only inhalation for 14 days at doses of 1.7, 6.4, and 13 mg/kg caused dose-related CNS clinical signs consistent with the pharmacology of loxapine. Treated animals in the mid and high dose groups showed significant gender-specific decreases in mean body weight and weight gain compared to animals in the control group. These changes are likely secondary to the primary clinical sign of lethargy observed throughout this study. At the end of the treatment period squamous metaplasia of the larynx was observed, but was likely related to particle impaction due to the route of administration. Mammary hyperplasia in both sexes, and ovarian follicular cysts and mucification of vaginal epithelium in females were observed and are likely extension of pharmacology. Mammary hyperplasia in males was no longer seen and ovarian follicular cysts in females were comparable to those seen in control animals by the end of the 14-day recovery period. The incidences of the remaining reproductive tissue changes were markedly reduced, indicating ongoing recovery. The NOAEL was considered to be 1.7 mg/kg/day based on persistence of clinical signs and slow recovery from body weight changes seen in animals receiving 6.4 and 13 mg/kg/day. This dose corresponds to loxapine plasma AUC values of 55.9 and 91.6 ng•hr/ml in males and females, respectively.

In beagle dogs treatment related findings were similar following 5 or 28 days of dosing. Administration of loxapine by oral inhalation caused clinical signs consistent with the pharmacology of loxapine and included decreased activity, lying on side, weakness, tremors, and lack of coordination. The incidence and severity of these observations

were dose related and decreased with repeated exposure. There were no treatment related changes in body weights and no toxicological effects on food consumption. There were no treatment related changes in clinical pathology or immunoglobulins and no treatment-related ocular or cardiac changes. No treatment related findings in organ weight, gross or histopathology. In addition, there were no treatment related changes during the recovery phase. In the 28 day dog study, the NOAEL was considered to be 1.8 mg/kg/day. This dose corresponds to loxapine plasma AUC values of 467 and 577 ng•hr/ml in males and females, respectively.

The genotoxicity of loxapine was investigated in *in vitro* studies by Alexza and in *in vitro* and *in vivo* studies as reported in the published literature. Based on a weight of evidence approach loxapine is deemed to be non-mutagenic.

No new non-clinical carcinogenicity studies were conducted by Alexza. In a 19-month dietary study in rats in support of the original NDA, loxapine showed no evidence of carcinogenicity at a daily dose of up to 11.6 mg/kg.

No new non-clinical reproductive and developmental toxicity studies of loxapine were conducted by Alexza. The studies contained in the approved NDA showed no effects on male reproductive performance or sperm morphology in rats or rabbits. No teratogenesis was observed in rats or rabbits. Loxapine disrupted estrous cycling in females rats, which is consistent with the known neuroendocrine effects of neuroleptics. High doses of loxapine, which resulted in marked maternal toxicity, caused an increase in resorptions, a low rate of dystocia, and reduced fetal weights indicative of developmental delay. Early neonatal death was observed when treated rats were allowed to deliver litters.

## 2 Drug Information

### 2.1 Drug: Staccato loxapine®

#### 2.1.1 CAS Registry Number: 1977-10-2

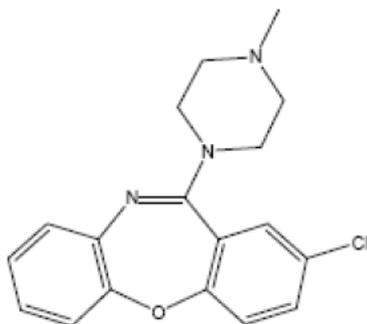
#### 2.1.2 Generic Name: oxilapine, loxitane

#### 2.1.3 Code Name: NA

#### 2.1.4 Chemical Name: 2-chloro-11-(4-methyl-piperazin-1-yl)-dibenzo[b,f][1,2]-oxazepine

**2.1.5 Molecular Formula/Molecular Weight:** C<sub>18</sub>H<sub>18</sub>ClN<sub>3</sub>O/328.81

**2.1.6 Structure**



**2.1.7 Pharmacologic class:** Antipsychotic

**2.2 Relevant IND/s, NDA/s, and DMF/s:** IND 073248, NDA 17-525, NDA 18-039

**2.3 Clinical Formulation:** A single-use, excipient-free, oral inhalation, drug-device combination product.

**2.3.1 Drug Formulation:** Aerosol for inhalation

**2.3.2 Comments on Novel Excipients:** NA

**2.3.3 Comments on Impurities/Degradants of Concern:** Impurities, degradants, and leachables were either qualified in non-clinical studies (i.e. (b) (4) and (b) (4) or deemed to be not of toxicological concern (i.e. (b) (4)

### 3 Studies Submitted

**3.1 Studies Reviewed:** All pivotal non-clinical studies submitted.

Pivotal non-clinical safety pharmacology and toxicology studies conducted by Alexza to support the NDA for *Staccato*<sup>®</sup> Loxapine.

Study Type/Duration	Species/ Strain	Method of Administration	Compound Administered	Study Number (CTD location)
Safety Pharmacology hERG Assay	hERG	In vitro	Loxapine succinate	D03.017/1 (m4.2.1.3)
	Dog/Beagle	Intravenous (bolus)	Loxapine succinate	ZCC00003 (m4.2.1.3)
Repeated Dose Toxicity 14 days	Rat/Sprague Dawley	Inhalation	Loxapine base	N106043 (m4.2.3.2)
	Dog/Beagle	Inhalation	Loxapine base	78670 (m4.2.3.2)
<b>Genotoxicity</b>				
<i>Active Pharmaceutical Ingredient</i>				
Microbial Mutagenesis Assay	<i>S. typhimurium</i> and <i>E. coli</i>	In vitro	(b) (4) loxapine base	AC14WV.503.BTL (m4.2.3.3.1)
Chromosomal Aberration Assay	Human peripheral blood lymphocytes	In vitro	(b) (4) loxapine base	AC14WV.341.BTL (m4.2.3.3.1)
Microbial Mutagenesis Assay	<i>S. typhimurium</i> and <i>E. coli</i> WP2uvrA	In vitro	(b) (4) loxapine base	AC29MU.503.BTL (m4.2.3.3.1)
Chromosomal Aberration Assay	Human peripheral blood lymphocytes	In vitro	(b) (4) loxapine base	AC29MU.341.BTL (m4.2.3.3.1)
<b>Metabolite</b>				
Microbial Mutagenesis Assay	<i>S. typhimurium</i> and <i>E. coli</i> WP2uvrA	In vitro	8-OH-loxapine	AC27XD.503.BTL (m4.2.3.3.1)
Chromosomal Aberration Assay	Human Peripheral Blood Lymphocytes	In vitro	8-OH-loxapine	AC27XD.341.BTL (m4.2.3.3.1)
<b>Impurities/Degradants/Leachables</b>				
Microbial Mutagenesis Assay	<i>S. typhimurium</i> and <i>E. coli</i> WP2uvrA	In vitro	(b) (4)	AC29GH.503.BTL (m4.2.3.3.1)
Chromosomal Aberration Assay	Human Peripheral Blood Lymphocytes	In vitro	(b) (4)	AC29GH.341.BTL (m4.2.3.3.1)
Microbial Mutagenesis Assay	<i>S. typhimurium</i> and <i>E. coli</i> WP2uvrA	In vitro	(b) (4)	963035 (m4.2.3.3.1)

**3.2 Studies Not Reviewed:** None.

**3.3 Previous Reviews Referenced:** SBA for NDA# 17-525, and NDA# 18-039

## 4 Pharmacology

### 4.1 Primary Pharmacology

The following is excerpted from Alexza's submission in support of the current NDA.

Table 3. Affinity of loxapine for neurotransmitter receptors ( $K_i$ , nM)

Receptor	Loxapine
D <sub>1</sub>	18
D <sub>2</sub>	9.8
5-HT <sub>2A</sub>	2
$\alpha_2$	250
M <sub>1</sub>	117

Source: [Natesan et al, 2005](#)  $K_i$ : inhibition constant

### 4.2 Secondary Pharmacology

See SBA reviews for approved NDAs.

### 4.3 Safety Pharmacology

See SBA reviews for approved NDAs.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

See SBA reviews for approved NDAs.

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

See SBA reviews for approved NDAs.

## 6.2 Repeat-Dose Toxicity

**Study title: Exploratory acute and 5 day repeat tox study of inhaled aerosol formulation of loxapine in beagle dogs (Study # N106043)**

### Key Study Findings:

No mortalities were observed at doses up to 2.3 mg/kg/day. The following clinical signs were observed after single doses of 2.0 and 2.7 mg/kg/day: reduced activity, the dog was lying on side, slight to moderate tremors, and/or weakness.. In addition to the above signs, lack of coordination and limited use of hind limbs were seen in drug groups following repeated dosing. These signs occurred immediately post dose but decreased by 9 hours post dose with complete recovery by 24 hours post dose except for “weakness” in HD dogs that was still present at 24 hours post dose. Body weight gain was slightly reduced together with slight decrease in food intake at 2.3 mg/kg/day. There were no gross or microscopic findings up to 2.3 mg/kg/day. The NOAEL in this is the high dose of 2.3 mg/kg/day.

**Study title: 28d tox/TK study with 14d recovery period of inhaled aerosol formulation of loxapine in beagle dogs.**

### Key Study Findings:

Dose related clinical signs were observed in all 3 drug groups and were likely an extension of the pharmacology of loxapine. These signs included hypoactivity and lack of coordination, tremors, lying on side, and weakness. The severity and duration of these signs decreased with repeated dosing. There were no other drug related effects on any of the measured parameters. TK parameters increased generally non-proportionally to increase in dose and values seemed to decrease with repeated dosing though accurate conclusion could not be made due to large variability of the data. Based on the minimal findings in this study, the NOAEL was considered to be 1.8 mg/kg/day. This dose corresponds to loxapine plasma AUC values of 467 and 577 ng•hr/ml in males and females, respectively.

**Study title: An escalating single dose and 14-day inhalation toxicity study of loxapine in Sprague-Dawley rats with a 14-day recovery period.**

Study no.:	N106043
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/06/2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	M0545, 100%

### Key Study Findings

The administration of loxapine by daily nose-only inhalation for 14 days at doses of 1.7, 6.4, and 13 mg/kg caused dose-related CNS clinical signs consistent with the pharmacology of loxapine. Treated animals in the mid and high dose groups showed significant gender-specific decreases in mean body weight and weight gain patterns compared to animals in the control group. These changes are likely secondary to the primary clinical sign of lethargy observed throughout this study.

At the end of the treatment period squamous metaplasia of the larynx was observed, but was likely related to particle impaction due to the route of administration; of note, while adverse pulmonary effects were observed in human subjects with asthma or COPD, a direct correlation and relevance to clinical safety can not be made. Mammary hyperplasia in both sexes, and ovarian follicular cysts and mucification of vaginal epithelium in females were observed and are likely an extension of pharmacology. Mammary hyperplasia in males was no longer seen and ovarian follicular cysts in females were comparable to those seen in control animals by the end of the 14 recovery period. The incidences of the remaining reproductive tissue changes were markedly reduced, indicating ongoing recovery by Day 29 of the study.

The NOAEL was considered to be 1.7 mg/kg/day based on persistence of clinical signs and slow recovery from body weight changes seen in animals receiving 6.4 and 13 mg/kg/day. This dose corresponds to Day 14 loxapine plasma AUC values of 55.9 and 91.6 ng•hr/ml in males and females, respectively.

#### Methods

Doses:	1.7, 6.4, 13 mg/kg/day
Frequency of dosing:	SID for 14 days
Route of administration:	Inhalation, 10 min
Dose volume/Formulation:	Loxapine was provided coated on stainless steel rolls
Vehicle:	Stainless steel roll with no drug
Species/Strain:	Rat/Sprague-Dawley Crl:CD (SD)
Number/Sex/Group:	10/sex/group
Age:	8-9 weeks
Weight:	172 to 300 g
Satellite groups:	An additional 5 animals/sex were allowed to recover for an additional 14 days to assess reversibility of drug related effects

Dose selection justification: The doses in this study were based on the results obtained from dose escalation study in which rats received 6.7-24 mg/kg. Rats were lethargic at all doses within 1 hr through the following morning. Clenched forelimb and tremors were observed in rats receiving 23 and 24 mg/kg/day. Based on these clinical signs the MTD was estimated to be 16 mg/kg. This was set as the target high dose for the repeat dose phase. Due to marked clinical observations and body weight loss in the high dose group during the current study, the exposure was lowered starting on day 2 for females and on day 3 for males.

