CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207930Orig1s000

PHARMACOLOGY REVIEW(S)

Pharmacology and Toxicology Secondary Review for NDA 207930

TO: NDA 207930 (UTIBRON NEOHALER; Combination of glycopyrrolate and indacaterol)

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DATE: October 1, 2015

NDA 207930 is a 505(b)(1) application for UTIBRON NEOHALER. UTIBRON NEOHALER is a fixed dose combination, in a dry powder inhaler, of glycopyrrolate (NVA237)15.6 mcg (salt) and indacaterol maleate (QAB149) 27.5 mcg (base). The combination was designated as QVA149. The formulation includes magnesium stearate and lactose monohydrate as excipients. The maximum recommended dose is one actuation twice daily (BID) or 31.2 mcg glycopyrrolate and 55 mcg indacaterol per day. UTIBRON NEOHALER is indicated for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema.

Dr. Sohn's reviews dated July 7, 2015 and September 24, 2015 focused on the nonclinical safety assessment of the glycopyrrolate monoproduct and the combination of glycopyrrolate and indacaterol. The Sponsor previously received FDA approval of the indacaterol monoproduct, (NDA 22383).

I concur with the recommendations of Dr. Sohn's reviews dated July 7, 2015 and September 24, 2015 that the nonclinical pharmacology and toxicology of the glycopyrrolate monoproduct and the combination of glycopyrrolate and indacaterol have been adequately studied and should be approved from the nonclinical perspective.

The Sponsor has complete nonclinical development programs for the glycopyrrolate and indacaterol monoproducts as well as the combination of glycopyrrolate and indacaterol. Thus, the NDA qualifies as a 505(b)(1) application.

Glycopyrrolate Monoproduct:

Pharmacology: Glycopyrrolate is a long-acting antimuscarinic antagonist (LAMA), which is often referred to under the more general term anticholinergic. The mechanism of action of glycopyrrolate is similar to that of tiotropium bromide, aclidinium bromide, and umeclidinium bromide, which are currently marketed anticholinergics. *In vitro* pharmacology studies show that glycopyrrolate is a high affinity, pan active muscarinic antagonist. In competitive binding assays, glycopyrrolate bound to the M1, M2, M3, M4 and M5 receptors with pKi values of 9.81, 9.05, 9.59, 9.05, and 8.96 nM, respectively. Glycopyrrolate was characterized as a competitive inhibitor at the M1, M2, and M3 receptors with pKi values of 9.60, 8.70, and 9.47, respectively, which were consistent

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with competitive kinetic assays. The pharmacologic effects of glycopyrrolate were assessed using in vitro and in vivo models. Using a rat isolated trachea model, glycopyrrolate produced a concentration-dependent inhibition of bethanechol-induced contraction. Glycopyrrolate inhibited methacholine-induced bronchoconstriction in rabbits.

ADME: See Dr. Sohn's review dated September 24, 2015 for more specific information.

Toxicology: Potential local (lung) and systemic toxicity (full organ and tissue evaluation) of glycopyrrolate were evaluated in inhalation toxicology studies with duration of 26 weeks in rats and 39 weeks in dogs.

In the 26-week toxicology study with a 4-week recovery period, rats were exposed to glycopyrrolate by nose-only inhalation. The target organs of toxicity were identified as the eyes, lungs, seminal vesicles, and urinary bladder. Eye findings consisted of unilateral and bilateral lenticular changes (anterior capsular opacity, anterior prominent suture line, anterior slight cataract) that were observed at the mid and high dose in males and females. These findings in the eyes were attributed to the anticholinergic action of glycopyrrolate and were partially reversible. Minimal epithelial hypertrophy was observed in the lungs at the mid and high dose and attributed to a local irritant effect. The cells within the areas of epithelial hypertrophy were not Type II pneumocytes. These findings in the lung were reversible following the 4-week recovery period. There were low incidence findings of seminal vesicle inflammation and urinary bladder inflammation in high dose males. Additional findings in the nasal cavity/sinuses, larynx, and Harderian gland were not considered clinically relevant. The NOAEL was identified as the low dose.

In the 39-week inhalation toxicology study with a 4-week recovery period, beagle dogs were exposed to glycopyrrolate. Target organs of toxicity included the eyes, lacrimal glands, heart, pharynx, and salivary mandibular gland. Test article-related ophthalmic findings observed at the high dose consisted of conjunctival hyperemia (bilateral), corneal opacity (bilateral), and focal nuclear opacity (bilateral). These ophthalmic findings were judged to be dose-limiting. Reductions of lacrimal secretion were measured in the high dose group at weeks 3 and 6, which was an expected pharmacological effect of the test article. Increased heart rates were observed in mid dose males and high dose males and females at weeks 13, 26, and 39; this was an expected pharmacological effect of the test article and monitorable in a clinical setting. In the pharynx, inflammation was observed in high dose males and females. Further, ectasia of the ducts and/or alveoli of the pharynx were observed for mid and high dose males and females. The findings in the pharynx were generally mild, reversible, and judged to be monitorable in a clinical setting. There were findings of hypertrophy in the lacrimal and salivary glands, which were not considered dose-limiting. The NOAEL was determined as the low dose; however, findings at the mid dose were judged to be monitorable in a clinical setting and/or not dose limiting.

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The NOAEL in the 26-week rat study and monitorable toxicity in the 39-week dog study provide adequate safety margins with respect to local (lung) and systemic toxicity for the clinical dose of glycopyrrolate.

Genetic Toxicity: Glycopyrrolate was negative in a standard battery of genotoxicity assays.

Carcinogenicity: There was no evidence of tumorigenic potential in a 2-year carcinogenicity study conducted in Wistar rats or in a 26 week carcinogenicity study in TgRasH2 mice. The study with TgrasH2 mice was for hazard identification purposes only; so exposure multiples relative to the clinical dose would not be calculated. Test article related non-neoplastic findings in the 2-year rat study were identified in the lungs (epithelial hypertrophy, eosinophilic cytoplasmic inclusions, macrophage accumulation), and tracheobronchial lymph nodes (pigment deposits). A dose dependent increase in anterior cortical subcapsular lens opacities was observed in both male and female rats. Based on dose dependent epithelial hypertrophy at all doses, no NOAEL was established. However, extensive clinical data is available from Phase 3 clinical trials and suggests that lung findings in rats are not predictive of human response.

Reproductive Toxicity: Impairment of fertility was observed in male and female rats at a subcutaneous dose of glycopyrrolate at 1.88 mg/kg/day based upon findings of decreased corpora lutea, implantation sites, and live fetuses. No effects on fertility and reproductive performance were observed in male and female rats at a subcutaneous dose of 0.63 mg/kg/day. Glycopyrrolate was not teratogenic in Wistar rats and New Zealand White rabbits at maternal inhaled doses up to 3.83 mg/kg/day in rats and up to 4.4 mg/kg/day in rabbits. Glycopyrrolate had no effects on peri-natal and post-natal developments in rats at subcutaneous doses up to 1.88 mg/kg/day.

Glycopyrrolate-Indacaterol Combination: Potential additive or synergistic toxicity of the combination of glycopyrrolate and indacaterol was assessed in a 13-week inhalation toxicology study with dogs.

In a 13-week inhalation study in dogs, the toxicity of combination of glycopyrrolate and indacaterol (QVA149) was evaluated and compared to its monoproducts constituents, glycopyrrolate (NVA237) and indacaterol (QAB149). Dogs were exposed to the air control, placebo control (lactose with 1% magnesium stearate), high-dose NVA237, high-dose QAB149, low-dose QVA149, mid-dose QVA149, or high-dose QVA149. Three dogs/sex/group were sacrificed after the 13-week exposure period and an additional two dogs/sex/group in the control and high dose groups were sacrificed after a 30-day recovery period. ECG data indicated that administration of QVA149 was associated with transient tachycardia in a synergistic manner compared to QAB149 or NVA237 alone. Mid- and high-dose QVA149 groups exhibited 45-70% (52-72 BPM) increases in heart rate at 30-60 minutes post-dose. While adverse and test article-related, the effect of QVA149 on heart rate was considered clinically monitorable. There were no dose-limiting histopathological findings in the dog study. Observed toxicity of

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QVA149 was generally consistent with the monoproducts, QAB149 and NVA237, and there was no evidence of significant additive or synergistic toxicity other than increased heart rate.

Indacaterol Monoproduct:
The toxicological profile of indacaterol was fully characterized under NDA 22-383 for ARCAPTA NEOHALER that was approved on July 1, 2011. A detailed description of the nonclinical program for indacaterol is available in the Pharmacology and Toxicology Review of NDA 22383 dated 8/25/09. Indacaterol maleate (indacaterol) is a long-acting beta2-adrenergic agonist (LABA). Pivotal general toxicology studies to support the use of indacaterol were 26- and 39-week inhalation studies in rats and dogs, respectively. NOAELs were identified in both studies. The target organs of toxicity for QAB149 in the rat were the nasal cavity (degeneration of the olfactory epithelium) and larynx (squamous metaplasia). The target organs of toxicity for QAB149 in the dog were the cardiovascular system (increased heart rates, decreased blood pressure, and myocardial fibrosis) and the liver (periportal liver hepatocyte vacuolation due to glycogen deposition). The heart and liver findings in dogs were considered class effects of B_2-adrenergic agonists. Indacaterol had no effects on fertility and reproductive performance in rats following subcutaneous administration. Indacaterol was not teratogenic following subcutaneous administration to rats and rabbits. Indacaterol had no effects on pre- and post-natal development in rats. Indacaterol did not show statistically significant increases in tumor formation in a 26-week carcinogenicity study with TgrasH2 mice or a 2-year carcinogenicity study with Wistar rats.

Labeling:
Dr. Sohn's reviews dated July 7, 2015 and September 24, 2015 recommend changes to product labeling in Indications and Usage under Highlights of Prescribing Information, Section 8.1 (Pregnancy), Section 8.3 (Nursing Mothers), Section 12.1 (Mechanism of Action), Section 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility), and Section 13.2 (Animal Toxicology – Eye findings with Glycopyrrolate). I concur with Dr. Sohn's recommendations for changes to the product label. See Dr. Sohn's reviews for additional details of changes to the product labeling.

Recommendation: From the nonclinical perspective, approval of the application is recommended.

There are no outstanding nonclinical issues,

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/s/

TIMOTHY W ROBISON
10/01/2015

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:	207923 and 207930
Supporting document/s:	SD-1, SD-10, SD-18, SD-20, SD-23, SD-24 (NDA 207923); SD-1, SD-10, SD-11, SD-19, SD-21, SD-24, SD-25 (NDA 207930)
Applicant's letter date:	12/29/14, 5/20/15, 8/12/15, 8/25/15, 9/21/15, 9/22/15 (NDA 207923); 12/29/14, 5/14/15, 5/27/15, 8/12/15, 8/25/15, 9/21/15, 9/22/15 (NDA 207930)
CDER stamp date:	12/29/14, 5/20/15, 8/12/15, 8/25/15, 9/21/15, 9/22/15 (NDA 207923); 12/29/14, 5/14/15, 5/27/15, 8/12/15, 8/25/15, 9/21/15, 9/22/15 (NDAs 207930)
Product:	Glycopyrronium bromide (NVA237) and Indacaterol maleate/Glycopyrronium bromide inhalation (QVA149) powder hard capsules
Indication:	Maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema
Applicant:	Novartis
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Jane J. Sohn, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D., DABT
Division Director:	Badrul A. Chowdhury, M.D., Ph.D.

Reference ID: 3824703

Project Manager: Christine Ford

Template Version: September 1, 2010

Disclaimer

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Any information or data necessary for approval of NDAs 207923 and 207930 that Novartis does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDAs 207923 and 207930.

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1 Executive Summary

1.1 Introduction

Novartis has submitted two applications under NDA 207923 and NDA 207930. The first is a 505 (b) (1) application under NDA 207923 for SEEBRI NEOHALER indicated for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema. SEEBRI NEOHALER is a dry powder inhaler that delivers 15.6 mcg glycopyrrolate (NVA237) per actuation with magnesium stearate and lactose monohydrate as excipients. The maximum recommended dose is one actuation, twice daily (BID), or 31.2 mcg glycopyrrolate per day.

The second application is a 505 (b) (1) application under NDA 207930 for UTIBRON NEOHALER for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema. UTIBRON NEOHALER is a fixed dose, dry powder inhaler that delivers a combination (QVA149) of glycopyrrolate 15.6 mcg (salt) and indacaterol maleate 27.5 mcg (base) indacaterol, (QAB149). The formulation includes magnesium stearate and lactose monohydrate as excipients. The maximum recommended dose is one actuation, twice daily (BID), or 31.2 mcg glycopyrrolate and 55 mcg indacaterol per day. Indacaterol is approved at 75 mcg per day as ARCAPTA NEOHALER for the same indication as the proposed products, under NDA 022383.

NDA 207923 included nonclinical studies with NVA237 alone. Studies to support the development of NVA237 were submitted previously and reviewed under IND 48655. In addition, studies were submitted to IND 48655 to support the use of magnesium stearate as an excipient through the inhalation route.

NDA 207930 included nonclinical studies with glycopyrrolate in combination with indacaterol. Studies to support the combination were submitted previously and reviewed under IND 76377.

The review below includes analysis of the reproductive and development toxicology program conducted with NVA237, a chronic dog study with NVA237, and labeling to support NVA237 under NDA 207923, and QVA149 under NDA 207930. A previous review of the carcinogenicity studies was submitted under NDA 207923 and NDA 207930 on July 7, 2015.

Throughout this review, concentrations for NVA237 are presented as a salt, unless otherwise indicated, which is consistent with the established common name of glycopyrrolate in other products, as recognized by USP. Concentrations of QAB149 and QVA149 are expressed as a base, unless otherwise indicated, which is also consistent with USP convention.

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1.2 Brief Discussion of Nonclinical Findings

The sponsor Novartis has conducted adequate nonclinical safety evaluations to support the approval of SEEBRI NEOHALER and UTIBRON NEOHALER from the nonclinical perspective. Nonclinical studies included pharmacology, safety pharmacology, ADME, general toxicology, genotoxicity, carcinogenicity, and reproductive toxicology. There are no outstanding nonclinical issues at this time.

The summary below is divided into sections for NVA237 (glycopyrrolate), QVA149 (QAB149/glycopyrrolate combination), QAB149 (indacaterol), and excipients. A complete summary for the nonclinical program of QAB149 for the inhalation route of administration is available under NDA 022383; a brief summary is provided below.

NVA237

NVA237 was tested primarily in mice, rats and dogs.

NVA237 is long-acting antimuscarinic antagonist (LAMA), which is often referred to under the more general term anticholinergic. The mechanism of action of NVA237 is similar to that of tiotropium bromide, aclidinium bromide, and umeclidinium bromide, which are currently marketed LAMAs.

In vitro pharmacology studies show that NVA237 is a high affinity, pan active muscarinic antagonist. NVA237 was assessed for muscarinic activity, binding and calcium mobilization at cloned human receptors. NVA237 is comprised of two enantiomers of glycopyrrolate, namely 3S, 2R (NVP-QBA608) and 3R, 2S (NVP-QBA609).

The pivotal general toxicology studies supporting the safety of NVA237 are chronic toxicology studies in rats and dogs. In the 26 week inhalation toxicology study, with a 4 week recovery in rats, animals received estimated achieved doses of 0 (air), 0 (vehicle: 1% magnesium stearate, 99% lactose monohydrate), 0.09, 0.67 and 4.98 mg/kg of NVA237 (salt). The target organs of toxicity were the eyes, lungs (epithelial hypertrophy), seminal vesicles (inflammation), and urinary bladder (inflammation). Eye findings consisted of bilateral and unilateral lenticular changes were observed at the MD and HD in both sexes, and were partially reversible. These changes were characteristic muscarinic receptor inhibition. Lung epithelial hypertrophy was observed at the MD and HD, but was minimal and completely reversible. The cells within the area where epithelial hypertrophy was observed were not Type II pneumocytes, and this hypertrophy may be due to a local irritant effect. The NOAEL was the low dose of 0.09 mg/kg (achieved dose), which is associated with an $AUC_{(0\text{-}24h)}$ of 13.8 ng*h/mL (salt).

In the 39 week inhalation toxicology study, beagle dogs were dosed with 0 (air), 0 (vehicle: 1% magnesium stearate, 99% lactose monohydrate), 0.030, 0.12, 0.33 mg/kg (estimated achieved doses in salt form) through the inhalation route of exposure (6/sex/group controls and HD; 4/sex/group LD and MD), with a 4 week recovery period (2/sex/group controls and HD). The vehicle group showed no notable differences compared to the air control. Test article related ophthalmic findings were conjunctival hyperemia (bilateral), corneal opacity (bilateral), and focal nuclear opacity (bilateral)

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observed at the HD. These ophthalmic findings were reversible. There was a reduction in lacrimal secretion at Weeks 3 and 6 at the HD, which reversed and is an expected pharmacological effect of the test article. Increased heart rate was noted in MD and HD males and females (+30-69%, compared to the pretest value on the same day) at Weeks 13, 26, and 39. This was an expected pharmacological effect of the test article. The targets organs of toxicity were the pharynx (inflammation, ectasia of the ducts and/or alveoli), lacrimal gland (hypertrophy), and mandibular salivary glands (hypertrophy). All findings reversed after the recovery period. The NOAEL was determined as the low-dose, however, some findings were determined to be clinically monitorable and/or not dose limiting. Therefore, the supporting dose was determined to be the mid dose of 0.12 mg/kg, based on ophthalmic findings. The supporting dose is associated with an AUC of 26.6 ng*h/mL in males and 42.0 ng.h/mL (salt) in females.

There are adequate safety margins for the proposed clinical dose of 31.2 mcg NVA37 for local toxicity in the lungs and systemic toxicity based on the NOAEL identified in the 26 week rat and the supporting dose in the 39 week dog toxicology studies.

Regarding genetic toxicology, NVA237 was negative in genetic toxicology testing based on results from the *in vitro* bacterial reverse mutation assay, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* rat micronucleus test.

Carcinogenicity assessments were performed in a traditional 2-year bioassay in rats, and a 26 week bioassay in transgenic mice (Tg.rasH2 mice). Both bioassays were negative for test-article related tumors. A safety margin of 330 is provided by the rat carcinogenicity study, based on AUC exposure.

The standard battery of reproductive and developmental toxicity studies were completed with NVA237 in rats and rabbits. To assess fertility, rats received subcutaneous (SC) doses of 0 (vehicle, 5% dextrose), 0.19, 0.63, 1.88 mg/kg/day (salt). Dosed animals had a higher percentage of abnormal estrous cycles (shortened, extended, prolonged, acyclic) compared to control, but this was not dose dependent (control 22%, LD 44%, MD 40%, HD 40%). Based on the high percentage of abnormal cycles in all groups, and the lack of a clear dose response, it is difficult to determine if this is a test article related finding. Impairments of fertility based upon decreases of implantation sites and live fetuses were observed at the high dose; therefore, a reproductive and fertility no adverse effect level was identified at the mid dose of 0.63 mg/kg.

Embryofetal development was investigated in gravid rats and rabbits. NVA237 was negative for teratogenicity.

Pre- and postnatal development (PPND) study was investigated in F0 female rats. F0 females developed a dose dependent decrease in body weight gain, showing that dosing was adequate. F0 dams showed no test article related effects on reproductive parameters. There were no negative test article related effects on viability of F1 offspring. F1 generation adults showed no changes in body weight, physical or neurological development, or reproductive parameters. The F2 generation was terminated at GD13, therefore there was no information collected regarding sex, body

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weight, or malformations. Overall, there were no teratogenic or non-teratogenic findings with NVA237.

QVA149 (NVA237 and QAB149)

In a 13-week inhalation study in dogs, the toxicity of combination product QVA149 was evaluated and compared to its monoproduct constituents QAB149 and NVA237. ECG data indicate that administration of QVA149 is associated with transient tachycardia in a synergistic manner compared to QAB149 or NVA237 alone. Mid- and high-dose QAV149 groups exhibited increased heart rate at 30-60 minutes post-dose. While adverse and test article-related, the effect of QAV149 on heart rate is considered clinically monitorable. There were no dose limiting histopathological findings in the dog study. Observed toxicity of QVA149 was generally consistent with the monoproducts, QAB149 and NVA237, and there was no evidence of additive or synergistic toxicity.

In an inhalation embryo-fetal development (EFD) study in rats, the maternal and embryo-fetal toxicity of the combination product QVA149 was evaluated and compared to its monoproducts constituents QAB149 and NVA237. There was no evidence of QVA149-related maternal or fetal toxicity in this study.

QAB149 (Indacaterol)

The toxicological profile of indacaterol has been characterized previously under NDA 22-383 for ARCAPTA NEOHALER, approved July 1, 2011. A summary of the nonclinical development of indacaterol is available in a review under NDA 22383, dated 8/25/09. Indacaterol maleate (indacaterol) is a long acting beta2-agonist (LABA). Pivotal general toxicology studies to support the use of indacaterol were 26 and 39 week inhalation studies in rats and dogs, respectively. NOAELs were identified in both studies. The target organs of toxicity for QAB149 in the rat are nasal cavity (degeneration of the olfactory epithelium) and larynx (squamous metaplasia). The target organs of toxicity in the dog are the cardiovascular system (increased heart rates), decreased blood pressure and myocardial fibrosis (class effects) and the liver (periportal liver hepatocyte vacuolation due to glycogen deposition; class effect).

With respect to teratogenic effects, indacaterol is designated Pregnancy Category C. Indacaterol was not teratogenic following subcutaneous administration to rats and rabbits at doses up to 1 mg/kg. It is not known if indacaterol is excreted in human milk.

Carcinogenicity was evaluated in a 26 week study in Tg.rasH2 mice using oral administration and in a 2 year rat study using inhalation administration. Indacaterol did not show statistically significant increases in tumor formation in mice or rats.

Excipients

The drug product includes lactose and magnesium stearate as excipients. There was no safety concern for either compound.

Conclusions

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The applicant has a complete nonclinical pharmacology and toxicology program for NVA237 under NDA 207923, for QAB149 as previously reviewed under NDA 22383, and for the QVA149 under NDA 207930. Therefore, there is adequate nonclinical support for the safety of the proposed clinical dose doses of NVA237 and QVA149.

1.3 Recommendations

1.3.1 Approvability

The applicant has provided complete nonclinical pharmacology and toxicology programs for NVA237 and QVA149, which support the safety of the proposed clinical dose of glycopyrrolate 15.6 mcg BID, and the fixed dose combination of glycopyrrolate 15.6 mcg and indacaterol 27.5 mcg BID, respectively, for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema. From the Pharm/Tox perspective, the application is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

See Labeling Recommendations

1.3.3 Labeling

Recommended line editing of the proposed text for the nonclinical sections of the UTIBRON NEOHALER and SEEBRI NEOHALER and labels are provided below. Changes are presented as strikethroughs for deletions or in red font for additions.

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UTIBRON NEOHALER

INDICATIONS AND USAGE (Highlights of Prescribing Information)

~~QVA149~~ UTIBRON NEOHALER is a combination of indacaterol, (b) (4) and glycopyrrolate, (b) (4) indicated for the long term, maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema. (1)

1 INDICATIONS AND USAGE

~~QVA149~~ UTIBRON NEOHALER® is a combination (b) (4) of indacaterol and glycopyrrolate indicated for the long-term, maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema.

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C:

(b) (4)

There are no adequate and well-controlled studies with UTIBRON~~QVA149~~ NEOHALER or its individual components, glycopyrrolate and indacaterol, in pregnant women. Animal reproduction studies were conducted with individual components, indacaterol and glycopyrrolate. Because animal reproduction studies are not always predictive of human response, UTIBRON~~QVA149~~ NEOHALER should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women should be advised to contact their physician if they become pregnant while taking UTIBRON NEOHALER.

(b) (4)

Indacaterol: Indacaterol was not teratogenic (b) (4) ~~).~~ in Wister rats and New Zealand rabbits at doses at approximately (b) (4) times, respectively, the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 1 mg/kg/day in rats and rabbits).

Glycopyrrolate: Glycopyrrolate was not teratogenic (b) (4) -in Wistar rats and New Zealand rabbits at approximately (b) (4) times, respectively, the MRHD in adults (on an AUC basis at maternal inhaled doses up to 3.83 mg/kg/day in rats and up to 4.4 mg/kg/day in rabbits).

Nonteratogenic Effects:

Indacaterol: There were no effects on peri-natal and post-natal developments in rats at approximately (b) (4) times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 0.3 mg/kg/day).

Glycopyrrolate: There were no effects on peri-natal and post-natal developments in rats at approximately (b) (4) times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 1.88 mcg/kg/day).

(b) (4)

8.3 Nursing Mothers

~~*QVA149*~~ *UTIBRON NEOHALER:* It is not known whether UTIBRON (b) (4) -NEOHALER is (b) (4) excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when ~~QVA149~~ UTIBRON NEOHALER is administered to a nursing woman. Since there are no data from well-controlled human studies on the use of UTIBRON~~QVA149~~ NEOHALER- (b) (4) by nursing mothers, based on the data for the individual components, a decision should be made whether to discontinue nursing or to discontinue UTIBRON~~QVA149~~ NEOHALER, taking into account the importance of UTIBRON~~QVA149~~ NEOHALER to the mother.

Indacaterol: It is not known whether indacaterol is excreted in human breast milk. Indacaterol (including its metabolites) have been detected in the milk of lactating rats.

Glycopyrrolate: It is not known whether glycopyrrolate is excreted in human breast milk. Glycopyrrolate (including its metabolites) have been detected in the milk of lactating rats and reached up to 10-fold higher concentrations in the milk than in the blood of the dam. (b) (4)

12.1 Mechanism of Action

QVA149 NEOHALER contains both indacaterol and glycopyrrolate. The mechanisms of action described below for the individual components apply to UTIBRON~~QVA149~~ NEOHALER. The~~se~~ drugs represent 2 different classes of medications (a LABA and an anticholinergic) that have different and additive effects on clinical and physiological indices.

Indacaterol: Indacaterol is a long-acting beta$_2$-adrenergic agonist (LABA). When inhaled, indacaterol acts locally in the lung as a bronchodilator. Although beta$_2$-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta$_1$-receptors are the predominant receptors in the heart, there are also beta$_2$-adrenergic receptors in the human heart comprising 10% to 50% of the total adrenergic receptors. The precise function of these receptors is not known, but their presence raises the possibility that even highly selective beta$_2$-adrenergic agonists may have cardiac effects.

The pharmacological effects of beta$_2$-adrenoceptor agonist drugs, including indacaterol, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic monophosphate). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells. In vitro studies have shown that indacaterol has more than 24-fold

greater agonist activity at $beta_2$-receptors compared to $beta_1$-receptors and 20-fold greater agonist activity compared to $beta_3$-receptors. The clinical significance of these findings is unknown.

Glycopyrrolate: Glycopyrrolate is a long-acting muscarinic antagonist (LAMA), which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical in vitro as well as in vivo studies, prevention of methacholine- (b) (4) -induced bronchoconstrictive effects was dose-dependent and lasted longer than 24 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of glycopyrrolate is predominantly a site-specific effect.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

~~QVA149~~ UTIBRON NEOHALER:

No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with ~~QVA149~~ UTIBRON NEOHALER; however, studies are available for the individual components, indacaterol and glycopyrrolate, as described below.

Indacaterol: (b) (4) Indacaterol produced no treatment-related increases in the incidence of tumors in a 2-year inhalation study in Wistar rats at inhaled doses up to 2.09 mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis, respectively). A 26-week oral (gavage) study in CB6F1/TgrasH2 hemizygous mice with indacaterol at doses up to 600 mg/kg/day did not show any evidence of tumorigenicity.

(b) (4)

Indacaterol tested negative in the following genotoxic assays: (b) (4) the *in vitro* Ames test, *in vitro* chromosome aberration test in V79 Chinese hamster cells, and *in vivo* rat bone marrow micronucleus test- (b) (4).

Indacaterol had no effects on fertility and reproductive performance in male and female Wistar rats at subcutaneous doses up to 2 mg/kg/day (approximately (b) (4) and (b) (4) times in males and females, respectively, the MRHD in adults on an AUC basis).

(b) (4)

Glycopyrrolate:- (b) (4)

Glycopyrrolate produced no treatment-related increases in the incidence of tumors in a 2-year inhalation study in Wistar rats at inhaled doses up to 0.56 mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis, respectively). A 26-week oral (gavage) study in male and female TgrasH2 mice that received glycopyrrolate at doses up to 93.8 and 125.1 mg/kg/day, respectively, did not show any evidence of tumorigenicity.

(b) (4) lycopyrrolate tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* rat bone marrow micronucleus assay.

(b) (4)

Impairment of fertility was observed in male and female (b) (4) rats at subcutaneous doses of 1.88 mg/kg/day (approximately (b) (4) and (b) (4) times (b) (4) the MRHD in adults on an AUC basis) based upon findings of decreased corpora lutea, implantation sites and live fetuses. No effects on fertility and reproductive performance in male and female rats were observed at a subcutaneous dose of 0.63 mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis).

13.2 Animal Toxicology

(b) (4) Eye findings were observed in male and female Wistar rats at inhaled doses of 0.67 (b) (4) mg/kg/day (approximately (b) (4) and (b) (4) times, respectively, the MRHD in adults on an AUC basis) based upon findings of anterior capsule opacity, prominent anterior suture line, and anterior cataract. No eye findings in (b) (4) Wistar rats were observed at inhaled doses of 0.09 mg/kg (approximately (b) (4) times the MRHD in adults on an AUC basis). Eye findings were observed in (b) (4) beagle dogs at inhaled doses of 0.33 mg/kg/day (approximately (b) (4) and (b) (4) times (b) (4) the MRHD in adults on an AUC basis) based upon findings of conjunctival hyperemia, corneal opacity, (b) (4) No eye findings in (b) (4) beagle dogs were observed at inhaled doses of 0.12 mg/kg/day (approximately (b) (4) times in (b) (4) the MRHD in adults on an AUC basis).

SEEBRI NEOHALER

INDICATIONS AND USAGE (Highlights of Prescribing Information)

SEEBRI NEOHALER is an anticholinergic indicated for the long-term, maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema. (1)

1 INDICATIONS AND USAGE

SEEBRI NEOHALER is [redacted] (b) (4) -indicated for the long-term, maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema.

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C:

[redacted] (b) (4) ~~ry~~

There are no adequate and well-controlled studies with SEEBRI NEOHALER in pregnant women. Because animal reproduction studies are not always predictive of human response, SEEBRI NEOHALER should only be used during pregnancy if the [redacted] (b) (4) -potential benefit [redacted] (b) (4) -justifies the potential risk to the fetus. Women should be advised to contact their physician if they become pregnant while taking SEEBRI NEOHALER.

Glycopyrrolate was not teratogenic in Wistar rats and New Zealand White rabbits at approximately [redacted] (b) (4) and [redacted] (b) (4) times, respectively, the MRHD in adults (on an AUC basis at maternal inhaled doses up to 3.83 mg/kg/day in rats and up to 4.4 mg/kg/day in rabbits).

Nonteratogenic Effects:

Glycopyrrolate had no effects on peri-natal and post-natal developments in rats at approximately [redacted] (b) (4) times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 1.88 mcg/kg/day).

[redacted] (b) (4)

[redacted]

8.3 Nursing Mothers

It is not known whether [redacted] (b) (4) -SEEBRI NEOHALER is excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when SEEBRI NEOHALER is administered to a nursing woman. Since there are no data from well-controlled human studies on the use of SEEBRI NEOHALER by nursing mothers, a decision should be

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made whether to discontinue nursing or to discontinue SEEBRI NEOHALER, taking into account the importance of SEEBRI NEOHALER to the mother.

It is now known whether glycopyrrolate is excreted in human breast milk. Glycopyrrolate (including its metabolites) have been detected in the milk of lactating rats and reached up to 10-fold higher concentrations in the milk than in the blood of the dam. (b) (4)

12.1 Mechanism of Action

(b) (4)

Glycopyrrolate is a long-acting muscarinic antagonist (LAMA), which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical in vitro as well as in vivo studies, prevention of methacholine- (b) (4) -induced bronchoconstrictive effects was dose-dependent and lasted longer than 24 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of glycopyrrolate is predominantly a site-specific effect.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Glycopyrrolate produced no treatment-related increases in the incidence of tumors in a 2-year inhalation study in Wistar rats at inhaled doses up to 0.56 mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis, respectively). A 26-week oral (gavage) study in male and female TgrasH2 mice that received glycopyrrolate at doses up to 93.8 and 125.1 mg/kg/day, respectively, did not show any evidence of tumorigenicity.

(b) (4)

lycopyrrolate tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* rat bone marrow micronucleus assay.

Impairment of fertility was observed in male and female rats at subcutaneous doses of 1.88 mg/kg/day (approximately (b) (4) and (b) (4) times (b) (4) the MRHD in adults on an AUC basis) based upon findings of decreased (b) (4) implantation sites and live fetuses. No effects on fertility and reproductive performance in male and female rats were observed at a subcutaneous dose of 0.63 mg/kg/day (approximately (b) (4) times the MRHD

in adults on an AUC basis) (b) (4)

13.2 Animal Toxicology

Eye findings were observed in (b) (4) Wistar rats at inhaled doses of 0.67 (b) (4) mg/kg/day (approximately (b) (4) times, (b) (4) the MRHD in adults on an AUC basis) based upon findings of anterior capsule opacity, prominent anterior suture line, and anterior cataract. No eye findings in (b) (4) Wistar rats were observed at inhaled doses of 0.09 mg/kg (approximately (b) (4) times the MRHD in adults on an AUC basis). Eye findings were observed in (b) (4) beagle dogs at inhaled doses of 0.33 mg/kg/day (approximately (b) (4) times (b) (4) the MRHD in adults on an AUC basis) based upon findings of conjunctival hyperemia, corneal opacity, (b) (4) No eye findings in male and female beagle dogs were observed at inhaled doses of 0.12 mg/kg/day (approximately (b) (4) times (b) (4) the MRHD in adults on an AUC basis.)

2 Drug Information

2.1 Drug

CAS Registry Number:
1) 596-51-0
2) 435273-74-8

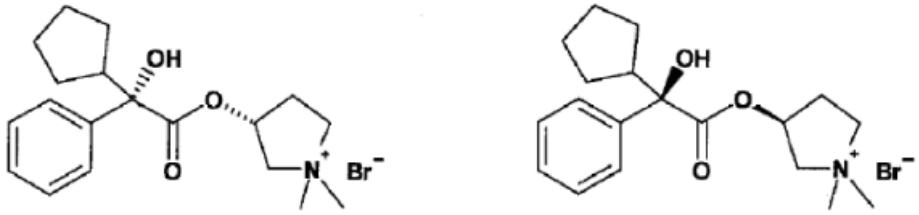
Generic Name:
1) Glycopyrrolate (salt form, the base is glycopyrronium bromide)
2) Indacaterol maleate (indacaterol)

Code Name:
1) NVA237 (base)
2) QAB149 (base), QAB149 maleate (salt form), QAB149-AFA.001 (maleate [redacted] (b) (4))

Chemical Name(s):
1) 3-[(cyclopentylhydrophenylacetyl) oxy)]-1, 1-dimethylpyrrolidinium bromide
2) (R)-5-[2-(5,6-Diethylindan-2-ylamino)-1-hydroxyethyl]-8-hydroxy-1H-quinolin-2-one maleate; 5-[(1R)-2-[(5,6)-Diethyl-2,3-dihydro-1H-inden-2-yl)amino]-1- hydroxyethyl]-8-hydroxy-2(1H)-quinolinone maleate

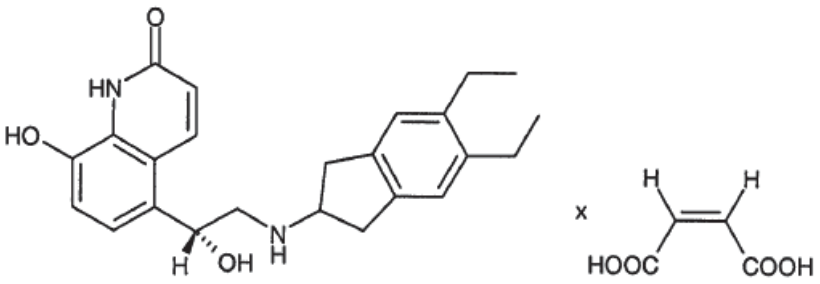
Molecular Formula/Molecular Weight:
1) $C_{19}H_{28}NO_3xBr$ (MW: 398.33, base)
2) $C_{24}H_{28}N_2O_3$. C4H4O4 (MW: 392.49 + 116.07 = 508.56)

Structure and Biochemical Description:
1) NVA237 is a racemic mixture of the *S,R* and *R,S* enantiomers [redacted] (b) (4) (CMC review dated September 25, 2012). The following figure shows the base, glycopyrronium bromide.



S,R *R,S*

2) QAB149



(Sponsor's figure, report DS_4001186)

Pharmacologic Class:
1) Anticholinergic
2) β_2-adrenergic agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 48655: Glycopyrrolate (NVA237)
IND 76377: Indacaterol maleate (QAB149) and Glycopyrrolate (QVA149)
NDA 022383: ARCAPTA NEOHALER (indacaterol maleate), approved July 1, 2011.
IND (b) (4): Indacaterol maleate (QAB149)

2.3 Drug Formulation

The sponsor is proposing products with NVA237 (glycopyrrolate), and QVA149 (NVA237 in combination with QVA149)

The proposed clinical formulation for NVA237 is shown below. The excipients are lactose and magnesium stearate. NVA237 will be delivered in a capsule by the Neohaler device, which is approved for use under NDA 022383 (ARCAPTA NEOHALER).

Table 1: Formulation for glycopyrronium bromide inhalation powder hard capsule

Ingredient	Amount per capsule (mg)	Function
Capsule fill		
Glycopyrronium bromide	0.01561#	Drug substance
Lactose monohydrate, USP	24.9469	(b) (4)
Magnesium stearate, USP	0.0375	
(Capsule fill weight)	(b) (4)	
Empty capsule shell		
Hypromellose	(b) (4)	(b) (4)

Printing Ink (see next section) (Capsule shell weight)	(b) (4)	Identification
Printing Ink		
(b) (4)		

Corresponds to 0.0125 mg of GP active moiety and a target delivered dose of (b) (4) mcg

QVA149 will be delivered in a capsule by the Neohaler device, which is approved for use under NDA 022383 (ARCAPTA NEOHALER). The proposed clinical formulation is shown in the table below.

Table 2: Formulation for Indacaterol maleate/glycopyrronium bromide inhalation powder hard capsule

Ingredient	Amount per capsule (mg)	Function
Capsule fill		
Indacaterol maleate	(b) (4)	Drug substance
Glycopyrronium bromide	0.01561	Drug substance
Lactose monohydrate, USP	24.9112	(b) (4)
Magnesium stearate, USP	0.0375	
(Capsule fill weight)	(b) (4)	
Empty capsule shell		
Hypromellose	(b) (4)	(b) (4)
(b) (4)		
Printing Ink (see next section) (Capsule shell weight)		
Printing Ink		
(b) (4)		

(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

There are no remaining concerns for impurities or degradants from the nonclinical perspective.

On July 6, 2015, CMC reviewer Dr. Arthur Shaw identified a potential concern with the proposed acceptance criteria of degradants (b) (4) at "(b) (4) %" in the original submission for NDA 207923. Novartis stated that the proposed levels of the degradants were supported by safety data from a 4 week rat inhalation study with the degradants, and the levels of the two degradants as metabolites of GP in general toxicology studies.

An Information Request was sent on July 30, 2015, requesting that Novartis decrease the acceptance criteria of both degradants from NMT (b) (4) % to NMT (b) (4) %, or provide a 13 week animal study with the degradants to support their safety for a chronic indication, consistent with ICH Guideline Q3B (R2): Impurities in New Drug Products. On August 12, 2015, Novartis repeated that the safety of the degradants was support by a 4 week rat inhalation study with the degradants, and the levels of the two degradants as metabolites of GP in general toxicology studies.

On August 18, 2015 a second Information Request was sent further explaining that for the proposed chronic indication, a 13 week study is considered appropriate, and that the "degradants should be administered by the inhalation route to address local toxicity resulting from deposition of the degradants in the respiratory tract. The presence of the degradants as metabolites does not qualify them for the local toxicity resulting from direct deposition of degradants in the lung airways and alveoli." Therefore the nonclinical justification provided by the sponsor was not considered acceptable. On August 25, 2015, Novartis agreed to acceptance criteria of NMT (b) (4) % for degradants (b) (4).

2.6 Proposed Clinical Population and Dosing Regimen

Adults with COPD, including chronic bronchitis and emphysema, are recommended to use one oral inhalation (one actuation) of SEEBRI NEOHALER, or UTIBRON NEOHALER, twice daily. One actuation of SEEBRI NEOHALER delivers 15.6 mcg of

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glycopyrrolate (NVA237). One actuation of UTIBRON NEOHALER delivers 15.6 mcg of glycopyrrolate and 27.5 mcg indacaterol (QVA149).

2.7 Regulatory Background

DPARP and Novartis held numerous meetings and exchanged written communications to discuss the development of NVA237 under IND 48655, and QVA149 under IND 76377. Safety for QAB149 is based on Novartis' right of reference to NDA 22383 for ARCAPTA NEOHALER, which is owned by Novartis.

Key interactions under INDs 48655 and 76377 are summarized in the table below.

Application	API	Key regulatory events	Dates
NDA 207923	NVA237	Filing of NDA	12/29/14
NDA 207930	QVA149	Filing of NDA	12/29/14
NDA 22383	QAB149	Approved	7/1/11
IND 48655	NVA237	PreIND meeting	6/9/04
		Rat carcinogenicity protocol agreement	10/5/06
		Written responses, nonclinical PK	11/30/06
		IND filing	4/27/07
		EOP2 meeting	7/15/08
		Type B EOP2A meeting	1/29/09
		Tg mouse carcinogenicity protocol agreement	2/24/10
		Type B meeting, support for NDA	9/28/11
IND 76377	QVA149	Pre-IND meeting	6/13/07
		IND filing	9/7/07
		Type B meeting	9/27/11
		EOP2 meeting	3/7/12
		Type C meeting, clinical development plan	12/11/12
		Written responses, clinical development plan	2/15/13
		Pre-NDA meeting	3/19/14

Under IND 48655, an EOP2 meeting was held on 7/15/08, and reference was made to the pre-IND meeting on 6/4/04 with the previous sponsor (not Novartis), whereby FDA recommended a 6 month inhalation study in rats and pending the outcome of the inhalation and genotoxic studies, a single carcinogenicity study may be required. This determination was in the context of Novartis submitting NDA 207923 as a 505 (b) (2) application, referencing an approved glycopyrrolate product. Glycopyrrolate had been approved in 1974 for the chronic treatment of peptic ulcers at a daily oral dose of 6 mg. No carcinogenicity studies had been conducted for the currently approved glycopyrronium formulations. As NDA 207923 has been submitted as a 505 (b) (1) application instead, a stand-alone nonclinical development program has been

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submitted, including chronic toxicity studies in the rat and dog, and carcinogenicity studies in the rat and mouse.

Under IND 76733, a pre-IND meeting was held on 6/13/07. Regarding the nonclinical development of QVA149, Novartis asked if "two 2-week inhalation studies in rats and dogs, one 13-week repeat dose inhalation toxicity study in dogs, as well as one embryo fetal development inhalation study for the combination, in addition to extensive toxicity data on the individual components, will be sufficient to support an application for a marketing authorization?" The Agency agreed pending the need for additional toxicity studies in the event of unexpected findings, and the development of a combination drug product (b) (4) It was also agreed the dog was the most appropriate species in which to conduct the 13-week inhalation toxicity study and the rat is the most appropriate species in which to conduct the embryo fetal development study. The Agency recommended the use of special stains (e.g., trichrome) or assessment of troponin T levels to more clearly delineate cardiac lesions.

Under IND 76733, a Type B meeting was held on 9/27/11. Novartis asked if the "non-clinical safety studies conducted with the combination of QAB149 and NVA237 at a ratio of 3:1 qualify the QVA149 combination for clinical use at an adjusted dose ratio of 1.1:1?" The Agency agreed.

3 Studies Submitted

3.1 Studies Reviewed

The tables of studies below reference previous reviews, and the current review. "NDA Review 1" refers to the review dated 7/7/15 under NDAs 207923/207930, which includes a carcinogenicity assessment. "NDA Review 2" refers to the current review.

NDA 207923

Review	Study no.	Title
		Pharmacology
		Primary Pharmacology
IND 48655, 6/20/2007	RD-2005-01557	Antibronchoconstrictor and cardiovascular effects of inhaled glycopyrronium, ipratropium and tiotropium in rhesus monkeys
IND 48655, 6/20/2007	RD-2006-01999	Potency, selectivity, duration of action and potential side effects of glycopyrronium in Brown Norway rats
IND 48655, 6/20/2007	RD-2006-02342	Duration of action of muscarinic receptor antagonism in rat isolated trachea
IND 48655, 6/20/2007	RD-2007-00209	In vitro muscarinic receptor activity of glycopyrronium
NDA 207923 2nd review	RD-2007-00409	In-vitro characterisation of the binding properties of the four isomers of glycopyrrolate plus NVP-QAM254 at human M1-5 receptors

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IND 48655, 6/20/2007	RD-2011-50035	Duration of action of glycopyrrolate (AD237) formulations on methacholine (MCh) induced bronchoconstriction in anaesthetized rabbits.
		Safety Pharmacology
NDA 207923 2nd review; IND 48655, 6/20/2007	R0510129	Intravenous (bolus) telemetry study in dogs including sighting phase
IND 48655, 6/20/2007	R0618559	Electrophysiological studies in the isolated rabbit heart
NDA 207923 2nd review	R0670652	A pharmacological assessment of the effect of QVA149 on the central nervous system and the respiratory system of the albino rat
NDA 207923 2nd review	R0770861	Effects of QAB149, NVA237, and Combination Mixture QVA149 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
		Pharmacokinetics
		Distribution
IND 48655, 6/20/2007	DMPK-r0600252	In vitro blood distribution and plasma protein binding of [14C]NVA237 including stability in blood and plasma of rat, dog, and human
NDA 207923 2nd review	DMPK-r1000614	Galactogenic transfer, pharmacokinetics and metabolism in milk and plasma after a single intravenous (4 mg/kg) administration of [14C6]NVA237 bromide
		Metabolism
IND 48655, 6/20/2007	DMPK-r0600285	In vitro biotransformation of [14C]NVA237 in rat, dog and human liver and lung microsomes, as well as in rat and human hepatocytes
		Excretion
NDA review 2	DMPK-r0900674	Disposition in male rats after single intravenous (4 mg/kg) and oral administration (30 mg/kg) of [14C6]NVA237 bromide and pharmacokinetics after intra-tracheal administration (1.3 mg/kg) of NVA237 bromide
NDA review 2	DMPK-r1000073	Disposition in male mice after single intravenous (3 mg/kg) and oral administration (25 mg/kg) of [14C6]NVA237 bromide
		Toxicology
		Repeat Dose
NDA review 2	r0670548	NVA237: A 39-week inhalation toxicity study of a powder formulation in dogs with a 4-week recovery period

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IND 48655, 9/15/2004, summary review	r0852240	AD 237: Glycopyrronium bromide dry powder: DRF Inhalation Toxicity Study in the Dog
IND 48655, 6/20/2007; 5/31/2007 memo; 9/15/2004 summary review	r0852241	AD 237: Glycopyrronium bromide dry powder: 4-week inhalation toxicity study in the dog
IND 48655, 3/30/2009 (supporting document 1/20/09), 9/25/2006 (13-week interim toxicity report)	r0580297/79031	A 26-week inhalation toxicity study of a powder formulation in the albino rat with a 28-day recovery period and a 13-week interim toxicity study
IND 48655, 9/15/2004, summary review	r0848191	AD 237: Glycopyrronium bromide dry powder: maximum repeatable dose study in rats by inhalation administration for 7 days
IND 48655, 6/20/2007; 5/31/2007 memo, IND 48655, 9/15/2004, summary review	r0848192	AD 237: Glycopyrronium bromide dry powder: 4 week inhalation toxicity study in rats followed by a 2 week recovery period
NDA review 2, summary review	r0870723	2-week subcutaneous dose range finding toxicity study in rats including sighting part
		Genotoxicity
IND 48655, 6/20/2007	r0225012-D6171	AD 237: Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*
IND 48655, 6/20/2007	r0225013-D6172	AD 237: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes
IND 48655, 6/20/2007	r0335014-D6172	AD 237: Induction of micronuclei in the bone marrow of treated rats
		Carcinogenicity
IND 48655, 2/24/2010	r0770666	1 week oral (gavage) DRF study in mice
NDA review 1	r770668	26-week oral gavage carcinogenicity in RasH1 mice
IND 48655,	r770669	A 4-week DRF toxicity study of NVA237 administered by

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2/24/2010		oral gavage to WT CB6F1 mice
IND 48655, 2/24/2010	r0970010	A repeat 4-week DRF toxicity of NVA237 administered by oral gavage to WT CB6F1 mice
NDA review 1	r670435	104-week inhalation carcinogenicity study in rats
		Reproductive and Developmental Toxicity
NDA review 2	r870596	A SC fertility and early embryonic study in rats
NDA review 2	r680005	An inhalation embryo fetal development dose-range finding study in rabbits
NDA review 2	r680006	An inhalation embryo fetal development study in rats
NDA review 2	r870597	An inhalation embryo fetal development study in the rabbit
NDA review 2	r870598	A subcutaneous pre and postnatal study in the rat
		Other PK Studies
IND (b) (4) (b) (4)	(b) (4)	26 week inhalation toxicity study with 1% (w/w) Mg-stearate in lactose monohydrate blend in Mg-stearate in rats

NDA 207930

Review	Study no.	Title
		Safety Pharmacology
NDA 2nd review	691877	A pharmacological assessment of the effect of QVA149 on the central nervous system and the respiratory system of the albino rat; Final report amendment no. 1
IND 76377, 7/31/2008	691878*	A pharmacological assessment of a single administration (inhalation) of QVA149 on the cardiovascular system in male beagle dog; Final report amendment no. 1 using telemetry *
NDA 2nd review	770861	Effects of QAB149, NVA237, and Combination Mixture QVA149 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
		Toxicology
		Repeat Dose
IND 76377, 7/31/2008	670547/79217	QVA149: A 14-day inhalation study of a combined powder formulation in the dog with a 14-day recovery
IND 76377, 9/18/2013	670756/79359	13-week combination inhalation dog study
IND 76377, 7/31/2008	670546/79298	A 2-Week Combination Inhalation Study in Wistar rats with a 2-Week Recovery Period
		Reproductive and Developmental Tox
IND 76377, 9/18/2013	670755/901312	EFD combination inhalation study in rats

* Note: This study was previously designated study #670639, and included "In-Life Evaluations" in the title.

Reference ID: 3824703

3.2 Studies Not Reviewed

NDA 207923

Study no.	Title
	Pharmacology
	Primary Pharmacology
RD-2008-00940	Magnesium stearate - solubility in survanta
RD-2010-00795	(b) (4) Pharmacology Data Report On Compound (b) (4) For Novartis Institutes for BioMedical Research
RD-2010-00825	(b) (4) s Pharmacology Data Report On Compound (b) (4) For Novartis Institutes for BioMedical Research
RD-2011-00111	In vitro pharmacology of NVP-QAM254 (glycopyrrolate) at muscarinic M4 and M5 receptors
RD-2011-50037	Evaluation of the bronchodilator activity of tiotropium on methacholine (MCh) induced bronchoconstriction in anaesthetized rabbits.
RD-2011-50356	NVP-QAW665: In vitro Safety Pharmacology Profile
RD-2012-00051	Profiling of NVA237 in a [35S]-GTPyS assay at the Muscarinic M1-M5 receptors.
	Secondary Pharmacodynamics
RD-2011-50439	NVP-QAX241: In vitro Safety Pharmacology Profile
RD-2012-50097	NVP-QCP745: In vitro Safety Pharmacology Profile
RD-2012-50099	NVP-QCP746: In vitro Safety Pharmacology Profile
	Safety Pharmacology
R0670653	A pharmacological assessment of a single administration (inhalation) of QVA149 on the cardiovascular system in male beagle dog using telemetry
	Pharmacokinetics
	Analytical Methods and Validation Reports
CDL101454	Validation of a method for the determination of NVA237 in rat plasma (Li heparin) by liquid chromatography-tandem mass spectrometry (LC-MS/MS)
DMPK-ARA-05	Validation of a LC-MS/MS bioanalytical method for the measurement of AD 237 in Han Wistar rat plasma
DMPK-R0500922	Quantitative determination of NVA237 (AD237) in plasma of dog by using on-line SPE coupled with LC-MS/MS
DMPK-R0500922A	Quantitative determination of NVA237 (AD237) in plasma of rat by using on-line SPE coupled with LC-MS/MS
DMPK-R0500922B	Quantitative determination of NVA237 (AD237) in plasma of rabbit by using on-line SPE coupled with LC-MS/MS
DMPK-R0500922C	Quantitative determination of NVA237 in mouse plasma by using on-line SPE and LC-MS/MS
DMPK-R0600034-01	[13CD3]NVA237 Synthesis and release analysis
DMPK-R0600086-01	[14C6]NVA237 Synthesis and release analysis

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DMPK-R0600354C	Quantitative determination of NVA237 in dog plasma by LC-MS/MS
DMPK-R0600354D	Quantitative determination of NVA237 in rat plasma by LC-MS/MS
DMPK-R0600354F	Quantitative determination of NVA237 in mouse plasma by LC-MS/MS
DMPK-R0600354H	Quantitative determination of QBA608 and QBA609 (enantiomers of NVA237) in dog plasma by LC-MS/MS
DMPK-R0600354I	Quantitative determination of QBA608 and QBA609 (enantiomers of NVA237) in rat plasma by LC-MS/MS
DMPK-R0700003-01	[13CD3]NVA237 Synthesis and release analysis
DMPK-R0900210	Transfer validation of a method for the quantification of NVA237 in mouse plasma by LC-MS/MS
DMPK-R0900301	[14C6]NVA237 Synthesis and release analysis
DMPK-R0900453	Quantitative determination of NVA237 in rabbit plasma and fetus by LC-MS/MS
DMPK-R0900453a	Quantitative determination of NVA237 in rat plasma by LC-MS/MS
DMPK-R09900938	[14C6]NVA237 Synthesis and release analysis
DMPK-R1000563	[13C6]CJL603 (metabolite of NVA237) Synthesis and release analysis
DMPK-R1000573	[13CD3]NVA237 Synthesis and release analysis
DMPK-R1100006	[14C6]NVA237 Synthesis and release analysis
DMPK-R1100006A	Validation of an LC-MS/MS Method for the Determination of CJL603 in Rat Plasma (K2EDTA)
DMPK-R1100006B	Quantitative determination of CJL603 in mouse plasma by LC-MS/MS
RCC-851688	Transfer validation of an analytical method for the determination of AD 237 in rat plasma
RCC-852647	Transfer validation of an analytical method for the determination of AD 237 in dog plasma
	Absorption
DMPK-r0800219	Pharmacokinetics of QBA608 and QBA609 in male rats after single intravenous administration of NVA237, QBA608 or QBA609 (2 mg/kg)
DMPK-r1000168	Disposition in male rats after single intravenous (4 mg/kg), intra-tracheal (1.7 mg/kg) and oral administration (30 mg/kg) of [14C6]NVA237 bromide
DMPK-r1000618a	Disposition in male rats after single intravenous (4 mg/kg), intra-tracheal (1.7 mg/kg) and oral administration (30 mg/kg) of [14C6]NVA237 bromide; Determination of NVA237 in rat plasma

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	Distribution
DMPK-r1000447	In vitro blood distribution and plasma protein binding of [14C6]NVA237 including stability in blood and plasma of male BALB/cBy x C57BL/6 F1 mice
DMPK-r1000650	In vitro blood distribution and plasma protein binding of [14C6]NVA237in rabbits
	Metabolism
DMPK-r0800737	In vitro hepatic intrinsic clearances of NVA237 enantiomers QBA608 and QBA609
DMPK-r1000375	In vitro interconversion of NVA237 enantiomers QBA608 and QBA609
DMPK-r1000397	In vitro metabolism of [14C6]NVA237 in mouse, rat, rabbit, dog and human hepatocytes
DMPK-r1000562	In vitro metabolism of [14C6]NVA237 in mouse and rabbit liver microsomes and rat and human intestinal microsomes and S9 fractions
	Excretion
	Other PK Studies
DMPK-r0800074	[3H4]Mg-stearate uptake in rat lung precise-cut slices
DMPK-r1070398	NVA237: A 28-day inhalation toxicokinetic study of a powder aerosol formulation in the rat; Determination and toxicokinetics of NVA237 in rat plasma
	Toxicology
	Immunotoxicity
r0670690	Assessment of contact sensitizing potential with the murine local lymph node assay (LLNA tier I)
	Impurities
r0870161	TOX2/NVA237: Mutagenicity test using Salmonella typhimurium (Batch control with impurities)
r0870162	TOX2/NVA237: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes
r0870163	NVA237 (spiked with CPMA): A 28-day inhalation toxicity study of a powder aerosol formulation in the rat
	Other PK Studies
r0520076	Magnesium stearate: 52-week inhalation toxicity study in the dog
r0670546	A 2-week combination inhalation study in Wistar rats with a 2-week recovery period
r0670547	QVA149: A 14-day inhalation study of a combined powder formulation in the dog with a 14-day recovery
r0670755	QVA149: An inhalation embryo fetal development study in rats
r0670756	QVA149: A 13-week inhalation study of a combined powder formulation in the dog with a 4-week recovery
r311736	Preliminary inhalation toxicity study with magnesium stearate in dog
r731575	One month inhalation toxicity study with magnesium stearate in rats

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r942504	Magnesium stearate: 4 weeks repeat dose inhalation toxicity study in dogs

NDA 207930

Study no.	Title
	Pharmacology
	Primary Pharmacology
rd-2012-00168	Additive effect of indacaterol and NVA237 in the guinea-pig isolated trachea
	Pharmacokinetics
	Analytical Methods and Validation Reports
DMPK R0300366H	Quantitative determination of QAB149 in dog plasma by LC-MS/MS: Method description and validation
DMPK R0300366H-01	Quantitative determination of QAB149 in dog plasma by LC-MS/MS: Method description and validation: Additional data, Amendment no. 01
DMPK R0300366I	Quantitative determination of QAB149 in rat plasma by LC-MS/MS: Method description and validation

3.3 Previous Reviews Referenced

NDAs 207923/207930: review dated 7/7/15 (carcinogenicity assessment)

IND 48655, reviews dated: 9/15/04, 2/3/05, 9/25/06, 5/51/07, 6/20/07, 10/4/07, 3/30/09, 2/24/10

IND 76377, reviews dated: 10/29/07, 7/31/08, 9/18/13

NDA 22383, reviews dated 8/25/09, 2/16/11

4 Pharmacology

4.1 Primary Pharmacology

See IND 48655, review dated 6/20/07

4.2 Secondary Pharmacology

See Integrated Summary.

4.3 Safety Pharmacology

Core safety pharmacology studies were conducted for potential effects of NVA237 on the central nervous, cardiovascular and respiratory systems, CNS effects were evaluated in study **#R0670652**. *In vitro* effects of NVA237 on the hERG channel were

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evaluated in study **#R0670317**. *In vitro* effects of NVA237 and QVA419 on the hERG channel were evaluated in study **#R0770861**. Additional *in vitro* and *in vivo* cardiovascular assessments and *in vivo* respiratory effect evaluations were previously reviewed under IND 48655 (see review dated 6/20/07).

Central nervous system effects (summary review previously recorded under IND 76377, review dated 7/31/08)

A pharmacological assessment of the effect of QVA149 on the central nervous system and the respiratory system of the albino rat, Final report amendment no. 1 (study # 0670652, GLP compliant, QA statement)

The effect of inhaled NVA237, QAB149, and QVA 149 on the central nervous system (CNS) in Phase 1, and respiratory system in Phase 2, was assessed in albino male rats. All animals were evaluated for mortality and signs of ill health or reaction to treatment twice daily (am and pm). In Phase 1, rats (8/group) were dosed with 0 (vehicle), QVA149 (NVA237/QAB149 at 0.144/0.507 mg/kg), NVA237 at 0.210 mg/kg, and QAB149 at 0.620 mg/kg, based on a theoretically achieved dose (salt, using a ratio of base: salt of 1.251). Animals were dosed over a 2 hour inhalation treatment. A functional observational battery (FOB) and assessments of grip strength, hind limb splay, and recording of body temperature were performed for animals predose, immediately following the treatment period, and at 2, 4 and 24 hours post dose. Pupil dilation was noted in animals that received NVA237 and QVA149 (8/8 moderately dilated, versus 8/8 pinhead in controls and QAB149 only), which is an expected pharmacological effect of NVA237. A slight decrease in motor activities was noted immediately post dose (1/7 slight and 7/8 moderate, versus 8/8 moderate in other groups), and 2 hrs following treatment with QAB149, but this was a transient effect.

In Phase 2, rats (5/group) were administered test articles at the same doses. Animals were placed in head-out plethysmographs and allowed to acclimate for approximately 15 minutes prior to data collection. Ventilatory parameters (tidal volume, respiratory rate, and derived minute volume) were measured up to 120-minute period predose (taken approximately 24 hours prior to dosing), continuously during the dosing period, and at 2, 4 and 24 hours post dose. One death was noted for animal #4104, but no gross lesions were noted, and the cause of death was undetermined and unclear. There were no clear effects on respiratory parameters.

In conclusion, QVA149 at 0.144/0.507 mg/kg (NVA237/QAB149), NVA237 at 0.210 mg/kg, and QAB149 at 0.620 mg/kg had no effects on respiratory parameters via the inhalation route of administration. At the same doses, there were no dose limiting effects on CNS parameters, although QAB149 induced a slight, transient decrease in motor activities immediately and 2 hrs post dose. Pupil dilation was noted in animals that received NVA237 and QVA149, but this is an expected pharmacological effect of NVA237.

Cardiovascular

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Effects of QAB149, NVA237, and Combination Mixture QVA149 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (study #0770861, GLP compliant, QA statement)

The *in vitro* effects of NVA237, QAB149, and QVA149 were evaluated in hERG-expressing human embryonic kidney (HEK293) cells. Vehicle was applied in triplicate for all experiments. In Phase 1 (concentration range determination), QAB149 at 30 µM was applied in triplicate. An inhibitory effect on hERG potassium current amplitude of 90.1% was observed. NVA237 at 300 µM was applied in triplicate, resulting in 30.6% inhibition. QVA149 (30 µM QAB149 and 300 µM NVA237) was applied in triplicate, resulting in 82.4% inhibition. In Phase 2 (concentration response determination), QAB149 was applied at doses of 1, 3, 10 and 30 µM. NVA 237 was applied at doses of 30, 100 and 300 µM. QVA149 was applied at the following concentrations:

(1) = 50mL of 1.875 µM QAB149 and 50mL 18.75 µM NVA237
(2) = 50mL of 3.75 µM QAB149 and 50mL 37.5 µM NVA237
(3) = 50mL of 3.75 µM QAB149 and 50mL 75 µM NVA237
(4) = 50mL of 30 µM QAB149 and 50mL 300 µM NVA237 (maximum solubility for both test articles in HB-PS + 0.3% DMSO)

The IC50 for the inhibitory effect of QAB149 on hERG potassium current was 3.1 µM. The IC50 for the inhibitory effect of NVA237 on hERG potassium current was not determined since hERG inhibition was < 50%. For QVA149, HERG inhibition at all four concentrations was statistically significant ($P<0.05$) when compared to vehicle control values, but a lower effect on hERG current than the high dose (30 µM) of QAB149. Therefore, QVA149 has no additive effect on hERG current inhibition compared to QAB149 alone. There were no findings of QTc prolongation in cardiovascular safety pharmacology or toxicology studies in dogs with QAB149 or QVA149.

5 Pharmacokinetics/ADME/Toxicokinetics

The absorption, distribution, metabolism, and excretion of NVA237 was investigated in mice, rats, dogs, rabbits, and humans.

Mice

Disposition in male mice after single intravenous (3 mg/kg) and oral administration (25 mg/kg) of [14C6]NVA237 bromide (study # DMPK-r1000073)

The goal of this study was to assess the absorption, distribution, metabolism, and excretion parameters of NVA237 after a single intravenous and oral administration of [14C6] NVA237 bromide to BALB/cBy x C57BL/6 F1 mice. Mice were dosed with 3.18 mg/kg IV (bolus) or 26.9 mg/kg PO (gavage) radiolabeled NVA237 (base) in 5% glucose. To quantify the kinetics and metabolism of NVA237, blood was collected at 0.083 (IV group only), 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 168 hr post dose, urine and feces were collected daily up to 72 hr, and 72-168h, and the carcass/cage wash was assessed at 168 h post dose. The radioactivity in aliquots of plasma, urine, feces, and

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carcass and other aliquots (dose solutions, cage wash) was measured by liquid scintillation counting. For distribution assessment, whole body radiography was conducted on mice dosed IV (sacrificed at 0.25, 1, 4, 24, 72, and 168 hrs post dose) and mice dosed PO (sacrificed at 24 and 168 hrs post dose). The concentrations of total radiolabeled components in the tissues, organs, and matrices were determined by means of quantitative whole-body autoradioluminography (QWBA). Metabolite profiles were assessed by HPLC on plasma, urine and feces. Metabolites were structurally characterized by LC-MS or LC-MS-MS.

No adverse effects were reported. The PK parameters are summarized in Table 3. The oral bioavailability of NVA237 was estimated at 0.625%. The minimal oral absorption was estimated at ~11%, based on urinary excretion data. The half-life of NVA237 was 3.9 h. The plasma clearance was 15.5 L/(h kg). The volume of distribution at steady state was moderate to high (4.51 L/kg) and approximately 5.6 times the total body water. NVA237 was distributed mostly into extravascular tissues after IV administration, with the longest elimination T1/2 values for the epididymis (25.2 h), eye choroid (94.0 h), eye vitreous body (23.0 h), fat (brown, 20.7 h), Harderian gland (54.2 h), kidney (cortex, 25.6 h), and liver (50.2 h). After a single PO and IV administration, the distribution of total radiolabeled components at 24 h and 168 h was comparable. Low levels of radiolabeled components were detected in the brain up to 1 hr post dose (0.08-fold), but the sponsor attributed this to vascular contamination. It is unclear if this represents true absorption through the blood brain barrier. Specific uptake/retention was noted into the pigmented eye choroid, but was partly reversible.

The primary biotransformation pathways for NVA237 in the mice were oxidative and included the addition of 1 or 2 oxygen atoms to the cyclopentane and phenyl rings and dehydrogenation on the cyclopentane ring. Hydrolysis of the ester linkage forms the corresponding carboxylic acid metabolite M9.

After IV dosing, NVA237 was the most prominent drug-related compound observed in urine and feces (60.1% at 168 h), and individual metabolites comprised less than 5% of the total excreted related compounds. In plasma (at 24 hrs), however, M9 (carboxylic acid metabolite; 23.05%) was the most prominent drug related compound, followed by NVA237 (18.9%). Following PO dosing, NVA237 represented the major proportion of radioactive material (62.5%) in excreta, with the majority in the feces (62.2% at 168 h); individual metabolites comprised less than 6% of the total excreted related compounds. In plasma (at 24 hrs), M9 was 89.7% of the total (AUC) drug related components, and NVA237 was only 0.248%. After IV administration, NVA237 was mainly excreted via urine (68%) and bile/feces (29%). After PO administration, NVA 237 was mainly recovered via feces (91.6%).

Rats

Disposition in male rats after single intravenous (4 mg/kg) and oral administration (30 mg/kg) of [14C6]NVA237 bromide and pharmacokinetics after intra-tracheal administration (1.3 mg/kg) of NVA237 bromide (study # DMPK-r0900674)

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The goal of this study was to assess the absorption, distribution, metabolism, and excretion parameters of NVA237 after single intravenous or oral administration of [$^{14}C_6$]NVA237 bromide and to assess basic pharmacokinetic parameters after single intra-tracheal administration in male rats (Han-Wistar for all experiments, except for Long Evans and Han Wister for distribution studies after IV dosing). Animals were dosed with 4.12 mg/kg IV (bolus), 35.7 mg/kg PO (gavage), or 2.17 mg/kg intra-tracheal (lactose dry powder) NVA237 (base). The vehicle for IV and PO dosing was 5% glucose.

Blood was collected sublingually or from the jugular vein for kinetics at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96 hrs post dose from IV and PO dosed animals. For metabolism and excretion, blood was collected at 0.083 (IV only), 0.25 (PO only), 0.5, 1, 4, 8, 24, and 48 hrs post dose in IV and PO dosed animals. For metabolism/excretion, excreta were collected daily up to 96 h, and at 96-168 hrs, and the carcass/cage wash was assessed at 168 hrs post dose. Tissue distribution studies were performed on IV dosed animals sacrificed at 24 and 168 hrs post dose, and on a second group of IV dosed animals at 0.25, 1, 4, 24, 72, 168 hrs postdose. Bile excretion was assayed up to 24 hrs in IV dosed animals; bile was collected (cannula) at 0-4, 4-8, 8-24, and 24-48 post dose, while urine/feces were collected daily to 48h, and the carcass/cage wash were assessed at 48 hrs. Intratracheally dosed animals were assessed for kinetics using blood collected at 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 30 hrs post dose. The concentrations of total radiolabeled components in the tissues, organs, and matrices were determined by means of quantitative whole-body autoradioluminography (QWBA). Radioactivity was measured by liquid scintillation counting. Metabolite profiles were assessed by HPLC on plasma, urine, feces and bile. Structural characterization of the metabolites was achieved using LC-MS and LC-MS/MS.

No adverse effects were reported. The PK parameters are summarized in Table 3. The minimal oral absorption was estimated at ~8.60%, based on urinary excretion data. Oral bioavailability was only 1.38%. The bioavailability after intratracheal administration was unclear, due to the compound being incorrectly administered to the stomach, and a high fraction of the dose being exhaled. The half-life of NVA237 was 23.0 hrs. The plasma clearance was 8.76 L/(h kg), which is about 2.6-fold greater than the liver blood flow indicating a non-hepatic elimination process (e.g., urinary excretion). The volume of distribution at steady state was 11.9 L/kg, approximately 18 times the total body water. NVA237 was distributed mostly into extravascular tissues after IV administration, with the longest elimination T1/2 values for the kidney (CMJ, 51.7 h) and liver (47.2 h), but even longer T1/2 values could be expected in pituitary gland and trigeminal nerve. No/trace radiolabeled components were detected in the brain, and specific uptake/retention into the pigmented eye choroid was observed, but was partly reversible.

The primary biotransformation pathways for NVA237 in the rat were oxidative and occurred primarily on the cyclopentane and phenyl rings. Hydrolysis of the ester linkage

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forms the corresponding carboxylic acid metabolite M9, the main drug-related compound after oral dosing.

After IV dosing, the most prominent drug-related compounds in plasma (up to 48 hrs) were NVA237 (43.9%) and unassigned compounds (25.2%), with each individual metabolite comprising less than 5% of the total AUC. In the urine (24 hrs) and feces (72 hrs), NVA237 (28.2%) and unassigned compounds (29.0%) were the most prominent structures in the excreta, which included 78.5% of the total radiolabeled dosage. Following intravenous administration, 67.7%, and 29% of the dose was found in urine and feces.

After PO dosing, the most prominent drug-related compounds in plasma (up to 48 hrs) were M9/M1 (72.6%), NVA237 (7.23%) and unassigned compounds (15.8%), with other individual metabolites each comprising less than 5% of the total AUC. In the urine (48 hrs) and feces (24 hrs), NVA237 (22.7%) was prominent, with other compounds represented at less than 5% each in the total excreta, which included 28.1% of the total radiolabeled dosage. After oral administration, 8.95% and 62.2% of the dose was found in urine and feces.

In IV dosed, bile duct-cannulated rats, 78.3% of the radiolabeled drug-related compounds were detected in the excreta at 48 hrs, comprised of the urine (67.5%), feces (3.34%), and bile (7.52%).

Dog

Single-dose intravenous (bolus) telemetry study in dogs including sighting phase (study #0510129; GLP)

PK parameters of NVA237 were assessed in beagle dogs in a sighting study, and a telemetry study (study # **R0510129**), which is also summarized under Safety Pharmacology. In the sighting study, animals received single intravenous doses of 0.01, 0.1 and 1 mg/kg. Toxicokinetic blood samples were sampled at pretest, 5 min, 15 min, and 0.1, 1, 2, 4, 8, and 24 hrs post dose. Samples were analyzed by LC-MS-MS. PK parameters at 1 mg/g are summarized in Table 3.

Human

See IND 48655, Review dated 6/20/07 for review of studies on plasma protein binding and biotransformation of [^{14}C] NVA237 in human blood and human liver and lung microsomes.

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Table 3: PK parameters in mice, rats, dogs, and humans

PK parameters (mean±SD) [a]	Mouse	Rat	Dog	Human
Nominal dose (mg/kg)	3	4	1	120 µg
AUC (ng·h/mL)	191	786 ± 201	330	2.8 ± 0.5
AUC / dose (ng·h/mL)/(mg/kg)	60	191 ± 48	330	1400 ± 250 [d]
AUC interval (h)	0-8	0-48	0-24	0-24
Vss (L/kg)	4.5	11.9 ± 7.4	5.4	1.2
T1/2 (h)	3.9	23 ± 12	4.4	~6
MRT (h) [b]	0.3	2.1 ± 1.3	1.7	1.7
CLp (mL/min/kg)	258.3	90 ± 25	53.3	11.7
Clearance (% of hepatic blood flow) [c]	~480	~280	~290	~100
References	[Table 2.6.5.3D-Study R1000073]	[Table 2.6.5.3A-Study R0900674]	[Table 2.6.3.4-Study R0510129]	[Study CNVA237A2108]

[a] selected list: the complete list of studies is given in PT-Table 10-8
[b] calculated by MRT= Vss/CLp
[c] calculated by:100 x fp/(1-H) x CLp / Qh, where Qh = hepatic blood flow and H = hematocrit from (Davies and Morris 1993)
[d] bodyweight adjustment for a 65 kg subject

(Sponsor's table)

Distribution (to milk)

Galactogenic transfer, pharmacokinetics and metabolism in milk and plasma after a single intravenous (4 mg/kg) administration of [14C6]NVA237 bromide (study # 1000614)

The goal of this study was to assess the transfer of NVA237 and/or its metabolites into the milk of lactating rats, and the PK in plasma and milk after single IV administration to lactating rats. Briefly, rats (n =6) were dosed with bolus IV 4 mg/kg (base; 5 mg/kg in salt form) [$^{14}C_6$]NVA237 at Day 8 after parturition. One control animal was not dosed, and was entered into the study at Day 11 after parturition. Blood and milk were collected from Group 1 NVA237 animals (n = 3) at 0, 0.25, 3, 24, 72 hrs after dosing, and from Group 2 NVA237 animals (n = 3) at 0, 1, 8, and 47 hrs after dosing. Milk was collected from the control animal 0 and 7 hrs after entering the study. Two hours before sample collection, the pups were separated from the dams. In order to stimulate milk secretion, 4 IU/kg oxytocin was administered intraperitoneally 3-5 minutes before the sample collection. Radioactivity was detected by liquid scintillation.

NVA237 and 16 metabolites were detected in milk. NVA237 and 18 metabolites were detected in the plasma of lactating rats. At 72 h post-dose, NVA237 and M9 were the only components observed in small amounts in plasma and milk. The metabolites appeared to have been formed by single and multiple hydroxylations, dehydrogenations as well as ester hydrolysis (M9) and sulfate conjugation of hydroxylated NVA237. PK parameters are summarized below:

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Matrix	Plasma		Milk	
Parameter	**Total radiolabeled components**	**NVA237**	**Total radiolabeled components**	**NVA237**
Actual dose (mg/kg) [a]:	4.00 ± 0.0647	-	-	-
C0 (µM):	4.90	1.91	-	-
Cmax (µM):	3.57	0.990	5.75[b]	2.00[b]
Tmax (h):	0.25	0.25	1[b]	1[b]
Tlast (h):	72	72	72	72
AUClast (µM·h):	12.7	3.54	102[b]	40.0[b]
AUClast/Dose (µM·h/(mg/kg)):	3.17	0.884	25.4[b]	10.0[b]
Excretion in milk (%):	-	-	~2	-
Range ratio milk/plasma concentrations	-	-	0.279-10.0	0.598-14.6
AUClast milk/plasma	-	-	8.03	11.3

a: referring to the free cation; b: Values might be biased as no milk samples could be withdrawn at 3 and 8 h p.d.

(Sponsor's table)

Based on AUC_{last}, the milk/plasma ratio was 11.3 for NVA237 and 8.03 for total radiolabeled components.

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose general toxicology studies were not submitted to NDAs 207923/207930.

6.2 Repeat-Dose Toxicity

ERRATA: 26-week inhalation toxicity study of a powder formulation in the albino rat with a 28-day recovery period and a 13-week interim toxicity study (study #79031)

Regarding the 26 week inhalation toxicology rat study, there was an error in the review dated 3/30/2009 under IND 48655. The NOAEL was determined to be the low dose of 0.009 mg/kg NVA237 (salt, pulmonary deposited dose), and the associated AUC(0-24hr) was stated to be 334 ng*h/mL (salt). The correct AUC(0-24hr) is 13.8 ng*h/mL (salt), an average of the male and female AUC values shown on page 1582 of the study report.

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Study title: NVA237: A 39-week inhalation toxicity study of a powder formulation in dogs with a 4-week recovery period

Study no.:	79334/0670548
Study report location:	NDA 207923, SD-1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	January 24, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NVA237, batch #X344 0906, 98%

Key Study Findings

- Beagle dogs were dosed with NVA237 at 0 (air), 0 (vehicle), 0.030, 0.12, 0.33 mg/kg (male/female, estimated achieved doses) through the inhalation route of exposure for 39 weeks (6/sex/group controls and HD; 4/sex/group LD and MD), with a 4 week recovery period (2/sex/group controls and HD). These correspond to pulmonary deposited doses of 0 (air), 0 (vehicle), 0.008, 0.03, and 0.08 mg/kg (male/female), respectively.
- The vehicle was 1% magnesium stearate, 99% lactose monohydrate, and showed no notable difference from air control.
- There were no mortalities.
- Clinical signs included red eyes, dry gums and dry muzzles. These findings are related to dryness, which is an expected pharmacological effect of the test article.
- There was a test article related decrease in body weight gain in males throughout the study at the MD and HD (Week 39: MD 75%, HD 75%), and in females at the MD and HD at the end of the dosing period (Week 39: MD 86%, HD 69%). This correlated with feed consumption, and reversed after the recovery period.
- Test article related ophthalmic findings were conjunctival hyperemia (bilateral) observed in 1/6 HD males and 1/6 HD females, corneal opacity (bilateral) observed in 1/6 HD females, and focal nuclear opacity (bilateral) observed in 1/6 HD males and 1/6 HD females.
- During the treatment period, there was a reduction in lacrimal secretion (defined by a value of < 10mm on the Schirmer tear test) at Week 3 and Week 6 at the HD in both sexes. This reversed after the recovery period, and is an expected pharmacological effect of the test article.
- Increased heart rate was noted in MD and HD males and females (+30-69%, compared to the pretest value on the same day) at Weeks 13, 26, and 39. This was an expected pharmacological effect of the test article.
- Test article related findings were identified in the pharynx, lacrimal gland, and mandibular salivary gland. In the pharynx, inflammation (grade 1) was noted at the HD (males 3/4, females 3/4), but was also observed in 1/4 control males. Ectasia of the ducts and or alveoli of the pharynx (grade 1 and 2) was observed in males (3/4 MD, 1/4 HD) and females (1/4 MD, 3/4 HD). All findings reversed

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after the recovery period. The findings in the pharynx were generally mild, reversible, and are clinically monitorable; therefore, these findings are not considered dose limiting. Hypertrophy was noted in the lacrimal and salivary glands, and is not a dose limiting finding.

- Systemic exposure (AUC) to the test article increased with dose, but was higher in HD males (80.9 ng.h/mL) than in HD females (32.9 ng.h/mL). The higher AUC values in males resulted from higher concentrations of plasma test article in 1 of 6 males compared other males and females.
- The NOAEL was determined as the low-dose, however, some findings were determined to be clinically monitorable and/or not dose limiting. Therefore, the supporting dose was determined to be the mid-dose of 0.12 mg/kg, based on ophthalmic findings. The supporting dose is associated with an AUC of 26.6 ng*h/mL in males and 42.0 ng.h/mL (salt) in females.

Methods

Doses:	0 (air), 0 (vehicle), 0.030, 0.10, 0.30 mg/kg
Frequency of dosing:	Daily
Route of administration:	Inhalation (face mask)
Dosing duration:	60 minutes
Formulation/Vehicle:	1% magnesium stearate, 99% lactose monohydrate
Species/Strain:	*Canis familiaris*/Beagle dog
Number/Sex/Group:	4/sex/group for main study, 2/sex/group for recovery in the control and HD groups
Age:	Approximately 8 to 9 months at the onset of treatment.
Weight:	6.9 to 9.7 kg (males) at the onset of treatment 6.0 to 8.8 kg (females) at the onset of treatment
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	There were no deviations that affected the validity of the study

Group no. identification	Target dose level* (mg/kg/day)	Animal number			
		Main study		Recovery study	
		Males	Females	Males	Females
1 Air control	0	101-104	151-154	105-106	155-156
2 Vehicle control	0	201-204	251-254	205-206	255-256
3 NVA237 low	0.030/0.024	301-304	351-354	-	-
4 NVA237 mid	0.10/0.080	401-404	451-454	-	-
5 NVA237 high	0.30/0.24	501-504	551-554	505-506	555-556

* Dose levels are expressed in salt form and cation form.

(Sponsor's table)

Observations and Results

Aerosol drug variables

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The particle size distribution shows that the test articles tested were within range for pulmonary deposition (MMAD of 1 – 5 microns).

(Sponsor's table)

Mortality

Observations for mortality were conducted twice daily. There were no mortalities.

Clinical Signs

Observations for clinical signs were conducted once prior to the start of dosing, and weekly throughout the treatment and recovery periods, and on the day of necropsy. Physical examinations were conducted immediately after dosing, focusing on the eyes, nose and mouth.

Test article related findings were noted in the eyes and the skin of the mouth area, related to dryness which is an expected pharmacological effect of the test article. Specifically, animals developed red eyes, dry gums and dry muzzles.

Table 4: 39 week dog study: Clinical signs

	Dose (mg/kg/day)									
	Males					Females				
	0 (Air)	0 (Vehicle)	0.031	0.12	0.33	0 (Air)	0 (Vehicle)	0.028	0.11	0.32
N	6	6	4	4	6	6	6	4	4	6
Eyeball red/left	0	0	0	0	5	0	0	0	4	6
Eyeball red/right	0	0	0	0	5	0	0	0	4	6
Gums dry	0	0	0	0	4	0	0	0	0	5
Skin Dry/muzzle	0	0	2	4	6	0	0	0	4	6

Body Weights

Body weights were recorded at pretest (Day -7), and weekly throughout the treatment and recovery periods, and on the day of scheduled sacrifice.

There was a test article related decrease in body weight gain in males throughout the study at the MD and HD (Week 39: MD 75%, HD 75%), and in females at the MD and HD at the end of the dosing period (Week 39: MD 86%, HD 69%). After the 4 week recovery period, this was fully reversible.