## Observations and Results

### Mortality

No treatment related mortalities were observed during the course of the study.

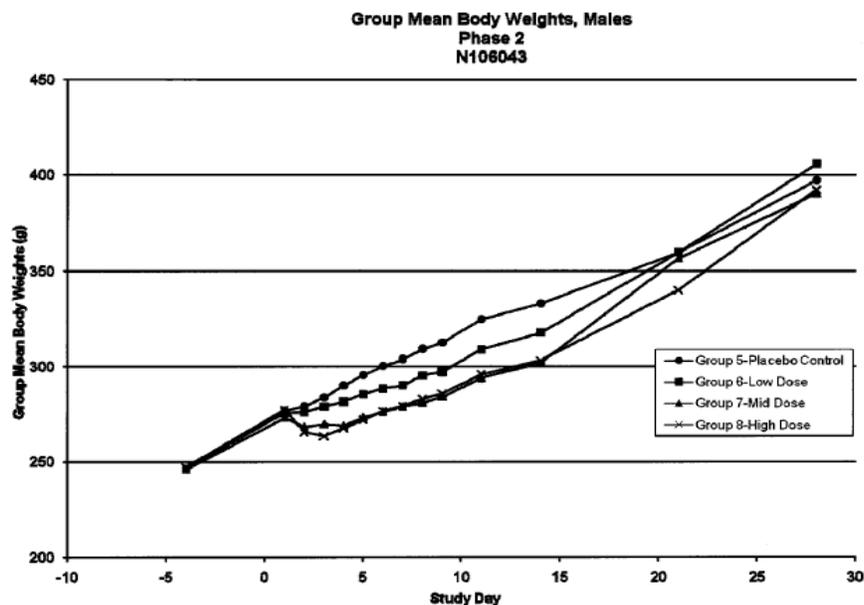
### Clinical Signs

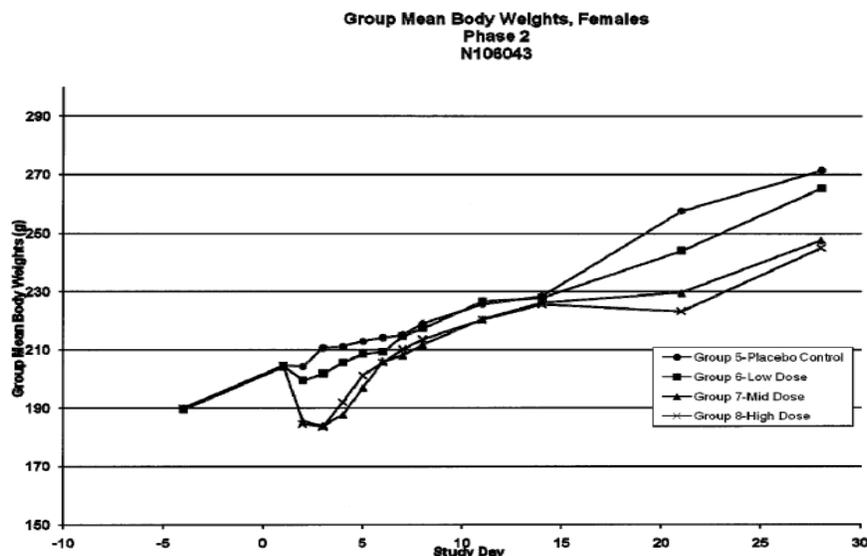
Treatment related CNS clinical signs noted were consistent with the known pharmacological activity of loxapine. All drug treated rats exhibited lethargy on all treatment days generally within 0.5-1 hr following exposure. On days 1-8 lethargy persisted until the following morning. Tremors were sporadically observed in low dose males and high dose males and females. Clenched forelimbs were observed in all drug treated groups. All clinical signs generally decreased in incidence and persistence with repeated exposures, and did not occur during the post treatment recovery period.

A check of the condition and behavior of all animals in main and recovery groups was made daily pre-dose and at 10 min, 30 min, 1 hr, 2 hr, 3 hr, and 5 hr after the end of exposure.

### Body Weights

Statistically significant body weight reduction was observed at the mid and high dose groups for both males (5% and 7%, respectively) and females (13% for both doses) as compared to controls. These bodyweight decreases were likely the result of the extended duration of lethargy that was observed following drug exposure leading to a probably decrease in food consumption (Food consumption was not measured).





Animals were weighed once at randomization, and prior to exposure on days 1, 2, 3, 4, 5, 6, 7, 8, 9 (males only), 11, 14; and during the recovery period on days 21 and 28.

### Feed Consumption

Not performed due to the addition of wet/moist food.

### Ophthalmoscopy

No treatment related ophthalmoscopic findings were observed in this study. The eyes of animals were examined, with an ophthalmoscope after application of a tropicamide solution, once prior to initiation of dosing, and on the main group and recovery animals on day 11 (males) or day 10 (females).

### ECG

Not performed

### Hematology

No treatment related abnormalities in the measured hematology parameters were noted in this study. Blood was collected from overnight fasted animals. Blood samples were collected on days 15 or 29 from the retro-orbital sinus for hematology and clinical chemistry, and via the posterior vena cava for coagulation parameters. Rats were anaesthetized with carbon dioxide/oxygen. A full battery of hematology and coagulation parameters were assessed.

## **Clinical Chemistry**

No treatment related abnormalities in the measured clinical chemistry parameters were noted in this study. Blood was collected from overnight fasted animals. Blood samples were collected on days 15 or 29 from the retro-orbital sinus for hematology and clinical chemistry, and via the posterior vena cava for coagulation parameters. Rats were anaesthetized with carbon dioxide/oxygen. A full battery of clinical chemistry parameters was assessed.

## **Urinalysis**

Decreased urine volume and increased specific gravity were observed in all drug treated groups. This was likely a secondary finding, due to the prolonged decrease in activity and a subsequent lack of water consumption observed in these rats. These changes were not observed following the 14 day recovery period. Urine was collected overnight prior to scheduled necropsy using metabolism cages on days 15 or 29.

## **Gross Pathology**

No treatment related gross lesions were noted in this study. Main study animals were necropsied on day 15, one day after final dose was given. Recovery animals were killed at the end of the recovery period. The animals were killed by exsanguination from the common carotid artery following carbon dioxide exposure.

## **Organ Weights**

All observed organ weight changes were relative to body weight. No absolute organ weight changes were noted. This paired with the lack of any histopathology correlates suggests that the changes were due to treatment related effects on bodyweight and not representative of organ-specific toxicity.

Adrenal glands (paired), brain, heart, kidneys (paired), liver, lungs, ovaries (paired), pituitary gland, prostate gland, spleen, testes (paired), thymus, thyroid, and uterus were weighed.

## **Histopathology**

The battery was adequate and a peer review was performed.

Treatment related changes were seen in the larynx, mammary glands, ovaries, and vagina. In the larynx of all drug treated rats, there was minimal squamous metaplasia of epithelium overlying the glands at the base of the epiglottis. This lesion is likely a non-specific change due to the impaction of particles in the back of the throat caused by the delivery method that is sometimes observed in inhalation studies in animals. However, a

correlation and relevance of this finding to the observed adverse effects on airways in asthma and COPD patients can not be made.

Minimal to mild mammary hyperplasia was seen in all drug treated females. Three high dose males and one mid dose male also displayed mammary hyperplasia. The ovaries of most treated females had small follicular cysts. The vaginal epithelium of most treated females was covered by mucous cells overlying squamous epithelium and was graded as minimal to mild mucification. This change resembled that typically observed in proestrus, however, the cornification present between the mucous and squamous layer normally present in proestrus was not present, and vaginal appearance did not correspond to the stage of cycle based upon the ovaries and uterus. These mammary and reproductive tract changes are likely due to action at the D2 receptor and subsequent alterations in prolactin, estrogen, and progesterone levels.

Following recovery, drug related changes were still observed in the mammary glands and vaginal epithelium of females and in the larynx of males, although there was a trend toward recovery in all instances.

### **Toxicokinetics**

$C_{max}$  was achieved 1 min post dose (the first time point taken). The high clearance values and high levels of metabolites formed indicate that loxapine was rapidly absorbed and metabolized into 7-OH loxapine, amoxapine, and loxapine N-oxide, with no 8-OH loxapine levels detected.

AUC parameters for amoxapine could not be reliably calculated. The  $C_{max}$  for amoxapine occurred between 0.5 and 1 hr post dose, with exposures higher in females than in males.

The  $C_{max}$  for 7-OH loxapine occurred between 0.5 and 1 hr post dose for males and between 0.5 and 4 hrs in females. 7-OH loxapine exposure was markedly higher in females compared to males throughout the dose range.

The  $C_{max}$  for loxapine N-oxide occurred 1 min post dose indicating that loxapine was rapidly absorbed and metabolized into loxapine N-oxide. On day 1 the  $C_{max}$  value was higher in females than in males in the low and mid dose groups. No marked difference was observed in the high dose group.

## Loxapine TK Exposure Parameters in Rats, Days 1 and 14

Sex /Dose <sup>a</sup>	Low dose	Mid dose	High dose	Low dose	Mid dose	High dose
Day 1						
C <sub>max</sub> (ng/mL)			AUC <sub>last</sub> (ng-hr/mL)			
<b>Loxapine</b>						
M	82.9	245	406	55.9	207	519
F	91.5	338	507	61.3	265	588
<b>Amoxapine</b>						
M	b	2.33	4.56	b	c	c
F	b	6.56	8.75	b	c	c
<b>7-OH-loxapine</b>						
M	11.4	21.4	30.1	44.5	117	340
F	21.3	54.1	121	234	847	1820
<b>Loxapine N-oxide</b>						
M	10.1	17.1	27.9	4.45	19.0	31.2
F	13.6	28.7	29.8	5.38	28.7	48.5
Day 14						
<b>Loxapine</b>						
M	72.7	252	437	55.9	209	354
F	118	262	860	91.6	269	543
<b>Amoxapine</b>						
M	b	4.04	7.80	b	c	28.6
F	4.15	12.1	15.0	c	c	62.4
<b>7-OH-loxapine</b>						
M	9.68	21.4	48.5	43.4	165	400
F	61.4	112	186	647	1360	2560
<b>Loxapine N-oxide</b>						
M	10.5	30.4	39.6	4.66	29.8	44.5
F	18.8	39.9	55.4	8.87	37.4	56.2

## 7 Genetic Toxicology

See original loxapine SBA review for details. Loxapine was tested in Salmonella typhimurium bacterial mutation assay up to 1000ug/plate in -/+S9 and in vivo mouse MN at 10, 20, or 40mg/kg/ day loxapine in DMSO; negative in both assays.

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

#### Study title: Evaluation of Loxapine in DMSO in the Bacterial Reverse Mutation Assay.

Study no.:	AC14WV.503.BTL
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	06/05/2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	60096-07-002, 100%

#### Key Study Findings

Loxapine free base was used in this study. Alexza concluded loxapine free base is negative, while this reviewer considered it mutagenic in *S. typhimurium* TA98 under the conditions used in this study in the presence of metabolic activation.

#### Methods

Strains: *Salmonella typhimurium*: TA98, TA100, TA1535, TA1537  
*Escherichia coli*: WP2 *uvrA*

Concentrations used in definitive study: Loxapine free base was used for these assays.  
Initial assay: 1.5, 5.0, 15, 50, 150, 500, 1500, 5000 µg/plate  
Confirmatory assay: 15, 50, 150, 500, 1500, 5000 µg/plate  
Follow up confirmatory assay: 15, 50, 150, 250, 500, 750, 1250, 1500, 5000 µg/plate

Basis of concentration selection: The highest dose of 5000 µg/plate was set in accordance with current guidelines and lower doses were set in a common ratio of 3. Toxicity was observed beginning between 500 and 5000 µg/plate with all strains in both the presence and absence of metabolic activation. The doses used in these assays were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All <i>Salmonella</i> Strains	Rat	2-aminoanthracene (b) (4)	1.0
WP2 <i>uvrA</i>		Lot No. 12317CE Exp. Date 01-Feb-2009 CAS No. (b) (4) Purity 99.9%	10

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98	None	2-nitrofluorene (b) (4) Lot No. 03926DC Exp. Date 18-Aug-2010 CAS No. (b) (4) Purity 98.1%	1.0
TA100, TA1535		sodium azide (b) (4) Lot No. 073K0119 Exp. Dates 31-Jul-2008 CAS No. (b) (4) Purity 99.9%	1.0
TA1537		9-aminoacridine (b) (4) Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. (b) (4) Purity >97%	75
WP2 <i>uvrA</i>		methyl methanesulfonate (b) (4) Lot No. 126K3721 Exp. Date 07-Jan-2011 CAS No. (b) (4) Purity 99.9%	1,000

**Incubation and sampling times:** The test system was exposed to loxapine free base via the plate incorporation method. Plates were incubated with solvent control, positive control or Loxapine free base for 48-72 hrs.