Table 5: 39 week dog study: Body weight analysis

	Dose (mg/kg/day)				Dose (mg/kg/day)			
Study Day	0 (Vehicle)	0.031	0.12	0.33	0 (Vehicle)	0.028	0.11	0.32

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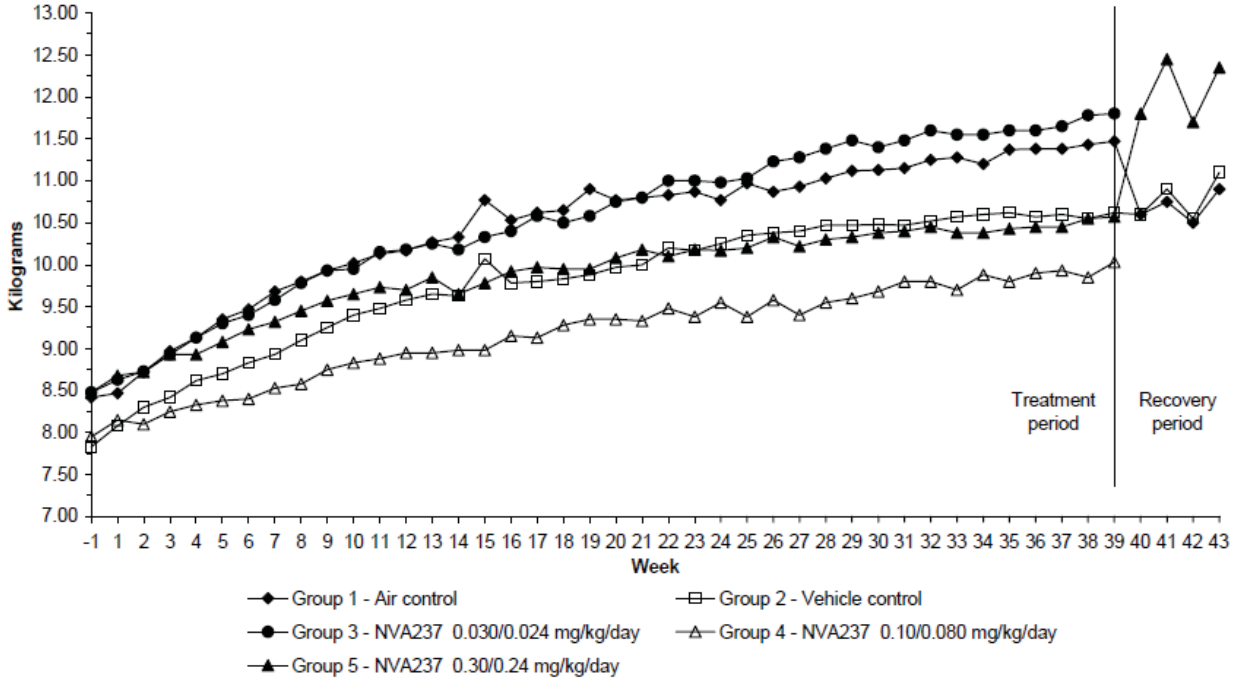
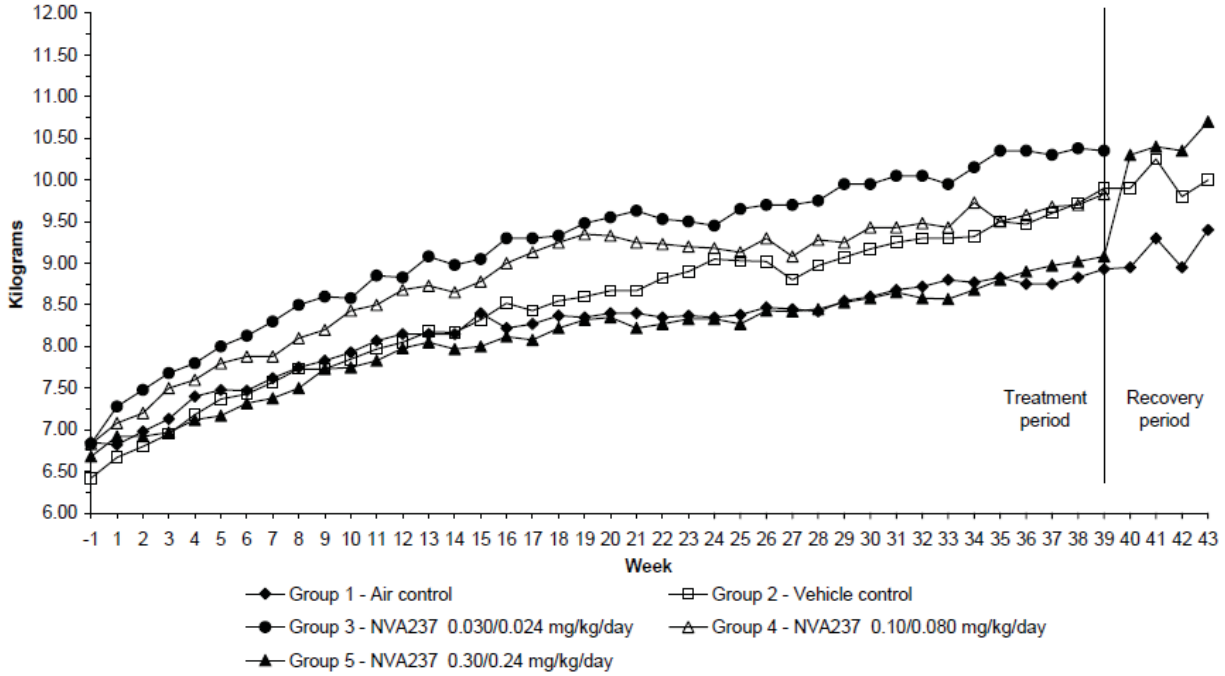
Pretest	7.83	8.48	7.95	8.48	6.42	6.83	6.83	6.68
Week 20	9.97	10.75	9.35	10.08	8.67	9.55	9.33	8.35
ΔBW (g)	2.14	2.27	1.40	1.60	2.25	2.72	2.50	1.67
BW gain, % initial	27%	27%	18%	19%	35%	40%	37%	25%
BW gain, % control	100%	106%	65%	75%	100%	121%	111%	74%
Week 39	10.62	11.8	10.03	10.57	9.9	10.35	9.83	9.08
ΔBW (g)	2.79	3.32	2.08	2.09	3.48	3.52	3.00	2.40
BW gain, % initial	36%	39%	26%	25%	54%	52%	44%	36%
BW gain, % control	100%	119%	75%	75%	100%	101%	86%	69%
Week 43 (recovery)	11.1	-	-	12.35	10	-	-	10.7
ΔBW (g)	3.27			3.87	3.58			4.02
BW gain, % initial	153%			242%	159%			241%
BW gain, % control	100%			118%	100%			112%

Figure 1: 39 week dog study: Body weight

Males



Females



Feed Consumption

Feed consumption was measured daily throughout the treatment and recovery periods.

There was a trend for decreased feed consumption at the HD in both males in females, but it was not clearly dose dependent (males Week 20: HD -12%; females Week 20: -11%, Week 39: -19%). There was no change in feed consumption at the HD, compared to vehicle control.

Table 6: 39 week dog study: Feed consumption, percent change compared to vehicle

	Dose (mg/kg/day)					
	Males			Females		
	0.031	0.12	0.33	0.028	0.11	0.32
Week 20	-8%	-8%	-12%	8%	6%	-11%
Week 39	-2%	-2%	-3%	-20%	1%	-19%
Week 43 (recovery)	-	-	-2%	-	-	-4%

Ophthalmoscopy

Ophthalmic exams were conducted once prior to the start of treatment (all animals) and again during Weeks 4, 8, 13, 20, 26 and 39 (before dosing). All main and recovery study animals were subjected to funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations. The mydriatic agent used was 1% mydriacyl.

Recovery animals were examined at the end of the recovery period since test article-related findings were observed during Week 39.

A Schirmer's tear test was performed on all animals once pre-study and again at 3, 6, 13, 29, and 39 weeks and at the end of recovery on main study and recovery animals in order to assess reduction of lacrimal secretion.

A board certified ophthalmologist identified the following test article related eye findings: "mucoid ocular discharge, conjunctival hyperemia, and faint corneal opacities, and evidence of previous corneal ulceration." One HD animal developed "slight unilateral diffuse corneal edema accompanied by aqueous flare and superficial corneal vascularization". The findings were not observed after the recovery period according to the report.

Only individual animal listings were provided for the ophthalmic data. An Information request was sent on September 16, 2015 requesting a summary table of the individual listings, indicating bilateral or unilateral findings. Incidence tables from the pretest period and Week 39 are shown below from the sponsor's response to IR, received on September 21, 2015:

Table 7: 39 week dog study: Ophthalmic findings, sponsor's tables

Pretreatment

Group 1 - Air control
Group 2 - Vehicle control
Group 3 - NVA237 0.030/0.024 mg/kg/day
Group 4 - NVA237 0.10/0.080 mg/kg/day
Group 5 - NVA237 0.30/0.24 mg/kg/day

		Group/Sex									
Clinical Sign	Site	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Conjunctival Hyperemia	Unilateral	.	1	.	.	.	.	.	.	.	.
Diffuse Dull Appearance of Tapetal Area : Normal Variant	Bilateral	.	.	.	.	.	.	1	.	.	.
Focal Atapetal Area(s)	Unilateral	.	.	.	.	1	.	.	.	1	.
Focal Nuclear Opacity	Unilateral	.	.	.	1	.	.	.	.	1	.
Focal Nuclear Opacity	Bilateral	.	.	1	1	.	.	.	.	.	1
Focal Posterior Capsular Opacity Infero-laterally	Unilateral	.	.	.	.	.	.	.	.	1	.
Medial Strabismus	Unilateral	1	.	.	.	.	.	.	.	.	.
Micropapilla	Bilateral	.	.	.	1	.	.	.	.	.	.
Mosaic-like Tapetum	Bilateral	1	.	.	.	.	.	.	.	.	.
Multifocal Pale Areas Along Inferomedial Retinal Vessels in Non-tapetal Area	Unilateral	.	.	.	.	1	.	.	.	.	.
Retinal Fold(s)	Unilateral	.	.	.	.	.	.	.	.	1	.
Serous Discharge	Unilateral	.	.	.	.	.	.	.	.	.	1

Week 39

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Group 1 - Air control
Group 2 - Vehicle control
Group 3 - NVA237 0.030/0.024 mg/kg/day
Group 4 - NVA237 0.10/0.080 mg/kg/day
Group 5 - NVA237 0.30/0.24 mg/kg/day

		Group/Sex									
Clinical Sign	Site	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Conjunctival Hyperemia	Unilateral	.	.	.	.	.	1	.	.	.	.
Conjunctival Hyperemia	Bilateral	.	.	.	.	1	.	.	.	.	1
Cornea Ghost Vessels Dorsal	Bilateral	.	.	.	.	.	.	.	.	.	1
Corneal Opacity	Unilateral	.	.	.	.	.	.	.	.	.	1
Corneal Opacity	Bilateral	.	.	.	.	.	.	.	.	.	1
Distichiasis	Unilateral	.	1	.	.	.	.	.	.	.	.
Distichiasis	Bilateral	.	.	1	.	.	.	.	.	.	.
Faint Diffuse Superficial Corneal Opacity with Mottled Appearance	Unilateral	.	.	.	.	.	.	.	.	.	1
Faint Superficial Paracentral Corneal Opacity	Unilateral	.	.	.	1	.	.	.	.	.	.
Focal Atapetal Area(s)	Unilateral	.	.	.	.	1	.	.	.	1	.
Focal Nuclear Opacity	Unilateral	.	.	.	.	.	.	.	.	.	1
Focal Nuclear Opacity	Bilateral	.	.	1	2	1	.	.	.	.	1
Focal Posterior Capsular Opacity Infero-laterally	Unilateral	.	.	.	.	.	.	.	.	1	.
Medial Strabismus	Unilateral	1	.	.	.	.	.	.	.	.	.
Micropapilla	Bilateral	.	.	.	1	.	.	.	.	.	.
Mosaic-like Tapetum	Bilateral	1	.	.	.	.	.	.	.	.	.
Multifocal Pale Areas Along Inferomedial Retinal Vessels in Non-tapetal Area	Unilateral	.	.	.	.	1	.	.	.	.	.
Multifocal Pinpoint Pigmented Anterior Capsular Opacities Axially	Unilateral	.	.	.	.	1	.	.	.	.	.
Ocular Discharge Mucous	Unilateral	.	.	.	.	.	.	.	.	1	.
Prolapsed Nictitans Gland	Unilateral	.	.	.	.	.	.	1	.	.	.
Serous Discharge	Unilateral	.	1	.	.	.	1	1	.	.	.
Serous Discharge	Bilateral	.	.	.	.	.	.	.	1	.	.

(Sponsor's tables)

Based on the tables above, treatment related findings were identified based on bilateral lesions that were observed at Week 39 and not during the pretest period in the same animal. Test article related findings included conjunctival hyperemia (bilateral) observed in 1/6 HD males and 1/6 HD females, corneal opacity (bilateral) observed in 1/6 HD females, and focal nuclear opacity (bilateral) observed in 1/6 HD males and 1/6 HD females. These ophthalmic findings were reversible. Regarding focal nuclear opacity (bilateral) in 1/4 MD males (#404), this animal a unilateral focal nuclear opacity during the pretest period, therefore it is difficult to determine if this specific finding is test article related.

During the treatment period, there was a reduction in lacrimal secretion (defined by

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a value of < 10mm on the Schirmer tear test) at Week 3 and Week 6 at the HD in both males and females, and a general reduction in the value on the Schimer tear test in dosed animals versus air and vehicle control animals throughout the study. This reversed after the recovery period, and is an expected pharmacological effect of the test article.

Table 8: 39 week dog study: Schimer tear test values (mm)

	Dose (mg/kg/day)									
	Males					Females				
	0 (Air)	0 (Vehicle)	0.031	0.12	0.33	0 (Air)	0 (Vehicle)	0.028	0.11	0.32
Pretest	17.5	17.17	14.25	18.25	17.58	15.67	15.58	15.5	18.63	18.6
Week 3	16.17	17.58	12.25	12.75	**9.25**	17.42	16.33	16.63	15.88	**9.5**
Week 6	17.17	20.17	12.38	14.25	**8.83**	20.08	16.67	16.25	18.25	**9.58**
Week 13	20.25	20	17.25	13.88	15.5	18.58	16.58	17.38	18.25	11.3
Week 29	20.75	22.33	19.63	15	13.67	21.5	17.92	18.75	16.75	12
Week 39	22.58	21.42	13	13.88	10.75	21.83	13.58	12.75	17.25	11.3
Week 43 (recovery)	24	25.25	-	-	22.25	26.5	17.5	-	-	20.5

Bold indicates statistically significant findings P < 0.05

ECG

Electrocardiograms were recorded once during pretreatment and again during Weeks 13, 26 and 39 (predose and near Tmax) of the treatment period, and at the end of the recovery period due to findings at Week 39. Recordings were performed using limb leads I, II, III, aVR, aVL, and aVF. A quantitative measurement of heart rate, PR, QRS, QT and RR intervals was performed using ECG waveform from leads II and/or III. QTc was calculated using Van de Waters' equation.

Respiratory minute volume was measured twice during pretreatment, once predose at the beginning of Week 2, and once predose at Weeks 13, 26 and 39, each animal's respiratory minute volume was continuously recorded for a period of 15 minutes. The overall mean of the 15-minute interval were determined for each animal. Measurements were made using the Buxco Biosystem XA.

Increased heart rate was noted in MD and HD males and females (+30-69%, compared to the pretest value on the same day) at Weeks 13, 26, and 39. This was an expected pharmacological effect of the test article.

Table 9: 39 week dog study: Heart rate (beats per minute, percent change)

			Week 13				Week 26				Week 39			
	Pretest		Male		Female		Male		Female		Male		Female	
	Male	Female	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Heart rate (beats per minute)														

Control (air)	105	102	103	118	118	107	98	103	108	98	112	113	110	117
Control (vehicle)	98	98	105	107	113	113	83	105	102	112	100	98	103	115
LD	115	95	130	118	130	110	128	115	115	115	120	118	123	110
MD	105	118	105	158	103	113	100	150	135	125	120	203	120	168
HD	108	107	133	188	122	190	127	170	132	188	140	182	137	200
Percent change compared to pretest on same day														
Control (air)			15%		-9%		5%		-9%		1%		6%	
Control (vehicle)			2%		0%		27%		10%		-2%		12%	
LD			-9%		-15%		-10%		0%		-2%		-11%	
MD			50%		10%		50%		-7%		69%		40%	
HD			41%		56%		34%		42%		30%		46%	

Hematology

Blood was collected following overnight food deprivation once prior to commencement of treatment and during Weeks 13, 26, and 39 of treatment, and at the end of the recovery period. A standard battery of hematology and coagulation parameters was measured.

There were no consistent dose dependent changes in hematology or coagulation parameters identified in both sexes, when comparing to the vehicle control group.

Clinical Chemistry

See Hematology for blood collection methods. A standard battery of clinical chemistry parameters was measured.

There were no consistent dose dependent changes in clinical chemistry parameters identified in both sexes, when comparing to the vehicle control group.

Urinalysis

Urine was collected overnight during which time the animals were deprived of food and water. Specific gravity, glucose, protein, bilirubin, ketones, urobilinogen, blood, pH, color, appearance, chloride, nitrites, potassium, sodium, volume, and microscopy of centrifuged sediment were examined. There were no treatment related changes in urinalysis parameters.

Gross Pathology

Animals were fasted overnight prior to scheduled euthanasia. Animals euthanized on completion of the treatment/recovery period underwent exsanguination by incision of the axillary or femoral arteries following anesthesia by intravenous injection of sodium pentobarbital. A sedative, ketamine HCl for injection, and xylazine, were administered

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by intramuscular injection before animals were transported from the animal room to the necropsy area. Necropsy consisted of an external examination, including identification of all clinically recorded lesions, as well as a detailed internal examination.

There were no clear treatment related gross findings.

Organ Weights

A standard battery of organs was weighed as specified in the sponsor's table shown under Histopathology.

There were dose dependent increases in spleen weight in both males (BW normalized: LD 4%, MD 9%, HD 109%) and females (BW normalized: LD 32%, MD 30%, HD 106%), with a trend for increase at the HD when normalized for brain weight. After the recovery period, there was reversal in spleen weight. There were no consistent microscopic changes observed in both males and females that corresponded to the increased spleen weight. Changes in organ weight were most likely effected by decreased body weight (see Body Weight), therefore it is difficult to interpret organ weight changes.

Other changes observed in adrenal, lung, pituitary and thyroid weight were only observed in one sex, but are shown below for completeness. There were no corresponding test article related microscopic changes in the adrenal glands, pituitary glands, or thyroid which corresponded to weight changes observed.

Table 10: 39 week dog study: Organ weight, percent change compared to vehicle control

		Dose (mg/kg)							
		Week 39						Week 43	
		Males			Females			Males	Females
		0.031	0.12	0.33	0.028	0.11	0.32	0.33	0.32
Absolute	ADRENAL	-8%	13%	20%	-14%	-12%	-5%	1%	4%
	LUNG	-7%	-2%	-2%	12%	10%	2%	-6%	13%
	PITUITARY	5%	-2%	-2%	21%	46%	38%	-30%	21%
	SPLEEN	16%	3%	97%	36%	27%	72%	-20%	-27%
	THYROID	-9%	-12%	-13%	18%	58%	35%	23%	81%
BW	ADRENAL	-17%	19%	27%	-17%	-10%	14%	-9%	-2%
	LUNG	-17%	3%	4%	8%	12%	23%	-16%	7%
	PITUITARY	-5%	3%	4%	17%	49%	65%	-37%	15%
	SPLEEN	4%	9%	109%	32%	30%	106%	-28%	-31%
	THYROID	-18%	-7%	-7%	14%	61%	61%	10%	71%
BrW	ADRENAL	-10%	9%	17%	-18%	-19%	-10%	-1%	10%
	LUNG	-10%	-6%	-4%	7%	1%	-3%	-8%	19%
	PITUITARY	3%	-6%	-4%	16%	34%	31%	-32%	28%

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	SPLEEN	12%	0%	93%	30%	16%	63%	-22%	-23%
	THYROID	-12%	-15%	-14%	13%	45%	28%	20%	91%

Histopathology

Adequate Battery Yes

Tissue list for collection, weighing (W) and/or processing (P)

W	P	Adrenal glands		P	Nasopharynx (included in the 3rd level of nasal cavities)[b, d]
	P	Aorta		P	Optic nerves[a]
	P	Bone and marrow (sternum)[b]	W	P	Ovaries and oviducts
W	P	Brain (cerebrum, cerebellum, midbrain and medulla oblongata)		P	Pancreas
	P	Cecum		P	Pharynx
	P	Colon	W	P	Pituitary
	P	Duodenum	W	P	Prostate
	P	Epididymides[a]		P	Rectum
	P	Esophagus		P	Salivary gland (mandibular, unilateral)
	P	Eyes[a]		P	Sciatic nerve
	P	Femur[b]		P	Skeletal muscle
	P	Gall bladder		P	Skin (inguinal)
W	P	Heart (including section of aorta)		P	Spinal cord (cervical, thoracic, lumbar)
	P	Ileum	W	P	Spleen
	P	Jejunum		P	Stomach
W	P	Kidneys			
	P	Larynx (3 levels)	W	P	Testes[a]
	P	Lacrimal glands	W	P	Thymus
W	P	Liver (sample of 2 lobes)	W	P	Thyroid lobes (and parathyroids)[c]
W	P	Lungs (all lobes)[d]		P	Tongue
	P	Lymph node – bronchial		P	Trachea
	P	Lymph node – mandibular		P	Urinary bladder
	P	Lymph node – unilateral		P	Ureters
	P	Lymph node – mesenteric	W	P	Uterus (horns, body and cervix)
	P	Mainstem bronchi		P	Vagina
	P	Mammary gland (inguinal)[c]		P	Macroscopic lesions
	P	Nasal cavities and sinuses (3 levels)[b, d]			Animal identification

a Fixed in modified Davidson's fluid (testes and epididymides); fixed in Davidson's fluid (eyes and optic nerves), (euthanized animals only).
b Bone decalcified prior to sectioning.
c Examined histopathologically only if present in routine sections of skin of males only (mammary gland). At least one parathyroid was examined.
d Infused with neutral buffered 10% formalin (all animals).
For all euthanized animals, 3 femoral bone marrow smears were prepared and stained for possible examination.

(Sponsor's table)

Peer Review: Yes, by a Novartis pathologist.

Histological Findings:

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There were no findings suggestive of systemic toxicity. There were no clear test article related findings in the lung, bronchus, trachea, or nasal/sinus cavity.

Test article related findings were identified in the pharynx, lacrimal gland, and mandibular salivary gland. In the pharynx, inflammation (grade 1) was noted at the HD (males 3/4, females 3/4), but was also observed in 1/4 control males. Ectasia of the ducts and or alveoli of the pharynx (grade 1 and 2) was observed in males (3/4 MD, 1/4 HD) and females (1/4 MD, 3/4 HD). All findings reversed after the recovery period. The findings in the pharynx were generally mild, reversible, and are clinically monitorable, therefore are not considered dose limiting. Hypertrophy was noted in the lacrimal and salivary glands, and is not a dose limiting finding.

Table 11: 39 week dog study: Histopathological findings

	Dose (mg/kg/day)							
	Males				Females			
	0 (Vehicle)	0.031	0.12	0.33	0 (Vehicle)	0.028	0.11	0.32
Observations/N	4	4	4	4	4	4	4	4
LACRIMAL GLAND								
hypertrophy	0	0	3	4	0	0	0	1
grade 1	0	0	2	3	0	0	0	1
grade 2	0	0	1	1	0	0	0	0
inflammation (grade 1)	0	0	0	0	0	0	0	1
Recovery	0	-	-	0	0	-	-	0
PHARYNX								
inflammation	1	0	3	3	0	1	1	3
grade 1	1	0	3	3	0	1	1	3
ectasia: ducts and/or alveoli	0	0	3	1	0	0	1	3
grade 1	0	0	2	1	0	0	1	3
grade 2	0	0	1	0	0	0	0	0
Recovery	0	-	-	0	0	-	-	0
SALIVARY GLAND MANDIBULAR								
hypertrophy (grade 1)	0	0	1	4	0	0	0	0
Recovery	0	-	-	0	0	-	-	0

Toxicokinetics

Blood samples (approximately 1 mL) were collected by jugular vein on Day 1, Day 28, and in Weeks 13, 26, and 39 at the following time points: 0 h (immediately post end of

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exposure), 30 min, 3 hrs, 7 hrs after the end of the inhalation period and 24 hrs after the beginning of the inhalation period. All samples received were analyzed for NVA237 by the sponsor using an LC-MS/MS method, and are expressed in quaternary cation of the bromide salt (base) form in the study report, but have been listed in both cation and salt form in this review. The ratio of the salt: quaternary cation of the bromide salt (base) is defined as 1.251 on page 1514 of the study report.

Systemic exposure (AUC) to the test article increased with dose, but was higher in HD males (80.9 ng.h/mL) than in HD females (32.9 ng.h/mL). The higher AUC values in males result from higher concentrations of plasma test article in 1/6 males compared to all other males and females. Tmax was generally estimated soon after the time of inhalation (0 to 0.13 hr after inhalation).

Concentrations of the test article were detected in control animals. Levels of the test article above the LLOQ (0.100 ng/mL) were detected in 4 out of 300 samples in Week 26 and Week 39 air control samples (0.120 ng/mL to 5.99 ng/mL) and 9 out of 300 samples in Day 28, Week 26 and Week 39 vehicle control samples (0.103 ng/mL to 0.727 ng/mL). The sponsor stated that these were most likely the result of external contamination. Based on the low level of test article, the study is still considered valid.

Table 12: 39 week dog study: Toxicokinetic parameters (quaternary cation of the bromide salt)

			Male			Female		
Time point	Parameter	Unit	LD	MD	HD	LD	MD	HD
Day 1	tmax	h	0 *	0 *	0 *	0 *	0 *	0 *
	Cmax	ng/mL	2.05	3.88	16.9	1.54	3.77	18.1
	AUC(0-24h)	ng.h/mL	6.6	8.97	47.8	4.04	10.4	41.8
Day 39	tmax	h	0 *	0 *	0 *	0 *	0.13 *	0 *
	Cmax	ng/mL	0.927	4.07	42.1	1.49	8.84	8.2
	AUC(0-24h)	ng.h/mL	4.64	21.3	80.9	4.63	33.6	32.8

* based on time after inhalation

Table 13: 39 week dog study: Toxicokinetic parameters (salt form)

			Male			Female		
Time point	Parameter	Unit	0.031	0.12	0.33	0.028	0.11	0.32
Day 1	tmax	h	0 *	0 *	0 *	0 *	0 *	0 *
	Cmax	ng/mL	2.56	4.85	21.1	1.93	4.72	22.6
	AUC(0-24h)	ng.h/mL	8.3	11.22	59.8	5.05	13.0	52.3
Day 39	tmax	h	0 *	0 *	0 *	0 *	0.13 *	0 *
	Cmax	ng/mL	1.16	5.09	52.7	1.86	11.06	10.3
	AUC(0-24h)	ng.h/mL	5.80	26.6	101.2	5.79	42.0	41.0

* based on time after inhalation

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Dosing Solution Analysis

The concentration of the test article was measured at the mask output weekly during the first 13 weeks and at least once every two weeks thereafter. A measured volume of test atmosphere was drawn through a glass fiber filter at a rate, which did not exceed 10 L/minute. Mask output concentrations of test article were determined by an HPLC method.

All 3 samples taken from all time points in the air control and vehicle control groups were below the LLOQ (lower limit of quantitation). Values are shown below under "Analytical" for the LD (1 sample taken at each time point), MD (2 samples taken at each time point), and the HD (3 samples are taken at each time point).

The average chamber conditions are presented in the table below:

Group	Gender	Mask concentrations (mg/L)		Particle size MMAD (µm) ± GSD	
		Gravimetric**	Analytical*	Gravimetric***	Analytical (salt)
1	Male Female	-	0	-	-
2	Male Female	0.0152 0.0139	0	2.7 ± 2.2	-
3	Male Female	0.0071 0.0069	0.00090/0.00067 0.00075/0.00058	2.2 ± 2.2	2.3 ± 2.0
4	Male Female	0.0160 0.0157	0.00309/0.00248 0.00295/0.00236	2.2 ± 2.3	2.2 ± 1.9
5	Male Female	0.0307 0.0301	0.00790/0.00631 0.00738/0.00590	2.0 ± 2.4	2.1 ± 1.8

\- Not measured.
* Dose levels are expressed in salt/cation form.
** As determined by using glass fiber filters.
*** As determined by using teflon filters.

(Sponsor's table)

7 Genetic Toxicology

IND 48655, review dated 6/20/07

8 Carcinogenicity

NDA 207923/207930, review dated 7/7/15

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9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A subcutaneous fertility and early embryonic development study in rats

Study no.:	0870596
Study report location:	NDA 207923, SD-1
Conducting laboratory and location:	Novartis Pharmaceuticals Corporation East Hanover New Jersey 07936. Bioanalytical analysis was conducted at [redacted] (b) (4)
Date of study initiation:	March 2, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NVA237, batch #0724007, 100.1%

Key Study Findings

- Rats received subcutaneous (SC) doses of 0 (vehicle, 5% dextrose), 0.19, 0.63, 1.88 mg/kg/day (salt) to assess fertility. Males were dosed at least 28 days prior to mating, during the 2 week mating period, and until terminal necropsy (Day 50-53). Females were dosed for 2 weeks prior to mating, through the mating period, and gestation days (GD) 0 to 6.
- Doses were based on the results of a 2 week SC dose range finding rat study (study 0870723), which showed decreased body weight gain at the HD of 6.255 mg/kg/day (salt).
- There were no mortalities.
- Males developed a dose dependent decrease in body weight gain that persisted throughout the study (Day 50: LD 87%, MD 82%, HD 69%), relative to the control. Females also developed decreased body weight gain during the premating period (LD 90%, MD 80%, HD 50%). During the gestation period, MD and HD females had decreased body weight gain (81-88%), which recovered by GD 13 after dosing was ceased. Based on the decreases in body weight, dosing appeared adequate.
- Dosed animals had a higher percentage of abnormal estrous cycles (shortened, extended, prolonged, acyclic) compared to control, but this was not dose dependent (control 22%, LD 44%, MD 40%, HD 40%). Based on the high percentage of abnormal cycles in all groups, and the lack of clear dose response, it is difficult to determine if this is a test article related finding.
- Systemic exposure (AUC and Cmax) were similar between the sexes at the LD and MD, but was higher in males at the HD. AUC increased in a roughly dose

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proportionate manner at the LD and MD, but was greater than dose proportional in HD males versus HD females

- Fertility index was generally low in all groups, including the control (control 72%, LD 60%, MD 84%, HD 60%). This effect was not dose dependent. The sponsor stated that studies conducted "recently" in the same facility had fertility rates that ranged from 84% to 96%, and noted that only the MD had a fertility rate within the expected range. Due the lack of dose response, this did not appear to be a test article related finding, but there is a concern that the cause of the decreased fertility rate may mask a test article related effect.
- At the MD and HD, there was a slight decrease in the litter mean number of corpora lutea (control 13.2, LD 13.6, MD 12.6, HD 11.0) and number of implantations (control 11.6, LD 12.3, MD 9.9, HD 7.9), which corresponded to a decreased number of live fetuses per litter (control 10.9, LD 11.6, MD 9.9, HD 7.9). Values, however, at the MD were within the historical control data submitted by the sponsor.
- The reproductive and fertility NOAEL was determined to be the MD of 0.63 mg/kg NVA237 (salt), which is associated with an $AUC_{0\text{-}24h}$ of 98.0 h*ng/mL (salt) in females and males.

Methods

Doses:	0 (vehicle), 0.19, 0.63, 1.88 mg/kg/day (salt)
Frequency of dosing:	Daily
Dose volume:	1.5 mL/kg
Route of administration:	Subcutaneous
Formulation/Vehicle:	5% Dextrose Injection, USP
Species/Strain:	Rat/Wistar Hannover, Crl:WI(Han)
Number/Sex/Group:	25/sex/group
Satellite groups:	None
Study design:	Males were dosed at least 28 days prior to mating, during the 2 week mating period, and until terminal necropsy (Day 50-53). Females were dosed for 2 weeks prior to mating, through the mating period, and gestation days (GD) 0 to 6. The 4 weeks and 2 weeks of dosing premating for males and females, respectively, is consistent with the recommendations in ICH S5(R2). Females with negative sperm results (from vaginal washing) and/or negative for a vaginal plug were dosed until 6 days after the mating period.
Deviation from study protocol:	1 LD male was treated during the study for neck wound. No description of the treatments/actions were provided in the study report, but were documented by the sponsor's raw data file (p. 14 of the study report).

Group	Number/ Sex	Animal numbers [a,b] males	Animal numbers [a,b] Females	Dose (mg/kg/day)[c] Base/Salt[d]	Concentration (mg/mL) Salt
1 Control	25	2-50	1-49	0	0
2 Low	25	56-104	55-103	0.15/0.19	0.13
3 Mid	25	110-158	109-157	0.5/0.63	0.42
4 High	25	164-212	163-211	1.5/1.88	1.25

[a]PSA computer assigns odd animal numbers to females and even numbers to males.
[b]Two studies were set up on the computer system. Study 0870596M was utilized for processing males, and 0870596F was utilized for processing females.
[c]Not corrected for purity.
[d]Salt/base ratio for NVA237 is 1.251.

(Sponsor's table)

Dose selection

Dose selection was based on results of a non-GLP 2 week SC rat dose range finding rat study (study #0870723). During the pilot (sighting) portion of the study, animals (2/sex/group) were dosed with 0.6255, 6.255, 9.3825 mg/kg/day (salt) for 4 days, and a single dose of 12.51 mg/kg (salt). Mortality, clinical signs, body weight and food consumption were assessed. In the main study, animals (5/sex/group) were dosed with 0 (vehicle), 0.6255, 1.8765, 6.255 mg/kg/day (salt) for 2 weeks (dose volume 3 mL/kg,

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vehicle 5% glucose). In the main study, assessment for mortality, clinical signs, body weight, and food consumption, as well as blood sampling for toxicokinetics, and necropsy with macroscopic examination were performed.

During the pilot phase, there were no mortalities. Summary table data were not presented for the pilot phase, therefore the sponsor's written summary data is captured here. Shallow respiration and other clinical signs were observed at 12.51 mg/kg, leading to discontinuation of treatment. Mydriasis was observed in all groups, and is an expected effect of the test article. The sponsor stated, that partially closed eyelids were observed at ≥ 6.255mg/kg/day. At 9.3825 mg/kg/day, all animals showed slightly decreased body weight (up to -7.4 %) on respective dosing days 2 to 4. Minimally to markedly decreased average (n = 2) food consumption was observed over 3 days at 9.3825 mg/kg/day (-17 % in males; -8.4 % in females) and over 1 day at 12.51 mg/kg (-68.4 % in males; -38.9 % in females)

In the 2-week main study, clinical signs included dose dependent partial closure of the eyelids (males: LD 1/5, MD 2/5, HD 4/5; females MD 3/5, HD 3/5), trembling (1/5 HD males), and tip-toe gait (1/5 HD males). Mydriasis (related to the pharmacology of the test article) was observed in all dosed males and females. Dosing site findings included discoloration (males: LD 1/5, MD 2/5, HD 5/5), scabbing (males: 1/5 MD, 5/5 HD), and swelling (males: 1/5 MD, 5/5 HD), and crusting (males: HD 1/5). At the MD (25%) and HD (-18%), there was dose dependent decrease in body weight gain, which coincided with decreased feed consumption at the MD (-9% Days 1-8), and HD (-23% Days 1-8, -10% Days 8-14). Necropsy findings included injection site reactions, which are clinically monitorable, and not relevant to the proposed clinical route of inhalation administration. There were also low incidence findings at the HD in the thymus (1/5 males, reddish discoloration), urinary bladder (2/5 males, dilatation), and liver (1/5 males, nodule). Based on the decreased body weight gain at the HD, the same doses were used in the rat fertility and rat PPND studies. Doses were higher in the rat EFD study.

Table 14: 2 week DRF rat study: Body weight assessment

	Dose (mg/kg/day)			
Day	0 (Vehicle)	0.6255	1.8765	6.255
Day 1	285	283	289	286
Day 6	296	297	292	284
ΔBW (g)	11	14	3	-2
BW gain, % initial	4%	5%	1%	-1%
BW gain, % control	100%	127%	27%	-18%

Table 15: 2 week DRF rat study: Feed consumption, compared to control

	Dose (mg/kg/day)		
Day	0.6255	1.8765	6.255
Days 1-8	-2%	-9%	-23%
Days 8-14	2%	-1%	-10%

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Observations and Results

Mortality

Animals were observed for mortality twice daily on weekdays (am and pm) and once daily on weekends. There were no deaths.

Clinical Signs

During the study period, males were observed twice daily, predose and within approximately 3 hours postdose. Females were observed twice daily during the dosing phase, predose and approximately 3 hours postdose. After the dosing phase, females we observed once daily.

Test article related clinical signs were limited to scabbing at the injection site in 4/25 HD males. This was not noted in females.

Body Weight

Animals were weighed once during the acclimation period. During the study period, males were weighed twice weekly until the initiation of terminal necropsies, after which they were weighed the day of necropsy. Females were weighed twice weekly until mated or sacrificed, and on GD 0, 3, 6, 9, and 13.

Male rats developed a dose dependent decrease in body weight gain that persisted throughout the study (Day 50: LD 87%, MD 82%, HD 69%), relative to the control. The decrease was observed at the first weighing in on Day 4.

Table 16: Fertility study: Body weight in males

	Dose (mg/kg/day) salt			
Study Day	0	0.19	0.63	1.88
Day 1	321	320	318	319
Day 18	366	357	354	347
ΔBW (g)	45	37	36	28
BW gain, % initial	14%	12%	11%	9%
BW gain, % control	100%	82%	80%	62%
Day 36	400	386	381	371
ΔBW (g)	79	66	63	52
BW gain, % initial	25%	21%	20%	16%
BW gain, % control	100%	84%	80%	66%
Day 50	420	406	399	387
ΔBW (g)	99	86	81	68
BW gain, % initial	31%	27%	25%	21%
BW gain, % control	100%	87%	82%	69%

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Females developed a dose dependent decrease in body weight gain during the premating period that was the most severe during the first week, with no body weight gain at the HD. Decreased body weight gain persisted (LD 90%, MD 80%, HD 50%) to the end of the 2 week dosing period, compared to control.

Table 17: Fertility study: Body weight in females, during the premating period

	Dose (mg/kg/day) salt			
Study Day	0	0.19	0.63	1.88
Day 1	202	205	203	205
Day 8	207	210	206	205
ΔBW (g)	5	5	3	0
BW gain, % initial	2%	2%	1%	0%
BW gain, % control	100%	100%	60%	0%
Day 15	212	214	211	210
ΔBW (g)	10	9	8	5
BW gain, % initial	5%	4%	4%	2%
BW gain, % control	100%	90%	80%	50%

During the gestation period, a slight decrease in body weight gain was observed during the first week at the MD (81%) and HD (88%), but this was not dose dependent. By GD13, a recovery of body weight gain was observed at the HD (107%), relative to control.

Table 18: Fertility study: Body weight in gestating females

	Dose (mg/kg/day)			
Study Day	0	0.15	0.5	1.5
GD 0	220	219	213	211
GD 6	246	244	234	234
ΔBW (g)	26	25	21	23
BW gain, % initial	12%	11%	10%	11%
BW gain, % control	100%	96%	81%	88%
GD 13	273	271	261	263
ΔBW (g) from GD 6	27	27	27	29
BW gain, % GD 6	11%	11%	12%	12%
BW gain, % control	100%	100%	100%	107%

Feed Consumption

Feeder weights were collected weekly on treatment Days 1, 8, 15, 22, and 29 in males, and on treatment Days 1, 8, 15 and GD 0, 3, 6, 9, and 13.

During the premating period, when males were dosed for 4 weeks, there was a slight decrease in male feed consumption at the HD on Days 1-8 (-13%), and Days 22-29 (-8%).

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Table 19: Fertility study: Feed consumption in males, percent change compared to control

	Dose (mg/kg/day) salt		
Study Day	0.19	0.63	1.88
Days 1-8	-4%	-4%	-13%
Days 8-15	0%	0%	-4%
Days 15-22	0%	-4%	-4%
Days 22-29	-4%	-4%	-8%

During the premating period (Days 1-15), when females were dose for 2 weeks, there was no notable difference in feed consumption. During the gestation period, there was a slight decrease in feed consumption at the MD and HD between GD3-6.

Table 20: Fertility study: Feed consumption in females, percent change compared to control

	Dose (mg/kg/day) salt		
Study Day	0.19	0.63	1.88
Days 1-8	0%	-7%	-7%
Days 8-15	0%	0%	0%
GD 0-3	-5%	-5%	-5%
GD 3-6	-5%	-10%	-10%
GD 6-9	-5%	-5%	-5%
GD 9-13	5%	0%	5%

Estrous Cycle Determination

Vaginal washings were performed daily during the premating period. Vaginal cytology data were recorded. A normal estrous cycle was defined as one lasting 4-5 days, which is consistent with the literature. Abnormal estrous were identified as “shortened” (2-days), prolonged (6-10 days), extended (3 days), and acyclic (10 days without estrous, or no estrous during the last 9 days of observations).

Dosed animals had a higher percentage of abnormal cycles (shortened, extended, prolonged, acyclic) compared to control, but it was not dose dependent (control 22%, LD 44%, MD 40%, HD 40%). All groups had a notably higher percent of abnormal cycles, compared to historical control data (0-12%) cited by the laboratory in similar fertility assessments. The sponsor provided no explanation for the overall increase in abnormal cycles in this study. Based on the high percentage of abnormal cycles in all groups, and the lack of a clear dose response, it is difficult to determine if this is a test article related finding.

Table 21: Fertility study: Estrous cycles

	Dose (mg/kg/day) salt

Study Day	0	0.19	0.63	1.88
Animals evaluated	25	25	25	25
Normal cycles number (%)	18 (72%)	14 (56%)	15 (60%)	15 (60%)
Abnormal cycles (%)	7 (22%)	11 (44%)	10 (40%)	10 (40%)
shortened	4	3	2	5
extended	2	6	3	3
prolonged	1	1	5	1
acyclic	0	1	0	1

(Adapted from sponsor's table)

Toxicokinetics

Blood (0.5 mL) was collected from the sublingual vein of isoflurane-anesthetized animals. Samples were collected from immediately prior to the mating period from 2/sex/group at each of the following time points: 0.5 1, 3, 7 and 24 hours postdose.

Systemic exposure to the test article was observed in all dose groups. Systemic exposure (AUC and Cmax) were similar between the sexes at the LD and MD, but higher in males at the HD. AUC increased in a roughly dose proportionate in males, but was greater than dose proportional in HD males.

All four control animals (2/sex) tested at 24 hrs post dose showed plasma concentrations of the test article (0.195 to 3.89 ng/mL). Based on the lack of positive signal before 24 hrs it appears that the source of the positive signal is *ex vivo* contamination. These results had no effect on the validity of the study.

Table 22: Fertility study: Toxicokinetic parameters (salt)

Dose (mg/kg/day, salt)	Gender	Tmax (h)	Cmax (ng/mL)	AUC_{0-24h} (salt) (h*ng/mL)
0.19	F	0.5	16.4	29.3
	M	0.5	18.1	30.8
	Both	0.5	17.3	30.0
0.63	F	0.5	67.8	93.8
	M	0.5	70.1	102.2
	Both	0.5	68.9	98.0
1.88	F	0.5	115	290
	M	0.5	156	519

* 0.5 hrs post dose was the first time point

Dosing Solution Analysis

Dosing solutions from Weeks 1, 5, and 7 were tested for test article. Dosing solutions containing test article were within 10% of the target concentration. Vehicle control samples were below the limit of quantification. Test article solutions in 5% Dextrose

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injection (0.13 to 1.25 mg/mL as a salt) were stable for at 35 days at 6 degrees Celsius and for at least 24 hours at room temperature. This is acceptable.

Necropsy

All animals were sacrificed by CO2 asphyxiation followed by exsanguination. Males were sacrificed after completion of the mating period (Days 50-53). Females were sacrificed at GD 13. Females negative for sperm were sacrificed 10 days following the end of the mating period. Gross examination was performed on "major viscera" according to the study report. No tissues were saved for histology based on the sponsor's evaluation that all organs/tissues were considered normal.

There were no dose dependent gross lesions identified. A mass on the epididymis was noted in 1 out of 25 males at the HD, but due to its low incidence, it is difficult to determine if this finding is test article related.

Sperm Counts

For male fertility parameters relating to sperm, the right and left testis and epididymis were weighed. The left testis was frozen for sperm counts. Testicular sperm was homogenized for head counts, with homogenization resistant testicular sperm counted manually. A sperm sample was collected from the vas deferens, and the videotaped to allow for manual determination of percent motile sperm. The right testis and epididymis were fixed, but subsequently discarded. The sponsor analyzed the testis and epididymis weight by normalizing to body weight, but according to Bailey *et al.* [1]normalizing of the testicular weight to body weight is an optimal endpoint. Therefore, the normalize values are not shown here.

There were no clear effects of the test article on sperm count or motility, considering the high variation in each parameter observed in the control group. There were no notable differences in absolute weights of the testes or epididymis.

Table 23: Fertility study: Sperm analysis

	Dose (mg/kg/day) salt			
Parameter	0	0.19	0.63	1.88
Number of males mated	25	25	25	25
Testis weight (g)	3.755	3.828	3.879	3.793
Epididymis weight (g)	1.432	1.426	1.448	1.418
Sperm count x 10^6 per gram testis	96.2 ± 34.5	99.0 ± 20.7	95.5 ± 23.1	91.0 ± 21.3
% Motility	79.8 ± 16.3	84.4 ± 7.2	83.1 ± 6.6	83.0 ± 9.9

[1] Bailey et al. Relationships Between Organ Weight and Body/Brain Weight in the Rat: What Is the Best Analytical Endpoint? Toxicologic Pathology, 32:448–466, 2004

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Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Males and females were cohabited in a 1:1 ratio for mating. Females underwent cesarean section on GD 13, at which time the number of corpora lutea from both ovaries was recorded for each pregnant female, and the uterine implant site was identified as either a live fetus or early resorption.

The time to mating (precoital interval) varied widely in control animals (2.9±3.3 days), making it difficult to determine if there was a biologically meaningful decrease in precoital interval in dosed animals. There were no dose dependent trends in mating and fertility index, or the number of pregnant rats (control 18/25, LD 15/25, MD 21/25, HD 15/25).

Fertility index was in general low in all groups, including the control (control 72%, LD 60%, MD 84%, HD 60%). This effect was not dose dependent. The sponsor stated that studies conducted "recently" in the same facility had fertility rates that ranged from 84% to 96%, and noted that only the MD had a fertility rate within the expected range. Due to the lack of dose response, this did not appear to be a test article related finding, but there is a concern that the cause of the decreased fertility rate may mask a test article related effect.

Upon further analysis, at the MD and HD, there was a slight decrease in the litter mean number of corpora lutea (control 13.2, LD 13.6, MD 12.6, HD 11.0) and number of implantations (control 11.6, LD 12.3, MD 9.9, HD 7.9), which corresponded to a decreased number of live fetuses per litter (control 10.9, LD 11.6, MD 9.9, HD 7.9). The decrease in implantations and live fetuses at the MD and HD may be a result of increased preimplantation loss at the MD and HD (control 12.1%, LD 9.9%, MD 19.8%, HD 22.4%), compared to the control and LD. Based on these dose dependent trends, it is difficult to rule out an effect of the test article on these parameters at the MD and HD.

In the study report, the sponsor had determined "slight but statistically significant decreases in the number of corpora lutea and implantation sites in females at 1.5 mg/kg/day [HD] which were attributed to the test article." Based on this determination, the reproductive and fertility NOAEL in female and male rats was considered to be the MD and HD, respectively, by the sponsor. An Information Request was sent on September 18, 2015 regarding the decreased number of corpora lutea, implantations, and live fetuses, which corresponded with increased pre implantation loss at the MD and HD compared to control. On September 22, 2015, the sponsor provided historical control data (see table below) for the number of corpora lutea, implantation sites, live fetuses and pre-implantation losses. All corresponding values at the MD were within historical control ranges. Fertility was judged to be impaired for HD males and females. It was not possible based upon the study design to determine if observed effects occurred in only sex as drug-treated males were paired with drug-treated females (e.g., the study did not included untreated females and males paired with drug-treated males and females, respectively).

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Table 24: Fertility study: Cesarean section data

	Dose (mg/kg/day) salt			
Study Day	0	0.19	0.63	1.88
Number of females mated	25	25	25	25
Precoital interval, days (mean ± SD)	2.9±3.3	2.0±0.09	2.0±1.1	2.2±1.2
Mating index % (no. examined)	72% (25)	60% (25)	84% (25)	60% (25)
Fertility index % (no. examined)	72% (25)	60% (25)	84% (25)	60% (25)
No. of pregnant rats	18	15	21	15
Litter mean no. of corpora lutea	13.2*	13.6*	12.6*	11.0
Litter mean no. of implantation	11.6*	12.3*	10.1*	8.5
Litter mean no. of live fetuses	10.9*	11.6*	9.9*	7.9
Live males %	NA	NA	NA	NA
No. of dead fetuses	0	0	0	0
Preimplantation loss %	12.1*	9.9*	19.8*	22.4*
Postimplantation loss %	6.1	5.4	2.5	8.1

NA = not available. The sponsor did not determine the sex of pups.
*within historical control range provided by sponsor

Table 25: Fertility study: Historical control data from reproductive studies in Wistar Hannover rats

		Reproductive parameter (mean)			
Study number	**Number of control litters**	**Corpora Lutea**	**Implantation sites**	**Live fetuses**	**% Pre implantation loss**
0570152	21	13.5	12.3	11.6	8.8
0970446	23	14.1	12.0	11.3	14.5
0770088	23	12.2	9.9	9.4	18.9
0970409[a]	21	14.0	13.0	12.4	7.5
0970409[a]	20	13.6	11.1	10.6	18.8
0270017	24	12.3	11.2	10.5	9.2
0770653	22	11.3	8.6	8.1	23.4
0670012	25	11.2	9.8	9.5	13.2
0670633	22	11.3	9.5	9.1	16.1
Total	201 litters				
Ranges		11.2 – 14.1	8.6 – 13.0	8.1 - 12.4	7.5 – 23.4
0870596 : 0.5 mg/kg		12.6	9.9	9.9	19.8

[a] This study had two mating trials. Control data is included from both trials.

(Sponsor's table)

9.2 Embryonic Fetal Development

RAT EFD STUDY

Study title: An inhalation embryo fetal development study in rats

Study no.:	900863/0680006
Study report location:	NDA 207923, SD-1
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	November 22, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NVA237 in 1% magnesium stearate, 99% lactose monohydrate, lot #X342 0906, 98% pure

Key Study Findings

- NVA237 at doses of 0 (air), 0 (vehicle: 1% magnesium stearate, 99% lactose monohydrate), 0.1, 0.68, and 3.83 mg/kg (estimated achieved dose, salt) was administered by inhalation in gravid rats to evaluate embryofetal development in rats. Animals were treated during gestation days (GD) 6 to 17, and sacrificed on GD 21.
- Dose dependent decreased body weight gain was observed at GD 12 at the MD (70%) and HD (27%), with partial reversal by GD 18 (MD 91%, HD 80%) and GD 21 (MD 100%, HD 91%). Based on decreased body weight gain, it appears that the dosing was adequate.
- Based on systemic exposure (AUC), exposure to NVA237 was roughly dose proportional, and was similar on GD6 and GD 17.
- There were no dose dependent changes in cesarean section parameters, or test article related malformations or variations up to the high dose of 3.83 mg/kg (salt), which is associated with an $AUC_{0\text{-}24h}$ of 388 h*ng/mL (salt) in gravid females.

Methods

Doses:	0 (air), 0 (vehicle), 0.1, 0.68, 3.83 mg/kg (estimated achieved dose, salt)
Frequency of dosing:	Daily, on GD 6 to 17 in gravid females
Dose volume:	120 min
Route of administration:	Inhalation (nose-only)
Formulation/Vehicle:	1% magnesium stearate, 91% lactose monohydrate
Species/Strain:	*Rattus norvegicus*/Wistar Hannover Crl:WI (han)

Reference ID: 3824703

Number/Sex/Group: 22 females/group main study
Satellite groups: Toxicokinetic animals: 3 females/group for Air and Vehicle groups, 9 females/group for test article dose groups
Study design: Dose levels of NVA237 0.1, 0.6 and 4 mg/kg/day (salt) were chosen for this study based on results from the 4-week inhalation study in rats followed by a 2-week recovery period (study no. 848192). Animals were mated at male: female ratio of 1:1. Females were examined daily for evidence of mating based on vaginal lavage for spermatozoa. Positive identification of spermatozoa was termed GD 0. Gravid females were dosed from GD 6 to 17 and sacrificed on GD 21.

Deviation from study protocol:

Group number identification	Target aerosol concentration (mg/L)	Target dose level (mg/kg/day)[a]	Female animal numbers	
			Main	Toxicokinetic
1/ Air control	NA	0	22	3
2/ Vehicle control	0.130	0	22	3
3/ NVA237	0.0046	0.1/0.08	22	9
4/ NVA237	0.027	0.6/0.48	22	9
5/ NVA237	0.183	4.0/3.2	22	9

a - expressed in active moiety (salt/quaternary cation of the bromide salt)
NA - Not applicable

The theoretical achieved doses of NVA237 (salt form) were calculated to be as follows:

Group	RMV (L/min)	Active conc'n (mg/L)	Exposure duration (min)	MMAD (µm)	Deposition fraction	Body weight (kg)[a]	Theoretical achieved dose (mg/kg/day)
3	0.167	0.0013	120	2.5	1	0.259	0.10
4	0.165	0.0088	120	2.2	1	0.255	0.68
5	0.162	0.0490	120	2.4	1	0.249	3.83

a Since no body weight was measured on gestation day 17, the average body weight was calculated from values measured on gestation day 18 and used for the calculation of theoretical achieved dose from day 6 to 17 of gestation.

(Sponsor's tables)

Observations and Results

Particle size

Particle size distribution analysis was performed at least once prior to the start of treatment and once weekly from each group during treatment, using a TE 20 800 cascade impactor. The mass median diameter and its geometric standard deviation (MMAD ± GSD) were calculated from the gravimetric and analytical data.

Reference ID: 3824703

The test article was within respirable range (between 1 – 5 microns). The MMAD ± GSD for NVA237 (salt) was 2.5 µm ± 2.1, 2.2 µm ± 1.8 and 2.4 µm ± 1.8 for the LD, MD and HD groups, respectively.

Mortality

All females were examined twice daily for mortality. There were no mortalities.

Clinical Signs

All females were examined twice daily for clinical signs. A detailed examination was performed on the days of body weight assessment, or as deemed appropriate by the conducting laboratory. There were no clear test article related clinical signs.

Body Weight

Individual body weights were measured on days GD 0, 3, 6, 9, 12, 15, 18 and 21. The air control group was used as reference for calculating percent body weight gain.

Dose dependent decreased body weight gain was observed at GD 18 at the MD (89%) and HD (79%). Based on decreased body weight gain, it appears that the dosing was adequate.

Table 26: Rat EFD study: Body weight gain, compared to Vehicle control

	Dose (mg/kg/day)				
Gestation Day	0 (Air)	0 (Vehicle)	0.1	0.6	4
Day 6	235.6	235.5	238.2	238.5	237.1
Day 12	252	250.6	253.9	249	241.2
ΔBW (g)	16.4	15.1	15.7	10.5	4.1
BW gain, % initial	7%	6%	7%	4%	2%
BW gain, % control	100%	92%	96%	64%	25%
Day 18	290.1	290.9	292.9	287.9	280.6
ΔBW (g)	54.5	55.4	54.7	49.4	43.5
BW gain, % initial	23%	24%	23%	21%	18%
BW gain, % control	100%	102%	100%	91%	80%
Day 21	327.1	327.6	330	330.2	320.7
ΔBW (g) from GD 18	37	36.7	37.1	42.3	40.1
BW gain, % GD 18	13%	13%	13%	15%	14%
BW gain, % control	100%	99%	100%	114%	108%

Feed Consumption

Individual food consumption was measured on GD 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18 and 18 to 21.

Reference ID: 3824703

There were no dose dependent effects on feed consumption.

Toxicokinetics

Blood samples (approximately 0.75 mL) were collected from the jugular vein of females. Samples were collected from animals assigned to the toxicokinetic phase on GD 6 and 7 at the following time points:

- Air control and vehicle control groups: 0 hour (immediately after the completion of inhalation) and 15 minutes after the completion of inhalation
- Test article dose groups: 0 hour (immediately after the completion of inhalation), 15 minutes, 1 hour, 3 hours and 7 hours after the completion of inhalation and 24 hours after the initiation of inhalation.

Samples from both air and Vehicle controls groups were below the LLOQ (0.100 ng/mL). Based on systemic exposure (AUC), exposure to NVA237 was roughly dose proportional, and was similar on GD6 and GD 17.

Table 27: Rat EFD study: Toxicokinetic parameters (salt)

Time point	Dose (mg/kg/day)	Cmax (ng/mL)	AUC_{0-24h} (h*ng/mL)
GD 6	0.1	4.3	14.6
	0.68	7.5	27.4
	3.83	270	459
GD 17	0.1	3.0	9.9
	0.68	19	66
	3.83	230	388

Dosing Solution Analysis

The concentration of NVA237 was determined by HPLC and atomic absorption spectroscopy (AAS). Nominal chamber concentrations were calculated from the airflow through the chamber and the quantities of test or vehicle control articles used.

On July 30, 2015, an Information Request was sent requesting clarification of the filter concentration data on page 118 of the study report, and gravimetric and analytical chamber test data presented on pages 36-48 of the study report. The sponsor provided satisfactory clarification.

Analytical analysis showed that control (air) and control (vehicle) groups had no detectable levels of NVA237 (<LLOQ). The mean analytical chamber concentration of NVA237 (salt form) was determined to be 0.0013, 0.0088 and 0.0490 mg/L (salt) for the LD, MD and HD respectively. Based on these concentrations, the estimated achieved dose was calculated, using a deposition fraction of 1.

Reference ID: 3824703

The theoretical achieved doses of NVA237 (salt form) were calculated to be as follows:

Group	RMV (L/min)	Active conc'n (mg/L)	Exposure duration (min)	MMAD (µm)	Deposition fraction	Body weight (kg)[a]	Theoretical achieved dose (mg/kg/day)
3	0.167	0.0013	120	2.5	1	0.259	0.10
4	0.165	0.0088	120	2.2	1	0.255	0.68
5	0.162	0.0490	120	2.4	1	0.249	3.83

a Since no body weight was measured on gestation day 17, the average body weight was calculated from values measured on gestation day 18 and used for the calculation of theoretical achieved dose from day 6 to 17 of gestation.

(Sponsor's table)

Necropsy

Animals were euthanized on GD 21 by CO_2 asphyxiation followed by exsanguination. A gross examination was conducted, including an external examination, identification of all clinically recorded lesions, and an internal examination. Gross abnormalities were collected and preserved, and tissues were collected from 2 gravid control females for future reference.

On GD21, the reproductive tracts of females were dissected, and the ovaries were removed and the corpora lutea were collected. The gravid uterus was weighed, the uterus contents, including the placentas, were examined and the number and position of live fetuses, dead fetuses and early, middle and late resorptions were recorded. For females that were determined to be not pregnant, the uterus was stained with 10% aq (v/v) ammonium sulfide solution and examined for implantation sites.

There were no clear test article related gross abnormalities.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
See Necropsy for methods.

Cesarean section data was reported by the sponsor as an average across all animals for some parameters. The average using the litter as a unit is recommended by ICH S5; therefore the litter average was calculated by the reviewer as indicated in the table below.

There were no dose dependent changes in cesarean section parameters.

Table 28: Rat EFD study: Cesarean section observations

	Dose (mg/kg/day)				
Parameter	0 (Air)	0 (Vehicle)	0.10	0.66	3.83
Number of females mated	22	22	22	22	22
No. of pregnant rats	22	22	22	20	22

Fertility index % (no. examined)[a]	100 (22/22)	100 (22/22)	100 (22/22)	90.9 (20.22)	100 (22/22)
Corpora lutea (litter mean no.)*	13.2	13.3	13.0	13.7	13.5
Implantations (litter mean no.)*	11.9	11.8	11.6	12.5	12.2
Live males % (litter mean no.)*	51.3	50.1	42.1	51.6	51.6
No. of live fetuses (litter mean no.)*	11.0	11.4	11.2	11.7	11.5
No. of dead fetuses (litter mean no.)*	0	0	0	0	0
Resorptions (litter mean no.)*	0.9	0.3	0.4	0.8	0.7
Gravid uterus weight (g; litter mean no.)*	77.2	77.0	77.7	79.7	77.5
Fetus weight (g; litter mean no.)	5.2	5.1	5.2	5.1	5.1
Preimplantation loss % (litter mean % ± S.D.)*[b]	10.0 ± 8.9	10.6 ± 12.1	10.3 ± 11.6	8.1 ± 10.9	9.0 ± 9.6
Postimplantation loss % (litter mean % ± S.D.)*[c]	7.4 ± 10.5	3.5 ± 6.2	3.4 ± 5.4	6.2 ± 10.3	6.0 ± 5.7

*calculated by reviewer
[a]Fertility Index (%) = (No. of pregnant females/No. of mated females) * 100 (sponsor stated "Pregnancy rate")
[b]Preimplantation loss (%) = [(No. of corpora lutea - no. of implants)/no. of corpora lutea] * 100
[c]Postimplantation loss (%) = [No. of implants - no. of live fetuses/no. of implants] * 100

Offspring (Malformations, Variations, etc.)

Each fetus was weighed externally examined, the sex determined based on external examination, and euthanized by SC injection of Euthanyl. An internal examination using a dissecting microscope was performed on approximately half of the fetuses in each litter. The heads of these fetuses were removed and placed in Bouin's fluid to allow for further examination. The remaining half of the fetuses in each litter were eviscerated and placed in 86% ethanol/15% methanol, and stained with alizarin red S for skeletal examination. Abnormalities were classified as major malformations, minor external visceral/skeletal abnormalities/common skeletal variants.

There were no dose dependent malformations or variations identified. The table below shows major malformations, visceral variations and skeletal variations. Incomplete ossifications are listed at the bottom of the table, and were considered to be normal background findings.

Table 29: Rat EFD study: Offspring observations

	Dose (mg/kg)

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Dosage	0 (Air)	0 (Vehicle)	0.1	0.6	4
Observations	Fetus (litter) affected				
Total examined fetuses (litters)					
External (EXT)	243 (22)	251 (22)	246 (22)	234 (20)	252 (22)
Visceral (VIS)	122 (22)	122 (22)	126 (22)	117 (20)	128 (22)
Skeletal (SKE)	121 (22)	129 (22)	121 (22)	117 (20)	124 (22)
Technique of Wilson (WT)	122 (22)	122 (22)	126 (22)	117 (20)	128 (22)
Fetal malformations	0	1 (1)	1 (1)	0	0
Head					
Cleft palate (EXT, WT)	0	0	1 (1)	0	0
Lower jaw absent (agnathia) (EXT, WT)	0	0	1 (1)	0	0
Mouth cavity reduced (microstomia) (WT)	0	0	1 (1)	0	0
Eye(s)					
Retinal folding (WT)	0	1 (1)	0	0	0
Lens(es) misshapen (WT)	0	1 (1)	0	0	0
Fetal visceral variations					
Head					
Tongue reduced (WT)	0	0	1 (1)	0	0
Liver					
Supernumary liver lobe (VIS)	3 (2)	2 (1)	3 (3)	0	1 (1)
Discoloration pale (VIS)	2 (2)	1 (1)	0	0	1 (1)
Fetal skeletal variations					
Vertebral column					
Extra presacra vertebrae	2 (2)	4 (3)	4 (2)	2 (2)	1 (1)
Ossification center on 1st lumbar or 14th thoracic vertebrae	17 (11)	18 (10)	17 (13)	22 (12)	16 (12)
Thoracic vertebrae centrum displaced from midline	0	0	0	1 (1)	0
Ribs					
Rudimentary 14th rib(s)	25 (12)	20 (12)	15 (10)	17 (9)	29 (14)
Extra 14th rib(s)	4 (4)	2 (2)	5 (2)	1 (1)	2 (2)
Ossification center(s) on 7th cervical vertebra	0	2 (2)	0	1 (1)	0
Rib(s) on 7th cervical vertebra	1 (1)	4 (3)	2 (2)	3 (2)	0
Extra 14th rib with contralateral rudimentary rib	4 (4)	0	1 (1)	3 (2)	2 (2)
Rudimentary 14th rib with contralateral ossification center	6 (4)	6 (4)	6 (4)	1 (1)	8 (5)
Incomplete ossification (unossified, incomplete ossification, bipartite, semi-bipartite)					
Skull					
Parietal bone(s): Incomplete ossification	0	4 (3)	4 (3)	4 (3)	1 (1)
Frontal bone(s): Incomplete ossification	0	1 (1)	0	0	0
Interparietal bone: Incomplete ossification	4 (4)	16 (9)	11 (7)	5 (4)	2 (1)

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Supraoccipital bone: Incomplete ossification	2 (2)	1 (1)	0	2 (1)	3 (2)
Hyoid bone: Incomplete ossification	2 (2)	2 (2)	1 (1)	1 (1)	0
Vertebral column					
Lumbar centrum semi-bipartite	0	0	0	0	1 (1)
Sacral vertebral centrum semi-bipartite	0	0	1 (1)	0	0
Pelvic Girdle					
Pubic bone(s): Incomplete ossification	0	1 (1)	0	0	0
Ischial bone(s): Incomplete ossification	0	1 (1)	0	0	0

RABBIT EFD STUDY

Study title: NVA237: An inhalation embryo fetal development study in the rabbit

Study no.: 901910/0870597
Study report location: NDA 207923 SD-1
Conducting laboratory and location: (b) (4)
Date of study initiation: April 15, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: NVA237, batch # X345 0906, 100% purity

Key Study Findings

- NVA237 at doses of 0 (vehicle, 1% magnesium stearate, 99% lactose monohydrate), 0.5, 1.6 and 4.4 mg/kg/day (estimated achieved dose, salt) was administered by inhalation in gravid rabbits to evaluate embryofetal development. Animals were treated during gestation days (GD) 7 to 19, and sacrificed on GD 29.
- One HD female (#4504) was euthanized on GD 25 with clinical signs that included decreased activity, reduced appetite, suspected dehydration and dilated pupils. Poor body condition was associated with a lack of body weight gain throughout the study. Necropsy findings included a pale firm liver, gelatinous material in the colon, and raised pail areas in the stomach. The mortality is considered to be test article related. Based on the TA related death at the HD, it appears that the MTD was exceeded.
- Gravid females developed a dose dependent decrease in body weight gain throughout the dosing period (GD19: LD 28%, MD 6%, HD -22%), compared to controls. There was a reversal of this trend after the dosing period at GD 29 with body weight gains at all doses. The decreased body weight gain corresponded to decreased feed consumption. Based on decreased body weight gain, it appears that the dosing was adequate.

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- In gravid females at GD19, systemic exposure (AUC), increased in a less than dose proportional manner, whereas Cmax increased in a roughly dose proportional manner. Fetal samples were negative for the test article (LLOQ of 0.500 ng/g).
- There were no test article related changes in uterine findings, or increases in malformations or variations up to the high dose of 4.4 mg/kg (salt), which is associated with an $AUC_{0\text{-}24h}$ of 148 h*ng/mL (salt) in gravid females.

Methods

Doses:	0 (vehicle), 0.5, 1.6 and 4.4 mg/kg/day (estimated achieved dose, salt)
Frequency of dosing:	Daily (GD 7 to 19)
Dose volume:	120 minutes
Route of administration:	Inhalation (nose-only)
Formulation/Vehicle:	1% magnesium stearate, 99% lactose monohydrate
Species/Strain:	*Oryctolagus cuniculus*, New Zealand White Rabbit (6 months at the start of treatment)
Number/Sex/Group:	20 rabbits/group
Satellite groups:	3 rabbits for control and 5/treated group for toxicokinetic sampling
Study design:	Dose levels of NVA237 0.5, 1.5 and 4.5 mg/kg/day (salt) were chosen for this study based on results from a dose range finding EFD study (900862/ 0680005) in which rabbits were dosed with 0.1, 0.7, 2.4 and 4.5 mg/kg/day (salt) from GD 7 to GD 19. For this DRF study, clinical signs, body weights, food consumption, toxicokinetic evaluation, gross observations at necropsy, uterine findings, fetal weight and external fetal findings. In the DRF study, the sponsor reported a reduction in body weight gain, and a decrease body weight at 4.5 mg/kg.
Deviation from study protocol:	No deviations were stated that affect study validity.

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Group number identification	Target dose level salt/active moiety [b, c] (mg/kg/day)	Target chamber concentration (mg/L)	Dosing duration (min)	Animal number main study females	Animal number toxicokinetic females
1/ Vehicle control [a]	0	0	120	1501 to 1505, 1507 to 1520, 1606	1521 to 1523
2/ Low NVA237	0.5/0.4	0.0109	120	2501 to 2520	2521 to 2525
3/ Mid NVA237	1.5/1.2	0.0326	120	3501 to 3515, 3616 to 3620	3521 to 3525
4/ High NVA237	4.5/3.6	0.0979	120	4501 to 4520	4521 to 4525

a Vehicle control was lactose with 1% magnesium stearate, and concentration was chosen to match dose level of magnesium stearate achieved for group 4

b It should be noted that for NVA237, the term 'quaternary ammonium cation of the bromide salt' is the correct terminology, rather than 'base'

c the salt/active moiety ratio for NVA237 is 1.251.

(Sponsor's table)

Observations and Results

Particle size

Particle size distribution analysis was performed at least once prior to the start of treatment and once weekly from each group during treatment, using a TE 20 800 cascade impactor. The mass median diameter and its geometric standard deviation (MMAD ± GSD) were calculated from the gravimetric and analytical data.

The test article was within respirable range (between 1 – 5 microns). The MMAD ± GSD for NVA237 (salt) determined chemically was 2.4 µm ± 1.7, 2.9 µm ± 1.8 and 2.5 µm ± 2.0 for the LD, MD and HD groups, respectively.

Mortality

One HD female (#4504) was euthanized on GD 25 with clinical signs that included decreased activity, reduced appetite, suspected dehydration and dilated pupils. Poor body condition was associated with a lack of body weight gain throughout the study. Necropsy findings included a pale firm liver, gelatinous material in the colon, and raised pail areas in the stomach. The mortality is considered to be test article related. Based on the TA related death at the HD, it appears that the MTD was exceeded.

Clinical Signs

Test article related clinical signs were identified in HD animal #4504, resulting in unscheduled euthanasia (see Mortality). There were no clear test article related clinical signs in other animals.

Clinical signs were noted by the sponsor in one LD female (#2511), and one MD female (#3505). Animal #2511 was noted to have a prominent backbone, thinness, and thin fur cover associated with low food consumption. Animal #3505 was observed to have

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moderately soft feces, and aborted tissue material including 9 fetuses and 4 placentas on GD 28. The sponsor stated in the study report text that animal # 3505 also displayed suspected dehydration, thinness, backbone prominent, skin pallor, decreased activity, but these were not listed in sponsor table 2.2 (Individual clinical observation data). Based on a lack of dose response, it is not clear if the clinical signs in animals #2511 and #3505 are test article related. No TK data are available for main study animals.

Body Weight

Gravid females developed a dose dependent decrease in body weight gain throughout the dosing period, illustrated by decreased body weight gain at GD 19 (LD 28%, MD 6%, HD -22%), compared to controls. The decreased body weight gain corresponded to decreased feed consumption in dosed groups. There was a reversal of this trend after the dosing period at GD 29 with body weight gains at all doses, which corresponded to increased feed consumption (see Feed Consumption). Based on decreased body weight gain, it appears that the dosing was adequate.

Table 30: Rabbit EFD study: Body weight analysis

	Dose (mg/kg/day)			
Gestation Day	0	0.5	1.6	4.4
Day 7	3.36	3.37	3.45	3.38
Day 13	3.45	3.36	3.43	3.32
ΔBW (kg)	0.09	-0.01	-0.02	-0.06
BW gain, % initial	3%	0%	-1%	-2%
BW gain, % control	100%	-11%	-22%	-67%
Day 19	3.54	3.42	3.46	3.34
ΔBW (kg)	0.18	0.05	0.01	-0.04
BW gain, % initial	5%	1%	0%	-1%
BW gain, % control	100%	28%	6%	-22%
Day 29	3.73	3.62	3.71	3.63
ΔBW (kg) from GD 19	0.19	0.2	0.25	0.29
BW gain, % GD 19	5%	6%	7%	9%
BW gain, % control	100%	105%	132%	153%

Feed Consumption

Gravid females developed a dose dependent decrease in feed consumption throughout the dosing period. Specifically, decreased feed consumption was noted at GD 7-13 (LD -40%, MD -47%. HD -55%) and at GD 14-19 (LD -37%, MD -38%, HD -49%). After dosing was ceased, there were no notable differences in feed consumption.

Table 31: Rabbit EFD study: Feed consumption

	Dose (mg/kg/day)		
Gestational Day	0.5	1.6	4.4
Days 7-13	-40%	-47%	-55%

Reference ID: 3824703

Days 14-19	-37%	-38%	-49%
Days 20-29	9%	15%	10%

Toxicokinetics

Blood samples (~1.0 mL) were collected via the auricular arteries on GD 19 at the following time points:

- 0 hour (immediately after the completion of inhalation), 15 minutes, 1 hour, 3 hours and 7 hours after the completion of inhalation and 24 hours after the initiation of inhalation in groups that received the test article.
- 0 hour (immediately after the completion of inhalation) and 15 minutes after the completion of inhalation in the vehicle control group

Samples were analyzed for the test article using LC-MS/MS.

Two animals (# 1521, 1523) in the vehicle control group were positive for NVA237 (0.287 - 0.795 ng/mL), with confirmation by reanalysis. For animal 1521, 0.373 ng/mL was measured at 0 h. For animal # 1523, 0.795 and 0.285 ng/mL were measured at 0 h and 15 min, respectively. The sponsor hypothesized that these readings were a result of *ex vivo* contamination, based on filter samples from the dosing chamber being negative for NVA237. Considering the relatively low concentration of NVA237 detected, these positive samples did not affect the validity of the study.

All animals in the NVA237 treated groups were positive for test article. Systemic exposure (AUC), increased in a less than dose proportional manner, whereas Cmax increased in a roughly dose proportional manner.

Fetal samples were negative for the test article (LLOQ of 0.500 ng/g).

Table 32: Rabbit EFD study: Toxicokinetic parameters in gravid females at GD19 (salt)

Dose (mg/kg/day)	Cmax (ng/mL)	AUC_{0-24h} (h*ng/mL)
0.5	5.68	33.5
1.6	24.9	74.2
4.4	56.7	148

Dosing Solution Analysis

The concentration of NVA237 was determined by HPLC and atomic absorption spectroscopy (AAS). Nominal chamber concentrations were calculated from the airflow through the chamber and the quantities of test or vehicle control articles used. Analytical data were collected on four replicates.

On July 30, 2015, an Information Request was sent requesting clarification of the filter concentration data on page 112 of the study report, and gravimetric and analytical

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chamber test data presented on pages 40-54 of the study report. The sponsor provided satisfactory clarification.

Analytical analysis showed that the control (vehicle) group had no detectable levels of NVA237 (<LLOQ). The mean analytical chamber concentrations of NVA237 (salt form) were determined to be 0.011, 0.033 and 0.092 mg/L (salt) for the LD, MD and HD respectively. Based on these concentrations, the estimated achieved dose was calculated, using a deposition fraction of 1.

The theoretical achieved doses of NVA237 (salt form) were calculated to be as follows:

NVA237 (salt form)

Group	Target dose level (mg/kg/day)	Body weight (kg)	RMV (L/min)	Overall exposure duration (min)	Mean analytical MMAD (µm)	Deposition fraction	Mean active conc'n (mg/L)	Theoretical achieved dose (mg/kg/day)
2	0.5	3.37	1.333	120	2.4	1	0.011	0.5
3	1.5	3.45	1.359	120	2.9	1	0.033	1.6
4	4.5	3.35	1.324	120	2.5	1	0.092	4.4

(Sponsor's table)

Necropsy

Main study females were euthanized on GD29. The reproductive tract was dissected out, the ovaries removed and the corpora lutea counted. The gravid uterus was weighed, the uterus contents, including the placentas, were examined and the number and position of live fetuses, dead fetuses and early, middle and late resorptions and/or empty implantation sites were recorded. The uterus of any animal judged to be nonpregnant was stained with 10% aq (v/v) ammonium sulfide solution and examined for implantation sites. The following organs were retained: abnormalities, larynx, lungs, main stem bronchi, nasal cavities and sinuses, ovaries, pharynx, and uterus.

Toxicokinetic females were euthanized on GD 20. The reproductive tract was dissected out, the ovaries removed and the corpora lutea counted. The gravid uterus was weighed, the uterus contents, including the placentas, were examined and the number and position of live fetuses, dead fetuses and early, middle and late resorptions and/or empty implantation sites were recorded.

Overall, there were no test article related changes in uterine findings.

There was a significant dose dependent increase in resorptions (control 0.1, LD 0.5, MD 0.6, HD 0.8, $P \leq 0.05$), but these were within the historical control range cited by the sponsor (early resorptions - 0.0-1.2, total resorptions - 0.1-1.9).

There was a significant dose dependent increase in percent post implantation loss (control 0.6, LD 5.1, MD 7.1, HD 9.5; $P \leq 0.05$), but these were within the historical control range cited by the sponsor (post implantation loss - 0.7-21.7%).

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

	Dose (mg/kg/day)			
Parameter	0 (Vehicle)	0.5	1.6	4.4
Number of females mated	20	20	20	20
No. of pregnant rabbits	18	20	20	19
Fertility index % (no. examined)[a]	90 (18/20)	100 (20/20)	100 (20/20)	95 (19/20)
Number of rabbits dying/euthanized	0	0	0	1
Number of rabbits aborting prior to scheduled caesarean	0	0	1	0
Corpora lutea (litter mean no.)*	8.8	9.4	9.4	9.3
Implantations (litter mean no.)*	8.3	8.4	8.4	8.7
Live males % (litter mean no.)*	48.6	44.3	54.9	48.6
No. of live fetuses (litter mean no.)*	8.3	7.9	7.8	7.9
No. of dead fetuses (litter mean no.)*	0	0	0	0
Resorptions (litter mean no.)*	0.1	0.5	0.6	0.8
Gravid uterus weight (g; litter mean no.)*	508.9	486.5	489.9	496.0
Fetus weight (g, litter mean no.)	44.08	44.29	44.50	45.23
Preimplantation loss % (litter mean % ± S.D.)*[b]	6.3	12.0	10.9	6.5
Postimplantation loss % (litter mean % ± S.D.)*[c]	0.6	5.1	7.1	9.5

Offspring (Malformations, Variations, etc.)

There were no dose dependent increases in malformations or variations. All offspring data are shown below for completeness.

	Dose (mg/kg)			
Dosage	0 (vehicle)	0.5	1.6	4.4
Observations	Fetus (litter) affected			
Total examined fetuses (litters)				
External (EXT)	149 (18)	157 (20)	148 (19)	143 (18)
Visceral (VIS)	149 (18)	157 (20)	148 (19)	143 (18)
Skeletal (SKE)	149 (18)	157 (20)	148 (19)	143 (18)
Fetal malformations	0	1 (1)	1 (2)	1 (1)
Heart				
Dilatation of the ascending aorta (VIS)	0	1 (1)	1 (2)	1 (1)

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Stenosis of the pulmonary trunk (VIS)	0	1 (1)	1 (2)	1 (1)
Interventricular septal defect (VIS)	0	1 (1)	1 (2)	1 (1)
Fetal visceral variations	1 (2)	1 (1)	0	1 (1)
Lungs and Thymus				
Accessory lung lobe absent (VIS)	1 (2)	0	0	0
Gallbladder				
Gallbladder absent (VIS)	0	1 (1)	0	0
Kidneys				
Reduction of the renal papilla(e) (VIS)	0	0	0	1 (1)
Fetal skeletal variations				
Skull				
Hyoid bone: Irregular ossification	5 (8)	5 (7)	4 (4)	0 (0)
Vertebral column				
Extra presacra vertebrae	1 (1)	0 (0)	0 (0)	0 (0)
25 Pre-sacral vertebrae	1 (1)	0 (0)	0 (0)	0 (0)
Ossification center on 1st lumbar	0 (0)	0 (0)	1 (2)	1 (1)
Cervical vertebral centrum absent	0 (0)	0 (0)	0 (0)	1 (1)
Cervical vertebral arch(es): Absent	0 (0)	0 (0)	0 (0)	1 (1)
Thoracic centrum misaligned	0 (0)	0 (0)	0 (0)	1 (1)
Thoracic vertebral arch(es) absent	0 (0)	0 (0)	0 (0)	1 (1)
Reduced number of caudal vertebrae	0 (0)	0 (0)	1 (1)	0 (0)
Sternebrae				
Sternebrae: Fused	1 (3)	4 (4)	1 (1)	1 (2)
Extra Sternebra(e)	5 (6)	3 (4)	0 (0)	0 (0)
Rib(s)				
Fused rib(s)	0 (0)	0 (0)	0 (0)	1 (1)
Notched rib(s)	0 (0)	0 (0)	1 (1)	1 (1)
Rib(s): Absent	0 (0)	0 (0)	0 (0)	1 (1)
Ossification center(s) on 7th cervical vertebra	2 (3)	0 (0)	2 (2)	1 (1)
Rib(s) on 7th cervical vertebra	3 (6)	1 (1)	2 (2)	0 (0)
Incomplete ossification (unossified, incomplete ossification, bipartite, semi-bipartite)				
Skull				
Parietal bone(s): Incomplete ossification	0 (0)	0 (0)	1 (1)	1 (1)
Frontal bone(s): Incomplete ossification	11 (16)	8 (13)	9 (17)	4 (6)
Hyoid bone: Incomplete ossification	11 (25)	14 (36)	5 (17)	11 (31)
Vertebral column				
Cervical vertebral arch(es): Incomplete ossification	0 (0)	0 (0)	0 (0)	1 (1)
Thoracic vertebral centrum semi-bipartite	5 (7)	4 (8)	3 (6)	4 (8)
Pelvic Girdle				
Pubic bone(s): Incomplete ossification	3 (3)	2 (6)	2 (2)	1 (1)

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Common Skeletal Variants	Litter mean (%)			
Unilateral 13th rib (extra/rudimentary)	17.15	17.23	12.03	11.13
Bilateral 13th rib (extra/rudimentary/ossification center)	30.37	29.17	26.00	29.81
Ribs - total 13th (unilateral and bilateral) extra/rudimentary/ossification center	33.05	24.26	29.01	34.64
Sternebrae (unossified/incomplete/semi-bipartite/bipartite)	24.11	27.48	33.56	34.84

9.3 Prenatal and Postnatal Development

Study title: NVA237: A subcutaneous pre and postnatal study in the rat

Study no.: Study 901945/0870598
Study report location: NDA 207923, SD-1
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: May 5, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: NVA237, Batch 0724007, 100.1%

Key Study Findings

- In a pre- and postnatal development (PPND) study, mated F_0 female rats received subcutaneous injections of the NVA237 at doses of 0 (vehicle), 0.19, 0.63, or 1.88 mg/kg/day (salt) from Gestation Day (GD) 6 to Postnatal Day (PND) 21-23.
- F_0 females developed a dose dependent decrease in body weight gain (LD 96%, MD 87%, HD 71%), compared to the control during the dosing period (GD 6 to GD 20), which correlated with decreased feed consumption. Therefore, dosing appears adequate.
- F_0 dams showed no test article related effects on reproductive parameters. There were no negative test article related effects on viability of F_1 offspring.
- F_1 generation male pups showed dose-dependent decreases in body weight at PND 0-7(LD 89%, MD 92%, HD 82%), which was not observed at the same magnitude in females (HD 94%). Male pups showed partial recovery by PND 21 (LD 95%, MD 99%, HD 92%). There were no effects on physical or neurological development in F_1 generation pups.
- F_1 generation adults showed no changes in body weight, physical or neurological development, or reproductive parameters up to the high dose of 1.88 mg/kg (salt).
- TK data are not yet available for this study. TK parameters are, however, available for the Rat Fertility Study (see above) in which animals were dosed up

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to 1.88 mg/kg (salt), which was associated with an AUC_{0-24h} of 290 h*ng/mL in females.

- The F_2 generation was terminated at GD13, therefore there was no information collected regarding sex, body weight, or malformations.

Methods

Doses:	0 (vehicle), 0.19, 0.63, 1.88 mg/kg/day (salt)
Frequency of dosing:	Daily (from GD 6 to PPD 21-23)
Dose volume:	1.5 mL/kg
Route of administration:	Subcutaneous
Formulation/Vehicle:	5% Dextrose Injection, USP
Species/Strain:	Wistar Han rats (Crl:WI[Han])
Number/Sex/Group:	24/group
Satellite groups:	None
Study design:	The F_2 generation was terminated at GD13, which is not an ideal study design. Due to the age of the fetuses, only viability was assessed. ICH S5A does not specify that F2 offspring should be kept to a certain age. Due to the lack of findings in the F1 generation of both EFD studies, and the below PPND study, there is no specific cause for concern for embryonic development.
Deviation from study protocol:	There were no deviations that affected the validity of the study.

Group number identification	Dose concentration (mg/mL) base/salt[a]	Dose level (mg/kg/day) base/salt[a]	Dose volume (mL/kg/day)	Animal nos.
1/ Vehicle control	0	0	1.5	1501 to 1524
2/ NVA237	0.10/0.13	0.15/0.19	1.5	2501 to 2524
3/ NVA237	0.33/0.42	0.5/0.63	1.5	3501 to 3524
4/ NVA237	1.0/1.25	1.5/1.88	1.5	4501 to 4524

a Salt/base ratio for NVA237 is 1.251. Dose concentrations were not corrected for purity.

(Sponsor's table)

Basis for Dose Selection

Doses were based partly on results from the non-GLP 2 week SC rat dose range finding (DRF) rat study (study #0870723), and preliminary results of an ongoing rat EFD study.

The 2 week SC rat DRF study is evaluated under "Dose selection" in the review for the rat fertility study (study #0870596, see above.) Briefly, animals (5/sex/group) were dosed with 0 (vehicle), 0.6255, 1.8765, 6.255 mg/kg/day (salt) for 2 weeks (vehicle 5% glucose). Clinical signs include dose dependent partial closure of the eyelids (males: LD 1/5, MD 2/5, HD 4/5; females MD 3/5, HD 3/5), trembling (1/5 HD males), and tip-toe

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gait (1/5 HD males). At the MD (25%) and HD (-18%), there was dose dependent decrease in body weight gain, which coincided with decreased feed consumption.

Based on those results, the fertility and early embryonic development study (study #0870596) was initiated using doses 0 (vehicle), 0.19, 0.63, 1.88 mg/kg/day (salt). During the gestation period, a slight decrease in body weight gain was observed during the first week at the 0.63 (81%) and 1.88 mg/kg/day (88%), but this was not dose dependent. The sponsor cited decreased mean body weight at 1.88 mg/kg/day by GD 9. Based on decreased mean body weight at the 1.88 mg/kg/day, the sponsor used the same doses in the reviewed PPND study.

Observations and Results (Optional Table)

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F_0 Dams

Survival: Animals were examined twice daily for mortality. There were no test article related mortalities. There was one unscheduled euthanasia at the MD.

Clinical signs: Animals were examined twice daily for ill health. Detailed examinations were performed on GD 0, 3, 6, 9, 12, 15, 18 and 20 and PPD 0, 4, 7, 10, 14, 17 and 21. There were no clear test article related clinical signs. At the HD, 6/24 animals showed thin fur cover on the lumbar region, but this finding is not considered to be a serious adverse effect, and may be a background finding.

Body weight: Body weights were recorded on GD 0, 3, 6, 9, 12, 15, 18 and 20 and PPD 0, 4, 7, 10, 14, 17 and 21. There was a dose dependent decrease in body weight gain (LD 96%, MD 87%, HD 71%), compared to the control during the dosing period (GD 6 to GD 20). During the lactation period, there was nearly full recovery of body weight at all doses.

	Dose (mg/kg/day)			
Study Day	0	0.19	0.63	1.88
Gestation				
GD 6	232.6	230.4	234.1	231.6
GD 20	326.5	320.1	316.2	298.1
ΔBW (g)	93.9	89.7	82.1	66.5
BW gain, % (GD 6)	40%	39%	35%	29%
BW gain, % control	100%	96%	87%	71%
Post Natal				
PND 0	256.0	248.5	250.2	233.0
PND 21	276.5	274.7	282.5	265.9
ΔBW (g)	20.5	26.2	32.3	32.9
BW gain, % initial (PND 1)	8%	11%	13%	14%
BW gain, % control	100%	128%	158%	160%

Body weight in dams during the gestation period

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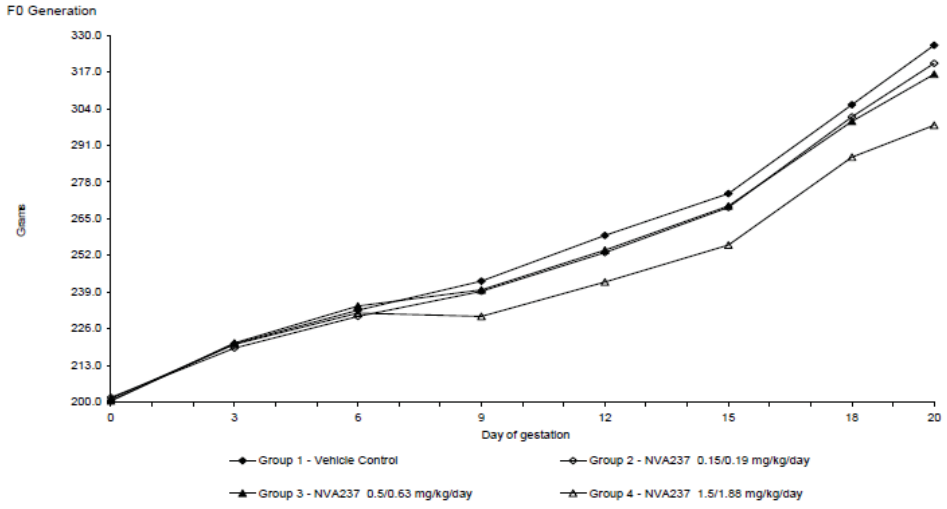
F0 Generation
Grams
330.0
317.0
304.0
291.0
278.0
265.0
252.0
239.0
226.0
213.0
200.0
0
3
6
9
12
15
18
20
Day of gestation
Group 1 - Vehicle Control
Group 2 - NVA237 0.15/0.19 mg/kg/day
Group 3 - NVA237 0.5/0.63 mg/kg/day
Group 4 - NVA237 1.5/1.88 mg/kg/day

Feed consumption: Feed consumption was measured on GD 3 to 6, 6 to 9, 9 to 12, 12 to 15 and 15 to 18, and PPD 0 to 4, 4 to 7, 7 to 10 and 10 to14. During the gestational dosing period, feed consumption was decreased in dosed animals (LD -7%, MD -5%, HD -13%), and was most pronounced at the HD. During the post natal period, feed consumption recovered and was comparable to controls.

	Dose (mg/kg/day)		
Study Day	0.01	0.06	0.18
Gestation			
GD 6-18	-7%	-5%	-13%
Post Natal			
PND 0-14	-2%	-1%	-5%

Uterine content: Dams were euthanized on PPD 22, 23 or 24 and the number of implantation site scars counted. The uterus of any animal judged to be nonpregnant was stained with 10% aqueous (v/v) ammonium sulfide solution and examined for implantation sites. See "Reproductive assessment".

Necropsy observation: Animals were euthanized by exsanguination from the abdominal aorta, following CO2 asphyxiation. A complete gross pathology examination (including examination of injection sites of the F0 generation females) of the carcass was conducted immediately and consisted of an external examination, including identification of all clinically recorded lesions, as well as a detailed internal examination. Abnormal tissues were retained. Seven females that did not litter were euthanized on Day 26 post coitum. Dams were euthanized on day 22, 23, or 24.

There were no test-article related necropsy findings.

Toxicokinetics: The sponsor stated, "Toxicokinetic data are not yet available for this study."

Dosing Solution Analysis: The concentrations of NVA237 in the dose formulations were determined by HPLC. Samples were taken from dose formulations prepared pre-study, Day 1, and Week 4. Samples were tested in duplicate. The individual test results ranged between 95.8 – 101% of the nominal concentration, and therefore were within 10% of the nominal concentration. This is acceptable.

Reproductive assessment: Females were observed 3 times each day from day 20 of gestation for signs of parturition. Where possible, parturition was observed, the time to complete of onset and completion of parturition was recorded. Pups/weanlings/juveniles were examined twice daily for mortality.

There were no test article related effects on reproductive parameters. On PPD 0, the pups were examined for malformations, sexed and the numbers of live and dead recorded. The live pups were weighed

individually. Dead pups were placed in Bouin's fluid for subsequent examination. Any pups found to be malformed were euthanized after approval of the study director, by a subcutaneous injection of a euthanasia solution. One female pup from the control maternal group was found dead on PPD 0, and was noted to have a misshapen and reduced tongue. There were no test article related findings in pups at PPD 0.

	Dose (mg/kg/day)			
Parameter	0	0.19	0.63	1.88
No. of mated females	24	24	24	24
No. of pregnant rats	23	21	21	24
No. of parturient rats*	23	21	21	24
Parturition index (%)	100.0	100.0	95.2	100.0
Mean gestation period (days)	21.6	21.3	21.6	21.5
Litter mean no. of implant sites	11.9	12.9	11.3	11.9
Litter mean no. of total newborns*	11.4	12.2	10.7	11.5
Litter mean no. of live newborns	11.3	11.8	10.6	11.5
Live males %	48.83	52.77	47.54	49.92
Viability of F_1 pups on Day 0 (%)[a*]	95%	91%	94%	97%
Viability of F_1 pups on Day 4 (%)[b]	99%	99%	100%	99%
Viability of F_1 pups on Day 7 (%)[c]	100%	100%	100%	100%

*determined by reviewer based on individual data
[a]Viability of F1 pups on Day 0 (%) = (No. live pups/Total No. implants) x 100
[b]Viability of F1 pups on Day 4 (%) = (No. live pups on day 4/ No. live pups on day 0) x 100
[c]Viability of F1 pups on Day 7(%) = (No. live pups on day 7/ No. live pups after adjustment on day 4) x 100

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F_1 Generation before mating

Survival: Animals were examined twice daily for mortality. There were no test article related deaths.

Clinical signs: The general condition of the pups was evaluated each day during the lactation period. There were no test article related clinical signs.

Body weight: Body weight was recorded at birth and on PPD 4, 7, 10, 14, and 21 before weaning.

Before weaning, males showed a dose dependent decrease in body weight gain from PND 0-7 (LD 89%, MD 92%, HD 82%), compared to control, with partial recovery by PND 21 (LD 95%, MD 99%, HD 92%). Females developed a slight decrease at the HD from PND 0-7 (HD 94%), without change at PND 21 (HD 95%); based on the slight decrease, this may reflect biological variation and not a test article related finding.

	Dose (mg/kg/day)			
Males				
Study Day	0	0.19	0.63	1.88
PND 0	6.29	5.98	5.98	**5.70**
PND 7	16.23	14.87	15.14	**13.87**
ΔBW (g)	9.94	8.89	9.16	8.17
BW gain, % initial	158%	149%	153%	143%
BW gain, % control	100%	89%	92%	82%
PND 14	22.43	**20.15**	20.57	**18.75**
ΔBW (g)	6.2	5.28	5.43	4.88
BW gain, % initial	99%	88%	91%	86%
BW gain, % control	100%	90%	92%	87%
PND 21	46.65	**41.93**	43.30	**38.9**
ΔBW (g)	24.22	21.78	22.73	20.15
BW gain, % initial	385%	364%	380%	354%
BW gain, % control	100%	95%	99%	92%
Females				
Study Day	0	0.19	0.63	1.88
PND 0	5.98	5.65	5.76	**5.38**
PND 7	15.59	14.24	14.88	**13.46**
ΔBW (g)	7	7.2	7	6.6
BW gain, % initial	106%	106%	103%	102%
BW gain, % control	100%	103%	100%	94%
PND 14	21.63	**19.48**	20.31	**18.30**
ΔBW (g)	14.4	14.2	13.6	13
BW gain, % initial	218%	209%	200%	200%
BW gain, % control	100%	96%	92%	92%
PND 21	45.35	**41.03**	42.79	**38.29**
ΔBW (g)	15.40	15.50	15.40	14.40
BW gain, % initial	233%	228%	226%	222%
BW gain, % control	100%	98%	97%	95%

Bold indicates P ≤ 0.05, compared to control

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Feed consumption: Not measured.

Physical development: Pinna unfolding was evaluated from PPD 1 until all pups in the litter had a positive response; tooth eruption was evaluated from PPD 7 and eye opening from PPD 12 until the pup tested showed signs of development.

There was no clear dose response with respect to pinna unfolding, tooth eruption or eye opening.

		Maternal Dose (mg/kg/day)			
		0	0.19	0.63	1.88
		Day of Development (PPD)			
Pinna unfolding	Males	2.31	2.64	2.54	2.69
	Females	2.22	2.57	2.49	2.64
	Total	2.24	2.60	2.49	2.66
Tooth eruption	Males	10.98	11.53	11.41	11.45
	Females	10.89	11.53	11.42	11.57
	Total	10.90	11.56	11.41	11.50
Eye opening	Males	14.16	14.52	14.05	14.46
	Females	13.98	14.19	13.95	14.08
	Total	14.04	14.35	13.98	14.34

Neurological assessment: Righting reflex was examined from PPD 2 until all pups in the litter had a positive response or until the pup tested showed signs of development, after individual identification (PPD 4 or 5); negative geotaxis from PPD 8, and the auricular startle response from PPD 12 until the pup tested showed signs of development.

There were no clear dose dependent increases in mean time to righting reflex, negative geotaxis, or auricular startle.

		Maternal Dose (mg/kg/day)			
		0	0.19	0.63	1.88
		Day of Development (PPD)			
Righting reflex	Males	2.18	2.4	2.48	2.38
	Females	2.42	2.6	2.53	2.49
	Total	2.33	2.45	2.52	2.44
Negative geotaxis	Males	8.74	9.03	8.95	9.34
	Females	8.98	8.86	8.78	9.16
	Total	8.88	8.97	8.91	9.26
Auricular startle	Males	12.44	12.66	12.45	12.61
	Females	12.4	12.48	12.29	12.44
	Total	12.43	12.56	12.38	12.53

Other: Any pups found dead or euthanized preterminally between PPD 0 and 7 were given a detailed internal examination or stored in Bouin's fluid

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for subsequent examination. Pups found dead or euthanized preterminally between days PPD 8 and 21 post were given a complete necropsy. Animals not selected for mating underwent a gross pathological examination, with abnormal tissues retained.

There were no test article related necropsy findings.

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F1 Generation (as adults)

Survival: Animals were examined twice daily for mortality. One female in each dosed group (LD 1/21, MD 1/20, HD 1/24) underwent unscheduled necropsy. Due to a lack of dose response, the unscheduled euthanasia does not appear to be test article related.

Clinical signs: Animals were examined twice daily for ill health. A complete detailed examination was performed at least weekly. There were no test article related clinical signs.

Body weight: After weaning, body weights were recorded pre-mating, mating and post mating. Specifically, males were weighed weekly from PND 28-105, and at PND 110; in females, body weights were recorded weekly from PND 28-77. Females were weighed on days 0, 3, 6, 9 and 13 of gestation.

After weaning and before gestation (in females), there were no test article related trends in body weight gain until PND 110 in males and PND 77 in females.

During gestation, there were no changes in body weight gain (data shown below).

	Dose (mg/kg/day)			
Study Day	0	0.19	0.63	1.88
GD 0	215.4	210.9	217.5	208.3
GD 6	234.1	228.8	235.6	227
ΔBW (g)	18.7	17.9	18.1	18.7
BW gain, % initial (GD 0)	9%	8%	8%	9%
BW gain, % control	100%	96%	97%	100%
GD 13	260.7	256.4	262.4	253.2
ΔBW (g)	26.6	27.6	26.8	26.2
BW gain, % initial (GD 0)	12%	13%	12%	13%
BW gain, % control	100%	104%	101%	98%

Feed consumption: Not measured.

Physical development: On PPD 21, the pupillary closure and visual placing responses of the F1 adult generation were tested. All animals showed pupillary closure and visual placing by PPD 21.

Vaginal opening was assessed from PPD 28 until development for females. Preputial separation was assessed from PPD 35 until development for males. There were no meaningful differences in time of vaginal opening or preputial separation.

Neurological assessment: Motor activity, auditory startle response, and memory (E water maze) were tested. Neurological performance was comparable in the groups

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dosed with the test article, compared to control.

Reproduction: At 85 days of age (±7), 1 female was placed with 1 male (sibling matings were not performed) in the same dosage group for up to 14 days. The females were examined for mating by examination of the vaginal lavage for spermatozoa. The day of positive identification of spermatozoa or presence of a vaginal plug was termed day 0 of gestation. The uterus of any animal judged to be nonpregnant was stained with 10% aq (v/v) ammonium sulfide solution and examined for implantation sites.

When animals failed to mate, they were not reassigned new partners; therefore it was not possible to determine the fertility or mating index for individual males versus individual females.

There were no changes in reproductive parameters that were dose responsive.

	Dose (mg/kg/day)			
Parameter	0	0.19	0.63	1.88
No of males/females mated	22	21	20	24
No. of pregnant rats	21	21	20	24
No. of parturient rats	21	21	20	24
Mating index (%)	95.5 (21/22)	100 (21/21)	100 (20/20)	100 (24/24)
Fertility index (%)	95.5 (21/22)	100 (21/21)	100 (20/20)	100 (24/24)
Mean no. of corpora lutea	14.2	13.1	13.8	13
Mean litter no. of implantation	13.2	12	13.3	12
Mean litter no. of live fetuses	12.6	11.4	12.7	11.4
Mean litter no. of dead fetuses	0	0.1	0	0
Early resorptions	0.6	0.6	0.6	0.6
Mean preimplantation loss per litter (%)	6.3	11.03	8.97	7.47
Mean postimplantation loss per litter (%)	4.33	9.67	4.92	4.38

Other: Animals underwent a gross pathological examination, with abnormal tissues retained. There were no notable test article related gross findings.

F_2 Generation

Survival: Females were euthanized on day 13 of presumed gestation. The number of corpora lutea (right and left ovaries) was recorded. The number of viable embryos and resorptions were recorded. (Page 27 of study report.) There was no further evaluation of the F2 generation. (See Reproduction for F_1 Generation Adults).

10 Special Toxicology Studies

None.

11 Integrated Summary and Safety Evaluation

Novartis has submitted two applications under NDA 207923 and NDA 207930. The first is a 505 (b) (1) application under NDA 207923 for SEEBRI NEOHALER indicated for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema. SEEBRI NEOHALER is a dry powder inhaler that delivers 15.6 mcg glycopyrrolate (NVA237, glycopyrronium bromide quaternary ammonium salt) per actuation, with in magnesium stearate and lactose monohydrate as excipients. The maximum recommended dose is one actuation, twice daily (BID), or 31.2 mcg glycopyrrolate per day.

The second application is a 505 (b) (1) application under NDA 207930 for UTIBRON NEOHALER for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema. UTIBRON NEOHALER is a fixed dose, dry powder inhaler that delivers a combination of glycopyrrolate 15.6 mcg and indacaterol (QAB149) 27.5 mcg (QVA149). The formulation includes magnesium stearate and lactose monohydrate as excipients. The maximum recommended dose is one actuation, twice daily (BID), or 31.2 mcg glycopyrrolate and 55 mcg indacaterol per day. Indacaterol is approved at 75 mcg per day as ARCAPTA NEOHALER for the same indication as the proposed products, under NDA 022383.

The sponsor Novartis has conducted adequate nonclinical safety evaluations to support the approval of SEEBRI NEOHALER and UTIBRON NEOHALER from the nonclinical perspective. Nonclinical studies included pharmacology, safety pharmacology, ADME, general toxicology, genotoxicity, carcinogenicity, and reproductive toxicology. There are no outstanding nonclinical issues at this time.

The summary below is divided into sections for NVA237 (glycopyrrolate), QVA149 (QAB149/glycopyrrolate combination), QAB149 (indacaterol), and excipients. A complete summary for the nonclinical program of QAB149 for the inhalation route of administration is available under NDA 022383; a brief summary is provided below. A labeling evaluation is provided after these summaries.

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11.1 NVA237 (Glycopyrrolate)

The nonclinical studies to support the safety of NVA237 adequately assessed the pharmacology, pharmacokinetics, and toxicology of NVA237 in animals. The relevant species tested were primarily mice, rats and dogs. The following summary is based upon the referenced reviews, and the reviews of studies presented here.

Pharmacology: NVA237 is a long-acting antimuscarinic antagonist (LAMA), which is often referred to under the more general term anticholinergic. The mechanism of action of NVA237 is similar to that of tiotropium bromide, aclidinium bromide, and umeclidinium bromide, which are currently marketed anticholinergics.

In vitro pharmacology studies show that NVA237is a high affinity, pan active muscarinic antagonist. NVA237 was assessed for muscarinic activity, binding and calcium mobilization at cloned human receptors. NVA237 (NVP-QAM254) is comprised of two enantiomers of glycopyrrolate, namely 3S, 2R (NVP-QBA608) and 3R, 2S (NVP-QBA609). In competitive binding assays (study **#RD-2007-00409**), NVA237 bound to M1, M2, M3, M4 and M5 receptors with pKi values of 9.81, 9.05, 9.59, 9.05, and 8.96 nM, respectively, using the CHO-cell lines. NVA237 was characterized as a competitive inhibitor at the M1, M2, and M3 receptors (IND 48655, review dated 6/20/07; study **#RD-2007-00209**), expressed by CHO cells, with pKi values of 9.60, 8.70, and 9.47, respectively, which were consistent with competitive kinetic assays.

An *in vitro* study using an animal model of contraction assessed the ability of NVA237 to induce bronchodilation. In rat isolated trachea (IND 48655, review dated 6/20/07; study **#RD-2006-02342**) with bethanechol-induced contraction, NVA237 caused a roughly concentration dependent tissue relaxation.

In vivo studies (IND 48655, review dated 6/20/07) measured the effect of NVA237 on methacholine-induced bronchoconstriction in anesthetized rabbits (study **#RD-2011-50035**), anesthetized Brown Norway rats and immobilized Rhesus monkeys. In rabbits, NVA237markedly inhibited (76%-93%) the bronchoconstriction response for 4- 6 hrs, and only minimally reduced the hypotensive response (2%-6%). In the Brown Norway rat study (study **#RD-2006-02342**), animals were sensitized to egg albumin and the response (bronchoprotection, salivation, hypotension and bradycardia) to intravenous methacholine was determined at 1, 6 and 24 hrs post dose. Bronchoconstriction was reduced by intratracheal installation of NVA237, and this effect was maintained 24 hrs post dose (study **#RD-2006-0199**). In immobilized rhesus monkeys (study #RD-2005-01557), NVA237 at inhalation doses of 0.05, 0.15, 0.31 and 0.61 ug/kg inhibited the bronchoconstriction response to methacholine, with response determined up to 285 min after the start of drug administration. Maximal effect occurred at 15 min post dose. By 225 min, the 0.05, 0.15, 0.31 ug/kg doses were minimally effective. At 285 min the inhibition by the 0.61 ug/kg dose was comparable to its maximal inhibition seen at 15 min.

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Safety Pharmacology: NVA237 was assessed for effects on the central nervous system (CNS), cardiovascular, and respiratory functions.

Central nervous system effects

The effect of inhaled NVA237, QAB149 and QVA149 on the central nervous system (CNS) and respiratory system was assessed in albino male rats (study **#0670652**). For CNS assessment, rats (8/group) were dosed with 0 (vehicle), QVA149 (NVA237/QAB149 at 0.144/0.507 mg/kg), NVA237 at 0.210 mg/kg, and QAB149 at 0.620 mg/kg, based on a theoretically achieved dose (salt). A functional observational battery (FOB) was performed for predose, immediately following the treatment period, and at 2, 4 and 24 post dose. Pupil dilation was noted in animals that received NVA237 and QVA149, which is an expected pharmacological effect of NVA237. A slight decrease in motor activities was noted immediately post dose and 2 hrs following treatment with QAB149, but this was a transient effect. In summary, no dose limiting effects were noted in CNS parameters.

In the respiratory assessment, rats (5/group) were administered test articles at the same doses. Ventilatory parameters (tidal volume, respiratory rate, and derived minute volume) were measured up to 120-minute period predose (taken approximately 24 hours prior to dosing), continuously during the dosing period, and at 2, 4 and 24 hours post dose. There were no clear effects on respiratory parameters in rats at inhaled doses of QVA149 (NVA237/QAB149 at 0.144/0.507 mg/kg), NVA237 at 0.210 mg/kg, and QAB149 at 0.620 mg/kg,

Cardiovascular

The *in vitro* effects of NVA237, QAB149, and QVA149 were evaluated in hERG-expressing human embryonic kidney (HEK293) cells (study #0770861). QAB149 was applied at doses of 1, 3, 10 and 30 μM. NVA 237 was applied at doses of 30, 100 and 300 μM. QVA149 was applied at the following concentrations:

(1) = 50mL of 1.875 μM QAB149 and 50mL 18.75 μM NVA237
(2) = 50mL of 3.75 μM QAB149 and 50mL 37.5 μM NVA237
(3) = 50mL of 3.75 μM QAB149 and 50mL 75 μM NVA237
(4) = 50mL of 30 μM QAB149 and 50mL 300 μM NVA237 (maximum solubility for both test articles in HB-PS + 0.3% DMSO)

The IC50 for the inhibitory effect of QAB149 on hERG potassium current was 3.1 μM. The IC50 for the inhibitory effect of NVA237 on hERG potassium current was not determined since hERG inhibition was < 50%. For QVA149, HERG inhibition at all four concentrations was statistically significant ($P<0.05$) when compared to vehicle control values, but a lower effect on hERG current than the high dose (30 μM) of QAB149. Therefore, QVA149 has no additive effect on hERG current inhibition compared to QAB149 alone. There were no findings of QTc prolongation in cardiovascular safety pharmacology or toxicology studies in dogs with QAB149 or QVA149.

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In vitro cardiac electrophysiological effects of NVA237 were determined in 6 isolated Langendorf perfused hearts (study **#0618559**; see IND 48655, review dated 6/20/07). The following parameters were measured: automaticity and escape cycle length, threshold stimulation current, coronary perfusion rate, ectopic activity, left ventricular septal and epicardial monophasic action potential duration at 30, 60 and 90% of repolarization, conduction time, triangulation, reverse use-potential, instability and dispersion of repolarization. NVA237 did not induce any cardiac electrophysiological changes at concentrations up to 30 uM.

In vivo cardiovascular effects of NVA237 were assessed in beagle dogs in a sighting study, and a telemetry study (study **#R0510129**; see IND 48655, review dated 6/20/07). In the sighting study, changes in EKG were assessed following single intravenous doses of 0.01, 0.1 and 1 mg/kg. EKGs and other parameters were recorded pretest and at 0.25, 0.5, and 1 and 4 hr post dose after all dose levels and at 8 and 24 hr following dosing with 0.1 and 1 mg/kg. In the telemetry group, single intravenous doses of 0.01 and 0.1 mg/kg were administered with a 7-day wash out period between each dosage. EKG and arterial blood pressure were recorded at 1 hr pretest, every 5 minutes up to 2 hr post dose, and thereafter every 15 min up to 22 hrs post test. In the sighting group, 0.01 mg/kg led to dry mucous mouth membranes on the first day. Doses of 0.1 and 1 mg/kg produced a slight decrease in food intake, mydriasis, tremor, and dry mucous mouth membranes up to 3 days post dose. In two animals at 1 mg/kg, there was a body weight loss of 0.2 and 0.3 kg. At 0.1 and 1 mg/kg, severe tachycardia up to 294 bpm occurred along with secondary shortening of the P, PQ, QRS and/or QT/QTc.

In the telemetry group, mydriasis persisted overnight and dry mucous mouth membranes were seen up to 6 hr post dose at 0.1 mg/kg, and directly after dosing at 0.01 mg/kg. A transient tachycardia of 221 and 231 bpm occurred at 5-10 min post dose at 0.01 and 0.1 mg/kg. Mean diastolic arterial blood pressure increased +20 mm Hg in 2/4 animals from 0.25 hr to 2.5 hr post 0.1 mg/kg administration. In conclusion, NVA237 induced tachycardia at 0.1 and 1.0 mg/kg, and increased diastolic arterial blood pressure at 1.0 mg/kg. Dryness was an expected anticholinergic effect of NVA237. No NOAEL was established for cardiovascular effects based on transient tachycardia at doses equal to and greater than 0.01 mg/kg, and increased diastolic arterial blood pressure at 0.1 mg/kg NVA237. Increased heart rate was known to be monitorable in a clinical setting.

In *vivo* cardiac and respiratory effects of NVA237 were evaluated in anesthetized male Rhesus monkeys (7 controls, 5/dose group) administered the test article by an endotracheal tube (study **#RD-2005-01557**; see IND 48655, review dated 6/20/07). This study is listed as a primary pharmacology study in the sponsor's submission, but also serves as a respiratory evaluation as part of the safety pharmacology battery for NVA237. Briefly, NVA237 was administered by inhalation at 0.05, 0.15, 0.31 and 0.61 mcg/kg (based on the sponsor's deposition factor) over 10 min, and 15 min prior to an initial administration of methacholine. The effects on cardiovascular parameters (heart rate, systolic and diastolic blood pressure), body temperature and respiratory rate were measured simultaneously. In regards to respiratory rate, spontaneous breathing was

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monitored with a pediatric pulmonary function unit, which is used clinically in human infants for 285 minutes after the start of dosing with NVA237. NVA237at all doses induced a dose dependent effect and significant ($P < 0.05$) effect on methacholine-induced bronchoconstriction at 15 min post dose. At the HD, significant inhibition ($P \leq 0.01$) was maintained up to the end of the observation period (285 minutes). There were no notable effects on heart rate, respiratory rate or blood pressure. Thus, NVA237 had no effect on respiratory rate in anesthetized monkeys dosed up to 0.61 mcg/kg via inhalation.

Respiratory

See summaries above for respiratory parameters assessed in albino male rats (study # **0670652**), and male Rhesus monkeys (study **#RD-2005-01557**; see IND 48655, review dated 6/20/07). There were no clear effects on respiratory parameters in rats at inhaled doses of QVA149 (NVA237/QAB149 at 0.144/0.507 mg/kg), NVA237 at 0.210 mg/kg, and QAB149 at 0.620 mg/kg, NVA237 had no effect on respiratory rate in anesthetized monkeys dosed up to 0.61 mcg/kg via inhalation.

ADME: The absorption, distribution, metabolism, and excretion of NVA237 was investigated in mice, rats, dogs, rabbits, and humans.

The half-lives of NVA237 in mice, rats, and dogs were 3.9, 23, and 4.4 hrs, respectively. The plasma protein binding in rats, dogs, and humans ranged from 23-41%, without a clear relation to concentration (study **#DMPK-R0600252**). Studies in mice, rats, and dogs suggest distribution in extravascular tissues, with moderate to strong clearance (258.3, 90, 53.3 mL/min/kg, respectively). Following IV administration in mice, the longest half-live values were noted in the epididymis, eye choroid, eye, fat (brown), Harderian gland, kidney (cortex), and liver. Following IV administration in rats, the longest half-live values were noted in the kidney and liver.

Metabolism was examined *in vitro* using rat, dog and human liver and lung microsomes, and rat and human hepatocytes. No notable metabolism was observed in pooled rat, dog and human lung microsomes. Extensive clearance was identified in rat and rabbit liver microsomes. Mouse, rat, rabbit, dog and human hepatocytes were found to metabolize NVA237 at different rates, but showed a similar primary pathway with the formation of multiple oxidized phase I metabolites. *In vivo*, the primary biotransformation pathways for NVA237 in the mouse and rat were oxidative and occurred primarily on the cyclopentane and phenyl rings. Hydrolysis of the ester linkage formed the corresponding carboxylic acid metabolite M9 (CJL603), which was the main metabolite (> 50% of the total plasma radioactivity AUC) in mouse, rat, and human plasma after oral dosing. When incubated with recombinant human CYP, low metabolism was shown with CYP2D6. There was no evidence for any unique human metabolites.

Excretion was examined in mice and rats. Following intravenous administration of 3.18 mg/kg (base) in mice, 67.7%, and 29% of the dose was found in urine and feces,

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respectively. After oral administration of 26.9 mg/kg (base) in mice, 8.95% and 62.2% of the dose was found in urine and feces. Following intravenous administration of 4.12 mg/kg (base) in rats, 46.4%, and 38.8% of the dose was found in urine and feces. Following intravenous administration of 4.12 mg/kg (base) in bile duct-cannulated rats, 67.5%, 7.27%, and 3.34% of the dose was found in the urine, bile and feces, respectively. After oral administration of 35.7 mg/kg (base) in rats, 2.86-4.37% and 98.5% of the dose was found in urine and feces.

General Toxicology: The pivotal general toxicology studies supporting the safety of NVA237 are chronic toxicology studies in rats and dogs.

In the 26 week inhalation toxicology study (see IND 48655, review dated 3/30/2009), with a 4 week recovery in rats, animals received estimated achieved doses of 0 (air), 0 (vehicle: 1% magnesium stearate, 99% lactose monohydrate), 0.09, 0.67 and 4.98 mg/kg of NVA237 (salt). The pulmonary deposited doses were 0, 0, 0,009, 0.067 and 0.50 mg/kg NVA237. Recovery animals received 0 (air), 0 (vehicle), and 4 mg/kg of NVA237 (estimated achieved doses). There was decreased body weight gained in both sexes, with males showing a greater decrease than females at the LD and MD. NVA237 induced mydriasis prior to the instillation of atropine and yet reduced the mydriatic effect of atropine at the MD and HD in both sexes. The target organs of toxicity were the eyes, lungs, seminal vesicles, and urinary bladder. Eye findings consisted of bilateral and unilateral lenticular changes (anterior capsular opacity, anterior prominent suture line, anterior slight cataract) were observed at the MD and HD in both sexes, and were partially reversible. These changes were characteristic muscarinic receptor inhibition. (Durand, G. et al. Toxicology Sciences: 66, 166-172, 2002). There were no histopathological changes in the eyes. Lung epithelial hypertrophy was observed at the MD and HD, but was minimal and completely reversible. The cells within the area where epithelial hypertrophy was observed were not Type II pneumocytes, and this hypertrophy may be due to a local irritant effect. Seminal vesicle inflammation was observed in 2/21 HD males, but was observed in 1/10 vehicle treated recovery males. Urinary bladder inflammation developed in 3/21 HD males only, and was completely reversible. Pathological changes were observed in the nasal cavity/sinuses, larynx and Harderian gland at all doses, but these findings are not clinically relevant due to the nose-only inhalation route of administration in rats, and the lack of the Harderian gland in humans. The inhalation toxicity NOAEL was the low dose of 0.09 mg/kg (achieved dose), which is associated with an $AUC_{(0\text{-}24h)}$ of 13.8 ng*h/mL (salt).

In the 39 week inhalation toxicology study, beagle dogs were dosed with 0 (air), 0 (vehicle: 1% magnesium stearate, 99% lactose monohydrate), 0.030, 0.12, or 0.33 mg/kg (male/female, estimated achieved doses) through the inhalation route of exposure (6/sex/group controls and HD; 4/sex/group LD and MD), with a 4 week recovery period (2/sex/group controls and HD). The vehicle group showed no notable differences compared to the air control. There were no mortalities. Clinical signs included red eyes, dry gums and dry muzzles. These findings are related to dryness, which is an expected pharmacological effect of the test article. There was a test article related decrease in body weight gain in both males throughout the study at the MD and

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HD, and in females at the MD and HD at the end of the dosing period. This correlated with feed consumption, and reversed after the recovery period. Test article related ophthalmic findings were conjunctival hyperemia (bilateral), corneal opacity (bilateral), and focal nuclear opacity (bilateral) observed at the HD. There was a reduction in lacrimal secretion (defined by a value of < 10mm on the Schirmer tear test) at Weeks 3 and 6 at the HD, which reversed and is an expected pharmacological effect of the test article. Increased heart rate was noted in MD and HD males and females (+30-69%, compared to the pretest value on the same day) at Weeks 13, 26, and 39. This was an expected pharmacological effect of the test article. The targets organs of toxicity were the pharynx, lacrimal gland, and mandibular salivary glands. In the pharynx, inflammation (grade 1) was noted at the HD (males 3/4, females 3/4), but was also observed in 1/4 control males. Ectasia of the ducts and or alveoli of the pharynx (grade 1 and 2) was observed in males (3/4 MD, 1/4 HD) and females (1/4 MD, 3/4 HD). All findings reversed after the recovery period. The findings in the pharynx were generally mild, reversible, and are clinically monitorable, therefore are not considered dose limiting. Hypertrophy was noted in the lacrimal and salivary glands, and is not a dose limiting finding. Systemic exposure (AUC) to the test article increased with dose, but was higher in HD males (80.9 ng.h/mL) than in HD females (32.9 ng.h/mL). The higher AUC values in males result from higher concentrations of plasma test article in 1 of 6 males compared other males and females. The NOAEL was determined as the low-dose, however, some findings were determined to be clinically monitorable and/or not dose limiting. Therefore, the supporting dose was determined to be the mid dose of 0.12 mg/kg, based on ophthalmic findings. The supporting dose is associated with an $AUC_{(0\text{-}24h)}$ of 26.6 ng*h/mL in males and 42.0 ng.h/mL (salt) in females.

Safety margins for the proposed clinical dose of 31.2 mcg NVA37 are calculated based on the NOAEL identified in the 26 week rat and the supporting dose in the 39 week dog toxicology studies. The AUC associated with the clinical dose is 0.111 ng*h/mL (base form, based on study # CQVA149A2107, discussion with Clinical Pharmacologist Dr. Lei He on May 15, 2015, which is equivalent to 0.139 ng*hr/mL (salt).

Table 33: Safety margins for the proposed clinical dose of NVA237 based on AUC

		Nonclinical supporting dose		Safety Margin for clinical dose of 31.2 mcg NVA237
Study	Sex	Achieved dose (mg/kg/day, salt form)	AUC (ng*hr/mL) (salt form)	AUC = 0.139 ng*hr/mL (salt form)
26 week rat	Both	0.09	13.8	99
39 week dog	Male	0.12	26.6	190
	Female	0.12	42.0	300

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Table 34: Safety margins for the proposed clinical dose of NVA237 based on mcg/g lung weight

		Nonclinical supporting dose		Safety Margin for clinical dose of 31.2 mcg NVA237
Study	Sex	Achieved dose (mg/kg/day, salt form)	Deposited dose (mg/g lung weight)	0.0312 mcg/g lung weight = 0.0000312 mg/g lung weight
26 week rat	Both	0.09	0.002	48
39 week dog	Both	0.12	0.003	87

*Assumes a pulmonary deposition factor of 0.1 for rats and 0.25 for dogs, and a body weight of 250 g for rats and 10 kg for dogs. Lung weight is 1.50 g for rat, 110 g for dog, and 1000 g for human.

Genetic toxicology: NVA237 was negative in genetic toxicology testing based on results from the *in vitro* bacterial reverse mutation assay, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* rat micronucleus test.

Carcinogenicity: The sponsor determined the carcinogenic potential of NVA237 in a traditional 2-year bioassay in rats, and a 26 week bioassay in transgenic mice (Tg.rasH2 mice). Both bioassays were negative for test-article related tumors.

In a 2-year bioassay in Wistar rats (50 animals/sex/group), animals received GP by inhalation (nose-only) at doses of 0 (air, on loading rack in restraint tubes in separate room), 0 (air, rotated on a flow through chamber), 0 (vehicle: 1% magnesium stearate and 99% lactose monohydrate), 0.07, 0.21, 0.56 mg/kg/day GP through 60-minute exposures (estimated achieved doses, salt form). The first air control group (Air1) showed increased body weight compared to all other groups, and was not used for analysis. Statistical analyses were done against the vehicle control and second air control (Air2) groups. No statistically significant neoplastic findings were observed in male or female rats. GP was associated with decreased body weight in males at all doses, and in HD females. NVA 237 had no effect on mortality. Systemic exposure (AUC) to GP was similar in both genders, and slight accumulation was observed at Week 52. Exposure was dose proportional between the LD and MD, but was slightly less than dose proportional between the MD and HD. Systemic exposures (AUC) at the HD exceeded the maximum anticipated clinical exposure in COPD patients by more than 300-fold.

In a 26-week bioassay, Tg.rasH2 mice (25/sex/group) received GP by oral gavage at 0 (vehicle: Deionized water), 12.5, 31.3, and 93.8 mg/kg/day in males, and 0 (vehicle), 12.5, 37.5, and 125.1 mg/kg/day in females, based on the salt form. Positive control mice were administered N-methyl-nitrosourea (MNU; 75 mg/kg) by a single intraperitoneal injection on Day 1. No statistically significant neoplastic findings were observed in male or female Tg.rasH2 mice. GP was associated with decreased body

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weight in male and female Tg.rasH2. NVA 237 had no effect on mortality in Tg.rasH2 mice. Due to inconsistent exposure to GP, exposure to the metabolite CJL603 was measured. Systemic exposure (AUC) for CJL603 was detected in at least 2 of 3 HD male and female Tg.rasH2 mice at 0.5, 1, 3, and 7 hrs post dose. Based on limited data for CJL603, AUC increased in a roughly dose proportional manner. As expected, MNU-treated Tg.rasH2 animals had increased mortality and neoplasia incidence, compared to control Tg.rasH2 animals.

Table 35: Safety margins for the proposed clinical dose of NVA237based on the AUC associated with the NOAEL in the rat carcinogenicity study

		Safety Margin for clinical dose of 31.2 mcg NVA237
Achieved dose (mg/kg; salt form)	AUC (ng*h/mL) (salt form)	AUC = 0.139 ng*hr/mL (salt form)
0.56	45.60	330

Reproductive and developmental toxicity: The standard battery of reproductive and developmental toxicity studies were completed with NVA237 in rats and rabbits.

To assess fertility, rats received subcutaneous (SC) doses of 0 (vehicle, 5% dextrose), 0.19, 0.63, 1.88 mg/kg/day (salt). Males were dosed at least 28 days prior to mating, during the 2 week mating period, and until terminal necropsy (Day 50-53). Females were dosed for 2 weeks prior to mating, through the mating period, and gestation days (GD) 0 to 6. There were no mortalities. Males developed a dose dependent decrease in body weight gain that persisted throughout the study (Day 50: LD 87%, MD 82%, HD 69%), relative to the control. Females also developed decreased body weight gain during the premating period (LD 90%, MD 80%, HD 50%). During the gestation period, MD and HD females had decreased body weight gain (81-88%), which recovered by GD 13 after dosing was ceased. Based on the decreases in body weight, dosing appeared adequate. Dosed animals had a higher percentage of abnormal cycles (shortened, extended, prolonged, acyclic) compared to control, but this was not dose dependent (control 22%, LD 44%, MD 40%, HD 40%). Based on the high percentage of abnormal cycles in all groups, and the lack of a clear dose response, it is difficult to determine if this is a test article related finding. Systemic exposure (AUC and Cmax) were similar between the sexes at the LD and MD, but was higher in males at the HD. AUC increased in a roughly dose proportionate manner at the LD and MD, but was greater than dose proportional in HD males versus HD females.

Fertility index was in general low in all groups, including the control (control 72%, LD 60%, MD 84%, HD 60%). This effect was not dose dependent. The sponsor stated that studies conducted “recently” in the same facility had fertility rates that ranged from 84% to 96%, and noted that only the MD had a fertility rate within the expected range. Due the lack of dose response, this did not appear to be a test article related finding, but there is a concern that the cause of the decreased fertility rate may mask a test article related effect. At the MD and HD, there was a slight decrease in the litter mean number

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of corpora lutea (control 13.2, LD 13.6, MD 12.6, HD 11.0) and number of implantations (control 11.6, LD 12.3, MD 9.9, HD 7.9), which corresponded to a decreased number of live fetuses per litter (control 10.9, LD 11.6, MD 9.9, HD 7.9). The decrease in implantations and live fetuses at the MD and HD may be a result of increased preimplantation loss at the MD and HD (control 12.1%, LD 9.9%, MD 19.8%, HD 22.4%), compared to the control and LD. Based on these dose dependent trends, it is difficult to rule out an effect of the test article on these parameters. Values, however, at the MD were within the historical control data submitted by the sponsor. Fertility was judged to be adversely effected for male and female rats at the HD of 1.88 mg/kg. The reproductive and fertility NOAEL was determined to be the MD of 0.63 mg/kg NVA237 (salt), which is associated with an $AUC_{(0\text{-}24h)}$ of 98.0 h*ng/mL (salt) in females and males.

To evaluate embryofetal development in gravid rats, animals received inhaled NVA237 at doses of 0 (air), 0 (vehicle: 1% magnesium stearate, 99% lactose monohydrate), 0.1, 0.68, and 3.83 mg/kg (estimated achieved dose, salt). Animals were treated during gestation days (GD) 6 to 17, and sacrificed on GD 21. Dose dependent decreased body weight gain was observed at GD 12 at the MD (70%) and HD (27%), with partial reversal by GD 18 (MD 91%, HD 80%) and GD 21 (MD 100%, HD 91%). Based on decreased body weight gain, it appears that the dosing was adequate. Based on systemic exposure (AUC), exposure to NVA237 was roughly dose proportional, and was similar on GD6 and GD 17. There were no dose dependent changes in cesarean section parameters, or test article related malformations or variations up to the high dose of 3.83 mg/kg (salt), which is associated with an $AUC_{0\text{-}24h}$ of 388 h*ng/mL (salt) in gravid females. NVA237 was not teratogenic in rats at exposures up to 388 ng*hr/mL.

To evaluate embryofetal development in gravid rabbits, animals received NVA237 at doses of 0 (vehicle, 1% magnesium stearate, 99% lactose monohydrate), 0.5, 1.6 and 4.4 mg/kg/day (estimated achieved dose, salt) by inhalation. Animals were treated during gestation days (GD) 7 to 19, and sacrificed on GD 29. One HD female (#4504) was euthanized on GD 25 with clinical signs that included decreased activity, reduced appetite, suspected dehydration and dilated pupils. Poor body condition was associated with a lack of body weight gain throughout the study. Necropsy findings included a pale firm liver, gelatinous material in the colon, and raised pail areas in the stomach. The mortality is considered to be test article related. Based on the TA related death at the HD, it appears that the MTD was exceeded. Gravid females developed a dose dependent decrease in body weight gain throughout the dosing period (GD19: LD 28%, MD 6%, HD -22%), compared to controls. There was a reversal of this trend after the dosing period at GD 29 with body weight gains at all doses. The decreased body weight gain corresponded to decreased feed consumption. Based on decreased body weight gain, it appears that the dosing was adequate. In gravid females at GD19, systemic exposure (AUC), increased in a less than dose proportional manner, whereas Cmax increased in a roughly dose proportional manner. Fetal samples were negative for the test article (LLOQ of 0.500 ng/g). There were no test article related changes in uterine findings, or increases in malformations or variations up to the high dose of 4.4

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mg/kg (salt), which is associated with an AUC_{0-24h} of 148 h*ng/mL (salt) in gravid females. NVA237 was not teratogenic in rabbits at exposures up to 148 ng*hr/mL.

In a pre- and postnatal development (PPND) study, mated F_0 female rats received subcutaneous injections of the NVA237 at doses of 0 (vehicle), 0.19, 0.63, 1.88 mg/kg/day (salt) from Gestation Day (GD) 6 to Postnatal Day (PND) 21-23. F_0 females developed a dose dependent decrease in body weight gain (LD 96%, MD 87%, HD 71%), compared to the control during the dosing period (GD 6 to GD 20), which correlated with decreased feed consumption. Therefore, dosing appears adequate. F_0 dams showed no test article related effects on reproductive parameters. There were no negative test article related effects on viability of F_1 offspring. F_1 generation male pups showed dose-dependent decreases in body weight at PND 0-7(LD 89%, MD 92%, HD 82%), which was not observed at the same magnitude in females (HD 94%). Male pups showed partial recovery by PND 21 (LD 95%, MD 99%, HD 92%). There were no effects on physical or neurological development in F_1 generation pups. F_1 generation adults showed no changes in body weight, physical or neurological development, or reproductive parameters up to the high dose of 1.88 mg/kg (salt). TK data are not yet available for this study. TK parameters are, however, available for the Rat Fertility Study (see above) in which animals were dosed up to 1.88 mg/kg (salt), which was associated with an AUC_{0-24h} of 290 h*ng/mL (salt) in females. The F_2 generation was terminated at GD13, therefore there was no information collected regarding sex, body weight, or malformations.

Exposure ratios from reproductive and developmental studies are shown in Section 11.5 (Labeling Evaluation).

11.2 QVA149 (NVA237 and QAB149)

Safety Pharmacology: The *in vitro* effects of QVA149, NVA237 and QAB149 were evaluated in hERG-expressing human embryonic kidney (HEK293) cells (study **#0770861**). QAB149 was applied at doses of 1, 3, 10 and 30 µM. NVA 237 was applied at doses of 30, 100 and 300 µM. QVA149 was applied at the following concentrations:

(1) = 50mL of 1.875 µM QAB149 and 50mL 18.75 µM NVA237
(2) = 50mL of 3.75 µM QAB149 and 50mL 37.5 µM NVA237
(3) = 50mL of 3.75 µM QAB149 and 50mL 75 µM NVA237
(4) = 50mL of 30 µM QAB149 and 50mL 300 µM NVA237 (maximum solubility for both test articles in HB-PS + 0.3% DMSO)

QVA149 was determined to have no additive effect on hERG current inhibition compared to QAB149 alone. There were no findings of QTc prolongation in cardiovascular safety pharmacology or toxicology studies in dogs with QAB149 or QVA149.

The *in vivo* effects of a single inhalation dose of QVA149 (NVA237/QAB149 combination), NVA237 and QAB149 on cardiovascular parameters was assessed the male beagle dog (study **#0670653/691878**, GLP). Note: This study was previously designated study #670639. Animals (4/dose) received 0 (vehicle), LD QVA149

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(0.046/0.124; NVA237/QAB149), HD QVA149 (0.183/0.487), NVA237 (0.186) and QAB149 (0.452) mg/kg (salt form). There were no mortalities. QVA149 induced a dose dependent increase in heart rate (LD +58%, HD +240%), compared to predose levels. This was associated with a decreased QTc interval at the HD (-30 msec) up to 6 hours post dose, and decreased systolic (LD -11%, HD -25%) and diastolic (LD -11%, HD -24%) pressure. Effects were less pronounced with NVA237 and QAB149 alone. NVA237 resulted in increased heart rate (+82%), and decreased QTc interval (-30 msec) up to 2 hrs of dosing. QAB149 induced an increased heart rate (+57%), and a decreased QTc interval (-20 msec) up to 2 hours post dose. Ventricular arrhythmias were observed in one dog in the QVA149 high dose group. In conclusion, the QVA149 induced more severe adverse cardiac effects including ventricular arrhythmias and decrease in heart rate, compared to NVA237 and QAB149 alone.

The effect of inhaled NVA237, QAB149 (QAB149), and QVA 149 on the central nervous system (CNS) and respiratory system was assessed in albino male rats (study **#0670652**), and previously summarized above under "NVA237" in the Integrated Summary. Briefly, rats were dosed with 0 (vehicle), QVA149 (NVA237/QAB149 at 0.144/0.507 mg/kg), NVA237 at 0.210 mg/kg, and QAB149 (QAB149) at 0.620 mg/kg, based on a theoretically achieved dose (salt). Pupil dilation was noted in animals that received NVA237 and QVA149, which is an expected pharmacological effect of NVA237. A slight decrease in motor activities was noted immediately post dose and 2 hrs following treatment with QAB149, but this was a transient effect. In summary, no dose limiting effects were noted in CNS parameters. There were no clear effects on respiratory parameters in rats.

General toxicology: The pivotal general toxicology study supporting the safety of QVA149 is a subchronic toxicology study in dogs.

In a 13-week inhalation study in dogs, the toxicity of combination product QVA149 was evaluated and compared to its monoproducts constituents QAB149 and NVA237. Animals were exposed to air control, placebo control (lactose with 1% magnesium stearate), high-dose NVA237 (estimated achieved dose 0.140 mg/kg/day), high-dose QAB149 (0.343 mg/kg/day), low-dose QVA149 (0.092 mg/kg/day QAB149 /0.032 mg/kg/day NVA237), mid-dose QVA149 (0.185/0.064 mg/kg/day), or high-dose QVA149 (0.370/0.128 mg/kg/day). Three animals per sex per group were sacrificed after three months and an additional two dogs per sex were sacrificed after a 30-day recovery period for the control and high-dose groups. QVA149 was not associated with mortality or changes in body weight, food consumption, laboratory parameters, or organ weights. Clinical signs noted at increased frequency in the high-dose (HD) QVA149 group included prepuce discharge, dry skin, and red gums. These findings were not considered adverse. ECG data indicate that administration of QVA149 is associated with transient tachycardia in a synergistic manner compared to QAB149 or NVA237 alone. Mid- and high-dose QVA149 groups exhibited 45-70% (52-72 BPM) increases in heart rate at 30-60 minutes post-dose. While adverse and test article-related, the effect of QVA149 on heart rate is considered clinically monitorable. There were no dose-limiting histopathological findings in the dog study. The supporting dose for the study

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was therefore determined to be the high-dose, corresponding to the estimated achieved dose of 0.370 mg/kg QAB149 and 0.128 mg/kg NVA237, which is associated with an AUC of 129.5/33.4 (QAB149/NVA237) ng*h/mL. Observed toxicity of QVA149 was generally consistent with the monoproducts, QAB149 and NVA237, and there was no evidence of additive or synergistic toxicity.

Reproductive and developmental toxicology: In an inhalation embryo-fetal development (EFD) study in rats, the maternal and embryo-fetal toxicity of the combination product QVA149 was evaluated and compared to its monoproducts constituents QAB149 and NVA237. Mated females were exposed to air control, placebo control (lactose with 1% magnesium stearate), high-dose NVA237 (pulmonary deposited dose 0.062 mg/kg/day), high-dose QAB149 (0.270 mg/kg/day), low-dose QVA149 (0.021 mg/kg/day QAB149 /0.007 mg/kg/day NVA237), mid-dose QVA149 (0.064/0.021 mg/kg/day), or high-dose QVA149 (0.212/0.071 mg/kg/day). 22 females per group were mated, exposed to drug on GD 5-17, and sacrificed on GD 21. There was no evidence of QVA149-related maternal or fetal toxicity in this study. Therefore, the NOAEL was considered as the high-dose group of 0.212 mg/kg/day QAB149 plus 0.071 mg/kg/day NVA237.

11.3 QAB149 (Indacaterol)

The toxicological profile of indacaterol has been characterized previously under NDA 22-383 for ARCAPTA NEOHALER, approved July 1, 2011. A summary of the nonclinical development of indacaterol is available in a review under NDA 22383, dated 8/25/09. Indacaterol maleate (indacaterol) is a long acting beta$_2$-agonist (LABA). Pivotal general toxicology studies to support the use of indacaterol were 26 and 39 week inhalation studies in rats and dogs, respectively. NOAELs were identified in both studies. The target organs of toxicity for QAB149 in the rat are nasal cavity, i.e. degeneration of the olfactory epithelium and larynx, i.e. squamous metaplasia. The target organs of toxicity in the dog are the cardiovascular system, i.e. increased heart rates, decreased blood pressure and myocardial fibrosis (class effects) and the liver, i.e. periportal liver hepatocyte vacuolation due to glycogen deposition (class effect).

With respect to teratogenic effects, indacaterol is designated Pregnancy Category C. Indacaterol was not teratogenic following subcutaneous administration to rats and rabbits at doses up to 1 mg/kg, approximately 130 and 260 times, respectively, the 75 mcg dose on a mg/m2 basis. It is not known if indacaterol is excreted in human milk.

Carcinogenicity was evaluated in a 26 week study in Tg.rasH2 mice using oral administration and in a 2 year rat study using inhalation administration. Indacaterol did not show statistically significant increases in tumor formation in mice or rats.

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11.4 Excipients

The drug product includes lactose and magnesium stearate as excipients (see Section 2.3 Formulation). There was no safety concern for either compound. Lactose is used as an excipient in a number of currently marketed inhalation drug products.

Magnesium stearate: To support the safety of magnesium stearate as an excipient, the sponsor submitted inhalation toxicity studies up to 26 week in rats (GSK Report# WD2004/00488 and WD2006/03154). These reports were reviewed by Dr. Luqi Pei (June 7, 2000). Briefly, no treatment-related effects were observed at pulmonary deposited doses up to 180 mcg/kg/day magnesium stearate. The NOAEL for inhaled magnesium stearate was 180 mcg/kg/day in rats. These studies did not reveal any safety concern about the inhalation administration of magnesium stearate. The expected exposure of patients to magnesium stearate is 37.5 mcg (BID) or a 75 mcg/day for SEEBRI NEOHALER and UTIBRON NEOHALER (1.25 mcg/kg/day for a 60 kg individual). The rat data provided a safety margin of 144 for the proposed clinical doses. The safety margin is considered adequate for the proposed use of magnesium stearate.

Conclusions: The applicant has a complete nonclinical pharmacology and toxicology program for NVA237 under NDA 207923, for QAB149 as previously reviewed under NDA 22383, and for the QVA149 under NDA 207930. Therefore, there is adequate nonclinical support for the safety of the proposed clinical dose doses of NVA237 and QVA149.

Recommendation: From the nonclinical perspective, the two applications are recommended for approval.

Unresolved toxicology issues (if any): None.

11.5 Labeling Evaluation

A review of the sponsor's proposed labeling and recommended labeling is provided here. The recommended established pharmacological classification is based on sponsor provided data, and consistency with products of the same class. The nonclinical sections presented below include the Indications and Usage (from the Highlights of Prescribing Information), Section 1 Indications and Usage (first paragraph), Sections 8.1 Pregnancy, Section 8.3 Lactation, 12.1 Mechanism of Action, 13 Nonclinical Toxicology.

The following labeling recommendation considers 31.2 mcg glycopyrrolate and 55 mcg indacaterol per day as the MRHD. The proposed doses of glycopyrrolate and indacaterol are associated with an AUC of 0.139 ng*h/mL (salt) and 0.515 ng*h/mL (base form, Clinical Pharmacologist Dr. Lei He), respectively, in humans.

The recommended labeling text for indacaterol includes changes for section 13.1 to delete (b) (4) and to clearly state that the results of the carcinogenicity assessment in rats

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and mice were negative, consistent with ECAC minutes under NDA 22383. (b) (4)

11.5.1 Labeling Recommendation for NDA 207930

INDICATIONS AND USAGE (Highlights of Prescribing Information)

UTIBRON NEOHALER is a combination of indacaterol, a long-acting beta$_2$-adrenergic agonist (LABA), and glycopyrrolate, an anticholinergic, indicated for the long-term, maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), (b) (4)

1 INDICATIONS AND USAGE

UTIBRON NEOHALER is a combination of indacaterol and glycopyrrolate indicated for the long-term, maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema.

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C

(b) (4)

There are no adequate and well-controlled trials with UTIBRON NEOHALER or its individual components, glycopyrrolate and indacaterol, in pregnant women. Animal reproduction studies were conducted with individual components, indacaterol and glycopyrrolate. Because animal reproduction studies are not always predictive of human response, UTIBRON NEOHALER should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women should be advised to contact their physician if they become pregnant while taking UTIBRON NEOHALER.

Indacaterol: Indacaterol was not teratogenic in Wister rats and New Zealand rabbits at doses at approximately (b) (4) times, respectively, the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 1 mg/kg/day in rats and rabbits).

Glycopyrrolate: Glycopyrrolate was not teratogenic in Wistar rats and New Zealand rabbits at approximately (b) (4) times, respectively, the MRHD in adults (on an AUC basis at maternal inhaled doses up to 3.83 mg/kg/day in rats and up to 4.4 mg/kg/day in rabbits).

Nonteratogenic Effects:

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Indacaterol: There were no effects on peri-natal and post-natal developments in rats at approximately (b) (4) times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 0.3 mg/kg/day).

Glycopyrrolate: There were no effects on peri-natal and post-natal developments in rats at approximately (b) (4) times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 1.88 mcg/kg/day).

8.3 Nursing Mothers

UTIBRON NEOHALER: It is not known whether UTIBRON NEOHALER is excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when UTIBRON NEOHALER is administered to a nursing woman. Since there are no data from well-controlled human studies on the use of UTIBRON NEOHALER by nursing mothers, based on the data for the individual components, a decision should be made whether to discontinue nursing or to discontinue UTIBRON NEOHALER, taking into account the importance of UTIBRON NEOHALER to the mother.

Indacaterol: It is not known whether indacaterol is excreted in human breast milk. Indacaterol (including its metabolites) have been detected in the milk of lactating rats.

Glycopyrrolate: It is not known whether glycopyrrolate is excreted in human breast milk. Glycopyrrolate (including its metabolites) have been detected in the milk of lactating rats and reached up to 10-fold higher concentrations in the milk than in the blood of the dam. (b) (4)

12.1 Mechanism of Action

UTIBRON NEOHALER contains both indacaterol and glycopyrrolate. The mechanisms of action described below for the individual components apply to UTIBRON NEOHALER. The drugs represent 2 different classes of medications (a LABA and an anticholinergic) that have different and additive effects on clinical and physiological indices.

<u>Indacaterol:</u> Indacaterol is a long-acting beta2-adrenergic agonist (LABA). When inhaled, indacaterol acts locally in the lung as a bronchodilator. Although beta2-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta1-receptors are the predominant receptors in the heart, there are also beta2-adrenergic receptors in the human heart comprising 10%-50% of the total adrenergic receptors. The precise function of these receptors is not known, but their presence raises the possibility that even highly selective beta2- adrenergic agonists may have cardiac effects.

The pharmacological effects of beta2-adrenoceptor agonist drugs, including indacaterol, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic monophosphate). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle. In vitro studies have shown that indacaterol has more than 24-fold greater agonist activity at beta2-receptors compared to beta1-receptors and 20-fold greater agonist activity compared to beta3-receptors. This selectivity profile is similar to formoterol. The clinical significance of these findings is unknown.

Glycopyrrolate: Glycopyrrolate is a long-acting, antimuscarinic agent (LAMA), which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of methacholine-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of glycopyrrolate is predominantly a site-specific effect.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment on Fertility

UTIBRON NEOHALER:
No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with UTIBRON NEOHALER; however, studies are available for individual components, indacaterol and glycopyrrolate, as described below.

Indacaterol: Indacaterol produced no treatment-related increases in the incidence of tumors in a 2-year inhalation study in Wistar rats at inhaled doses up to 2.09 mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis, respectively). A 26-week oral (gavage) study in CB6F1/TgrasH2 hemizygous mice with indacaterol at doses up to 600 mg/kg/day did not show any evidence of tumorigenicity.

Indacaterol tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* chromosome aberration test in V79 Chinese hamster cells, and in vivo rat bone marrow micronucleus assay.

Indacaterol had no effects on fertility and reproductive performance in male and female Wistar rats at subcutaneous doses up to 2 mg/kg/day (approximately (b) (4) and (b) (4) times in males and females, respectively, the MRHD in adults on an AUC basis).

Glycopyrrolate: Glycopyrrolate produced no treatment-related increases in the incidence of tumors in a 2-year inhalation study in Wistar rats at inhaled doses up to 0.56

mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis, respectively). A 26-week oral (gavage) study in male and female TgrasH2 mice that received glycopyrrolate at doses up to 93.8 and 125.1 mg/kg/day, respectively, did not show any evidence of tumorigenicity.

Glycopyrrolate tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* rat bone marrow micronucleus assay.

Impairment of fertility was observed in male and female (b) (4) rats at subcutaneous doses of 1.88 mg/kg/day (approximately (b) (4) and (b) (4) times in (b) (4) the MRHD in adults on an AUC basis) based upon findings of decreased corpora lutea, implantation sites and live fetuses. No effects on fertility and reproductive performance in male and female rats were observed at a subcutaneous dose of 0.63 mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis).

13.2 Animal Toxicology

(b) (4) Eye findings were observed in (b) (4) Wistar rats at inhaled doses of 0.67 (b) (4) mg/kg/day **(**approximately (b) (4) and (b) (4) times, respectively, the MRHD in adults on an AUC basis) based upon findings of anterior capsule opacity, prominent anterior suture line, and anterior cataract. No eye findings in (b) (4) Wistar rats were observed at inhaled doses of 0.09 mg/kg (approximately (b) (4) times the MRHD in adults on an AUC basis). Eye findings were observed in (b) (4) beagle dogs at inhaled doses of 0.33 mg/kg/day (approximately (b) (4) and (b) (4) times in (b) (4) the MRHD in adults on an AUC basis) based upon findings of conjunctival hyperemia, corneal opacity (b) (4). No eye findings in (b) (4) e beagle dogs were observed at inhaled doses of 0.12 mg/kg/day (approximately (b) (4) times in (b) (4), the MRHD in adults on an AUC basis).

11.5.2 Labeling Recommendation for NDA 207923

INDICATIONS AND USAGE (Highlights of Prescribing Information)

SEEBRI NEOHALER is an anticholinergic indicated for the long-term, maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema.

1 INDICATIONS AND USAGE

SEEBRI NEOHALER is indicated for the long-term, maintenance treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema.

8.1 Pregnancy

Teratogenic Effects: Pregnancy Category C

There are no adequate and well-controlled trials of SEEBRI NEOHALER in pregnant women. Because animal reproduction studies are not always predictive of human response, SEEBRI NEOHALER should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women should be advised to contact their physician if they become pregnant while taking SEEBRI NEOHALER.

Glycopyrrolate was not teratogenic in Wistar rats and New Zealand White rabbits at approximately (b) (4) and (b) (4), respectively, the MRHD in adults (on an AUC basis at maternal inhaled doses up to 3.83 mg/kg/day in rats and up to 4.4 mg/kg/day in rabbits).

Nonteratogenic Effects:

Glycopyrrolate had no effects on peri-natal and post-natal developments in rats at approximately (b) (4) times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 1.88 mcg/kg/day).

8.3 Nursing Mothers

It is not known whether SEEBRI NEOHALER is excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when SEEBRI NEOHALER is administered to a nursing woman. Since there are no data from well-controlled human studies on the use of SEEBRI NEOHALER by nursing mothers, a decision should be made whether to discontinue nursing or to discontinue SEEBRI NEOHALER, taking into account the importance of SEEBRI NEOHALER to the mother.

It is not known whether glycopyrrolate is excreted in human breast milk. Glycopyrrolate (including its metabolites) have been detected in the milk of lactating rats and reached up to 10-fold higher concentrations in the milk than in the blood of the dam. (b) (4)

12.1 Mechanism of Action

(b) (4)

Glycopyrrolate is a long-acting, antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of methacholine -induced bronchoconstriction effects was dose-dependent and lasted longer than 24

hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of glycopyrrolate is predominantly a site-specific effect.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment on Fertility

(b) (4)

Glycopyrrolate produced no treatment-related increases in the incidence of tumors in a 2-year inhalation study in Wistar rats at inhaled doses up to 0.56 mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis, respectively). A 26-week oral (gavage) study in male and female TgrasH2 mice that received glycopyrrolate at doses up to 93.8 and 125.1 mg/kg/day, respectively, did not show any evidence of tumorigenicity.

Glycopyrrolate tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* rat bone marrow micronucleus assay.

Impairment of fertility was observed in male and female rats at subcutaneous doses of 1.88 mg/kg/day (approximately (b) (4) and (b) (4) times in (b) (4) the MRHD in adults on an AUC basis) based upon findings of decreased (b) (4) implantation sites and live fetuses. No effects on fertility and reproductive performance in male and female rats were observed at a subcutaneous dose of 0.63 mg/kg/day (approximately (b) (4) times the MRHD in adults on an AUC basis).

13.2 Animal Toxicology

Eye findings were observed in (b) (4) Wistar rats at inhaled doses of 0.67 (b) (4) mg/kg/day **(**approximately (b) (4) times, (b) (4) the MRHD in adults on an AUC basis) based upon findings of anterior capsule opacity, prominent anterior suture line, and anterior cataract. No eye findings in (b) (4) Wistar rats were observed at inhaled doses of 0.09 mg/kg (approximately (b) (4) times the MRHD in adults on an AUC basis). Eye findings were observed in (b) (4) beagle dogs at inhaled doses of 0.33 mg/kg/day (approximately (b) (4) times in (b) (4) the MRHD in adults on an AUC basis) based upon findings of conjunctival hyperemia, corneal opacity, (b) (4). No eye findings in male and female beagle dogs were observed at inhaled doses of 0.12 mg/kg/day (approximately (b) (4) times in (b) (4) the MRHD in adults on an AUC basis.)

11.5.3 Labeling Discussion

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Introduction
The following review evaluates the nonclinical sections of the UTIBRON NEOHALER and SEEBRI NEOHALER labeling submitted to this NDA. Recommendations include editing the proposed text for wording, and updating dose ratios between animals and humans to reflect the Agency's findings.

Dose Ratios
The following labeling recommendation considers 31.2 mcg glycopyrrolate and 55 mcg indacaterol per day as the MRHD. The proposed doses of glycopyrrolate and indacaterol are associated with an AUC of 0.139 ng*h/mL (salt) and 0.515 ng*h/mL (base; Clinical Pharmacologist Dr. Lei He), respectively, in humans.

Dose ratios for glycopyrrolate
Dose ratios for glycopyrrolate (NVA237) are based on the sponsor provided AUC values that correspond to the relevant dose in each Reproductive and Developmental Toxicity study. All doses for glycopyrrolate are indicated in the salt form.

Table 36: Dose ratios for glycopyrrolate (NVA237)

					Dose Ratio	Rounded	
					Clinical	Clinical	
Study	Route	Sex	Dose (mg/kg)	AUC (ng.h/mL)	AUC = 0.139 (ng.h/mL)	AUC = 0.139 (ng.h/mL)	
Carcinogenesis							Study #
2 year rat	IH	Both	0.56	45.6	328.1	330	r670435
Reproductive and Development							
Fertility in rats	SC	Male	1.88	519	3733.8	3700	r870596
Fertility in rats	SC	Female	1.88	290	2086.3	2100	r870596
Fertility in rats	SC	Both	0.63	98.0	705.0	710	r870596
EFD in rats	IH	Female	3.83	388	2791.4	2800	r680006
EFD in rabbits	IH	Female	4.4	148	1064.7	1100	r870597
PPND in rats	SC	Female	1.88	290	2086.3	2100	r870598*

* Fertility study #870596 was used for AUC extrapolation due to lack of TK data in the PPND study
SC = subcutaneous
IH = inhalation

Dose ratios for indacaterol

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Dose ratios for indacaterol were based on the studies and corresponding doses identified in the review for NDA 22383, dated 2/16/11 (page 27), and the corresponding summaries of the studies in the review for NDA 22383 dated 8/25/09. All doses for indacaterol are in the base form. A summary of the reproductive and developmental studies is shown below as a reference:

Type	Species	Route	Treatment period	QAB149 (mg/kg) (base)	Major Findings	Study #
Fertility	Rats	SC	Females: 2 weeks before mating, through mating period, to GD 6. Males: 28 days prior to mating, during the 2 week mating period, until necropsy	0, 0.2, 0.6, 2.0	No effects on fertility or reproductive performance. Dose selection adequate.	270074
EFD	Rats	SC	GD 6-17	0, 0.1, 0.3, 1.0	No teratogenic or reproductive effects	0270037
EFD	Rats	IH	GD 6-17	Combination study w/a single QAB149 only group at 2.70 mg/kg (estimated achieved dose, base).	No teratogenic or reproductive effects	670755
EFD	Rabbits	SC	GD 7-20	0, 0.1, 1.0, 3.0 (base)	No reproductive effects. An increase in one skeletal variation (full supernumerary ribs) in the fetuses was noted at the HD.	270038
PPND	Rats	SC	GD 0 to end of lactation for F0 females. PPD 4-20 for F1 offspring.	0, 0.1, 0.3, 1.0 (base)	Increase in F0 stillborn pups at the HD. Decreased number of F1 generation pregnant dams at the HD.	270185

The proposed labeling for indacaterol uses dose ratios based on systemic exposure (AUC). The approved label for ARCAPTA NEOHALER uses dose ratios based on mg/m^2, using doses at the appropriate NOAEL. Dose ratios calculated from systemic exposure (AUC) are the preferred method when data from toxicology studies are available.

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Table 37: Dose ratios for indacaterol (QAB149)

					Dose Ratio	Rounded	
					Clinical	Clinical	
Study	Route	Sex	Dose (mg/kg; base)	AUC (ng.h/mL; base)	AUC = 0.515 (ng.h/mL; base)	AUC = 0.515 (ng.h/mL; base)	
Carcinogenesis							Study #
2 year rat	IH	Both	2.09	116	225.2	230	320002
Reproductive and Development							
Fertility in rats	SC	Male	2.0	921	1788.3	1800	270074
Fertility in rats	SC	Female	2.0	694	1347.6	1300	270074
EFD in rats	SC	Female	1.0	345	669.9	670	0270037
EFD in rabbits	SC	Female	1.0	795	1543.7	1500	670755
PPND in rats	SC	Female	0.3	114	221.4	220	270038*

* study #0270037 was used for AUC extrapolation in the absence of toxicokinetic data
IH = inhalation
SC = subcutaneous
Calc = calculated
Round = rounded for label

Glycopyrrolate (NVA237)

Nonclinical information relevant to the glycopyrrolate portion of the labels is discussed below. Nonclinical data include reproductive and developmental toxicity, mechanism of action, mutagenicity, and carcinogenicity.

Overall Reproductive and Developmental Toxicity

The standard battery of reproductive and developmental toxicity studies were completed with glycopyrrolate in rats and rabbits. There were test article related effects on fertility (see next section, Impairment of Fertility). Glycopyrrolate was not teratogenic in rats or rabbits, and it did not have any pre- or postnatal effects in rats.

Teratogenicity & Post-natal development

Glycopyrrolate was not teratogenic in rats or rabbits, based on the lack of test article related malformations. Glycopyrrolate had no postnatal effects in rats.

The sponsor submitted the following text under Section 8.1:

"Pregnancy Category C

(b) (4)

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

(b) (4)

Current labeling practices allow for the statements regarding teratogenic and nonteratogenic effects to be captured separately in Section 8.1. The following recommended language reflects this practice. In addition, it provides dose ratios that are consistent with those calculated in this review for the EFD and PPND studies. Doses are provided in the salt form, to remain consistent with the proposed dose of glycopyrrolate (15.6 mcg), which is expressed in the salt form. Nonteratogenic effects are listed for future use in PLLR labeling.:

"Teratogenic Effects: Pregnancy Category C

There are no adequate and well-controlled studies with SEEBRI NEOHALER in pregnant women. Because animal reproduction studies are not always predictive of human response, SEEBRI NEOHALER should only be used during pregnancy if the potential benefit justifies the potential risk to the fetus. Women should be advised to contact their physician if they become pregnant while taking SEEBRI NEOHALER.

Glycopyrrolate was not teratogenic in Wistar rats and New Zealand White rabbits at approximately (b) (4) and (b) (4) times, respectively, the MRHD in adults (on an AUC basis at maternal inhaled doses up to 3.83 mg/kg/day in rats and up to 4.4 mg/kg/day in rabbits).

Nonteratogenic Effects:
Glycopyrrolate had no effects on peri-natal and post-natal developments in rats at approximately (b) (4) times the MRHD in adults (on an AUC basis at maternal subcutaneous doses up to 1.88 mcg/kg/day)."

Excretion in Milk

Glycopyrrolate has been shown to be present in the milk of lactating rats administered a single dose of 5 mg/kg radiolabeled NVA237 (salt) in study #1000614. Based on AUClast of NVA237 in milk and blood samples collected up to 72 hrs after dosing, the milk/plasma ratio was 11.3 for NVA237 and 8.03 for total radiolabeled components. The value of 8.03 should be rounded to 10, as shown in the sponsor's submitted text, which is consistent with the guidelines for rounding numbers in labels. (b) (4)

(b) (4)

The sponsor submitted the following text for Section 8.3:

> "It is not known whether (b) (4) is excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when SEEBRI NEOHALER is administered to a nursing woman. Since there are no data from well-controlled human studies on the use of SEEBRI NEOHALER by nursing mothers, a decision should be made whether to discontinue nursing or to discontinue SEEBRI NEOHALER, taking into account the importance of SEEBRI NEOHALER to the mother. Glycopyrrolate (including its metabolites) have been detected in the milk of lactating rats and reached up to 10-fold higher concentrations in the milk than in the blood of the dam."

The general statements proposed by the sponsor regarding SEEBRI NEOHALER have been retained in the recommended labeling. The second paragraph contains more specific information from the sponsor's study in lactating rats, (b) (4)

> "It is not known whether SEEBRI NEOHALER is excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when SEEBRI NEOHALER is administered to a nursing woman. Since there are no data from well-controlled human studies on the use of SEEBRI NEOHALER by nursing mothers, a decision should be made whether to discontinue nursing or to discontinue SEEBRI NEOHALER, taking into account the importance of SEEBRI NEOHALER to the mother.
>
> It is now known whether glycopyrrolate is excreted in human breast milk. Glycopyrrolate (including its metabolites) have been detected in the milk of lactating rats and reached up to 10-fold higher concentrations in the milk than in the blood of the dam. (b) (4)

Impairment of fertility

Fertility was assessed in male and female rats dosed with 0 (vehicle) , 0.19, 0.63, and 1.88 NVA237 (salt) subcutaneously. The fertility index was in general low in all groups, including the control (control 72%, LD 60%, MD 84%, HD 60%). This effect was not dose dependent. The sponsor stated that studies conducted "recently" in the same facility had fertility rates that ranged from 84% to 96%, and noted that only the MD had a fertility rate within the expected range. Due the lack of dose response, this did not appear to be a test article related finding, but there is a concern that the cause of the decreased fertility rate may mask a test article related effect.

At the MD and HD, there was a slight decrease in the litter mean number of corpora lutea (control 13.2, LD 13.6, MD 12.6, HD 11.0) and number of implantations (control 11.6, LD 12.3, MD 9.9, HD 7.9), which corresponded to a decreased number of live fetuses per litter (control 10.9, LD 11.6, MD 9.9, HD 7.9). The decrease in implantations

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and live fetuses at the MD and HD may be a result of increased preimplantation loss at the MD and HD (control 12.1%, LD 9.9%, MD 19.8%, HD 22.4%), compared to the control and LD. Based on these dose dependent trends, it is difficult to rule out an effect of the test article on these parameters. Values, however, at the MD were within the historical control data submitted by the sponsor. The reproductive and fertility NOAEL was determined to be the MD of 0.63 mg/kg NVA237 (salt), which is associated with an $AUC_{0\text{-}24h}$ of 98.0 h*ng/mL (salt) in females and males. Fertility was adversely affected for male and female rats at the HD of 1.88 mg/kg/day.
The sponsor provided the following labeling:

(b) (4)

The recommended text reflects the findings in the rat fertility study:

> "Impairment of fertility, (b) (4) was observed in male and female rats at subcutaneous doses of 1.88 mg/kg/day (approximately (b) (4) and (b) (4) times in males and females, respectively, the MRHD in adults on an AUC basis)."

Mechanism of Action

Glycopyrrolate is a long-acting antimuscarinic agent, which is often referred to under the more general term anticholinergic. The mechanism of action of glycopyrrolate is similar to that of tiotropium bromide, aclidinium bromide, and umeclidinium bromide, which are currently marketed LAMAs. The following recommendations are based upon data from primary pharmacology studies conducted with glycopyrrolate, and an effort to keep the label consistent with marketed LAMAs.

Overall, rewording of the proposed label has been recommended to be consistent with the label for tiotropium bromide and aclidinium bromide inhalation powder (see below).

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Table 38: Section 12.1: Text of Marketed LAMAs

SPIRIVA (tiotropium bromide) NDA 021395 Approved 10/11/2005	TUDORZA (aclidinium bromide) NDA 202450 Approved 7/23/2012
Tiotropium is a long-acting, antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors, M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3-receptors at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of methacholine-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The bronchodilation following inhalation of tiotropium is predominantly a site-specific effect.	Aclidinium bromide is a long-acting antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical *in vitro* as well as *in vivo* studies, prevention of acetylcholine-induced bronchoconstriction effects was dose-dependent and lasted longer than 24 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of aclidinium bromide is predominantly a site-specific effect.

ANORO ELLIPTA (umeclidinium bromide) NDA 203975 Approved 12/18/2013
Umeclidinium is a long-acting, antimuscarinic agent, which is often referred to as an anticholinergic. It has similar affinity to the subtypes of muscarinic receptors M1 to M5. In the airways, it exhibits pharmacological effects through inhibition of M3 receptor at the smooth muscle leading to bronchodilation. The competitive and reversible nature of antagonism was shown with human and animal origin receptors and isolated organ preparations. In preclinical in vitro as well as in vivo studies, prevention of methacholine and acetylcholine-induced bronchoconstrictive effects was dose-dependent and lasted longer than 24 hours. The clinical relevance of these findings is unknown. The bronchodilation following inhalation of umeclidinium is predominantly a site-specific effect.

(In-use labeling from DARRTS)

The sponsor submitted labeling which is consistent with the above labels. The only recommendation is to delete [redacted] (b) (4) :

> "In preclinical in vitro as well as in vivo studies, prevention of methacholine [redacted] (b) (4) induced bronchoconstrictive effects was dose-dependent and lasted longer than 24 hours."

The following text is recommended, omitting [redacted] (b) (4) :

> "In preclinical in vitro as well as in vivo studies, prevention of methacholine-induced bronchoconstrictive effects was dose-dependent and lasted longer than 24 hours."

Mutagenesis

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Glycopyrrolate tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* rat bone marrow micronucleus assay.

The sponsor proposed the following statement:

> [redacted] (b) (4) ."

The recommended statement provides the tests that were conducted, which is consistent with currently labeling practice.

> "Glycopyrrolate tested negative in the following genotoxicity assays: the *in vitro* Ames assay, *in vitro* human lymphocyte chromosomal aberration assay, and *in vivo* rat bone marrow micronucleus assay."

Carcinogenesis

Glycopyrrolate was negative for carcinogenicity in 2-year bioassay conducted in rats and 26-week bioassay in transgenic mice. In both studies, there were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis.

The sponsor proposed the following statement regarding the carcinogenicity assessment:

> [redacted] (b) (4)

A statement regarding the lack of carcinogenicity data with the relevant proposed product (UTIBRON NEOHALER or SEEBRI NEOHALER) is recommended. The ECAC considers the [redacted] (b) (4) .

The following recommended text reflects this, and also provides the relevant dose of NVA237 (salt) in the rat carcinogenicity study, and the associated dose ratio:

> "No studies of carcinogenicity, mutagenicity, or impairment of fertility were conducted with SEEBRI NEOHALER; however, studies are available for glycopyrrolate, as described below.
>
> Glycopyrrolate produced no treatment-related increases in the incidence of tumors in a 2-year inhalation study in Wistar rats at inhaled doses up to 0.56 mg/kg/day (approximately [redacted] (b) (4) times the MRHD in adults on an AUC basis, respectively). A 26-week oral (gavage) study in male and female TgrasH2 mice that received glycopyrrolate at doses up to 93.8 and 125.1 mg/kg/day, respectively, did not show any evidence of tumorigenicity."