**Study validity:** Plates were run in triplicate for the main assays. Loxapine free base was considered to be mutagenic if it caused a dose-related increase in the mean revertants per plate of at least one strain over a minimum of two increasing concentrations of test article. Tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3 times the mean vehicle control value. Tester strains TA98, TA100, and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2 times the mean vehicle control value. The positive controls all fell within the historical control range data provided. The

negative controls in some of the test were at or below the minimum acceptable value and were therefore retested.

**Results:** A 3-fold increase in revertants was observed with TA98 in the presence of metabolic activation. Alexza did not consider this response mutagenic however as there was no dose response, the maximum increase in colonies was within the historical vehicle control range, and the vehicle control value was at the minimum acceptable value. In a confirmatory assay a 3.5-fold increase in revertants was observed with tester strain TA98 in the presence of metabolic activation. In a retest of the confirmatory assay no mutagenic response was observed with TA98 in the presence of metabolic activation. This reviewer does not agree with Alexza that the response is not mutagenic. While the vehicle control was low for the first assay, it was well within the historical control range in the second confirmatory assay, in which a 3.5-fold increase was observed. Moreover, a doubling or close to a doubling of revertants was observed at concentrations greater than or equal to 5 µg suggesting a dose-response effect. In the confirmatory assay a dose response was observed as well, although no other concentrations resulted in a doubling of mean revertants. Therefore, this reviewer deems loxapine free base mutagenic in *S. typhimurium* strain TA98 following metabolic activation under the conditions present in this assay.

**Report title: Bacterial Reverse Mutation Assay of Loxapine in DMSO**

**Test Article:** Loxapine Free Base

**Test for Induction of:** Reverse mutation in bacterial cells  
**Strains:** *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2uvrA

**No. of Independent Assays:** 2

**Study No.** AC14WV.503.BTL

**No. of Replicate Cultures:** 2 for toxicity-mutagenicity assay, 3 for confirmatory assay

**Metabolizing System:** Aroclor 1254-induced rat liver (10% S9)

**Vehicle:** For Test Article and For Positive Controls: Dimethyl sulfoxide (DMSO)

**GLP Compliance:** Yes

**Treatment:** Plate incorporation

**Dates of Treatment:**

**Cytotoxic Effects:** beginning at 500 to 5000 µg per plate

05Jun2008, 19Jun2008, 29Jul2008

**Genotoxic Effects:** None

**Toxicity-Mutation Assay**

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100 <sup>a</sup>	TA1535	TA1537	WP2uvrA
Without Activation	Vehicle (DMSO)	0	15 ± 3	123 ± 14	14 ± 1	5 ± 4	18 ± 3
		2.2	21 ± 16	90 ± 2	18 ± 4	5 ± 0	9 ± 0
		5.0	27 ± 13	94 ± 8	15 ± 4	6 ± 0	15 ± 7
		15	12 ± 9	118 ± 1	16 ± 4	6 ± 4	17 ± 18
		50	12 ± 1	136 ± 22	20 ± 3	4 ± 1	25 ± 1
		150	15 ± 1	125 ± 1	12 ± 1	9 ± 1	8 ± 11
		500	18 ± 4	0 ± 0 <sup>b</sup>	12 ± 3	8 ± 1	15 ± 1
		1500	5 ± 6	0 ± 0 <sup>b</sup>			
		5000	0 ± 0 <sup>b</sup>	0 ± 0 <sup>b</sup>	0 ± 0 <sup>b</sup>	0 ± 0 <sup>b</sup>	0 ± 0 <sup>b</sup>
		Positive Controls	2-nitrofluorene	1.0	203 ± 18		
Sodium Azide	1.0			569 ± 13	207 ± 65		
9-aminoacridine	75					1203 ± 64	
Methyl methanesulfonate	1000						205 ± 1

**Footnotes:**

<sup>a</sup>Background lawn moderately reduced to absent

<sup>b</sup>Precipitate present

<sup>c</sup>Nominal low dose for this strain was 1.5 µg/plate

## Study No. AC14WV.503.BTL (cont'd)

## Test Article: Loxapine Free Base

## Toxicity-Mutagenicity Assay Continued

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100 <sup>f</sup>	TA1535	TA1537	WP2uvrA
With Activation	Vehicle (DMSO)	0	10 ± 4	122 ± 3	10 ± 3	7 ± 1	18 ± 1
	Loxapine Free Base	2.2	17 ± 2	92 ± 8	11 ± 8	9 ± 1	22 ± 2
		5.0	20 ± 14	95 ± 8	9 ± 1	6 ± 2	25 ± 1
		15	18 ± 1	123 ± 6	5 ± 5	10 ± 5	27 ± 2
		50	27 ± 1	110 ± 1	4 ± NA	7 ± 2	24 ± 0
		150	30 ± 4 <sup>*</sup>	108 ± 5	5 ± 1	9 ± 1	19 ± 0
		500	22 ± 6	92 ± 4	9 ± 1	6 ± 3	19 ± 1
		1500	9 ± 6 <sup>a</sup>	0 ± 0 <sup>a</sup>	4 ± 6	0 ± 0 <sup>a</sup>	9 ± 1 <sup>a</sup>
		5000	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
	Positive Controls	2-aminoanthracene	1.0	351 ± 40	434 ± 161	82 ± 28	50 ± 3
	2-aminoanthracene	10					

## Footnotes:

<sup>a</sup>Background lawn moderately reduced to absent<sup>b</sup>Precipitate present<sup>f</sup>Nominal low dose for this strain was 1.5 µg/plate<sup>\*</sup> 3.0-fold increase in revertants seen with TA98 with S9 activation; since there was no dose response, maximum increase in revertant colonies was within normal vehicle control range for this strain, and vehicle control value was at the minimum acceptable value, this was not considered indicative of mutagenic activity

NA: Not applicable. The second plate was not counted due to a procedural error

## Study No. AC14WV.503.BTL (cont'd)

## Test Article: Loxapine Free Base

## Confirmatory Mutagenicity Assay

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	Vehicle (DMSO)	0	11 ± 2	144 ± 2	24 ± 5	11 ± 3	19 ± 2
	Loxapine Free Base	15	14 ± 7	130 ± 6	23 ± 3	11 ± 1	19 ± 1
		50	13 ± 3	123 ± 12	19 ± 8	7 ± 3	15 ± 5
		150	16 ± 7	134 ± 12	22 ± 5	7 ± 2	20 ± 1
		500	11 ± 7	89 ± 26 <sup>a</sup>	12 ± 1 <sup>a</sup>	9 ± 4 <sup>a</sup>	17 ± 2
		1500	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	4 ± 3 <sup>a</sup>
		5000	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>
Positive Controls	2-nitrofluorene	1.0	120 ± 14				
	Sodium Azide	1.0		547 ± 8	419 ± 10		
	9-aminoacridine	75				1608 ± 190	
	Methyl methanesulfonate	1000					240 ± 13
With Activation	Vehicle (DMSO)	0	18 ± 3	148 ± 16	17 ± 4	13 ± 5	33 ± 3
	Loxapine Free Base	15	21 ± 5	152 ± 6	17 ± 2	12 ± 3	22 ± 7
		50	25 ± 5	143 ± 12	20 ± 4	9 ± 4	21 ± 7
		150	33 ± 4	136 ± 26	14 ± 2	10 ± 3	23 ± 7
		500	63 ± 23 <sup>*</sup>	156 ± 13	8 ± 0	12 ± 2	18 ± 7
		1500	20 ± 7 <sup>a</sup>	0 ± 0 <sup>a</sup>	5 ± 2 <sup>a</sup>	2 ± 2 <sup>a</sup>	5 ± 3
		5000	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>
Positive Controls	2-aminoanthracene	1.0	1031 ± 819	947 ± 570	153 ± 14	118 ± 15	331 ± 80
	2-aminoanthracene	10					

## Footnotes:

<sup>a</sup>Background lawn moderately reduced to absent<sup>b</sup>Precipitate present<sup>\*</sup> A 3.5-fold increase observed with tester strain TA98 in the presence of S9 activation required retesting to clarify response

**Confirmatory Mutagenicity Assay—Retest of TA98 with Activation**

Metabolic Activation	Test Article	Concentration ( $\mu\text{g}/\text{plate}$ )	Mean $\pm$ SD Revertants per Plate	
				TA98
With Activation	Vehicle (DMSO) Loxapine Free Base	0		18 $\pm$ 2
		15		18 $\pm$ 2
		50		19 $\pm$ 0
		150		16 $\pm$ 3
		250		17 $\pm$ 2
		500		18 $\pm$ 2
		750		19 $\pm$ 1
		1250		10 $\pm$ 1 <sup>a</sup>
		1500		9 $\pm$ 1 <sup>a</sup>
		5000		0 $\pm$ 0 <sup>a,b</sup>
Positive Controls	2-aminoanthracene	1.0		509 $\pm$ 49

**Footnotes:**<sup>a</sup>Background lawn moderately reduced to absent<sup>b</sup>Precipitate present

**Study title: Evaluation of (b) (4) loxapine base in the Bacterial Reverse Mutation Assay.**

Study no.: AC29MU.503.BTL

Study report location:

Conducting laboratory and location: (b) (4)

Date of study initiation: 07/02/2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: A-080160, 99.9%

**Key Study Findings**

Loxapine free base was used in this study. Loxapine free base is deemed to be nonmutagenic under the conditions used in this study.

**Methods**

**Strains:** *Salmonella typhimurium*: TA98, TA100, TA1535, TA1537  
*Escherichia coli*: WP2 *uvrA*

**Concentrations used in definitive study:** Loxapine free base was used for these assays.  
Initial assay: 1.5, 5.0, 15, 50, 150, 500, 1500, 5000  $\mu\text{g}/\text{plate}$   
Confirmatory assay: *Salmonella strains*; 5, 15, 50, 150, 500, 1500  $\mu\text{g}/\text{plate}$   
*E. coli*: WP2 *uvrA*; 15, 50, 150, 500, 1500, 5000  $\mu\text{g}/\text{plate}$

**Basis of concentration selection:** The highest dose of 5000  $\mu\text{g}/\text{plate}$  was set in accordance with current guidelines and lower doses were set in a common ratio of 3.

Toxicity was observed beginning between 500 and 5000 µg/plate with all strains in both the presence and absence of metabolic activation. The doses used in these assays were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535, TA1537	Rat	2-aminoanthracene (b) (4)	1.0
TA100			2.0
WP2 <i>uvrA</i>		Lot No. 03403ED Exp. Date 22-Jan-2012 CAS No. (b) (4) Purity 99.8%	10
TA98	None	2-nitrofluorene (b) (4) Lot No. 03319JD Exp. Date 28-Feb-2011 CAS No. (b) (4) Purity 98.1%	1.0
TA100, TA1535		sodium azide (b) (4) Lot No. G24R025 Exp. Date 10-Feb-2010 CAS No. (b) (4) Purity 99% min.	1.0
TA1537		9-aminoacridine (b) (4) Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. (b) (4) Purity >97%	75
WP2 <i>uvrA</i>		methyl methanesulfonate (b) (4) Lot No. 06823KH Exp. Date 04-Jun-2011 CAS No. (b) (4) Purity 99.9%	1,000

Incubation and sampling times: The test system was exposed to loxapine free base via the plate incorporation methodology. Plates were incubated with solvent control, positive control or Loxapine free base for 48-72 hrs.

Study validity: Plates were run in triplicate for the main assays. Loxapine free base was considered to be mutagenic if it caused a dose-related increase in the mean revertants per plate of at least one strain over a minimum of two increasing concentrations of test article. Tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3 times the mean vehicle control value. Tester strains TA98, TA100, and

WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2 times the mean vehicle control value. The positive and negative controls all fell within the historical control range data provided.