<u>Indacaterol</u>

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The recommended labeling text for indacaterol generally follows the approved label under NDA 22383 (see Pharmacology/Toxicology Labeling Review dated 4/22/2013). The dose multiples for indacaterol are changed to reflect the proposed dose of indacaterol, which is 27.5 mcg BID, or 55 mcg/day, compared to ARCAPTA NEOHALER, which is recommended at 75 mcg OD.

Three deviations from the approved label for ARCAPTA NEOHALER are recommended, under Section 13.1, and Section 8.3 as detailed below.

Under Section 13.1, the recommendation is to delete [redacted] (b) (4) and to clearly state that the result of the carcinogenicity assessment in rats and mice were negative, consistent with ECAC minutes under NDA 22383. [redacted] (b) (4) The current text in the label for ARCAPTA NEOHALER is shown below as a reference:

> "**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**
>
> Long-term studies were conducted in transgenic mice using oral administration and in rats using inhalation administration to evaluate the carcinogenic potential of indacaterol maleate. Indacaterol did not show a statistically significant increase in tumor formation in mice or rats.
>
> Lifetime treatment of rats resulted in increased incidences of benign ovarian leiomyoma and focal hyperplasia of ovarian smooth muscle in females at doses approximately 270-times the dose of 75 mcg once-daily for humans (on a mg/m2 basis).
>
> A 26-week oral (gavage) study in CB6F1/Tg.rasH2 hemizygous mice with indacaterol did not show any evidence of tumorigenicity at doses approximately 39,000-times the dose of 75 mcg once-daily for humans (on a mg/m2 basis).
>
> Increases in leiomyomas of the female rat genital tract have been similarly demonstrated with other beta2-adrenergic agonist drugs. The relevance of these findings to human use is unknown.
>
> Indacaterol was not mutagenic or clastogenic in Ames test, chromosome aberration test in V79 Chinese hamster cells, and bone marrow micronucleus test in rats.
>
> Indacaterol did not impair fertility of rats in reproduction studies."

The recommendation is for the following new text under Section 13.1:

> "<u>Indacaterol:</u> Indacaterol produced no treatment-related increases in the incidence of tumors in a 2-year inhalation study in Wistar rats at inhaled doses up to 2.09 mg/kg/day (approximately [redacted] (b) (4) times the MRHD in adults on an AUC basis, respectively). A 26-week oral (gavage) study in CB6F1/TgrasH2

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> hemizygous mice with indacaterol at doses up to 600 mg/kg/day did not show any evidence of tumorigenicity.
>
> Indacaterol tested negative in the following genotoxicity assays: the in vitro Ames assay, in vitro chromosome aberration test in V79 Chinese hamster cells, and in vivo rat bone marrow micronucleus assay.
>
> Indacaterol had no effects on fertility and reproductive performance in male and female rats at subcutaneous doses up to 2 mg/kg/day (approximately (b) (4) and (b) (4) times in males and females, respectively, the MRHD in adults on an AUC basis)."

In the proposed labeling for UTIBRON NEOHALER, the sponsor stated under Section 8.3:

> "Indacaterol: Indacaterol (including its metabolites) have been detected in the milk of lactating rats."

The approved labeling for ARCAPTA NEOHALER states:

> "It is not known that the active component of ARCAPTA NEOHALER, indacaterol, is excreted in human milk. Because many drugs are excreted in human milk and because indacaterol has been detected in the milk of lactating rats, caution should be exercised when ARCAPTA NEOHALER is administered to nursing women."

Under the sponsor's Nonclinical Overview, submitted under NDA 207930, the sponsor states, "Indacaterol and/or its metabolites passed the placenta-blood-barrier in pregnant rats and were transferred rapidly into the milk of lactating rats (Study R02-0220) (Study R0700884)." An Information Request was sent on 9/18/15 requesting the location of the study report under NDA 207930. On 9/22/15, the sponsor replied that the studies were submitted to NDA 22383, which is referenced by NDA 207930. Indeed, study R0700884 was reviewed under NDA 22383 (nonclinical review dated 8/25/09), and it was determined that drug-related radioactivity was detected in fetuses from dams dosed with radiolabeled QAB149 from GD 12-17. Study R02-0220 was also summarized in the same review, and showed that QAB149 was found in the milk of dams dosed with a single dose of radiolabeled QAB149 from PPD 8-9. Thus, the sponsor's proposed labeling statement is acceptable.

Animal Findings

Eye findings were identified in both pivotal general toxicology studies supporting the safety of NVA237 are chronic toxicology studies in rats and dogs. In the 26 week inhalation toxicology study, with a 4 week recovery in rats, animals received estimated achieved doses of 0 (air), 0 (vehicle: 1% magnesium stearate, 99% lactose monohydrate), 0.09, 0.67 and 4.98 mg/kg of NVA237 (salt). Eye findings consisted of bilateral and unilateral lenticular changes (anterior capsular opacity, anterior prominent suture line, anterior slight cataract) that were observed at doses of 0.67 mg/kg and

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above (approximately 440 times and above the MRHD in adults on an AUC basis.) These findings were partially reversible.

In the 39 week inhalation toxicology study, beagle dogs were dosed with 0 (air), 0 (vehicle: 1% magnesium stearate, 99% lactose monohydrate), 0.030, 0.12, 0.33 mg/kg (estimated achieved doses in salt form) through the inhalation route of exposure (6/sex/group controls and HD; 4/sex/group LD and MD), with a 4 week recovery period (2/sex/group controls and HD). Test article related ophthalmic findings were bilateral conjunctival hyperemia, corneal opacity, and focal nuclear opacity observed at 0.33 mg/kg (approximately 770 and 290 times in males and females, respectively, the MRHD in adults on an AUC basis). These findings were reversible.

Table 39: Dose ratios for nonclinical eye findings

		Nonclinical dose corresponding to eye finding		Dose ratio for clinical dose of 31.2 mcg NVA237
Study	Sex	Achieved dose (mg/kg/day, salt form)	AUC (ng*hr/mL) (salt form)	AUC = 0.139 ng*hr/mL (salt form)
26 week rat	Both	4.98	334	2400
	Both	0.67	61.6	440
	Both	0.09	13.8	100
39 week dog	Male	0.33	101.2	730
	Female		41.0	290
	Male	0.12	26.6	190
	Female		42.0	300

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12 Appendix/Attachments

The following appendix includes a list of abbreviations used the review, and previous reviews of pivotal toxicology studies for glycopyrrolate (NVA237) and the combination of glycopyrrolate and indacaterol (QVA149).

Note: The review of the carcinogenicity studies for NVA237 was previously completed under NDAs 207923/207930 on 7/7/15. Reviews for indacaterol (QAB149) were completed under NDA 22383.

Appendix #	Content	Date (for reviews/minutes)
1	Abbreviations	Not applicable
2	Review under IND 48655 (13 week DRF study in rats)	9/25/2006
3	Review under IND 48655 (Pharmacology, genetic toxicology)	6/20/2007
4	Review under IND 48655 (26 week NVA237 rat study)	3/30/2009
5	Review under IND 48655 (4 week DRF study in mice)	2/24/2010
6	Review under IND 76377 (Safety Pharmacology, 14 day combination dog study)	7/31/2008
7	Review under IND 76377 (13 week combination dog study, combination EFD rat study)	9/18/2013

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Appendix 1

Abbreviations

API	active pharmaceutical ingredient
AUC	area under the curve
bmp	beats per minute
BW	body weight
BrW	brain weight
calc	calculated value for label
carc	carcinogenesis
C_{max}	maximum concentration
CMC	chemistry, manufacturing and controls
d	day
DPI	dry powder inhaler
ECG	electrocardiogram
EFD	embryofetal development
EOF2	end of phase 2
F	females
GD	gestation day
HD	high dose
HLQ	higher limit of quantification
hr(s)	hour(s)
IH, Ihn	inhalation
IP	intraperitoneal
IC_{50}	half maximal inhibitory concentration
ICH	International Conference on Harmonization
IV	intravenous
kg	kilograms
LABA	long-acting β2-agonist
LAMA	long-acting muscarinic antagonist
LD	low dose
LLOQ	lower limit of quantification
M	males
mcg	micrograms
MD	mid dose
min	minutes
mg	milligrams
MRHD	maximum recommended human dose
PC	post coitum
PND	post natal day
PPND	peri- and post-natal development

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PO	per oral dosing
round	rounded value for label
SC	subcutaneous
SD	study day
sec	second(s)
T_{max}	time at maximum
VPC	ventricular premature complex(es)

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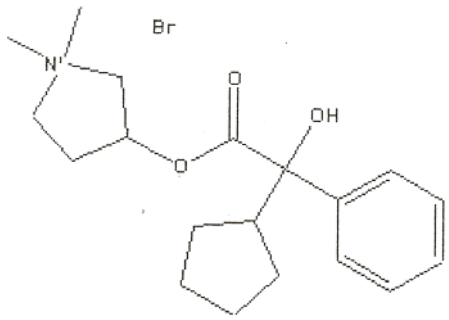
PHARMACOLOGY/TOXICOLOGY REVIEW

IND number: 48,655
Sequence number/date/type of submission: 8/29/06/SX
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Novartis Pharmaceutical Corporation

Reviewer name: Lawrence F. Sancilio, Ph.D.
Division name: Pulmonary and Allergy Products
Review completion date: 9/25/06

Drug:

Trade name: Unknown
Generic name: Glypyrronium bromide, glycopyrrolate, USP
Code name: NVA237
Chemical name: 3-[(cyclopentylhydroxyphenylacetyl)oxy]-1, 1-dimethylpyrrolidinum bromide
CAS registry number: 596-51-0
Molecular formula/molecular weight: $C_{19}H_{28}BrNO_3$/ 398.34
Structure:



Drug class: Quaternary ammonium antimuscarinic agent.

Intended clinical population: COPD.

Proposed Clinical formulation: Capsules containing 30, 60, 120 and 240 mcg of glycopyrronium bromide in a [redacted] (b) (4) formulation containing lactose and magnesium stearate and administered by the inhaled route with a [redacted] (b) (4). This formulation consists of [redacted] (b) (4)% glycopyrronium bromide, [redacted] (b) (4)% magnesium stearate and [redacted] (b) (4) lactose. An inhalation dose tested in a clinical pharmacokinetics study was 5 mcg/kg.

Route of administration: Inhalation.

Introduction and Drug History

This is a special request for an assessment of a rat carcinogenicity protocol for glycopyrronium bromide based on a 13-week inhalation toxicity study in rats. The proposed inhalation doses are (b) (4) mg/kg of the salt. Glycopyrronium bromide has long been marketed as an injectable muscarinic receptor antagonist used as a premedication during anesthesia, topically for hyperhidrosis and orally for the treatment of peptic ulcers. It is being developed as an inhalation product in view of its ability to antagonize cholinergic bronchoconstriction and mucus secretion. One of the excipients, magnesium stearate in the drug product, has been only approved for oral use. For approval of glycopyrronium bromide for inhalation, the Agency recommended at the 6/9/04 PIND meeting that the safety of this formulation (compound and excipient) be tested in a 6-month bridging inhalation toxicity study in the most relevant specie (rats). Pending the results of this study, most notably the presence of preneoplastic/neoplastic lesions particularly in the respiratory tract, and the results of genotoxicity assessment, a carcinogenicity assay in one specie may be warranted. However, the sponsor has not submitted the results of a 6-month inhalation toxicity study and elected to conduct a carcinogenicity assay in rats with this special protocol assessment, proposing inhalation doses based on a 3-month inhalation toxicity study in rats.

The sponsor summarized the properties of glycopyrronium bromide. It is a quaternary ammonium antimuscarinic agent that is poorly absorbed orally (7%) and by inhalation (10%) in humans. In animal studies, glycopyrronium bromide poorly enters the central nervous system and poorly crosses the placenta. Orally, when administered as a radioactive substance, it (radioactivity) is excreted predominantly unchanged in the feces and very little radioactivity is present in the urine. Glycopyrronium bromide is metabolized by hydroxylation and oxidation and was not genotoxic in the Ames, Human Lymphocyte Chromosomal Aberration and Rat Micronucleus assays.

Toxicology

Summary

In a 13-week complete interim inhalation toxicity study of a 26-week toxicity study in rats, the formulation used was a dry powder consisting of 8% glycopyrronium bromide, 1% magnesium stearate and 91 % lactose monohydrate. The inhaled doses of glycopyrronium bromide salt were 0.1 (LD), 0.6 (MD) and 4 (HD) mg/kg and that for the magnesium stearate control vehicle group was 0.5 mg/kg, the amount exposed to the HD glycopyrronium bromide group. Analytically, the ranges of MMAD were: 2.1-3.1 um for glycopyrronium bromide and 1.7 -2.3 um for magnesium stearate, and gravimetrically, the ranges of MMAD were: 3.3-3.8 um for glycopyrronium bromide and 2.8-4.3 um for magnesium stearate. No toxicity was seen in the vehicle treated group. A dose related decrease in body weight gained occurred in both sexes at the MD and HD; in the HD males, there was an increase in the white blood cell and neutrophil count. In the HD males, there was a decrease in thymus and adrenal weight and an increase in lung weight. These were not supported by histopathological findings and, therefore, were not

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considered biologically significant. Dose related histopathological changes were seen in the nasal cavity/sinuses (male and female, all doses), Hardarian gland (male and female, all doses), goblet cells (male, all doses; female, MD and HD) and larynx (male, MD and HD; female, all doses). Most severely affected was the olfactory epithelium of the nasal cavity sinuses. The pharmacokinetics was similar in both sexes; the Cmaxs and AUCs were doses related and not dose proportional, and there was no accumulation. The MTD was 0.6 mg/kg based on a decrease in body weight gained.

Review

Toxicology

Study title: 13-Week inhalation toxicity study in rats (DRAFT).

Key findings:

- No toxicity was seen with the excipient, magnesium stearate.
- Both sexes showed a decrease in body weight gained at the MD and HD.
- HD males showed increased levels of white blood cells and neutrophils.
- Histopathology was seen in the nasal cavity/sinuses, Hardarian gland, goblet cells and larynx.
- The inhalation MTD was 0.6 mg/kg based on decreased body weight gained.

Study no: No. 79031
Volume # and page #: vol. 1, p 99.
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/10/06
GLP compliance: Yes.
QA report: yes (X)
Drug, lot #, and % purity: 8% glycopyrronium bromide, 1% magnesium stearate, 91% lactose monohydrate. VR6107.
Control vehicle, Formulation/Vehicle: 1% magnesium stearate, 99% lactose monohydrate. VAR5905.

Method
Dosing:
Species/strain: Wister Hanover Crl: WI (Han).Charles River Sprague-Dawley Crl: CD (SD) IGS BR rats.
#/Sex/group: 10 (Main Group): toxicokinetics group; 5.
Age: 8 Weeks.
Weight: M, 196-241g; F, 152-187 g.
Route: Inhalation; exposure was by nose only for 120 min/day.
Particle size: Range of MMAD determined analytically: 2.1-3.1 um for glycopyrronium bromide and 1.7 -2.3 um for magnesium stearate; gravimetrically, the ranges of MMAD were: 3.3-3.8 um for glycopyrronium bromide and 2.8-4.3 um for magnesium stearate.

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Doses of glycopyrronium bromide (salt) and excipient (magnesium stearate) are summarized in the following tables.

Glycopyrronium bromide

Group	Target Dose mg/kg
Air Control	0
Mg Stearate/Lactose (vehicle) Control	0
LD	0.1
MD	0.6
HD	4

Magnesium Stearate

Group	Target Dose mg/kg
Air Control	0
Mg Stearate/Lactose (Vehicle) Control	0.5
LD	0.0125
MD	0.075
HD	0.5

Observations:

Analysis of Formulation: Weekly.
Mortality: Daily.
Clinical signs: Daily.
Body weights: Prior to and weekly after initiating treatment.
Food consumption: Prior to and weekly after initiating treatment.
Ophthalmoscopy: Both air and vehicle control and HD groups: Week 13.
Hematology: Week 13.
Clinical chemistry: Week 13.
Urinalysis: Week 13.
Toxicokinetics: Day 28 and week 13. Time points: Both control groups: Immediately and 15 min after completion of exposure: LD, MD and HD, 15 min, 3, 7 and 24 hrs following exposure.
Assay: On-line solid phase extraction followed by a liquid chromatography and tandem mass spectrometry using electrospray ionization as interface in positive ion mode. The LLOQ was 0.1 ng/ml.
Gross pathology: 13 Weeks, all animals.
Organs weighed: Adrenals, brain, kidneys, heart, liver, lungs, ovaries, pituitary prostate, spleen, testes, thymus, thyroid and parathyroids and uterus.

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Histopathology: The following tissues in the two control and HD groups were examined: Abnormal lesions, adrenals, aorta, femur, bone and marrow from sternum, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, Hardarian glands, heart, ileum, jejunum, kidneys, Lacrimal glands, larynx, liver, lungs, lymph nodes (tracheobronchial, mandibular, unilateral, mesenteric) bronchi, nasal cavities and sinuses, optic nerves, ovaries, pancreas, pharynx, pituitary, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, testes, thymus, thyroid and parathyroid glands, tongue, trachea, urinary bladder, uterus and vagina,

For the LD and MD groups, the following tissues were examined:

Abnormalities, lungs, larynx, bronchi, nasal cavities and sinuses, pharynx, trachea and lymph node (tracheobronchial).

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

Results

Particle size: Analytically, the ranges of MMAD were: 2.1-3.1 um for glycopyrronium bromide and 1.7 -2.3 um for magnesium stearate, and gravimetrically, the ranges of MMAD were: 3.3-3.8 um for glycopyrronium bromide and 2.8-4.3 um for magnesium stearate.

Mortality: None.

Clinical signs: None.

Body weight: Day 91: % decrease from vehicle control: Males, MD, -8% ($p < 0.05$)
HD, -16% ($p<0.05$)
Females, MD, -3% ($p>0.05$)
HD, -8% ($p<0.05$)

Note: There was no statistical difference between the air- and vehicle controls. See attachments for growth curves that were excerpted from the sponsor's submission.

Body Weight Gained: % decrease from vehicle control: Males, MD, -15% ($p>0.05$)
HD, -32% ($p<0.05$)
Females, MD, -15% ($p>0.05$)
HD, -27% ($p<0.05$)

Note: There was no statistical difference between the air- and vehicle- controls.

Food Consumption (Day 91): % decrease from air control: Males, vehicle control, -15%.

Hematology: % increase from vehicle control: Male, HD, White blood cell count, +45%.
Male, HD, Neutrophil count, +82%.

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Clinical Chemistry: No effect.

Urine analysis: No effect.

Toxicokinetics

Sex/Day/Parameter	LD	MD	HD
Males			
Day 28			
Cmax, ng/ml	2.14	7.06	72
$AUC_{0\text{-}24\ hr}$ ng.hr/ml	8.65	36.9	198
Tmax, hr	2	2.25	2
Males			
Day 91			
Cmax, ng/ml	1.57	8.27	52.4
$AUC_{0\text{-}24\ hr}$ ng.hr/ml	9.94	34.5	187
Tmax, hr	2.25	2	2
Females			
Day 28			
Cmax, ng/ml	2.44	18.5	91.8
$AUC_{0\text{-}24\ hr}$ ng.hr/ml	9.81	61.5	230
Tmax, hr	2	2	2
Females			
Day 91			
Cmax, ng/ml	1.57	16.2	44.8
$AUC_{0\text{-}24\ hr}$ ng.hr/ml	5.63	48.4	166
Tmax, hr	2.25	2	2

Necropsy: No gross abnormalities.

Organ Weight (difference from vehicle control):

Males:

Thymus, HD, Absolute wt., -23 %.

Adrenals, HD, Relative wt., -24%

Lung, HD, Relative wt., +31%

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Histopathology: The results are summarized in the following tables.

Males

Organ/Pathology	Incidence				
	C-air	C-vehicle	LD	MD	HD
Nasal Cavity/Sinuses					
Olfactory epithelium					
Eosinophilic globules	0/10	0/10	2/10	9/10	10/10
Respiratory epithelium					
Eosinophilic globules	0/10	0/10	0/10	7/10	10/10
Metaplasia, squamous	0/10	0/10	0/10	0/10	2/10
Goblet cells					
Hyperplasia/hypertrophy	0/10	0/10	0/10	7/10	9/10
Hardarian gland					
Increased porphyrin deposition	0/10	0/10	2/10	6/10	9/10
Larynx					
Metaplasia, squamous	0/10	0/10	3/10	7/10	8/10

Females

Organ/Pathology	Incidence				
	C-air	C-vehicle	LD	MD	HD
Nasal Cavity/Sinuses					
Olfactory epithelium					
Eosinophilic globules	0/10	0/10	1/10	9/10	10/10
Respiratory epithelium					
Eosinophilic globules	0/10	0/10	0/10	8/10	10/10
Goblet cells					
Hyperplasia/hypertrophy	0/10	0/10	0/10	4/10	9/10
Hardarian gland					
Increased porphyrin deposition	0/10	0/10	1/10	6/10	5/10
Inflammation	0/10	0/10	0/10	0/10	2/10
Larynx					
Metaplasia, squamous	0/10	0/10	2/10	9/10	9/10

The severity of the microscopic findings at the HD were the highest in the olfactory epithelium (graded scores ranging from 2-4 of a 1-4 range).

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OVERALL CONCLUSIONS AND RECOMMENDATIONS

This is a Special Rat Carcinogenicity Protocol Assessment of proposed doses based on the results of a 13-week inhalation toxicity study. The inhaled doses of glycopyrronium bromide salt tested in this study were 0.1, 0.6 and 4 mg/kg. The sponsor proposed oral doses of 0.1, 0.3 and 1 mg/kg. In addition to an air control group, there was a vehicle control group which had magnesium stearate 0.5 mg/kg as an excipient in addition to lactose. The sponsor proposed inhalation doses of (b) (4) mg/kg. No toxicity was seen with the vehicle control group. The report indicated that there were no treatment related clinical signs. However, in the Investigational Brochure, persistent mydriasis was the only significant treatment related clinical sign seen in a summarized 28-day inhalation toxicity study in rats at the same doses used in this 13-week inhalation toxicity study in rats. This indicates that the anticholinergic effect (mydriasis) was present and not reported. Glycopyrronium bromide produced a decrease in body weight gained (MD and HD) in both sexes, an increase the white blood and neutrophil count (HD, males) and in both sexes, histopathological changes in the nasal cavity/sinuses, Hardarian gland, and larynx. The pharmacokinetics was similar in both sexes. The MTD was the MD (0.6 mg/kg) based on decreased body weight gained. The AUC for the MTD was 46 times the AUC for a clinical dose of 5 mcg/kg.

RECOMMENDATION

Pending the Exec-CAC concurrence that the MTD was identified as 0.6 mg/kg based on a 15% decrease in body weight gained, the proposed inhalation HD of (b) (4) mg/kg should be lowered to 0.6 mg/kg.

Reviewer's signature: _____

Supervisor's signature:

Concurrence - _____

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Figure 1 Summary of body weight data - males

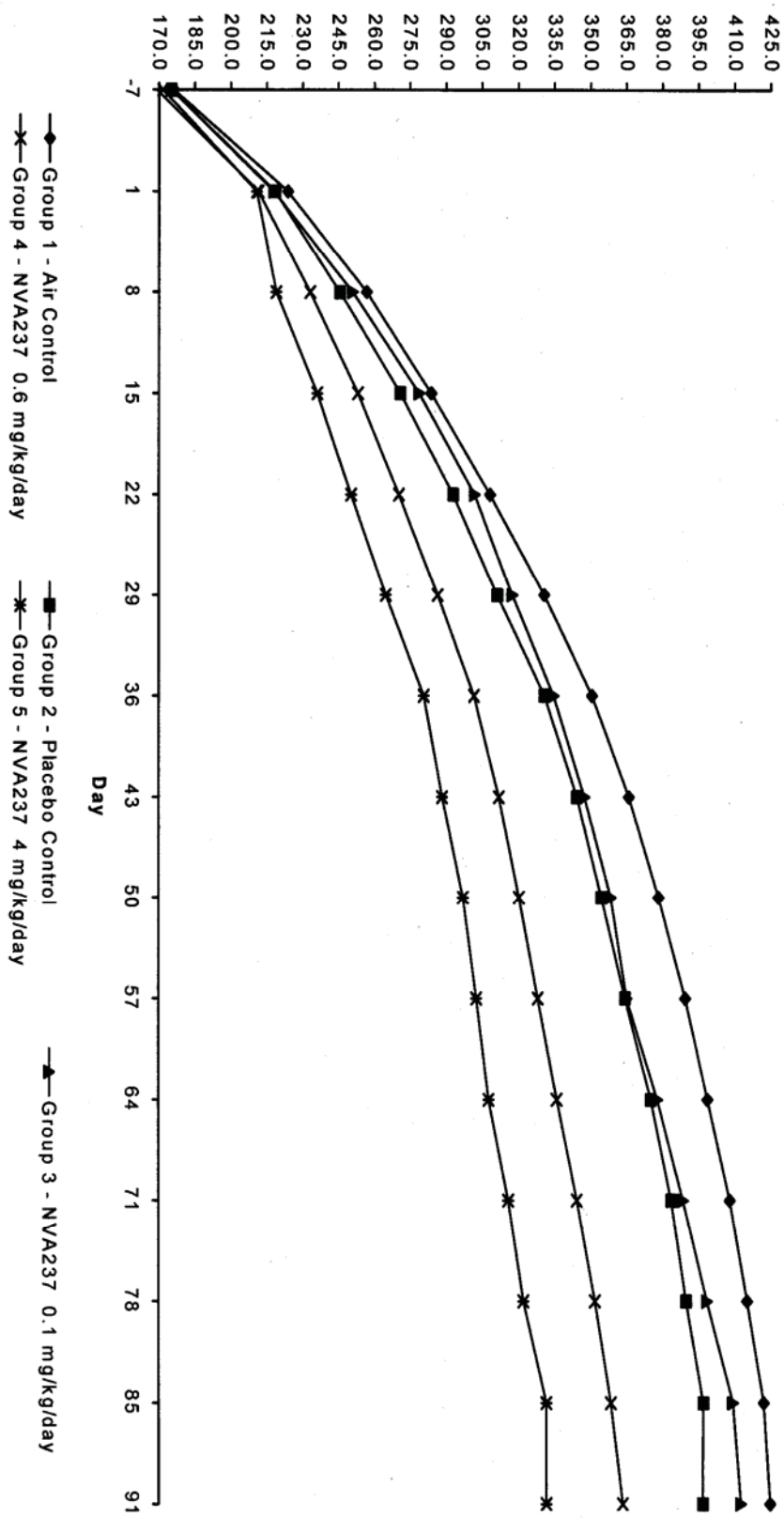
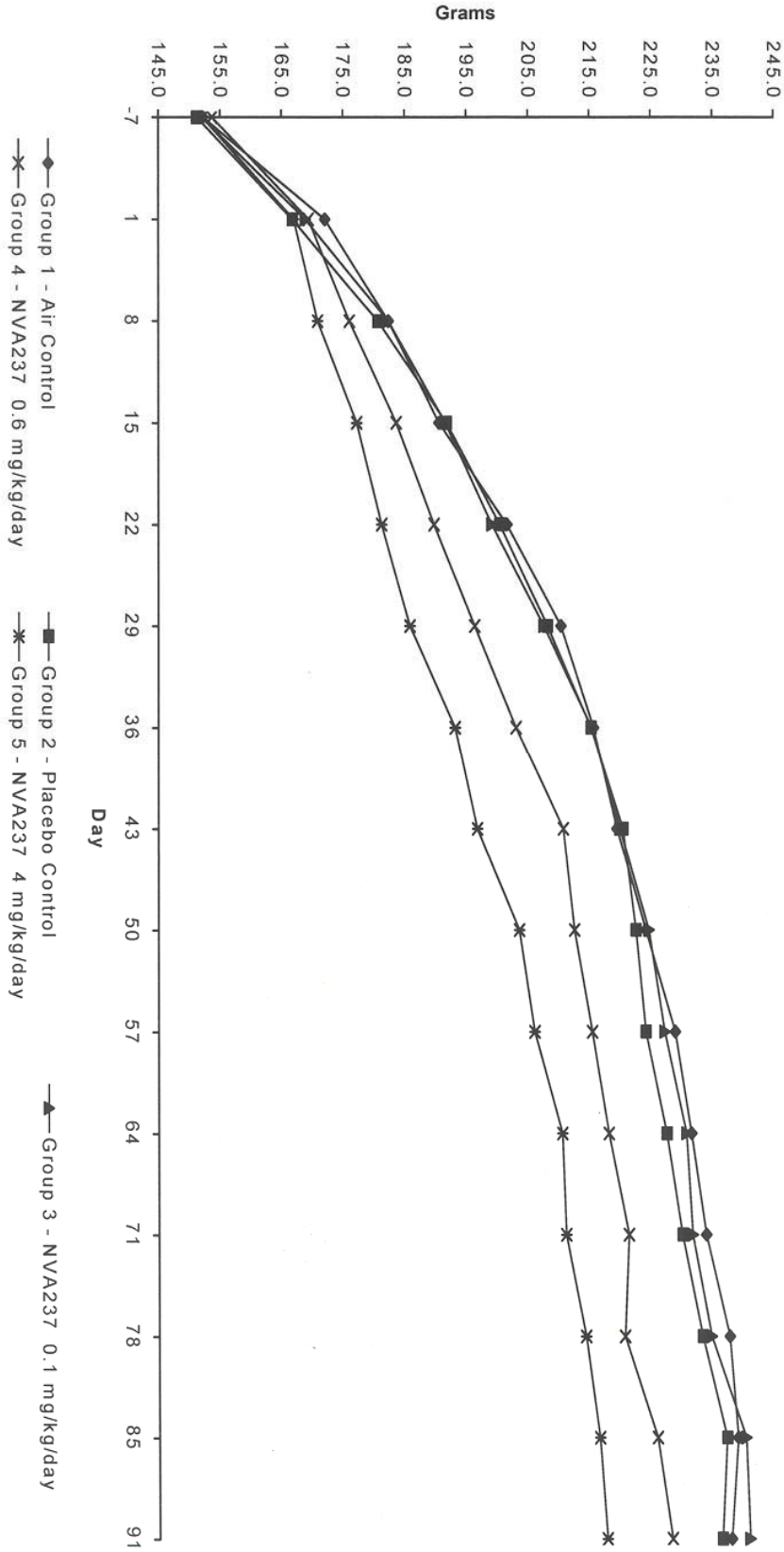


Figure 2 Summary of body weight data - females



APPEARS THIS WAY ON ORIGINAL

Reference ID: 3824703

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/s/

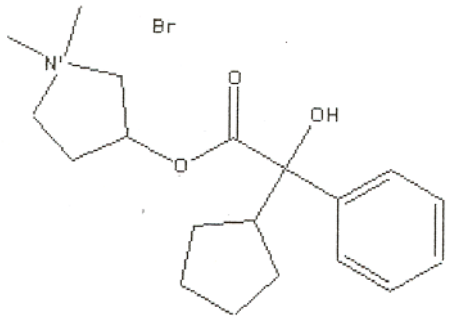
Lawrence Sancilio
9/25/2006 01:55:40 PM
PHARMACOLOGIST

Joseph Sun
9/25/2006 02:09:32 PM
PHARMACOLOGIST
I concur.

PHARMACOLOGY/TOXICOLOGY REVIEW

IND number: 48,655
Sequence number/date/type of submission: 000, 4/27/07,original
Review No.: 2
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Novartis Pharmaceutical Corporation

Reviewer name: Lawrence F. Sancilio, Ph.D.
Division name: Pulmonary and Allergy Products
Review completion date: 6/20/07.
Drug: Trade name: Unknown
Generic name: Glypyrronium bromide, glycopyrrolate, USP
Code name: NVA237; NVP-NVA237**;** NVP-QAM254-DC-1.
Chemical name: R, S, 3-[(cyclopentylhydroxyphenylacetyl)oxy]-1, 1-dimethylpyrrolidinum bromide
CAS registry number: 596-51-0
Molecular formula/molecular weight: $C_{19}H_{28}BrNO_3$/ 398.34
Structure:



Relevant INDs/NDAs/DMFs:

Drug class: Quaternary ammonium antimuscarinic agent.

Intended clinical population: COPD.

Clinical formulation: Hard capsules containing 12.5, 25, 50, 100 and 200 mcg of glycopyrronium bromide in a powder formulation containing lactose and magnesium stearate and administered by the inhaled route [redacted] (b) (4). This formulation consists of glycopyrronium bromide, [redacted] (b) (4) mg of lactose and [redacted] (b) (4) of magnesium stearate.

Route of administration: Inhalation.

Proposed clinical protocol:
This is a randomized double-blind, ,placebo controlled, parallel group, 40 center study, to assess the safety and tolerability of 28-days treatment with glycopyrronium (100 or 200 mcg daily) in 240 male and female patients aged 40 years and older with moderate to severe COPD.

Previous clinical experience:
Glycopyrronium bromide has long been marketed as an injectable muscarinic receptor antagonist used as a premedication during anesthesia, topically for hyperhidrosis and orally for the treatment of peptic ulcers. There was no reported experience by the inhaled route.

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

Studies reviewed within this submission:

Pharmacology

In vitro muscarinic receptor activity of glycopyrronium, No. RD-2007-00209, vol. 2, p 255.
Duration of action of muscarinic receptor antagonism in rat isolated trachea, No. RD-2006-02342, vol. 2, p 278.
Duration of action of glycopyrronium formulations on methacholine-induced bronchcoconstriction in anesthetized rabbits, No. 82, vol. 2, p 167.
Evaluation of tiotropium on methacholine-induced bronchcoconstriction in anesthetized rabbits, No. 95, vol. 2, p 186.
Evaluation of glycopyrronium, ipratropium and tiotropium on methacholine-induced bronchcoconstriction in anesthetized rabbits (supplement to report 95), No. 117, vol. 2, p 205.
Potency, selectivity, duration of action and potential side effects of glycopyrronium in Brown Norway rats, No. RD-2006-01999, vol. 2, p 240.
Antibronchoconstrictor and cardiovascular effects of inhaled glycopyrronium, ipratropium and tiotropium in rhesus monkeys, No. RD-2005-01557, vol. 2, p 212.

Safety Pharmacology

Cardiovascular
Antibronchoconstrictor, respiratory and cardiovascular effects of inhaled glycopyrronium, ipratropium and tiotropium in rhesus monkeys, No. RD-2005-01557, vol. 2, p 212.
Intravenous (bolus) telemetry study in dogs including sighting phase, R050129, vol. 2, p 343.
Electrophysiological studies in the isolated rabbit heart, No. 0618559, vol. 3. p 157.

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Respiratory
Antibronchoconstrictor respiratory and cardiovascular effects of inhaled glycopyrronium, ipratropium and tiotropium in rhesus monkeys, No. RD-2005-01557, vol. 2, p 212.

Toxicokinetics/Pharmacokinetics

Distribution
In vitro blood distribution and plasma protein binding of [^{14}C] glycopyrronium and its stability in the blood and plasma of rat, dog and human, No. DMPK-R0600252, vol. 2, p 288.
Metabolism
In vitro biotransformation of [^{14}C] in rat, dog and human liver and lung microsomes and in rat and human hepatocytes, No. DMK-R0600285, vol. 2, p 317.

Toxicology

Multi-dose toxicity
4-Week inhalation study in rats followed by a 2-week recovery, No. 848192, vol. 3, p 276
4-Week inhalation study in dogs followed by a 2-week recovery, No. 852441, vol. 9, p 276

Genetic toxicity
Reverse bacterial mutation assay, No.2250/12-D6171, vol. 11, p 1.
Chromosome Aberration assay in human lymphocytes, No.2250/13-D6172, vol. 11, p 56.
Micronucleus assay in rats, No.2250/14-D6172, vol. 11, p 106.

Studies not reviewed within this submission:

The following submitted reports were previously reviewed.
A 13-week interim toxicity report of a 26-Week inhalation toxicity study of the powder formulation in the rat with a 28-day recovery period (No. 0580297, vol.6, p 63). See review of L. Sancilio of IND 48,655; submission date, 8/29/06; review date 9/25/06. The 26 week report has not yet been submitted.
26-Week inhalation toxicity study with 1% (w/w) Mg stearate in lactose monohydrate blend in rats, No. 724353, vol. 12, p 1. See review of L. Pei of IND (b) (4) ,submission date, 427/00; review date 6/7/00.

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

In vitro M1, M2 and M3 muscarinic binding studies using the CHO-cell lines, glycopyrronium binds to all 3 receptors. In comparison with tiotropium, glycopyrronium was longer acting in its binding capacity. In the isolated rat tracheal strip preparation, glycopyrronium was longer acting than tiotropium but shorter than ipatropium in blocking the contraction response to the M3 agonist, bethanechol.
Antagonism of the methacholine induced bronchoconstriction and hypotension was determined in anesthetized rabbits, anesthetized Brown Norway rats and immobilized Rhesus monkeys. In rabbits, glycopyrronium was administered intratracheally as a dry powder (10 and 20 ug), aqueous solution (20 ug) and a controlled release formulation (10 ug). For reference, tiotropium (3 ug) and ipatropium (20 ug) were included in the study. All glycopyrronium preparations markedly inhibited (76%-93%) the bronchoconstriction response for 4- 6 hrs and only minimally reduced the hypotensive response (2%-6%). Both reference compounds blocked the bronchoconstriction (84%-95%); however, ipratropium inhibited the hypotensive response by 85% and tiotropium by 18% showing a difference between glycopyrronium and the two reference compounds. In a similar rabbit study, the ED50s for blocking the bronchoconstriction were 2.27 ug/kg for glycopyrronium, 1.41 ug/kg for ipratropium and 0.21 ug/kg for tiotropium and for inhibiting the hypotensive response, the ED50s were >20 ug/kg for glycopyrronium, > 20 ug/kg for ipratropium and 0.56 ug/kg for tiotropium showing a difference between tiotropium and glycopyrronium and ipotropium. In the Brown Norway rat study, the animals were sensitized to egg albumin. The response (bronchoprotection, salivation, hypotension and bradycardia) to intravenous methacholine was determined at 1, 6 and 24 hrs post dose. By the intratracheal route, glycopyrronium was 1.8 times more potent than tiotropium at 1 hr and less potent at 6 and 24 hr in blocking the methacholine induced bronchoconstriction. However, relative to it ability to block the methacholine-induced bronchoconstriction, glycopyrronium was less active than tiotropium and ipratropium at 1, 6 and 24 hr in inhibiting the salivation, hypotension and bradycardia response to methacholine indicating a less systemic and a more local effect. In immobilized rhesus monkeys, glycopyrronium at inhalation doses of 0.05, 0.15, 0.31 and 0.61 ug/kg inhibited the bronchoconstriction response to methacholine. Maximal effect occurred at 15 min post dose. By 225 min, the 0.05, 0.15, 0.31 ug/kg doses were minimally effective while at 285 min the inhibition by the 0.61 ug/kg dose was comparable to its maximal inhibition seen at 15 min.
In safety pharmacology studies, blood pressure, heart and respiratory rates were measured in the immobilized Rhesus monkey study. At inhalation doses up to 0.61 ug/kg, there were no effect on the blood pressure, heart and respiratory rates. In an intravenous study in dogs, there was 1 group of animals in a sighting phase receiving 0.01, 0.1 and 1 mg/kg, intravenously and in another group with telemetry received 0.01 and 0.1 mg/kg, intravenously. In the sighting group, clinical signs, EKG, body weight and food consumption were determined while in the telemetry group clinical signs, body weight, EKG, body weight, systolic and diastolic pressures were measured. In the sighting group, signs of anticholinergic activity and tremors were observed at the higher doses, i.e.

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mydriasis, dry mucous membrane, tachycardia and decreased food intake. Two animals in the HD lost weight. In the telemetry group, there was mydriasis, dry mucous membrane transient tachycardia, and a transient increased diastolic pressure. Electrophysiological studies in isolated perfused rabbit's heart revealed no changes at concentrations up to 30 uM.

2.6.2.2Primary pharmacodynamics

Mechanism of action:

In vitro muscarinic receptor activity of glycopyrronium, No. RD-2007-00209, vol. 2, p 255.

The binding affinity constants (pK_1), association rate (K_{on}) and dissociation rate (k_{off}) were determined for glycopyrronium (NVP-QAM254) and tiotropium (NVP-XQA024) on the M1, M2 and M3 CHO (Chinese hamster ovary cell membranes)-cell lines. The results are presented in the following table excerpted from the submission.

Table 3-3 Kinetic parameters of NVP-QAM254-DC-1 and NVP-XQA024-DC-7 at the M_1 receptor

	k_{on} ($M^{-1}min^{-1}$)	K_{off} (min^{-1})	Kinetic pK_d	Equilibirum pK_i
NVP-QAM254-DC-1	$1.16 \pm 0.14 \times 10^8$	0.05 ± 0.002	9.46 ± 0.09	9.60 ± 0.03
NVP-XQA024-DC-7	$3.37 \pm 0.20 \times 10^8$	0.0193 ± 0.002	10.23 ± 0.02	10.34 ± 0.03

Data are mean ± s.e.mean for the least separate experiments

Table 3-4 Kinetic parameters of NVP-QAM254-DC-1 and NVP-XQA024-DC-7 at the M_2 receptor

	k_{on} ($M^{-1}min^{-1}$)	K_{off} (min^{-1})	Kinetic pK_d	Equilibirum pK_i
NVP-QAM254-DC-1	$6.03 \pm 1 \times 10^8$	0.646 ± 0.04	8.72 ± 0.09	8.70 ± 0.04
NVP-XQA024-DC-7	$8.03 \pm 1 \times 10^8$	0.064 ± 0.006	10.13 ± 0.08	10.05 ± 0.05

Data are mean ± s.e.mean for the least four separate experiments

Table 3-5 Kinetic parameters of NVP-QAM254-DC-1 and NVP-XQA024-DC-7 at the M_3 receptor

	k_{on} ($M^{-1}min^{-1}$)	K_{off} (min^{-1})	Kinetic pK_d	Equilibirum pK_i
NVP-QAM254-DC-1	$9 \pm 0.8 \times 10^7$	0.07 ± 0.004	9.34 ± 0.04	9.47 ± 0.02
NVP-XQA024-DC-7	$2.29 \pm 0.05 \times 10^8$	0.015 ± 0.002	10.20 ± 0.03	10.37 ± 0.04

Data are mean ± s.e.mean for the least four separate experiments

Conclusion: Glycopyrronium like the reference, tiotropium, shows a high affinity for the M1, M2 and M3 muscarinic receptors. Based on the dissociation constants (K_{off}), glycopyrronium shows a long duration of binding to the three receptors.

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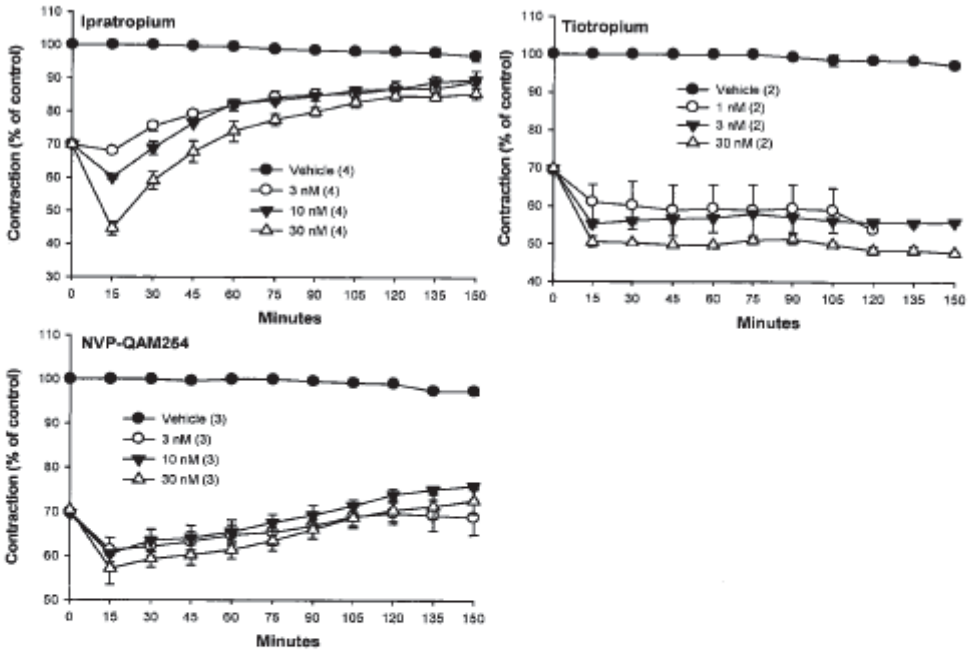
Duration of action of muscarinic receptor antagonism in rat isolated trachea, No. RD-2006-02342, vol. 2, p 278.

In vitro studies were conducted on isolated tracheas from male Norway Brown rats. The test compound was added to the bath when 80 % of the maximum response to the M_3, agonist, bethanechol, was achieved. When the tissue relaxed by 30% of its response, the bath was washed, and fresh bethanechol was added. This was repeated at 15 minutes intervals over 150 minutes. Tiotropium and ipratropium served as reference compounds. Glycopyrronium was labeled as QVP-QAM254. The results are shown in the following figures and table excerpted from the submission.

Figure 3-1 Duration of action of muscarinic receptor antagonists in rat trachea



Shown are the changes in contractile response to bethanechol (30 µM) following treatment with ipratropium, tiotropium and NVP-QAM254 to achieve 30 % blockade of the response to bethanechol and repeated washing at 15 minute intervals. Means ± sem (range for n = 2) are presented for the numbers of individual experiments indicated in parenthesis.

Table 3-1 The duration of action of muscarinic receptor antagonists

Compound	Initial rate of response recovery (slope)[1]	Maximum return in 150 minutes (%)[2]
NVP-QAM254 (10 nM)	0.120	39
Ipratropium (30 nM)	0.770	74
Tiotropium (10 nM)	0.048	-3.3

[1] The rate of change in tension during the period 15-45 minutes after the first washout.

[2] The degree of reversal at the 150 minute time point.

Data are derived from mean curves in Figure 3-1.

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Conclusion: At comparable doses, Glycopyrronium was longer acting than ipratropium and less than tiotropium based on the maximum the return of the tracheal response in 150 min to the M_3 agonist, bethanechol.

Drug activity related to proposed indication:

Duration of action of glycopyrronium formulations on methacholine-induced bronchoconstriction in anesthetized rabbits, No. 82, vol. 2, p 167.
Evaluation of tiotropium on methacholine-induced bronchoconstriction in anesthetized rabbits, No. 95, vol. 2, p 186.

To anesthetized rabbits, compounds were administered intratracheally 30 min prior to the intravenous administration of methacholine. Glycopyrronium was administered in different formulations while tiotropium and ipatropium were administered as an aquous solution. Thereafter, methacholine was administered intravenously every 30 min until the response to the cholinergic agonist had returned. The results are summarized in the following table excerpted from the submission.

Table 1. Anaesthetized rabbits: data showing the maximum change and duration on the meth (10 µg kg-1, i.v.) evoked response in the presence of different glycopyrrolate, tiotropium and ipratropium formulations (aq – aqueous, dp – dry powder and cr – controlled release. Glycopy and ipatropium data extracted from study number 82).

	Max. Inhibition of hypotension (%)	Max. % Change in Bronchoconstriction	Duration Action (Hrs)
Ipatropium (aq) 20 µg	18	95	6
GP (aq) 20µg	2	92	6
Tiotropium (aq) 3 µg	85	84	5.5
GP (dp) 20µg	4	89	6
GP (dp) 10 µg	4	93	4
GP (cr) 10 µg	6	76	6

The hypotension produced by methacholine was unaffected by glycopyrronium and inhibited by ipratropium and tiotropium.

Conclusion: Various formulations of glycopyrronium administered intratracheally blocked the bronchoconstriction of intravenous methacholine and unlike tiotropium did

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not significantly affect the cardiovascular effects of methacholine indicating a local effect in contrast to a weak systemic effect.

Evaluation of glycopyrronium, ipratropium and tiotropium on methacholine-induced bronchcoconstriction in anesthetized rabbits (supplement to report 95).

The intratracheal ED50s for glycopyrronium, ipratropium and tiotropium for inhibiting the intravenous methacholine-induced bronchoconstriction and bradycardia are presented in the following table.

Compound	ED50, ug/kg , intratracheally	
	Inhibition of Bronchoconstriction	Inhibition of Bradycardia
Glycopyrronium	2.27	>20
Ipratropium	1.41	>20
Tiotropium	0.21	0.56

Conclusion: Glycopyrronium, ipratropium and tiotropium in decreasing order of ED50s blocked methacholine-induced bronchoconstriction, and only tiotropium significantly blocked the methacholine-induced bradycardia.

Potency, selectivity, duration of action and potential side effects of glycopyrronium in Brown Norway rats, No. RD-2006-01999, vol. 2, p 240.

Groups of 3- 5 male Brown Norway rats (200-300g) were sensitized to egg albumin. After 21 days, under anesthesia, glycopyrronium was administered intratracheally at doses ranging from 0.1 to 30 ug/kg to determine the ED50s for inhibiting the responses to 30 ug/kg of methacholine intravenously given 1, 6 and 24 hr following the administration of glycopyrronium and the reference, tiotropium. The results are shown in the following table excerpted from the submission.

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Table 3-1 Potency and therapeutic indexes for NVP-NVA237 and tiotropium given intratracheally in the Brown Norway rat

		Bronchoprotection	Salivation		Hypotension		Bradycardia	
		ED_{50} ($\mu g\ kg^{-1}$)	ED_{50} ($\mu g\ kg^{-1}$)	Therapeutic index	ED_{50} ($\mu g\ kg^{-1}$)	Therapeutic index	ED_{50} ($\mu g\ kg^{-1}$)	Therapeutic index
1 hour								
	NVP-NVA237	0.31 ± 0.02	2.73 ± 0.41	8.8	6.04 ± 1.39	19.5	8.70 ± 1.72	28.0
	Tiotropium	0.55 ± 0.03	1.95 ± 0.69	3.5	0.83 ± 0.03	1.5	2.32 ± 0.09	4.2
6 hours								
	NVP-NVA237	0.41 ± 0.03	11.73 ± 4.53	28.6	> 100	> 250	> 100	> 250
	Tiotropium	0.28 ± 0.01	1.92 ± 0.26	6.8	4.56 ± 1.49	16.3	5.45 ± 0.20	19.4
24 hours								
	NVP-NVA237	1.20 ± 0.41	46.12 ± 15.65	38.4	> 100	> 75	> 100	> 75
	Tiotropium	0.14 ± 0.01	2.83 ± 0.32	20.2	9.89 ± 2.87	70.6	4.67 ± 1.54	33.3

Results are expressed as mean ± s.e.m. of the number of animals presented in (Figure 3-1), (Figure 3-2) and (Figure 3-3). ED_{50} values were calculated by fitting the drug's inhibitory dose-response obtained against a dose of 30 $\mu g\ kg^{-1}$ of methacholine. Therapeutic indexes were calculated against the ED_{50} values for the inhibition of methacholine-induced bronchoconstriction (bronchoprotection).

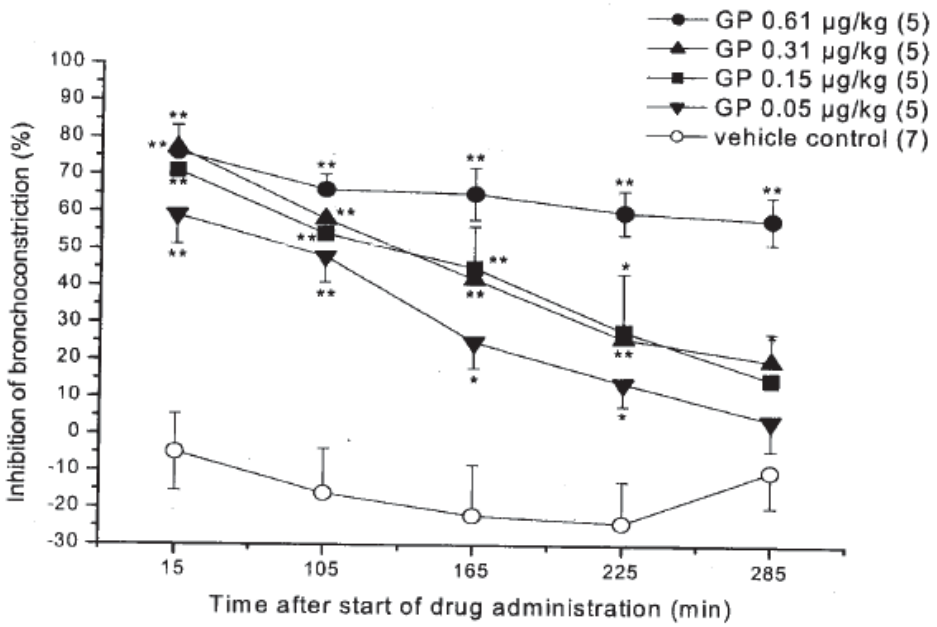
Conclusion: Glycopyrronium was 1.8 times more potent than tiotropium in blocking the methacholine induced bronchoconstriction at 1 hr post dose and was less potent than

tiotropium at 6 and 24 hrs. However, glycopyrronium was less potent at 1, 6 and 24 hrs than tiotropium in blocking the salivation, hypotension and bradycardia effects of methacholine.

Antibronchoconstrictor cardiovascular and respiratory effects of inhaled glycopyrronium, ipratropium and tiotropium in rhesus monkeys, No RD-2005-01557, vol. 2, p 212.

Glycopyrronium was administered by an endotracheal tube to immobilized male rhesus monkeys and anesthesia maintained with thiopental. Each treated group consisted of 5 animals, and the control group contained 7 animals. Glycopyrronium was administered by inhalation over a 10 min period 15 min prior to the intratracheal administration of methacholine. Subsequent administration of methacholine were 105, 165 225 and 285 min following the administration of glycopyrronium. The data for glycopyrronium are presented in the following figure excerpted from the submission. The report indicated that the doses stated were the deposited doses assuming that the deposition factor was 30%.

Figure 1-1 **Effect of different doses of glycopyrrolate on methacholine-induced bronchoconstriction in the Rhesus monkey**



Conclusion: Glycopyrronium produced a dose related inhibition of the bronchoconstriction response to methacholine in the Rhesus monkey.

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2.6.2.3Secondary pharmacodynamics: No report submitted.

2.6.2.4Safety pharmacology

Neurological effects: No report submitted.

Cardiovascular effects:

Antibronchoconstrictor, cardiovascular and respiratory effects of inhaled glycopyrronium, ipratropium and tiotropium in rhesus monkeys, No RD-2005-01557, vol. 2, p 212.

Glycopyrronium was administered by an endotracheal tube to immobilized male rhesus monkeys and anesthesia maintained with thiopental. Each treated group consisted of 5 animals, and the control group contained 7 animals. Glycopyrronium was administered by inhalation doses of 0.05, 0.15, 0.31 and 0.61 ug/kg over a 10 min period 15 min prior to the administration of methacholine. The report indicated that the doses stated were the deposited doses assuming that the deposition factor was 30%. Non-invasive measurement of the blood pressure and heart rate were monitored periodically during the 285 min duration of the study.
Conclusion: Glycopyrronium did not affect the blood pressure and heart rate of immobilized rhesus monkeys.

Intravenous (bolus) telemetry study in dogs including sighting phase, R050129, vol. 2, p 343.

Two groups of 4 male beagle dogs (7.7-13.4 kg) were used in the study, a sighting group and a telemetry group. The sighting group was for observing clinical signs, body weight changes and food consumption and changes in EKG following the single intravenous administration of 0.01, 0.1 and 1 mg/kg. The vehicle (2ml/kg) was 5% glucose for both groups. There was an 8 day washout period between the 0.01 and 1 mg/kg doses. The animals were observed for clinical signs, and EKGs were recorded once prior to and at 0.25, 0.5, and 1 and 4 hr after all dose levels and at 8 and 24 hr following the administration of 0.1 and 1 mg/kg.
In the telemetry group, single intravenous doses of 0.01 and 0.1 mg/kg were administered with a 7-day wash out period between each dosage. Clinical signs, body weight, heart rate, systolic and diastolic arterial blood pressure, EKG intervals and morphology and core body temperature were recorded. Each animal served as its own control. At 1 hr prior to intravenous administration, the EKG, arterial blood pressure were recorded for 2 min every 5 minutes up to 2 hr post dose. Then the recordings were for 2 min every 15 min up to a total recording period of 22 hr.

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Results

In the sighting group, intravenous doses of 0.1 and 1 mg/kg produced a slight decrease in food intake, mydriasis, tremor, dry mucous mouth membranes up to 3 days post dose. In two animals at 1 mg/kg, there was a body weight loss of 0.2 and 0.3 kg. At 0.01 mg/kg, only dry mucous mouth membranes were observed on the first day. At 0.1 and 1 mg/kg, severe tachycardia up to 294 bpm occurred along with secondary shortening of the P, PQ, QRS and/or QT/QT_c.
In the telemetry group, mydriasis persisted overnight and dry mucous mouth membranes were seen up to 6 hr post 0.1 mg/kg, intravenously and only directly after dosing with 0.01 mg/kg, intravenously. A transiently tachycardia of 221 and 231 bpm occurred at 5-10 min post dose at 0.01 and 0.1 mg/kg. Mean diastolic arterial blood pressure increased +20 mm Hg in 2/4 animals from 0.25 hr to 2.5 hr post 0.1 mg/kg administration. No other effects were noted.
Conclusion: In both dog studies, the changes seen after single intravenous doses up to 1 mg/kg of glycopyrronium produced no significant cardiovascular effects other than tachycardia.

Electrophysiological studies in the isolated rabbit heart, No. 0618559, vol. 3. p 157.

The cardiac electrophysiological effects of glycopyrronium were determined in 6 isolated Langendorf perfused hearts. The hearts were exposed for 30 min. to 0.3, 0.9, 3, 9, and 30 uM of glycopyrronium. The following parameters were measured: automaticity and escape cycle length, threshold stimulation current, coronary perfusion rate, ectopic activity, left ventricular septal and epicardial monophasic action potential duration at 30, 60 and 90% of repolarization, conduction time, triangulation, reverse use-potential, instability and dispersion of repolarization.
Conclusion: Glycopyrronium did not induce any cardiac electrophysiological changes at concentrations up to 30 uM.

Pulmonary effects:

Antibronchoconstrictor respiratory and cardiovascular effects of inhaled glycopyrronium, ipratropium and tiotropium in rhesus monkeys.

Glycopyrronium was administered by an endotracheal tube to immobilized male rhesus monkeys and anesthesia maintained with thiopental. Each treated group consisted of 5 animals, and the control group contained 7 animals. Glycopyrronium was administered by inhalation doses of 0.05, 0.15, 0.31 and 0.61 ug/kg over a 10 min period 15 min prior to the initial administration of methacholine. The report indicated that the doses stated were the deposited doses assuming that the deposition factor was 30%. Non-invasive measurement of the respiratory rate was monitored periodically during the 285 min duration of the study.
Conclusion: Glycopyrronium did not affect the respiratory rate.

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Renal effects: No report submitted.

Gastrointestinal effects: No report submitted.

Abuse liability: NA.

Other: NA

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1Brief summary

In 4-week inhalation toxicity studies in rats and dogs, the daily doses were 97, 617 and 4,240 ug/kg in the rat and 32, 96 and 317 ug/kg in the dog. In the rat, the AUCs were similar in both sexes, dose related and not dose proportional, and there was no accumulation. In dogs, the AUCs were similar in both sexes, dose related, dose proportional and accumulation did not occur. In in vitro stability studies with blood and plasma from rat dog and humans, glycopyrronium was more stable in blood than in plasma with respect to time and binding to the plasma proteins was low (27%-32%). Metabolism studies were conducted in vitro with rat, dog and human liver and lung microsomes and in rat and human hepatocytes. The decreasing order of metabolism in liver microsomes was the rat, dog and humans; 8, 7 and 4 metabolites were detected from rat, dog and human microsomes, respectively. Only 1 metabolite was detected in the dog and human lung microsomes. In the hepatocyte study, rats metabolized glycopyrronium to a greater degree than humans.

2.6.4.2Methods of Analysis

Analysis was determined by LC-MS/MALES; the lower limit of quantitation was 0.05 to 0.10 ng/ml.

2.6.4.3Absorption

Study title: **4-Week inhalation study in rats**

The inhalation doses were 97 (LD), 617 (MD) and 4,240 (HD) ug/kg. AUCs were determined on days 1 and 27. The results are shown in the following table excerpted from the submission.

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Dose level	Males AUC8h (ng·h/ml)			Females AUC8h (ng·h/ml)		
	Day 28	Day 1	Ratio	Day 28	Day 1	Ratio
low	43.0	21.0	**2.0**	30.1	22.7	**1.3**
medium	45.4	40.5	**1.1**	52.6	52.3	**1.0**
high	250.0	153.1	**1.6**	186.8	180.4	**1.0**

Conclusion: In a 4-week inhalation study in rats, the AUCs were similar in both sexes, dose related and not dose proportional and there was no accumulation.

Study title: **4-Week inhalation study in dogs**

The inhalation doses were 32 (Group 3), 96 (Group 4) and 317 (Group 5) ug/kg. AUCs were determined on days 1 and 27. The results are shown in the following table excerpted from the submission.

Mean C_{max}, T_{max} and $AUC_{(0-23.5h)}$ values for males and females on Days 1 and 27

Day	1						27					
Sex	Male			Female			Male			Female		
Group	3	4	5	3	4	5	3	4	5	3	4	5
C_{max} (ng/mL)	2.5	8.9	26.2	2.6	9	25.0	2.9	9.7	39.1	4.4	14.7	49.9
T_{max} (hours)	0	0	0	0	0	0	0	0	0	0	0	0
$AUC_{(0-6.5h)}$ (ng.h/mL)	4.5	13.1	38.7	4.5	11.8	35.5	5.7	15.6	64.5	6.9	20.8	70

Conclusion: In a 4-week inhalation study in dogs, the AUCs were similar in sexes, dose related, dose proportional and accumulation did not occur.

2.6.4.4Distribution

In vitro blood distribution and plasma protein binding of [^{14}C] glycopyrronium and its stability in the blood and plasma of rat, dog and human, No. DMPK-R0600252, vol. 2, p 288.

Blood was taken from male rats, dogs and humans. Distribution was determined in heparinized blood, protein binding in plasma and stability at 1, 3, 7, 24 and 30 hr in plasma and 0, 12, 3 ann7 hrs in blood. Levels of glycopyrronium were determined by LC/MS.
At concentrations of 10, 100, 1,000 and 10,000 ng/ml, distribution of glycopyrronium in the blood was 61-63% in rat, 49-50% in dog and 53-54% in human.

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Stability studies in plasma and blood decreased with time. The greatest change took place after 7 hr in plasma and after 3 hr in blood as seen in the following table.

Species/ Conc., ng/ml	Range of Stability (%)	
	Plasma	Blood
Rat, 100	106-71	97-69
Dog, 100	105-64	98-92
Human, 100	108-53	90-97[a]
Human, 1000	93-51	90-88

[a] Stability increased with time

Plasma protein binding in rat, dog and humans is presented in the following table excerpted from the submission.

Rat				
Nominal concentration [ng/mL]	Actual concentration		Bound fraction [%]	Unbound fraction [%]
	Plasma [ng/mL]	Ultrafiltrate [ng/mL]		Mean ± SD
10000	9745	7094	27.2	72.8 ± 0.4
1000	984	714	27.4	72.6 ± 0.9
100	103	74.3	27.7	72.3 ± 1.3
10	9.93	6.95	30.0	70.0 ± 0.3

Dog				
Nominal concentration [ng/mL]	Actual concentration		Bound fraction [%]	Unbound fraction [%]
	Plasma [ng/mL]	Ultrafiltrate [ng/mL]		Mean ± SD
10000	10147	7611	25.0	75.0 ± 0.5
1000	1020	746	26.9	73.1 ± 0.9
100	107	75.8	28.9	71.1 ± 0.2
10	10.5	7.45	28.9	71.1 ± 2.8

Human 1				
Nominal concentration [ng/mL]	Actual concentration		Bound fraction [%]	Unbound fraction [%]
	Plasma [ng/mL]	Ultrafiltrate [ng/mL]		Mean ± SD
10000	10321	7507	27.3	72.7 ± 1.3
1000	1006	688	31.7	68.3 ± 0.4
100	105	69.8	33.3	66.7 ± 1.1
10	10.1	6.53	35.6	64.4 ± 0.4

In rat, dog and human plasma, only 25-35% was bound to plasma proteins. Further studies with human plasma show that 16-22% of the plasma protein binding was bound to albumin and very little were bound to α_1 acid glycoprotein.

The distribution in blood in decreasing order was rat, dog and human. The stability of glycopyrronium in plasma and blood decreased with time. Plasma protein binding in decreasing order was rat, dogs and humans.

Conclusion: In rats, dogs and humans, glycopyrronium was more stable in blood than in plasma and protein binding was equally low, 27-32%.

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2.6.4.5Metabolism

In vitro biotransformation of [^{14}C] in rat, dog and human liver and lung microsomes and in rat and human hepatocytes, No. DMK-R0600285, vol. 2, p 317.

Glycopyrronium was incubated with liver and lung microsomes from rats, dogs and humans and from hepatocytes from rats and humans. Incubation times were 60 min for microsomes at 5 uM concentration and 3 and 6 hr at 10 uM for hepatocytes. Analysis was by HPLC and LC-MS instrumentations. The results are presented in the following tables excerpted from the submission.

Metabolite or compound [No]	HPLC rt [min]	Proportion of radioactivity [% of total]						
			Liver microsomes			Lung microsomes		
		Control *	Rat	Dog	Human	Rat	Dog	Human
M1	31.9		2.0					
M9	32.4	0.4		0.8	0.9		0.9	1.1
M2	35.1		1.3					
M3	38.9		8.4	0.5				
M4	39.1			0.4	0.3			
M5	44.4		16.3	1.4	2.4			
M6	45.7		10.9	1.2				
M7	49.6		23.6	3.0	0.7			
M8	50.5		24.2	0.9				
NVA237	59.6	93.9	5.9	87.9	90.7	95.8	95.0	94.6

*, control incubation without microsomes;
Blank cell, not detected.

Hepatocytes

Compound/ metabolite	HPLC rt # [min]	Proportion of radioactivity [% of total]				
		Control 6 h*	Rat 3 h	Rat 6 h	Human 3 h	Human 6 h
M9	32.0	2.5	0.8		0.4	0.5
M1	35.0		5.9	6.7		
M2	38.5		0.7	1.7		
M3	42.6		4.7	7.6	0.8	3.1
M4	43.2			0.6	1.4	3.2
M5	48.1		8.4	11.3	4.2	10.1
M6	49.2		5.5	8.8	1.5	4.8
M7	51.7		7.3	9.6	1.9	4.9
M8	52.5		7.8	12.1		
NVA237	63.0	93.4	50.1	29.2	84.2	65.0

*, control incubation without hepatocytes;
#, rt [min] differing from Table 7-2; peak allocation was confirmed by LC-MS and co-chromatography;
Blank cell, not detected.

Eight metabolites were identified from the liver microsomes study. The decreasing order of the number of metabolites based on radioactivity was rat, dog and humans. In the lung

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microsome study, only 1 metabolite (M9) was identified in the dog and human. Most of the radioactivity was the parent compound, glycopyrronium, in the microsomes and hepatocytes in all species. In the rat and human hepatocyte study, a higher rate of metabolism was seen in the rat as compared to the human,

Conclusion: In vitro studies with liver microsomes from rats, dogs and humans, extensive metabolism was observed in rats in comparison to the dog and human; extensive metabolism also occurred with the rat hepatocytes. With dog and human lung microsomes, little metabolism occurred and none in the rat.

2.6.4.6Excretion: No report submitted.

2.6.4.7Pharmacokinetic drug interactions: No report submitted.

2.6.4.8 Other Pharmacokinetic Studies: None submitted.

2.6.4.9Discussion and Conclusions

In the toxicokinetics of the 4-week inhalation studies in rats and dogs, both studies show that there was no difference between sexes, there was a dose response and accumulation did not occur. However, in the dog, there was dose proportionality and not in the rat. In stability studies with plasma and blood from rats, dogs and humans, glycopyrronium was more stable in blood than in rats with respect to time of exposure. Plasma protein was equally low in rats, dogs and humans. In human plasma, binding was bound to albumin. In vitro metabolism with rat, dog and human liver and lung microsomes, show that the rat liver microsomes metabolizes glycopyrronium to a greater degree than dog and human microsomes; 8 metabolites were detected. Only 1 metabolite was detected in the dog and human from lung microsome. With hepatocytes, the rat metabolizes glycopyrronium to a greater degree than human with respect to the number (8 vs 6) and proportion of metabolites (rat, 50%-71%; human, 16%-35%).

2.6.4.10 Tables and figures to include comparative TK summary: None submitted.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY: No tables submitted.

2.6.6 TOXICOLOGY

2.6.6.1Overall toxicology summary

General toxicology:

Four week inhalation toxicity studies were conducted in rats and dogs with the powder formulation containing lactose and magnesium stearate. In rats the doses were 97, 617 and 4,240 ug/kg. Clinical sign was mydriasis in all treated animals, characteristic of the

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anticholinergic pharmacological action of glycopyrronium. Reversible decreased body weight gain was seen in the MD and HD males and HD females. Immunologically, there was no effect on the antibody response to sheep red blood cells, and no change in the leucocyte subset population. The AUCs in both sexes were similar, and there was no accumulation. All the changes were completely or partially reversible. Histologically, at the MD and/or HD, there were changes in the nasal cavity, parotid and lacrimal glands and larynx, and at all doses, there was porphyrin deposition in the Hardarian glands. These were the results of typical anticholinergic activity. No pathology was observed in the lower respiratory tract. Since this marketed oral product will be administered by the inhaled route, focus on its toxicity was on the lower respiratory tract. Therefore, the inhaled lower respiratory tract NOAEL was 4,240 ug/kg.
In dogs, the inhaled doses of glycopyrronium in the formulation were 32, 96 and 317 ug/kg. Reversible corneal opacity and inhibition of the pupil reaction was observed at the HD. There was transient tachycardia accompanied by transient changes in the EKG. The AUCs were similar in both sexes, dose related and dose proportional and accumulation did not occur. Histologically, The HD males showed hepatocellular hypertrophy and acinar hypertrophy of the sublingual and lacrimal glands. In the HD in males and in all doses in females, there were changes in the muzzle (snout) area of the skin. Other than hepatocellular hypertrophy, these findings were the results of typical anticholinergic activity. Males also showed changes in the prostate, testes and epididymidis which was attributed to the immaturity of the dogs. No pathology was observed in the lower respiratory tract. Since this marketed oral product will be administered by the inhaled route, focus on its toxicity was on the lower respiratory tract. Therefore, the inhaled lower respiratory tract NOAEL was 317 ug/kg.

Genetic toxicology:

Glycopyrronium was negative in the Reverse Bacterial Mutation and Human Lymphocyte Chromosomal Aberration assays and in the Micronucleus assay in rats. All were valid assays as the maximal acceptable concentration (5000 ug/kg/ml) was tested in the Reverse Bacterial Mutation assay, the maximal acceptable concentration (10 uM) was tested in the Human Lymphocyte Chromosomal Aberration assay and a toxic oral dose (1000 mg/kg) was tested in the Micronucleus assay in rats.

2.66.2 Single-dose toxicity: No reports submitted.

2.6.6.3Repeat-dose toxicity

Study title: 4-Week inhalation study in rats followed by a 2-week recovery

Key study findings:

- Decreased body weight gained occurred in the MD and HD males and in the HD females. This effect was reversible.
- Histopathology occurred in the nasal cavity, larynx, parotid, Hardarian and lacrimal glands with partial recovery. These findings were the results of typical anticholinergic activity.

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- The inhalation NOAEL regarding the lower respiratory tract was 4.24 mg/kg.

Study no.: No. 848192
Volume # and page #: 3, p 276
Conducting laboratory and location: (b) (4)
Date of study initiation: 7/09/03
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity: VRD030709MRA, 97.1%

Methods

Doses: 0 (Air), 0 (vehicle, 0.25% magnesium stearate + 99.75 % lactose), LD, 0.097 mg/kg; MD, 0.617 mg/kg; HD, 4.24 mg/kg

Species/strain: Male and Female Wistar rat, HanBrl outbred (SPF)

	No. of animals/sex/group		
Group	Main Study	Recovery Group	Pharmacokinetics
0 (Air)	12	8	
0(Vehicle),	12	8	3
LD	12		8
MD	12		8
HD	12	8	

Route, formulation, volume: Inhalation (snout only), 5% glycopyrronium, 0.25% magnesium stearate, 94.75 % lactose.

Particle size:

Achieved Dose mg/kg	MMAD um
LD, 0.097	1.43-1.83
MD, 0.617	1.79-2.53
HD, 4.24	2.50-3.09

Age: Males, 7 weeks; females, 9 weeks

Weight: mean males 184 g; females, 210-211 g

Sampling times: Days 1 and 28: -2 hr, 0.5, 1, 2 and 6 hr post dose.

Unique study design or methodology (if any): None.

Observations

Clinical signs: Prior to and twice weekly post dose.
Body weights: Day 1 and twice weekly.
Food and water consumption: Day 1 and twice weekly.
Ophthalmoscopy: Day 1 and week 4.
Hematology: During weeks 4 and 6.
Clinical chemistry: During weeks 4 and 6.
Urinalysis: During weeks 4 and 6.
Immunology: Week 4, Serum was tested for IgM antibody against sheep red blood cells from C (air) and HD, and the distribution of the leucocyte population in all groups.
Gross pathology: Necropsy.
Organ weights (specify organs weighed if not in histopath table):

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Histopathology: specify groups examined, special stains, etc
Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)
All the C (air), C (vehicle) and HD tissues were examined. A number of tissues in the LD and MD was also examined.

Results:

Mortality: 1 Male C (air); 1 LD female; 1 HD male. The control male was found dead in the restraining cage. The HD male was found dead in the cage; no clinical signs were seen prior to its death. The female was found dead on day 20 in the restraining tube; on day 18, the animal was injured after falling from the cage. Deaths were not due to treatment.

Clinical signs: Mydriasis was seen in all treated animals.

Body weight gain: Male, MD, -24%; HD, -54%; Recovery period: HD, +60%
Female, HD, -86%; Recovery period: HD, +77%
Food consumption: No change.

Water consumption: No change.

Ophthalmoscopy: No change.

Hematology: No change.

Clinical chemistry: Females HD, Phosphate, -32%; Recovery period, no change.

Urinalysis: No change.

Immunology: Antibody response to sheep red blood cells: No change.
Distribution of the leucocyte subset population: No change.

Toxicokinetics: The results are shown in the following table.

Dose level	Males AUC8h (ng·h/ml)			Females AUC8h (ng·h/ml)		
	Day 28	Day 1	**Ratio**	Day 28	Day 1	**Ratio**
low	43.0	21.0	**2.0**	30.1	22.7	**1.3**
medium	45.4	40.5	**1.1**	52.6	52.3	**1.0**
high	250.0	153.1	**1.6**	186.8	180.4	**1.0**

Gross pathology: None.

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Organ weights: Male HD, mandibular salivary gland, absolute +20%, relative. +17%; recovery period, no increase in organ weight.

Histopathology:

The results are shown in the following tables.

Organ Observation	Incidence /Mean Severity Score (1, 2, 3)				
	Males				
	C(air)	C (veh.)	LD	MD	HD
Nasal Cavity, Level 3					
Hyaline inclusions[a]	0/12	0/12	0/12	5/12 1.0	10/12 1.1
Nasal Cavity, Level 4					
Hyaline inclusions[a]	0/12	0/12	0/12	0/12	6/12 1.3
Larynx, Level 5					
Squamous metaplasia	0/12	0/5	0/11	0/11	4/12 1.0
Larynx, Level 6					
Squamous metaplasia[a]	0/11	0/11	0/10	0/12	8/12 1.3
Parotid Gland					
Acinar hypertrophy[a]	0/12	0/12	0/12	9/12 1.8	11/12 2.2
Hardarian Gland					
Porphyrin deposition[a]	5/12 1.0	4/12 1.0	8/12 1.1	9/12 1.0	11/12 1.1
Hypertrophy	0/12	0/12	1/12	4/12	6/12
Lacrimal Glands					
Atrophy[a]	0/12	0/12	0/12	0/12	3/12 1.7

[a] Partial recovery

Organ Observation	Incidence /Mean Severity Score (1, 2, 3)				
	Females				
	C(air)	C (veh.)	LD	MD	HD
Nasal Cavity, Level 3					
Hyaline inclusions[a]	0/12	0/12	0/12	8/12 1.0	12/12 1.14
Nasal Cavity, Level 4					
Hyaline inclusions[a]	0/12	1/12	0/12	6/12 1.2	11/12 1.3
Parotid Gland					
Acinar hypertrophy[a]	1/12 1.0	0/12	2/12	5/12 1.0	12/12 1.8
Hardarian Glands					
Porphyrin deposition[al]	3/12 1.0	5/12 1.0	11/12 1.0	12/12 1.0	7/12 1.0
Hypertrophy	0/12	0/12	0/12	2/12	5/12
Lacrimal Glands					
Atrophy	0/12	0/12	0/12	1/10 1.0	6/12 1.5

[a] Partial recovery

Conclusion: Other than typical anticholinergic effects and local nasal changes, no toxicity was observed in the lower respiratory tract. The NOAEL was the highest test dose, 4.24 mg/kg based on lower respiratory effects.

Reference ID: 3824703

Study title: **4-Week inhalation study in dogs followed by a 2-week recovery period**

Key study findings:

- Hepatocellular hypertrophy occurred in HD males.
- Corneal opacity was seen at the HD males and females.
- Male reproductive changes were attributed to immaturity of the dogs.
- Targeted organs were the lacrimal glands, skin and sublingual glands. The changes were typical of anticholinergic activity.
- The inhalation NOAEL with respect to the lower respiratory tract was 0.317 mg/kg.

Study no.: No. 852241
Volume # and page #: 9, p 1
Conducting laboratory and location: (b) (4)
Date of study initiation: 3/19/04
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity: VRD040113MRA, 97.1%

Methods

Doses (estimated): 0 (Air), 0 (vehicle, 0.25% magnesium stearate, 99.75 % lactose). LD, 0.032 mg/kg; MD, 0.096 mg/kg; HD, 0.317 mg/kg
Species/strain: Beagle dogs

Group	Main Study		Recovery Group	
	M	F	M	F
0 (Air)	3	3	2	2
0(vehicle),	3	3	2	2
LD	3	3		
MD	3	3		
HD	3	3	2	2

Route, formulation: Inhalation (face mask, duration, 28-30 min), 5% glycopyrronium, 0.25% magnesium stearate, 94.75 % lactose.
Particle size: Achieved Dose MMAD
Gravimetric mg/kg um

C (vehicle)	2.45
LD, 0.032	2.31
MD, 0.096	2.20
HD, 0.317	2.31

Age: 6-8 months
Weight: 7.3-10.3 kg
Sampling times: Days 1 and week 4: pre-dose. Immediately after, 0.5, 1, 2 and 6hr post dose.
Unique study design or methodology (if any): None.

Reference ID: 3824703

Observation and Times:
Mortality: Daily.
Clinical signs: Prior to and twice weekly post dose.
Body weights: Daily.
Food and consumption: Daily.
Ophthalmoscopy: Pre-dosing and weeks 4 and 6 (recovery group).
Pupil reaction: Pre- dosing and weeks 4 and 6 (recovery group).
EKG: Pre- dosing and weeks 3 and 6 (recovery group).
Respiratory function (rate, tidal vol. and minute vol.): Pre- dosing and weeks 4, during treatment period, before and immediately after exposure, within 15 min and week 6 (recovery group).
Hematology: Pre-dosing and weeks 4 and 6 (recovery group).
Clinical chemistry: Pre-dosing and weeks 4 and 6 (recovery group).
Urinalysis: Pre-dosing and weeks 4 and 6 (recovery group).
Gross pathology: Necropsy.
Organ weights: See histopath table:
Histopathology: specify groups examined, special stains, etc
Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)
All the C (air), C (vehicle) and HD tissues were examined. A number of tissues in the LD and MD was also examined.

Results:

Mortality: None.

Clinical signs: None.

Body weight: No change.

Food and consumption: No change.

Ophthalmoscopy: Corneal opacity, male, C (veh.), 0/3; HD 1/3; female, C (veh.), 0/3; HD 2/3. Recovery period: no corneal opacity.

Pupil reaction: Males, C 0/6; HD, 1/3 and females, C, 06; HD. 2/6 inhibition. Response returned at 24 hr post dose.

Heart Rate: Weeks 1 and 3, Males and females, MD and HD, tachycardia occurring immediately post treatment, which after 4 hr, the heart rates were normal.

EKG:
Males
Week 1, HD, decrease in P-Q interval occurring immediately post treatment, which after 4 hr, the P-Q interval was normal.

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Week 3, Males, MD and HD, decrease in P-Q interval occurring immediately post treatment, which after 4 hr, the P-Q interval was normal.
Females
Weeks 1 and 3, MD and HD, decrease in P-Q interval occurring immediately post treatment, which after 4 hr, the P-Q interval was normal.

Respiratory function: Week 4, before treatment, No change.
After treatment, males, minute vol., +44%; females, no effect.
Recovery group: Normal.

Hematology: Females, platelets, +44%, full recovery. Males, no change.

Clinical chemistry: No change.

Urinalysis: No change.

Gross pathology: None.

Organ weights: Testes and prostate: HD, Abs. wt. -33%; prostate, -50%.
Rel. body wt. -25%; prostate, -33%
Rel. brain wt. -37%; prostate, -52%
Testes and prostate: full recovery

Toxicokinetics

The results are summarized in the following table. There were no differences in the toxicokinetics between sexes.

Mean C_{max}, T_{max} and $AUC_{(0-23.5h)}$ values for males and females on Days 1 and 27

Day	1						27					
Sex	Male			Female			Male			Female		
Group	3	4	5	3	4	5	3	4	5	3	4	5
C_{max} (ng/mL)	2.5	8.9	26.2	2.6	9	25.0	2.9	9.7	39.1	4.4	14.7	49.9
T_{max} (hours)	0	0	0	0	0	0	0	0	0	0	0	0
$AUC_{(0-6.5h)}$ (ng.h/mL)	4.5	13.1	38.7	4.5	11.8	35.5	5.7	15.6	64.5	6.9	20.8	70

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Histopathology

Organ Observation	Incidence /Mean Severity Score (1, 2, 3)				
	Males				
	C(air)	C (veh.)	LD	MD	HD
Liver					
Hepatocellular hypertrophy	0/3	1/3	0/3	1/3 1.0	3/3 1.3
Lacrimal Glands					
Acinar hypertrophy	0/3	0/3	0/3	0/3	2/3 1.5
Skin (muzzle)					
Hyperkeratosis [a]	1/3	0/3	0/3	0/3	3/3 1.0
Epithelial hyperplasia	0/3	0/3	0/3	0/3	2/3 1.5
Inflammation	0/3	0/3	0/3	0/3	1/3 2.0
Scabs	0/3	0/3	0/3	0/3	2/3 1.5
Erosion	0/3	0/3	0/3	0/3	2/3 1.5
Sublingual gland					
Acinar hypertrophy [a]	0/3	0/3	0/3	0/3	2/3 1.5
Prostate					
Immature	0/3	0/3	1/3	2/3	2/3
Testes					
Peripubertal	0/3	0/3	2/3	0/3	3/3
Epididymidis					
Peripubertal	0/3	0/3	2/3	0/3	3/3
Reduced spermatocytes	1/3	1/3	2/3	0/3	3/3

[a] Partial recovery

Organ Observation	Incidence /Mean Severity Score (1, 2, 3)				
	Females				
	C(air)	C (veh.)	LD	MD	HD
Liver					
Hepatocellular hypertrophy	0/3	0/3	1/3 1.0	0/3	1/3 1.0
Lacrimal Glands					
Acinar hypertrophy	0/3	0/3	0/3	0/3	0/3
Skin (muzzle)					
Hyperkeratosis [a]	0/3	3/3 1.0	3/3 1.0	1/3 1.0	3/3 1.0
Epithelial hyperplasia	0/3	0/3	1/3 1.0	2/3 1.5	1/3 2.0
Inflammation	0/3	0/3	1/3 1.0	1/3 1.0	2/3 2.0
Scabs	0/3	0/3	0/3	0/3	2/3 2.0
Erosion	0/3	0/3	0/3	0/3	2/3 1.5
Sublingual gland					
Acinar hypertrophy	0/3	0/3	0/3	0/3	2/3 1.0

[a] Partial recovery

Reference ID: 3824703

Conclusion: Inhaled glycopyrronium was not toxic to the respiratory tract.

Histopathology inventory (optional)

Study	4-week Oral	4-week Oral	4-week Oral
Species	Rat	Rat	Dog
Report No.	848192		852241
Groups Examined	C, C, HD + recovery	LD, MD +recovery	All
Adrenals	X		X*
Aorta	X		X
Bone Marrow smear	X		X
Bone (femur)			X
Brain	X		X*
Bronchi			X
Carina	X	X	
Cecum	X		X
Cervix			
Colon	X		X
Duodenum	X		X
Epididymis	X		X*
Epiglottis			X
Esophagus	X		X
Eye	X		X
Fallopian tube			
Gall bladder			X
Gross lesions	X		X
Harderian gland	X		
Heart	X	X	X*
Ileum	X		X
Injection site			
Jejunum	X		X
Kidneys	X	X	X*
Lachrymal glands	X	X	X
Larynx	X	X	X
Liver	X	X	X*
Lungs	X*	X	X*
Lymph nodes, Mediastinal	X*		
Lymph nodes Retropharyngeal			X
Lymph nodes	X*		

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mandibular			
Lymph nodes tracheobronchial	X	X	X
Lymph nodes, mesenteric	X*		X
Mammary Gland	X		X
Nasal cavity 4 levels	X	X	X
Optic nerves	X		
Oro-nasal pharynx			X
Ovaries	X*		X*
Pancreas	X		X
Parathyroid	X*		X*
Peripheral nerve			
Pharynx	X		
Pituitary	X*		X*
Prostate	X		X*
Rectum	X		X
Salivary glands	X*	X	X*
Sciatic nerve	X		X
Seminal vesicles	X		
Skeletal muscle	X		X
Skin	X		X
Spinal cord	X		X
Spleen	X*		X*
Sternum	X		X
Stomach	X		X
Testes	X*		X*
Thymus	X*		X*
Thyroid	X*		X*
Tongue	X		X
Trachea	X	X	X
Urinary bladder	X		X
Uterus	X		X*
Vagina	X		
Zymbal gland			

X, histopathology performed
*, organ weight obtained

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2.6.6.4Genetic toxicology

Study title: **Reverse bacterial mutation assay.**

Key finding:

- Glycopyrronium was not mutagenic in the Reverse bacterial mutation assay at the maximal acceptable concentration (5,000 mcg/ml).

Study no.: **2250/12-D6171**
Volume and page #: 11, p 1
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/4/04
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, Batch # and % purity: glycopyrronium, 030381 and 100.2%.
Methods

Strains/species/cell line: Salmonella typhimurium TA1535, TA1537, TA98, TA100 and TA 102.

Doses used in definitive study: -S9:156.5, 312.5, 625, 1250, 2500 and 5000 ug/plate
+S9: 31.25, 62.5, 125, 250 and 500 and 1000 ug/plate.
Basis of dose selection: With Salmonella typhimurium TA100, concentrations of 1.6, 8, 40, 200, 1000 and 5000 ug/ml tested in triplicate in the presence and absence of S9 for toxicity were not toxic, and therefore, concentrations up to 5000 ug/ml, the acceptable maximum concentration, were tested.

Vehicle: Purified water.

S9: Commercially available from livers male Sprague-Dawley rats treated with Araclor 1254.

Reference ID: 3824703

Positive controls:

Organism	With S9, Conc. (µg/plate)	Without S9, Conc. (µg/plate)
Salmonella typhimurium		
TA98	Benzo[a]pyrene, 10	2-Nitrofluoorene, 5
TA102	2-Aminoanthracene, 20	Gluteraldehyde, 25 ug
TA100	2-Aminoanthracene, 5	Sodium azide, 2 ug
TA1535	2-Aminoanthracene, 5	Sodium azide, 2 ug
TA 1537	2-Aminoanthracene, 5	9-Aminoacridine, 50

Incubation and sampling times: Preincubated for 60 min at 37◦ C followed by incubation at 37◦ C for 3 days.

Results

Study validity: The test was conducted in triplicate twice in the presence and absence of S9. The number of revertant cells was counted with a Seescan Colony counter or manually. The background lawn was inspected for signs of toxicity.

For a valid assay, the following criteria were met.

1. The mean negative control counts fell within the normal historical ranges.
2. The positive control induced a clear increase in the number of revertant cells confirming discrimination between different strains in the presence and absence of S9.
3. No more than 5% of the plates were lost through contamination or some other unforeseen event.

For a significant mutagenic response, the following criteria were met.

1. The assay was valid.
2. Dunnett's t test gave a significant response ($p \leq 0.01$), and the data set(s) showed a significant dose correlation.

Study outcome: Glycopyrronium was not mutagenic at concentrations up to 5000 ug/ml in the presence and absence of metabolic activation, and the positive controls produced a clear increase in the number of revertant colonies.

Chromosome Aberration assay in human lymphocytes

Key findings

- Glycopyrronium was negative in the presence and absence of S9 in the Human lymphocyte chromosomal assay at the maximal acceptable concentration (10 mM).

Study no.: **No.2250/13-D6172**

Volume and page #: 11, p 56.NA.

Reference ID: 3824703

Conducting laboratory and location: (b) (4)
Date of study initiation: 4/19/04.
GLP compliance: yes (X) no ( ).
QA reports: yes (X) no ( ).
Drug, Batch #, and % purity: 030381, 99.6%.

Methods:

Strains/species/cell line: Human peripheral lymphocytes.
Dose selection criteria:
Basis of dose selection: Maximal acceptable concentration of 10 mM.
Stability: Not stated.
Metabolic activation system: Livers from rats pretreated with Aroclor.
Controls:
Vehicle: Purified water.
Negative controls: Purified water.
Positive controls: Dimethylsulfoxide.
Positive controls: For -S9, 4-Nitroquinolone 1-oxide (NQO), 2.5 mcg/ml; for S9+, Cyclophosphamide, and 6.25 mcg/ml.
Exposure conditions:
Incubation (hr) + harvest times (hr): Exp. 1 -S9, 3+17 hr.
+S9, 3+17 hr.
Exp. 2 -S9, 20+0 hr.
+S9, 3+17 hr.
Test concentrations:
Exp. 1 -S9, 3+17 hr.: 2039, 2549, 3983 ug/ml.
+S9, 3+17 hr.: 2549, 3186, 3983 ug/ml.
Exp. 2 -S9, 20+0 hr.:2878, 3386, 3983 ug/ml.
+S9, 3+17 hr.: 2878, 3386, 3983 ug/ml.

Colchicine (1µg/ml) was added at 1.5 hr prior to harvesting.

Study design:
In each experiment, blood was taken from 3 healthy female volunteers and pooled. The blood was processed according to OECD Guideline 473 (1997) and the ICH Tripartite Harmonised Guideline on Genotoxicity: Specific Aspects of Regulatory Tests (1995). Each experiment was conducted twice in duplicate scoring a total number of 200 cells in each experiment per concentration. Approximately 2 hr prior to harvesting, colchicine was added to give a final concentration of 1 ug/ml.

Analysis:
No. of replicates: 2
Counting method: From slides with microscope. 100 cells from each duplicate culture were analyzed for structural aberration. Mitotic index was determined by analyzing 1000 cells per duplicate culture.

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Criteria for a valid assay:

- The binomial dispersion test demonstrated acceptable heterogeneity.
- The proportion of cells with structural aberration (excluding gaps) in negative control culture fell within normal range.
- At least 160 cells out of the intended 200 cells were analyzable at each dose level.
- the positive controls induced statistical increase in the number of cells with structural aberration.

Criteria for positive results:

1. Statistically increase in the proportion of cells with structural aberration (excluding gaps) increased at one or more concentrations.
2. The proportion of cells with structural aberration at such doses at such doses exceeded the normal range in both replicate cultures.
3. The results were confirmed in a second experiment.

Results

The results with the highest concentrations, exposure and harvest times, the percent change in the Mitotic Index at the highest concentrations were 0% (-S9) and 19% (+S9) in the first experiment and 37% (-S9) and 21 % (+S9). This was not acceptable based on inhibition; however, the highest concentration (10 mM) is the maximal acceptable concentration for an acceptable assay.

Study Outcome

Glycopyrronium was negative in the presence and absence of S9 in the Human lymphocyte chromosomal assay at the maximal acceptable concentration (10 mM).

Study Title: Micronucleus assay in rats

Key findings

- Glycopyrronium was negative in the Micronucleus assay in male rats at a dose that was toxic.

Study no.: **2250/14-D1672**
Volume and page #: 11, p 106.
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/21/04.
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: glycopyrronium, 030381, 99.6%

Methods

Body weight: Daily

Reference ID: 3824703

Plasma levels: Day 1 at 0.25, 0.5 and 4 h post dose
Strains/species/: Male (range finder, 205-245g; main study, 138-177 g) and female (165-180 g), Han Wistar Crl:WI(Glx/BRL)IGS BR rats.
Doses: Preliminary toxicity evaluation (vehicle, purified water):
Range-finder test: 700 (3 females), 1000 (3 males and 3 females) and 1400 mg/kg, orally (3 males) twice, 24 hr apart
Definitive test: 250, 500 and 1000 mg/kg (6 males /group).
Satellite animals: 3 rats in the 1000 mg/kg main study group. Blood was removed at 0.25, 0.5 and 4 h post dose on day 1.
Positive control: Cyclophosphamide, 20 mg/kg, orally (6 males /group) on day 2; vehicle: 0.9% saline.
Route, volume: Oral, 10 ml/kg all groups.

Basis of dose selection: In a range finding study, 700 mg/kg produced weight loss, exophthalmus, increased respiration and piloerection; 1000 mg/kg produced lethargy, abnormal gait, weight loss and piloerection; 3/3 females were killed in extremis; 1400 mg/kg produced bradynpea, piloerection and weight loss.

Rats were killed 24 hr after the last dose of glycopyrronium. The positive control animals were sacrificed 24 after administration. Bone marrows were removed from the femurs, processed and scored using fluorescence microscopy. From the bone marrow of each animal, 1000 cells were examined to determine the ratio of the polychromatic erythrocytes (PCE) to the number of micronucleated polychromatic (MPCE).The counting continued until 2000 PCEs were counted to determine the percent of micronucleated PCEs.

Criteria for a valid assay:

1. The incidence and distribution of micronucleated PCE in the vehicle control group was consistent with the historical vehicle control data.
2. At least 5 animals out of each group were available for analysis.
3. The positive control induced a statistically significant increase in the frequency of micronucleated PCE.

Criteria for a positive response.

1. A statistically significant increase in the frequency of micronucleated PCE.
2. The incidence and distribution of micronucleated PCE within the group exceeded the historical vehicle control group.

Results

Body weight: Decreased body weight at all doses.

Plasma levels: The results are shown in the following table excerpted from the submission.

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Calibration Range: 0.10 – 102.3 ng/ml			
Limit of Detection (LOD): 0.05 ng/ml Lower Limit of Quantification (LLOQ): 0.10 ng/ml			
Group [mg/kg/day]	Animal [ID]	Time [h]	AD237 [ng/ml]
1000	681	0.25 h	416.6[1]
		0.5 h	623.7[1]
		4 h	282.5[1]
	682	0.25 h	11018.9[1]
		0.5 h	5328.2[1]
		4 h	2658.5[1]
	683	0.25 h	8640.0[1]
		0.5 h	3483.8[1]
		4 h	1315.4[1]

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Study validity: This is an acceptable study it met the requirements for a valid study, and the test dose was not toxic to the bone marrow. Glycopyrronium at 1000 mg/kg orally did not increase the percent of micronucleated PCE.

Study outcome: Glycopyrronium was not genotoxic in the Micronucleus test in rats.

2.6.6.5Carcinogenicity: No reports were submitted.

2.6.6.6Reproductive and developmental toxicology: No reports were submitted.

2.6.6.7Local tolerance: No reports were submitted.

2.6.6.8 Special toxicology studies: No reports were submitted.

2.6.6.9Discussion and Conclusions

Four week inhalation toxicity studies were conducted in rats and dogs with the powder formulation containing lactose and magnesium stearate. In rats, the inhaled doses were 97, 617 and 4,240 ug/kg. Decreased body weight gained occurred at the MD and HD. Mydriasis and histological changes in the parotid, Hardarian and lacrimal glands were related to the anticholinergic pharmacological action of glycopyrronium. The changes in the nasal areas and larynx were not clinically relevant since the glycopyrronium formulation will be administered clinically by the inhaled route and concern was for toxicity was in the lower respiratory tract. In this study, there was no toxic manifestations to the lower respiratory tract. The lower respiratory tract NOAEL was 4,240 ug/kg.
In the dog study, the inhaled doses were 32, 96 and 317 ug/kg. Corneal opacity occurred in both sexes at the HD which was reversible. The decreased pupil reaction, transient tachycardia with changes in the EKG and histopathologic changes in the lacrimal and sublingual glands and skin were related to the anticholinergic pharmacological action of glycopyrronium. The hepatocellular hypertrophy in males suggests enzyme induction.

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Males showed immature prostate and, peripubertal testes and epididymidis which were related to the age of the dogs. The lower respiratory tract NOAEL was 317 ug/kg. Glycopyrronium was negative in the Reverse Mutation Bacterial and Human Lymphocyte Chromosomal Aberration assays and in the Micronucleus assay in rats.

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY: NA

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

Glycopyrronium, a quaternary ammonium anticholinergic compound, possesses in vitro binding capacity to the M1, M2 and M3 cholinergic receptors. Based on its Koff kinetics, glycopyrronium possesses a long duration of action. This was supported in its antagonism of the isolated the rat tracheal muscle contraction to the M_3 antagonist, bethanechol. Several studies were conducted to show that glycopyrronium antagonized the brochonstriction to methacholine. In anesthetized rabbits, different formulations of glycopyrronium (aqueus, dry powder and control led release) administered intratracheally markedly inhibited methacholine bronchoconstriction. However, unlike the references, tiotropium and ipratropium, glycopyrronium did not significantly inhibit the hypotension to methacholine indicating that glycopyrronium showed minimal systemic effect. Further, the ED50s for glycopyrronium (highest and least potent), ipotropium and tiotropium in inhibiting the methacholine induced bronchoconstriction were 2.27, 1.41 and 0.21 ug/kg, respectively as compared to > 20, > 20 and 0.56 ug/kg, respectively, for inhibiting the methylcholine induced bradycardia. In sensitized Brown Norway rats, the inhibition of the bronchoconstriction, salivation, and hypotension and bradycardia response to methacholine was compared to tiotropium. Relative to its ability to block the bronchoconstriction response to methacholine, glycopyrronium was less potent than tiotropium and ipratropium in blocking the effects (salivation, and hypotension and bradycardia) of methacholine. In immobilized anesthetized monkeys, glycopyrronium showed sustained inhibition of methacholine induced bronchoconstriction.
In immobilized anesthetized monkeys, glycopyrronium administered by intratracheally did not affect blood pressure, heart rate and respiratory rate. In the isolated perfused rabbit heart preparation, glycopyrronium did not induce any electrophysiological changes. By the intravenous route, glycopyrronium at doses up to 1 mg/kg in dogs produced the classical anticholinergic effects, i.e., mydriasis, dry mucous membranes and tachycardia.
In inhalation toxicity studies in rats and dogs, plasma levels were determined at the beginning and the end of the 4-week study. In both species, there was no difference in the AUCs between sexes and accumulation was not evident. In the rat, the AUCs were dose related and not dose proportional while in the dog there was dose proportionality. In in vitro studies, glycopyrronium was more stable in blood than in plasma of rat dog and human and in all 3 species, binding to plasma proteins was low. In in vitro metabolism studies with rat, dog and human, and human microsomes, and in rat and human hepatocytes, rats metabolized glycopyrronium to a greater degree than the dog and

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human. Eight metabolites were detected in addition to the parent compound. However, in the lung, only 1 metabolite and glycopyrronium were detected in the dog and human showing a difference in tissue metabolic capability.
Four week inhalation toxicity studies of the formulation were conducted in rats and dogs with the formulation. The excipient, magnesium stearate, although approved in oral products was found to be safe by the inhaled route as shown in a 6-month inhalation toxicity study in rats (See review of L. Pei of IND (b) (4); submission date, 4/27/00; review date, 6/7/2000). In the rat, histopathology was seen in the nasal cavity. However, this was not of clinical concern since the glycopyrronium formulation will be administered by inhalation, and nasal changes are clinically monitorable. The pathology seen in the lacrimal, Hardarian and parotid glands was attributed to the anticholinergic action of glycopyrronium. However, in the DRAFT interim 13-week rat inhalation toxicity study of a 26- week study (See review of L. Sancilio of IND 48,655; submission date, 8/29/06; review date 9/25/06), only the Hardarian gland was affected indicating that the lacrimal and parotid gland changes were not confirmed. In the dog, histopathology seen in the lacrimal and parotid glands and skin was attributed to the anticholinergic activity. However, in only male dogs, hepatocellular hypertrophy was observed suggesting enzyme induction. In rats and dogs no toxicity was observed in the lower respiratory tract. The lower respiratory tract NOAEL was 4,240 ug/kg in the rat and 317 ug/kg in the dog.
Glycopyrronium was negative in the Reverse Bacterial Mutation and Human Lymphocyte Chromosomal Aberration assays and in the Micronucleus assay in rats.

Since the concern was for toxicity of the lower respiratory tract, the Safety Index was based on the lung burden derived from the NOAEL. The doses cited in the report did not address the deposition factor. The following table shows that The Safety Indexes (determined using the deposition factor) in the 4-week inhalation toxicity studies in the rat and dog support the proposed clinical trial of 200 ug/day.

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Species, Study Deposition Dose	Lung Burden mg/g	Safety Index Rat or Dog/Human
4-Weeks Rat NOAEL: 4.24 mg/kg Total dose: 1.06 mg 10% Deposition Deposited dose: 0.106 mg	0.71	2367
Dog NOAEL: 0.317 mg/kg Total dose, 3.17 mg 20% Deposition Deposited dose: 0.634 mg	0.006	20
Human Dose: 0.2 mg Total dose: 0.2 mg 100% Deposition Deposited dose: 0.2 mg	0.0003	

Dose divided by lung weight (10 kg dog, 110 g; 250 g rat, 1.5 g; 50 kg Human, 714 g)

Conclusion:

The preclinical data support the proposed clinical 28-day trial of 200 ug/day.

Internal comments: None.

Recommendation:

From a preclinical standpoint, there is no objection to proposed clinical 28-day trial of 200 ug/day. Changes if any between the 13-week rat DRAFT inhalation toxicity and the finalized report should be submitted.

External comments

Letter to the sponsor:

Your April 27, 2007 submission of IND 48,655 has been reviewed. We have the following comment. The 13-week rat inhalation toxicity study (No.79031) submitted on 8/29/06 was a DRAFT and in this submission, it is the finalized report. Provide any differences between the DRAFT and finalized reports. If none, so state it.

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Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

Appendix/attachments: None.

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/s/

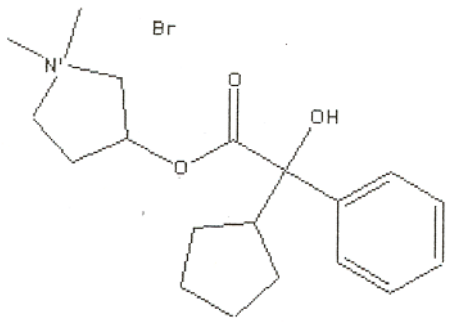
Lawrence Sancilio
6/20/2007 02:30:29 PM
PHARMACOLOGIST

Joseph Sun
6/20/2007 02:47:01 PM
PHARMACOLOGIST
I concur.

PHARMACOLOGY/TOXICOLOGY REVIEW

IND number: 48,655
Sequence number/date/type of submission: Ser. No. 35, 2/12/09 (SPA)
Review No.: 2
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Novartis Pharmaceutical Corporation

Reviewer name: Lawrence F. Sancilio, Ph.D.
Division name: Pulmonary and Allergy Products
Review completion date: 3/27/09
Drug: Trade name: Unknown
Generic name: Glypyrronium bromide, glycopyrrolate, USP
Code name: NVA237; NVP-NVA237**;** NVP-QAM254-DC-1.
Chemical name: R, S, 3-[(cyclopentylhydroxyphenylacetyl)oxy]-1, 1-dimethylpyrrolidinum bromide
CAS registry number: 596-51-0
Molecular formula/molecular weight: $C_{19}H_{28}BrNO_3$/ 398.34
Structure:



Relevant INDs/NDAs/DMFs: None

Drug class: Quaternary ammonium antimuscarinic agent.

Intended clinical population: COPD patients.

Drug History and Introduction:

This is a special protocol assessment (SPA) to conduct the above 52-week inhalation clinical study in COPD patients. In this submission, a 6-month rat inhalation toxicity study was submitted. In the minutes of the July 15, 2008 meeting, FDA indicated that clinical trials longer than 4-weeks requires support from inhalation toxicity studies in rats and dogs of adequate duration. Based on the recommendation from a PIND meeting with Arakis on June 9, 2004, as cited in the minutes of the July 15, 2008 meeting

only a 6-month rat inhalation toxicity study was required to support this 52-week clinical study. This submission contains the recommended 6-month rat inhalation toxicity study to support the proposed 52-week clinical study.

Clinical Protocol: This is a 52-week treatment, randomized, double blind, placebo-controlled, with open label tiotropium, parallel group study to assess the efficacy, safety and tolerability of inhaled 50 µg/day of glycopyrrolate in COPD patients.
This is a 3 arm study with a randomization ratio of 2:1:1 glycopyrrolate, placebo, tiotropium. The study will consist of 1065 adult male and female patients, ≥ 40 years old, with stable to severe COPD and a smoker for ≥ 10 years. The goal is to have 745 patients to complete the study.

Route of administration: Inhalation.

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

Studies reviewed within this submission:

Toxicology

Multi-dose toxicity

26-Week inhalation study in rats followed by a 4-week recovery period, No. 79031

Studies not reviewed within this submission: None**.**

Review

Toxicology

Multi-dose toxicity

Study title: 26-Week inhalation study of a powder formulation in the albino rat with a 13-week interim sacrifice followed by a 4-week recovery period
Note: A 13-week interim sacrifice was previously reviewed (review of 8/29/06 submission of IND 48,655 by L. Sancilio on 9/25/06).

Key findings:

- Decreased body weight gained was dose related at all doses in the males and at the MD and at the HD in females.
- Glycopyrrolate induced lenticular changes at the MD and HD in both sexes. They were partially reversible at the end of the recovery period.
- The pharmacokinetics showed that the AUCs were dose related and not dose proportional and there was no accumulation.

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- Histopathology changes occurred in the lungs at the MD and HD in both sexes, and they were reversible.
- The Inhalation NOAEL was 10 µg/kg with an average AUC of 334 ng*hr/ml.

Study no: No. 79031
Volume # and page #: EDR.
Conducting laboratory and location: (b) (4)
Date of study initiation: 12/23/05
GLP compliance: Yes.
QA report: yes (X)
Drug, lot #, and % purity: VAR6105 and 8% glycopyrronium bromide, 1% magnesium stearate, 91% lactose monohydrate; purity, 104%.
Control vehicle, Formulation/Vehicle: Lot No. VAR9505 and 1% magnesium stearate, 99% lactose monohydrate.
Methods
Species/strain: Wister Hanover Crl: WI (Han) rats.
#/Sex/group: Main group, C_{air}, C_{veh} HD, 30; LD, MD, 20: toxicokinetics group; 5; 28-day recovery group, 10.
Age: 8 Weeks.
Weight: M, 196-241g; F, 152-187 g.
Route: Inhalation; exposure was by nose only for 120 min/day in an inhalation chamber.
Particle size: Ranges of MMAD determined chemically: 2.1-3.0 µm for glycopyrronium bromide and 1.9 -2.4 µm for magnesium stearate; the ranges of GSD were: 1.9-2.4 µm for glycopyrronium bromide and 2.2-2.9 µm for magnesium stearate.

Targeted and achieve doses of glycopyrronium bromide (salt) are summarized in the following table. The dose of the magnesium citrate was 1/8 that of the glycopyrrolate. Calculation of the achieved doses was determined from the following formula excerpted from the submission. The difference between the achieved and targeted doses were: LD, -10%, MD, +11% and HD, +25%.

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Calculation of achieved dosage:[1]

The achieved dose of active ingredient (mg/kg/day) for each treatment level was determined as follows:

$$\text{Achieved dose of active ingredient (mg/kg/day)} = \frac{\text{RMV x Active Concentration x T}}{\text{BW}}$$

Where RMV (L/min) = respiratory minute volume calculated[2]

Active concentration (mg/L) = chamber concentration of active test material determined by chemical analysis

T (min) = treatment time (up to maximum of 120 minutes)

BW (kg) = mean body weight per sex per group from the regular body weight occasions during treatment

1 Total body dose assuming a deposition fraction of 100%

2 0.499 x [body weight (kg)]$^{0.809}$ L/min (Bide, R.W. et al 2000). It is assumed that this parameter is unaffected by exposure to the test article

The 26 week recovery groups were air control, vehicle control and HD.

Glycopyrronium bromide

Group	Target Dose mg/kg	Average Achieved Dose mg/kg/kg	Deposited Dose[a] mg/kg
Air Control	0	0	0
Mg Stearate/Lactose (vehicle) Control	0	0	0
LD	0.1	0.09	0.009
MD	0.6	0.67	0.067
HD	4	4.98	0.50

[a] Based on 10% deposition of the average achieved dose

Observations:

Analysis of Formulation: 104% pure.
Mortality: Twice daily observation.
Clinical signs: 2 x Daily.
Body weights: Day 1 and weekly after initiating treatment.
Food consumption: Prior to and weekly during the treatment and recovery periods.

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Ophthalmoscopy: Prior to treatment, during week 13 (before dosing groups air control, vehicle control and HD and post dosing for LD and MD), 0.5% atropine was instilled in the eyes to determine the effect of glycopyrrolate on the mydriatic effect. This was followed by fundiscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examination.
Pupil diameter: Week 26 before dosing and the last week of the recovery period, the following scoring system was used to evaluate mydriasis before and after the instillation of the mydriatic, (0.5% atropine sulfate). The volume instilled was not reported. Following examination of the pupil size post mydriasis, the eyes were subject to fundiscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp examination) examination.

1= miosis (0% to 20% dilation)
2= slight dilation (20%-50% dilation)
3= mid dilation (50%-80% dilation)
4= complete dilation (80%-100% dilation)

Hematology: At the end of week 26 and at the end of the 4-week recovery period. A complete hematology battery was assessed.
Clinical chemistry: At the end of week 26 and at the end of the 4-week the recovery period. A complete clinical chemistry battery was assessed.
Urinalysis: At the end of week 26 and at the end of the 4-week the recovery period. A complete urinalysis was evaluated.
Toxicokinetics: Day 28 and in weeks 13 and 26. Blood was removed at the following time points: 0 hr (immediately following inhalation), 0.25 hr, 1 hr, 3 hr, 7 hr and 24 hr post dosing. Data from day 28 and 13 were previously reported in the 13-week report (review of 8/29/06 submission of IND 48,655 by L. Sancilio on 9/25/06)

Gross pathology: Week 26 and at the end of the recovery period. A full necropsy was conducted.
Organs weighed: Adrenals, brain, kidneys, heart, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and parathyroids and uterus.

Histopathology: Tissues in the two control groups and HD groups and all animals found dead or euthanized prior to examination were stained with hematoxylin and eosin and examined. In some instances, the LD tissues were examined. The tissues examined were: those showing abnormal lesions, adrenals, aorta, femur, bone and marrow from sternum, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, Harderian glands, heart, ileum, jejunum, kidneys, lacrimal glands, larynx, liver, lungs, lymph nodes (tracheobronchial, mandibular, unilateral, mesenteric) mainstem bronchi, nasal cavities and sinuses, optic nerves, ovaries, pancreas, pharynx, pituitary, prostate, rectum, salivary gland (mandibular, unilateral), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid and parathyroid glands, tongue, trachea, urinary bladder, uterus (horns, body and cervix) and vagina.

For the LD and MD groups, only the following tissues were examined:

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Macroscopic abnormalities and any compound –related changes noted in the HD group, lungs, larynx, pharynx, main stem bronchi, nasal cavities and sinuses, trachea and lymph node (tracheobronchial).

Special immunohistochemistry evaluation: Sections from the lungs from 2 control animals/sex and 6 treated animals/sex were examined using the immunostaining Surfactant B (SPI-8811-B) stain as marker for type II pneumocytes.

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

Results

Mortality: None, due to treatment.
1 LD female was found dead on day 31 due to tube restraint procedure.
2 HD males died or were sacrificed, day 179 and day 183; death was attributed to the presence of bacteria and urolithiasis in the urinary tract.

Clinical signs: None.

Body Weight: The growth curves of males and females excerpted from the submission from the submission are presented in the following 2 figures. The males were more affected than the females. This was evident when the body weight gained was determined as seen below.

Figure 1 Summary of body weight data - males

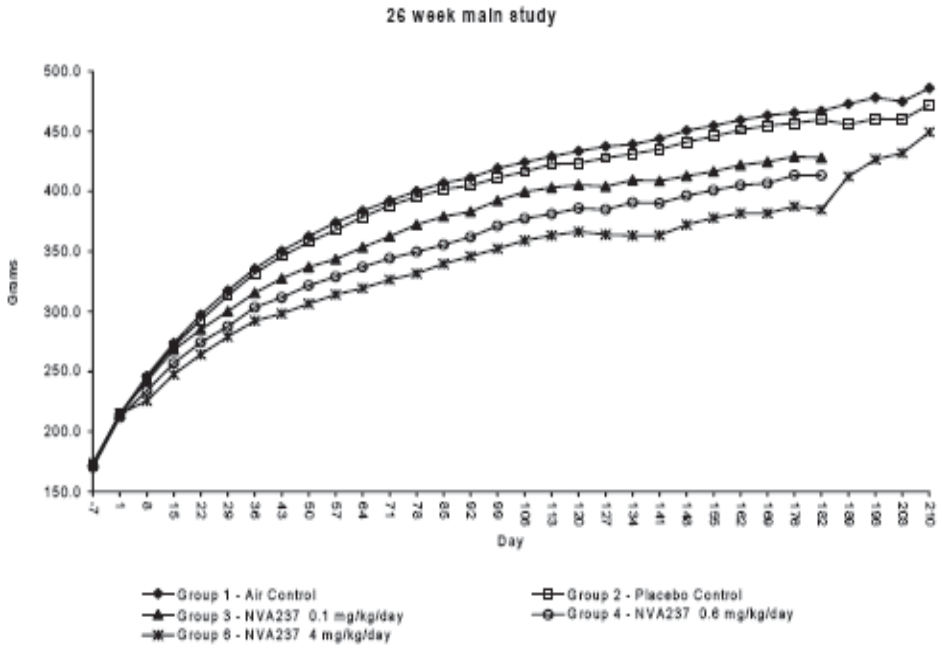

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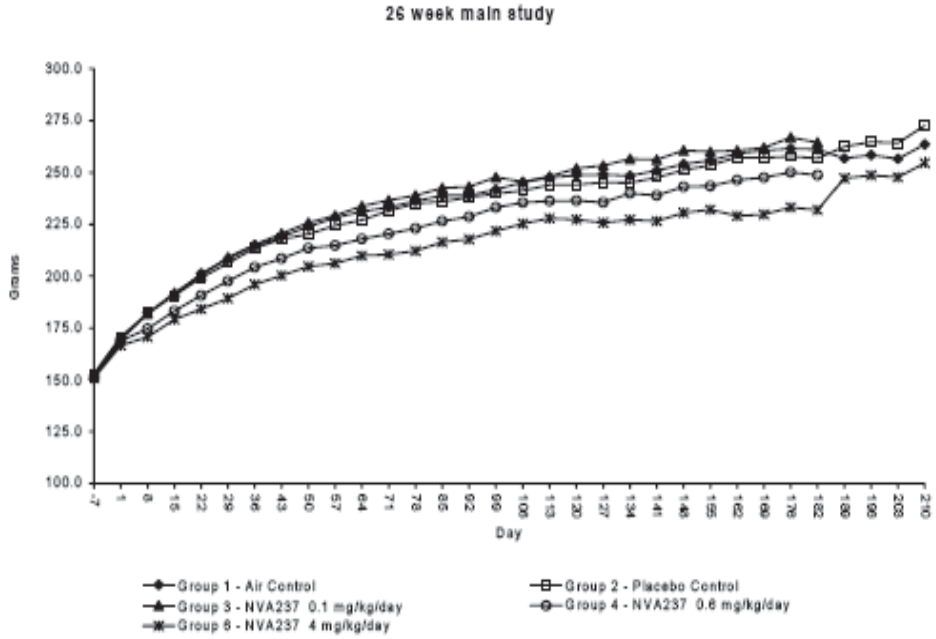
Figure 2 Summary of body weight data - females



The change in body weight gained from the $C_{veh.}$ group on day 182 and at the end of the 4-week recovery period are presented in the following table. There was a dose related decrease in body weight gained in both sexes. Recovery at the HD in males was greater than that observed in the control groups. The HD female group showed comparable decrease in body weight to the HD of males; in the recovery HD males, the increase was markedly higher than the increase seen in females.

Group	Percent Change from C_{veh} (P<0.05)			
	Day 1- 182		Day 182- 210, Recovery Period	
	Male	Female	Male	Female
C_{air}	NC	NC	NC	NC
LD	-15	NC	NT	NT
MD	-20	-12	NT	NT
HD	-33	-32	+188	NC

NC, No change from C_{veh}
NT, Not Tested

Food Consumption: No effect due to treatment. In the recovery group, the HD males on days 203 to 210 showed a 17% increase in food consumption.

Ophthalmoscopy:

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In the 26-week evaluation of the effect on atropine induced mydriasis in both eyes, scores were 1 prior to and 4 after the instillation of atropine. The incidences of changes in the scores are presented in the following table. There were no changes in the C_{air} control group. In the left eye of the female C_{veh} group, 1/30 showed a decrease in the mydriatic score from 4 to 3 post atropine. The changes occurred predominantly at the MD and HD in both sexes and in the post mydriatic dose at the LD in the right eye in females. The results show that glycopyrrolate alone was mydriatic (mydriasis scores increased from 1 to 2s and 3s) and decreased the mydriatic activity of atropine (mydriasis scores decreased from 4 to 2s and mainly 3s). There was full recovery from the effect on atropine.

Group	Incidence of Change in Mydriatic Score							
	Males				Females			
	Left Eye		Right Eye		Left Eye		Right Eye	
	Pre- A Score, >1	Post- A Score, <4	Pre- A Score, >1	Post- A Score, <4	Pre- A Score, >1	Post- A Score, <4	Pre- A Score, >1	Post- A Score, <4
LD	0/20	0/20	0/20	0/20	0/19	0/19	0/19	3/19
MD	1/20	5/20	2/20	4/20	2/20	9/20	2/20	8/20
HD	30/30	30/30	30/30	30/30	27/30	30/30	30/30	30/30

There were unilateral or bilateral lenticular changes. None was observed in the two control and LD groups. These changes and incidence in the MD and HD are shown in the following table.

Treatment Period

Lenticular Changes	**Incidence**			
	MD		**HD**	
	M	**F**	**M**	**F**
Anterior Capsular Opacity	1/20	4/20	2/30	3/30
Anterior Prominent Suture Line	0/20	0/20	2/30	12/30
Anterior Slight Cataract (suture lines, capsular or subscapular)	0/20	0/20	1/30	2/30

At the end of the recovery period as presented in the following table, these lenticular changes were still present. As cited by (b) (4) (Durand, G. et al. Toxicology Sciences: 66, 166-172, 2002) this effect is characteristic of muscarinic receptor inhibition.

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Recovery (HD group only)

Lenticular Changes	Incidence			
	MD		HD	
	M	F	M	F
Anterior Capsular Opacity	NT	NT	1/10	0/10
Anterior Prominent Suture Line	NT	NT	2/10	3/10
Anterior Slight Cataract (suture lines, capsular or subscapular)	NT	NT	1/10	3/10

NT, Not tested.

Hematology and Clinical Chemistry: The results are summarized in the following table. They were reversible.

Parameter	% Change from C_{veh} (P<0.05)							
	C_{air}		LD		MD		HD	
	M	F	M	F	M	F	M	F
Neutrophils	ND	ND	ND	ND	+17[a]	ND	+81	ND
APTT	ND	ND	ND	+6 [a]	ND	+31[a]	ND	+37
Direct Bilirubin	ND	ND	ND	ND	+18	ND	+26	ND

ND, No significant difference from C_{veh}
[a] P>0.05

Urine analysis: Potassium, males, MD,-24%; HD, -22% Recovery group, HD, -13%
Potassium, females, MD,-22%; HD, -27% Recovery group, HD, full recovery.

Toxicokinetics: The results of the 28, 91 and 182 days are shown in the following tables. The achieved doses on days 28, 91 and 182 were slightly different. The AUCs were dose related and not dose proportional and there were no sex differences; however, accumulation occurred at the HD in females.

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Table 7-4 Toxicokinetic parameters of NVA237 in rat plasma on study day 28 and 91

Dose (mg/kg/day)	t_{max} (h)	C_{max} (ng/mL)	C_{max}/dose (ng/mL)/(mg/kg/day)	$AUC_{(0-24h)}$ (ng.h/mL)	$AUC_{(0-24h)}$/dose (ng.h/mL)/(mg/kg/day)
			Study day 28 - Male		
0.11	2	2.14	24.3	8.65	98.4
0.71	2.25	7.06	12.4	36.9	65.0
4.83	2	72	18.7	198	51.2
			Study day 28 - Female		
0.11	2	2.44	27.8	9.81	112
0.76	2	18.5	30.4	61.5	101
5.11	2	91.8	22.5	230	56.4
			Study day 91 - Male		
0.06	2.25	1.57	32.7	9.94	207
0.52	2	8.27	19.9	34.5	82.8
3.62	2	52.4	18.1	187	64.8
			Study day 91 - Female		
0.07	2.25	1.57	28.0	5.63	100
0.56	2	16.2	36.2	48.4	108
3.88	2	44.8	14.5	166	53.6

Table 7-3 TK parameters of NVA237 in rat plasma on study day 182

Achieved dose	t_{max} (h)	C_{max} (ng/mL)	C_{max}/dose (ng/mL)/(mg/kg/day)	$AUC_{(0-24h)}$ (ng.h/mL)	$AUC_{(0-24h)}$/dose (ng.h/mL)/(mg/kg/day)
			Male		
0.08	2	1.97	30.8	10.1	158
0.62	2.25	10.5	21.2	55.0	111
4.06	2	128	39.5	353	109
			Female		
0.09	2	1.90	26.4	17.5	244
0.67	2	16.1	30.0	68.1	127
4.38	2	102	29.1	315	90.1

The achieved doses are expressed in mg /kg/day of NVA237as salt

The dose-normalized parameters are calculated using the achieved doses expressed in mg /kg/day of NVA237 as base

Necropsy: None.

Organ Weights: Males, adrenals, HD, relative weight, +15%
Recovery period, HD, +27%
Brain, relative weight, MD +15%; HD, +19%
Recovery period, HD, +6%
Testes, relative weight, HD, +16%.
Recovery period, full recovery
Females, adrenals, HD, relative weight, +24%
Recovery period, full recovery
Brain, relative weight, HD, +14%
Recovery period, HD, +14%

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Test for pneumocytes Type II in the lung: Negative.

Histopathology: The results are summarized in the following tables. The severity was graded from 1 (minimal), 2 (slight), 3 (moderate), 4, (marked), 5 (severe). The severity average is presented in the table below next to the incidences of each observation. In the HD males, tissues from 21 instead of 20 animals were examined. This number may have been an error since in the methodology, the number cited in the group was 20. In both sexes, histopathology was seen in the respiratory tract. The severity in most groups was minimal in the 1-2 range with the exception of the eosinophilic globules in the olfactory epithelium and goblet cells of the nasal cavity/sinuses where the scores were higher.

End of Treatment Period-Males

Organ/Pathology	Incidence/ Mean Severity Score				
	C-air	C-vehicle	LD	MD	HD
Nasal Cavity/Sinuses					
Olfactory epithelium					
Eosinophilic globules	0/20	0/20	5/20 1.4	20/20 2.3	21/21 3.6
Respiratory epithelium					
Eosinophilic globules	0/20	0/20	0/20	14/20 1.3	21/21 1.5
Exudate	0/20	0/20	0/20	2/20 2.0	4/21 1.3
Metaplasia, squamous	0/20	0/20	0/20	3/20 2.0	3/21 2.7
Goblet cells/hypertrophy	0/20	0/20	7/20 1.0	19/20 1.6	21/21 2.4
Degeneration					
Olfactory epithelium	0/20	0/20	0/20	1/20 1.0	2/21 1.5
Harderian Gland					
Increased porphyrin deposition	0/20	0/20	6/20 1.0	9/20 1.3	15/21 1.6
Larynx					
Metaplasia, squamous	0/20	0/20	10/20 1.1	16/20 1.1	16/21 1.2
Lung					
Epithelium hypertrophy of bronchioloalveolar junction	0/20	0/20	0/20	2/20 1.0	13/21 1.0
Hyperplasia of alveolar epithelium	0/20	0/20	0/20	0/20	1/20 2.0
Seminal vesicles					
Inflammation	0/20	0/20	NE	NE	2/21 2.5
Urinary bladder	1/20 1.0	0/20	NE	NE	3/21 4.3
Inflammation					

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End of Recovery Period-Males

Organ/Pathology	Incidence/ Mean Severity Score		
	C-air	C-vehicle	HD
Nasal Cavity/Sinuses			
Olfactory epithelium			
Eosinophilic globules	0/10	0/10	9/9 3.8
Respiratory epithelium			
Eosinophilic globules	0/10	0/10	8/9 1.4
Exudate	0/10	0/10	0/9
Metaplasia, squamous	0/10	0/10	0/9
Goblet cells/hypertrophy	0/10	0/10	8/9 1.0
Degeneration			
Olfactory epithelium	0/10	0/10	1/9 1.0
Harderian Gland			
Increased porphyrin deposition	0/10	0/10	0/9
Larynx			
Metaplasia, squamous	1/10 1.0	0/10	3/9 1.0
Lung			
Epithelium hypertrophy of bronchioloalveolar junction	0/10	0/10	0/9
Hyperplasia of alveolar epithelium	0/10	0/10	0/9
Seminal vesicles			
Inflammation	0/10	1/10 1.0	1/9 3.0
Urinary bladder	0/10	0/10	0/9
Inflammation			

End of Treatment Period-Females

Organ/Pathology	Incidence/ Mean Severity Score				
	C-air	C-vehicle	LD	MD	HD
Nasal Cavity/Sinuses					
Olfactory epithelium					
Eosinophilic globules	0/20	0/20	2/19 1.0	20/20 2.2	20/20 3.7
Respiratory epithelium					
Eosinophilic globules	0/20	0/20	1/19 1.0	14/20 1.1	20/20 1.7
Metaplasia, squamous	0/20	0/20	0/19	0/20	1/20 2.0
Goblet cells/hypertrophy	0/20	0/20	5/19 1.0	20/20 1.6	20/20 2.7
Degeneration					
Olfactory epithelium	0/20	0/20	0/19	1/20 1.0	4/20 1.3

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Organ/Pathology	Incidence/ Mean Severity Score				
	C-air	C-vehicle	LD	MD	HD
Harderian Gland					
Increased porphyrin deposition	0/20	0/20	2/19 1.0	9/20 1.1	12/20 1.3
Inflammation	2/20 1.0	0/20	0/19	1/20 1.0	5/20 1.2
Larynx					
Metaplasia, squamous	0/20	0/20	10/19 1.0	9/20 1.0	17/19 1.4
Lung					
Hypertrophy of epithelium bronchioloalveolar junction	0/20	0/20	0/19	3/20 1.0	17/20 1.0

End of Recovery Period-Females

Organ/Pathology	Incidence/ Mean Severity Score		
	C-air	C-vehicle	HD
Nasal Cavity/Sinuses			
Olfactory epithelium			
Eosinophilic globules	0/10	0/10	10/10 3.5
Respiratory epithelium			
Eosinophilic globules	0/10	0/10	10/10 1.2
Metaplasia, squamous	0/10	0/10	0/10
Goblet cells/hypertrophy	0/10	0/10	8/10 1.1
Degeneration			
Olfactory epithelium	0/10	0/10	2/10 1.0
Harderian Gland			
Increased porphyrin deposition	0/10	0/10	0/10
Inflammation	1/10 2.0	0/10	0/10
Larynx			
Metaplasia, squamous	0/10	0/10	3/10 1.0
Lung			
Hypertrophy of epithelium bronchioloalveolar junction	0/10	0/10	0/10

OVERALL SUMMARY AND CONCLUSIONS AND RECOMMENDATIONS

Summary: In a 26-week inhalation study in rats, they were treated with C (air) and C (veh.-lactose plus magnesium stearate) and the pulmonary deposited doses of 0.01 (LD), 0.06 (MD) and 0.4(HD) mg/kg. The achieved deposited doses were: 0.009, 0.067 and 0.05 mg/kg. The 4-week recovery groups involved the C (air) and C (veh.) and the (HD) groups. There was decreased body weight gained in both sexes with males showing a greater decrease than females at the LD and MD. Glycopyrrolate produced mydriasis prior to the instillation of atropine and yet reduced the mydriatic effect of atropine. This

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occurred predominantly at the MD and HD of both sexes. At these doses at week 26, there were also lenticular changes which were not completely reversible. These changes were characteristic muscarinic receptor inhibition, the pharmacological action of glycopyrrolate. (Durand, G. et al. Toxicology Sciences: 66, 166-172, 2002). There were no histopathological changes in the eyes. In males, there were increased neutrophil (MD and HD) levels and increased direct bilirubin levels (MD and HD), and in females there was an increase in APTT levels (MD and HD). These changes were reversible. In both sexes, increased urinary excretion of potassium was not accompanied by a change in serum potassium or renal pathology. The pharmacokinetics changed as the HD females showed accumulation.
Histologically, both sexes showed pathological changes in the nasal cavity/sinuses, Harderian gland and larynx at all doses and in the lung at the MD and HD. The severity of the lung histopathology in both doses was minimal with an average score of 1. The change in the lung was completely reversible. The cells within the area where epithelial hypertrophy was observed were not Type II pneumocytes, and this hypertrophy may be due to a local irritant effect of the inhaled glycopyrrolate. The only clinically relevant histopathology was the epithelium hypertrophy of the bronchioalveolar lung tissue since this product was administer via the nose and clinically will be administered by the oral route; the changes in the larynx are common in the rat in inhalation studies. The inhalation NOAEL was the LD, 0.009 mg/kg (what was the actual dose not the targeted dose) which is associated with an average AUC of 334 ng.h/ml.

Conclusion**:** Inhaled glycopyrrolate was minimally irritating to the lungs of rats. The effects seen in the eyes were monitorable or related to the pharmacological action of glycopyrrolate. The other changes were monitorable or not clinically relevant.

RECOMMENDATION: The proposed 52- week clinical study is supported, since from the 6-month rat inhalation study, the NOAEL was 9 mcg/kg; based on the proposed clinical dose of 0.050 mg (1 mcg/kg), there was acceptable Safety Margin of 9 (NOAEL (9 mcg/kg) ÷ proposed human dose (1 mcg/kg).

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Linked Applications	Sponsor Name	Drug Name / Subject
IND 48655	NOVARTIS PHARMACEUTICALS CORP	NVA237 GLYCOPYRRONIUM BROMIDE

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

/s/

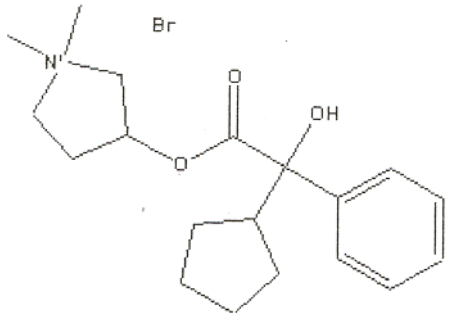
LAWRENCE F SANCILIO
03/30/2009

MOLLY E SHEA
03/30/2009
I concur.

PHARMACOLOGY/TOXICOLOGY REVIEW

IND number: 48,655
Sequence number/date/type of submission: DN 57, 1/15/10 SX
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Novartis Pharmaceutical Corporation

Reviewer name: Lawrence F. Sancilio, Ph.D.
Division name: Pulmonary and Allergy Products
Review completion date: 2/24/10
Drug: Trade name: Unknown
Generic name: Glypyrronium bromide, glycopyrrolate, USP
Code name: NVA237; NVP-NVA237**;** NVP-QAM254-DC-1.
Chemical name: R, S, 3-[(cyclopentylhydroxyphenylacetyl)oxy]-1, 1-dimethylpyrrolidinum bromide
CAS registry number: 596-51-0
Molecular formula/molecular weight: $C_{19}H_{28}BrNO_3$/ 398.34
Structure:



Relevant INDs/NDAs/DMFs: None.

Drug class: Quaternary ammonium antimuscarinic agent.

Intended clinical population: COPD.

Route of administration: Inhalation.

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

Drug History and Introduction

Novartis Pharmaceuticals is developing a clinical inhalation formulation of glycopyrronium bromide for the long term treatment of chronic obstructive pulmonary disease (COPD). The original IND was submitted 4/27/07. To date, the sponsor has completed 4-, 13, and 26 week inhalation toxicity studies in rats and 4 and 39-week inhalation toxicity in dogs, and has completed 6 clinical studies in COPD patients, 2 up to 400/480 mcg in a single dose study, 1 up to 100 mcg/day for 7 days, 1 at 50 mcg/day for 7 days and 2 up to 200/240 mcg for 28 days. A protocol was submitted on November 19, 2009 to conduct a 26- week inhalation study in COPD patients at a daily dose of 50 mcg. Currently, Novartis is conducting a two-year rat inhalation carcinogenicity at doses of 0.06, 0.2 and 0.6 mg/kg/day. The Executive Carcinogenicity Assessment Committee (ECAC) recommended these doses based on the MTD (decrease in body weight gained) on 10/6/06.

Glycopyrronium was approved in 1974 for the chronic treatment of peptic ulcers at a daily oral dose of 6 mg. No carcinogenicity studies have been conducted for the currently approved glycopyrronium formulations. In the 7/15/08 meeting with the sponsor, reference was made to the pre-IND meeting on 6/4/04 with the previous sponsor (Askaris) whereby FDA recommended a 6 month inhalation study in rats and pending the outcome of the inhalation and genotoxic studies, a single carcinogenicity study may be required.

In the current submission, the sponsor seeks concurrence for their proposed mouse carcinogenicity protocol and oral doses of (b) (4) mg/kg/day (submitted on January 15, 2010). (b) (4) The sponsor proposes to conduct a mouse carcinogenicity assay in the alternative TgrasH2 mouse. This assay is used for non-genotoxic and genotoxic compounds. In support of this proposal, two 4-week oral toxicity assays were conducted. This review will be submitted to the ECAC for concurrence of this reviewer's different recommended doses.

Studies reviewed within this submission:

A 7-day oral (gavage) dose range finding study in mice, No. 0770666 (Summary)

A 4-week dose oral (gavage) dose range finding study in Wild-Type CBF1 mice, No. 0770669
A 4-week dose oral (gavage) dose range finding study in Wild-Type CBF1 mice, No. 0970010

Study not reviewed within this submission:

A summary of the nonclinical pharmacokinetic data and the genetic toxicology studies are provided based on information summarized in the Investigator's Brochure (IB).

Reference ID: 3824703

Additionally, the following study does not contribute to the proposed mouse carcinogenicity and will be reviewed at a later date.

A 2-week subcutaneous dose range finding study in rats including sighting part, No. 0870723

Reference ID: 3824703

PHARMACOKINETICS/TOXICOKINETICS

Novartis has not conducted mouse pharmacokinetic studies. The publically available literature indicates that there was no reported bioavailability in animals. Plasma protein binding is low for humans, dogs and rats with unbound fractions between 51 and 75%. Only a minor fraction (<10%) distributes into blood cells. No lung metabolism was observed in lung microsomal incubations of rat, dog and human. In the dog and human liver microsomes, no major metabolism was detected. However, in the rat, extensive clearance was seen mainly by P450-mediated oxidative metabolism. The metabolic profiles of NVA237 in rat and human hepatocytes were comparable to the respective liver microsomal incubations, with up to nine phase 1 metabolites, including 5 mono and two bis hydroxylated metabolites. Based on in vitro data using pooled human liver microsomes no inhibition of cytochrome P450 enzymes at therapeutic concentrations of NDA237 is expected

In humans glycorpyrronium had a low oral bioavailability (<7%). After IV administration, it was found to be rapidly distributed and eliminated, with an elimination half life of 1 to 1.5 h. Following IV doses, mass balance urine and bile was almost complete with 85% of the dose excreted in urine, mostly as unchanged drug. Thus, GP is mainly eliminated renally and metabolism plays a minor role in the elimination process of systemically available drug.

TOXICOLOGY

Overall toxicology summary

The sponsor conducted a 1 week oral dose ranging toxicity study in Crl:CD1(Icr) male and female mice to determine doses for the pivotal 4-week assay in Wild-Type CBF1 mice. Glycopyrronium bromide was administered by gavage at doses of 0, 30 (LD), 100 (MD) and 300 (HD) mg/kg to groups of 10/sex/group. Glycopyrronium bromide was lethal to 1 male and 2 females at 300 mg/kg. These animals showed diarrhea, hunched posture and/or non-weight bearing of the left hindlimb. No clinical signs were seen at the 30 and 100 mg/kg doses. Morphological changes were seen in salivary glands (acinar hypertrophy), lungs (acute- subacute focal and multifocal inflammation), liver (increase in inflammatory foci), large intestine (mucosal hypertrophy/hyperplasia, inflammatory cell infiltration) and thymus (lymphoid depletion, lymphocytolysis) at ≥ 100 mg/kg/day. The NOAEL was 30 mg/kg with an associated AUC of 857 ng*h/ml and 26 ng*h/mL in males and females, respectively. MTD was considered <300 mg/kg. From this study, the doses of 30, 75 and 150 mg/kg were selected for a 4-week oral toxicity study in Wild-Type CBF1 mice.

Two 4-week oral toxicities were conducted in Wild-Type CBF1 mice. In the first study, the sponsor concluded that no toxicity was observed up to 150 mg/kg/day oral doses. The NOAEL was 150 mg/kg with an associated AUC of 94. ng.h/ml and 58.5 ng.h/ml in males and females, respectively.

Reference ID: 3824703

A second 4-week oral toxicity study was conducted in Wild-Type CBF1 mice at doses of 0 (control), 225, 300 and 750 mg/kg in males and 0 (control), 300, 500 and 750 mg/kg in females in view of the lack of toxicity in the 4-week study. In this study, a number of animals in all the treated groups died or were moribund and euthanized. The initial oral doses were 300 and 750 mg/kg (HD) in males and females. Due to toxicity and deaths occurring after a single dose (7/19 males and 6/20 females occurring between 2 and 5 days) at the 750 mg/kg dose, dosing stopped and the remaining animals sacrificed on day 7. A third dose was added after 1 week: 225 mg/kg males and 500 mg/kg females. In females receiving 500 mg/kg, there was severe toxicity and deaths (6/10) occurring between 1 and 4 days and dosing was stopped after 5 days, and the surviving 4 mice were euthanized on day 6. Both the 750 mg/kg and 500 mg/kg treated animals showed decreased activity, prostration, labored and shallow breathing, hunched posture, rigidity upon handling, little or no feces, urine stain, apparent hypothermia, dehydration, and ptosis; these signs began to be seen on day 2. Surviving males receiving 225 and 300 mg/kg showed decreased fecal output and ptosis and females receiving 300 mg/kg showed no clinical signs. These groups showed no change in body weight gain, although females manifested increased food consumption. At necropsy, the most notable change in organ weight was a decrease in the absolute (40%) and relative (35%) thymus weight in males at the 300 mg/kg dose. In the histopathology evaluation, organs from the animals at termination and from the animals that died or were euthanized were reported separately. At the 29 day termination, where the doses were 225 and 300 mg/kg in males, pathology with the exception of the liver was only seen at the 300 mg/kg dose. The targeted organs were the adrenal glands, kidney, lacrimal gland, liver, lung, mesenteric lymph node, prostate, spleen, thymus and urinary bladder. In females, the targeted organs in the 300 mg/kg group were the adrenal glands, mesenteric lymph node, mandibular salivary gland, spleen, thymus and parotid and salivary gland. The incidences in the males were higher than the females which may be attributed to the exposure of glycopyrronium bromide being higher in males. In the 750 and 500 mg/kg treated animals where euthanasia was scheduled and in all treated groups that were dead or moribund and euthanized, the target organs were similar to those seen in organs examined from the lower dosed animals at termination (day 29). In addition, the heart and bone marrow were targeted in animals that were dead or moribund and euthanized. In this study, there were deaths at all doses. Therefore, MTD was exceeded.

Genetic Toxicology: In a complete genetic toxicology battery (reverse bacterial mutagenicity assay, chromosomal aberration assay and an in vivo rat micronucleus assay), glycopyrronium was negative.

Study title: **A 7-Day (gavage) dose range finding study in mice, No. 0770666**

Key findings:

- Oral doses were 30, 100 and 300 mg/kg.
- Mortality occurred at 300 mg/kg/day.
- Morphological changes were seen in salivary glands (acinar hypertophy), lungs (acute- subacute focal and multifocal inflammation), liver (increase in

Reference ID: 3824703

inflammatory foci), large intestine (mucosal hypertrophy/hyperplasia, inflammatory cell infiltration) and thymus (lymphoid depletion, lymphocytolysis) at ≥ 100 mg/kg/day
- Males showed higher AUCs than females.
- The oral NOAEL was 30 mg/kg with an associated AUC of 857 ng*h/ml and 26 ng*h/ml in males and females, respectively.

Study no: No. 077066
Volume # and page #: EDR.
Conducting laboratory and location: Development, preclinical safety, Novartis Pharma AG, Basel Switzerland
Date of study initiation: NA
GLP compliance: No
QA report: No
Drug, Batch No. and % purity: NVA237 (glycopyrronium bromide), 0623005, 100%.
Control vehicle: Milli-Q Gradient A10 water
Methods
Species/strain: Mouse, Crl: CD1 (Icr)
#/Sex/group: 10/sex/group
Age: 10 Weeks.
Weight: F, 26-35 g; M, 34-42 g.
Doses of glycopyrronium bromide are those of the base. The dosing groups were C, 10 ml/kg, 30 mg/kg (LD), 100 mg/kg (MD), 300 mg/kg (HD).

Observations:

Mortality: Daily.
Clinical signs: Daily.
Body weights: Measured using an electronic balance.
Food consumption: Mean feed was measured but time was not indicated.
Ophthalmoscopy: Not examined
Hematology: Not examined
Clinical chemistry: Not examined.
Urinalysis: None conducted.
Toxicokinetics: After the 1st administration and at the end of the study, 2 animals/sex/group/time point were samples: 0.5, 1, 3, 7 and 24 h post-dose and assessed for plasma levels of NVA237 using HPLC/MS/MS (LLOQ = 0.1 ng/L).
Gross pathology: Animals were necropsied at the end of the dosing period.
Organ weights: The brain, heart, liver and testes were weighed.
Histopathology: Tissues were sampled from all animals tested. All gross lesions were also sampled and examined. As this was a dose range findings study only a sample of tissues were examined microscopically.
Adequate Battery: yes ( ), no (X)
Peer review: yes ( ), no (X)

Reference ID: 3824703

Results
Mortality: Drug related deaths were observed in HD animals. One main study animal (male no. 4005) died after the 7th dose and one male (no. 4001) was sacrificed on day 6. One main group females (no. 4507) was found dead after treatment on Day 3. Additionally, one satellite animals was found dead after the first dose (No. 4513).

Clinical signs: Clinical signs of piloerection, diarrhea (no. 4005 only), hunched posture (no. 4001 only) and non-weight bearing left hind limb (animal no. 4510 only) were observed at 300 mg/kg/day dose.

Body Weight: There was slight body weight loss in the HD males and females. HD, mean ±SD, males: C, 0.7±1.5 g; T, -3.4±2.3 g; females: C, 0.1±1.3 g; T, -1.5±1.8 g.

Food Consumption: Females reduced food intake in the HD group.

Toxicokinetics: NA

Necropsy: See summary below.

Organ Weights: There were no drug-related changes in organ weights.

Histopathology: In females, the target organs were: lungs (MD, HD), salivary gland (MD, HD), large intestine (HD), spleen (MD, HD), thymus (MD, HD) and liver (LD, MD, HD). The severity was minimal to moderate. The report considered the lesions seen in the lymphoid system, liver and large intestine to be not treatment related but due to stress and poor condition. This is questionable since these animals showed no clinical signs and a decrease in body weight gained (HD, mean ±SD, males: C, 0.7±1.5 g; T, -3.4±2.3 g; females: C, 0.1±1.3 g; T, -1.5±1.8 g) that was not considered significant because of the high variability.

Study title: A 4-week dose range finding study of NVA237 administered by oral gavage in Wild-Type CB6F1 mice

Key findings:

- Oral doses were 30, 75 and 150 mg/kg.
- 1 HD female died on Day 26 that the sponsor attributes to stress.
- No significant toxicity was observed.
- Males showed higher AUCs than females.
- The oral NOAEL was 150 mg/kg with an associated AUC of 94. ng.h/ml and 58.5 ng.h/ml in males and females, respectively.

Study no: No. 077069
Volume # and page #: EDR.

Reference ID: 3824703

Conducting laboratory and location: (b) (4)

Date of study initiation: 6/26/08
GLP compliance: Yes.
QA report: yes (X)
Drug, Batch No. and % purity: glycopyrronium bromide, 0724006, 100%.
Control vehicle: Milli-Q Gradient A10 water
Methods
Species/strain: Wild-Type CB6F1 mice.
#/Sex/group: Main group, 10: toxicokinetics group; C, 4; T, 12.
Age: 11 Weeks.
Weight: M, 26-32g; F, 21-26 g.
Doses of glycopyrronium bromide are those of the base. The dosing groups were C, 5 ml/kg, 30 mg/kg (LD), 75 mg/kg (MD), 150 mg/kg (HD).
Selection of doses: In a 7-day oral dose range finding toxicity study in CD-1 mice, 30 mg/kg was well tolerated; doses ≥ 100 mg/kg showed some histopathology and 300 mg/kg was lethal.
Duration of dosing: 28 days.

Observations:

Analysis of Formulation: Dose formulations were determined on day -1 and day 28.
Mortality: Daily.
Clinical signs: Daily.
Body weights: Days -2/-1, 1 and weekly thereafter.
Food consumption: Weekly.
Ophthalmoscopy: Days -8/-7 and on day 27.
Hematology: At the scheduled sacrifice of 28 days. In the method section, the time point was day 92, a probable mistake. Analysis was from 5 /sex/group. A complete analysis was conducted.
Clinical chemistry: At the scheduled sacrifice of 28 days. In the method section, the time point was day 92, a probable mistake. Evaluation was made on unscheduled sacrificed animals. Analysis was from 5 /sex/group. A complete analysis was conducted.
Urinalysis: None conducted.
Toxicokinetics (Day 28): Control blood sample was taken at 0 hour; for treated animals, blood was taken at 0, 0.5, 1, 3, 7 and 24 hours post treatment. At each time point, blood was taken from 2 males and 2 females and pooled. Analysis of the plasma was a liquid-solid extraction followed by analysis of the extract by LC-M/MS using etectrospray ionization. The LLOQ was 0.10 ng/ml.
Gross pathology: At necropsy (day 29), examination was made of the carcass, skeletal muscle, external surfaces and orifices, cranial cavity and external surfaces of the brain and thoracic, abdominal and pelvic cavities with their associated organs.
Organs weighed and tissues examined microscopically are presented in the following table. Histopathology was conducted on all tissues in the control and HD groups and in animals euthanized in a moribund condition and to a limited number in the LD and MD animals.

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Tissue list for collection, weighing (W) and/or processing (P)

W	P	Adrenal gland (paired)		P	Mammary gland
	P	Aorta		P	Nerve, optic (paired)[b]
	P	Bone, femur		P	Nerve, sciatic
	P	Bone, sternum	W	P	Ovary with oviduct (paired)
	P	Bone marrow, sternum		P	Pancreas
	P	Bone marrow smear[a]	W[d, e]	P	Parathyroid gland[d]
W	P	Brain (cerebrum, cerebellum, brain stem, medulla)	W[e]	P	Pituitary gland
	P	Cervix	W	P	Prostate gland
	P	Epididymis (paired)		P	Salivary gland (paired)
	P	Esophagus		P	Seminal vesicle (paired)
	P	Eye (paired)[b]		P	Skeletal muscle (thigh)
		Gallbladder		P	Skin (mammary)
	P	Harderian gland (paired)		P	Spinal cord (cervical, thoracic, lumbar)
W	P	Heart	W	P	Spleen
	P	Intestine, cecum		P	Stomach (glandular and nonglandular)
	P	Intestine, colon	W	P	Testis (paired)
	P	Intestine, duodenum	W	P	Thymus
	P	Intestine, ileum with Peyer's patch[c]	W[d, e]	P	Thyroid gland (paired)
	P	Intestine, jejunum		P	Tongue
	P	Intestine, rectum		P	Trachea
W	P	Kidney (paired)		P	Ureter (paired; cross section)
	P	Lachrymal gland (paired)		P	Urinary bladder
	P	Larynx (cross section)	W	P	Uterus
W	P	Liver		P	Vagina
	P	Lung		P	Macroscopic lesions
	P	Lymph node – mandibular			Animal identification
	P	Lymph node – mesenteric			

[a]Bone marrow smears were collected from the femur at scheduled necropsies for possible examination.
[b]Preserved in Davidson's fixative and then transferred to 10% neutral buffered formalin.
[c]Examined only if present in the routine section.
[d]The thyroid and parathyroid glands were weighed together.
[e]Post-fixed weight.

Adequate Battery: yes (X), no ( )
Peer review: yes (X), no ( )

Results

Analysis of formulations: Dose formulations were within the acceptable limits of ± 10% error.
Mortality: None, due the treatment. One female in the HD was moribund and was euthanized on day 26. This animal showed decreased activity, few feces and dyspnea. Lymphoid depletion was the only histopathology seen, and the sponsor attributed the death to experimental manipulation during dosing. Considering clinical signs of toxicity were observed in this animal, this death may be related to drug. The deaths of the 2 mice in the toxicokinetics group that died occurred shortly after dosing. Most likely, this was attributed to the dosing procedure. Gross necropsy did not indicate the cause of death.

Clinical signs: None.

Body Weight: No effect.

Food Consumption: No effect.

Ophthalmoscopy: No effect.

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Hematology: No effect.

Clinical Chemistry: No effect.

Toxicokinetics: The results are presented in the following table excerpted from the submission. The results show that there was a dose related increase in the AUC in the LD and MD males and there was no increase at the HD suggesting saturation; the LD, MD and HD females showed a doses relationship. The increase was supbproportional by 1.34 between the MD and HD and supraproportional by 59 between the LD and MD. Males showed higher AUCs than females.

Table 4-1 Mean toxicokinetic parameters of NVA237 in mouse plasma

Parameter	Units	Males	Females
	Dose: 30 mg/kg/day		
t_{max}	h	7	1
C_{max}	ng/mL	0.995	0.269
C_{max}/dose	(ng/mL)/(mg/kg/day)	0.0332	0.00897
$AUC_{(0-24h)}$	ng.h/mL	11.8	0.738
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	0.394	0.0246
	Dose: 75 mg/kg/day		
t_{max}	h	7	0.5
C_{max}	ng/mL	9.11	75.8
C_{max}/dose	(ng/mL)/(mg/kg/day)	0.121	1.01
$AUC_{(0-24h)}$	ng.h/mL	100	43.5
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	1.33	0.579
	Dose: 150 mg/kg/day		
t_{max}	h	0.5	3
C_{max}	ng/mL	144	15.5
C_{max}/dose	(ng/mL)/(mg/kg/day)	0.960	0.103
$AUC_{(0-24h)}$	ng.h/mL	94.0	58.5
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	0.627	0.390

$AUC_{(0-24h)}$/dose was determined in order to normalize $AUC_{(0-24h)}$ to a dose of 1 mg/kg/day (dose expressed as quaternary cation of bromide salt). The results are presented in the figure below:

Necropsy: None.

Organ Weights: Males, Absolute: Thymus, MD, -22%; HD, -27%.
Relative: Thymus, MD, -20%; HD, -22%.
Absolute: Ovaries/Oviducts, LD, -14; MD, -25%; HD, -22%.
Relative: Ovaries/Oviducts, LD, -14; MD, -22%; HD, -18%.

Histopathology: The HD female (no. 9355) that died on Day 26 showed mild lymphoid depletion at death. No other histopathology was observed.

Study title: A repeat 4-week dose oral (gavage) dose range finding study in Wild-Type CBF1 mice

Key findings:

Reference ID: 3824703

- Test oral doses were 225 (28 days), 300 (28 days), and 750 (1 day) mg/kg in males and 300 (28 days), 500 (5 days), and 750 (1 day) mg/kg in females.
- At all doses, there was lethality. The incidences and days of deaths were: males: 225 mg/kg, 3/20 (day 2); 300 mg/kg, 8/20 (day 2), 750, 7/20 (day 1); females, 300 mg/kg, and 2/20 (days of deaths, not stated), 500, 10/20 (day 1); 750 mg/kg, 6/10 (day 1).
- Males showed higher AUCs than females by 176 fold at 300 mg/kg.
- At the scheduled termination, targeted organs were the adrenal glands, kidney, lacrimal gland, liver, lung, mesenteric lymph node, salivary and parotid salivary glands, prostate, spleen, thymus and urinary bladder.
- In scheduled euthanasia animals at 5 and 7 days, the target organs were the adrenal glands, lacrimal gland, liver, lung, mesenteric and mandibular lymph nodes, salivary and parotid salivary glands, spleen and thymus.
- In the unscheduled euthanized animals and animals found dead, the targeted organs were the adrenal glands, bone marrow, heart, kidney, liver, lung, mesenteric and mandibular lymph nodes spleen, thymus, mandibular and parotid salivary glands, urinary bladder and uterus.
- The MTD was exceeded.

Study no: No. 0970010
Volume # and page #: EDR.
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/2/09
GLP compliance: Yes.
QA report: yes (X)
Drug, Batch No. and % purity: glycopyrronium bromide, 0724008, 99.4%.
Control vehicle and volume: Milli-Q Gradient A10 water, 5 ml/kg.
Methods
Species/strain: Wild-Type CB6F1 mice.
#/Sex/group: Main group, 10; in each of the 2 control groups, there were 5. In the toxicokinetics group there were 4 in each of the control groups and 10 in each of the treated groups.
Age: 9 Weeks.
Weight: M, 26-31g; F, 21-24 g.
Doses of glycopyrronium bromide are those of the free base.
Doses: There were 2 separate studies: Study 1 and 2
In Study 1: C (Group 1), 300 mg/kg (Group 3, both sexes) and 750 mg/kg (Group 4, both sexes).
In Study 2: C (Group 2), 225 mg/kg (Group 5, males) and 500 mg/kg (Group 5, females).
This study was conducted 1 week after Study 1 was initiated as described as follow.

Study 1, 4/9/09: C , 300 mg/kg and 750 mg/kg (males, toxicity)
C, 300 mg/kg and 750 mg/kg (toxicokinetics, both sexes)
4/10/09: C, 300 mg/kg and 750 mg/kg (females, toxicity)
Study 2, 4/16/09: C and 225 mg/kg (males, toxicity)

Reference ID: 3824703

C and 225 mg/kg (males, toxicokinetics)
C and 500 mg/kg (females, toxicokinetics)
4/17/09, C and 500 mg/kg (females, toxicity)

Selection of doses: In a 28-day oral toxicity study No. 077066 in Wild-Type CB6F1 mice, there was no notable systemic toxicity at 150 mg/kg.
Duration of dosing: 28 days.

Observations:

Analysis of Formulation: Days -1, 7 and 20.
Mortality: Daily.
Clinical signs: Daily.
Body weights: Determined on the following days, excerpted from the submission.

Pretest period:	Day -9 (Group 2 and 5 females), Day -8 (Group 2 and 5 males), Day -2 (Group 1, 3, and 4 females), and Day -1 (Group 1, 3, and 4 males)
Dosing period:	Days 1, 8, 15, 22, 28, and 29 (Group 1/2 and 3; Group 5 females)
	Day 1 and 6 (Group 5 females)
	Day 1 and 7 (Group 4)

Food consumption: Determined on the following days, excerpted from the submission.

Food consumption measurements were recorded on Days 1, 8, 15, 22, and 28 for Groups 1/2 and 3 and Group 5 females, on Days 1 and 6 for Group 4, and on Days 1 and 7 for Group 5 males.

Ophthalmoscopy: Determined on the following days, excerpted from the submission.

Pretest:	Days -11 (Group 2 and 5 females)/Day -10 (Group 2 and 5 males)/Day -4 (Group 1, 3, and 4 females)/Day -3 (Group 1, 3, and 4 males)
Week 13:	Day 25 (surviving females)/26 (surviving males)

Hematology and Clinical Chemistry: The scheduled times for evaluation are listed in the following table excerpted from the submission.

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Group nos.	Time point	Hematology	Clinical chemistry
5 (females only)	Day 6	x	x
4 (males and females)	Day 7	x	x
1, 2, 3, 5 (males only)[a]	Day 29	x	x
Unscheduled sacrifice	Prior to euthanasia	--	x

Note: x = scheduled sample collection.
[a]Approximately one-half of the animals per sex in each group were bled for hematology and the other half per sex in each groups were bled for clinical chemistry. If the remaining number of animals per sex in a group was uneven, the weighting was on clinical chemistry.

Urinalysis: None conducted.

Toxicokinetics (Day 28): Control blood sample was taken at 0 hour; for treated animals, blood was taken at 0, 0.5, 1, 3, 7 and 24 hours post treatment. At each time point, blood was taken from 2 males and 2 females and pooled. Analysis of the plasma was a liquid-solid extraction followed by analysis of the extract by LC-M/MS using electrospray ionization. The LLOQ was 0.10 ng/ml.

Gross pathology: The scheduled days of necropsy are listed in the following table excerpted from the submission. At necropsy, examination was made of the carcass, skeletal muscle, external surfaces and orifices, cranial cavity and external surfaces of the brain and thoracic, abdominal and pelvic cavities with their associated organs.

Group no.	No. of male/female mice	Scheduled euthanasia day	Pathology procedures			Histopathology
			Necropsy	Tissue collection	Organ weights	
1	5/5	29	x	x	x	Full
2	5/5	29	x	x	x	Full
3	10/10	29	x	x	x	Limited
4	10/10	7	x	x	x	--
5	10 male	29	x	x	x	Full
5	10 female	6	x	x	x	Full
Animals found dead or euthanized moribund			x	x	--	Full

Note: x = procedure conducted.

Organs weighed and tissues processed are presented in the following table excerpted from the submission. Tissues examined were from Groups 1, 2, 3 and 5 and from animals that died or were moribund and euthanized.

Adequate Battery: yes (X), no ( )
Peer review: yes (X), no ( )

Reference ID: 3824703

Tissue list for collection, weighing (W) and/or processing (P)

W[a]	P	Adrenal gland (paired)		P	Mammary gland[b]
	P	Aorta		P	Nerve, optic (paired)[b, c]
	P	Bone, femur[d]		P	Nerve, sciatic
	P	Bone, sternum[d]	W	P	Ovary with oviduct (paired)
	P	Bone marrow, sternum		P	Pancreas
	P	Bone marrow smear[e]	W[a, f]	P	Parathyroid gland
W	P	Brain (cerebrum, cerebellum, brain stem, medulla)	W[a]	P	Pituitary gland
	P	Cervix	W	P	Prostate gland
	P	Epididymis (paired)		P	Salivary gland (mandibular, parotid, sublingual)[g]
	P	Esophagus		P	Seminal vesicle (paired)
	P	Eye (paired)[c]		P	Skeletal muscle (thigh)
		Gallbladder		P	Skin (inguinal)
	P	Harderian gland (paired)[c]		P	Spinal cord (cervical, thoracic, lumbar)
W	P	Heart	W	P	Spleen
	P	Intestine, cecum		P	Stomach (glandular and nonglandular)
	P	Intestine, colon	W	P	Testis (paired)[h]
	P	Intestine, duodenum	W	P	Thymus
	P	Intestine, ileum with Peyer's patch	W[a, f]	P	Thyroid gland (paired)
	P	Intestine, jejunum		P	Tongue
	P	Intestine, rectum		P	Trachea
W	P	Kidney (paired)		P	Ureter (cross section)[g]
	P	Lachrymal gland (paired)		P	Urinary bladder
	P	Larynx (cross section)	W	P	Uterus
W	P	Liver		P	Vagina
	P	Lung (*en bloc*)[i]		P	Macroscopic lesions
	P	Lymph node, mandibular			Animal identification
	P	Lymph node, mesenteric			

[a]Post-fixed weight.
[b]Examined only if present in the routine sections of eyes (optic nerves) or skin (mammary gland) of males only.
[c]Preserved in Davidson's fixative and then transferred to 10% neutral buffered formalin.
[d]Bone decalcified prior to sectioning
[e]Bone marrow smears were collected from the femur at scheduled necropsies for possible examination.
[f]The thyroid and parathyroid glands were weighed together.
[g]Bilateral for collection, unilateral for histopathology.
[h]Preserved in modified Davidson's fixative and then transferred to 10% neutral buffered formalin.
[i]Infused with 10% neutral buffered formalin.

Results

Analysis of formulation: Analysis of Formulation: Dose formulations were within the acceptable limits of 10 % error.

Mortality: The following tables excerpted from the submission present a summary of the deaths in the toxicity and toxicokinetics phases and the day of their deaths. Administration of a single dose to Group 4 (750 mg/kg) resulted in immediate toxicity and deaths; dosing ceased and both males and females were euthanized on day 7. Group 5 females (500 mg/kg) were euthanized on day 6 after 5 days of dosing due to severe clinical signs and mortality.

Reference ID: 3824703

Table 4-1 Summary of mortality – toxicity and toxicokinetic phases

	Dose level (mg/kg/day)			No. dead/no. dosed	
Group no.	Base	Salt	Sex	Toxicity phase	Toxicokinetic phase
1/2[a]	0	0	Male	0/10	0/8
			Female	0/10	1/8[b]
3	300	375	Male	3/10	5/10
			Female	0/10	2/10
4	750[c]	938	Male	4/9[d] (5/10)	3/10
			Female	2/10	4/10
5	225	281	Male	2/10	1/10
	500[e]	625	Female	6/10	4/10

[a] The control group was stagger-started as Groups 1 and 2 which were later combined into a single group designated as Group 1/2.
[b] Although the definitive cause of death could not be determined, the death appeared to be related to a liver finding rather than a dosing error.
[c] These results are based on a single dose administration; dosing was ceased. Group 4 males and females were terminated on Day 7.
[d] One gavage error was observed in a single Group 4 male.
[e] These results are based on repeated dose administration; however, based on mortality/moribundity, the Group 5 females were terminated on Day 6 (no dosing holiday prior to termination).

Table 4-2 Individual mortality and fate – toxicity phase

		Dose level (mg/kg/day)				
Sex	Group no.	Base	Salt	Animal no.	Day of death	Fate
Males	3	300	375	7848	2	Found dead
				7843	3	Found dead
				7849	3	Found dead
	4	750	938	7854	1	Found dead/ accidental injury
				7858	5	Moribund
				7855	3	Moribund
				7860	2	Moribund
				7861	4	Moribund
	5	225	281	7872	2	Found dead
				7868	4	Moribund
Females	4	750	938	7895	4	Found dead
				7900	2	Moribund
	5	500	625	7903	3	Found dead
				7905	2	Found dead
				7907	1	Found dead
				7910	2	Found dead
				7911	4	Found dead
				7912	3	Found dead

Clinical signs: Group 4 males and females and Group 5 females that were terminated showed decreased activity, prostration, labored and shallow breathing, hunched posture, rigidity upon handling, little or no feces, urine stain, apparent hypothermia, dehydration, and ptosis starting on day 2. Groups 3 and 5 males that survived until day 29 showed decreased fecal output and ptosis, while Group 3 females showed no clinical signs.

Body Weight Gained: There was no effect on body weight gain

Food Consumption: Day 22-28, Males, 225 and 300 mg/kg: No effect; Females, 300 mg/kg, + 22%.

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Ophthalmoscopy: No effect. No data was submitted except that examination of the eyes was made by a board- certified veterinary ophthalmologist.

Hematology: The results are summarized in the following tables.

Males

There was no meaningful data in the 300 mg/kg group since there was only one available sample; 2 samples clotted and could not be analyzed. In the 750 and 500 mg/kg group, termination was made on days 7 and 5, respectively. There were 4 samples in the control and 225 mg/kg groups

Parameter	**% Change from Control**
	225 mg/kg
Reticulocytes	-15.0
Eosinophils	-39.3

Females

There was no meaningful data in the 500 mg/kg group since termination occurred on day 6 and in the 750 mg/kg group termination occurred on day 7. There were 5 samples in the 300 mg/kg group and 4 samples in the control group.

Parameter	**% Change from Control P<0.05**
	300 mg/kg
Eosinophils	-31.7

Clinical Chemistry: The results are summarized in the following tables.

Males

There was no meaningful data in the 750 mg/kg group since termination occurred on day 7 due to morbidity/mortality. There were 4 males in each treated group and 6 males in each control group. The changes in the globulin levels and A/G ratios were minor. Significant increase in the phosphorous levels occurred at the 300 mg/kg dose, and there was an increasing trend based on what was observed at the 225 mg/kg dose

Parameter	**% Change from Control, P<0.05**	
	225 mg/kg	**300 mg/kg**
Globulin	+8.3	+10.6
A/G	-0.7	-10.3
Phosphorous	+10.9 NS	+35.4

NS, Not significant

Reference ID: 3824703

Females

There were no meaningful data in the 500 and 750 mg/kg group since early termination occurred due to morbidity/mortality. There were 6 females in the control group and 5 mice in the 300 mg/kg group. Meaningful increases occurred in the total bilirubin levels.

Parameter	**% Change from Control P<0.05**
	300 mg/kg
Total Bilirubin	+138
Cholesterol	+13
Magnesium	+12

Toxicokinetics: The results are presented in the following table excerpted from the submission. The results show that there was a dose related increase in the AUC in the 225 and 300 mg/kg males. The increases were not dose proportional; they were superproportional, by 19 fold. Males at 300 mg/kg showed a higher AUC than the AUC for 300 mg/kg in females. The 6690 ng.h/ml AUC for the 300 mg/kg dose in males was unexpectedly high because of high inter-animal variability. Reanalyzing 4 random samples showed a difference from the original sample of 2.5%, -13.7%, -12.5% and -5.3% indicating that these were true values.