**Results:** In the initial mutagenicity assay, the maximum dose concentration of loxapine evaluated was 5000 µg per plate. Precipitate was observed at 5000 µg per plate with all strains in the presence of rat S9 and with TA1535 and TA1537 in the absence of rat S9. Toxicity was observed beginning at 500 or 1500 µg per plate with all strains in the presence and absence of rat S9. No positive mutagenic responses were observed with loxapine in any of the tester strains in either the presence or absence of rat S9 activation.

In the confirmatory mutagenicity assay, the maximum concentration of loxapine evaluated was 5000 µg per plate for WP2 *uvrA* and 1500 µg per plate for all *Salmonella* strains. No precipitate was observed. Toxicity was observed beginning at 500, 1500 or at 5000 µg per plate with all strains in the presence and absence of rat S9. No positive mutagenic responses were observed with loxapine in any of the tester strains in either the presence or absence of rat S9 activation.

Report title: Evaluation of (b) (4) Loxapine Base in the Bacterial Reverse Mutation Assay

Test Article: Loxapine Base

Test for Induction of: Reverse mutation in bacterial cells  
Strains: *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2*uvrA*

No. of Independent Assays: 2

Study No. AC29MU.503.BTL

No. of Replicate Cultures: 2 for toxicity-mutagenicity assay, 3 for confirmatory assay

Metabolizing System: Aroclor 1254-induced rat liver (10% S9)

Vehicle: For Test Article and For Positive Controls except Sodium Azide: Dimethyl

GLP Compliance: Yes

sulfoxide (DMSO)

For Sodium Azide: Water

Treatment: Plate incorporation

Dates of Treatment: 08 July 2009, 22 July 2009

Cytotoxic Effects: beginning at 500 to 5000 µg per plate

Genotoxic Effects: None

#### Toxicity-Mutation Assay

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
Without Activation	Vehicle (DMSO)	0	19 ± 0	124 ± 14	16 ± 4	7 ± 2	29 ± 5
	Loxapine Base	1.5	24 ± 1	126 ± 16	14 ± 4	8 ± 4	33 ± 1
		5.0	25 ± 6	104 ± 13	9 ± 4	4 ± 1	25 ± 2
		15	23 ± 15	105 ± 0	13 ± 1	5 ± 4	26 ± 7
		50	23 ± 5	120 ± 8	14 ± 2	7 ± 3	32 ± 11
		150	20 ± 4	116 ± 20	13 ± 6	7 ± 4	28 ± 0
		500	12 ± 4 <sup>a</sup>	0 ± 0 <sup>a</sup>	10 ± 1 <sup>a</sup>	7 ± 3 <sup>a</sup>	40 ± 5
		1500	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	12 ± 6 <sup>a</sup>
5000	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>		
Positive Controls	2-nitrofluorene	1.0	273 ± 0				
	Sodium Azide	1.0		686 ± 32	527 ± 45		
	9-aminoacridine	75				1028 ± 187	
	Methyl methanesulfonate	1000					532 ± 29

#### Footnotes:

<sup>a</sup>Background lawn moderately reduced to absent

<sup>b</sup>Precipitate

## Toxicity-Mutagenicity Assay Continued

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2uvrA
With Activation	Vehicle (DMSO)	0	29 ± 1	127 ± 3	12 ± 2	8 ± 1	46 ± 4
	Loxapine Base	1.5	32 ± 9	139 ± 3	14 ± 1	8 ± 1	46 ± 6
		5.0	23 ± 3	134 ± 7	12 ± 4	8 ± 3	40 ± 21
		15	31 ± 2	130 ± 9	10 ± 3	11 ± 2	45 ± 1
		50	35 ± 0	121 ± 5	16 ± 3	6 ± 2	37 ± 1
		150	34 ± 5	119 ± 13	14 ± 2	3 ± 2	31 ± 8
		500	29 ± 2	120 ± 2 <sup>a</sup>	11 ± 0 <sup>a</sup>	8 ± 3 <sup>a</sup>	31 ± 15
		1500	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	17 ± 6 <sup>a</sup>
5000	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>	0 ± 0 <sup>ab</sup>		
Positive Controls	2-aminoanthracene	1.0	741 ± 114		120 ± 7	102 ± 25	
	2-aminoanthracene	2.0		1552 ± 61			
	2-aminoanthracene	10					494 ± 42

## Footnotes:

<sup>a</sup>Background lawn moderately reduced to absent<sup>b</sup>Precipitate

## Confirmatory Mutagenicity Assay

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	Vehicle (DMSO)	0	27 ± 3	96 ± 15	16 ± 4	6 ± 2	34 ± 4
	Loxapine Base	5.0	23 ± 4	112 ± 2	8 ± 3	7 ± 2	
		15	22 ± 2	101 ± 9	10 ± 4	6 ± 3	33 ± 7
		50	19 ± 4	89 ± 21	10 ± 1	7 ± 2	30 ± 12
		150	22 ± 7	79 ± 6	10 ± 4	7 ± 2	34 ± 1
		500	14 ± 3 <sup>a</sup>	16 ± 7 <sup>a</sup>	9 ± 3 <sup>a</sup>	4 ± 4 <sup>a</sup>	34 ± 9 <sup>a</sup>
		1500	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	4 ± 7 <sup>a</sup>
		5000					0 ± 0 <sup>a</sup>
Positive Controls	2-nitrofluorene	1.0	184 ± 15				
	Sodium Azide	1.0		672 ± 57	460 ± 38		
	9-aminoacridine	75				1004 ± 92	
	Methyl methanesulfonate	1000					404 ± 64
With Activation	Vehicle (DMSO)	0	29 ± 1	102 ± 16	10 ± 4	7 ± 3	29 ± 3
	Loxapine Base	5.0	30 ± 10	110 ± 23	10 ± 1	7 ± 5	
		15	28 ± 5	95 ± 9	9 ± 2	9 ± 2	31 ± 4
		50	33 ± 1	116 ± 6	9 ± 4	8 ± 3	36 ± 3
		150	35 ± 9	83 ± 13	11 ± 3	6 ± 2	37 ± 5
		500	29 ± 7	66 ± 14	11 ± 2	4 ± 1	24 ± 5
		1500	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	17 ± 7
		5000					0 ± 0 <sup>a</sup>
Positive Controls	2-aminoanthracene	1.0	509 ± 163		102 ± 19	52 ± 7	
	2-aminoanthracene	2.0		1292 ± 146			
	2-aminoanthracene	10					263 ± 29

## Footnotes:

<sup>a</sup>Background lawn moderately reduced to absent

Study title: Evaluation of 8-OH-Loxapine in the Bacterial Reverse Mutation Assay.

Study no.: AC27XD.503.BTL  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: 05/18/2009  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: AARD RS0109, 99.76%

## Key Study Findings

This study was conducted to evaluate the mutagenic potential of the metabolite 8-OH-loxapine. 8-OH-loxapine is deemed to be nonmutagenic under the conditions used in this study.

## Methods

Strains: *Salmonella typhimurium*: TA98, TA100, TA1535, TA1537  
*Escherichia coli*: WP2 *uvrA*

### Concentrations used in definitive study:

Initial assay: 1.5, 5.0, 15, 50, 150, 500, 1500, 5000 µg/plate

Confirmatory assay: 15, 50, 150, 500, 1500, 5000 µg/plate

Basis of concentration selection: The highest dose of 5000 µg/plate was set in accordance with current guidelines and lower doses were set in a common ratio of 3. Toxicity was observed beginning between 1500 and 5000 µg/plate with all strains in both the presence and absence of metabolic activation. The doses used in these assays were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535, TA1537	Rat	2-aminoanthracene (b) (4)	1.0
TA100		Lot No. 03403ED	2.0
WP2 <i>uvrA</i>		Exp. Date 22-Jan-2012 CAS No. (b) (4) Purity 99.8%	10
TA98	None	2-nitrofluorene (b) (4)	1.0
		Lot No.03319JD Exp. Date 28-Feb-2011 CAS No. (b) (4) Purity 98.1%	
TA100, TA1535		sodium azide (b) (4)	1.0
		Lot No. G24R025 Exp. Date 10-Feb-2010 CAS No. (b) (4) Purity 99% min.	
TA1537		9-aminoacridine (b) (4)	75
	Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. (b) (4) Purity >97%		
WP2 <i>uvrA</i>		methyl methanesulfonate (b) (4)	1,000
		Lot No. 06823KH Exp. Date 04-Jun-2011 CAS No. (b) (4) Purity 99.9%	

Incubation and sampling times: The test system was exposed to 8-OH-loxapine via the plate incorporation methodology. Plates were incubated with solvent control, positive control or 8-OH-loxapine free base for 48-72 hrs.

Study validity: Plates were run in triplicate for the main assays. 8-OH-loxapine was considered to be mutagenic if it caused a dose-related increase in the mean revertants per plate of at least one strain over a minimum of two increasing concentrations of test article. Tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3 times the mean vehicle control value. Tester strains TA98, TA100, and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2 times the mean vehicle control value. The positive and negative controls all fell within the historical control range data provided.

Results: In the initial mutagenicity assay, the maximum dose concentration of 8-OH-loxapine evaluated was 5000 µg per plate. No precipitate was observed. Toxicity was observed beginning at 1500 µg per plate with all strains in the presence and absence of rat S9. No positive mutagenic responses were observed with 8-OH-loxapine in any of the tester strains in either the presence or absence of rat S9 activation.

In the confirmatory mutagenicity assay, the maximum concentration of 8-OH-loxapine evaluated was 5000 µg per plate. No precipitate was observed. Toxicity was observed beginning at 1500 µg per plate with all strains in the presence and absence of rat S9. No positive mutagenic responses were observed with 8-OH-loxapine in any of the tester strains in either the presence or absence of rat S9 activation.

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Report title: Evaluation of 8-OH-Loxapine in the Bacterial Reverse Mutation Assay

Test Article: 8-OH-Loxapine

Test for Induction of: Reverse mutation in bacterial cells  
Strains: *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2*uvrA*  
Metabolizing System: Aroclor 1254-induced rat liver (10% S9)  
Vehicle: For Test Article and Positive Controls except Sodium Azide: Dimethyl sulfoxide (DMSO)  
For Sodium Azide: Water  
Treatment: Plate incorporation  
Cytotoxic Effects: beginning at 1500 to 5000 µg per plate  
Genotoxic Effects: None

No. of Independent Assays: 2  
No. of Replicate Cultures: 2 for toxicity-mutagenicity assay, 3 for confirmatory assay

Study No. AC27XD.503.BTL

GLP Compliance: Yes

Dates of Treatment: 20 May 2009, 03 June 2009

Toxicity-Mutation Assay

Mean ± SD Revertants per Plate

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
Without Activation	Vehicle (DMSO) 8-OH-Loxapine	0	15 ± 1	113 ± 16	11 ± 3	5 ± 0	25 ± 2
		1.5	16 ± 2	115 ± 13	15 ± 1	4 ± 1	22 ± 8
		5.0	14 ± 1	103 ± 12	14 ± 0	4 ± 3	29 ± 7
		15	11 ± 2	123 ± 4	12 ± 3	3 ± 1	21 ± 0
		50	11 ± 3	134 ± 20	16 ± 1	4 ± 1	24 ± 8
		150	13 ± 1	117 ± 1	15 ± 1	9 ± 0	30 ± 6
		500	16 ± 3	113 ± 10	15 ± 4	3 ± 0	28 ± 2
		1500	11 ± 1*	49 ± 9*	6 ± 0*	3 ± 1*	21 ± 1
		5000	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
Positive Controls	2-nitrofluorene Sodium Azide 9-aminoacridine Methyl methanesulfonate	1.0	187 ± 21				
		1.0		688 ± 76	570 ± 80		
		75				761 ± 160	
		1000					401 ± 86

Footnotes:

\*Background lawn moderately reduced to absent

## Toxicity-Mutagenicity Assay Continued

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2uvrA
With Activation	Vehicle (DMSO)	0	19 ± 6	124 ± 8	11 ± 6	6 ± 1	29 ± 1
	8-OH-Loxapine	1.5	19 ± 3	132 ± 11	15 ± 4	11 ± 3	34 ± 11
		5.0	19 ± 2	149 ± 11	14 ± 2	7 ± 1	32 ± 7
		15	24 ± 1	152 ± 25	12 ± 4	5 ± 1	35 ± 4
		50	21 ± 2	118 ± NA	12 ± 7	7 ± 1	33 ± 8
		150	19 ± 4	109 ± 8	16 ± 4	4 ± 1	24 ± 8
		500	24 ± 2	118 ± 11	11 ± 1	11 ± 1	26 ± 1
		1500	12 ± 1*	60 ± 3*	12 ± 1	5 ± 2	16 ± 3
5000	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*		
Positive Controls	2-aminoanthracene	1.0	379 ± 91		87 ± 11	45 ± 1	
	2-aminoanthracene	2.0		1267 ± 118			
	2-aminoanthracene	10					433 ± 36

## Footnotes:

\*Background lawn moderately reduced to absent

NA: Not applicable. The second plate was not counted due to contamination.