Parameter	Units	Males	Females
	Dose: 225 mg/kg/day		
t_{max}	h	0.5	---
C_{max}	ng/mL	238	---
C_{max}/dose	(ng/mL)/(mg/kg/day)	1.06	---
$AUC_{(0-24h)}$	ng.h/mL	350	---
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	155	--
	Dose: 300 mg/kg/day		
t_{max}	h	0.5	0.5
C_{max}	ng/mL	11900	12.2
C_{max}/dose	(ng/mL)/(mg/kg/day)	39.7	0.0407
$AUC_{(0-24h)}$	ng.h/mL	6690	37.9
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	22.3	0.126

Necropsy (Day 29)

Organ Weight: The results are presented in the following tables: Organ weight relative to brain weight was similar in both sexes to organ weight relative to body weight and therefore, only changes relative to body weight were reported. Based on absolute and relative weights, activity was seen in both sexes at 300 mg/kg. In males, the heart, kidneys and thymus were decreased; the change in the thymus gland was moderate while those in the heart and kidneys were small. Similarly, in females the decrease in heart and kidney weights was small. The decrease in the ovaries/oviduct and pituitary was moderate.

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Males

Organ	% Change from Control, P<0.05			
	225 mg/kg		300 mg/kg	
	Absolute	Relative to BW	Absolute	Relative to BW
	N=7		N=8	
Heart	-5 NS	-1.1 NS	-8	-0.6 NS
Kidneys	-5 NS	-1.5 NS	-12	-4 NS
Thymus	-6 NS	-1.1 NS	-40	-35

Females

Organ	% Change from Control, P<0.05	
	300 mg/kg	
	Absolute	Relative to BW
	N=10	
Heart	-11	-6.2 NS
Kidneys	-10	-5.2
Ovaries/Oviducts	-22	-17
Pituitary	-26	-23

NS, P>0.05

Histopathology

The results are summarized in the following tables.

Terminal sacrifice on Day 29

The results are presented in the following tables. The following scoring system was used to present the severity of the lesion: 1, minimal, 2, mild, 3, moderate, 4, marked. The results are presented as the mean severity score ( ). In males, histopathology was seen mainly in the 300 mg/kg group. Targeted organs were the adrenal glands, kidney, lacrimal gland, liver, lung, mesenteric lymph node, prostate, spleen, thymus and urinary bladder. In the 225 mg/kg group, the liver was the only targeted organ. In the 300 mg/kg treated females, not all the tissues were examined. The target organs were the adrenal glands, liver, mesenteric lymph node, mandibular and parotid salivary glands, spleen and thymus.

Reference ID: 3824703

Organ Observation	Incidence (mean severity score)				
	Male			Female	
	C	Group 3 225 mg/kg	Group 5 300 mg/kg	C	Group 3 300 mg/kg
	N=10	N=8	N=7	N=10	N=10
Adrenal glands					
Hypertrophy: diffuse	0	0	7(1.0)	0	10(1.8)
Kidney					
Infarction: focal	0	0	1(3.0)	0	NE
Liver					
Inflammation: chronic, focal	3 (1.0)	4(1.0)	6(1.1)	6(1.0)	9(1.0)
Depletion (glycogen)	0	0	1(2.0)	0	0
Lymph node, mesenteric					
Hyperplasia: follicular	3 (1.0)	6(1.5)	4(1.5)	3 (1.3)	4(1.5)
Lymphoid: necrosis	0	0	6(1.0)	3	0
Prostate					
Inflammation: subacute, focal	0	0	1 (3.0)	-	-
Salivary gland: mandibular					
Hypertrophy: cellular, diffuse	0	0	0	0	7(1.4)
Spleen					
Hematopoiesis	0	0	1 (1.0)	0	1(1.0)
Necrosis: lymphoid	0	0	2 (1.0)	0	0
Thymus					
Necrosis: lymphoid	0	0	2(1.0)	0	2 (1.0)
Depletion: lymphoid, diffuse	0	0	6(1.3)	0	0
Salivary gland, Parotid					
Atrophy: cellular, diffuse	0	0	0	0	4(1.5)

NE, Not examined

Scheduled Euthanasia

Group 4 animals were euthanized on day 4 after a single dose of 750 mg/kg, and Group 5 animals were euthanized after 5 daily doses of 500 mg/kg on day 6. For Group 4, not all the organs were examined. The target organs were similar in both sexes with the exception of the mandibular lymph node which was not targeted in males; the targeted organs were: adrenal gland, liver, mesenteric lymph node, mandibular and parotid salivary glands, spleen and thymus gland. In Group 5, the target organs were: adrenal gland, lacrimal gland, liver, lung, mesenteric and mandibular lymph node, mandibular and parotid salivary glands, and spleen and thymus gland.

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Organ Observation	**Incidence** (mean severity score)		
	Male	**Female**	
	Group 4 750 mg/kg	Group 5 500 mg/kg	Group 4 750 mg/kg
	N=5	N=4	N=8
Adrenal gland			
Hypertrophy: Diffuse	5(1.5)	2(1.5)	7(1.7)
Pigmentation	1(1.0)	0	0
Lacrimal gland			
Atrophy	NE	1(2.0)	NE
Inflammation: chronic, multifocal	NE	1(2.0)	NE
Liver			
Inflammation: chronic, focal	1(1.0)	1(1.0)	7(1.0)
Depletion: glycogen	4(1.3)	0	7(1.3)
Lung			
Inflammation: chronic, focal	NE	1(1.0)	NE
LN, Mesenteric			
Hyperplasia: follicular	2(1.5)	1(1.0)	2(1.0)
Necrosis: lymphoid	2(2.0)	0	2(1.5)
LN Mandibular			
Hyperplasia: follicular	0	3(1.5)	1(1.0)
Salivary gland, Mandibular			
Hypertrophy: cellular, diffuse	2(1.0)	3(2.0)	7(2.1)
Hypertrophy: cellular, multifocal	0	1(1.0)	0
Spleen			
Hematopoiesis	5(2.0)	3(1.7)	7(2.3)
Pigmentation	1(2.0)	2(2.0)	1(2.0)
Thymus			
Depletion: lymphoid, diffuse	4(2.8)	3(3.3)	6(1.7)
Necrosis: lymphoid	4(2.5)	1(1.0)	3(1.0)
Salivary gland, Parotid			
Inflammation: acute, diffuse	0	2(2.5)	0
Atrophy: cellular, diffuse	4(1.8)	3(2.1)	7(1.1)
Inflammation: chronic, focal	1(1.0)	0	0

NE, Not examined

Moribund Euthanasia/Found Dead
Autolysis in some animals precluded evaluation

Not all the tissues were examined. In males, the target organs were: adrenal glands, bone marrow, heart, kidney, liver, lung, mesenteric and mandibular lymph nodes, parotid and mandibular salivary glands, spleen, thymus and urinary bladder. In females, the same organs were targeted except kidney which was not examined; in addition, the uterus was targeted.

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Organ Observation	Incidence (mean severity score)				
	Male			Female	
	Group 3 225 mg/kg	Group 4 300 mg/kg	Group 5 750 mg/kg	Group 4 500 mg/kg	Group 5 750 mg/kg
	N=2	N=3	N=5	N=6	N=2
Adrenal gland					
Congestion	0	0	0	4(2.0)	0
Hypertrophy: Diffuse	0	2 (2.0)	4(1 5)	3(1.7)	2 (2.5)
Necrosis	0	0	0	0	1(1.0)
Bone Marrow					
Congestion	1(2.0)	1(2.0)	NE	3(2.0)	NE
Heart					
Necrosis: myocardial , focal	1(1.7)	1 (1.0)	0	1 (1.0)	0
myocardial , multifocal	0	0	3(1.7)	0	0
Kidney					
Congestion	0	2 (2.0)	NE	0	NE
Liver					
Congestion	1(2.0)	2 (2.0)	0	0	0
Depletion: glycogen	1(2.0)	0	3(2 1)	2 (2.0)	2(2.5)
Lung					
Congestion	1(3.0)	3(3.0)	NE	5(3.0)	NE
LN Mesenteric					
Depletion: lymphoid	1(2.0)	1(4.0)	1(2.0)	1(2.0)	0
Necrosis: lymphoid	1(1.0)	3(2.0)	4(2 5)	0	1(2.0)
LN Mandibular					
Depletion: lymphoid	1(2.0)	2(2.0)	0	0	0
Necrosis: lymphoid	0	1(2.0)	3(2.0)	2(2.0)	1(2.0)
Salivary gland, Mandibular					
Hypertrophy: cellular, diffuse	0	1(1.0)	2(2.0)	6(3.1)	2(3.5)
Hypertrophy: cellular, multifocal	1(2.0)	0	2(1.0)	0	0
Spleen					
Congestion	0	0	1(2.0)	0	0
Depletion: lymphoid	1(3.0)	3(2.7)	2(2.0)	1(2.0)	0
Necrosis: lymphoid	1(2.0)	2(2.0)	4(2.0)	2(2.0)	2(2.5)
Thymus					
Depletion: lymphoid, diffuse	1(4.0)	3(2 3)	5(3.4)	2(2.0)	2(1.5)
Necrosis: lymphoid	2(2.5)	3(3 3)	5(2.8)	4(2.5)	2(3.5)
Urinary bladder					
Dilation	0	3(2.7)	NE	0	NE
Salivary gland, Parotid					
Atrophy: cellular, diffuse	2(1.5)	3(1.7)	5(1.8)	6(1.5)	1(1.0)
Vacuolation	1(1.0)	0	0	1(2.0)	0

NE, Not examined

Genetic toxicology

Three GLP genetic toxicity studies have been completed using NVA237. GP did not induce mutations in the presence or absence of S-9 mix up to 5000 mcg/plate in the reverse mutation assay. In the chromosomal aberration assay conducted in human peripheral lymphocytes, GP did not induce clastogenicity in the presence or absence of S-9 mix up to 3983 mcg/ml. In the in vivo bone marrow micronucleus assay conducted in rats, GP did not induce clastogenicity at oral doses up to 1000 mg/kg/day. Therefore, GP was negative for inducing genetic toxicity.

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OVERALL SUMMARY, DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Summary and Discussion

This was a request for a special protocol assessment to support doses in the alternative TgRasH mouse carcinogenicity assay based on two 4-week oral toxicity studies in the Wild-TypeCB6F1 mice. To determine the doses for the 4-week toxicity study, a 7-day oral (gavage) dose finding toxicity study was conducted in Crl:CD1(Icr) mice at oral doses were 30, 100 and 300 mg/kg (10 mice/sex/group). One male and 2 female died. Although there were no clinical signs in the surviving animals, histopathology was seen at 100 and 300 mg/kg involving the liver and the lungs, large intestine, spleen, thymus, mesenteric lymph nodes and salivary gland. At 10 mg/kg, the liver and thymus were targeted. From these results, doses of 30, 75 and 150 mg/kg were selected for the 4-week oral toxicity study.

In the first 4-week toxicity study conducted in Wild-Type CB6F1 mice, no clinical signs of toxicity or significant histopathology were observed. Three deaths were observed in the HD group (1 main group female and two toxicokinetic animals). The sponsor considered the HD main group female death to be a result of stress as no significant histopathology was observed. This animal did exhibited clinical signs of toxicity and had lymphoid depletion in the thymus. The other two deaths were attributed to dosing administration error. The sponsor defined the NOAEL as 150 mg/kg/day. Due to observations of deaths in the second study at doses ≥ 225 mg/kg/day (the lowest dose tested), this death may be related to drug.

A second 4-week oral toxicity study was conducted in the Wild-TypeCB6F1 mice using higher doses to demonstrate toxicity. The oral doses were vehicle, 225, 300 and 750 mg/kg in males and 300, 500 and 750 mg/kg in females. The 225 mg/kg dose in males and the 500 mg/kg dose in females were administered 7 days after the other 2 doses were administered. In the 750 mg/kg treated males and females, dosing was stopped after 1 dose due to toxicity and the animals were sacrificed on day 7. In the 500 mg/kg females, 4 died after 5 doses resulting in stopping the dosing. During the 4-weeks, 8 males (3, toxicity phase and 7, toxicokinetics phase) and 2 females (toxicokinetics phase) at 300 mg/kg and 3 males (2 toxicity phase and 1 toxicokinetics phase) at 225 mg/kg were found dead or in a moribund state. At the end of 4 weeks, the surviving groups were the 225- and 300- mg/kg treated males and the 300 mg/kg-treated females. There was no effect on body weight changes but an increase in food consumption in the 300 mg/kg females. At the scheduled terminal sacrifice (day 29), the target organs in either or both sexes were the adrenal glands, kidney, lacrimal gland, lung, liver, mesenteric lymph node, prostate, mandibular salivary gland, spleen, thymus and urinary bladder. The AUC for the 300 mg/kg dose in males was markedly higher than the AUC for the 300 mg/kg dose in females which may account for the increased incidence of histopathology and mortality in males over females. In the scheduled euthanized group (750 mg/kg, both sexes; 500 mg/kg, females), and in the animals that died or were euthanized, the targeted

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organs were similar except that the heart and bone marrow was also targeted. The MTD was exceeded in this study.

Glycopyrronium bromide is a quaternary ammonium compound and it is known to be poorly absorbed orally. Despite this, lethality was seen in males at doses ≥ 225 mg/kg and at ≥ 300 mg/kg in females indicating that there was adequate systemic absorption. No notable toxicity was seen at 150 mg/kg in both sexes. The toxicokinetics of the two 4-week studies show that the AUCs in males at comparable doses were consistently higher than the AUCs in females, and this was a reflection in the difference in the incidence of mortality. To compensate for this difference, the highest dose in males should be lower than that in females. Based on the mortality results, the oral MTD is 75 mg/kg in males and 100 mg/kg in females

Table 4-1 Mean toxicokinetic parameters of NVA237 in mouse plasma (Day 28)

Dose (mg/kg/day)	Gender	Tmax (h)	Cmax (ng/mL)	AUC0-24h (ng.h/mL)	Cmax/Dose (ng/mL) / (mg/kg/day)	AUC0-24h/Dose (ng.h/mL) / (mg/kg/day)
30	M	7	0.995	11.8	0.0332	0.394
30	F	1	0.269	0.738	0.00897	0.0246
75	M	7	9.11	100	0.121	1.33
75	F	0.5	75.8	43.5	1.01	0.579
150	M	0.5	144	94.0	0.960	0.627
150	F	3	15.5	58.5	0.103	0.390
225	M	0.5	238	350	1.1	1.6
300	M	0.5	11900	6690	40	22
300	F	0.5	12.2	37.9	0.041	0.13

All animals dosed with NVA237 were exposed to NVA237 (where samples were available). Inter-animal variability of NVA237 concentrations was very high. Exposure to NVA237 was the lowest in the 30 mg/kg/day dose group and similar in the 75 mg/kg/day and 150 mg/kg/day dose groups. A Cmax value of 11900 ng/mL was detected in male animals at 0.5h following 300 mg/kg/day treatment. This Cmax value was about 1000-fold higher than in the female group and 50-fold higher than in the male group following 225 mg/kg/day treatment. This high concentration had also a significant impact on AUC0-24h in the male 300 mg/kg/day dose group.

(b) (4)

Taking the data as a whole, the reviewer considers the sponsor's proposed doses as inadequate for the 26-week carcinogenicity study. The reviewer recommends male doses of 8, 25, and 75 mg/kg/day as the LD, MD and HD groups, respectively. For females, the reviewer recommends 10, 30 and 100 mg/kg/day as the LD, MD and HD groups, respectively.

The animal/human dose ratios based on the proposed HD and the recommended HD are presented in the following table. Due to extreme variability in AUC data, these values are estimations only.

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Dose, mg/kg	AUC, ng.h/ml	Ratio	
		mg/kg, A/B	AUC A'/B'
Proposed Mouse	A'		
(b) (4)			
Recommended	A'		
Males 75 A	62	75,000	134
Females 100	41	100,000	88
Males, 25	32	25,000	69
Females, 30	9	30,000	19
Males, 8	22	8,000	47
Females, 10	0.3	10,000	0.6
Human, 0.05 mg 1 mcg/kg B 0.464 ng.h/ml B' 14-day study			

AUCs were determined by regression using the AUCs for 30, 75 and 150 mg/kg

Internal Comment

The Execute Carcinogenicity Assessment Committee (ECAC) met on February 23, 2010 to discuss the sponsor's proposed doses. The following is excerpted from the Meeting Minutes that were faxed to the sponsor on February 24, 2010.

Reference ID: 3824703

(b) (4)

External comments: None.

Reference ID: 3824703

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-48655	ORIG-1	NOVARTIS PHARMACEUTICALS CORP	NVA237 GLYCOPYRRONIUM BROMIDE

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

/s/

LAWRENCE F SANCILIO
02/24/2010

MOLLY E SHEA
02/24/2010
I concur.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 76,377
Review number: 01
Sequence number/date/type of submission: 000/September 27, 2007/original review
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Novartis Pharmaceuticals Corporation
Manufacturer for drug substance: Novartis Pharma AG, Lichtstrasse 35, CH-4056 Basle, Switzerland

Reviewer name: Virgil Whitehurst, Ph.D.
Division name: Division of Pulmonary and Allergy Products
Review completion date: July 31, 2008

Drug:

Trade name: QVA149 (indacaterol maleate and glycopyrronium bromide) Inhalation Powder

Generic name: None

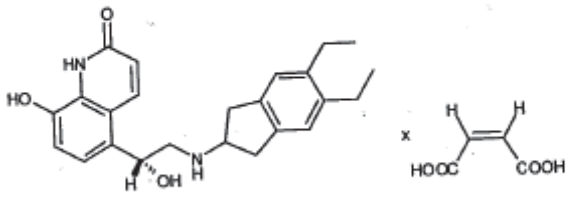
Code name: QAB149 (indacaterol maleate) and NVA237 (glycopyrronium bromide)

Chemical name: **Indacaterol maleate**: 5-[(1R)-2-[5, 6-diethyl-2, 3-dihydro-1H-inden-2-yl) amino]-1-hydroxyethyl]-8-hydroxy-2(1H)-quinolinone maleate (9CL)
Glycopyrronium bromide: 3-[(cyclopentylhydrophenylacetyl) oxy)]-1, 1-dimethyl-pyrrolidinium bromide

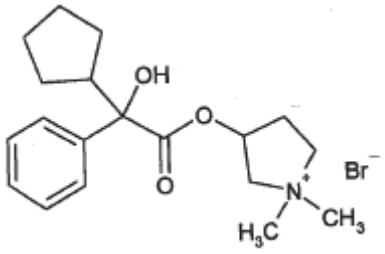
CAS registry number: **Indacaterol maleate**: 435273-74-8 and **Glycopyrronium bromide:** 596-51-0

Molecular formula/molecular weight: **Indacaterol maleate:** $C_{24}H_{28}N_2O_3xC_4H_4O_4$/ 508.56 and **Glycopyrronium bromide:** $C_{19}H_{28}NO_3xBr$/398.33

Structure:



Indacaterol maleate



Glycopyrronium bromide

Relevant INDs/NDAs/DMFs: INDs 48, 649 and (b) (4) (indacaterol dry powder formulations), (b) (4)

Drug class: Long-acting beta 2 adrenergic agonist (indacaterol maleate) and long acting muscarinic acetycholine antagonist (glycopyrronium bromide)

Intended clinical population: Treatment of COPD

Clinical formulation: The potential formulations include QAB149 to NVA237 ratios of 12:1, 6:1, 3:1, AND 1.5:1.

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Table 3-1 Composition of QVA149 inhalation powder hard capsules

Ingredient	Amount per [redacted] (b) (4) mg capsule [mg]	Amount per [redacted] (b) (4) mg capsule [mg]	Amount per mg [redacted] (b) (4) mg capsule [mg]	Amount per [redacted] (b) (4) mg capsule [mg]	Amount per [redacted] (b) (4) mg capsule [mg]	Amount per [redacted] (b) (4) mg capsule [mg]	Function
Capsule fill	[redacted] (b) (4)						
Indacaterol Maleate (QAB149)							Drug substance
Glycopyrronium bromide (NVA237)							Drug substance
Lactose monohydrate							[redacted] (b) (4)
Magnesium stearate							
Capsule fill weight							
Capsule shell							
Hypromellose							
Total capsule weight	74.0	74.0	74.0	74.0			

Route of administration: Oral inhalation

Proposed clinical protocol: The sponsor is planning a phase II program consisting of 2 studies evaluating safety tolerability and bioavailability.
Study 1 will be a randomized, double-blind, cross-over, multi center study to evaluate the efficacy of a fixed dose combination of 6:1 formulation of QVA149 (300µg/50µg) administered via a SDDP inhaler in patients with moderate to severe COPD, QAB149 (300 and 600 µg) and placebo. The 140 male and female patients, ≥ 40 years of age and older will be administered all 4 treatments (each treatment is daily exposure for 7 days) with a 7 day washout period between treatments. This study will involve serial pharmacokinetic sampling performed on a subgroup of patients (~30).

Study 2 will be a randomized, double-blind, placebo controlled, multi-centered study to assess the safety and tolerability of QVA149 after 14 days of treatment in moderate and severe male and female COPD patients (250), ≥40 year of age and older. The oral inhalation doses will be 6:1, 3:1 and 1.5:1 (QAB149:NVA237) formulations of QVA149 600/100, 300/100 mcg and 150/100 mcg. The drug will be administered daily for 14 days.

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Previous clinical experience: There has been no human experience with the QVA149. However, there is extensive human experience with indacaterol and some previous experience with inhaled glycopyrronium bromide administered separately:

Indacaterol:

Indacaterol has not yet been approved but is under development in multiple INDs as a single drug component as well as in combination with (b) (4) (IND 76,376). 960 asthma patients and 800 COPD patients have been exposed to indacaterol.

Single doses ranged from 25-3000 mcg while repeat doses range from 50-800 mcg daily for up to 28 days.

Glycopyrronium bromide:

Single doses of glycopyrronium bromide (GB) have been administered to 358 COPD/ healthy volunteers. The doses were up to 480 µg. Multiple doses of glycopyrronium bromide up to 240 µg have been administered to COPD patients daily for 28 days. Clinical trials with glycopyrronium bromide have been conducted in the United Kingdom and Germany. No clinical trials with GB have been conducted under an IND. GB is marketed in several countries, e.g., United Kingdom and Germany

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

Studies reviewed within this submission:

Pharmacology:

Results for (b) (4) General Pharmacology Screen for NVP_QAB149- Volume 4, page 8-1

Antagonist Effect of NVPQAB149-AF-1 on the Alpha 1D-Adrenoceptor of Rat Aorta-Volume 4, page 8-127

Antibronchoconstrictor and Cardiovascular Effects of Inhaled Glycopyrronium (NVPQAM254), Tiotropium Bromide (NVP-XQA204) and Ipratropium Bromide in Rhesus Monkeys-Volume 4, page 8-135

In vitro Muscarinic Receptor Pharmacology of NVP-QAM254-DC-1 (Glucopyrronium Bromide)-Volume 4, page 8-163

Assessment of Potency, Selectivity, Duration of Action and Potential Side Effects of a Muscarinic Receptor Antagonist, NVP-NVA237 in the Sensitized Brown Norway Rat-Volume 4, page 186

Assessment of the Duration of Action of Muscarinic Receptor Antagonists in the Rat Isolated Trachea-Volume 4, page 8-201

Evaluation of the Bronchodilator Activity of Glycopyrrolate, Ipratropium and Tiotropium on Methacholine (MCh) Induced Bronchoconstriction in Anesthetized Rabbits (Supplement)-Volume 4, page 8-211

Duration of Action of Glycopyrrolate (AD237) Formulation on Methacholine (MCh) Induced Bronchoconstriction in Anesthetized Rabbits-Volume 4, page 8-216

Evaluation of the Bronchodilator Activity of Tiotropium on Methacholine (MCh) Induced Bronchoconstriction in Anesthetized Rabbits-Volume, Page 8-231

Safety Pharmacology:

A Single Oral (Gavage) Dose General and Neurobavioral Activity Study in Male Mice-Volume 4, page 8-291

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Single Dose Intravenous (Bolus) Telemetry Study in Dogs Including Sighting Phase-Volume 5, page 8-1
Single Dose Intravenous (Bolus) Telemetry Study in Dogs Including Sighting Phase-Volume 5, page 8-6
Effect of QAB149 on HERG Currents Recorded From Stably Transfected HEK293 Cells-Volume 4, page 8-247
Electrophysiological Investigations in the Isolated Rabbit Heart-Volume 5, 8-189
A Pharmacological Assessment of a Single Administration (Inhalation) of QVA149, NVA237 and QAB149 on the Cardiovascular System in Male Beagle Dog Using Telemetry-Volume 6, page 8-1
A Pharmacological Assessment of a Single Administration (Inhalation) of QVA149, NVA237 and QAB149 on the Cardiovascular System in Male Beagle Dog Using Telemetry-Volume 6, page 8-6
A Pharmacological Assessment of the Effect of QVA149 on the Central Nervous System and the Respiratory System of the Albino Rat-Volume 7, page 8-1
A Pharmacological Assessment of the Effect of QVA149 on the Central Nervous System and the Respiratory System of the Albino Rat-Volume 7, page 8-8
Comparison of Tiotropium Once Daily, Formoterol Twice Daily and Both Combined Once Daily in Patients with COPD-Volume 7, page 354
Effects of Tiotropium with and Without Formoterol on Airflow Obstruction and Resting Hyperinflation in Patients With COPD- Volume 7, page 363
Pharmacological Assessment of the Duration of Action of Glycopyrrolate vs Tiotropium and Ipratropium in Guinea pig and Human Airways- Volume 7, page 372
Formoterol and Tiotropium Both Improve Lung Function in Stable COPD Patients With Some Additional Benefit When Given Together-Volume 7, Page 8-360

Toxicology:

A 2-Week Combination Inhalation Study in Wistar rats with a 2-Week Recovery Period-Volume 8, page 8-200
A 14- day Inhalation Study of a Combination Powder Formulation in the Dog With a 14-Day Recovery Period- Volume 11, Page 8-1

Studies not reviewed within this submission: None

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

QAB149

QAB 149 activity involves interaction with active sites of the beta receptor via the membrane lipid biulayer stimulating cAMP formation. QAB 149, salmeterol and formoterol demonstrated efficacy and duration of action in isolated guinea pig trachea preparations. The comparison of EC_{50} values in guinea pig trachea reveals a ß1/ß2 ratio in which salmeterol > QAB 149 > formoterol.

Previously submitted *in vivo* and *in vitro* studies show that QAB149 is a β_2 adrenergic agonist with bronchodilation activity. The drug has a fairly rapid onset (approximately 5 minutes) with an extended duration of action up to 24 hours. Major side-effects with QAB149 include tachycardia and myocardial necrosis.

The mechanism of action for QAB149 involves the interaction with active sites of the receptor via the membrane lipid bilayer stimulating cyclic AMP formation and activation of cyclic AMP-dependent protein kinase which promotes phosphorylation and smooth muscle relaxation of the airways. The lipid bilayer acts as a depot for B_2 adrenergic agonists which are available to interact with the active sites of the receptor. The duration of action of long-acting β_2 adrenergic agonists is determined by the physiochemical interaction between the drug and the receptor membrane lipid bilayer.

Safety pharmacology studies with QAB 149 was conducted to evaluate the effect of this drug on general and neurobehavioral in mice (oral dose of 2000 mg/kg) and an in vitro study to investigate the potential of QAB149 to inhibit the hERG tail current (concentrations to 1.5 mcg/mL). QAB149 had no significant effects on neurobehavioral or hERG tail current (IND (b) (4) - Pharmacology review dated May 6, 2008). QAB149 administered by inhalation to dogs daily for 4 weeks using doses to 0.24 mg/kg had no significant effects on the renal or the GI systems (IND (b) (4) - Pharmacology review dated May 6, 2008). The effects of QAB 149 on the respiratory parameters were not investigated although no notable effects were observed in toxicology studies in rats and dogs of up to 28 days (IND 48,649- Pharmacology review dated April 27, 2004) or in previously conducted clinical trials. The sponsor was informed that the effects of QAB149 on CNS should be completed in a letter regarding IND 48, 649 dated May 21, 2004.

Glycopyrronium Bromide:

Glycopyrronium bromide (NVA237) binds to muscarinic receptors in the bronchial smooth musculature and inhibits the cholinergic (bronchoconstrictive) effects of acetylcholine released from the parasympathetic nerve endings. NVA237 is highly selective for the human M3 receptors, the principal muscarinic receptors involved in bronchoconstriction. NVA237 inhibits agonist or electrical stimulated- induced contractions of rat, guinea pig and human airways. NVA237 has a long duration of action.

Safety pharmacology studies to evaluate the effects of NVA237 on the CNS, GI, cardiovascular and respiratory systems were conducted in the dog. NVA237 was

administered intravenously (doses = 5-10 mcg/kg) and orally (doses= 0.5-5 mg/kg. NVA237 administered intravenously and orally to dogs at these doses had no significant effects on the CNS, GI, respiratory and cardiovascular systems. The effect of NVA237 on the renal system was evaluated as part of a 4 week inhalation toxicity study in the rat. The high dose in the study was 4.24 mg/kg. NVA237 had no significant effect on the renal system in the rat

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Results For (b) (4) General Pharmacology Screen For NVA-QAB149 ((b) (4) Reports PT#:1004640)

The objective of this study was to assess the specificity of action of QVA149 in the (b) (4) Spectrumscreen. At 10 µM, NVP-QAB149 was active against a large number of receptors and especially targeted receptors, beta-2 adrenoceptor (Ki 12 nM), the alpha adrenorecptor α_{1D}, (Ki 210 nM) and α_{1A} (Ki 454 nM). NVP-QAB149 inhibits these receptors by 80=100 %. Formoterol reacted with only beta-2 adrenoceptor (Ki 3.6 nM). The Ki and IC50 data for NVP-QAB149 at receptors selected after the general pharmacology screen are shown below:

Table 3: Ki or IC_{50} data for NVP-QAB149 at receptors selected after general pharmacology screen.

Receptor	Species	Activity	
		NVP-QAB149-AA-4	formoterol
Adrenergic α_{1A}	rat	K_i 0.454 ± 0.053 µM	K_i 1.17 ± 0.107 µM
Adrenergic α_{1B}	rat	K_i 1.09 ± 0.147 µM	K_i 2.70 ± 0.364 µM
Adrenergic α_{1D}	human	K_i 0.201 ± 0.058 µM	K_i 2.98 ± 0.236 µM
Adrenergic α_{2B}	human	K_i 1.94 ± 0.419 µM	IC_{50} > 10 µM
Adrenergic β_1	human	K_i 1.13 ± 0.182 µM	K_i 2.18 ± 0.265 µM
Adrenergic β_2	human	K_i 0.012 ± 0.005 µM	K_i 3.58 ± 1.52 nM
Adrenergic β_3	human	K_i 0.872 ± 0.275 µM	K_i 1.62 ± 0.458 µM
Calcium Channel Type L, Benzothiazepine	rat	IC_{50} > 1.00 µM*	IC_{50} > 10 µM
Calcium Channel Type L, Phenylalkylamine	rat	K_i 1.20 ± 0.074 µM	K_i 11.2 ± 1.19 µM
Dopamine D_3	human	IC_{50} >1.00 µM*	IC_{50} > 10 µM
Dopamine $D_{4.2}$	human	IC_{50} >1.00 µM*	IC_{50} > 10 µM
Muscarinic M_5	human	IC_{50} 1.34 µM	IC_{50} > 10 µM
Serotonin 5-HT_{1A}	human	IC_{50} >1.00 µM*	IC_{50} > 10 µM
Serotonin 5-HT_{1B}	human	IC_{50} >10.0 µM*	IC_{50} > 10 µM
Serotonin 5-HT_{1D}	human	IC_{50} >10.0 µM*	IC_{50} > 10 µM
Serotonin 5-HT_2	rat	IC_{50} >10.0 µM*	IC_{50} 4.53 µM
Serotonin 5-HT_{2A}	human	IC_{50} >1.00 µM*	IC_{50} 1.31 µM
Serotonin 5-HT_4	guinea pig	IC_{50} >1.00 µM*	IC_{50} > 10 µM
Serotonin 5-HT_{5A}	human	IC_{50} >10.0 µM*	IC_{50} > 10 µM
Sigma σ_1	guinea pig	IC_{50} 4.32 µM	IC_{50} 1.63 µM
Sigma σ_2	rat	IC_{50} > 10.0 µM*	IC_{50} > 10 µM

*Poorly defined dose response curve, maximum concentration tested did not achieve >50% inhibition, data reinterpreted from (b) (4)

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Drug activity related to proposed indication:

Antagonist Effect of NVPQAB149-AF-1 on the Alpha 1D- Adrenoceptor of Rat Aorta

The in vitro study was conducted to define the potency of NVPQAB149 at the α_{1D} adrenoceptor in the rat aorta (male-Brown-Norway rats) and to determine whether NVPQAB149 is an agonist or an antagonist at this receptor. After repeated washings with phenylephrine and/or 5-hydroxytryptamine, the aorta tissues were incubated with NVPQAB149 (10-6 M) for 30 minutes. NVPQAB149 induced a shift to the right of the concentration-response curve to phenylephrine. NVPQAB149 had no response to 5-hydroxytryptamine. NVPQAB149 was determined to be an antagonist at the A_{1D} adrenoceptor in the rat aorta.

Antibronchoconstrictor and Cardiovascular Effects of Inhaled Glycopyrrolate (NVP-QAM254), Tiotropium Bromide (NVP-XQA024) and Ipratropium Bromide in Rhesus Monkeys.

The objective of this study was to assess the ability of glycopyrrolate to inhibit methacholine-induced bronchoconstriction in male anesthetized monkeys (n=3-6). Heart rate, systolic and diastolic, blood pressure, body temperature and respiratory rate were also evaluated. Glycopyrrolate was administered by inhalation using doses of 0.05, 0.15, 0.31 and 0.61 µg/kg (pulmonary deposited doses (25% deposition) 0.0125, 0.0375, 0.078 and 0.15 µk/kg). Tiotropium and ipratropium bromide was included in the study as positive controls. The results of this study reveal that glycopyrrolate induced dose and time dependent inhibition of methacholine–induce bronchoconstriction. The inhibition ranged from 60-80%. The drug had a rapid onset but a short duration of action (3-4 hours). Glycopyrrolate, at the doses in this study had no effect on heart rate, blood pressure body temperature or respiratory rates.

In Vitro Muscarinic Receptor Pharmacology of NVP-QAM254-DC-1 (Glycopyrrolate Bromide)

The objective of this study is to describe the characterization of the in vitro pharmacology of glycopyrrolate bromide (GB) on human M_1, M_2 and M_3 muscarinic receptors (equilibrium and kinetics). Parasympathetic nerves are the major bronchoconstrictor neural pathway in airways and cholinergic tone is the major reversible component in COPD. The stimulation of these nerves results in the release of acetylcholine that acts at multiple muscarinic receptor subtypes. There are M2 and M3 receptors expressed in the smooth muscles of the lungs in a ratio of ~4:1. CHO cells expressing human M_1, M_2 and M_3 muscarinic receptors were incubated with glycopyrrolate (~5 nM) and the kinetics and dissociation rate were calculated. The association and dissociation rates for GB were $1.58 \pm 0.2 \times 10^8$ M^{-1} min and 0.0015 ± 0.0002 min^{-1} respectively resulting in an equilibrium pK_d of 11.10 ± 0.04 at the M_3 receptor. These data show that glycopyrrolate has a greater equilibrium and binding selectivity for M_3 over M_2.

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Assessment of Potency, Selectivity, Duration of Action and Potential Side Effects of a Muscarinic Receptor Antagonist, NVP-NVA237 in the Sensitized Brown Norway Rat

The objective of this study was to evaluate the potency, duration of action and potential for side effects of NVP-NVA237 using lung function and cardiovascular parameters in the anesthetized male Brown Norway rat. The rats were sensitized using ovalbumin on days, 1, 15 and 21 of the study. NVP-NVA237was administered intratracheally, 1, 6 and 24 hours before being challenged with methacholine. The doses of NVP-NVA237 were 0.1, 0.3, 1, 3, 10 and 30 µg/kg. NVP-NVA237 inhibited bronchoconstriction ~30-60% in the anesthetized rat at doses of 3 µg/kg and higher for up to 24 hours after the methacholine challenge. NVP-NVA237 was not effective against methacholine induced hypotension, salivation and bradycardia in the sensitized rat.

Assessment of Duration of Action of Muscarinic Receptor Antagonists in the Rat Isolated trachea

The objective of this study is to describe the duration of action of NVP-NVA237, a muscarinic receptor antagonist whose mechanism of action is via the M_3 receptor, using rat isolated trachea from male Brown Norway. After contraction with bethanechol, a selective muscarinic receptor agonist to induce 80% contraction, the trachea tissues were exposed to 3, 10 and 30 nM of NVP-NVA237 in order to achieve a 30% reduction of sustained contractile response. The tracheal tissues were then washed every 15 minutes to determine the duration of action via maximum contraction return observed in 150 minutes or 10 washings after 10 washings, NVP-NVA237 at a 10 nM concentration inhibited bethanechol- induced contractions by 39%.

Pharmacological Assessment of the Duration of Glycopyrrolate vs Tiotropium and Ipratropium in Guinea Pigs and Human Airways. GinoVilletti et al: British J Pharmacol. (2006)148, 291-298.

The objective of this study was to investigate the duration of the bronchodilator action of the antimuscarinic drug glycopyrrolate compared with tiotropium and ipratropium. Tracheas were isolated from male albino Dunkin-Hartley guinea pigs (n=5) while human lung tissues were isolated from males (n=4), ages 48-66 years, who were undergoing surgery for lung cancer. The human and guinea pig tissues were then treated with carbachol (0.3 and1.0 nM) and then the guinea pig trachea tissues were treated with either glycopyrrolate, tiotropium and ipratropium (10 nM). The human lung tissues were also treated with glycopyrrolate (3.0 nM), tiotropium (1.0 nM) and ipratropium (10 nM), respectively. In the in vivo part of this study, male guinea pigs (number not given) were anesthetized and bronchoconstriction was induced via acetylcholine administration. The test compounds, glycopyrrolate (0.3-3.0 nM/kg), tiotropium (0.13-1.3 nM/kg) and ipratropium (0.15-01.5 nM/kg) were administered intratracheally. In the in vitro studies glycopyrrolate was effective in inhibiting carbachol induced bronchoconstriction in guinea pig tracheal tissues and human lung tissues ~50 and ~100 %, respectively. In the in vivo studies glycopyrrolate was also effective in inhibiting acetycholine-induced bronchoconstriction ~88% at the 3 hour evaluations. Glycopyrrolate had a longer duration of action than ipratropium but not as long as tiotropium. The duration of action for glycopyrrolate was ~3-16 hours.

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Evaluation of the Bronchodilator Activity of Bronchodilator Activity of Glycopyrrolate, Ipratropium and Tiotropium on Methacholine (MCh) Induced Bronchoconstriction in Anesthetized Rabbits (Supplement)

The objective of this study was to evaluate the efficacy of glycopyrrolate on methacholine induced bronchoconstriction in the anesthetized rabbits (n=3). Pretreatment with glycopyrrolate dry powder (0.3- 20 µg) administered intra-tracheally inhibited the increase in pulmonary inflation pressure evoked by methacholine administration by 80-90%. The intra-tracheal administration of ipratropium (0.3-20 µg) and tiotropium (0.03-3 µg) produced comparable results as those observed for GB. The ED_{50} values for GB was 2.27 µg, for ipratropium 1.41 µg and 0.21 µg for tiotropium.

Duration of Action of Glycopyrrolate (AD237) Formulations on Methacholine (MCh) Induced Bronchoconstriction in Anesthetized Rabbits

The objective of this study was to evaluate the efficacy and the duration of action of three different formations of glycopyrrolate (aqueous, dry powder and controlled release) on methacholine induced bronchoconstriction in the anesthetized rabbits (n=3). Pretreatment with aqueous glycopyrrolate (20 µg), glycopyrrolate dry powder (10 and 20 µg) and glycopyrrolate (10 µg) administered intra-tracheally inhibited the increase in pulmonary inflation pressure evoked by methacholine administration. The inhibition was 76-93% (for all 3 formulations) and the duration of inhibition was 4-6 hours; these results were comparable to those for ipratropium. The glycopyrrlate formulations had no effect on methacholine-induced decreases in heart rate and fall in blood pressure. The data on bronchoconstriction inhibition and duration of action are shown below.

Table 1. Anaesthetized rabbits: data showing the maximum change and duration on the methacholine (10 µg kg-1, i.v.) evoked response in the presence of different glycopyrrolate and ipratropium formulations (aq – aqueous, dp – dry powder and cr – controlled release)

	Max. Inhibition of hypotension (%)	Max. % Change in Bronchoconstriction	Duration Action (Hrs)
Ipatropium (aq) 20 µg	18	95	6
GP (aq) 20µg	2	92	6
GP (dp) 20µg	4	89	6
GP (dp) 10 µg	4	93	4
GP (cr) 10 µg	6	76	6

Evaluation of the Bronchodilator Activity of Tiotropium on Methacholine (mCh) Induced Bronchoconstriction in Anesthetized Rabbits

The objective of this study was to evaluate the efficacy and the duration of action of tiotropium on methacholine induced bronchoconstriction in the anesthetized rabbits (n=3). Tiotropium administered intra-tracheally at a dose of 3 µg inhibited the increase

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in pulmonary inflation pressure evoked by methacholine administration by 84% for approximately 6 hours.

2.6.2.3 Secondary pharmacodynamics
There were no secondary pharmacodynamic studies submitted in this IND submission.

2.6.2.4 Safety pharmacology

Neurological effects:
A Single Oral (Gavage) Dose General and Neurobehavioral Activity Study in Male Mice
The objective of this study was to investigate the effects of QAB149 on the general and neurobehavioral activity of male mice. A single oral dose of 2000 mg/kg was administered orally to male mice (n=10). There were no general or neurobehavioral effects induced in mice by QAB149 at a dose of 2000 mg/kg.

A Pharmacological Assessment of the Effect of QVA149 on the Central Nervous System and the Respiratory System of the Albino Rat
The aim of this study was to evaluate the pharmacological effects of a single inhalation dose of QVA149, NVA237 and QAB149 on the CNS and respiratory system of the albino rat. In phase 1- the CNS part of the study, there were 8 male Wistar rats in each dose group while in phase 2-the respiratory part of the study, there were 5 male Wistar rats in each dose group. The achieved inhalation doses for phase 1 and phase 2 are shown below. The pulmonary deposition doses (10 % deposition) are QVA149: 0.0115/0.0405 mg/kg, NVA 237:0.0168 mg/kg and QAB1490.0496 mg/kg. The CNS parameters measured were functional battery, assessment of grip strength, hind limb play and body temperature. The respiratory assessments include tide volume, respiratory rate, and derived minute volume.

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Phase 1 Central nervous system:

Group/identification	Targeted dose level expressed in base (salt) (mg/kg)	Theoretical achieved dose (mg/kg)[a]	No. of males
1/ Control	0	0	8
2/ High QVA149 (NVA237/QAB149)	0.128/0.370 (0.160/0.480)	0.115/0.405	8
3/ High NVA237	0.128 (0.160)	0.168	8
4/ High QAB149	0.370 (0.480)	0.496	8

Phase 2 Respiratory system:

Group/identification	Targeted dose level expressed in base (salt) (mg/kg)	Theoretical achieved dose (mg/kg)[a]	No. of males
1/ Control	0	0	5
2/ High QVA149 (NVA237/QAB149)	0.128/0.370 (0.160/0.480)	0.115/0.405	5
3/ High NVA237	0.128 (0.160)	0.168	5
4/ High QAB149	0.370 (0.480)	0.496	5

[a] Achieved dose levels reported represent the free base and quaternary ammonium cation for QAB149 and NVA237 respectively.

Single doses of QVA149 (up to 0.128/0.370 mg/kg), NVA237 (0.128 mg/kg) and QAB149 (0.370 mg/kg) had no significant effects on the respiratory or the CNS of the albino rats.

Cardiovascular effects:

The Effect of QAB149 on HERG Currents Recorded From Stably Transfected HEK293 Cells

The effect of QAB149 on the tail current recorded from HEK293 cells stably transfected with HERG cDNA was investigated. Compounds which inhibit HERG current have been shown to prolong the cardiac action potential and hence QT interval in man. QAB149 was evaluated at concentrations of 0.5, 1 and 5 µg/ml. The results of this study show that QAB149 inhibits HERG tail current at the highest concentration, 5.0 µg/ml. The frequency-dependence of QAB149 inhibition of HERG current was investigated at a concentration of 5.0 µg/ml. As part of the study, depolarizing pulses were applied to cells every 2 or 15 seconds. QAB149 did not inhibit frequency-dependence of HERG current. QAB149, at concentrations of 0.5 and 1.0 µg/ml did not inhibit HERG channels stably expressed in HEK293 cells. E-4031, the positive control inhibited (100 nM) inhibited HERG tail current by 86.1 %.

Single –Dose Intravenous (Bolus) Telemetry Study in Dogs Including Sighting Phase

The purpose of this study was to determine the acute effects of NVA237 on the cardiovascular system of the Beagle dog when administered intravenously. The study included a sighting phase to determine the maximum tolerated dose for the subsequent telemetry part of the study.

In the sighting part, 2 groups composed of 2 male dogs were administered intravenous doses of 0.01, 0.1 and 1.0 mg/kg alternating with an 8 hour washout period between

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doses. EKGs were recorded at 0.25, 0.5, 1.0 and 4.0 hours after dosing. Blood was also collected at several time points beginning 5 minutes after dosing and continuing for period up to 24 hours. The results of this study revealed that doses 0.01 and 0.1 were suitable for the telemetry part of the study. NVA237 induced dose-related increases in heart rates, tremors, dry mouth and nose tissues, reduction in food intake and marginal body weight losses. The $AUCs_{(0\text{-}4h)}$ were 2.36, 32.2 and 268 ng/h/mL, respectively.
In the telemetry part of the study, 4 male dogs were administered intravenous doses 0.01 and 0.1 mg/kg. There was a 7 day washout period between doses. The results of this study were similar to those observed in the sighting part of the study. Increases in heart rates observed 5-10 minutes postdosing ranged from 25-150 % and remained elevated for approximately 24 hours. Secondary shortening of the P, PQ, QRS and/or QT/QTc was observed at the highest heart rates. The NOAEL in the study was determined to be 0.1 mg/kg.

NVA237: ElectroPhysiological Investigations in the isolated Rabbit Heart
The purpose of this study was to examine the electrophysiological effects of NVA237 on 6 isolated hearts taken from Langendorff perfused female rabbit. The hearts were exposed to 0.3, 0.9, 3, 9 and 30 µM of NVA237 for 30 minutes. The following parameters were then measured: automaticity and escape length, threshold stimulation current, coronary perfusion rate, ectopic activity, left ventricular septal and epicardial monophasic action potential duration at 30, 60 and 90% repolarization, conduct time, triangulation reverse use- dependence and instability. NVA237 at the concentrations tested did not have any electrophysiological effects on the isolated rabbit hearts.

A Pharmacological Assessment of a Single administration (Inhalation) of QVA149, NVA237 and QAB149 on the Cardiovascular System in Male Beagle Dogs Using Telemetry in Life Examinations
The objective of this study was to assess the pharmacological effects of a single inhalation dose of QVA 149 (NVA237/QAB149), NVA237 and QAB149 on the hemodynamic and electrocardiographic parameters in male dogs via telemetry. The drugs were administered by inhalation and each dog was administered 5 single doses of either vehicle, QVA149 (low dose), QVA149 (high dose), NVA237 or QAB149. The dogs (4/dose) were dosed as followed:

Dose/identification	Targeted dose level[a] (mg/kg)	Theoretical achieved dose (mg/kg)[a]	No. of males
1/ Vehicle/Control[b]	0	0	4
2/ QVA149 (Low dose)	0.032/0.092 (0.040/0.120)	0.037/0.096	4
3/ QVA149 (High dose)	0.128/0.370 (0.160/0.480)	0.146/0.376	4
4/ NVA237 (High dose)	0.128 (0.160)	0.149	4
5/ QAB149 (High dose)	0.370 (0.480)	0.349	4

[a] Doses were corrected for salt/base ratio.
[b] Due to a computer failure this dose was repeated as dose 6. Data from original occasion is retained on file but not reported.

Reference ID: 3824703

All animals had the following parameters recorded: mortality and signs of ill health or reaction to treatment twice daily (am and pm) except on day of transfer and release from study, arterial blood pressures (mean arterial pressure, systolic blood pressure and diastolic blood pressure, pulse pressure) heart rate, electrocardiographic intervals (P width, PR, RR, QRS, QT and QTc), qualitative evaluation of the electrocardiographic waveforms once prior to each dose and at approximately 1, 3, 5, 7, 9, 12 and 24 hours post dose for each dose level.

There were no mortalities in the study. QVA149, in a dose-related manner increased heart rate and shortened the PR, P width, QT and QTc intervals. Ventricular arrhythmias were observed in one dog in the QVA149 high dose group. Increases in heart rates were less severe when NVA237 and QAB149 were administered individually. The results of this study are shown below:

Dose/identification	Theoretical achieved dose (mg/kg[b]	Findings
1/ Vehicle/Control[a]	0	None
2/ QVA149 (Low dose)	0.037/0.096	Increased heart rate 58% during the dosing period, remained elevated approximately 23% at 24 hours postdose.
		Decreased systolic and diastolic pressures, approximately 11% and 11% out to 10 hours following dosing.
3/ QVA149 (High dose)	0.146/0.376	Increased heart rate of ~240%, relative to predose levels, was noted from the start of dosing until 1 hour 30 minutes post start of dosing. At 24 hours postdose values remained elevated at approximately 97% relative to baseline.
		Decreased QTc, up to 30 msec over the 6 hours postdose.
		Decreased systolic and diastolic pressures, approximately 25% and 24% following dosing.
4/ NVA237 (High dose)	0.149	Increased heart rate of ~82% was noted from the start of dosing until 1 hour 30 minutes post start of dosing. At 7 hours postdose had returned to baseline.
		Decreased QTc, up to 30 msec over the first 2 hours postdose.
5/ QAB149 (High dose)	0.349	Increased heart rate of 57% was noted from the start of dosing until 1 hour 30 minutes poststart of dosing. At 24 hours postdose heart rate still elevated by 25%.
		Decreased QTc, up to 20 msec over the first 2 hours postdose.

[a] Doses were corrected for salt/base ratio.

[b] Due to a computer failure this dose was repeated as dose 6. Data from original occasion is retained on file but not reported.

Pulmonary effects:

Reference ID: 3824703

The effects of QVA149, NVA237 and QAB149 on pulmonary parameter were reviewed above under CNS effects. The name of the study is "**A Pharmacological Assessment of the Effect of QVA149 on the Central Nervous System and the Respiratory System of the Albino Rat".**

Renal effects:
No renal safety studies were submitted in this IND submission, however indacaterol and glycopyrrolate admininistered individually had no renal effects in experimental animals.

Gastrointestinal effects:
No GI safety studies were submitted in this IND, however indacaterol and glycopyrrolate, admininistered individually, had no GI effects in experimental animals.

Abuse liability:
There were no data related to abuse liability in this submission.

2.6.2.5 Pharmacodynamic drug interactions

No pharmacology tabulated summary for the fixed combination of indacaterol and glycopyrrolate was submitted in this IND submission.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Toxicokinetic studies were conducted as part of 2-week inhalation toxicity studies in the rat and the dog. In the Wistar rat, pulmonary deposited doses for the fixed combination (glycopyrrolate/ indacaterol) were 3.3/10, 6.6/20 and 13/40 mcg/kg, 17 mcg/kg (glycopyrrolate) and 48 mcg/kg (indacaterol). In the Beagle dog, pulmonary deposited doses for the fixed combination (glycopyrrolate/indacaterol) were 9/25, 20/48 and 32/102 mcg/kg, 30 mcg/kg (glycopyrrolate) and 104 mcg/kg (indacaterol). In both studies, systemic exposure increased with increased dose regardless of gender. The increases in systemic exposure were less than proportional in the rat and greater than proportional in the dogs. The TK parameters for the fixed combination were similar to the toxicokinetics of mometasone and indacaterol when these components were administered alone.

The ADME data for Indacaterol was previously reviewed in IND (b) (4) Pharmacology review dated May 6, 2008. The ADME data for glycopyrrolate is from " Gross, NJ (1999): Anticholinergic Agents as Bronchodilators. Lung biology in Health and Disease 134 (Anticholinergic Agents in the Upper and Lower), 59-72. Airways

2.6.4.3 Absorption

Indacaterol:

Oral absorption was 20-34% in the rat after a single 0.5 mg/kg dose, ~53% in mice after a single, oral 0.5 mg/kg dose and 72% in the dog following a 0.1 single oral dose.

Reference ID: 3824703

Glycopyrrolate:
It is unlikely that inhaled glycopyrrolate is absorbed from the GI tract. (Ali-Melkkila: Pharmacokinetics and Related Pharmacodynamics of Anticholinergic Drugs. Acta Anesthiol. Sans. 37, 633-642, 1993). A PK study in healthy male volunteers confirms this finding; only 10% bioavailability was observed after inhalation administration.

2.6.4.4 Distribution
Indacaterol:
Following intravenous dosing of indacaterol to rats, the radioactivity was found in all tissues except the brain, spinal column, testis and lymph nodes with the highest concentration observed in the lungs. Indacaterol was found in the milk of lactating rats. The plasma protein binding of indacaterol in rats, dogs and humans ranged from 91-96%.
Glycopyrrolate:
Following intravenous administration of labeled glycopyrrolate (10 mcg/kg) to Beagle dogs, very low levels of radioactivity was found in the tissues of the dogs. Glycopyrrolate did not cross the blood-brain barrier. When administered to pregnant dogs, very low levels of radioactivity crossed the placenta-blood barrier. Glycopyrrolate is not very lipid soluble contributing to the abovementioned results observed in the dog.

2.6.4.5 Metabolism
Indacaterol:
Rat, dog liver slices and human hepatocytes indicate that indacaterol is metabolized via direct phenolic O-glucuronidation. No metabolism occurs in the human lung. Enzyme kinetics experiments in human liver microsomes identified UGT1A1 as the main UGT enzyme response for indacaterol glucuronidation and CYP3A4 as the main P450enzyme response for oxidative metabolism of indacaterol.
Glycopyrrolate:
Rat, dog and human lung and liver microsomes were used to identify the metabolic pathway for glycopyrrolate. Data from these in vitro studies reveal that glycopyrrolate is not metabolized in the lung. In the dog and human liver microsomes no major metabolism of glycopyrrolate was detected. Glycopyrrolate was metabolized in rat liver microsomes mainly via P450 –mediated oxidative metabolism.

2.6.4.6 Excretion
Indacaterol:
In mice, rats and dogs, the predominant route of elimination for indacaterol is via the feces regardless of route of administration.
Glycopyrrolate:
Studies in rats administered glycopyrrolate orally reveal that the drug is excreted mainly in the feces~95%.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Reference ID: 3824703

Two week inhalation toxicity studies were conducted in the rat and the dog with the fixed combination of indacaterol /glycopyorrolate. The objective of these studies was to determine whether the administration of a fixed combination of indacaterol and glycopyrrolate would result in potentiation of known target organ toxicities for each agent and/or induced unanticipated target organ toxicities. Pulmonary deposited doses in the Wistar rat study were air control, placebo control (lactose- magnesium stearate), QVA149: 3.3/10.1, 6.56/20.1 and 13.2/40.2 mcg/kg; glycopyrronium bromide: 17.0 mcg/kg; and indacaterol maleate: 47.9 mcg/kg. No significant drug-related effects were observed. The administration of the fixed combination did not significantly affect the systemic exposure of the individual components. The NOAEL was identified as the high combination dose of 13.2/40.2 mcg/kg.

Pulmonary deposited doses in the Beagle dog, for the fixed combination (glycopyrrolate/indacaterol) were QVA 149: 7.75/25.2, 15.5/48.2 and 31.5/95.0 mcg/kg; glycopyrronium bromide: 30.1 mcg/kg; indacaterol maleate: 104 mcg/kg; air control and placebo control (lactose-magnesium stearate). There were no mortalities in the study. Microscopic analyses revealed minimal fibrosis in the papillary muscles of the left ventricle in some dogs in the QAB149 and the high dose of the fixed combination. Minimal cytoplasmic rarefaction was observed in the liver of some dogs in the QAB149 alone and in some dogs in all dose groups of the fixed combination. This effect was due to the presence of periportal glycogen. This effect was reversible. There were no additive or potentiating effects in the dogs in this study. The NOAEL for this study was identified as mid combination dose of 15.5/48.2 mcg/kg.

The interaction studies with the fixed combination in the rats and the dog are valid studies. The objective of these studies was to determine whether the administration of a fixed combination of indacaterol and glycopyrronium would result in potentiation of known target organ toxicities for each agent and/or induced unanticipated target organ toxicities. The highest achieved dose of the fixed combination 131.6/ 402.3 used in the rat study (glycopyrrolate/indacaterol) was based in part on previous toxicity studies. High single, individual doses of the combination in rats (100 mg/kg subcutaneously, indacaterol induced skin lesions only and these were thought to be due mostly to the vehicle-50% hydroxypropyl-beta cyclodextrin). Glycopyrrolate (100 mg/kg subcutaneously was the minimum lethal dose but no target organ toxicity was reported). In fact, a review of the single studies showed no significant toxicity when oral glycopyrrolate (681 mg/kg) and indacaterol (1600 mg/kg) are administered individually. And finally, the young rat is not a good animal model to investigate the toxicity profile for the indacaterol/ glycopyrronium combination. The NOAEL doses in the 2 week toxicity studies in the rat and dog provide adequate margin of safety for the doses proposed for the clinical study.

The class target organ for beta 2 adrenoceptor agonist (indacaterol) and the muscarinic antagonist (glycopyrrolate) is the cardiovascular system.

Genetic toxicology:

Reference ID: 3824703

No genetic assays have been conducted with the fixed combination. However, genetic assays have been conducted with indacaterol and glycopyrrolate separately.

Indacaterol:

Indacaterol was negative in the Ames, chromosomal aberration and the rat bone marrow micronucleus genetic assays (IND (b) (4) - Pharmacology review dated May 6, 2008) .

Glycopyrrolate:

Glycopyrrolate was negative in the Ames (Williams L. AD237: Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhimurium*. (b) (4) Report Number 2250/12-D6171 Arakis Ltd, August 2004), chromosomal aberration (Lloyd, M. AD 237: Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes. (b) (4) Report Number 2250/13-D6172 Arakis Ltd, September, 2004) and the rat bone marrow micronucleus (Beevers C. AD237: Induction of Micronuclei in the Bone Marrow of Treated rats. (b) (4) Report Number 2250/6172. Arakis LTD, October , 2004) genetic assays.

Carcinogenicity:

No carcinogenicity studies have been conducted with the fixed combination. However carcinogenicity studies have been conducted or are underway with indacaterol and glycopyrrolate separately.

Indacaterol:

Indacaterol did not induce neoplastic findings in CB6F1-TgHras transgenic mice in 26-week carcinogenicity study (sponsor ‘ summary). Indacaterol was administered orally with doses to 600 mg/kg. A 2 year inhalation carcinogenicity study is currently being conducted in SD rats.

Glycopyrrolate:

No carcinogenicity studies have been conducted with inhaled glycopyrrolate. An inhalation carcinogenicity study in rats is currently being conducted.

Reproductive studies

No reproductive studies have been conducted with the fixed combination. However, reproductive studies have been conducted with indacaterol and glycopyrrolate separately.

Indacaterol:

Reference ID: 3824703

Table 4-18 Reproduction toxicity studies in rats and rabbits

Study Type	Species	Method of admin. (Vehicle/ Formulation)	Dose of indacaterol base (mg/kg/day)	Findings
Dose range finding EFD Non GLP [Doc 27]	Rat	Subcutaneous (maleate in aq. 50% w/w 2-hydroxypropyl -beta-cyclodextrin)	3, 10, 30 and 100 6/group	Skin and underlying muscle lesions at injection sites in controls and at doses ≥ 3 mg/kg No teratogenicity at 3 and 10 mg/kg NOEL for pregnant rat not established
Follow-up Dose range finding EFD Non GLP [Doc 28]	Rat	Subcutaneous (maleate in PEG400, NF/ 0.9% saline 20:80 (v/v))	0.1, 1 single dose; 1 b.i.d. 6/group	Skin lesions at injection sites at 1 mg/kg single dose and b.i.d. ↑ Body weight gain at 1 mg/kg single dose and b.i.d. NOEL for pregnant rat =0.1 mg/kg single dose NOEL for fetus =1 mg/kg single dose and b.i.d.
EFD GLP study [Doc 29]	Rat	Subcutaneous (maleate in PEG400, NF/ 0.9% saline 20:80 (v/v))	0.1, 0.3 and 1 b.i.d. 25/group	Skin lesions in the regions of the injection sites at doses ≥ 0.1 mg/kg ↑ Body weight at doses ≥ 0.3 mg/kg ↑ Body weight gain at doses ≥ 0.3 mg/kg NOEL for pregnant rat not established NOEL for fetus =1 mg/kg; no teratogenicity
Dose range finding EFD Non GLP [Doc 30]	Rabbit	Subcutaneous (maleate in aq. 50% w/w 2-hydroxypropyl -beta-cyclodextrin)	3, 10, 30 and 100 6/group	Skin lesions at injection sites in controls and at doses ≥ 3 mg/kg 30 and 100 mg/kg groups terminated due to extensive skin lesions ↓ Body weight parameters at 10 mg/kg ↓ Food consumption at doses ≥ 10 mg/kg No teratogenicity at 3 or 10 mg/kg NOEL for pregnant rabbit not established
Follow-up Dose range finding EFD Non GLP [Doc 31]	Rabbit	Subcutaneous (maleate in aq. 5% w/w 2-hydroxypropyl -beta-cyclodextrin)	0.1, 1, 3 single dose; 1 b.i.d. 5/group	Skin lesions at injection sites at 1 and 3 mg/kg single dose and 1 mg/kg b.i.d. ↓ Food consumption at 1 and 3 mg/kg single dose and 1 mg/kg b.i.d. NOEL for pregnant rabbit =0.1 mg/kg NOEL for fetus =3 mg/kg
EFD GLP study [Doc 32]	Rabbit	Subcutaneous (maleate in aq. 5% w/v 2-hydroxypropyl -beta-cyclodextrin)	0.1, 1, 3 20/group	Skin lesions at injection sites at 1 and 3 mg/kg ↓ Food consumption at 3 mg/kg ↑ Full supernumerary rib (variation) at 3 mg/kg NOEL for pregnant rabbit =1 mg/kg NOEL for fetus =1 mg/kg; no teratogenicity
FEED GLP study [Doc 33]	Rat	Subcutaneous (maleate in PEG400, NF/ 0.9% saline 20:80 (v/v))	0.2, 0.6, 2 bid 25/sex/group	Skin lesions at injection sites at dose ≥ 0.6 mg/kg ↑ Body weight parameters and food consumption at doses ≥ 0.2 mg/kg NOEL for males and females not established No effect on fertility, reproductive performance or early embryonic development

Study Type	Species	Method of admin. (Vehicle/ Formulation)	Dose of indacaterol base (mg/kg/day)	Findings
PPND GLP [Doc 34]	Rat F_0 (F) F_1 (M+F)	Subcutaneous (PEG400, NF/0.9% saline 20:80 v/v) treatment of the F_0 and F_1	0.1, 0.3 and 1.0 (base) 24/group	F_0 effects: Increases in body weight parameters and discoloration at sites of injection at doses ≥ 0.3 mg/kg/day; transient increases in food consumption at 1 mg/kg/day. F_1 effects: reversible decreases in body weight parameters at doses ≥ 0.3 mg/kg/day; decrease in number of animal reaching criterion for acquisition/learning in males at 1 mg/kg/day; and fertility and fecundity were affected with a decrease in the number of pregnant animals at 1 mg/kg/day with no effect on mating of other parameters of reproductive performance. NOEL: 0.1 mg/kg/day
Neonatal and juvenile development dose range finding non-GLP [Doc 35]	Rat (M+F)	Subcutaneous (PEG400, NF/0.9% saline 20:80 v/v) post partum day 4 through 20	0.1, 0.3, 1.0 (base) low/mid dose 23/group high dose 24/group	≥ 0.1 mg/kg/day: increase in body weight , no treatment related mortalities, clinical signs or necropsy findings NOEL: not established

EFD = embryo-fetal development; FEED = fertility and early embryonic development; NOEL= no observed effect level; PPND = pre- and post-natal development

Glycopyrrolate:

Table 4-18 Reproduction toxicity studies in rats and rabbits

Study Type	Species	Method of admin.	Dose of NVA237salt (mg/kg/d)	Findings
EFD Non GLP [Franko et al, 1970]	Rat	Diet	Up to 130	No teratogenic effects of GP although diminished rates of conception and survival were seen.
EFD Non GLP [Robinul® NDA, 1974]	Rabbit	i.m.	0.05, 0.5	There were no effects on implantation rates, fetal survival or any significant treatment-related fetal abnormalities. Maternal weight gains were reduced during the dosing period that probably reflected maternal toxicity.
Multi-generation study [Robinul® NDA, 1974]	Rat	oral	Up to 65	No teratogenic effects although there was a dose-related reduction in rate of conception and survival at weaning
Pre- and post natal development studies [Kagiwada et al, 1973]	Rat Mouse	oral	4, 25, 150 4, 20, 100	No effects of GP on pre- and post-natal development

EFD = embryo fetal development

The reproduction studies in rats and rabbits with GB were obtained from published literature.

Special toxicology:
No special studies have been conducted with the fixed combination. However, special studies have been conducted to assess the contact allergic potential of indacaterol. Indacaterol, in the local lymph node assay was identified as a weak skin sensitizer based on changes in the skin-draining lymph nodes. In the Buehler assay, indacaterol was not a sensitizer in guinea pigs (IND 48,649-Pharmacology review dated April 27, 2004). Special studies with indacaterol are shown below:

Reference ID: 3824703

Table 4-19 Special studies

Study Type	Species (Strain)	Site of topical admin. (Vehicle)	Concentration of indacaterol (w/v)	Duration of dosing (Gender and no./group)	Remarks
LLNA tier I Non GLP [Doc 36]	Mice (BALB/c)	Topical, dorsum of pinnae (di-methyl formamide)	0%, 0.3%, 3%, 30% [Positive control, DNCB = 0.5%]	3 days (6f)	30%: ↑ lymph node weight and cell count. TC = 16.1% (weak lymph node activator). NOEL = 3%.
LLNA tier II GLP study [Doc 37]	Mice (BALB/c)	Induction: dorsum Challenge: dorsum of pinnae (di-methyl formamide)	Induction: 0%, 25%, [0.5% DNCB] Challenge: 0%, 10%, 25%, [0.5% DNCB] [Positive control]	3 days; 12-days between induction and challenge (6f)	≥ 10%: ↑ lymph node weight and cell count. Skin sensitizer (weak contact allergen).
Buehler test GLP study [Doc 38]	Guinea pigs	Induction: topical (left flank), once a week for 3 weeks (3 treatments) of corn oil or indacaterol Challenge: topical, 2 weeks after last induction dose (each animal received corn oil (upper right) and indacaterol (lower right)	DRF: 10, 50, 150, 250 mg/mL Main study: 250 mg/mL	DRF: single dose (2f) main study (10f controls; 20f indacaterol)	No response observed during 3-week induction or subsequent challenge with indacaterol at 250 mg/mL, the maximum practicable concentration. Indacaterol is considered not to be a skin sensitizer in guinea pigs.
Respira-tory/ Sensitisa-tion Non-GLP study [Doc 39]	Guinea pigs	Induction: SC Challenge: IT Indacaterol in 20% PEG400 in saline Ovalbumin with $Al(OH)_3$ in saline (Positive control)	Induction: Indacaterol 200 µg (400 µg/mL) Ovalbumin 10 µg (20 µg/mL + 20 mg/mL $Al(OH)_3$) Challenge: Indacaterol 50 µg (500 µg/mL) Ovalbumin 30 or 0.1 µg (300 or 1 µg/mL)	Induction: days 0, 7 and 14 Challenge: Day 21 10m/group	No acute bronchospasm; no pulmonary eosinophilia Indacaterol is considered not to be a respiratory sensitizer in guinea pigs

LLNA = Local lymph node assay; TC = threshold concentration; DNCB = dinitrochlorobenzene; NOEL= no observed effect level

No special studies have been conducted with glycopyrrolate.

2.6.6.2 Single-dose toxicity

No single –dose toxicity studies were conducted with the fixed combination. However, single dose studies have been conducted with indacaterol and glycopyrrolate separately. The oral MTD for indacaterol in the rat is ~1600 mg/kg. Single inhalation doses of indacaterol, micronized (3.0 mcg/kg) and with HFA (3.7 mcg/kg) were administered to rats. There was no target organ toxicity in the rat at these doses.
The sponsor's summary table is shown below.

Indacaterol:

Reference ID: 3824703

Table 4-12 Single dose toxicity studies

Species (Strain) [Doc]	Method of administration (Vehicle/ Formulation)	Dose (mg base/kg)	Gender and No. per Group	Remarks
Rat (Han Wistar) GLP study [Doc 1]	Oral gavage (maleate in aq. 1% Na CMC)	1600	5m/5f	No adverse effect of treatment. LD_{50} > 1600 mg/kg
Mouse (CD-1) GLP study [Doc 2]	Oral gavage (maleate in aq. 1% Na CMC)	1600	5m/5f	No adverse effect of treatment. LD_{50} > 1600 mg/kg
Rat (Han Wistar) GLP study [Doc 3]	Subcutaneous (maleate in aq. 50% w/w 2-hydroxypropyl-beta-cylocdextrin)	5, 50, 100, 200	5m/5f	Skin lesions at injection sites (exacerbated by vehicle) Min lethal dose = 200 mg/kg Max non-lethal dose = 100 mg/kg
Mouse (CD-1) GLP study [Doc 4]	Subcutaneous (maleate in aq. 50% w/w 2-hydroxypropyl-beta-cylocdextrin)	5, 50, 100, 200	5m/5f	Skin lesions at injection sites of indacaterol-treated mice Min lethal dose (m/f) = 50 / 200 mg/kg Max non-lethal dose (m/f) = 5 / 100 mg/kg
Rat (Han Wistar) GLP study [Doc 5]	Inhalation (snout-only) (maleate, micronized powder and HFA formulations	3.07 (micronized) 3.57 (HFA formulation)	10m	At comparable inhalable doses, exposure of rats to aerosols generated from a micronized powder or HFA formulation resulted in similar systemic bioavailability and lung concentrations of indacaterol. Micronized powder of indacaterol: Aerosol conc. = 0.103 mg/L, MMAD = 2.06 µm, aerodynamic diameter < 4.2 µm = 75.8% particles. AUC24 = 99.7 ng.h/mL, lung conc. = 6.19 & 2.08 mg/g at 6 & 23 h post exposure. HFA formulation: Aerosol conc. = 0.119 mg/L, MMAD = 2.50 µm, aerodynamic diameter < 4.2 µm = 51.5% particles. AUC24 = 78.0 ng.h/mL, lung conc. = 5.82 & 1.81 mg/g at 6 & 23 h post exposure.

f=female; m=male
Na CMC = Sodium carboxymethylcellulose

Glycopyrrolate:
The oral MTD for GB in the rat is ~ 681 mg/kg. The oral LD_{50} for rats is 1715.5 mg/kg, 937.8 mg/kg in mice and 2360 mg/kg in rabbits. The sponsor's summary table is shown below.

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Table 4-13 Single dose toxicity studies

Species	Method of Admin	Remarks [1]
Mouse and Rat [Franko et al, 1970]	Oral	The maximum non-lethal dose of GP was 681 mg/kg in rats. The half-maximal lethal dose (LD_{50}) at 72 hours after dosing was estimated to be 1150 mg/kg in male rats, 1280 mg/kg in female rats and 550 mg/kg in male mice.
Mouse, Rat and Rabbit [Saito et al 1973]	Oral	In a separate study, no sex differences were apparent and a mean LD_{50} value of 1715.5 mg/kg in rats, 937.8 mg/kg in mice and 2360 mg /kg in rabbits were reported.
Mouse and Rat [Franko et al, 1970]	i.p.	The maximum non-lethal dose of GP was 144 mg/kg in female rats and 79 mg/kg in female mice. The LD_{50} at 72 hours after dosing was estimated to be 196 mg/kg in female rats, 107 mg/kg in female mice and 112 mg/kg in male mice.
Mouse and Rat [Saito et al, 1973]	i.p.	In a separate study, no sex differences were apparent and a mean LD_{50} value of 263.6 mg/kg in rats and 120 mg/kg in mice was reported
Mouse, Rat rabbit, dog and cats [Franko et al, 1962]	i.v.	The half-maximal lethal dose (LD_{50}) at 72 hours after dosing was estimated to be 14.6 mg/kg in female rats, 14.7 mg/kg in male mice, 25 mg/kg in rabbits and between 15 and 30 mg/kg in dogs and cats.
Mouse and rabbit [Saito et al, 1973]	i.v.	In a separate study, no sex differences were apparent and a mean LD_{50} value of 18 mg/kg in mice and 29.1 mg /kg in rabbits was reported

i.p. = intraperitoneal; i.v. = intravenous; [1] doses referenced are expressed in NVA237 salt

2.6.6.3 Repeat-dose toxicity

Study title: A **2-Week Combination Inhalation Study in Wistar Rats with a 2-Week Recovery Period**

Key study findings:
Pulmonary deposited doses, air control, placebo control (lactose- magnesium stearate), QVA149 in 1:3 formulation (glycopyrronium:QAB149) ratios: 3.3/10.1, 6.56/20.1 and 13.2/40.2 mcg/kg glycopyrronium bromide: 17.0 mcg/kg and indacaterol maleate: 47.9 mcg/kg were administered to rats daily for 14 days.
There were no mortalities in the study.
There were no significant treatment related effects on clinical signs, body weight, food consumption, ophthalmology, hematology, urinalysis, organ weights, or histopathology in the fixed combination, NVA237 or QAB149 rats.
There were no additive or potentiating effects in the rats treated with the fixed combination.
The administration of the fixed combination did not significantly affect the systemic exposure of the individual components

The NOAEL was the high combination dose of 13.2/40.2 mcg/kg
The interaction study with the fixed dose combination in the rat is a valid study. The objective of these studies was to determine whether the administration of a fixed combination of indacaterol and mometasone would result in potentiation of known target organ toxicities for each agent and/or induced unanticipated target organ toxicities. High

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doses of the individual components of the indacaterol/ glycopyrronium did not induced significant toxicity on the rat. Inhalation dose selection for this study was selected from toxicology studies conducted individually with indacaterol and GB, potential human exposure and limitations imposed by the exposure apparatus.

Study no.: 79298
Volume #, and page #: Volume 8, page 8-200
Conducting laboratory and location: (b) (4)
Date of study initiation: November 21, 2006
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: QVA149 (QAB149/NVA237 192 +64 mg/g) (19.2% QAB149 (free base), 6.4 %NVA237 (free base), 1% magnesium stearate, 73.4% lactose)/X3480906/97%

Methods

Doses: Air control, placebo control (magnesium and lactose), achieved doses (glycopyrrolate-indacaterol): 32.9/100.6, 65.6/200.5 and 131.6/402.3 mcg/kg, glycopyrrolate bromide: 170.0 mcg/kg and indacaterol maleate: 479 mcg/kg

Pulmonary deposited doses (based on 10% deposition): air control, placebo control (lactose- magnesium stearate), QVA149: 3.3/10.1, 6.56/20.1 and 13.2/40.2 mcg/kg glycopyrronium bromide: 17.0 mcg/kg and indacaterol maleate: 47.9 mcg/kg.

Species/strain: Rat, Wistar

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

Route, formulation, volume, and infusion rate: Nose only inhalation/ QVA149 (QAB149/NVA237 192 +64 mg/g) (19.2% QAB149 (free base), 6.4 %NVA237 (free base), 1% magnesium stearate, 73.4% lactose)/ 0.125L/min./24L/min

Satellite groups used for toxicokinetics or recovery: TK: 6/sex/dose group and recovery: 5/sex/dose group

Age: 6 weeks

Weight: Males: 103-190g and females: 108-148g

Sampling times:

Prior to the study and weekly, representative filter samples of the test article formulations used to produce test aerosols were analyzed for glycopyrrolate, QAB149, lactose and magnesium stearate based on GC or HPLC methods.

Unique study design or methodology (if any):

The achieved doses were calculated as followed:

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Calculation of achieved dosage: [1]	The achieved dose of NVA237, QAB149 and Magnesium Stearate (mg/kg/day) for each treatment level was determined as follows: Achieved dose of active ingredient (µg/kg/day) $= \frac{\text{RMV x Active Concentration x T x D}}{\text{BW}}$ Where RMV (L/min) = respiratory minute volume calculated[2] Active concentration (µg/L) = aerosol concentration of active test material determined by chemical analysis T (min) = treatment time D (Deposition) = 100% was assumed for total aerosol deposition fraction, or according to the particle size BW (kg) = mean body weight per sex per group from the regular body weight occasions during treatment [1] Total body dose assuming a deposition fraction of 100%. [2] 0.499 x [body weight (kg)]$^{0.809}$ L/min (Bide, R.W. et al 2000). It is assumed that this parameter is unaffected by exposure to the test article.

Mean MMAD values for QVA149, NVA237 and QAB149 determined via a cascade impactor ranged 1.5-2.8. The overall mean analytical particle size analysis data for NVA237 and QAB149 and the combination are shown below:

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Group ID	Chamber concentrations		Particle size MMAD (µm) ± GSD		Nominal concentrations (mg/L)
	Gravimetric (mg/L)	Analytical (mg/L)	Gravimetric	Analytical	
Magnesium stearate content					
2/ Placebo control	0.0109	0.00032	2.4 ± 2.7	1.1 ± 2.2	0.09
3/ Low dose QVA149 (NVA237/QAB149)	0.0120	0.00015	2.0 ± 2.2	1.5 ± 1.6	0.06
4/ Mid dose QVA149 (NVA237/QAB149)					
5/ High dose QVA149 (NVA237/QAB149)					
6/ High NVA237 64 mg/g	0.0074	0.00020	2.8 ± 2.6	1.7 ± 2.0	0.027
7/ High QAB149 192 mg/g	0.0129	0.00024	2.0 ± 2.3	1.4 ± 1.5	0.05
*NVA237 content					
3/ Low dose QVA149 (NVA237/QAB149)	0.0171	0.0016	3.0 ± 2.3	2.3 ± 2.1	0.06
4/ Mid dose QVA149 (NVA237/QAB149)					
5/ High dose QVA149 (NVA237/QAB149)					
6/ High NVA237 64 mg/g	0.0097	0.0020	3.7 ± 2.5	2.2 ± 1.8	0.27
**QAB149 content					
3/ Low dose QVA149 (NVA237/QAB149)	0.0174	0.0048	2.6 ± 2.3	2.3 ± 2.0	0.06
4/ Mid dose QVA149 (NVA237/QAB149)					
5/ High dose QVA149 (NVA237/QAB149)					
7/ High QAB149 192 mg/g	0.0163	0.0057	2.9 ± 2.4	2.8 ± 2.0	0.05

Oxygen levels measured on day 2 was 20.9%, respectively for groups 1, 2, 6 & 7.
Oxygen levels measured on day 2 was 20.8%, respectively for groups 3, 4 & 5.
* NVA237 reported as quaternary ammonium cation of the bromide salt.
** QAB149 reported as free base.

Clinical signs: Clinical signs were conducted twice daily.
Body weights: Bodyweights were evaluated weekly during treatment and recovery.
Food consumption: Food consumption was evaluated during the final week of the study and during the recovery period.
Ophthalmoscopy: Eye examinations were conducted at the start of and at the end of the second week.

Hematology: At termination, hematology parameters were evaluated on all main and recovery rats.
Clinical chemistry: At termination, clinical chemistry parameters were evaluated on all main and recovery rats.

Urinalysis: At termination, urinalyiss parameters were evaluated on all main and recovery rats.

Gross pathology: At termination and after the recovery period, gross pathology was examined
Organ weights: The following organs were weighed for each rat:
Adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and parathyroids and uterus.

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Histopathology:
Adequate Battery: yes (x), no ( ) microscopic examinations were conducted for all the rats in air and placebo control groups, the high dose QVA149 dose group, the NVA237 and QAB149 dose groups as well as the tissues of rats found dead or were euthanized prior to the scheduled necropsy. Respiratory tissues (lungs, larynx, pharynx, main stem bronchi, trachea, tracheobronchial lymph nodes and nasal cavities and sinuses), all macroscopic abnormalities and any compound-related changes were examined microscopically in the rats in the low and mid QVA 149 dose groups.
Peer review: yes (x), no ( )

Toxicokinetics: Blood samples were drawn on days 1 and 14 (2 rats/sex/dose group) at timepoints 0, 30 and 60 minutes, 3 and 8 hours after the completion of dosing and 24 hours after the initiation of dosing.

Results:

Mortality: There were no mortalities in this study.

Clinical signs: There were no treatment–related clinical signs observed in the rats in this study.

Body weights: There was no significant body weight gains observed in the rats in this study.

Food consumption: Food consumption was similar in the rats in all dose groups.

Ophthalmoscope: There was no treatment–related eye changes observed in the rats in this study.

Hematology: Hematology parameters were similar in the rats in all dose groups in this study.

Clinical chemistry: Mean urea values were increased in the females in all QVA 149 dose groups compared with the air control. The increases ranged from 17-32%. Mean urea values were also increased in the females in the QAB149 and NVA 237 dose group ~23%. Mean urea values were similar in all dose groups after the recovery period.

Urinalysis: Urinalysis parameters were similar in the rats in all dose groups in this study.

Gross pathology: No significant gross pathology was identified in the rats treated with QVA 149, QAB149 and QVA237.

Reference ID: 3824703

Organ weights: The organ weights were comparable in the rats in all dose groups in this study.

Histopathology: Microscopic examinations of the tissues of the rats treated with QVA149, NVA237 and QAB149 reveal no significant histological changes compared with the air and placebo control animal tissues.

Toxicokinetics:
All rats were exposed to NVA237 and QAB149. The administration of the fixed combination did not significantly affect the systemic exposure of the individual components (indacaterol and glycopyrrolate). The systemic exposure was slighter higher for glycopyrrolate and indacaterol on day 14 than on day 1. AUC values (0-24) were similar in males and females. Systemic exposure (day 14) for QAB149 increased proportionally with dose in the female rat and greater than proportionally in the male rat. Systemic exposure for NVA237 did not increase proportionally with dose in males or females. The mean toxicokinetic parameters for NVA237 and QAB149 are shown below:

Reference ID: 3824703

Table 2-10 Mean toxicokinetic parameters of QAB149 in rat plasma

Parameter	Units	Day 1	Day 14	Day 1	Day 14
		Males	Males	Females	Females
Group 3 (NVA237/QAB149)	QAB149 target dose (mg/kg/day)	0.092	0.092	0.092	0.092
Mean achieved dose	mg/kg/day	0.074	0.106	0.078	0.112
Time of inhalation	h	0.5	0.5	0.5	0.5
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	2.71	3.36	4.00	5.09
C_{max} / dose	(ng/mL) / (mg/kg/day)	36.6	31.7	51.3	45.4
$AUC_{(0-24h)}$	ng.h/mL	5.22	4.39	5.47	7.94
$AUC_{(0-24h)}$ / dose	(ng.h/mL) / (mg/kg/day)	70.5	41.4	70.1	70.9
Group 4 (NVA237/QAB149)	QAB149 target dose (mg/kg/day)	0.185	0.185	0.185	0.185
Mean achieved dose	mg/kg/day	0.149	0.210	0.156	0.226
Time of inhalation	h	1	1	1	1
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	3.42	5.38	5.25	7.52
C_{max} / dose	(ng/mL) / (mg/kg/day)	23.0	25.6	33.7	33.3
$AUC_{(0-24h)}$	ng.h/mL	9.27	15.9	9.98	16.2
$AUC_{(0-24h)}$ / dose	(ng.h/mL) / (mg/kg/day)	62.2	75.9	64.0	71.6
Group 5 (NVA237/QAB149)	QAB149 target dose (mg/kg/day)	0.370	0.370	0.370	0.370
Mean achieved dose	mg/kg/day	0.299	0.425	0.308	0.448
Time of inhalation	h	2	2	2	2
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	4.42	6.35	5.65	7.75
C_{max} / dose	(ng/mL) / (mg/kg/day)	14.8	14.9	18.3	17.3
$AUC_{(0-24h)}$	ng.h/mL	17.4	26.2	19.9	27.7
$AUC_{(0-24h)}$ / dose	(ng.h/mL) / (mg/kg/day)	58.2	61.6	64.7	61.8
Group 7 (QAB149)	QAB149 target dose (mg/kg/day)	0.370	0.370	0.370	0.370
Mean achieved dose	mg/kg/day	0.435	0.389	0.457	0.416
Time of inhalation	h	2	2	2	2
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	8.88	8.74	8.30	9.17
C_{max} / dose	(ng/mL) / (mg/kg/day)	20.4	22.5	18.2	22.0
$AUC_{(0-24h)}$	ng.h/mL	31.2	36.5	33.6	38.8
$AUC_{(0-24h)}$ / dose	(ng.h/mL) / (mg/kg/day)	71.7	93.8	73.4	93.3

*: end of inhalation

Reference ID: 3824703

Table 2-9 Mean toxicokinetic parameters of NVA237 in rat plasma

Parameter	Units	Day 1	Day 14	Day 1	Day 14
		Males	Males	Females	Females
Group 3 (NVA237/QAB149)	NVA237 target dose (mg/kg/day)	0.032	0.032	0.032	0.032
Mean achieved dose	mg/kg/day	0.026	0.033	0.027	0.035
Time of inhalation	h	0.5	0.5	0.5	0.5
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	0.920	0.884	1.12	1.64
C_{max} / dose	(ng/mL) / (mg/kg/day)	35.4	26.8	41.5	46.9
$AUC_{(0-24h)}$	ng.h/mL	1.57	2.55	1.54	3.36
$AUC_{(0-24h)}$ / dose	(ng.h/mL) / (mg/kg/day)	60.4	77.2	56.9	95.9
Group 4 (NVA237/QAB149)	NVA237 target dose (mg/kg/day)	0.064	0.064	0.064	0.064
Mean achieved dose	mg/kg/day	0.052	0.065	0.054	0.070
Time of inhalation	h	1	1	1	1
t_{max}	h	0.5 **	0 *	0 *	0 *
C_{max}	ng/mL	0.971	1.02	1.66	4.22
C_{max} / dose	(ng/mL) / (mg/kg/day)	18.7	15.7	30.7	60.3
$AUC_{(0-24h)}$	ng.h/mL	2.06	5.74	3.89	8.09
$AUC_{(0-24h)}$ / dose	(ng.h/mL) / (mg/kg/day)	39.6	88.4	72.0	116
Group 5 (NVA237/QAB149)	NVA237 target dose (mg/kg/day)	0.128	0.128	0.128	0.128
Mean achieved dose	mg/kg/day	0.104	0.132	0.107	0.139
Time of inhalation	h	2	2	2	2
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	2.14	1.64	2.56	2.45
C_{max} / dose	(ng/mL) / (mg/kg/day)	20.6	12.4	23.9	17.6
$AUC_{(0-24h)}$	ng.h/mL	8.87	8.80	8.44	10.0
$AUC_{(0-24h)}$ / dose	(ng.h/mL) / (mg/kg/day)	85.3	66.6	78.9	72.3
Group 6 (NVA237)	NVA237 target dose (mg/kg/day)	0.128	0.128	0.128	0.128
Mean achieved dose	mg/kg/day	0.184	0.187	0.194	0.200
Time of inhalation	h	2	2	2	2
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	2.59	4.70	3.10	6.20
C_{max} / dose	(ng/mL) / (mg/kg/day)	14.1	25.1	16.0	31.0
$AUC_{(0-24h)}$	ng.h/mL	9.95	16.4	11.2	22.0
$AUC_{(0-24h)}$ / dose	(ng.h/mL) / (mg/kg/day)	54.1	87.5	57.5	110

*: end of inhalation
**: after the end of inhalation

Study title: **QAB149: A 14-day Inhalation Study of a Combination Powder Formulation in the Dog With a 14 Day Recovery**

Key study findings:
Pulmonary deposited doses: NVA 237: 30.8 mcg/kg, QAB149: 104 mcg/kg and QVA149 (glycopyrrolate/indacaterol) in 1:3 formulation ratios: 8.5/25.3, 15.5/48.3 and 31.5/95 mcg/kg were administered daily for 14 days
There were no mortalities in this study.
Microscopic analyses revealed minimal fibrosis in the papillary muscles of the left ventricle in some dogs in the QAB149 dose group and the high dose group of the fixed combination.

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Minimal cytoplasmic rarefaction was observed in the liver of all dogs in the QAB149 alone and in dogs in all dose groups of the fixed combination except the low dose. This effect was due to the presence of periportal glycogen. This effect was reversible after a 14 day recovery period.
The administration of the combination (indacaterol/glycopyrrolate) did not induce additive or potentiating effects.
Systemic exposure was similar in males and females. The administration of the fixed combination did not significantly affect the systemic exposure of the individual components.

The NOAEL was the mid-dose of 15.5/48.2 mcg/kg due to minimal fibrosis in the papillary muscles of the left ventricle in some dogs in the high dose group of the fixed combination.

Study no.: 79217
Volume #, and page #: Volume 11, page 8-1
Conducting laboratory and location:
Date of study initiation: November 13, 2006
GLP compliance: (b) (4)

QA report: yes (x) no ( )
Drug, lot #, and % purity: QVA149 (QAB149/NVA237 192 +64 mg/g) (19.2% QAB149 (free base), 6.4 %NVA237 (free base), 1% magnesium stearate, 73.4% lactose)/X3480906/97%

Methods
Doses: Air control, vehicle control (lactose and magnesium stearate), NVA237: 123 mcg/kg, QAB149: 416 mcg/kg and QVA149 (glycopyrrolate/indacaterol): 34/101, 62/193 and 126/380 mcg/kg
Pulmonary deposited doses based on 25% deposition: NVA 237: 30.8 mcg/kg, QAB149: 104 mcg/kg and QVA149 (glycopyrrolate/indacaterol): 8.5/25.3, 15.5/48.3 and 31.5/95 mcg/kg.

Species/strain: Dog, Beagle
Number/sex/group or time point (main study): 3/sex/dose group
Route, formulation, volume, and infusion rate: Face mask inhalation/ QVA149 (QAB149/NVA237 192 +64 mg/g) (19.2% QAB149 (free base), 6.4 %NVA237 (free base), 1% magnesium stearate, 73.4% lactose)/10 L/min

Satellite groups used for toxicokinetics or recovery: Recovery: 2/sex/dose group and TK: 3/sex/dose group
Age: 8 months
Weight: Males: 8.0-11.0 kg and females: 5.3-8.0 kg

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Sampling Times: Filter samples were taken 4 times per treatment and on days 1 and 14 of the study. The samples were analyzed for indacaterol and glycopyrrolate via HPLC.

Unique study design or methodology (if any):

The achieved dose of active ingredient for each treatment group was determined as follows:

Calculation of achieved dosage: The achieved dose of active ingredient (mg/kg/day) for each treatment level was determined as follows:

$$\text{Achieved Dose of Active Ingredient (mg/kg/day)} = \frac{\text{RMV x Active Concentration x T x D}}{\text{BW}}$$

Where RMV (L/min)	=	respiratory minute volume measured twice during the pretreatment period*
Active Concentration (mg/L)	=	aerosol concentration of active ingredient determined by chemical analysis
T (min)	=	treatment time
D	=	assumed to be 100 % for total aerosol deposition fraction (not according to particle size)
BW (kg)	=	mean body weight per sex per group from the regular body weight occasions during treatment

* Measured during the pretreatment period using the Buxco Bio System XA.

Clinical signs: Clinical signs were conducted twice daily.

Body weights: Bodyweights were evaluated weekly during treatment and recovery.

Food consumption: Food consumption was evaluated during the final week of the study and during the recovery period.

Ophthalmoscopy: Eye examinations were conducted at the start of and at the end of the second week.

EKG: EKG tracings were conducted pretreatment, end of week and after the recovery period.-30 minutes and 1 and 24 hours after dosing.

Hematology: Hematology parameters were evaluated pretreatment, end of week 2 and after the recovery period.

Clinical chemistry: Clinical chemistry parameters were evaluated pretreatment, end of week 2 and after the recovery period.

Urinalysis: Urinalysis was evaluated pretreatment, end of week 2 and after the recovery period.

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Gross pathology: Gross pathology was evaluated at the end of the treatment period and the recovery period

Organ weights: The following tissues were weighted for each dog:
Adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen,

Histopathology:
Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )
Microscopic examinations were conducted for all dogs in all dose groups. A number of slides from two dogs in the air control and the high dose of QAB149 were stained with PAS and evaluated for the presence of glycogen in the liver.

Results:

Mortality: There were no mortalities in this study.

Clinical signs: An increase in heart rate (~20-30%) was observed in the dogs in the QAB149 dose group on day 1. Partly digested food and brown materials were noted in some female dogs in the high dose QVA149 group (day 12) and in some dogs in the QAB149 dose group (day 2). Some female dogs in the low QVA149 dose group and in QAB149 dose group had soft feces on day 12.

Body weights: Body weight gain was similar in the dogs in all groups in this study.

Food consumption: There were food consumption decreases in the dogs in the NVA237 dose group during week 1 (~44%). During the second week and during recovery, the food consumption of these dogs was comparable with the air controls. During week 1, the mid and high dose QVA 149 dogs had decreases in food consumption by approximately 19%. During week 2 and the recovery period, the food consumption was similar to food consumption to the dogs in the air controls.

Ophthalmoscopy: There were no treatment-related eye changes observed in the dogs in this study.

EKG: Heart rates were increased in the dogs in the NVA237, QAB149 and the mid and high dose QVA149 during the 3 and 8 hour evaluations. The increases were 20-55%. The increases in heart rate were reversed by 24 hours.

Hematology: There were no significant treatment-related changes in hematology parameters in the dogs in this study.

Clinical chemistry: There were no significant treatment-related changes in clinical chemistry parameters in the dogs in this study.

Reference ID: 3824703

Urinalysis: There were no significant treatment-related changes in urinalysis parameters in the dogs in this study.

Gross pathology: No significant gross pathology was identified in the dogs treated with QVA 149, QAB149 and NVA237.

Organ weights The organ weights were comparable in the dogs in all dose groups in this study.

Histopathology:
Microscopic examinations revealed microscopic changes in the liver and the heart. Fibrosis of the papillary muscle (moderate) was observed in one female and one male dog (minimum) in the high dose QVA149 group as well as one male (minimum) and one female dog (minimum in the QAB149 dose group. These changes in the papillary muscle were still present following a 14 day recovery period.
Minimal periportal cytoplasmic rarefaction was found in the liver of the dogs in all QVA149 dose groups except the females in the low dose QVA149 dose group as well as all dogs in the QAB149 dose group. The liver changes were reversible after a 14 day recovery period. The incidence and severity of the liver changes in the dog are shown below:

Table 4-6 Incidence and severity of QAB149 and QVA149-related histopathological changes in the liver (main study)

Tissue/Finding Sex	Male						Female					
QAB149 dose (mg/kg/day)	-[a]	-[b]	0.416	—	—	—	-[a]	-[b]	0.416	—	—	—
QVA149 dose (mg/kg/day NVA237/QAB149)	-[a]	-[b]	—	0.034/ 0.101	0.062/ 0.0193	0.126/ 0.380	-[a]	-[b]	—	0.034/ 0.101	0.062/ 0.193	0.126/ 0.380
Liver no. examined	3	3	3	3	3	3	3	3	3	3	3	3
Cytoplasmic rarefaction												
Total no. affected	—	—	3	1	2	2	—	—	2	—	3	1
Minimal	—	—	3	1	2	2	—	—	2	—	3	1

[a] Air control
[b] Placebo control magnesium stearate/lactose

The NVA237 dose group was not included in the table above because glycogen –related changes in the liver are thought to be induced by indacaterol. There were no significant toxicities induced by GB.

Toxicokinetics:
All dogs were exposed to NVA237 and QAB149. The administration of the fixed combination did not significantly affect the systemic exposure of the individual components (indacaterol and glycopyrrolate). The systemic exposure was slighter higher for NVA237 on day 14 than on day 1. Systemic exposure for NVA237 increased proportionally with dose in the female and male dog. Systemic exposure for QAB149 in males and females increased slightly greater than proportionally in comparison to dose. AUC values (0-24) were similar in males and females. The mean toxicokinetic parameters for NVA237 and QAB149 are shown below:

Reference ID: 3824703

Table 4-5 Mean toxicokinetic parameters of QAB149 in dog plasma

Parameter	Units	Day 1	Day 14	Day 1	Day 14
		Males	Males	Females	Females
Group 4	Achieved dose (mg/kg/day)	0.416	0.416	0.416	0.416
Mean achieved dose	mg/kg/day	0.435	0.295	0.447	0.306
Time of inhalation	h	0.67	0.67	0.67	0.67
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	55.0	20.3	79.3	29.4
C_{max}/dose	(ng/mL)/(mg/kg/day)	127	68.9	177	96.3
$AUC_{(0-24h)}$	ng.h/mL	198	109	246	121
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	455	371	549	394
Group 5	Achieved dose (mg/kg/day)	0.101	0.101	0.101	0.101
Mean achieved dose	mg/kg/day	0.098	0.100	0.116	0.119
Time of inhalation	h	0.17	0.17	0.17	0.17
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	6.89	7.70	7.21	6.17
C_{max}/dose	(ng/mL)/(mg/kg/day)	70.4	77.0	62.1	51.9
$AUC_{(0-24h)}$	ng.h/mL	24.6	39.6	25.9	31.4
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	251	396	223	263
Group 6	Achieved dose (mg/kg/day)	0.193	0.193	0.193	0.193
Mean achieved dose	mg/kg/day	0.160	0.180	0.204	0.230
Time of inhalation	h	0.33	0.33	0.33	0.33
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	17.2	14.5	18.5	20.8
C_{max}/dose	(ng/mL)/(mg/kg/day)	107	80.4	90.6	90.5
$AUC_{(0-24h)}$	ng.h/mL	61.0	66.0	66.9	76.1
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	381	367	328	331

Parameter	Units	Day 1	Day 14	Day 1	Day 14
		Males	Males	Females	Females
Group 7	Achieved dose (mg/kg/day)	0.380	0.380	0.380	0.380
Mean achieved dose	mg/kg/day	0.343	0.350	0.404	0.406
Time of inhalation	h	0.67	0.67	0.67	0.67
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	45.2	24.9	52.2	31.6
C_{max}/dose	(ng/mL)/(mg/kg/day)	132	71.2	129	77.8
$AUC_{(0-24h)}$	ng.h/mL	173	111	179	115
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	504	318	443	284

*: end of inhalation

Reference ID: 3824703

Table 4-4 Mean toxicokinetic parameters of NVA237 in dog plasma

Parameter	Units	Day 1	Day 14	Day 1	Day 14
		Males	Males	Females	Females
Group 3	Achieved dose (mg/kg/day)	0.123	0.123	0.123	0.123
Mean achieved dose	mg/kg/day	0.126	0.130	0.122	0.124
Time of inhalation	h	0.67	0.67	0.67	0.67
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	9.39	9.23	13.1	7.21
C_{max}/dose	(ng/mL)/(mg/kg/day)	74.5	71.0	107	58.1
$AUC_{(0-24h)}$	ng.h/mL	19.9	27.0	26.3	25.1
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	158	208	215	203
Group 5	Achieved dose (mg/kg/day)	0.034	0.034	0.034	0.034
Mean achieved dose	mg/kg/day	0.026	0.025	0.031	0.030
Time of inhalation	h	0.17	0.17	0.17	0.17
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	3.66	5.43	4.21	4.25
C_{max}/dose	(ng/mL)/(mg/kg/day)	141	217	136	142
$AUC_{(0-24h)}$	ng.h/mL	3.86	7.49	4.00	6.03
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	149	300	129	201
Group 6	Achieved dose (mg/kg/day)	0.062	0.062	0.062	0.062
Mean achieved dose	mg/kg/day	0.051	0.064	0.065	0.082
Time of inhalation	h	0.33	0.33	0.33	0.33
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	7.48	7.41	8.54	17.0
C_{max}/dose	(ng/mL)/(mg/kg/day)	147	116	131	207
$AUC_{(0-24h)}$	ng.h/mL	9.54	14.1	10.7	16.8
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	187	220	165	205

Parameter	Units	Day 1	Day 14	Day 1	Day 14
		Males	Males	Females	Females
Group 7	Achieved dose (mg/kg/day)	0.126	0.126	0.126	0.126
Mean achieved dose	mg/kg/day	0.107	0.114	0.126	0.132
Time of inhalation	h	0.67	0.67	0.67	0.67
t_{max}	h	0 *	0 *	0 *	0 *
C_{max}	ng/mL	25.4	10.7	24.9	22.4
C_{max}/dose	(ng/mL)/(mg/kg/day)	237	94.4	198	170
$AUC_{(0-24h)}$	ng.h/mL	36.7	25.6	35.1	31.8
$AUC_{(0-24h)}$/dose	(ng.h/mL)/(mg/kg/day)	343	224	279	241

*: end of inhalation

2.6.6.9 Discussion and Conclusions

Fixed combination of glycopyrrolate (NVA237) and indacaterol maleate (QAB149) dry powder was administered to SD rats and the Beagle dogs in 2 week toxicity studies in a 1:3 ratio to determine whether the administration of a fixed combination of indacaterol and glycopyrrolate would result in potentiation of known target organ toxicities for each agent and/or induce unanticipated target organ toxicities. Neither study provided evidence of an interactive effect under the conditions tested although the doses tested in rats failed to produce any drug-related toxicity. In the toxicity study in the rat, the nose only inhalation doses were: air control, vehicle (lactose-magnesium stearate), pulmonary deposited doses (based on 10% deposition) were QVA149: 3.3/10.1,6.56/20.1 and 13.2/40.2 mcg/kg glycopyrronium bromide: 17.0 mcg/kg and indacaterol maleate: 47.9 mcg/kg. There were no mortalities in the study. There were no significant treatment related effects on clinical signs, body weight, food consumption, ophthalmology, hematology, urinalysis, organ weights, or histopathology. There were no additive or

Reference ID: 3824703

potentiating effects in the rats treated with the fixed combination. The administration of the fixed combination did not significantly affect the systemic exposure of the individual components. The NOAEL was the high dose combination of 13.2/40.2 mcg/kg.

In the dog, face mask only pulmonary deposited doses based on 25% deposition: NVA 237: 30.8 mcg/kg, QAB149: 104 mcg/kg and QVA149 (glycopyrrolate/indacaterol): 8.5/25.3, 15.5/48.3 and 31.5/95 mcg/kg. As in the rat study, the drug ratio was 1:3. Microscopic analyses revealed minimal fibrosis in the papillary muscles of the left ventricle in some dogs in the QAB149 and the high dose of the fixed combination. Minimal, reversible cytoplasmic rarefaction was observed in the liver of all dogs in the QAB149 alone and in dogs in all dose groups of the fixed combination except the low dose. This effect was due to the presence of periportal glycogen. The administration of the combination (indacaterol/glycopyrrolate) did not induce additive or potentiating effects. Systemic exposure was similar in males and females. The administration of the fixed combination did not significantly affect the systemic exposure of the individual components. The NOAEL was the mid combination dose of 15.5/48.2 mcg/kg.

The inhalation toxicity studies in the rat and dog with the fixed combination of indacaterol/glycopyrrolate were designed to address interaction (potentiation of toxicity). The inhalation studies had two shortcomings that would have provided more comprehensive interaction data. (1) the inhalation doses in the rat study did not induce toxicity and (2) the studies tested only 3:1 ratio while clinical range will be 6:1. As mentioned above, the use of the fixed combination did not induce potentiation of known target organ toxicities in the rat or the dog. The toxicities identified in these studies were associated with one or the other drug from previous studies. The ratios of the individual drugs used in the toxicity studies provide an adequate margin of safety for the proposed doses for the clinical studies.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:
QVA149 [indacaterol maleate (QAB149)/glycopyrronium bromide (NVA237)] inhalation powder is a fixed long acting muscarinic antagonist/long acting beta adrenergic agonist combination for use in the treatment of asthma and COPD. The combination will be administered via a (b) (4). The NVA237/QAB149 fixed combination has not been administered to humans. However, there is extensive human experience with indacaterol and glycopyrrolate administered separately. A total 960 asthma patients and 800 COPD patients have been exposed to indacaterol. Single doses ranged from 25-3000 mcg while repeat doses range from 50-800 mcg daily for up to 28 days. A total of 358 COPD patients have been treated with glycopyrrolate. Single inhalation ranged from 60 to 480 mcg while repeat doses ranged from 30-240 mcg administered daily for 28 days.

Reference ID: 3824703

In the current IND, the sponsor plans to conduct two clinical studies. The first study will include a fixed dose combination of 6:1 formulation of QVA149 (300µg/50µg) administered via a SDDP inhaler in patients with moderate to severe COPD daily for 7 days. QAB149 (300 and 600 µg) and placebo will also be included in the study.
In the second study, oral inhalation fixed combination ratios will be 6:1, 3:1 and 1.5:1 (QAB149:NVA237) utilizing doses of 600/100, 300/100 mcg and 150/100 mcg. The drug will be administered daily for 14 days.

In order to determine whether the administration of a fixed combination of indacaterol and glycopyrrolate would result in potentiation of known target organ toxicities for each agent and/or induce unanticipated target organ toxicities, 2–week toxicity studies were conducted in the SD rat and Beagle dog using a 1:3 combination of glycopyrrolate and indacaterol. These studies reveal that the fixed combination of indacaterol/glycopyrrolate did not induce additive or potentiating effects under the conditions tested. No toxicity was identified in the rat study and the dog study produced cardiac and liver toxicity similar to that identified previously for QAB149. The NOAELs from the studies were 13.2/40.2 mcg/kg in the rat and 15.5/48.2 mcg/kg in the dog.

The class target organ for the beta 2 adrenoceptor agonist (indacaterol) is the cardiovascular system and the liver and the liver is also the target organ for the muscarinic antagonist (glycopyrrolate).

There are adequate margins based on mg/kg body weight comparisons for the proposed clinical doses to be used in the proposed clinical trial as are shown below:

Species	NOAEL dose (glycopyrronium/indacaterol) (mcg/kg)	Human dose (glycopyrronium/indacaterol) (mcg/kg)	Margin of safety
Rat	13.2/40.2	1/6	13/7*
Rat	13.2/40.2	1/6	44/22**
Dog	15.5/48.2	1/6	16/8*
Dog	15.5/48.2	1/6	28/15**

*mcg/kg BW basis
** lung mass basis

Internal comments:

Preclinical data supports the use of the fixed combination of indacaterol and mometasone via (b) (4) in the proposed single dose clinical trial. Therefore, the proposed clinical trial is considered safe to proceed.

External comments (to sponsor): None

Reference ID: 3824703

Linked Applications	Sponsor Name	Drug Name
IND 76377	NOVARTIS PHARMS	QVA149 ORAL INHILATION POWDER

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

/s/

VIRGIL E WHITEHURST
07/31/2008

TIMOTHY J MCGOVERN
07/31/2008
I concur.

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	76,377
Supporting document/s:	20, 104, 109, 110, 117, 126, 129, 130, 144, 158
Sponsor's letter date:	6/14/2010, 9/19/2012, 11/09/2012, 11/09/2012, 12/20/2012, 2/21/2013, 3/11/2013, 3/12/2013, 5/31/2013, 8/20/2013
CDER stamp date:	6/14/2010, 9/19/2012, 11/09/2012, 11/09/2012, 12/20/2012, 2/21/2013, 3/11/2013, 3/12/2013, 5/31/2013, 8/20/2013
Product:	QVA149
Indication:	COPD
Sponsor:	Novartis
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products (DPARP)
Reviewer:	Andrew Goodwin, PhD
Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	CDR Christine Chung, RPh

Template Version: September 1, 2010

Reference ID: 3825700

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1 Executive Summary

1.1 Introduction

QVA149 is a combination product being developed by Novartis under IND 76,377 consisting of indacaterol maleate (QAB149, a Long-acting Beta-2 Agonist [LABA]) and glycopyrronium bromide (NVA237, a Long-acting muscarinic antagonist [LAMA]) administered via the inhalation route of exposure for the treatment of COPD. This review evaluates a 13-week combination inhalation toxicology study in dogs and a combination inhalation embryo-fetal development (EFD) study in rats that were conducted to support the phase 3 development program and potential registration of QVA149 in the United States.

1.2 Brief Discussion of Nonclinical Findings

In a 13-week inhalation study in dogs, the toxicity of combination product QVA149 was evaluated and compared to its monoproducts constituents QAB149 and NVA237. Animals were exposed to air control, placebo control (lactose with 1% magnesium stearate), high-dose NVA237 (pulmonary deposited dose 0.035 mg/kg/day), high-dose QAB149 (0.086 mg/kg/day), low-dose QVA149 (0.025 mg/kg/day QAB149 /0.008 mg/kg/day NVA237), mid-dose QAV149 (0.053/0.018 mg/kg/day), or high-dose QVA149 (0.096/0.031 mg/kg/day). Three animals per sex per group were sacrificed after three months and an additional two dogs per sex were sacrificed after a 30-day recovery period for the control and high-dose groups.

QVA149 was not associated with mortality or changes in body weight, food consumption, laboratory parameters, or organ weights. Clinical signs noted at increased frequency in the high-dose (HD) QVA149 group included prepuce discharge, dry skin, and red gums. These findings were not considered adverse.

ECG data indicate that administration of QVA149 is associated with transient tachycardia in a synergistic manner compared to QAB149 or NVA237 alone. Mid- and high-dose QAV149 groups exhibited 45-70% (52-72 BPM) increases in heart rate at 30-60 minutes post-dose. While adverse and test article-related, the effect of QAV149 on heart rate is considered clinically monitorable.

There were no dose-limiting histopathological findings in the dog study. The NOAEL for the study was therefore determined to be the high-dose, corresponding to pulmonary deposited doses of 0.096 mg/kg QAB149 and 0.031 mg/day NVA237.

In an inhalation embryo-fetal development (EFD) study in rats, the maternal and embryo-fetal toxicity of the combination product QVA149 was evaluated and compared to its monoproducts constituents QAB149 and NVA237. Mated females were exposed to air control, placebo control (lactose with 1% magnesium stearate), high-dose NVA237 (pulmonary deposited dose 0.062 mg/kg/day), high-dose QAB149 (0.270 mg/kg/day), low-dose QVA149 (0.021 mg/kg/day QAB149 /0.007 mg/kg/day NVA237),

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mid-dose QAV149 (0.064/0.021 mg/kg/day), or high-dose QVA149 (0.212/0.071 mg/kg/day). 22 females per group were mated, exposed to drug on GD 5-17, and sacrificed on GD 21.

There was no evidence of QVA149-related maternal or fetal toxicity in this study. Therefore, the NOAEL was considered as the high-dose group of 0.212 mg/kg/day QAB149 plus 0.071 mg/kg/day NVA237.

1.3 Recommendations

1.3.1 Clinical Studies Safe to Proceed: Yes

2 Drug Information

2.1 Drug

Trade Name: QVA149 (indacaterol maleate and glycopyrronium bromide) inhalation powder

Indacaterol maleate

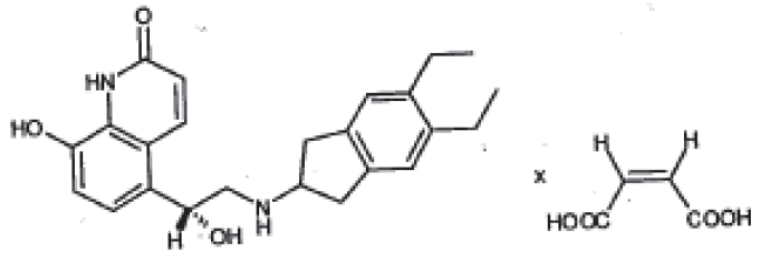
CAS Registry Number: 435273-74-8

Code Name: QAB149

Chemical Name: 5-[(1R)-2-[5, 6-diethyl-2, 3-dihydro-1H-inden-2-yl) amino]-1-hydroxyethyl]-8-hydroxy-2(1H)-quinolinone maleate (9Cl)

Molecular Formula/Molecular Weight: $C_{24}H_{28}N_2O_3xC_4H_4O_4$ (MW: 508.56)

Structure:



Pharmacologic Class: Long-acting Beta-2 Agonist (LABA)

Glycopyrronium bromide

Reference ID: 3825700

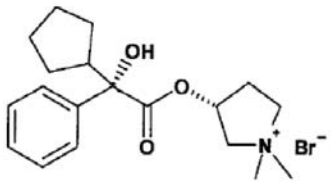
CAS Registry Number: 596-51-0

Code Name: NVA237

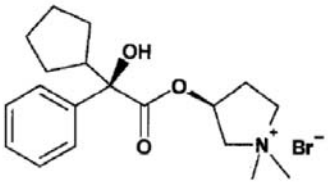
Chemical Name: 3-[(cyclopentylhydrophenylacetyl) oxy)]-1, 1-dimethylpyrrolidinium bromide

Molecular Formula/Molecular Weight: $C_{19}H_{28}NO_3xBr$ (MW: 398.33)

Structure**:



S,R



R,S

**According to CMC reviewer Dr. Craig Bertha (review memo dated 25 September 2012), NVA237 is a racemic mixture of the *S,R* and *R,S* enantiomers (b) (4)

Pharmacologic Class: Long-acting muscarinic antagonist (LAMA)

2.2 Relevant INDs, NDAs, BLAs and DMFs

- Indacaterol maleate (QAB149): NDA-022383 and IND's 48649, (b) (4), (b) (4)
- Glycopyrronium bromide (NVA237): IND 48655

Each of the above IND's was opened with DPARP for development of these drugs in asthma and/or COPD.

2.3 Drug Formulation

The lower-dose formulations of QVA149 to be used in the proposed clinical trials were reviewed and found to be acceptable by CMC reviewer Dr. Craig Bertha (memo dated 25 September 2012). QVA149 capsule will be delivered by the Concept1 device which is approved for use under NDA-022383 (ARCAPTA NEOHALER). Capsule compositions are summarized in the table below.

Reference ID: 3825400

Table 1. QVA149 Capsule Composition

Ingredient	Amount per capsule (mg)		Function	Reference to standards
	27.5/12.5 mcg [1]	27.5/25 mcg [1]		
Capsule fill				
Indacaterol maleate (QAB149)	(b) (4) [2]	(b) (4)	Drug substance	Novartis monograph
Glycopyrronium bromide (NVA237)	0.0156 [3]		Drug substance	Novartis monograph
Lactose monohydrate	24.9113		(b) (4)	Ph. Eur./NF + Novartis monograph [5]
Magnesium stearate	0.0375			Ph. Eur./NF + Novartis monograph [4]
Capsule fill weight	(b) (4)			
Empty capsule shell				
Capsule shell (theoretical weight) [6]	(b) (4)			Novartis monograph
Total capsule weight	**74.0000**	**74.0000**		

1 Amounts are represented as rounded values
2 Corresponds to 27.5 mcg QAB149 active moiety; Salt Corrective factor: (b) (4)
3 Corresponds to 12.5 mcg NVA237 active moiety; Salt Corrective factor: (b) (4)
(b) (4)
5 Novartis monograph contains additional tests as provided in Section [2.1.P.4]
6 The components of the capsule shell are given in Table 2-2 below

Capsule shell components	Reference to standards
Hypromellose (main component)	Ph. Eur. / USP / JP
(b) (4)	

No changes have been made to the placebo product, which consists of a hypromellose capsule containing 24.963 mg lactose monohydrate and 0.0375 mg magnesium stearate (see CMC review memo by Dr. Craig Bertha dated 10 October 2007).

All inactive ingredients are considered qualified from the nonclinical perspective.

2.4 Comments on Novel Excipients

QVA149 contains magnesium stearate at levels corresponding to a maximum daily exposure of 75 ug. Magnesium stearate is contained in the approved inhalation products Foradil Certihaler (NDA 21-592, daily exposure level of 56 ug/day) and Breo Elipta (NDA 204275, daily exposure of 125 ug/day). The Sponsor has submitted nonclinical data to IND (b) (4) and NDA 21-592 including a 6-month inhalation toxicology study of magnesium stearate with a NOAEL of 180 ug/kg/day (see review by

Reference ID: 3825400

Dr. Timothy Robison, 9/29/2003). The NOAEL from the chronic rat study provides a 23.2-fold safety margin to the maximum clinical dose on a mg/kg basis. Magnesium stearate is considered qualified for safety in the QVA149 inhalation drug product at the current clinical dose levels.

2.5 Comments on Impurities/Degradants of Concern

The following comments result from personal communication with CMC reviewer Dr. Craig Bertha. The Sponsor's individual degradant limit level of (b) (4) % is more stringent that than required by ICH Q3B for doses of less than 1 mg. Two degradants (b) (4) of the NVA237 parent molecule are listed in the specifications but are negative for structural alerts. Compound (b) (4) process impurity for NVA237, (b) (4) form (b) (4), which does have a structural alert. However, compound (b) (4) is not detected in the NVA237 drug substance (threshold of (b) (4) %) and there is no safety concern.

Figure 1. Key Structures for QVA149 Impurity and Degradant Evaluation

(b) (4)

All figures c/o Craig Bertha

On May 31, 2013, the Sponsor submitted Supporting Document #144 to the IND, to provide discussion of a "quality event" impacting QVA149 (b) (4)

Reference ID: 3825400

(b) (4)

From the nonclinical perspective, the Reviewer concurs with the Sponsor's assessment that this quality event does not represent a safety risk.

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2.6 Clinical Trials

The Sponsor submitted clinical trial protocols in Supporting Documents 104, 109, and 110 (September-November 2012) proposing to evaluate QVA149 at a twice-daily dose of 27.5 ug indacaterol maleate / 12.5 ug glycopyrronium bromide for 52 weeks. Further discussion around the details of the development program occurred between the Sponsor and the DPARP clinical team at a December 2012 Type C meeting and via written responses provided in February 2013. Subsequently, a 12-week 27.5/12.5 ug protocol (SD#130, March 2013) and a pharmacokinetic equivalence study comparing QVA149 to its constituent monoproducts QAB149 and NVA237 (SD#140, May 2013) were also submitted to the IND.

The objective of the recent clinical protocols proposed by the Sponsor and discussed with the DPARP clinical team is to support eventual licensure of QVA149 in the U.S. (b) (4) all studies involve a maximum 55/25 (b) (4) ug total daily dose that is equal to or lower than QVA149 doses used in previous late stage trials.

The following updated status of ongoing QVA149 clinical trials, as of May 30, 2013, was recently provided by the Sponsor:

- Study CQVA149A2336: 12-week study has enrolled 115 patients at 50 U.S. centers and recruitment continues.
- Study CQVA149A2337: 12-week study has enrolled 281 patients at 57 U.S. centers and recruitment continues.
- Study CQVA1492340: 52-week study that has completed enrollment of 615 patients at 88 U.S. centers.

2.7 Regulatory History

- Arcapta Neohaler (Indacaterol maleate) was approved on July 1, 2011 for the treatment of COPD at a clinical dose of 75 µg/day.
- Glycopyrrolate is found in the following approved products: Cuvposa, Glycopyrrolate, Robinul, and Robinul Forte.

3 Studies Submitted

3.1 Studies Reviewed

1) QVA149: A 13-week inhalation study of a combined powder formulation in the dog with a 4-week recovery (Final report amendment number 1 dated 30 July 2012). Sponsor reference #0670756 and test facility study #79359.

Reference ID: 3825700

The amended study report was filed to IND 76,377 as supporting document 126 on 21 February 2013. The original final report was released on 26 August 2008 and was filed to IND 76,377 as supporting document 20 on 14 June 2010. Changes effected in the amendment did not impact the interpretation of study data.

2) QVA149: An inhalation embryo fetal development study in rats (Final report dated 24 July 2008). Sponsor reference #0670755 and test facility study #901312.

3.3 Previous Reviews Referenced

- Dr. Virgil Whitehurst, IND 76,377 review of two-week QVA149 combination inhalation toxicology studies in rats and dogs, 31 July 2008.
- Dr. Virgil Whitehurst, NDA 22-383 review for indacaterol maleate, 15 December 2008.
- Dr. Lawrence Sancilio, IND 48,655 review of NVA237 six-month rat toxicology study, 27 March 2009.
- Dr. Lawrence Sancilio, IND 48,655 review of NVA237 four-week rat and dog toxicology studies, 20 June 2007.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: QVA149: A 13-week inhalation study of a combined powder formulation in the dog with a 4-week recovery

Study no.:	Sponsor reference #0670756 Test facility study #79359
Study report location:	EDR #126 (Amendment 1, 30 July 2012) EDR #20 (Original, 26 August 2008)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	First treatment 19 April 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	**QAB149** batch T005 0107, 101.4% purity; salt correction factor (b) (4) **NVA237** batch X344 0906, 98% purity; salt correction factor (b) (4) **QVA149** batch T004 0107, 99-100% purity

Key Study Findings

- In the inhalation study in dogs, the toxicity of combination product QVA149 was evaluated and compared to its monoproducts constituents QAB149 and NVA237.

Reference ID: 3825700

Animals were exposed to air control, placebo control (lactose with 1% magnesium stearate), high-dose NVA237 (pulmonary deposited dose 0.035 mg/kg/day), high-dose QAB149 (0.086 mg/kg/day), low-dose QVA149 (0.025 mg/kg/day QAB149 /0.008 mg/kg/day NVA237), mid-dose QAV149 (0.053/0.018 mg/kg/day), or high-dose QVA149 (0.096/0.031 mg/kg/day).

- Clinical signs associated with QVA149 exposure included increased dry skin (males) and red gums (males and females). These findings were not adverse.
- Mid- and high-dose QVA149 groups exhibited marked 45-70% (52-72 BPM absolute change) increases in heart rate at 30-60 minutes post-dose. There did not appear to be any dose-response between the MD and HD groups despite a two-fold difference in active ingredient doses. This represents an adverse synergistic effect of the individual effects of QAB149 and NVA237 that is considered clinically monitorable.
- There were no dose-limiting histopathological findings in the study. The NOAEL was therefore determined to be the high-dose, corresponding to theoretical achieved daily dose of 96 ug/kg QAB149 and 31 ug/day NVA237.
- At the NOAEL, exposure ($AUC_{0\text{-}24h}$) values of 129.5/33.4 (QAB149/NVA237) ng*h/mL were observed.

Methods

Target Doses:	**Group 1)** 0 (air control) **2)** 0 (lactose with 1% magnesium stearate; placebo control) **3)** 0.128 mg/kg/day NVA237 (high NVA237) **4)** 0.37 mg/kg/day QAB149 (high QAB149) **5)** 0.092/0.032 mg/kg/day QVA149 (expressed as QAB149/ NVA237; low-dose) **6)** 0.185/0.064 mg/kg/day QVA149 (mid-dose) **7)** 0.37/0.128 mg/kg/day QVA149 (high-dose)
Frequency of dosing:	Once daily
Route of administration:	Inhalation
Formulation/Vehicle:	99% lactose / 1% magnesium stearate (placebo) Lactose with 1% magnesium stearate (active)
Species/Strain:	Beagle dog
	Source: (b) (4)
Number/Sex/Group:	3/sex/group
Age:	9-10 months
Weight:	8.8-12.0 kg (males); 5.6-8.6 kg (females)
Satellite groups:	2/sex/group for Groups 1, 2, and 7 for 4-week recovery sacrifice.
Unique study design:	All groups were exposed to air/vehicle/aerosol for 40 minutes per day except QVA149 LD (10 minutes) and MD (20 minutes).
Deviation from study protocol:	No deviations that impacted study data interpretation

Reference ID: 3825700

Observations and Results

Inhalation Procedures and Atmosphere Evaluation

Group housing of animals was used in this study. An acclimation period of approximately three weeks was included. Animals were acclimated to the harness, restraint platform, and exposure mask used in the study prior to the dosing period.

Harnessed animals were restrained on a dosing platform and fitted with a mask placed over the muzzle during inhalation exposure. Powdered test or control article was generated via a positive-flow delivery system, introduced into the mixing chamber with pre-dried compressed air, and delivered to the mask. Desired placebo, QAB149, NVA237, and QVA149 concentrations in the delivered atmosphere were controlled by adjusting the rate of introduction in the feed nozzle of the T-section and the length of exposure.

Characterization of the control and test article aerosols was performed by measuring deposits on gravimetric filters pre-study and on Days 1, 2, 3, 5, 7, 9, 13, 16, 20, 23, 26, 27, 30, 34, 37, 41, 44, 48, 51, 54, 55, 58, 62, 65, 69, 72, 76, 79, 82, 83, 86 and 89. Parameters evaluated included QAB149, NVA237, and magnesium stearate concentration, homogeneity of the chamber atmosphere, and aerosol particle size distribution.

Achieved atmospheric conditions for QAB149, NVA237, and magnesium stearate are summarized in the table below. Geometric standard deviations for MMAD values were ≤1.9 uM in all groups. Separate measurements were not performed for Groups 5 and 6 because they involved the same chamber run at the same conditions as Group 7.

Table 2. 13-week Dog Study: Atmospheric Conditions

Group	Active concentration (mg/L)			MMAD (uM)		
	QAB149	NVA237	MgS	QAB149	NVA237	MgS
2: Placebo	NA	NA	0.0007	NA	NA	1.9
3: NVA237	NA	0.0054	0.0007	NA	2.3	1.7
4: QAB149	0.0157	NA	0.0007	2.1	NA	1.5
5: low QVA149	0.0163	0.0053	0.0006	2.4	2.5	1.9
6: mid QVA149	0.0161	0.0053	0.0007	2.4	2.5	1.9
7: high QVA149	0.0161	0.0052	0.0007	2.4	2.5	1.9

MMAD: mass median aerodynamic diameter
MgS: magnesium stearate
NA: not applicable

Targeted and achieved doses are summarized in the table below. Pulmonary deposited doses of magnesium stearate were approximately 0.004 mg/kg/day for Groups 2, 3, 4, and 7 and about two- and four-fold lower in Groups 6 and 5 as predicted (calculations not shown)

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Table 3. 13-week Dog Study: Calculation of Pulmonary Deposited Doses

Group	Target Dose (mg/kg/day)		Sex	Body Weight (kg)[a]	RMV (L/min)	Active Concentration (mg/L)		Exposure duration (min)	Deposition fraction	Pulmonary Deposited Dose (mg/kg/day)[b]	
	QAB149	NVA237				QAB149	NVA237			QAB149	NVA237
3: High NVA237	NA	0.128	M	10.70	6.88	NA	0.0054	40	0.25	NA	0.035
			F	6.96	4.50						0.035
4: High QAB149	0.37	NA	M	10.80	5.57	0.0157	NA	40	0.25	0.081	NA
			F	8.10	4.65					0.090	
5: Low QVA149	0.092	0.032	M	11.70	6.77	0.0163	0.0053	10	0.25	0.024	0.008
			F	7.30	4.67					0.026	0.008
6: Mid QVA149	0.185	0.064	M	11.12	6.32	0.0161	0.0053	20	0.25	0.046	0.015
			F	7.02	5.21					0.060	0.020
7: High QVA149	0.37	0.128	M	10.95	5.95	0.0161	0.0052	40	0.25	0.087	0.028
			F	7.60	4.97					0.105	0.034

NA: Not applicable; M: male; F: female; RMV: respiratory minute volume
[a]Body weight represents average over days 1-92.
[b]Calculated via the following formula: (RMV)*(Active concentration in air)*(duration of exposure)*(deposition factor of 0.25 for dogs)/(body weight)

Mortality

Animals were observed for mortality twice daily (AM and PM). There were no unscheduled deaths in the study.

Clinical Signs

Animals were observed for signs of ill health twice daily (AM and PM) and underwent detailed exams during the pre-treatment period and then weekly during the treatment and recovery periods.

Treatment with QVA149 by inhalation did not result in any dose-limiting or adverse clinical signs. As summarized in the table below, the combination product was associated with increased dry skin in males as well as red gums in both sexes. These clinical observations were not considered adverse. Red gums, attributed to vasodilation induced by B_2 agonist activity was not noted in the QAB149 group in this study but was observed in the QAB149 monoproduct toxicology studies in dogs (IND (b) (4) review dated 5/8/2008 by Virgil Whitehurst). This finding is therefore considered unlikely to be a true QVA149-related effect.

Table 4. 13-week Dog Study: Clinical Signs

Observation - Males	Air	Placebo	QAB149	NVA237	QVA LD	QVA MD	QVA HD
Number in group	*5*	*5*	*3*	*3*	*3*	*3*	*5*
Skin dry, forepaw left	0	1	0	1	1	1	5
Skin dry, forepaw right	2	0	0	1	1	1	5
Skin dry, hindpaw left	0	0	0	1	1	0	3
Skin dry, hindpaw right	1	0	0	1	1	0	3
Skin dry, muzzle	0	0	0	0	0	0	3
Skin red, gums	0	0	0	1	1	2	2
Observation - Females	**Air**	**Placebo**	**QAB149**	**NVA237**	**QVA LD**	**QVA MD**	**QVA HD**
Skin red, gums	0	0	0	0	2	1	4

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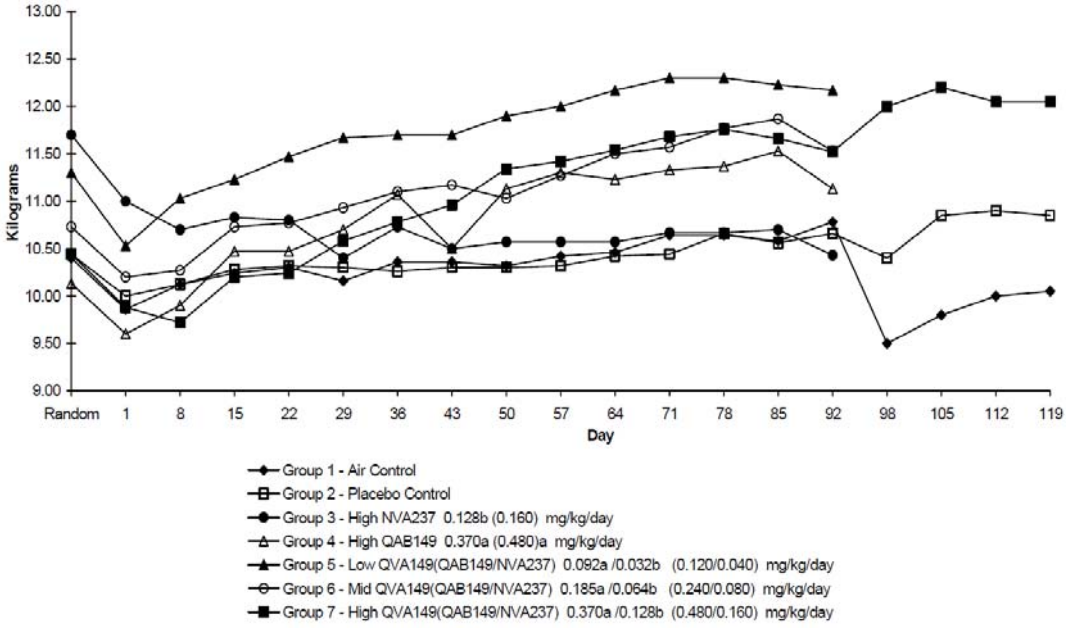
Body Weights

Animals were weighed in the pre-treatment period and were randomized on this basis. Body weights were recorded weekly during the treatment and recovery periods.

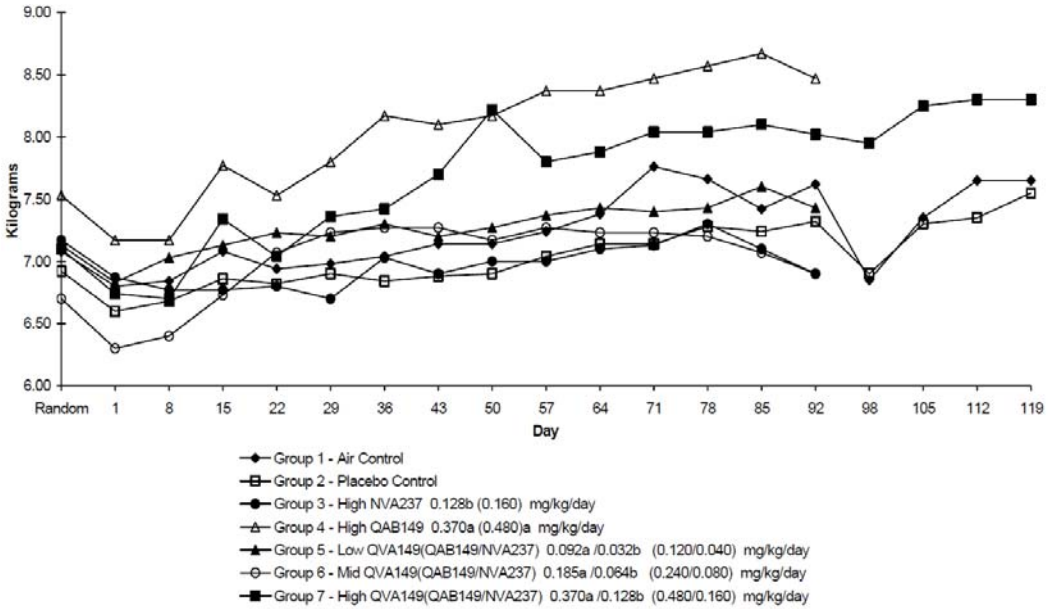
Increased body weights have been associated with studies of B_2 agonists such as QAB149. In males, animals receiving QAB149 (alone or any dose level of QVA149) gained 13-17% body weight over the course of the 13-week study in contrast to little or no weight gain in control or NVA237 animals. Females receiving the highest dose of QAB149 (alone or as high-dose QVA149) gained 18-19% body weight during the study compared to 0-12% increases in other groups. There were not any body weight effects attributed to the combination test article.

Figure 3. Effect of QVA149 on Body Weight: Males



Reference ID: 3825400

Figure 4. Effect of QVA149 on Body Weight: Females



Feed Consumption

Individual food consumption was recorded daily starting one week prior to dosing and throughout the treatment and recovery periods.

Decreased food consumption was noted in animals receiving NVA237 and MD/HD QVA149 during the first three weeks of dosing (see figure below). This finding is likely secondary to inhibition of salivary gland secretion associated with LAMAs such as NVA237. Supplementation with warm water beginning in Week 4 makes numerical comparisons inconclusive for the remainder of the study. The Sponsor stated that food consumption “generally returned to normal with supplementation during the treatment and recovery periods.” Animals in the QAB149 group experienced increased food consumption, typical of findings with other B_2 agonists.

There was no increased effect on food consumption with QVA149 compared to its monoproduct constituents and these effects were not considered adverse or dose-limiting.

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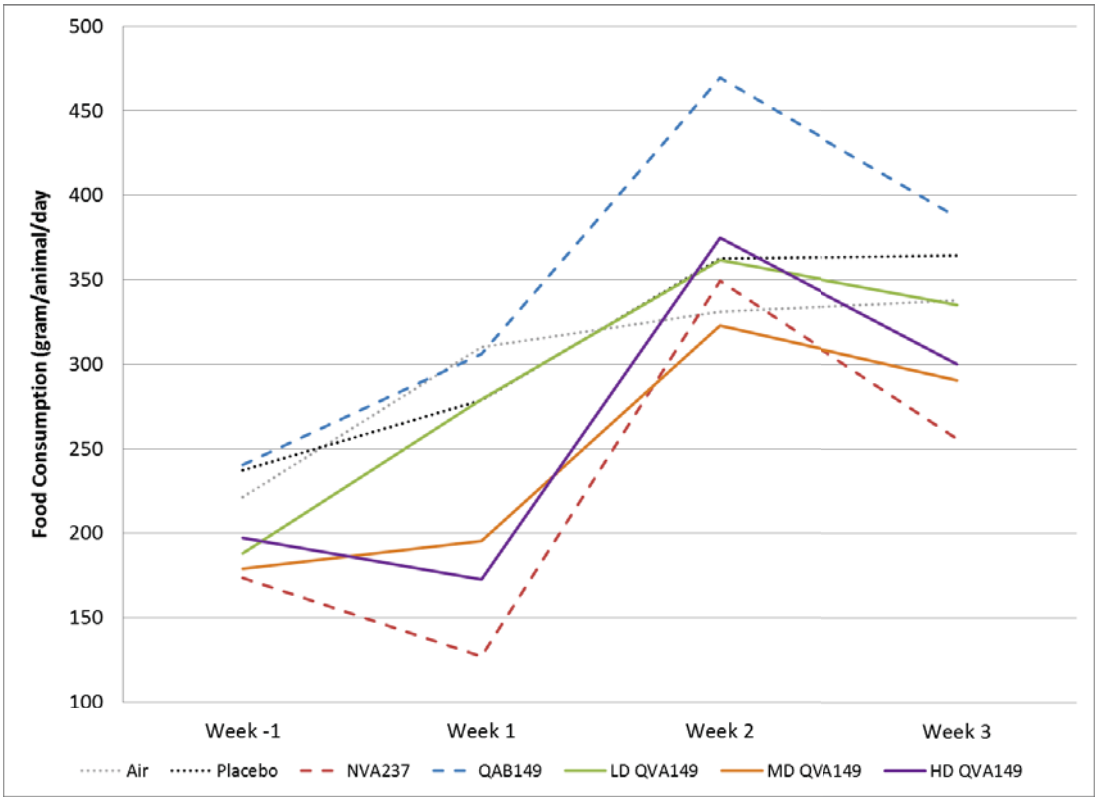
Figure 5. Effect of QVA149 on Food Consumption



Ophthalmoscopy

A board certified veterinary ophthalmologist performed funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations on all animals during the pre-treatment period at and the end of Week 13. 1% mydriacyl was used.

There were no test-article related ophthalmoscopic findings.

ECG

A board certified veterinary cardiologist performed electrocardiography recordings on all animals during the pre-treatment period and at the end of Week 13. Measurements were obtained pre-dose and 30 minutes, 1 hour, 24 hours, and 48 hours (recovery only) post-dose using limb leads I, II, III, aVR, and aVF. PR, QRS, QT, and RR (to calculate heart rate) intervals were measured quantitatively and qualitative assessment of the trace was performed for rhythm and abnormalities. QTC was calculated via the Van de Waters method, which is considered appropriate by the Reviewer for this study in beagle dogs. Respiratory minute volume was measured twice for each animal during the pre-treatment period and was assumed by the Sponsor to be unaffected by treatment.

Animals receiving air or placebo control inhalation treatments (gray and black dotted lines, respectively, on chart) experienced very slight increases in HR 30-60 minutes

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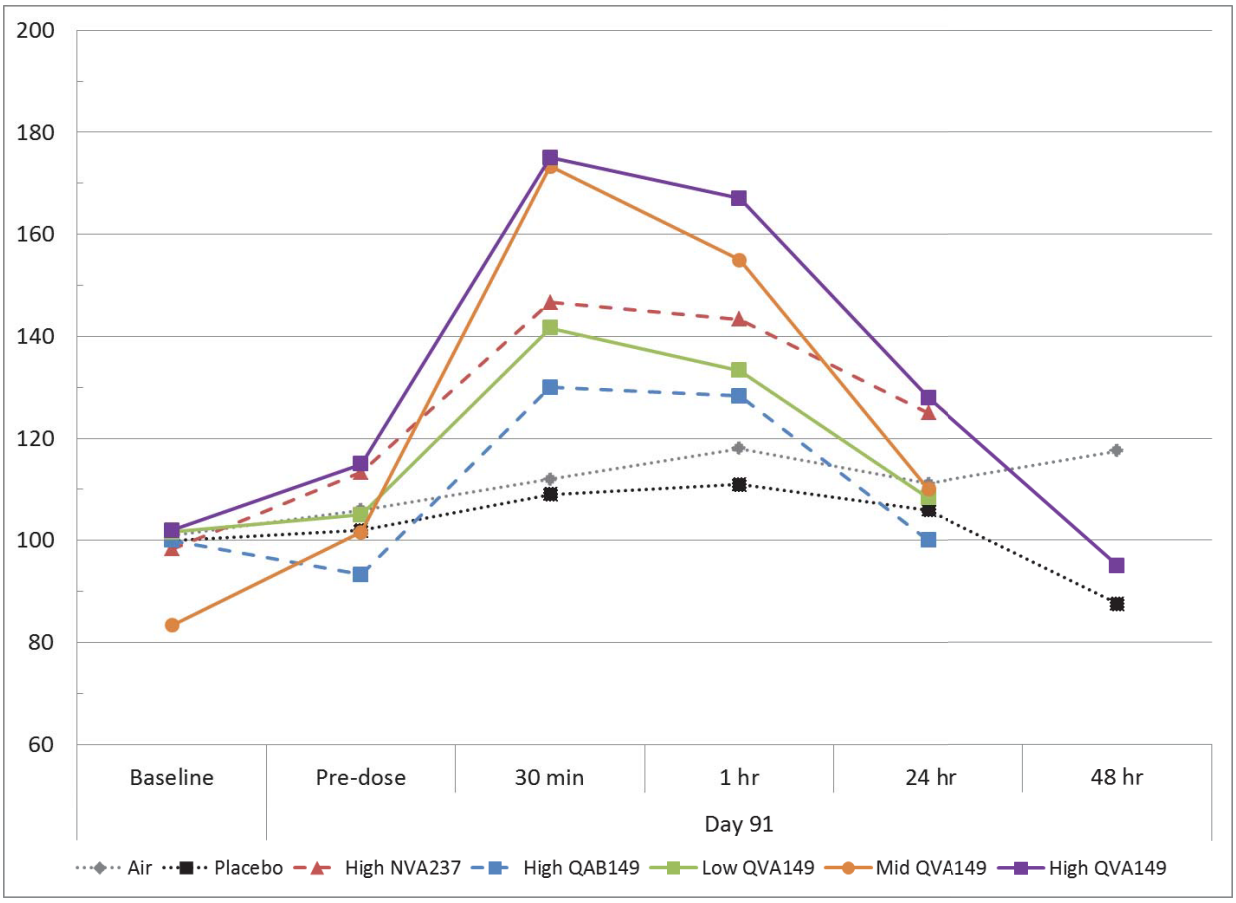
post-dose. High-dose NVA237 and QAB149 alone (red and blue dashed lines, respectively, on chart) were each associated with 26-38% (30-37 BPM absolute change) increases in HR at 30-60 minutes post-dose, consistent with prior nonclinical experience with these drugs. HR values were within 10% of baseline by 24 hours post-dose.

Combination inhalation treatment with QVA149 resulted in a synergistic effects on HR compared to QAB149 or NVA237 alone. Animals receiving low-dose QVA149 (solid green line on chart) experienced a similar degree of tachycardia as the monoproducts controls despite four-fold lower doses of each constituent. The mid- (solid orange line) and high-dose (solid purple line) QVA149 groups exhibited marked 45-70% (52-72 BPM absolute change) increases in HR at 30-60 minutes post-dose. There did not appear to be any dose-response between the MD and HD groups despite a two-fold difference in active ingredient doses.

Figure 6. Effect of QBA149, NVA237, and QVA149 on Heart Rate in Dogs



Y-axis denotes heart rate (beats per minute). Plotted values are group means at each time points (n=3 or 5 per sex per group). 48 hour timepoint includes only recovery sacrifice animals (n=2 per sex per group).

In summary, QVA149 and its monoproducts constituents QAB149 and NVA237 induced transient tachycardia in dogs after 13 weeks of treatment, with heart rates peaking at 30

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minutes post-dose and largely returning to pre-dose baseline levels by 24 hours post-dosing. Effects were similar in males and females. The persistence of the effect is unclear due to the lack of recordings between 1 and 24 hours post-dose. The tachycardia finding in the MD and HD QVA149 groups is considered to be adverse but acceptably monitorable in the clinical setting. There were no test article-related effects on any other ECG parameters evaluated.

Hematology

Blood samples were collected from the jugular vein of all animals once during the pre-treatment period and again during Week 13 (main study groups) or Week 17 (recovery groups). The following parameters were evaluated:

EDTA anticoagulant

- Hematocrit
- Blood cell morphology
- Hemoglobin
- Red blood cell count
- Erythrocyte indices (MCV, MCH, MCHC, RDW)
- Mean platelet volume
- Reticulocyte count (absolute and percent)
- Platelet count
- White blood cell count (total, absolute, and percent differential)

Citrate anticoagulant

- Activated partial thromboplastin time
- Prothrombin time
- Fibroinogen

There were no test article-related changes in any of the hematology or coagulation parameters measured in this study.

Clinical Chemistry

Blood samples were collected from the jugular vein of all animals once during the pre-treatment period and again during Week 13 (main study groups) or Week 17 (recovery groups). Animals were fasted overnight prior to sample collection. The following parameters were evaluated:

- A/G ratio (calculated)
- calcium
- inorganic phosphorus
- alanine aminotransferase
- cholesterol
- potassium
- albumin
- chloride
- sodium
- alkaline phosphatase
- creatinine
- bilirubin (total, direct, indirect)
- aspartate aminotransferase
- globulin (calculated)
- total protein
- blood urea nitrogen
- glucose
- triglycerides

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- creatine kinase

Increased creatinine (+36-58%) levels compared to placebo control groups were generally noted in QVA149-treated groups (see table below). These effects were similar in magnitude to those observed for QAB149 alone, either in this study or in previous studies reviewed as part of the indacaterol NDA (Dr. Virgil Whitehurst, 15 December 2008). There was no consistent association between increasing QVA149 doses and increasing effects on these parameters. Further, there were no correlating histopathological findings in the kidney in this study. These effects were judged to be neither adverse nor related to the combination product test article QVA149.

Table 5. 13-week Dog Study: Clinical Chemistry Parameters

Parameter - Males	**Air**	**Placebo**	**NVA237**	**QAB149**	**QVA LD**	**QVA MD**	**QVA HD**
Number of animals in group	*5*	*5*	*3*	*3*	*3*	*3*	*5*
Creatinine (mg/dL)	0.76	0.66	0.67	0.90	0.90	**1.03**	**1.04**
Percent change		-	+2%	+36%	+36%	+56%	+58%
Parameter - Females	**Air**	**Placebo**	**NVA237**	**QAB149**	**QVA LD**	**QVA MD**	**QVA HD**
Number of animals in group	*5*	*5*	*3*	*3*	*3*	*3*	*5*
Creatinine (mg/dL)	0.66	0.66	0.53	0.97	0.77	0.87	**0.88**
Percent change		-	-20%	+47%	+17%	+32%	+33%

Percent change is displayed relative to placebo control group.
Bold text denotes statistically significant difference vs. placebo control

Urinalysis

Urine was collected overnight from all animals while food deprived once during pre-treatment and in Week 13 (main study groups) or Week 17 (recovery groups). The following parameters were evaluated:

- Appearance
- ketones
- specific gravity
- Bilirubin
- microscopy of centrifuged deposit
- urobilinogen
- Blood
- nitrites
- volume
- Color
- pH
- Glucose
- proteins

There were no test article-related changes in any urinalysis parameters measured.

Gross Pathology

At the end of the main study or recovery periods, animals were fasted overnight, sedated with Ketamine/Xylazine, anesthetized with sodium pentobarbital injection, and euthanized by exsanguination via the axillary or femoral arteries. Necropsies were supervised by a pathologist and consisted of external and internal examinations and identification of all clinically recorded lesions.

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Observations of small thymus were noted for 1-2 males per groups in the QAB149 and all QVA149 dose groups at the main study sacrifice only. Corresponding thymic lymphoid atrophy was noted in some of these animals. In addition, thymic lymphoid atrophy plus low organ weight in the absence of a gross pathological correlate was observed in one LD QVA149 female. There was no dose-dependent increase in the incidence or severity of these findings in the QVA149 groups, and no evidence of a synergistic effect compared to the QAB149 group. These effects do not appear to be attributable to the combination test article.

Organ Weights

The following organs were dissected free of fat and weighed (paired organs were weighed together):

- adrenal glands
- brain
- heart
- kidneys
- liver
- lungs
- ovaries
- pituitary
- prostate
- spleen
- testes
- thymus
- thyroid and parathyroids
- uterus

Absolute and body weight-adjusted thymus organ weights were generally decreased in drug-treated groups. Correlating observations of small thymus and microscopic evidence of thymic lymphoid atrophy were noted in some animals as described above. The similar magnitude of effect seen in animals receiving the monoproducts NVA237 and QAB149 and the lack of any relationship to QVA149 dose indicate that this finding is not attributable to the combination test article.

Table 6. 13-week Dog Study: Organ Weights

Parameter - Males	Placebo	NVA237	QAB149	QVA LD	QVA MD	QVA HD
Number of animals in group	*5*	*3*	*3*	*3*	*3*	*5*
Thymus weight (grams)	4.36	3.69	1.56	1.74	3.13	2.67
Absolute change	-	-15%	-64%	-60%	-28%	-39%
Body weight-adjusted change	-	-16%	-68%	-65%	-36%	-42%
Parameter - Females	**Placebo**	**NVA237**	**QAB149**	**QVA LD**	**QVA MD**	**QVA HD**
Number of animals in group	*5*	*3*	*3*	*3*	*3*	*5*
Thymus	7.21	3.59	5.84	3.25	3.50	4.22
Absolute change	-	-50%	-19%	-55%	-52%	-42%
Body weight-adjusted change	-	-44%	-32%	-54%	-46%	-43%

Histopathology

<u>*Adequate Battery*</u>: Yes. The following tissues and organs were fixed/preserved:

- Abnormalities
- animal identification
- adrenals
- aorta (thoracic)

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- bone and marrow (sternum)
- brain (forebrain, midbrain, cerebellum, and medulla oblongata)
- cecum
- colon
- duodenum
- epididymides
- esophagus
- eyes
- gallbladder
- heart (including section of aorta)
- ileum
- jejunum
- kidneys
- lacrimal gland
- larynx (three levels)
- liver (sample of two lobes)
- lungs (all lobes)
- lymph nodes (tracheobronchial, mandibular, unilateral; mesenteric)
- mainstem bronchi
- mammary gland (inguinal)
- nasal cavities and sinuses (three levels)
- nasopharynx
- optic nerves
- ovaries
- pancreas
- pharynx
- pituitary
- prostate
- salivary gland (submandibular)
- sciatic nerve
- skeletal muscle
- skin (inguinal)
- spinal cord (cervical)
- spleen
- stomach
- testes
- thymus
- thyroid lobes (and parathyroids)
- tongue
- trachea
- ureters
- urinary bladder
- uterus (horns, body and cervix)
- vagina

All tissues were embedded, sectioned, and stained with hematoxylin and eosin for examination. PAS staining (2 per sex for air control and high-dose QVA149 groups) was employed to evaluate glycogen presence in the liver and trichrome staining of heart tissue was conducted. In addition, three femoral bone marrow smears were prepared from each animal and stained with eosin-thiazin.

Peer Review: A second pathologist from the test facility performed a peer review according to internal standard operating procedures. All tissues from one (groups 1-4) or two (group 7) animals per sex were examined, along with a representative number of liver and thymus slides from all groups. The pathology report reflects the discussion and consensus of the two test facility pathologists. In addition, a Sponsor pathologist reviewed the pathology report and examined selected tissues (no report provided).

Histological Findings

Microscopic histopathological findings associated with QVA149 are summarized in the table below. Potential target organs of toxicity for the combination product include the adrenals, , lung, mesenteric and tracheobronchial lymph nodes, nasopharynx, , and skin. Liver (glycogen rarefaction) and heart (myocardial fibrosis) findings noted in the

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QAB149 (indacaterol) pivotal toxicology package were less common in each of the QVA149 dose groups compared to the QAB149 control arm.

All other findings described below do not correlate to those described in reviews of QAB149 toxicity studies in dogs of up to 39 weeks (Whitehurst, NDA review) or a 6-month NVA237 toxicity study in rats (Sancilio review 3/2009). The Sponsor has completed a 39-week NVA237 study in dogs, though this study was determined to not be required for the NVA237 development program. The final study report (Novartis reference #0670548) was submitted to IND 48,655 on 8/12/2013 as SD#247 following an information request by the Reviewer. This study has not been reviewed by Agency staff.

Table 7. 13-week Dog Study: Histopathological Findings

Group / Finding - Males	Control		NVA 237	QAB 149	QVA LD	QVA MD	QVA HD
	Air	Veh.					
Main Study (# examined)	*3*	*3*	*3*	*3*	*3*	*3*	*3*
LN, mesenteric							
Hyperplasia, lymphoid (1)	0	0	0	0	0	0	1
Nasopharynx							
Inflammation, neutrophilic, submucosal gland (1)	0	0	0	0	0	0	2
(2)	0	0	0	0	0	1	0
Skin							
Inflammation, follicular/ perifollicular (1)	0	0	0	0	0	0	1
Group / Finding - Females	**Control**		**NVA 237**	**QAB 149**	**QVA LD**	**QVA MD**	**QVA HD**
	Air	**Veh.**					
Number of animals in group	*3*	*3*	*3*	*3*	*3*	*3*	*3*
Adrenal							
Cyst, single, bilateral (1)	0	0	0	0	0	0	1
LN, tracheobronchial							
Deposits, pigment (1)	0	0	0	0	0	0	1
Pancreas							
Inflammation, chronic, multifocal (1)	0	0	0	0	0	0	1

Veh: placebo vehicle control
LN: lymph node
Number in parentheses indicated grade: 1) minimal; 2) slight; 3) moderate; 4) marked

Findings in the QVA149 high-dose group in the skin (inflammation) and tracheobronchial lymph node (pigment deposits) were considered likely to be incidental. Other findings at the QVA149 HD including lymphoid hyperplasia of the mesenteric lymph node and adrenal cyst are less common background findings and are of uncertain relationship to the combination product. Nasopharynx inflammation showed dose-dependent incidence, but not severity in male dogs receiving QVA149. According to the Sponsor's study summary, findings of minimal inflammation in the submucosal pharyngeal glands was observed in the 39-week NVA237 dog study. While absent from NVA237 animals in the present study, this finding is considered unlikely to be attributable to the combination test article.

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A single female in the HD QVA149 group (#751) was observed to have chronic multifocal inflammation in the pancreas. It is noted that this animal had relatively high steady-state exposure to both QAB149 (AUC_{0-24h} 164 hg*h/mL) and NVA237 (43.0 ng*h/mL). When considering all exposed animals (6 per sex total per test article) in Group 3 (high NVA237), Group 4 (high QAB149), and Group 7 (HD QVA149), the QAB149 exposure in animal #751 was the highest of all treated animals and the NVA237 exposure was highest among females and second-highest overall. Historical control data was requested from and provided by the Sponsor. This finding was observed in 1/39 control females dogs aged 6-11 months across 10 studied at the test facility. The 33% incidence in the present study is higher than the 20% maximum single study incidence in the historical control data, though this comparison is of limited value with only a single case. Chronic inflammation of the pancreas is also described as a "sporadic" finding in young Beagle dogs (Greaves, *Histopathology of Preclinical Toxicity Studies*), though the cited study mentions a single animal with confirmed inflammation in the context of pancreatic atrophy out of a population of thousands. In conclusion, this finding is noted but is not clearly test article-related and is not considered dose-limiting.

In summary, no histopathological findings were judged to be dose limiting and the NOAEL in this inhalation toxicity study of QVA149 was considered as the high-dose of 0.096/0.031 (QAB149/NVA237) mg/kg/day.

Special Evaluation

Troponin T

In the last week of treatment (predose) and in the last week of recovery, blood samples (approximately 0.5 mL) were collected from the jugular vein of all animals for Troponin T analysis. Blood was allowed to clot, centrifuged, and serum was stored at -80˚C until analysis.

No test article-related effect on troponin T levels was identified, as levels were undetectable in nearly all animals.

Trichrome staining

Trichrome staining of heart tissue was included in the protocol based on discussion in the May 2007 meeting minutes. No pathological findings were noted.

Conclusion

Ideally the troponin T evaluation might have completed early in the treatment period in order to avoid any effects being masked by animals becoming tolerant to the test article over the 13-week study period. However, in totality there is sufficient data available to conclude that QVA149 does not induce cardiac histopathological lesions in dogs. No effects on troponin were noted at early times points in the 39-week QAB149 inhalation toxicity study in dogs and there were no heart findings in either the 2- or 13-week QVA149 studies. In addition it is noted that patients in the clinical program will be carefully monitored based on the known effect of this drug combination on heart rate.

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Toxicokinetics

Blood samples (1.4 mL) were collected from the jugular vein from main study animals on Day 1 and at study conclusion on Day 89 (0 hour immediately after inhalation, 30 minutes, 1 hour, and 8 hours post-completion of inhalation, and 24 hours after initiation of inhalation). Resulting plasma samples were analyzed for QAB149 and NVA237 by LC/MS/MS (LLOQ 0.1 ng/mL in 100 and 50 uL plasma, respectively). Drug concentrations, C_{max}, T_{max}, and AUC_{0-24h} were determined.

Pharmacokinetic parameters for QAB149 and NVA237 for each treatment group are summarized in the table below.

Table 8. 13-week Dog Study: Toxicokinetics

Parameter	Day	Sex	Group 3 NVA237	Group 4 QAB149	Group 5 LD QVA149	Group 6 MD QVA149	Group 7 HD QVA149
Length of inhalation (h)			0.67	0.67	0.17	0.33	0.67
QAB149							
T_{max} (h)[a]	1	M		0	0.33	0	0
	89	M		0	0.33	0	0.17
	1	F		0	0.17	0	0
	89	F		0	0.17	0	0.17
C_{max} (ng/mL)	1	M		15.3	6.44	12.9	36.1
	89	M		30.5	7.67	37.6	31.4
	1	F		21.3	6.21	15.0	40.1
	89	F		29.3	9.97	26.2	28.2
AUC_{0-24h} (ng*h/mL)	1	M		52.5	26.7	55.8	131
	89	M		110	42.8	83.1	131
	1	F		67.5	24.0	57.7	167
	89	F		95.3	38.6	94.1	128
NVA237							
T_{max} (h)[a]	1	M	0		0	0	0
	89	M	0		0	0	0
	1	F	0		0	0	0
	89	F	0		0	0	0
C_{max} (ng/mL)	1	M	11.8		2.96	5.81	22.7
	89	M	9.40		8.38	15.8	21.1
	1	F	12.0		3.72	7.88	19.1
	89	F	9.11		5.08	14.7	20.9
AUC_{0-24h} (ng*h/mL)	1	M	27.6		4.52	10.1	33.1
	89	M	26.0		11.6	18.8	34.5
	1	F	29.6		3.97	9.45	33.6
	89	F	23.3		9.35	20.3	32.6

[a]T_{max} value represents time after the end of inhalation
M: male; F: female
N=3 per sex per group except for Group 7 (high-dose QVA149) on Day 1 which had n=2.

QAB149 exposure in plasma was noted in this 13-week dog study with T_{max} in the range of 0-0.33 hours after completion of inhalation. Modest accumulation between Day 1 and Day 89 was noted for the HD QAB149, LD QVA149, and MD QVA149 groups, but not

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the HD QVA149 group. QAB149 exposure increased in an approximately dose-proportional manner across the range of QVA149 doses. At the conclusion of the study, toxicokinetic data suggest that combination with NVA237 is associated with a modest increase in QAB149 exposure compared to its monoproduct control group.

NVA237 exposure in plasma was associated with T_{max} corresponding to the end of the inhalation period. Modest accumulation between Day 1 and Day 89 was noted in the LD and MD QVA149 groups only. NVA237 exposure at the conclusion of the study was similar to slightly higher in combination with QAB149 compared to its monoproduct control arm.

The NOAEL in this 13-week combination inhalation toxicity study evaluating QVA149 (QAB149/NVA237) in dogs was considered the high-dose level, corresponding to AUC_{0-24h} values of 129.5/33.6 (QAB149/NVA237) ng*h/mL.

9 Reproductive and Developmental Toxicology

9.2 Embryonic Fetal Development

Study title: An inhalation embryo fetal development study in rats

Study no.:	Test facility #901312 Sponsor reference #0670755
Study report location:	EDR SD#20, 6/14/2012
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Protocol signed 4/16/2007 First treatment 4/17/2007 Last cesarean 5/7/2007 Report signed 7/24/2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	**QAB149** batch T005 0107, 101.4% purity; salt correction factor (b) (4) **NVA237** batch X344 0906, 98% purity; salt correction factor (b) (4) **QVA149** batch T004 0107, 99-100% purity Certificates of analysis provided.

Key Study Findings

- In the inhalation EFD study in rats, the maternal and embryo-fetal toxicity of the combination product QVA149 was evaluated and compared to its monoproducts constituents QAB149 and NVA237. Mated females were exposed to air control, placebo control (lactose with 1% magnesium stearate), high-dose NVA237

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(theoretical achieved dose 0.062 mg/kg/day), high-dose QAB149 (0.270 mg/kg/day), low-dose QVA149 (0.021 mg/kg/day QAB149 /0.007 mg/kg/day NVA237), mid-dose QAV149 (0.064/0.021 mg/kg/day), or high-dose QVA149 (0.212/0.071 mg/kg/day).

- There was no evidence of QVA149-related maternal or embryofetal toxicity in this study. The study failed to produce a minimum level of maternal toxicity as described in the ICH S5(R2) Guideline, although the study is considered acceptable as discussed in the Integrated Summary below.
- The NOAEL for both maternal and embryofetal toxicity was considered as the high-dose group of 0.212 mg/kg/day QAB149 plus 0.071 mg/kg/day NVA237.
- Exposure ($AUC_{0\text{-}24h}$) at the NOAEL, as measured on GD 17, was 160 ng*h/mL (QAB149) and 71.5 ng*h/mL (NVA237).

Methods

Target Doses:	**Group 1**: Air control **Group 2**: Placebo control (dry powder blend containing 1% w/w magnesium stearate in lactose) **Group 3**: Low-dose QVA149 (0.30 QAB149 / 0.10 NVA237 mg/kg/day) **Group 4**: Mid-dose QVA149 (0.90 QAB149 / 0.30 NVA237 mg/kg/day) **Group 5**: High-dose QVA149 (3.0 QAB149 / 1.0 NVA237 mg/kg/day) **Group 6**: NVA237 (1.0 mg/kg/day) **Group 7**: QAB149 (3.0 mg/kg/day)
Frequency of dosing:	Once daily
Route of administration:	Nose-only inhalation
Formulation/Vehicle:	1% magnesium stearate, 99% lactose powder
Species/Strain:	Han Wistar rats (b) (4) 76-81 days old at start of treatment 206-267 g at start of treatment
Number/Sex/Group:	22 mated females per group. Day 0 of gestation denoted upon positive identification of spermatozoa in vaginal lavage.
Satellite groups:	Toxicokinetics: 3 per control group, 9 per drug-treated group
Study design:	Individual housing (except for mating) with a 13-day acclimation period. Randomization based on body weight at GD 3. Drug exposure on GD 6-17.
Deviation from study protocol:	None that affect study interpretation

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Observations and Results

Inhalation Procedures and Atmospheric Evaluation

Standard stainless steel cylindrical "flow-through" nose-only inhalation chambers were utilized. Powdered test articles and vehicle control were generated using an extended duration powder delivery system and introduced into a mixing chamber via pre-dried compressed air under slight negative pressure to prevent outward leakage. For groups 1 (air control), 6 (high NVA237), and 7 (high QAB149), compressed air flow rate from the EDPDS was 40 L/min and the extract air flow rate was 50 L/min. For groups 2 (vehicle control), 3, (low QVA149), 4 (mid QVA149), and 5 (high QVA149), the respective air flow rates were 50 and 60 L/min. The various test article and vehicle control concentrations were achieved by altering the length of time that animals were connected to the chamber (see table for details). Separate ventilated walk-in fume hoods were used to house control and test article chambers in order to prevent contamination. Animals were rotated to different locations on the chamber weekly to compensate for any local atmospheric variations.

Actual chamber concentrations of total aerosol were measured from all groups at least twice on each day of treatment. The concentration of QAB149, NVA237, and magnesium stearate on the gravimetric filters was determined via validated HPLC and AA methods twice during the pre-study period and weekly during the treatment phase. Particle size distribution analysis was performed at least once pre-study and once weekly during the treatment phase. Homogeneity of chamber atmosphere conditions was also assessed at least once for each group based on samples collected at 2-3 equidistant ports around the circumference of the mixing chamber.

Observed atmospheric conditions and calculated theoretical achieved doses for each active treatment group are shown in the table below.

Table 9. Rat EFD Study: Atmospheric Conditions and Pulmonary Deposited Doses

Group	Target Dose (mg/kg/day)		MMAD (um)	Body Weight (kg)[a]	RMV (L/min)	Active Concentration (mg/L)		Exposure duration (min)	Deposition fraction	Pulmonary Deposited Dose (mg/kg/day)[b]	
	QAB149	NVA237				QAB149	NVA237			QAB149	NVA237
3: Low QVA149	0.3	0.1	2.6	0.26	0.17	0.0219	0.0073	15	0.1	0.021	0.007
4: Mid QVA149	0.9	0.3	2.6	0.26	0.17	0.0219	0.0073	45	0.1	0.064	0.021
5: High QVA149	3	1	2.6	0.26	0.17	0.0219	0.0073	150	0.1	0.212	0.071
6: High NVA237	NA	1	3.0	0.25	0.16	NA	0.0063	150	0.1	NA	0.062
7: High QAB149	3	NA	2.2	0.27	0.17	0.0281	NA	150	0.1	0.270	NA

NA: Not applicable; RMV: respiratory minute volume; MMAD: mass median aerodynamic diameter
[a]Body weight represents corrected body weight values from GD 21.
[b]Calculated via the following formula: (RMV)*(Active concentration in air)*(duration of exposure)*(deposition factor of 0.1 for rats)/(body weight)

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Mortality

All females were examined twice daily for mortality. There were no unscheduled deaths in this study.

Clinical Signs

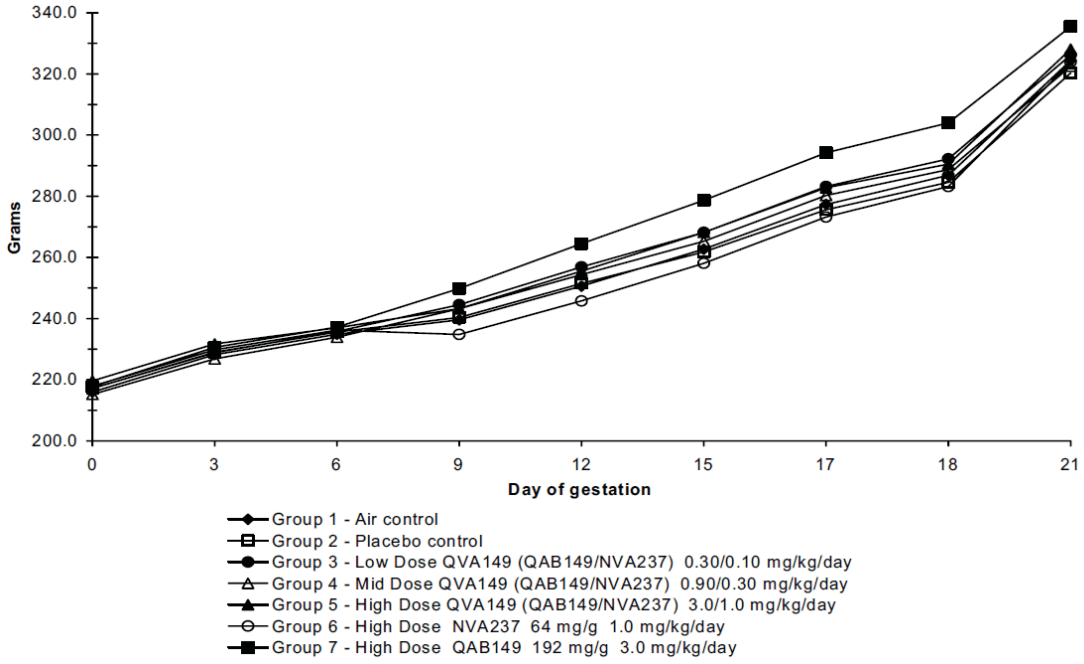
All females were observed twice daily and a complete detailed examination was performed on days 0, 3, 6, 9, 12, 15, 17, 18, and 21. There were no clinical signs associated with QVA149 treatment.