## Confirmatory Mutagenicity Assay

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	Vehicle (DMSO)	0	18 ± 3	111 ± 9	23 ± 2	20 ± 3	32 ± 5
	8-OH-Loxapine	15	19 ± 4	110 ± 10	23 ± 4	19 ± 4	44 ± 6
		50	19 ± 1	118 ± 12	19 ± 2	20 ± 1	41 ± 7
		150	22 ± 2	125 ± 10	23 ± 4	20 ± 5	36 ± 3
		500	24 ± 3	130 ± 16	17 ± 3	17 ± 3	30 ± 6
		1500	0 ± 0*	27 ± 47*	7 ± 4*	4 ± 4*	26 ± 7
		5000	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
		Positive Controls	2-nitrofluorene	1.0	200 ± 40		
	Sodium Azide	1.0		676 ± 34	543 ± 45		
	9-aminoacridine	75				1159 ± 43	
	Methyl methanesulfonate	1000					398 ± 32
With Activation	Vehicle (DMSO)	0	27 ± 2	155 ± 13	11 ± 2	20 ± 4	28 ± 4
	8-OH-Loxapine	15	24 ± 3	148 ± 10	12 ± 6	20 ± 7	34 ± 6
		50	31 ± 1	110 ± 5	15 ± 0	21 ± 4	35 ± 4
		150	31 ± 4	138 ± 10	13 ± 3	17 ± 5	32 ± 2
		500	35 ± 5	121 ± 12	13 ± 2	17 ± 5	35 ± 6
		1500	19 ± 5*	50 ± 87*	8 ± 3*	3 ± 1*	26 ± 8
		5000	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
		Positive Controls	2-aminoanthracene	1.0	461 ± 64		83 ± 11
	2-aminoanthracene	2.0		1333 ± 56			
	2-aminoanthracene	10					441 ± 36

## Footnotes:

\*Background lawn moderately reduced to absent

Study title: Evaluation of (b) (4) in the Bacterial Reverse Mutation Assay.

Study no.: AC29GH.503.BTL

Conducting laboratory and location: (b) (4)

Date of study initiation: 07/20/2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: G1D375, 100%

## Key Study Findings

This study was conducted to evaluate the mutagenic potential of the impurity (b) (4) is deemed to be nonmutagenic under the conditions used in this study.

## Methods

Strains: *Salmonella typhimurium*: TA98, TA100, TA1535, TA1537  
*Escherichia coli*: WP2 *uvrA*

Concentrations used in definitive study:

Initial assay: 1.5, 5.0, 15, 50, 150, 500, 1500, 5000 µg/plate

Confirmatory assay: *Salmonella strains*; 5, 15, 50, 150, 500, 1500 µg/plate

*TA100 & E. coli*: WP2 *uvrA*; 15, 50, 150, 500, 1500, 5000 µg/plate

Basis of concentration selection: The highest dose of 5000 µg/plate was set in accordance with current guidelines and lower doses were set in a common ratio of 3. Toxicity was observed beginning between 500 and 5000 µg/plate with all strains in both the presence and absence of metabolic activation. The doses used in these assays were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535, TA1537	Rat	2-aminoanthracene (b) (4)	1.0
TA100		Lot No. 03403ED Exp. Date 22-Jan-2012 CAS No. (b) (4) Purity 99.8%	2.0
WP2 <i>uvrA</i>			10
TA98	None	2-nitrofluorene (b) (4) Lot No. 03319JD Exp. Date 28-Feb-2011 CAS No. (b) (4) Purity 98.1%	1.0
TA100, TA1535		sodium azide (b) (4) Lot No. G24R025 Exp. Date 10-Feb-2010 CAS No. (b) (4) Purity 99% min.	1.0
TA1537		9-aminoacridine (b) (4) Lot No. 106F06682 Exp. Date 08-Nov-2009 CAS No. (b) (4) Purity >97%	75
WP2 <i>uvrA</i>		methyl methanesulfonate (b) (4) Lot No. 06823KH Exp. Date 04-Jun-2011 CAS No. (b) (4) Purity 99.9%	1,000

**Incubation and sampling times:** The test system was exposed to (b) (4) via the plate incorporation methodology. Plates were incubated with solvent control, positive control or (b) (4) free base for 48-72 hrs.

**Study validity:** Plates were run in triplicate for the main assays. (b) (4) was considered to be mutagenic if it caused a dose-related increase in the mean revertants per plate of at least one strain over a minimum of two increasing concentrations of test article. Tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3 times the mean vehicle control value. Tester strains TA98, TA100, and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2 times the mean vehicle control value. The positive and negative controls all fell within the historical control range data provided.

**Results:** In the initial mutagenicity assay, the maximum dose concentration of (b) (4) evaluated was 5000 µg per plate. No precipitate was observed. Toxicity was observed beginning at 500, 1500, or 5000 µg per plate with all strains in the presence and absence of rat S9. No positive mutagenic responses were observed with

(b) (4) in any of the tester strains in either the presence or absence of rat S9 activation.

In the confirmatory mutagenicity assay, the maximum concentration of (b) (4) evaluated was 5000 µg per plate for TA100 and WP2 *uvrA* and 1500 µg per plate for the remaining *Salmonella* strains. No precipitate was observed. Toxicity was observed beginning at 1500 µg per plate with all strains in the presence and absence of rat S9. No positive mutagenic responses were observed with (b) (4) in any of the tester strains in either the presence or absence of rat S9 activation.

Report title: Evaluation of (b) (4) in the Bacterial Reverse Mutation Assay  
 Test Article: (b) (4)

Test for Induction of: Reverse mutation in bacterial cells  
 Strains: *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2*uvrA*  
 Metabolizing System: Aroclor 1254-induced rat liver (10% S9)  
 Vehicle: For Test Article and For Positive Controls except Sodium Azide: Dimethyl sulfoxide (DMSO)  
 For Sodium Azide: Water  
 Treatment: Plate incorporation  
 Cytotoxic Effects: beginning at 500 to 5000 µg per plate  
 Genotoxic Effects: None  
 No. of Independent Assays: 2  
 No. of Replicate Cultures: 2 for toxicity-mutagenicity assay, 3 for confirmatory assay  
 Study No. AC29GH.503.BTL  
 GLP Compliance: Yes  
 Dates of Treatment: 21 July 2009, 04 August 2009

Toxicity-Mutation Assay

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
Without Activation	Vehicle (DMSO) (b) (4)	0	13 ± 4	105 ± 21	12 ± 2	11 ± 4	36 ± 3
		1.5	18 ± 4	79 ± 4	11 ± 1	11 ± 4	35 ± 3
		5.0	20 ± 5	114 ± 4	13 ± 1	10 ± 1	28 ± 0
		15	18 ± 4	75 ± 4	10 ± 3	10 ± 1	30 ± 1
		50	19 ± 3	87 ± 21	15 ± 4	14 ± 1	26 ± 0
		150	20 ± 1	135 ± 6	12 ± 4	7 ± 1	28 ± 1
		500	9 ± 4*	98 ± 8	6 ± 3*	4 ± 1*	26 ± 4
		1500	0 ± 0*	100 ± 3	0 ± 0*	0 ± 0*	3 ± 3
Positive Controls	2-nitrofluorene Sodium Azide 9-aminoacridine Methyl methanesulfonate	1.0	164 ± 0		525 ± 108		
		1.0		668 ± 23			
		75				602 ± 103	
		1000					604 ± 13

Footnotes:

\*Background lawn moderately reduced to absent

Toxicity-Mutagenicity Assay Continued

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA98	TA100	TA1535	TA1537	WP2 <i>uvrA</i>
With Activation	Vehicle (DMSO) (b) (4)	0	24 ± 1	154 ± 7	18 ± 5	11 ± 5	41 ± 6
		1.5	27 ± 4	142 ± 16	12 ± 1	16 ± 5	42 ± 14
		5.0	15 ± 2	97 ± 28	14 ± 4	13 ± 4	37 ± 3
		15	26 ± 0	144 ± 8	17 ± 7	14 ± 3	43 ± 6
		50	24 ± 2	144 ± 20	12 ± 4	10 ± 5	39 ± 4
		150	23 ± 3	121 ± -- <sup>b</sup>	15 ± 0	11 ± 1	26 ± 4
		500	7 ± 3	70 ± 1	13 ± 1	7 ± 1	29 ± 2
		1500	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	5 ± 2
		5000	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
		Positive Controls	2-aminoanthracene	1.0	826 ± 18		119 ± 35
2.0				1703 ± 137			
10							291 ± 44

Footnotes:

\* Background lawn moderately reduced to absent

<sup>b</sup> Standard deviation not applicable. The second plate was not evaluated due to water damage on the plate.

		Confirmatory Mutagenicity Assay					
		Mean $\pm$ SD Revertants per Plate					
Metabolic Activation	Test Article	Concentration ( $\mu\text{g}/\text{plate}$ )	TA98	TA100	TA1535	TA1537	WP2 <sub>uvrA</sub>
Without Activation	Vehicle (DMSO) (b) (4)	0	12 $\pm$ 2	114 $\pm$ 18	11 $\pm$ 5	3 $\pm$ 2	23 $\pm$ 2
		5.0	12 $\pm$ 6		10 $\pm$ 2	4 $\pm$ 3	
		15	10 $\pm$ 1	123 $\pm$ 19	12 $\pm$ 2	5 $\pm$ 2	30 $\pm$ 9
		50	9 $\pm$ 3	107 $\pm$ 4	11 $\pm$ 3	3 $\pm$ 2	27 $\pm$ 7
		150	13 $\pm$ 2	87 $\pm$ 9	7 $\pm$ 2	5 $\pm$ 2	32 $\pm$ 4
		500	10 $\pm$ 1*	0 $\pm$ 0*	1 $\pm$ 1*	0 $\pm$ 0*	15 $\pm$ 7
		1500	0 $\pm$ 0*	0 $\pm$ 0*	0 $\pm$ 0*	0 $\pm$ 0*	0 $\pm$ 0*
		5000		0 $\pm$ 0*			0 $\pm$ 0*
Positive Controls	2-nitrofluorene	1.0	185 $\pm$ 25				
	Sodium Azide	1.0		516 $\pm$ 16	302 $\pm$ 17		
	9-aminoacridine	75				212 $\pm$ 47	
	Methyl methanesulfonate	1000					317 $\pm$ 14
With Activation	Vehicle (DMSO) (b) (4)	0	16 $\pm$ 5	87 $\pm$ 10	7 $\pm$ 1	4 $\pm$ 1	29 $\pm$ 9
		5.0	14 $\pm$ 3	68 $\pm$ 3	12 $\pm$ 3	5 $\pm$ 4	
		15	20 $\pm$ 8	74 $\pm$ 9	10 $\pm$ 6	3 $\pm$ 1	26 $\pm$ 2
		50	20 $\pm$ 3	68 $\pm$ 6	11 $\pm$ 2	4 $\pm$ 2	30 $\pm$ 3
		150	21 $\pm$ 0	87 $\pm$ 10	13 $\pm$ 3	9 $\pm$ 3	35 $\pm$ 6
		500	1 $\pm$ 1*	11 $\pm$ 11*	5 $\pm$ 3*	0 $\pm$ 0*	28 $\pm$ 6
		1500	0 $\pm$ 0*	0 $\pm$ 0*	0 $\pm$ 0*	0 $\pm$ 0*	2 $\pm$ 1
		5000					0 $\pm$ 0*
Positive Controls	2-aminoanthracene	1.0	617 $\pm$ 75		80 $\pm$ 12	137 $\pm$ 70	
	2-aminoanthracene	2.0		1226 $\pm$ 199			
	2-aminoanthracene	10					150 $\pm$ 4

**Footnotes:**

\*Background lawn moderately reduced to absent

Study title: (b) (4) Bacterial Mutation test.

Study no.: 963035

Conducting laboratory and location: (b) (4)

Date of study initiation: 09/28/2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: AARD RS0114, 91.6%

**Key Study Findings**

This study was conducted to evaluate the mutagenic potential of the impurity (b) (4). (b) (4) is deemed to be nonmutagenic under the conditions used in this study.

**Methods**

**Strains:** *Salmonella typhimurium*: TA98, TA100, TA1535, TA1537  
*Escherichia coli*: WP2 *uvrA*

**Concentrations used in definitive study:**Initial assay: 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000  $\mu\text{g}/\text{plate}$ Confirmatory assay: 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000  $\mu\text{g}/\text{plate}$

Basis of concentration selection: The highest dose of 5000 µg/plate was set in accordance with current guidelines and lower doses were set in a common ratio of 3. Toxicity was observed beginning at 500 µg/plate. The doses used in these assays were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

In the Absence of S9 Mix;  
Sodium azide, 0.5 µg/plate  
9-Aminoacridine, 50 µg/plate  
2-Nitrofluorene, 1 µg/plate  
4-Nitroquinoline N-oxide, 0.5 µg/plate

In the Presence of S9 Mix;  
2-Aminoanthracene, 5, 15 µg/plate  
Benzo[a]pyrene, 5 µg/plate

Incubation and sampling times: The test system was exposed to (b) (4) via the plate incorporation methodology. Plates were incubated with solvent control, positive control or (b) (4) for 48-72 hrs.

Study validity: Plates were run in triplicate for the main assays. (b) (4) was considered to be mutagenic if it caused a dose-related increase in the mean revertants per plate of at least one strain over a minimum of two increasing concentrations of test article. Tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3 times the mean vehicle control value. Tester strains TA98, TA100, and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2 times the mean vehicle control value. The positive and negative controls all fell within the historical control range data provided.

Results: In the initial mutagenicity assay, the maximum dose concentration of (b) (4) evaluated was 5000 µg per plate. No precipitate was observed. Toxicity was observed beginning at 500 µg per plate with all strains in the presence and absence of rat S9. No positive mutagenic responses were observed with (b) (4) in any of the tester strains in either the presence or absence of rat S9 activation.

In the pre-incubation confirmatory mutagenicity assay, the maximum concentration of (b) (4) evaluated was 5000 µg per plate. No precipitate was observed. Toxicity was observed beginning at 50 µg per plate with all strains in the presence and absence of rat S9. No positive mutagenic responses were observed with (b) (4) in any of the tester strains in either the presence or absence of rat S9 activation.

Report title: (b) (4) Bacterial Mutation Test  
 Test Article: (b) (4)

**Test for Induction of:** Reverse mutation in bacterial cells  
**Strains:** *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2uvrA  
**Metabolizing System:** Phenobarbital/5,6-benzoflavone-induced rat liver (10% v/v S9)  
**Vehicle:** For Sodium Azide and 9-Aminoacridine: Water  
 For Test Article and other Positive Controls: Dimethyl sulfoxide (DMSO)  
**Treatment:** Plate incorporation and Pre-incubation  
**Cytotoxic Effects:** Yes  
**Genotoxic Effects:** No

**No. of Independent Assays:** 2  
**No. of Replicate Cultures:** 3 for plate incorporation and pre-incubation assays

**Study No.** 963035  
**GLP Compliance:** Yes<sup>b</sup>  
**Dates of Treatment:** 28 September 2009

**Plate Incorporation Assay**

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA1535	TA1537	TA98	TA100	WP2uvrA
Without Activation	Vehicle (DMSO) (b) (4)	0	21 ± 3	9 ± 2	24 ± 5	135 ± 5	41 ± 3
		1.58		15 ± 3		140 ± 14	
		5.0		12 ± 8		140 ± 13	
		15.8		11 ± 5		149 ± 15	
		50	25 ± 2	12 ± 4	35 ± 5	134 ± 5	49 ± 7
		158	19 ± 8	10 ± 5	27 ± 8	123 ± 6	52 ± 7
		500	20 ± 2	a	27 ± 2	a	51 ± 10
		1581	22 ± 7	a	26 ± 1	a	41 ± 9
		5000	17 ± 7	a	32 ± 4	a	42 ± 6
		Positive Controls	Sodium Azide	0.5	335 ± 25		
	9-Aminoacridine	50		211 ± 86			
	2-Nitrofluorene	1			179 ± 26		
	4-Nitroquinoline N-oxide	0.5				183 ± 5	

Footnotes:

- a Background lawn incomplete or absent (toxicity)
- b GLP exceptions: No formulation analysis was performed. Stability and test article characterization were performed non-GLP

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**Plate Incorporation Assay Continued**

Metabolic Activation	Test Article	Concentration (µg/plate)	Mean ± SD Revertants per Plate				
			TA1535	TA1537	TA98	TA100	WP2uvrA
With Activation	Vehicle (DMSO) (b) (4)	0	22 ± 5	18 ± 5	43 ± 1	136 ± 16	52 ± 7
		1.58		18 ± 2	42 ± 6	158 ± 7	
		5.0		15 ± 4	45 ± 6	161 ± 21	
		15.8		17 ± 2	46 ± 8	161 ± 14	
		50	21 ± 4	17 ± 0	46 ± 0	161 ± 10	56 ± 13
		158	27 ± 6	19 ± 5	32 ± 11	133 ± 19	58 ± 11
		500	22 ± 5	a	a	a	56 ± 4
		1581	24 ± 1	a	a	a	53 ± 12
		5000	20 ± 3	a	a	a	50 ± 11
		Positive Controls	2-Aminoanthracene	5	457 ± 19		
	2-Aminoanthracene	15				194 ± 32	
	Benzo[a]pyrene	5		87 ± 5	361 ± 8	948 ± 31	

Footnotes:

- a Background lawn incomplete or absent (toxicity)

		Pre-incubation Assay					
		Mean $\pm$ SD Revertants per Plate					
Metabolic Activation	Test Article	Concentration ( $\mu$ g/plate)	TA1535	TA1537	TA98	TA100	WP2uvrA
Without Activation	Vehicle (DMSO)	0	18 $\pm$ 3	15 $\pm$ 7	26 $\pm$ 4	135 $\pm$ 8	37 $\pm$ 9
	(b) (4)	1.58		8 $\pm$ 4	33 $\pm$ 3	151 $\pm$ 7	
		5.0		13 $\pm$ 4	31 $\pm$ 5	156 $\pm$ 13	
		15.8		13 $\pm$ 2	34 $\pm$ 7	146 $\pm$ 7	
		50	18 $\pm$ 5	a	29 $\pm$ 2	a	45 $\pm$ 7
		158	20 $\pm$ 3	a	32 $\pm$ 5	a	54 $\pm$ 3
		500	22 $\pm$ 6	a	a	a	48 $\pm$ 7
		1581	17 $\pm$ 4	a	a	a	45 $\pm$ 3
		5000	20 $\pm$ 2	a	a	a	39 $\pm$ 7
		Positive Controls	Sodium Azide	0.5	359 $\pm$ 16		596 $\pm$ 31
	9-Aminoacridine	10		793 $\pm$ 245			
	2-Nitrofluorene	1		170 $\pm$ 17			
	4-Nitroquinoline N-oxide	0.5				1291 $\pm$ 16	
With Activation	Vehicle (DMSO)	0	21 $\pm$ 6	22 $\pm$ 1	43 $\pm$ 4	135 $\pm$ 8	60 $\pm$ 4
	(b) (4)	1.58		25 $\pm$ 9	46 $\pm$ 7	151 $\pm$ 5	
		5.0		21 $\pm$ 4	38 $\pm$ 9	142 $\pm$ 11	
		15.8		17 $\pm$ 1	43 $\pm$ 3	151 $\pm$ 15	
		50	24 $\pm$ 5	17 $\pm$ 5	38 $\pm$ 5	146 $\pm$ 5	63 $\pm$ 3
		158	19 $\pm$ 3	18 $\pm$ 7	44 $\pm$ 4	142 $\pm$ 6	65 $\pm$ 4
		500	22 $\pm$ 3	a	a	a	56 $\pm$ 7
		1581	23 $\pm$ 6	a	a	a	57 $\pm$ 8
		5000	26 $\pm$ 10	a	a	a	47 $\pm$ 6
		Positive Controls	2-Aminoanthracene	5	291 $\pm$ 10		
	2-Aminoanthracene	15		87 $\pm$ 8	282 $\pm$ 17	780 $\pm$ 22	
	Benzo[a]pyrene	5				228 $\pm$ 16	

Footnotes:

1. Background lawn incomplete or absent (toxicity)

## 7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

**Study title:** *In vitro* mammalian chromosome aberration test of loxapine in DMSO using human peripheral blood lymphocytes.

Study no.: AC14WV.341.BTL

Conducting laboratory and location: (b) (4)

Date of study initiation: 05/15/2008

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: 60096-07-002, 100%

### Key Study Findings

Loxapine free base was found to be negative for the induction of structural chromosome aberrations and numerical chromosome aberrations in either the absence or presence of metabolic activation under the condition of this study.

### Methods

**Cell line:** Human peripheral blood lymphocytes were obtained from a healthy non-smoking 28 year old adult male.

**Concentrations used in definitive study:**

Treatment Condition	Treatment Time	Recovery Time	Dose Levels of Loxapine Free Base ( $\mu\text{g/mL}$ )
Non-activated	4 hr	16 hr	12.5, 25, 50, 75, 100, 125, 150
	20 hr	0 hr	12.5, 25, 50, 75, 100, 125, 150
S9-activated	4 hr	16 hr	12.5, 25, 50, 75, 100, 125, 150

Basis of concentration selection: The selection of the dose levels was based upon a preliminary toxicity test. The highest dose level selected was the dose that induced at least 50% toxicity as measured by mitotic inhibition relative to solvent control. The concentrations used in the main study were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

Study without metabolic activation: Mitomycin (0.3 and 0.6  $\mu\text{g/ml}$ )

Study with metabolic activation: Cyclophosphamide monohydrate (20 and 40  $\mu\text{g/ml}$ )

Incubation and sampling times: In the non-activated study, cells were incubated with solvent control, positive control, or loxapine free base for 4 or 20 hrs in the absence of metabolic activation. Following the 4 hr incubation, the cells were washed and incubated in fresh medium for an additional 16 hrs. In the activated study, cells were incubated with solvent control, positive control, or loxapine free base for 4 hrs in the presence of metabolic activation. Following the 4 hr incubation, the cells were washed and incubated in fresh medium for an additional 16 hrs.

Study validity: 200 metaphase spreads containing 46 centromeres per dose were examined for structural and numerical aberrations. For structural aberrations, chromatid-type, chromosome-type aberrations or fragmentation were recorded. For numerical aberrations, polyploidy and endoreduplicated cells were evaluated per 100 metaphase cells for each dose level. The test was deemed positive when the frequency of cells having chromosomal aberrations was significantly and dose dependently increased in any treatment group when compared with the negative control group. The negative and positive controls all fell within the historical control range data provided.

Results: Negative.

Loxapine free base incubation did not induce chromosomal aberrations in human peripheral blood lymphocytes, in the absence or presence of metabolic activation up to doses that produced a greater than 50% reduction in mitotic index under the conditions of this study. It should be noted that mitomycin C cytotoxicity was low in this study as compared to the vehicle control which may indicate lower sensitivity of the assay.

***In Vitro* Mammalian Chromosome Aberration Test of Loxapine in DMSO using Cultured Human Peripheral Blood Lymphocytes**

**Test Article:** Loxapine free base

**Test for Induction of:** Numerical and Structural Chromosomal Aberrations    **No. of Independent Assays:** 1    **Study No.** AC14WV.341.BTL  
**Test System:** Human Peripheral Blood Lymphocytes    **No. of Replicate Cultures:** 2  
**Metabolizing System:** Aroclor 1254-induced rat liver (2% S9)    **No. of Cells Analyzed/Culture:** 100 except as noted  
**Vehicles:** For Test Article: Dimethyl Sulfoxide (DMSO)    For Positive Controls: Water    **GLP Compliance:** Yes  
**Treatment:** 4-hour treatment with and without S9; 20-hour treatment without S9    **Treatment Date(s):** 29 May 2008  
**Cytotoxic Effects:** Dose-related reductions in mitotic index  
**Genotoxic Effects:** None

Test Article	Concentration (µg/mL)	4-hour Treatment Without Metabolic Activation				4-hour Treatment With Metabolic Activation			
		Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell	Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural			Numerical	Structural	
Vehicle (DMSO)	-	100%	0	0	0	100%	0	0	0
Loxapine Free Base	25	97%	0	0	0	92%	0	0	0
50		86%	0	0	0	86%	0	0.5	0.005
100		44%	0	0.5	0.005	47%	0	0	0
Mitomycin C <sup>b</sup> 0.6		73%	0	17.0**	0.270	ND	ND	ND	ND
Cyclophosphamide <sup>b</sup> 20		ND	ND	ND	ND	69%	0	18.0**	0.230

**Footnotes:**

a: Based on mitotic inhibition    b: 50 cells analyzed/culture    Fisher's exact test: \*\* p ≤ 0.01    ND: Not Done

**Study No.** AC14WV.341.BTL (cont'd)

**Test Article:** Loxapine free base

Test Article	Concentration (µg/mL)	20-hour Treatment Without Metabolic Activation			
		Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0	0	0
Loxapine Free Base	12.5	91%	0	0	0
25		95%	0	0	0
75		44%	0	0	0
Mitomycin C <sup>b</sup> 0.3		70%	0	19.0**	0.310

**Footnotes:**

a: Based on mitotic inhibition    b: 50 cells analyzed/culture    Fisher's exact test: \*\* p ≤ 0.01

**Study title: *In vitro* mammalian chromosome aberration test of [REDACTED] (b) (4) loxapine base using human peripheral blood lymphocytes.**

Study no.: AC29MU.341.BTL  
Conducting laboratory and location: [REDACTED] (b) (4)  
Date of study initiation: 07/01/2009  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: A-080160, 99.9%

**Key Study Findings**

Loxapine free base was deemed to be negative for the induction of structural chromosome aberrations and numerical chromosome aberrations in either the absence or presence of metabolic activation under the condition of this study.

**Methods**

Cell line: Human peripheral blood lymphocytes sampled from 1 healthy non-smoking 23 year old adult female.

Concentrations used in definitive study: 5, 10, 20, 40, 80, 100, 120, 140, 160, 180, 200 µg/ml.

Basis of concentration selection: The selection of the dose levels was based upon a previous chromosome aberration test (Study # AC14WV.341.BTL). The concentrations used in the main study were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

Study without metabolic activation: Mitomycin (0.3 and 0.6 µg/ml)

Study with metabolic activation: Cyclophosphamide monohydrate (10 µg/ml)

Incubation and sampling times: In the non-activated study, cells were incubated with solvent control, positive control, or loxapine free base for 4 or 20 hrs in the absence of metabolic activation. Following the 4 hr incubation, the cells were washed and incubated in fresh medium for an additional 16 hrs. In the activated study, cells were incubated with solvent control, positive control, or loxapine free base for 4 hrs in the presence of metabolic activation. Following the 4 hr incubation, the cells were washed and incubated in fresh medium for an additional 16 hrs.

Study validity: 200 metaphase spreads containing 46 centromeres per dose were examined for structural and numerical aberrations. For structural aberrations, chromatid-type, chromosome-type aberrations or fragmentation were recorded. For numerical aberrations, polyploidy and endoreduplicated cells were evaluated per 100

metaphase cells for each dose level. The test was deemed positive when the frequency of cells having chromosomal aberrations was significantly and dose dependently increased in any treatment group when compared with the negative control group. The negative and positive controls all fell within the historical control range data provided.

**Results:** Negative.

Loxapine free base incubation did not induce chromosomal aberrations in human peripheral blood lymphocytes, in the absence or presence of metabolic activation up to doses that produced a greater than 50% reduction in mitotic index under the conditions of this study.

It should be noted that mitomycin C cytotoxicity is in the acceptable range in this study unlike the level of cytotoxicity observed in the above study.

*In Vitro* Mammalian Chromosome Aberration Test of Loxapine Base in DMSO using Cultured Human Peripheral Blood Lymphocytes  
Test Article: Loxapine Base

Test for Induction of: Numerical and Structural Chromosomal Aberrations      No. of Independent Assays: 1      Study No. AC29MU.341.BTL  
Test System: Human Peripheral Blood Lymphocytes      No. of Replicate Cultures: 2  
Metabolizing System: Aroclor 1254-induced rat liver (2% S9)      No. of Cells Analyzed/Culture: 100 except as noted  
Vehicles: For Test Article: Dimethyl Sulfoxide (DMSO)      For Positive Controls: Water      GLP Compliance: Yes  
Treatment: 4-hour treatment with and without S9; 20-hour treatment without S9      Treatment Date(s): 09 July 2009  
Cytotoxic Effects: Dose-related reductions in mitotic index  
Genotoxic Effects: None

Test Article	Concentration (µg/mL)	4-hour Treatment Without Metabolic Activation			
		Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.0	0
Loxapine Base	20	78%	0.0	0.0	0
	40	66%	0.0	0.0	0
	100	46%	0.0	0.0	0
Mitomycin C <sup>b</sup>	0.6	45%	0.0	21.0**	0.250

Footnotes:

a: Based on mitotic inhibition      b: 50 cells analyzed/culture

Fisher's exact test: \*\* p ≤ 0.01      ND: Not Done

Study No. AC29MU.341.BTL (cont'd)

Test Article: Loxapine Base

Test Article	Concentration (µg/mL)	4-hour Treatment With Metabolic Activation			
		Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.0	0
Loxapine Base	20	101%	0.0	0.0	0
	40	102%	0.0	0.5	0.005
	120	46%	0.0	0.0	0
Cyclophosphamide <sup>b</sup>	10	29%	0.0	22.0**	0.260

Footnotes:

a: Based on mitotic inhibition

b: 50 cells analyzed/culture

Fisher's exact test: \*\* p ≤ 0.01

Study No. AC29MU.341.BTL (cont'd)

Test Article: Loxapine Base

Test Article	Concentration (µg/mL)	20-hour Treatment Without Metabolic Activation			
		Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.0	0
Loxapine Base	5	91%	0.0	0.0	0
	10	76%	0.0	0.0	0
	40	49%	0.0	0.0	0
Mitomycin C <sup>b</sup>	0.3	53%	0.0	20.0**	0.340

**Footnotes:**

a: Based on mitotic inhibition

b: 50 cells analyzed/culture

Fisher's exact test: \*\* p ≤ 0.01

**Study title: *In vitro* mammalian chromosome aberration test of 8-OH-loxapine using human peripheral blood lymphocytes.**

Study no.: AC27XD.341.BTL

Conducting laboratory and location:

(b) (4)

Date of study initiation: 05/17/2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: AARD RS0109, 99.76%

**Key Study Findings**

8-OH-loxapine was deemed to be negative for the induction of structural chromosome aberrations and numerical chromosome aberrations in either the absence or presence of metabolic activation under the condition of this study.

**Methods**

Cell line: Human peripheral blood lymphocytes obtained from a healthy non-smoking 32 year old adult female.

Concentrations used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose Levels of 8-OH-Loxapine (µg/mL)
Non-activated	4 hr	16 hr	25, 50, 100, 130, 140, 150, 175
	20 hr	0 hr	10, 25, 40, 50, 65, 75, 85, 100
S9-activated	4 hr	16 hr	25, 50, 100, 130, 140, 150, 175

Basis of concentration selection: The selection of the dose levels was based upon a preliminary toxicity test. The highest dose level selected was the dose that induced at least 50% toxicity as measured by mitotic inhibition relative to solvent control. The concentrations used in the main study were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

Study without metabolic activation: Mitomycin (0.3 and 0.6 µg/ml)

Study with metabolic activation: Cyclophosphamide monohydrate (20 and 40 µg/ml)

Incubation and sampling times: In the non-activated study, cells were incubated with solvent control, positive control, or 8-OH-loxapine for 4 or 20 hrs in the absence of metabolic activation. Following the 4 hr incubation, the cells were washed and incubated in fresh medium for an additional 16 hrs. In the activated study, cells were incubated with solvent control, positive control, or 8-OH-loxapine for 4 hrs in the presence of metabolic activation. Following the 4 hr incubation, the cells were washed and incubated in fresh medium for an additional 16 hrs.

Study validity: 200 metaphase spreads containing 46 centromeres per dose were examined for structural and numerical aberrations. For structural aberrations, chromatid-type, chromosome-type aberrations or fragmentation were recorded. For numerical aberrations, polyploidy and endoreduplicated cells were evaluated per 100 metaphase cells for each dose level. The test was deemed positive when the frequency of cells having chromosomal aberrations was significantly and dose dependently increased in any treatment group when compared with the negative control group. The negative and positive controls all fell within the historical control range data provided.

Results: Negative.

8-OH-loxapine incubation did not induce chromosomal aberrations in human peripheral blood lymphocytes, in the absence or presence of metabolic activation up to doses that produced a greater than 50% reduction in mitotic index under the conditions of this study.

*In Vitro* Mammalian Chromosome Aberration Test of 8-OH-Loxapine in DMSO using Cultured Human Peripheral Blood Lymphocytes

Test Article: 8-OH-Loxapine

Test for Induction of: Numerical and Structural Chromosomal Aberrations  
 Test System: Human Peripheral Blood Lymphocytes  
 Metabolizing System: Aroclor 1254-induced rat liver (2% S9)  
 Vehicles: For Test Article: Dimethyl Sulfoxide (DMSO) For Positive Controls: Water  
 Treatment: 4-hour treatment with and without S9; 20-hour treatment without S9  
 Cytotoxic Effects: Dose-related reductions in mitotic index  
 Genotoxic Effects: None

No. of Independent Assays: 1  
 No. of Replicate Cultures: 2  
 No. of Cells Analyzed/Culture: 100 except as noted  
 GLP Compliance: Yes  
 Treatment Date: 03 June 2009 (Definitive Assay)

## 4-hour Treatment Without Metabolic Activation

Test Article	Concentration (µg/mL)	Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.0	0
8-OH-Loxapine	25	91%	0.0	0.5	0.005
	100	76%	0.0	0.0	0
	140	45%	1.0	0.5	0.005
Mitomycin C <sup>b</sup>	0.6	44%	0.0	28.0**	0.360

## Footnotes:

a: Based on mitotic inhibition

b: 25 cells analyzed/culture

Fisher's exact test: \*\* p ≤ 0.01

Study No. AC27XD.341.BTL (cont'd)

Test Article: 8-OH-Loxapine

## 4-hour Treatment With Metabolic Activation

Test Article	Concentration (µg/mL)	Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.0	0
8-OH-Loxapine	25	99%	0.0	0.0	0
	50	75%	0.0	0.0	0
	130	49%	0.5	0.5	0.005
Cyclophosphamide <sup>b</sup>	20	27%	0.0	16.0**	0.170

## Footnotes:

a: Based on mitotic inhibition

b: 50 cells analyzed/culture

Fisher's exact test: \*\* p ≤ 0.01

Study No. AC27XD.341.BTL (cont'd)

Test Article: 8-OH-Loxapine

Test Article	Concentration (µg/mL)	20-hour Treatment Without Metabolic Activation			
		Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.5	0.005
8-OH-Loxapine	10	107%	0.5	0.5	0.010
	25	104%	0.0	0.5	0.005
	85	42%	0.0	1.0	0.010
Mitomycin C <sup>b</sup>	0.3	17%	0.0	26.0**	0.300

**Footnotes:**

a: Based on mitotic inhibition

b: 25 cells analyzed/culture

Fisher's exact test: \*\* p ≤ 0.01

**Study title:** *In vitro* mammalian chromosome aberration test of (b) (4) using human peripheral blood lymphocytes.

Study no.: AC29GH.341.BTL

Conducting laboratory and location: (b) (4)

Date of study initiation: 07/07/2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: G1D375, 100%

## Key Study Findings

(b) (4) was deemed to be negative for the induction of structural chromosome aberrations and numerical chromosome aberrations in either the absence or presence of metabolic activation under the condition of this study.

## Methods

Cell line: Human peripheral blood lymphocytes obtained from a healthy non-smoking 27 year old adult female.

Concentrations used in definitive study:

Treatment Condition	Treatment Time	Recovery Time	Dose Levels of Amoxapine (µg/mL)
Non-activated	4 hr	16 hr	5, 10, 20, 25, 35, 50, 65, 75, 100
	20 hr	0 hr	1, 5, 10, 15, 20, 25, 30, 35, 50
S9-activated	4 hr	16 hr	1, 5, 10, 20, 25, 30, 35, 40, 45, 50, 60

Basis of concentration selection: The selection of the dose levels was based upon a preliminary toxicity test. The highest dose level selected was the dose that induced at least 50% toxicity as measured by mitotic inhibition relative to solvent control. The concentrations used in the main study were appropriate.

Negative controls: DMSO

Positive controls: Appropriate controls were used.

Study without metabolic activation: Mitomycin (0.3 and 0.6 µg/ml)

Study with metabolic activation: Cyclophosphamide monohydrate (10 and 15 µg/ml)

Incubation and sampling times: In the non-activated study, cells were incubated with solvent control, positive control, or (b) (4) for 4 or 20 hrs in the absence of metabolic activation. Following the 4 hr incubation, the cells were washed and incubated in fresh medium for an additional 16 hrs. In the activated study, cells were incubated with solvent control, positive control, or (b) (4) for 4 hrs in the presence of metabolic activation. Following the 4 hr incubation, the cells were washed and incubated in fresh medium for an additional 16 hrs.

Study validity: 200 metaphase spreads containing 46 centromeres per dose were examined for structural and numerical aberrations. For structural aberrations, chromatid-type, chromosome-type aberrations or fragmentation were recorded. For numerical aberrations, polyploidy and endoreduplicated cells were evaluated per 100 metaphase cells for each dose level. The test was deemed positive when the frequency of cells having chromosomal aberrations was significantly and dose dependently increased in any treatment group when compared with the negative control group. The negative and positive controls all fell within the historical control range data provided.

Results: Negative.

(b) (4) incubation did not induce chromosomal aberrations in human peripheral blood lymphocytes, in the absence or presence of metabolic activation up to doses that produced a greater than 50% reduction in mitotic index under the conditions of this study.

*In Vitro* Mammalian Chromosome Aberration Test of (b) (4) in DMSO using Cultured Human Peripheral Blood Lymphocytes

Test Article: (b) (4)

<b>Test for Induction of:</b> Numerical and Structural Chromosomal Aberrations	<b>No. of Independent Assays:</b> 1	<b>Study No.:</b> AC29GH.341.BTL
<b>Test System:</b> Human Peripheral Blood Lymphocytes	<b>No. of Replicate Cultures:</b> 2	
<b>Metabolizing System:</b> Aroclor 1254-induced rat liver (2% S9)	<b>No. of Cells Analyzed/Culture:</b> 100 except as noted	
<b>Vehicles:</b> For Test Article: Dimethyl Sulfoxide (DMSO)	<b>For Positive Controls:</b> Water	<b>GLP Compliance:</b> Yes
<b>Treatment:</b> 4-hour treatment with and without S9; 20-hour treatment without S9		<b>Treatment Date:</b> 23 July 2009 (Definitive Assay)

**Cytotoxic Effects:** Dose-related reductions in mitotic index  
**Genotoxic Effects:** None

4-hour Treatment Without Metabolic Activation					
Test Article	Concentration (µg/mL)	Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.0	0
(b) (4)	5	92%	0.0	0.0	0
	10	72%	0.0	0.0	0
	50	45%	0.0	0.0	0
Mitomycin C <sup>b</sup>	0.6	44%	0.0	17.0**	0.250

**Footnotes:**

a: Based on mitotic inhibition      b: 50 cells analyzed/culture      Fisher's exact test: \*\* p ≤ 0.01

Study No. AC29GH.341.BTL (cont'd)

Test Article: (b) (4)

4-hour Treatment With Metabolic Activation					
Test Article	Concentration (µg/mL)	Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.0	0
(b) (4)	10	100%	0.0	0.0	0
	20	83%	0.0	0.0	0
	50	50%	0.0	0.0	0
Cyclophosphamide <sup>b</sup>	10	27%	0.0	15.0**	0.210

**Footnotes:**

a: Based on mitotic inhibition      b: 50 cells analyzed/culture      Fisher's exact test: \*\* p ≤ 0.01

Study No. AC29GH.341.BTL (cont'd)

Test Article: (b) (4)

Test Article	Concentration (µg/mL)	20-hour Treatment Without Metabolic Activation			
		Cytotoxicity <sup>a</sup> (% of Control)	% Aberrant Cells		Mean Abs/Cell
			Numerical	Structural	
Vehicle (DMSO)	-	100%	0.0	0.0	0
(b) (4)	1	97%	0.0	0.0	0
	5	82%	0.0	0.0	0
	25	47%	0.0	0.0	0
Mitomycin C <sup>b</sup>	0.3	43%	0.0	18.0**	0.230

**Footnotes:**

a: Based on mitotic inhibition

b: 50 cells analyzed/culture

Fisher's exact test: \*\* p ≤ 0.01

## 8 Carcinogenicity

Done only in rats using succinate salt at 0.17, 0.56, 1.84, and 6.13 mg/kg/ oral daily doses that were increased after 5 months to 0.3, 1.06, 3.4, and 11.6 mg/kg/day due to lack of toxicity. No signs of carcinogenicity were observed.

## 9 Reproductive and Developmental Toxicology

Loxapine was not teratogenic in both the rat and rabbit.

## 11 Integrated Summary and Safety Evaluation

The drug substance, loxapine, is an FDA approved and marketed drug since 1975. Therefore, all required nonclinical studies have been conducted, reviewed, and evaluated for safety. All this information can be found in the SBA for the corresponding drug application. In this application Alexza has conducted bridging studies for its drug product in the rat and dog and single and repeat dose studies up to 14 days as well as assessed its genetic toxicity potential. Pharmacological studies conducted in support of NDA 17-525 evaluating the CNS effects of loxapine demonstrated the typical antipsychotic profile, with *in vitro* and *in vivo* antagonist activity at dopamine and serotonin receptors. Effects of Staccato loxapine on the cardiovascular and pulmonary systems were studied in dogs and revealed no adverse effects at an IV dose of 0.5 mg/kg (mean peak venous plasma levels of 1,398 ng/mL) and only transient hypotension at an IV dose of 1.5 mg/kg (mean peak plasma level of 1,438 ng/ml). The C<sub>max</sub> observed with IV doses of 0.5 to 1.5 mg/kg was approximately 5 fold greater than that measured in healthy human volunteers administered a single 10 mg dose of Staccato Loxapine.

In rats, dogs, and humans loxapine was rapidly absorbed after inhalation administration. 8-OH-loxapine is a disproportionate metabolite in humans, following inhalation exposure. Alexza examined the genotoxic potential of this metabolite and found it to be non-mutagenic. The terminal elimination half-life ( $T_{1/2}$ ) for loxapine after inhalation ranged from 1.2 to 5.4 hours in rat, 0.8 – 6.6 hours in dog, and 7.6 – 8.2 hours in human.

The toxicity of loxapine was assessed *in vivo* using nose-only and oral inhalation, oral, and parenteral administration. Alexza conducted acute inhalation toxicity studies in rats and dogs using nose-only and oral inhalation administration, respectively, pharmacological effects on the CNS (lethargy or decreased activity, weakness and tremors) were noted at mean doses of 6.7 mg/kg and higher in rats and 0.68 mg/kg and higher in dogs. Repeat dose oral toxicity studies of up to 1, 12, and 19 months were previously conducted in mice, rats and dogs, respectively. CNS effects such as decreased locomotor activity, sedation, catalepsy, ptosis and/or convulsions were observed in all three species. In rats, audiogenic seizures increased with prolongation of treatment time. In dogs, the recovery time from onset of loxapine-induced decrease in locomotor activity shortened as dosing progressed.

Loxapine administered to rats by inhalation for 14 consecutive days at doses of 1.7 to 13 mg/kg/day resulted in dose-related CNS clinical signs consistent with the extended pharmacology of loxapine. Dose-related decreases in body weight and changes in urinary parameters were considered secondary to reduced activity and lethargy. Histological changes related to the extended pharmacology of loxapine included mammary hyperplasia in both sexes, and ovarian follicular cysts and mucification of vaginal epithelium in females; mammary hyperplasia in males and ovarian cysts were no longer present at the end of recovery, while mammary hyperplasia in females and vaginal mucification were seen with a decreased incidence at the end of recovery. Based on the persistence of the clinical signs until the morning following dosing, the slow recovery of body weight decreases and the partial recovery of the histological findings at the high dose, the NOAEL was 1.7 mg/kg/day, the low dose in the study. The NOAEL value is approximately 0.6-fold the maximum proposed clinical dose in a 24 hour period on a  $\text{mg}/\text{m}^2$  basis (10 mg *Staccato*<sup>®</sup> loxapine and 60 kg body weight assumed). The NOAEL corresponds to Day 14 loxapine plasma AUC values of 55.9 and 91.6 ng•hr/mL in male and female rats, respectively, which are approximately 0.18 and 0.29-fold the loxapine exposure (AUC) in a clinical study with a 3 x 10 mg *Staccato*<sup>®</sup> Loxapine doses 4 hours apart (approximately 315 ng•hr/mL, Study 004-102).

When administered to beagle dogs via inhalation for 28 days, loxapine induced clinical signs noted at dose levels ranging from 0.12 to 1.8 mg/kg/day included decreased activity, weakness, tremors and lack of coordination. These effects are likely an exaggeration of the pharmacological activity of loxapine. The NOAEL was 1.8 mg/kg/day, approximately 2-fold the maximum proposed clinical dose in a 24 hour period of 0.5 mg/kg, on a  $\text{mg}/\text{m}^2$  basis (10 mg *Staccato* loxapine and 60 kg body weight assumed). Mean plasma exposure (AUC) in males and females at the NOAEL was 467 and 577 ng•hr/mL in male and female dogs, respectively, which are approximately 1.5

and 1.8-fold the loxapine exposure (AUC) in a clinical study with a 3 x 10 mg *Staccato* Loxapine doses 4 hours apart (approximately 315 ng•hr/mL, Study 004-102).

No local irritation was observed with repeated inhalation exposure at dose levels up to 1.8 mg/kg/day in the dog. In the rat with repeated inhalation exposure for 14 consecutive days, minimal squamous metaplasia of the larynx was seen at doses of 1.7, 6.4 and 13 mg/kg/day. This is likely a non-drug specific effect due to particle impaction per the route of administration. While, adverse pulmonary effects were observed in human subjects with asthma or COPD, a direct correlation between these adverse events and this non-clinical finding cannot be made at this time. Therefore its clinical relevance is unclear at this time.

Based on a *weight of evidence* approach, loxapine was found to be non-genotoxic *in vitro* and *in vivo*. However, loxapine in +S9 was mutagenic in TA98 bacterial mutation assay in 2 assays but negative in the 3<sup>rd</sup> repeat assay. The disproportionate human metabolite 8-OH-loxapine was non-genotoxic in *in vitro* studies conducted by Alexza.

Alexza did not conduct reproductive and developmental toxicity studies of loxapine. The studies contained in the approved NDAs indicate that the reproductive and developmental toxicity profiles of loxapine are similar to those of other compounds with dopamine antagonist and/or serotonergic activity. There was no nonclinical indication of effects on male reproductive performance or sperm morphology in rats, rabbits and/or mice, or teratogenesis in rats or rabbits. Loxapine disrupted estrous cycling in female rats, which is consistent with the known neuroendocrine effects of many neuroleptics in this species, and high doses of loxapine that resulted in marked maternal toxicity also produced increased resorptions, a low rate of dystocia, and/or reduced fetal weights and other indicators of developmental delay, as well as early neonatal death when treated rats were allowed to deliver their litters.

Assessments of components of the *Staccato* device, and of potential impurities, degradants and leachables in the loxapine aerosol were adequate and raise no safety concerns.

From a Pharmacology/Toxicology perspective, the non-clinical studies that supported the approval of the innovator product in combination with published literature, and the additional bridging studies conducted by Alexza are considered adequate to support the current submission.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22549	ORIG-1	ALEXZA PHARMACEUTICA LS INC	Staccato (loxapine) for Oral Inhalation

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/s/

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DARREN B FEGLEY  
09/14/2010

AISAR H ATRAKCHI  
09/14/2010

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

NDA/BLA Number: 022549 Applicant: Alexza Pharm.

Stamp Date: 12/11/2009

Drug Name: Staccato Loxapine NDA/BLA Type: 505b2

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		Standard nonclinical studies were not done under NDA22-549, but completed under the FDA marketed oral and intramuscular formulations of the drug (NDA17-525 & NDA18-039). However, appropriate nonclinical studies conducted under the current NDA have been submitted and reviewed.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m <sup>2</sup> 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		
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable.
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.

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Reviewing Pharmacologist Date

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Team Leader/Supervisor Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22549	ORIG-1	ALEXZA PHARMACEUTICA LS INC	Staccato (loxapine) for Oral Inhalation

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/s/

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DARREN B FEGLEY  
03/02/2010

AISAR H ATRAKCHI  
03/02/2010