Body Weight

Individual body weights were recorded on days GD 0, 3, 6, 9, 12, 15, 17, 18, and 21.

As summarized in the figure below, QVA149 was not associated with any effect on body weight gain in this study. The study failed to produce a minimum level of maternal toxicity as described in the ICH S5(R2) Guidance.

Figure 7. Rat EFD Study: Maternal Body Weight Gain



Feed Consumption

Individual food consumption was measured on days GD 3-6, 6-9, 9-12, 12-15, 15-18, and 18-21.

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A statistically significant increase (up to 20%) in food consumption was noted in all treated groups vs. air and/or placebo controls for the GD18-21 period. This may reflect a pharmacological effect of B_2 agonism but was also noted in the NVA237 monotherapy arm. This finding was not considered toxicologically significant.

Toxicokinetics

Blood samples (~0.5 mL) were obtained from the jugular vein of TK animals and collected in lithium heparin tubes on GD 17. Samples were collected from 3 animals/group (2 timepoints per animal) at the following intervals: 0 hour (immediately after completing inhalation), 30 minutes, 1 hour, 3 hours, and 8 hours after completing inhalation, as well as 24 hours after initiation of inhalation. For control groups 1 and 2, only the 0 hour and 30 minutes post-inhalation timepoints were conducted. Resulting plasma samples were analyzed by Novartis Pharma for QAB149 and NVA237 levels by LC-MS/MS (LLOQ 0.2 and 0.1 ng/mL, respectively in 50 uL plasma samples). Concentration, C_{max}, T_{max}, and AUC_{0-24h} were calculated and expressed in terms of the base for QAB149 and the quaternary cation of the bromide salt for NVA237.

Table 10. Rat EFD Study: Toxicokinetic Parameters

Parameter	Group 3 LD QVA149	Group 4 MD QVA149	Group 5 HD QVA149	Group 6 NVA237	Group 7 QAB149
Length of inhalation (h)	0.25	0.75	2.5	2.5	2.5
QAB149					
T_{max} (h)[a]	0	0	0		0
C_{max} (ng/mL)	21.9	24.4	32.1		74.4
AUC_{0-24h} (ng*h/mL)	25.4	50.9	160		267
NVA237					
T_{max} (h)[a]	0	0	0	0	
C_{max} (ng/mL)	5.14	7.77	21.1	10.8	
AUC_{0-24h} (ng*h/mL)	10.2	19.6	71.5	55.0	

[a]T_{max} value represents time after the end of inhalation

All treated animals were exposed to NVA237 and/or QAB149 as appropriate and neither drug was detected above the LLOQ in any sample from either the air or vehicle control groups. QAB149 exposure increased in a slightly less than dose-proportional manner in the LD, MD, and HD QVA149 groups. Exposure to QAB149 was decreased in the HD QVA149 compared to the monoproduct QAB149 group with the same target concentration. Exposure to NVA237 increased in a roughly dose-proportional manner across the LD, MD, and HD QVA149 groups. In contrast to the results seen with QAB149, NVA237 C_{max} and AUC_{0-24h} values were slightly higher in the HD QVA149 vs. the monoproduct QVA237 group.

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Necropsy

Scheduled euthanasia occurred on GD 21 and proceeded in random order. Animals were asphyxiated via CO2 before exsanguination. Gross pathology examination was then conducted immediately. The reproductive tract was dissected out, ovaries removed, and corpora lutea counted. Gravid uterus weights were recorded and uterus contents as well as placentae were examined.

There were no gross findings at necropsy that were judged to be test article-related.

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

The number and position of live fetuses, dead fetuses, and early/middle/late resorptions were recorded. The uterus of any animal judged to be nonpregnant was stained with 10% ammonium sulfide and examined for implantation sites.

Table 11. Rat EFD Study: Cesarean Section Findings

Parameter	Treatment Group						
	Air Control	Vehicle Control	QVA LD[b]	QVA MD	QVA HD	NVA 237	QAB 149
Pregnant Animals[a]	21	21	22	21	22	21	21
Corpora Lutea (litter mean)	13.0	12.5	12.5	12.6	13.9	13.6	12.8
# of Implantations (litter mean)	11.9	11.0	10.8	10.1	11.4	12.1	10.7
Preimplantation loss (litter mean %)	8.96	12.17	13.84	20.89	17.22	10.85	16.56
Litter size	11.2	10.5	9.6	9.6	10.7	11.5	10.0
Early resorptions (litter mean % of implants)	0.6	0.4	1.2	0.5	0.5	0.6	0.6
Middle resorptions (litter mean % of implants)	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Late resorptions (litter mean % of implants)	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Dams with any resorptions (%)	47.6	42.9	68.2	42.9	40.9	57.1	42.9
Postimplantation loss (litter mean %)	5.25	4.63	15.16	6.58	5.90	5.37	5.61
Sex ratio (litter mean % live male fetuses)	51.0	47.6	48.2	54.5	46.1	53.9	49.8
Gravid uterus weight (mean, g)	75.6	71.5	70.4	66.9	70.0	77.3	68.0
Fetal body weight (litter mean, g)	5.13	5.15	5.20	5.25	4.87	5.04	5.10

[a] Number (out of 22) that were found to be pregnant and survived to schedule cesarean section
[b] Includes one female in this group with total resorptions.

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22 females per group were mated and no more than one animal per group was determined to be nonpregnant. There was no association between QVA149 and pregnancy rates. One female in the LD QVA149 group was noted with total resorptions. There were no notable body weight, food consumption, clinical, or necropsy observations in this animal. This event was considered spontaneous due to the low incidence and lack of relationship to QVA149 dose.

Offspring (Malformations, Variations, etc.)

Fetuses were weighed and external examinations were conducted, including recording of the gender. Fetuses were then euthanized via SC injection of Euthanyl solution. Half the fetuses in each litter underwent detailed internal examinations using a dissecting microscope, including preserving the head via the Wilson method. The other half of the fetuses were eviscerated, fixed in 85% ethanol/ 15% methanol, stained with alizarin red S (modified Dawson's technique), and underwent skeletal examination. Abnormalities were classified as major malformations, minor external/skeletal anomalies, or common skeletal variations.

Table 12. Rat EFD Study: Fetal Examination Findings

	Treatment Group						
Parameter	**Air Control**	**Vehicle Control**	**QVA LD[b]**	**QVA MD**	**QVA HD**	**NVA 237**	**QAB 149**
External examination	21/235[a]	21/220	21/211	21/202	22/235	21/241	21/211
Visceral examination	21/116	21/113	21/106	21/103	22/119	21/119	21/106
Skeletal examination	21/119	21/110	21/105	21/100	22/117	21/122	21/105
Technique of Wilson	21/116	21/113	21/106	21/102	22/118	21/119	21/106
Major malformations	**0/0**	**1/4**	**0/0**	**0/0**	**1/1**	**1/1**	**0/0**
Cranium, acephaly					1/1		
Head, cleft palate		1/3					
Face, pinna malpositioned					1/1		
Face, astomia		1/1					
Face, agnathia		1/1					
Face, aglossia		1/1					
Heart, transposition of major vessels					1/1		
Heart, dilitation of ascending aorta					1/1		
Heart, tricuspid valves absent					1/1		
General, situs inversus						1/1	
Limbs, radius bent		1/3					
Limbs, ulna bent		1/3					
External/visceral variations	**0/0**	**1/1**	**1/1**	**0/0**	**2/2**	**0/0**	**1/2**
Skeletal variations	**19/84**	**20/75**	**19/52**	**18/47**	**18/52**	**19/74**	**16/44**
Frontal bone: incomplete ossification	0/0	1/1	1/1	1/1	4/6	0/0	0/0

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Sternebrae 1-4: unossified/ incomplete/ semi-bipartite / bipartite	0.0%[b]	0.0%	0.8%	0.0%	5.7%	0.0%	0.8%

[a]Denotes number of litters / fetuses evaluated or affected
[b]Denotes mean % affected fetuses per litter

Major malformations were rare in this study, as summarized in the table above. Four fetuses from one placebo litter had craniofacial abnormalities (cleft palate, astomia, aglossia, agnathia). One HD QVA149 fetus was noted with acephaly, transposition of the major vessels of the heart, dilatation of the ascending aorta and absence of tricuspid valves. Finally, one NVA237 fetus had situs inversus of all thoracic and abdominal organs. There was no association between QVA149 and fetal malformations. Minor external and visceral variations were noted in no more than two litters and two pups per treatment group as summarized in the table above. There was no relationship between QVA149 and the total incidence of these findings or any individual variation.

Skeletal variations were noted with similar frequency across all treatment groups. In terms of individual findings, the incidence of incomplete ossification of the frontal bone in the HD QVA149 group was statistically significantly increased compared to control (18.2% of litters and 5.13% of fetuses affected). These results fall outside of the Sponsor's maximum historical control incidences of 4.76% litters and 0.91% fetuses affected. A statistically significant increase in the incidence of sternebrae variants (unossified, incomplete, semi-bipartite, or bipartite) was also noted in the HD QVA149 group compared to controls. The 5.7% litter mean incidence in this study was well above the 2% maximum litter mean incidence in the Sponsor's historical control data covering 275 litters from the test facility.

These two individual findings in the HD QVA149 group are noted and were detected at incidences outside of test facility historical control ranges. However, due to the overall low incidence, lack of an overall signal of test article-related skeletal findings, and lack of findings in monoproduct EFD studies with QAB149 and NVA237, the Reviewer did not consider the findings to be test article-related embryofetal toxicity. In addition, these findings of delayed ossification would not be expected to have any impact of fetal survival. Therefore, the NOAEL is considered as the high-dose of 0.212 mg/kg/day QAB149 and 0.071 mg/kg/day NVA237. Exposure ($AUC_{0\text{-}24h}$) at the NOAEL, as measured on GD 17, was 160 ng*h/mL (QAB149) and 71.5 ng*h/mL (NVA237).

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11 Integrated Summary and Safety Evaluation

The major nonclinical support for the clinical development of QVA149 has been conducted with the two monoproduct constituents, indacaterol maleate (QAB149) and glycopyrronium bromide (NVA237), and reviewed separately under NDA 22-383 and IND 48,655, respectively. The Sponsor submitted two pivotal toxicology studies evaluating multiple dose levels of QVA149 (using a 3:1 QAB149:NVA237 ratio) in parallel with high doses of QAB149 and NVA237: a 13-week repeat-dose inhalation toxicology study in dogs and an EFD inhalation study in rats.

In the 13-week dog study, there were no dose-limiting histopathological findings. Therefore the NOAEL was considered as the high-dose group with pulmonary deposited doses of 0.096 mg/kg/day QAB149 and 0.031 mg/kg/day NVA237. Marked synergy in terms of the induction of transient tachycardia in MD and HD QVA149 groups was observed. This finding was considered adverse but adequately monitorable in the clinical setting. Other findings typical of the single-agent QAB149 and NVA237 toxicology programs were identified at equal or lower incidences in QVA149-treated animals.

In the embryo-fetal development study in rats, no adverse findings were noted with respect to either maternal or fetal toxicity. The study failed to produce a minimum level of maternal toxicity as described in the ICH S5(R2) Guideline, although the study results are still considered potentially useful. Based on dose levels being greater to those utilized in the pivotal inhalation toxicology study, wide margins to predicted human exposure levels, and the extensive characterization of the developmental and reproductive toxicology of the monoproducts, the study was considered to be acceptable to support the ongoing QVA149 phase 3 development program. The NOAEL was determined to be the high-dose level of 0.212 mg/kg/day QAB149 and 0.071 mg/kg/day. There is no evidence that QVA149 poses a teratogenic risk to human subjects.

Safety margins for QVA149 are summarized in the table below. Multiples between animals NOAEL data and human exposure are expressed in terms of 1) systemic drug exposure ($AUC_{0\text{-}24h}$); 2) on a mg/kg body weight basis; and 3) on a ug/g lung weight basis reflecting a comparison of local toxicity.

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Table 13. Safety Margin Calculations for QVA149

Parameter	Human	Dog	Rat
Study	14-day PK study in healthy volunteers[a]	13-week inhalation toxicity NOAEL	Inhalation EFD study NOAEL
Dose Levels			
ug/kg/day	QAB149: 1.8	96	212
	NVA237: 0.8	31	71
ug/g lung weight	QAB149: 0.11[b]	12.2[c]	Not meaningful
	NVA237: 0.05	3.95	
Exposure			
$AUC_{0\text{-}24h}$ (ng*h/mL)	QAB149: 2.02	129.5	160
	NVA237: 0.57	33.6	71.5
Safety Margins			
ug/kg/day	QAB149:	53.3	118
	NVA237:	38.8	88.8
ug/g lung weight	QAB149:	111	Not meaningful
	NVA237:	79.0	
$AUC_{0\text{-}24h}$ (ng*h/mL)	QAB149:	64.1	79.1
	NVA237:	58.9	126

[a]Safety margins calculations based on human QVA149 PK data reported in the Investigator Brochure consisting of 14-day exposure to 110 ug QAB149 / 50 ug NVA237 in healthy subjects.
[b]Human pulmonary dose calculated based on 110/50 ug daily dose and 1000 gram lung weight
[c]Pulmonary dose calculated based on reported body and lung weights at sacrifice, calculated separately for males and females and then averaged.

The nonclinical repeat-dose and EFD toxicology studies conducted with QVA149 provide ample safety margins compared to anticipated human exposures, as shown above. Compared to steady-state exposures following daily dosing for 14 days with QVA149 at doses of 110 ug QAB149 (1.8 ug/kg/day) and 50 ug NVA237 (0.8 ug/kg/day), exposures at the NOAEL in the 13-week dog study provide 39-111X safety margins depending on the method of comparison. No evidence of maternal of fetal toxicity was observed in the rat EFD study at exposure levels 72-160 times that observed in humans. It is further noted that ongoing phase 3 clinical trials of QVA149 involve even lower human doses of QAB149 (55 ug daily), and in some cases NVA237 (25 or 50 ug daily), than referenced in the table above, rendering the above estimates conservative.

In summary, the general and reproductive toxicology studies submitted by the Sponsor, in conjunction with the established toxicological profiles of QAB149 and NVA237, provide adequate nonclinical support for the ongoing phase 3 clinical trials of the QVA149 combination product.

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/s/

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09/18/2013

TIMOTHY W ROBISON
09/18/2013
I concur

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/s/

JANE J SOHN
09/24/2015

TIMOTHY W ROBISON
09/24/2015
I concur

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:	207923 and 207930
Supporting document/s:	SD1 (NDAs 207923 and 207930), SD10 (NDA 207930; Nonclinical IR response), SD10 (NDA 207923, Nonclinical IR response, survival data), SD 11 (NDA 207930, Nonclinical IR response, ophthalmic findings)
Applicant's letter date:	12/29/14, 5/14/15, 5/20/15, 5/27/15
CDER stamp date:	12/29/14, 5/14/15, 5/20/15, 5/27/15
Product:	Glycopyrronium bromide and Indacaterol/Glycopyrronium bromide inhalation powder hard capsules
Indication:	Maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema
Applicant:	Novartis
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Jane J. Sohn, Ph.D.
Supervisor/Team Leader:	Timothy Robison, Ph.D., DABT
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Project Manager:	Christine Ford

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDAs 207923 and 207930 are owned by Novartis or are data for which Novartis has obtained a written right of reference.

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1 Executive Summary

1.1 Introduction

Novartis has submitted a 505 (b) (1) application under NDA 207923 for Seebri Neohaler glycopyrrolate (GP; NVA237) 15.6 mcg (glycopyrronium bromide quaternary ammonium salt) BID for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema. Seebri Neohaler is a dry powder inhaler that delivers a dry powder of GP in magnesium stearate and lactose monohydrate.

Under NDA 207930, Novartis has submitted a 505 (b) (1) application for 27.5 mcg Indacaterol in combination with 15.6 mcg GP for inhalation via the Neohaler BID for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema.

Two-year rat carcinogenicity and 26-week Tg.rasH2 transgenic mouse carcinogenicity studies assessing NVA237 are included in this review. Special protocol assessments were conducted under IND 48655 for the rat carcinogenicity protocol (ECAC minutes October 5, 2006), and the mouse carcinogenicity protocol (ECAC minutes February 24, 2010).

Carcinogenicity studies with Indacaterol alone were previously reviewed under NDA 22383.

1.2 Brief Discussion of Nonclinical Findings

Two-year rat carcinogenicity and 26-week Tg.rasH2 transgenic mouse carcinogenicity studies were analyzed. Both the rat and mouse protocols were reviewed through special protocol assessments.

Glycopyrrolate was negative in genetic toxicology testing based on results from the *in vitro* bacterial reverse mutation, *in vitro* human peripheral lymphocyte chromosomal aberration, and *in vivo* rat micronucleus assays.

The sponsor conducted a 2-year Wistar rat bioassay (50 animals/sex/group). Animals received NVA237 by inhalation (nose-only) at doses of 0 (air, on loading rack in restraint tubes in separate room), 0 (air, rotated on a flow through chamber), 0 (vehicle: 1% magnesium stearate and 99% lactose monohydrate), 0.07, 0.21, 0.56 NVA237 mg/kg/day (estimated achieved doses, quaternary ammonium salt). Decreased body weight was observed in HD males (85%) and females (91%). There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis. Thus, NVA237 was determined to not be tumorigenic in either male or female Wistar rats.

Test article related non-neoplastic findings in rats were identified in the lungs (epithelial hypertrophy, eosinophilic cytoplasmic inclusions, macrophage accumulation), and

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tracheobronchial lymph nodes (pigment deposits). A dose dependent increase in anterior cortical subcapsular lens opacities was observed in both male and female rats. These findings should be listed in the product label in Section 13.2. Based on dose dependent epithelial hypertrophy at all doses, no NOAEL was established. However, extensive clinical data is available from Phase 3 clinical trials and suggests that lung findings in rats are not predictive of human response.

In the 26-week oral gavage carcinogenicity study in Tg.rasH2 mice (25 animals/sex for NVA237 dosed groups), males received NVA237at 0 (vehicle: Deionized water), 12.5, 31.3, and 93.8 mg/kg/day and females received NVA237 at 0 (vehicle), 12.5, 37.5, and 125.1 mg/kg/day, based on the concentration of the quaternary ammonium salt. Positive control mice were administered N-methyl-N-nitrosourea (MNU; 75 mg/kg) by a single intraperitoneal injection on Day 1. Tg.rasH2 mice treated with NVA237 showed a dose dependent decrease in body weight. There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis in Tg.rasH2 dosed up to 93.8 and 125.1 mg/kg/day in males and females, respectively. NVA237 was not tumorigenic in either male or female Tg.rasH2 mice.

NVA237-related nonneoplastic lesions in mice were identified in the liver. Liver necrosis (focal and multifocal) was observed at the HD. Liver atrophy (Grade 3) was noted in 3 females (1/25 LD, 2/25 HD) and 1/25 MD male. Based on the severity of the liver atrophy, it is unclear if there is a NOAEL for this finding. The clinical significance of these low incidence findings is unclear. Due to the inconsistent exposure data to NVA237 in Tg.rasH2 animals, the metabolite CJL603 was examined. Systemic exposure (AUC) for CJL603 increased in a roughly dose proportional manner. Although CJL603 was not detected in all tested animals at all time points, CJL603 remained detectable at 24 hrs post dose in males, and at 7 hrs post dose in females.

In conclusion, NVA237 was determined to not be tumorigenic based on evaluation in Wistar rats and Tg.rasH2 mice.

Exposure margins achieved, based on the high-dose in the rat carcinogenicity study, provide safety margins greater than 300-fold over clinical exposure in COPD patients.

1.3 Recommendations

Conclusions from the Executive CAC meeting conducted on May 26, 2014.

Rat:

- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female rats.

Mouse:

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- The Committee found that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either male or female Tg.rasH2 mice.

2 Drug Information

2.1 Drug

CAS Registry Number: 596-51-0

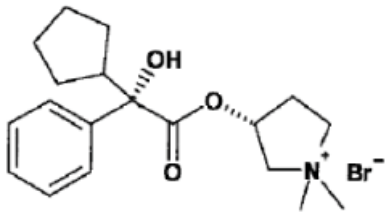
Generic name: Glycopyrrolate

Code Name: NVA237

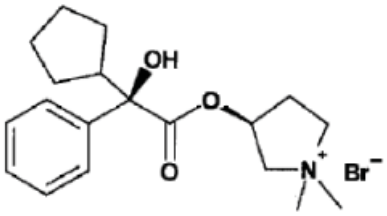
Chemical Name: 3-[(cyclopentylhydrophenylacetyl) oxy)]-1, 1-dimethylpyrrolidinium bromide

Molecular Formula/Molecular Weight: $C_{19}H_{28}NO_3xBr$ (MW: 398.33)

Structure and Biochemical Description: NVA237 is a racemic mixture of the *S,R* and *R,S* enantiomers (b) (4)
(CMC review dated September 25, 2012).



S,R



R,S

Pharmacologic Class: Muscarinic acetylcholine receptor (mAChR) antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 48655: Glycopyrrolate (NVA237)
IND 76377: Indacaterol and Glycopyrrolate (NVA237)

2.3 Drug Formulation

The sponsor is proposing products with glycopyrrolate (GP; NVA237) alone, and GP in combination with Indacaterol.

The proposed clinical formulation and the formulation used in the rat carcinogenicity are

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similar for GP alone. The excipients are lactose and magnesium stearate. GP will be delivered in a capsule by the Neohaler device, which is approved for use under NDA 022383 (ARCAPTA NEOHALER). The proposed clinical formulation is shown in the table below.

Table 1: Formulation for glycopyrronium bromide inhalation powder hard capsule

Ingredient	Amount per capsule (mg)	Function
Capsule fill		
Glycopyrronium bromide Lactose monohydrate, USP Magnesium stearate, USP (Capsule fill weight)	0.01561# 24.9469 0.0375 (b) (4)	(b) (4)
Empty capsule shell		
Hypromellose (b) (4) Printing Ink (see next section) (Capsule shell weight)	(b) (4)	
Printing Ink		
(b) (4)		

Corresponds to 0.0125 mg of GP active moiety and a target delivered dose of (b) (4) mcg

GP in combination with Indacaterol will be delivered in a capsule by the Neohaler device, which is approved for use under NDA 022383 (ARCAPTA NEOHALER). The proposed clinical formulation is shown in the table below.

Table 2: Formulation for Indacaterol maleate/glycopyrronium bromide inhalation powder hard capsule

Ingredient	Amount per capsule (mg)	Function

Capsule fill		
Indacaterol maleate	(b) (4)	(b) (4)
Glycopyrronium bromide	0.01561	
Lactose monohydrate, USP	24.9112	
Magnesium stearate, USP	0.0375	
(Capsule fill weight)	(b) (4)	
Empty capsule shell		
Hypromellose (b) (4)	(b) (4)	
Printing Ink (see next section)		
(Capsule shell weight)		
Printing Ink		
(b) (4)		

3 Studies Submitted

3.1 Studies Reviewed

1. NVA237: A 104-week inhalation carcinogenicity study of a powder formulation in rats (study #0670435/79032).
2. NVA237: A 26-week oral gavage carcinogenicity study in Tg.rasH2 mice (study #0770668/N109087).

4 Carcinogenicity

2-year rat study

Study title: NVA237: A 104-week inhalation carcinogenicity study of a powder formulation in rats

Reference ID: 3788692

Study no.:	0670435/79032
Study report location:	SD-1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 5, 2006 (page33 of study report)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NVA237, batch #X3440906 and X3450906, 98%.
CAC concurrence:	Yes

Key Study Findings

- In the 2-year carcinogenicity study, Wistar rats received NVA237 by inhalation (nose-only) at doses of 0 (air, on loading rack in restraint tubes in separate room), 0 (air, rotated on a flow through chamber), 0 (vehicle: 1% magnesium stearate and 99% lactose monohydrate), 0.07, 0.21, 0.56 NVA237 mg/kg/day (estimated achieved doses, quaternary ammonium salt), consistent with doses recommended by the ECAC.
- Treatment with NVA237 was not associated with a dose-dependent increase in mortality.
- There was a dose dependent decrease in body weight gain in males that was noted in the sponsor's figures around Week 14, which corresponded to a decrease in body weight at the end of the study (LD 95%, MD 93%, HD 85%), compared to vehicle controls. The decreased body weight for HD males suggests a MTD was achieved. HD females also had decreased body weight (91%), compared to vehicle controls. Dose dependent pupil dilation was also noted in males.
- A dose dependent increase in anterior cortical subcapsular lens opacities was observed in both male (vehicle 0, LD 8, MD 14, HD 22) and female rats (vehicle 0, LD 3, MD 9 HD 12).
- Neoplastic findings were not statistically significant by both trend and pair-wise analysis.
- Test article related non-neoplastic findings were identified in the nasal cavity/sinuses, larynx, lungs, and tracheobronchial lymph nodes. Findings in the nasal cavity/sinuses were not deemed clinically relevant as rats are obligate nose breathers, and humans will be dosed via oral inhalation. Squamous metaplasia in the larynx was a result of irritation, and not considered a clinically relevant finding because of rat-specific anatomy/physiology.
- In the lung, epithelial hypertrophy was noted at the bronchioloalveolar junction, with a dose dependent increase in incidence and severity. Hypertrophic epithelia were noted to have eosinophilic inclusions. Macrophage accumulation (all types) was increased with a dose dependent increase in incidence in females, and a dose dependent increase in severity in both sexes. Pigment deposits were noted

in the tracheobronchial lymph nodes in HD males and MD/HD females. Based on dose dependent epithelial hypertrophy at all doses, no NOAEL was established.

- Systemic exposure (AUC) to NVA237 was similar in both genders at all dose groups, with slight accumulation at Week 52. Exposure was dose proportional between the LD and MD, but was slightly less than dose proportional between the MD and HD.
- NVA237 was not tumorigenic in Wistar rats.

Adequacy of Carcinogenicity Study

- The duration of treatment at 104 weeks is adequate.
- The doses were recommended by the ECAC (minutes dated October 5, 2006).

Appropriateness of Test Models

- The ECAC concurred with the protocol for the presented carcinogenicity study. The Wistar rat is a standard model for assessment of carcinogenic potential.

Evaluation of Tumor Findings

- There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis.
- NVA237 was not tumorigenic in rats.

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Methods

Doses:	Estimated achieved doses, based on quaternary ammonium salt: Group 1: 0 (air, on loading rack in restraint tubes), in separate animal room from Group 2-6 animals. Group 2-6: Animals were exposed to air, vehicle, or the test article in a flow through chamber Group 2: 0 (air, rotated on a flow through chamber) Group 3: 0 (vehicle: 1% magnesium stearate and 99% lactose monohydrate) Group 4: 0.07 (low dose/LD) mg/kg/day Group 5: 0.21 (mid dose/MD) mg/kg/day Group 6: 0.56 (high dose/HD) mg/kg/day
Frequency of dosing:	Daily
Dose volume:	60 minutes
Route of administration:	Nose only
Formulation/Vehicle:	1% magnesium stearate in lactose monohydrate
Basis of dose selection:	A 13-week dose selection study was conducted rats and evaluated by the ECAC (minutes 10/5/06). Histopathological lesions were observed in the nasal cavity/sinuses involving the respiratory and olfactory epithelium and goblet cells, the Harderian gland and larynx. Decreased body weight gain was observed at 0.6 mg/kg/day of glycopyrronium bromide base. Based on the decreased body weight gain, the ECAC recommended inhalation doses of 0.06, 0.2 and 0.6 mg/kg of glycopyrronium bromide quaternary ammonium salt, which is consistent with the doses used in the 2 year rat study.
Species/Strain:	*Rattus norvegicus*/Wistar Hannover Crl: WI (Han)
Number/Sex/Group:	50 rats/sex/group
Age:	Approximately 7 weeks
Animal housing:	Animals will be social housed (2 or 3 animals of the same sex and same dosing group together) in stainless steel perforated floor cages equipped with an automatic watering valve.
Paradigm for dietary restriction:	None.
Dual control employed:	Two air control groups were employed, but Group 1 (Air 1) was housed in a separate room

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	from other animals, and exposed on loading racks in restraint tubes, which was unique to this group. Group 2 (Air 2) was housed in the same room as animals exposed to the test article, and similarly rotated through the flow through chamber, which was the same method of exposure for Groups 3-6. Air 1 was exposed to the test article differently than other groups and showed increased body weight compared to both Air 2 and vehicle treated animals; therefore, the two air-treated groups were not combined in this analysis. Statistical analyses were done with vehicle control and Air 2 groups, and not with the Air 1 group due to the body weight and handing differences in the Air 1 group.
Interim sacrifice:	None.
Satellite groups:	Toxicokinetics: 6 rats/sex/group in Groups 1, 2 and 3, and 9 rats/sex/group in Groups 4, 5 and 6.
Deviation from study protocol:	On one day, the HD group was dosed for the first 30 minutes with the concentration intended for the MD group. This does not appear to have affected the validity of the study.

Study Design and Aerosol Dosing

The sponsor calculated estimated achieved doses as shown below. The doses were 0 (vehicle), 0.07 (low dose/LD), 0.21 (mid dose/MD), and 0.56 (high dose/HD) mg/kg/day (estimated achieved doses, quaternary ammonium salt). The salt doses are used throughout this review, unless noted.

Reference ID: 3788692

Group no. Identification	Overall estimated achieved dose* expressed in active moiety (salt/cation) (mg/kg/day)	Main study		Toxicokinetic study	
1/ Air control**	0	1001-1050	1501-1550	1051-1056	1551-1556
2/ Air control	0	2001-2050	2501-2550	2051-2056	2551-2556
3/ Vehicle control	0	3001-3012, 3113, 3014-3050	3501-3550	3051-3056	3551-3556
4/ NVA237 low	0.07/0.06	4001-4050	4501-4550	4051-4056, 4157, 4058, 4059	4551- 4553, 4654, 4555-4559
5/ NVA237 mid	0.21/0.17	5001-5050	5501-5550	5051-5059	5651, 5552-5559
6/ NVA237 high	0.56/0.45	6001-6050	6501-6550	6051-6059	6551-6559
7/ Health screen	-	7001-7010	7501-7510	-	-

* Dose levels for inhalation exposure are expressed in salt/quaternary cation of bromide salt form.
** Group 1 air control group animals were housed and exposed in a separate animal room from the other groups of animals.

Animal nos. 3013 (pretreatment), 4057, 4554 and 5551 (day 1) were found dead and replaced by animal nos. 3113, 4157, 4654 and 5651.

(Sponsor's table, page 28 of the study report)

The test article was within respirable range (between 1 – 5 microns), based on the reported MMAD ± GSD:

Group ID	Chamber concentrations (mg/L)		Particle size MMAD (µm) ± GSD	
	Gravimetric	Analytical*	Gravimetric	Analytical*
2/ Air control	-	0	-	-
3/ Vehicle control	0.0447	0	4.7 ± 3.5	-
4/ NVA237 Low dose	0.0144	0.0018	2.8 ± 2.6	2.4 ± 2.2
5/ NVA237 Mid dose	0.0427	0.0058	2.7 ± 2.7	2.5 ± 2.1
6/ NVA237 High dose	0.0596	0.0152	2.8 ± 3.2	2.3 ± 1.8

* Concentrations given refer to the salt form of NVA237

(Sponsor's table)

Observations and Results

Mortality

Animals were checked for mortality twice daily after arrival, and once on the day of necropsy.

There were no test article related effects on mortality, based on lack of dose response and using Air 2 and Vehicle groups as baselines for comparison. The survival data provided under Table 3 corresponds to the sponsor's summary mortality table provided on page 46 of the study report. These summary data did not appear to correspond to the summary mortality table provided by the sponsor in the statistical report, with a difference of 2 deaths. An IR was sent to the sponsor on May 15, 2015 regarding this discrepancy, and the sponsor responded on May 20, 2015 (NDA 207923, SD-10):

> "Table 4-4 on page 46 of the study report provides the survival in each treatment group on completion of the scheduled 104-week treatment period. Two animals subsequently died during the scheduled necropsy period in Week 105 (Group 2M, No. 2037 on day 733) in Week 107 (Group 6F, No. 6530 on day 744). As

described in the statistical report…the first day of the scheduled necropsy was used in the mortality analyses to exclude all animals which died during days 729-749, inclusively."

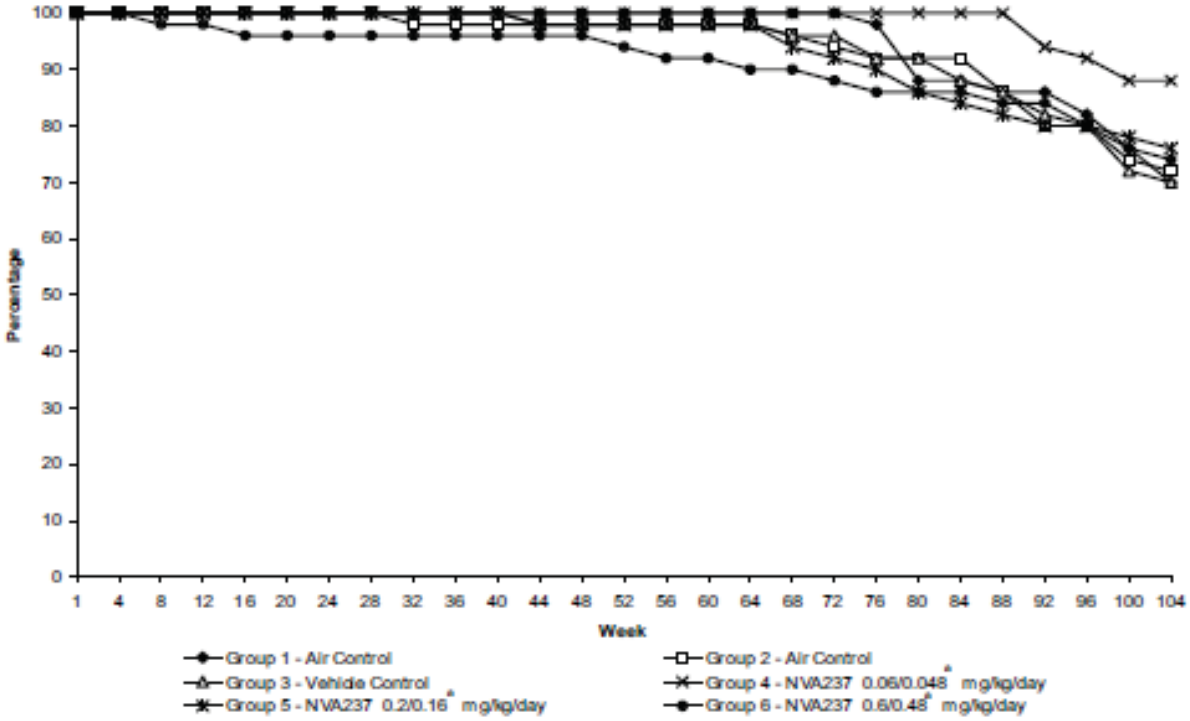
Statistical analysis showed no trend (dose-dependent) for statistically significant differences in survival across males or females. There was a statistically significant increase in deaths in LD females (P<0.0001), compared to vehicle control, but there was no dose-dependence.

Table 3: Rat carcinogenicity study: Survival analysis

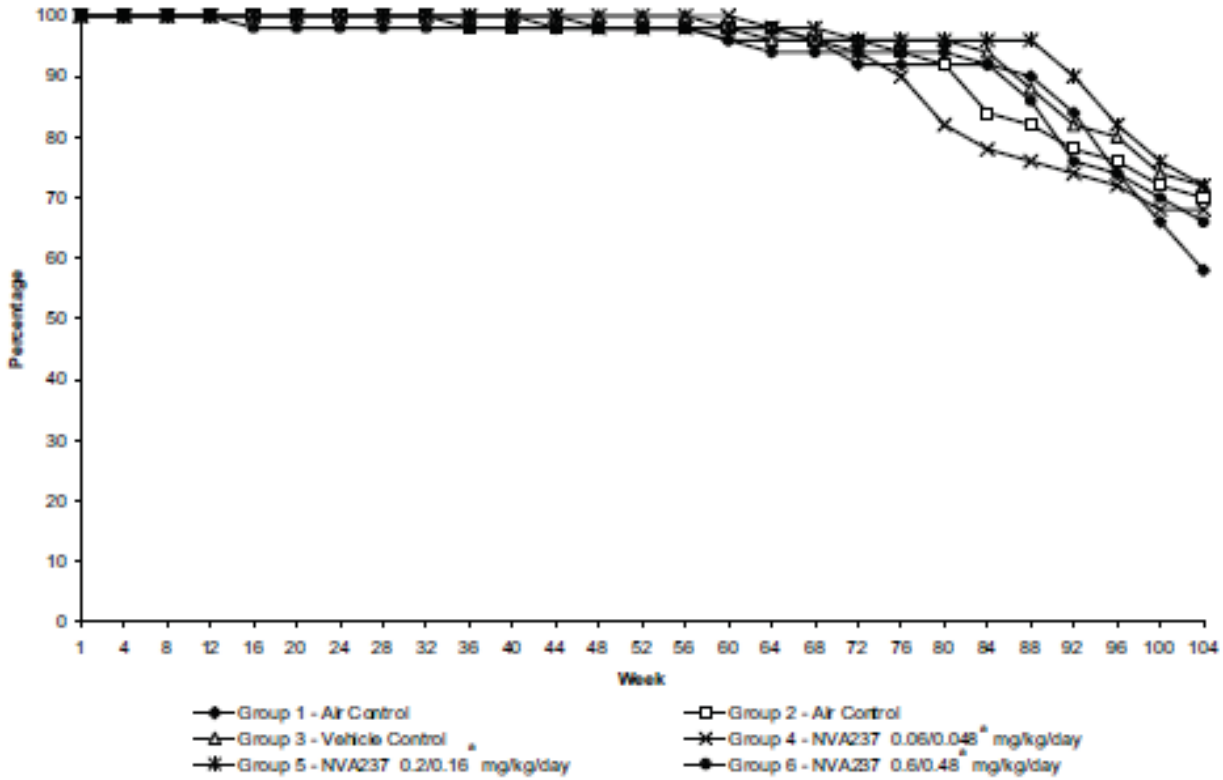
	Males		Females	
Group/Dose (mg/kg/day)	# Surviving/Total	% Survival	# Surviving/Total	% Survival
Air 1	35/50	70	29/50	58
Air 2	36/50	70	35/50	70
Vehicle	35/50	70	36/50	72
0.07	44/50	88	34/50	68
0.21	38/50	76	36/50	72
0.56	37/50	74	33/50	64

Figure 1: Rat carcinogenicity study: Survival curves

Males



Females



(Sponsor's figures)

Clinical Signs

Clinical examinations were performed once during the pretreatment period, and weekly during the treatment period for main study animals (excluding TK animals). Main study animals were also examined for palpable masses during the weekly clinical examination. The site, size and appearance of the masses were recorded when first detected, and the presence or disappearance of the mass was noted thereafter.

Dose dependent pupil dilation was noted in males only (vehicle 0/0, LD 1/1, MD 2/2, HD 7/3). Yellow staining of fur was noted in females (vehicle 19/5, LD 18/6, MD 53/9, HD 201/13), but was not observed in a dose-dependent manner in males.

There were no dose dependent increases in palpable masses.

Table 4: Rat carcinogenicity study: Clinical signs

		Dose (mg/kg/d)					
Observation	Sex	0 (Air 1)	0 (Air 2)	0 (Vehicle)	0.07	0.21	0.56
Pupil dilated	Males	0/0	0/0	0/0	1/1	2/2	7/3
	Females	0/0	0/0	0/0	2/2	0/0	4/2
Fur staining yellow	Males	41/11	3/2	21/8	9/5	2/2	14/5
	Females	4/2	61/5	19/5	18/6	53/9	201/13

Note: Shading indicates lack of dose response

Table 5: Rat carcinogenicity study: Palpable masses

		Males		Females	
Group#	Dose (mg/kg/d)	Incidence	%	Incidence	%
1	0 (Air 1)	17/50	34	13/50	26
2	0 (Air 2)	24/50	48	12/50	24
3	0 (Vehicle control)	22/50	44	20/50	40
4	0.07	13/50	26	14/50	28
5	0.21	16/50	32	14/50	28
6	0.56	8/50	16	13/50	26

Body Weights

Individual body weights were measured weekly from the day of randomization for the first 14 weeks of treatment, and every 4 weeks thereafter until Week 78. After Week 78 until Week 104, body weights were measured every 2 weeks.

There was a dose dependent decrease in body weight gain in males that was noted in the sponsor's figures around Week 14, which corresponded to a decrease in percent body weight at the end of the study (LD 95%, MD 93%, HD 85%), compared to vehicle controls. The decreased body weight for HD males suggests a MTD was achieved. HD females also a decrease in body gain (91%), compared to vehicle controls.

Notably, Air 1 controls consistently had increased body weight over the course of the study compared to all groups; therefore the Air 1 group was not used primarily for comparisons to the text article treated groups by the sponsor or the reviewer.

Figure 2: Rat carcinogenicity study: Body weight

Males

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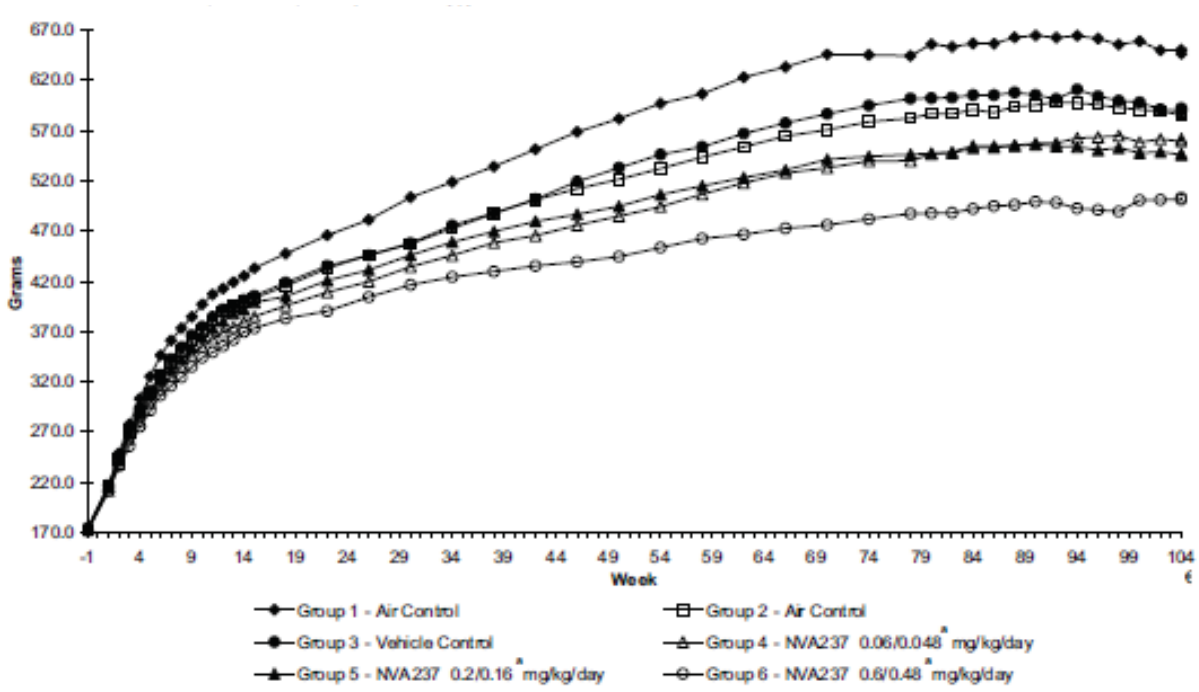
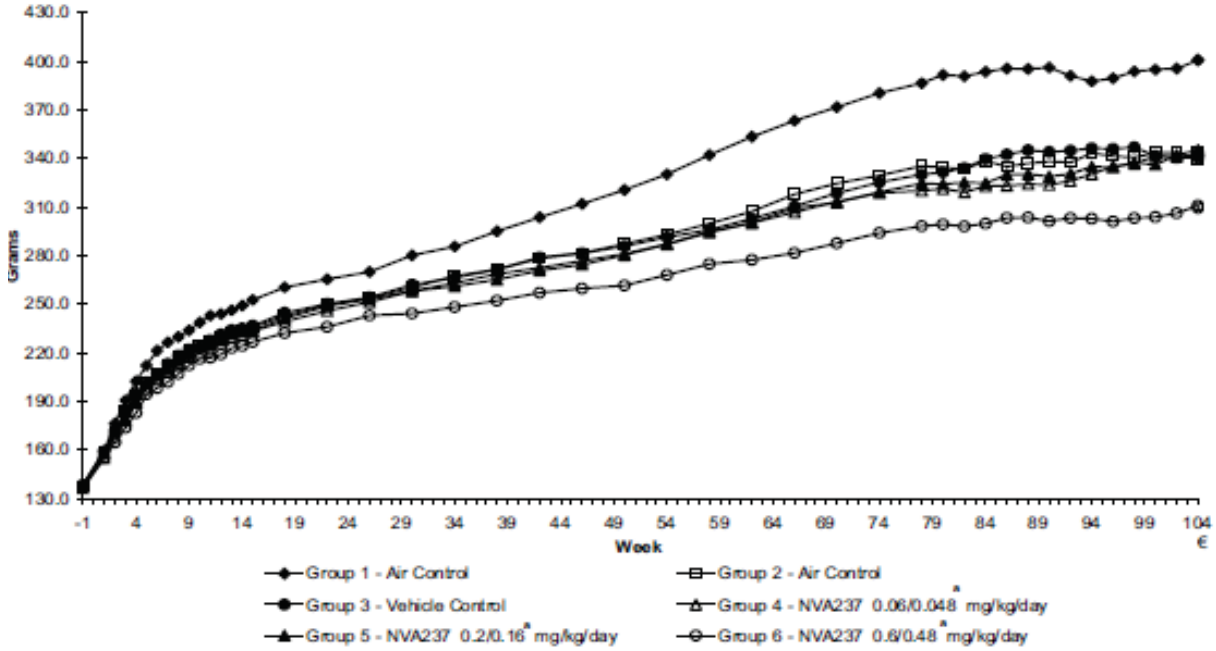


Females



(Sponsor's figures)

Table 6: Rat carcinogenicity study: Body weight analysis, percent change compared to vehicle control

Males

Dose (mg/kg/d)					
Male					
0 (Air 1)	0 (Air 2)	0 (Vehicle)	0.07	0.21	0.56

Week -1	171.0	171.6	173.9	172.4	173.4	171.5
Week 26	481.4	446.2	445.8	419.7	420.7	390.2
Absolute BW, % Control	108%	100%	100%	94%	94%	88%
Δ (g)	310.4	274.6	271.9	247.3	247.3	218.7
BW gain, % initial	182%	160%	156%	143%	143%	128%
BW gain, % control	114%	101%	100%	91%	91%	80%
Week 54	597.2	543.7	554.0	494.2	506.5	453.5
Absolute BW, % Control	108%	98%	100%	89%	91%	82%
Δ (g)	426.2	372.1	380.1	321.8	333.1	282
BW gain, % initial	249%	217%	219%	187%	192%	164%
BW gain, % control	112%	98%	100%	85%	88%	74%
Week 78	644.7	582.5	602.0	540.1	564.4	487.4
Absolute BW, % Control	107%	97%	100%	90%	94%	81%
Δ (g)	473.7	410.9	428.1	367.7	391	315.9
BW gain, % initial	277%	239%	246%	213%	225%	184%
BW gain, % control	111%	96%	100%	86%	91%	74%
Week 104	650.7	585.9	590.0	559.5	546.0	501.4
Absolute BW, % Control	110%	99%	100%	95%	93%	85%
Δ (g)	479.7	414.3	416.1	387.1	372.6	329.9
BW gain, % initial	281%	241%	239%	225%	215%	192%
BW gain, % control	115%	100%	100%	93%	90%	79%

Females

	Dose (mg/kg/d)					
	Female					
	0 (Air 1)	0 (Air 2)	0 (Vehicle)	0.07	0.21	0.56
Week -1	138.1	137.4	138.5	136.7	138.5	137.2
Week 26	270.0	253.9	254.2	251.4	253.2	243.1
Absolute BW, % Control	106%	100%	100%	99%	100%	96%
Δ (g)	131.9	116.5	115.7	114.7	114.7	105.9
BW gain, % initial	96%	85%	84%	84%	83%	77%
BW gain, % control	114%	101%	100%	99%	99%	92%
Week 54	330.3	293.1	291.5	287.2	287.1	268.2
Absolute BW, % Control	113%	101%	100%	99%	98%	92%
Δ (g)	192.2	155.7	153	150.5	148.6	131
BW gain, % initial	139%	113%	110%	110%	107%	95%
BW gain, % control	126%	102%	100%	98%	97%	86%
Week 78	386.5	335.4	330.2	320.1	324.7	298.2
Absolute BW, % Control	117%	102%	100%	97%	98%	90%

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Δ (g)	248.4	198	191.7	183.4	186.2	161
BW gain, % initial	180%	144%	138%	134%	134%	117%
BW gain, % control	130%	103%	100%	96%	97%	84%
Week 104	400.7	339.2	340.4	342.6	345.2	311.0
Absolute BW, % Control	118%	100%	100%	101%	101%	91%
Δ (g)	262.6	201.8	201.9	205.9	206.7	173.8
BW gain, % initial	190%	147%	146%	151%	149%	127%
BW gain, % control	130%	100%	100%	102%	102%	86%

Feed Consumption

Individual food consumption was measured weekly in main study animals from the week prior to treatment initiation through the first 14 weeks of treatment, and every 4 weeks thereafter until Week 78. After Week 78, measurements were performed every 2 weeks.

There were no persistent dose dependent changes in food consumption. There was a slight decrease in food consumption at Week 78 in males at the MD (-3%) and HD (-7%), but this trend was not observed throughout the study.

Table 7: Rat carcinogenicity study: Food consumption, percent change compared to vehicle control

Males

	Dose (mg/kg/d)					
	Male					
	0 (Air 1)	0 (Air 2)	0 (Vehicle)	0.07	0.21	0.56
Week -1	22.2	22.2	22.6	22.0	22.3	22.1
Percent change	-2%	-2%		-3%	-1%	-2%
Week 26	24.0	23.9	24.4	23.3	24.3	23.4
Percent change	-2%	-2%		-5%	0%	-4%
Week 54	23.1	25.2	23.6	23.5	23.8	23.0
Percent change	-2%	7%		0%	1%	-3%
Week 78	22.5	24.7	24.0	24.2	23.2	22.4
Percent change	-6%	3%		1%	-3%	-7%
Week 104	22.6	24.7	23.8	27.0	22.7	22.9
Percent change	-5%	4%		13%	-5%	-4%

Females

	Dose (mg/kg/d)					
	Female					
	0 (Air 1)	0 (Air 2)	0 (Vehicle)	0.07	0.21	0.56
Week -1	16.5	16.8	16.7	17.0	17.1	16.7
Percent change	-1%	1%		2%	2%	0%
Week 26	16.3	17.9	18.4	18.7	18.5	18.0

Reference ID: 3788692

Percent change	-11%	-3%		2%	1%	-2%
Week 54	17.1	18.6	18.8	18.9	18.9	18.4
Percent change	-9%	-1%		1%	1%	-2%
Week 78	18.0	19.5	18.6	19.3	18.5	18.9
Percent change	-3%	5%		4%	-1%	2%
Week 104	18.5	22.6	18.7	19.5	19.1	20.3
Percent change	-1%	21%		4%	2%	9%

Ophthalmology

Ophthalmic examinations were performed on all animals during the pre-treatment period, and once during Weeks 52-53, except animal no. 6511 which was examined at Week 57. Animals were administered mydriatic drops, and examined by biomicroscopy (slit-lamp examination) and funduscopy (indirect ophthalmoscopy) by a board certified veterinary ophthalmologist.

A dose dependent increase in anterior cortical subcapsular lens opacities was observed in both male (vehicle 0, LD 8, MD 14, HD 22) and female mice (vehicle 0, LD 3, MD 9 HD 12). Summary data were requested in an Information Request sent on May 11, 2015, and received on May 27, 2015 (SD-11, NDA 207930).

Table 8: Rat carcinogenicity study: Ophthalmic findings

	Dose (mg/kg/d)											
	Male						Female					
	0 Air1	0 Air2	0 Vehicle	0.07	0.21	0.56	0 Air1	0 Air2	0 Vehicle	0.07	0.21	0.56
Observations	50	49	49	50	50	49	49	49	50	50	49	49
Lens Opacity Anterior Subcapsular	1	4	0	8	14	22	0	0	0	3	9	12

Gross Pathology

Animals euthanized at the end of the treatment period, or for humane reasons before scheduled euthanasia, were exsanguinated from the abdominal aorta following isoflurane anesthesia. Animals were fasted overnight before scheduled necropsy.

All main study animals found dead during the study were subject to necropsy and tissues samples were preserved.

TK animals found dead or euthanized (isoflurane anesthesia) prior to scheduled TK sampling were subjected to gross necropsy to assist in the determination of death with

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no tissue retention. These animals were discarded after completion of the gross necropsy examination.

Dark foci were noted in the lungs of HD females (6/50, animal numbers 6503, 6512, 6516, 6517, 6521, 6545), which was associated with microscopic macrophage accumulation with pigment in 5/6 animals (animal numbers 6503, 6516, 6517, 6521, 6545).

Table 9: Rat carcinogenicity study: Gross lesions

		Dose (mg/kg/d)					
		0 (Air 1)	0 (Air 2)	0 (Vehicle)	0.07	0.21	0.56
	N=	50	50	50	50	50	50
Males	LUNG - Foci dark	0	0	0	1	0	1
Females	LUNG - Foci dark	1	0	0	0	1	6

Histopathology

The following tissues were fixed in 10% neutral buffered formalin, unless other indicated.

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P	Adrenal	P	Optic nerves[ce]
P	Aorta (thoracic)	P	Ovaries
P	Bone and marrow (sternum)[b]	P	Oviduct
P	Brain (cerebrum, midbrain, cerebellum and medulla oblongata)	P	Pancreas
		P	Pharynx
P	Cecum	P	Pituitary
		P	Preputial gland/clitoral gland
P	Colon	P	Prostate
P	Draining lymph node of clinically observed external masses [h]	P	Rectum
P	Duodenum	P	Salivary gland (submandibular; sublingual;parotid;-unilateral)
P	Epididymis[c]	P	Sciatic nerve
P	Esophagus	P	Seminal vesicles
P	Eyes[c]	P	Skeletal muscle
		P	Skin (inguinal)
P	Femorotibial joint[b]	P	Spinal cord (cervical; thoracic; lumb
		P	Spleen
P	Harderian glands	P	Stomach
		P	Testes[c]
P	Heart (including section of aorta)	P	Thymus [e]
P	Ileum	P	Thyroid lobes (and parathyroids)[e]
P	Jejunum		
P	Kidneys	P	Tongue
P	Lacrimal gland	P	Trachea
P	Larynx (3 levels)	P	Ureters
P	Liver (sample of 2 lobes)	P	Urinary bladders
P	Lung (*all lobes*)[d]	P	Uterus (horns, body, cervix)
		P	Vagina
P	Lymph node – tracheobronchial		Animal identification [g]
P	Lymph node – mandibular, unilateral	P	Abnormalities
P	Lymph node – mesenteric		
P	mainstem bronchi		
P	Mammary gland (inguinal-females only) [e]		
P	Nasal cavities and sinuses (4 levels) [bd]		

b Bone decalcified prior to sectioning.
c Fixed in Davidson's fluid (testes and epididymides); fixed in Davidson's fluid (eyes and optic nerves), (euthanized animals only, excluding in extremis euthanasia).
d Infused with neutral buffered 10% formalin (all animals).
e Examined histopathologically only if present in routine sections of eyes (optic nerves), thyroid lobes (parathyroid glands), skin (mammary gland), thymus (thymic lymphoid tissue).
g retained but not processed
h retained only if gross lesion present

(Sponsor's table)

Peer Review: A Peer Review was conducted on macroscopic and microscopic findings in the nasal cavity and sinus, larynx, lung, and trachea, including 100% of neoplasms plus "select hyperplastic changes" by [redacted] (b) (4) (page 2917 of the study report.)

Neoplastic

Neoplasias were analyzed by organ, and tumors that shared common cell origins in the same organ were combined for analysis. Specific tumors that arise from systemically distributed cells were identified by incidence per animal. Specifically, hemangioma and hemangiosarcoma, lymphomas, leukemias, mesotheliomas, and histiocytic sarcomas were analyzed by incidence per animal, if they occurred in the study. Brix *et al.* state, "Such systemic neoplasms that might be considered in this fashion would include all blood cell neoplasms, including histiocytic sarcoma, as well as neoplasms in which the cell type is present in many different organs, such as hemangiosarcomas and malignant mesotheliomas[1]."

Common tumor findings were considered statistically significant at P-values at or below 0.005 and 0.01 for tests of dose response relationship and pairwise comparison, respectively, as recommended by the ECAC. Rare tumor findings were considered statistically significant at P-values at or below 0.025 and 0.05 level for tests of dose response relationship and pairwise comparison, respectively, as recommended by the Executive Carcinogenicity Assessment Committee (ECAC).

As discussed under Body Weight, the Air 1 control consistently showed increased body weight over all other groups. In addition, the Air 1 group was housed in a separate room, and exposed differently to air on a loading rack, compared to rotation through a flow through chamber. Therefore, the Air 1 group was not used primarily for interpreting findings in this study.

A statistical analysis was performed between the Air 2 and vehicle group to determine if there were any significant differences in tumor incidence related to the vehicle. There were no significant differences for pairwise or trend analysis. In addition, the proposed level of magnesium stearate (0.15% w/v) results in a maximum exposure of 75 mcg/day, which is lower than the daily exposure of magnesium stearate from approved inhalation drugs for similar indications. Further, magnesium stearate is a common food additive that was determined to be GRAS at levels of 300 mg or less per day for adults.

There were no neoplastic lesions that were statistically significant for pairwise or trend analysis when comparing test article dose groups versus the vehicle control.

[1] Brix, A.E. J.F. Hardisty, and E.E. McConnell. Combining neoplasms for evaluation of rodent carcinogenesis studies. In: Hsu and Stedeford (eds.) Cancer Risk Assessment, John Wiley and Sons, Inc., 2010: 699-715.

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Non Neoplastic

The majority of findings were related to local toxicity from the inhalation route of administration. Specifically, test article related non-neoplastic findings were identified in the nasal cavity/sinuses, larynx, lungs and tracheobronchial lymph nodes.

Findings in the nasal cavity/sinuses are shown in the table below, but were not deemed clinically relevant because rats are obligate nose breathers, and humans will be dosed via oral inhalation. The larynx of both males and females developed squamous metaplasia (males: vehicle 1/50, LD 1/50, MD 11/50, HD 9/50; females: vehicle 1/50, LD 2/50, MD 9/50, HD 15/50), which is rat-specific effect due to the physiology of the rat respiratory tract, and resulting irritation to the larynx. This was not considered a clinically relevant finding.

In the lung, epithelial hypertrophy was noted at the bronchioloalveolar junction, with a dose dependent increase in incidence and severity (males: vehicle 1/50, LD 3/50, MD 16/50, HD 25/50; females: vehicle 0/50, LD 7/50, MD 23/50, HD 36/50). Hypertrophic epithelia were noted to have eosinophilic inclusions, which the sponsor hypothesized to be Clara cells responsible for surfactant production. Macrophage accumulation (all types) was increased overall in dosed animals (males: vehicle 12/50, LD 36/50, MD 32/50, HD 34/50) with a dose dependent increase in incidence in females (vehicle 17/50, LD 18/50, MD 25/50, HD 30/50), and a dose dependent increase in severity in both sexes. The sponsor noted that macrophages occasionally contained small foci containing “clear, speculate, cholesterol-like clefts.” The sponsor did not have a hypothesis regarding the biological significance of the clefts. The clear vacuoles may be indicative of phospholipidosis, but it is not clear. Pigment deposits were noted in the tracheobronchial lymph nodes in HD males (7/50) and MD and HD females (MD 14/50, 12/50). Based on dose dependent epithelial hypertrophy at all doses, no NOAEL was established.

Table 10: Rat carcinogenicity study: Microscopic findings

	Dose (mg/kg/d)											
	Male						Female					
	Air 1	Air 2	Vehicle	0.07	0.21	0.56	Air 1	Air 2	Vehicle	0.07	0.21	0.56
Observations/N	50	50	50	50	50	50	50	50	50	50	50	50
CAVITY NASAL/SINUSES	50	50	50	50	50	50	50	50	50	50	50	50

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- Eosinophilic globules: olfactory epithelium	4	8	7	13	23	45	4	0	6	5	22	49
- Hyperplasia: respiratory epithelium	0	0	0	4	4	6	0	0	0	0	0	1
LARYNX	50	50	50	50	50	50	50	50	50	50	50	50
- Metaplasia: squamous	0	0	1	1	11	9	0	1	1	2	9	17
LUNG	50	50	50	50	50	50	50	50	50	50	50	50
- Epithelial hypertrophy: bronchioloalveolar junction	0	0	1	3	16	25	0	0	0	7	23	36
minimal	0	0	1	2	16	17	0	0	0	7	21	20
slight	0	0	0	0	0	8	0	0	0	0	2	15
moderate	0	0	0	0	0	0	0	0	0	0	0	1
- Eosinophilic cytoplasmic inclusions bronchiolar	0	0	0	3	5	11	0	0	0	1	3	6
minimal	0	0	0	3	5	10	0	0	0	1	3	6
slight	0	0	0	0	0	1	0	0	0	0	0	0
- Macrophage accumulation w/o clefts	14	18	11	31	27	22	7	13	17	16	21	23
minimal	10	13	10	28	22	18	6	13	14	15	16	14
slight	4	4	1	3	5	4	1	0	3	1	4	8
moderate	0	1	0	0	0	0	0	0	0	0	1	1
- Macrophage accumulation with clefts	1	3	1	5	5	12	0	2	0	2	4	7
minimal	1	3	1	4	5	10	0	2	0	2	4	6
slight	0	0	0	1	0	1	0	0	0	0	0	1
moderate	0	0	0	0	0	1	0	0	0	0	0	0
- All macrophage accumulation	15	21	12	36	32	34	7	15	17	18	25	30
L.N.TRACHEOBRONCHIAL	42	42	44	43	40	43	44	46	44	42	44	45
- Deposits: pigment	1	2	1	3	0	7	7	7	7	9	14	12

With respect to systemic toxicity, there were no clear test article related findings. Low incidence findings of uterus dilatation and acinar epithelial hyperplasia of the prostate are shown below for completeness:

Table 11: Rat carcinogenicity study: Low incidence microscopic findings

	Dose (mg/kg/d)											
	Male						Female					
	Air 1	Air 2	Vehicle	0.07	0.21	0.56	Air 1	Air 2	Vehicle	0.07	0.21	0.56
Observations/N	50	50	50	50	50	50	50	50	50	50	50	50
UTERUS							50	50	50	50	50	50
- Dilatation							2	6	4	3	3	8
PROSTATE	50	50	50	50	50	50						
- Hyperplasia: acinar epithelium	0	0	0	0	0	2						

Reference ID: 3788692

Toxicokinetics

Blood (0.8 mL) was collected via the jugular vein on Day 1 and during Week 52. In the animals dosed with the test article, blood was collected (3 animals/sex/group) at the following time points: 0 min (immediately after dosing), 15 min, 1 hr, 3 hrs, and 7 hrs after the completion of inhalation dosing, and 24 hrs after the initiation of dosing. In control groups dosed with air or vehicle, blood was collected at the following time points: 0 min (immediately after dosing), and 15 min after the completion of inhalation dosing. The concentration of the test article was determined after extraction and LC-MS/MS analysis.

All samples from Air 2 and vehicle control groups showed no detectable test article concentrations (LLOQ 0.100 ng/mL). In the Air 1 control group, 3/21 samples had concentrations above the LLOQ (2 samples on Day 1 and 1 sample in Week 52). The concentrations ranged from 0.333 to 0.074 ng/mL, which was less than the Cmax of the LD on both sampling days. Taking into considering the low frequency and low concentration of the samples, the Air 1 group is still considered to valid with respect to dosing overall in the study. Importantly, the Air 1 group was not used primarily to interpret findings in this review.

Systemic exposure (AUC) to NVA237 was similar in both genders at all dose groups, with slight accumulation at Week 52. Exposure was dose proportional between the LD and MD, but was slightly less than dose proportional between the MD and HD. Cmax and AUC values are expressed in bromide salt form.

Table 12: Rat carcinogenicity study: Toxicokinetic parameters

			Dose (mg/kg/d)					
			Male			Female		
Time point	Parameter	Unit	0.07	0.21	0.56	0.07	0.21	0.56
Day 1	tmax	h	0 *	0 *	0 *	0 *	0 *	0 *
	Cmax	ng/mL	2.93	7.09	19.5	3.21	5.72	9.39
	AUC(0-24h)	ng.h/mL	6.7	16.3	35.2	6.88	15.3	24.4
Week 52	tmax	h	0 *	0 *	0 *	0 *	0 *	0 *
	Cmax	ng/mL	2.28	5.01	11.4	1.93	6.29	12.3
	AUC(0-24h)	ng.h/mL	8.5	23.2	34.6	7.88	21.1	38.3
Both sexes (average)								
Day 1	tmax	h	0 *	0 *	0 *			
	Cmax	ng/mL	3.07	6.41	14.45			
	AUC(0-24h)	ng.h/mL	6.79	15.80	29.80			
Week 52	tmax	h	0 *	0 *	0 *			
	Cmax	ng/mL	2.105	5.65	11.85			
	AUC(0-24h)	ng.h/mL	8.19	22.15	36.45			

* end of inhalation

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Dosing Solution Analysis

During dosing, chamber concentrations of aerosol were determined gravimetrically from open-face glass fiber or Teflon filter samples collected at a representative animal breathing point, at least once daily (except from air control groups). Chamber concentrations of NVA237 were determined analytically by [redacted] (b) (4) from the deposit on the gravimetric filters on 2 time points prestudy, and weekly during the first 13 weeks of the treatment period, and generally once every 2 weeks thereafter.

The sponsor calculated for estimated achieved doses as shown below. The doses were 0 (vehicle), 0.07 (low dose/LD), 0.21 (mid dose/MD) and 0.56 (high dose/HD) mg/kg/day, based on the quaternary ammonium cation of the bromide salt. The achieved dose was within 17% of the nominal dose. Although this is not optimal, the study is valid.

Table 13: Rat carcinogenicity study: Achieved dose

	Dose mg/kg/day		
Group	Nominal (salt)	Achieved (salt)	% of target
Low dose/LD	0.06	0.07	117%
Mid dose/MD	0.2	0.21	105%
High dose/HD	0.6	0.56	93%

The test article was within respirable range (between 1 – 5 microns), based on the reported MMAD ± GSD:

Group ID	Chamber concentrations (mg/L)		Particle size MMAD (µm) ± GSD	
	Gravimetric	Analytical*	Gravimetric	Analytical*
2/ Air control	-	0	-	-
3/ Vehicle control	0.0447	0	4.7 ± 3.5	-
4/ NVA237 Low dose	0.0144	0.0018	2.8 ± 2.6	2.4 ± 2.2
5/ NVA237 Mid dose	0.0427	0.0058	2.7 ± 2.7	2.5 ± 2.1
6/ NVA237 High dose	0.0596	0.0152	2.8 ± 3.2	2.3 ± 1.8

* Concentrations given refer to the salt form of NVA237

(Sponsor's table)

Analysis of the aerosol particle size distribution for groups dosed with vehicle and NVA237 was performed using a cascade impactor. The mass median diameter (MMAD) and its geometric standard deviation (GSD) were calculated. Homogeneity of the chamber atmosphere was determined for the vehicle control, LD and HD groups. Filter samples were collected in duplicate for gravimetric analysis from 3 equidistantly spaced sampling ports located at the top and bottom of the animal exposure levels. Samples were also collected from a reference port to assess total and within port variation of test article distribution within the inhalation chamber.

The test article was within respirable range (between 1 – 5 microns), based on the reported MMAD ± GSD:

Group ID	Chamber concentrations (mg/L)		Particle size MMAD (µm) ± GSD	
	Gravimetric	Analytical*	Gravimetric	Analytical*
2/ Air control	-	0	-	-
3/ Vehicle control	0.0447	0	4.7 ± 3.5	-
4/ NVA237 Low dose	0.0144	0.0018	2.8 ± 2.6	2.4 ± 2.2
5/ NVA237 Mid dose	0.0427	0.0058	2.7 ± 2.7	2.5 ± 2.1
6/ NVA237 High dose	0.0596	0.0152	2.8 ± 3.2	2.3 ± 1.8

* Concentrations given refer to the salt form of NVA237

(Sponsor's table)

Homogeneity varied up to 20.6%, as shown in the table below. While this is not optimal, the study is valid.

	Reference port		Overall	
Group	**Mean aerosol NVA237 concentration (mg/L)**	**C.V.%**	**Mean aerosol NVA237 concentration (mg/L)**	**C.V.%**
Vehicle control	0.0629 ± 0.00985	15.7	0.0539 ± 0.00993	18.4
Low concentration	0.0072 ± 0.00154	21.4	0.0069 ± 0.00142	20.6
High concentration	0.0555 ± 0.00326	5.9	0.0552 ± 0.00399	7.2

(Sponsor's table, page 31 of the study report)

<u>26-week transgenic mouse study</u>

Study title: NVA237: A 26-week oral gavage carcinogenicity study in Tg.rasH2 mice

Study no.: 0770668/N109087
Study report location: SD-1
Conducting laboratory and location: (b) (4)
Date of study initiation: 31-Mar-2010
GLP compliance: Y
QA statement: Y
Drug, lot #, and % purity: NVA237, Batch no. 0724007, 100.0%
CAC concurrence: Yes

Key Study Findings

- In the 26-week oral gavage carcinogenicity study in Tg.rasH2 mice, males received NVA237 at 0 (vehicle: Deionized water), 12.5, 31.3, and 93.8 mg/kg/day and females received NVA237 at 0 (vehicle), 12.5, 37.5, and 125.1 mg/kg/day, based on the concentration of the salt. Positive control mice were administered N-methyl-N-nitrosourea (MNU; 75 mg/kg) by a single intraperitoneal injection on Day 1.
- There were no effects on survival in NVA237 treated animals, based on a lack of dose response and overall low incidences of mortality. MNU-treated Tg.rasH2 animals had increased mortality (males 15/25, females 5/25), compared untreated Tg.rasH2 animals (males 0/25, females 0/25).

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- Hunched posture was observed for 2 or 3 males at the MD and HD, 1 female each at the LD and MD, and 8 females at the HD. Lethargy was noted in HD females, and 2-3 males at all doses.
- Tg.rasH2 mice treated with NVA237 showed a dose dependent decrease in body weight (males: LD 95%, MD 90%, HD 82%; females: MD 98%, HD 93%). Wild-type mice treated with NVA237 also developed decreased body weight (males 75%, females 86%). Transient decreases in food consumption were noted in HD Tg.rasH2 and WT animals.
- There were no neoplastic lesions that had a dose-dependent relationship with NVA237 in male and female Tg.rasH2 mice. There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis in Tg.rasH2 mice dosed up to 93.8 and 125.1 mg/kg/day in males and females, respectively.
- NVA237-related nonneoplastic lesions were identified in the forestomach/limiting ridge, and the liver. Findings in the forestomach were determined to be not relevant to human. In the liver, focal and multifocal necrosis was observed at the HD (males 3/25, females 3/25, up to Grade 2) and was not observed in control animals. A clarification was requested in an Information Request sent on May 11, 2015, and the sponsor confirmed that focal and multifocal necrosis were pooled, and did not provide information to separate the findings based on distribution. Liver atrophy (Grade 3) was noted in 3 females (1/25 LD, 2/25 HD) and 1/25 MD male, and was not noted in control animals. The severity of the liver atrophy is a concern, and it is unclear if there is a NOAEL for this finding. Published historical control data do not show any incidence of liver atrophy[2]. Glycogen increase was noted in females (control 0/0, LD 1/25, MD 0/0, HD 3/25), but this was not considered a dose-limiting finding.
- Exposure to NVA237 was not consistently shown in transgenic animals. Due to the inconsistent exposure data in Tg.rasH2 animals, the metabolite CJL603 was examined. Mice had notably higher concentrations of CJL603 compared to NVA237 (1800 to 13000-fold increase). At several time points, CJL603 was not detected in all tested animals. Systemic exposure (AUC) for CJL603 increased in a roughly dose proportional manner. CJL603 remained detectable at 24 hrs post dose.

Adequacy of Carcinogenicity Study

- The duration of treatment at 26 weeks is adequate.
- The doses were recommended by the ECAC (minutes dated February 24. 2010).

Appropriateness of Test Models

- The Tg.rasH2 transgenic mouse strain is an acceptable model for evaluating the carcinogenic potential of pharmaceuticals per ICH *Guidance for Industry S1B*

[2] Kanno *et al*. Historical Background data in CB6F1-Tg-rasH2 mice and CB6F1-nonTg-rasH2 mice over a 26-week experimental period. J Toxicol Pathol 2003: 16:267-274.

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Testing for Carcinogenicity of Pharmaceuticals. The Tg.rasH2 is recognized as a model for cancer hazard identification[3].

Evaluation of Tumor Findings

- There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis in Tg.rasH2 dosed up to 93.8 and 125.1 mg/kg/day in males and females, respectively, based on the concentration of the salt.
- The MNU-treated positive control group developed multicentric lymphoma (males), and neoplasias in the Harderian gland (adenoma), skin (squamous cell papilloma, carcinoma and keratoacanthoma), non-glandular stomach (squamous cell papilloma and carcinoma), and uterus (endometrial inflammatory polyp). These are expected effects of MNU-treatment in Tg.rasH2 mice.
- Thus, NVA237 was determined to not be tumorigenic in male and female Tg.rasH2 mice.

[3] Mortan, D *et al*. The Tg rasH2 Mouse in Cancer Hazard Identification. Toxicological Pathology, vol 30, no 1, pp 139–146, 2002.

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Methods

Doses:	0 (vehicle), 12.5, 31.3, 93.8 mg/kg/day of NVA237 for transgenic males and 0 (vehicle), 12.5, 37.5, 125.1 mg/kg/day of NVA237 for transgenic females, with concentration based on the salt; wild type animals were administered doses of 0 (vehicle), 93.8 (males), and 125.1 (females) mg/kg/day of NVA237. Positive control mice were administered the MNU formulation by a single intraperitoneal injection at a volume of 10 mL/kg on day 1
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Deionized water
Basis of dose selection:	Two 4-week oral toxicity studies were conducted in mice and evaluated by the ECAC (minutes 2/24/10). In the first study, mice were dosed with 30, 75 and 150 mg/kg, but no toxicity was observed. In the second study, the MTD was exceeded at 225 mg/kg/day for males, and 300 mg/kg/day for females, based on mortality. Therefore, the ECAC recommended doses of 0, 10, 25, and 75 mg/kg/day in males and 0, 10, 30, and 100 mg/kg/day in females. The doses in the reviewed 6 month transgenic mouse study are consistent with the recommended doses. (Concentrations are listed in terms of the base under “Basis of dose selection”).
Species/Strain:	Mouse, CByB6F1-Tg(HRAS)2Jic
Number/Sex/Group:	25/sex/group for Tg.rasH2 mice and WT main study mice.
Age:	8 weeks (at initiation)
Animal housing:	Individually housed in polycarbonate cages with hardwood bedding
Paradigm for dietary restriction:	None
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Toxicokinetic animals: 3/sex/group scheduled at 0.5, 1, 3, 7, and 24 hours post-dose for each group dosed with the TA (groups 2, 3, 4, and 7); 2/sex/group at 0.5 hour post-dose for vehicle (group 1) and MNU positive control (group 6).

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Deviation from study protocol: There were no deviations that affected the validity of the study.

The number of transgenic hemizygote rasH2 mice per group and dose levels were as follows.

Group	Dose base (salt)[a] (mg/kg/day)		Concentration base (salt)[a] (mg/mL)		Total number of mice	Number of mice designated for main study	Number of mice designated for toxicokinetics
	M	F	M	F			
1 Vehicle	0	0	0	0	54 (27M/27F)	25/sex	2/sex
2 NVA237	10 (12.5)	10 (12.5)	2 (2.5)	2 (2.5)	80 (40M/40F)	25/sex	15/sex
3 NVA237	25 (31.3)	30 (37.5)	5 (6.3)	6 (7.5)	80 (40M/40F)	25/sex	15/sex
4 NVA237	75 (93.8)	100 (125.1)	15 (18.8)	20 (25.0)	80 (40M/40F)	25/sex	15/sex
5 MNU (Positive control)	75[b] (dosed IP on Day 1 only)		7.5[b]		50 (25M/25F)	25/sex	0/sex

a. Salt/base ratio for NVA237 is 1.251.

b. Dose and concentration is MNU.

The numbers of wild-type mice per group and dose levels were as follows:

Group	Dose base (salt)[a] (mg/kg/day)		Concentration base (salt)[a] (mg/mL)		Total number of mice	Number of mice designated for main study	Number of mice designated for toxicokinetics
	M	F	M	F			
6 Vehicle	0	0	0	0	54 (27M/27F)	25/sex	2/sex
7 NVA237	75 (93.8)	100 (125.1)	15 (18.8)	20 (25.0)	80 (40M/40F)	25/sex	15/sex

a. Salt/base ratio for NVA237 is 1.251.

(Sponsor's tables)

Observations and Results

Mortality

Animals were checked for mortality at least twice daily throughout the study.

The overall mortality for Tg.rasH2 mice was low. There was a slight increase in deaths in Tg.rasH2 animals dosed with the NVA237 (LD 2/25 females, MD 2/25 males and 2/25 females, HD 3/25 males and 2/25 females, compared to controls (males 0/25, females 0/25). There were no statistically significant dose relationships in Tg.rasH2 mice for mortality, and no statistically significant increased mortality was noted in any NVA237-treated Tg.rasH2 group compared to the Tg.rasH2 control group.

There was no notable difference in mortality in WT treated versus control animals. As expected, MNU-treated Tg.rasH2 animals had increased mortality (males 15/25, females 5/25), compared to control Tg.rasH2 animals (males 0/25, females 0/25). The incidence and causes of death are shown below.

Table 14: Mouse carcinogenicity study: Mortalities

			Deaths		% Survival	
Dose (mg/kg)	Mice	N/sex	Males	Females	Males	Females
0	Tg.rasH2	25	0	0	100	100
12.5	Tg.rasH2	25	0	2	100	92
31.3/37.5	Tg.rasH2	25	2	2	92	92
93.8/125.1	Tg.rasH2	25	3	2	88	92
0	WT	25	0	0	100	100
93.8/125.1	WT	25	0	1	100	96
MNU	Tg.rasH2	25	15	5	40	80

Table 15: Mouse carcinogenicity study: Cause of death

	Dose (mg/kg)	Mice	Sex	Animal#	Cause of death	Day
2	12.5	rasH2	female	2502	Bone: osteosarcoma	173
				2517	Spinal cord: epidermal cyst, grade 4	168
3	31.3/37.5	rasH2	male	3007	Skeletal muscle: hemangiosarcoma	169
				3024	Kidneys: nephropathy, grade 3	160
			female	3503	Systemic: malignant lymphoma	106
				3507	Systemic: malignant lymphoma	177
4	93.8/125.1	rasH2	male	4001	Not determined	178
				4014	Not determined	184
				4019	Not determined	169
			female	4513	Not determined	153
				4515	Not determined	18
7	93.8/125.1	WT	female	7518	Not determined	53

Clinical Signs

Animals were examined no less than 2 hours post-dose for clinical signs, at least once per week. Palpable mass examinations were performed beginning at Week 2 and once every 2 weeks thereafter. Cage-side observations were performed approximately 2 to 4 hours post-dose, at least once daily.

Hunched posture was noted in male (MD 2/25, HD 3/25) and female (LD 1/25, MD 1/25, HD 8/25) transgenic animals, with highest incidence at the HD. Lethargy was noted in males (LD 2/25, MD 2/25, HD 3/25) and HD females (3/25).

Table 16: Mouse carcinogenicity study: Clinical signs

Dose (mg/kg)		
rasH2	WT	rasH2

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		0	12.5	31.3/37.5	93.8/125.1	0	93.8/125.1	MNU
	N=	25	25	25	25	25	25	25
Appearance Abnormality/Hunched	Males	0	0	2	3	0	1	9
	Females	0	1	1	8	0	1	1
Activity Abnormality/Lethargy	Males	0	2	2	3	0	0	3
	Females	0	0	0	3	0	0	0

In animals dosed with NVA237, there were no clear dose-dependent palpable masses. An increased incidence of palpable masses was associated with MNU administration at the body, ear, genitalia, head/neck and tail. All palpable masses are shown below for completeness.

Table 17: Mouse carcinogenicity study: Palpable masses in transgenic mice

Males					
Dose (mg/kg)	Mass	Animals affected	1st day	Last day	Total number
0	-	-	-	-	-
12.5	-	-	-	-	-
31.3/37.5	Tail	1	92	183	8
93.8/125.1	-	-	-	-	-
MNU	Body Ventral	4	107	183	5
	Genitalia	15	92	183	72
	Head/Neck	1	183	183	1
	Tail	2	36	174	6
Females					
Dose (mg/kg)	Mass	Animals affected	1st day	Last day	Total number
0	-	-	-	-	-
12.5	Tail	3	64	183	24
31.3/37.5	-	-	-	-	-
93.8/125.1	-	-	-	-	-
MNU	Ear	1	22	42	3
	Genitalia	14	78	183	71
	Tail	6	50	183	46

Body Weights

Body weights were recorded once during the pre-test period, and weekly during the study.

Transgenic mice treated with NVA237 showed a dose dependent decrease in body weight midway through the study (Day 92) that was more pronounced in males (LD 92%, MD 90%, HD 81%) than females (LD 99%, MD 98%, HD 94%). At Day 176, transgenic animals continued to have a decreased body weight (males: LD 95%, MD 90%, HD 82%; females: MD 98%, HD 93%). The data are shown in Table 18 and Figure 3.

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Table 18: Mouse carcinogenicity study: Body weight analysis in transgenic mice

	Dose (mg/kg)							
	Male				Female			
	0	12.5	31.3/37.5	93.8/125.1	0	12.5	31.3/37.5	93.8/125.1
Day 1	24.4	23.9	23.8	23.9	18.9	18.9	18.8	18.7
Day 92	31.8	29.1	28.5	25.8	22.5	22.3	22	21.1
Absolute BW, % Control	100%	92%	90%	81%	100%	99%	98%	94%
Δ (g)	7.4	5.2	4.7	1.9	3.6	3.4	3.2	2.4
BW gain, % initial	30%	22%	20%	8%	19%	18%	17%	13%
BW gain, % control	100%	70%	64%	26%	100%	94%	89%	67%
Day 176	35.1	33.3	31.6	28.8	24.1	24.4	23.7	22.5
Absolute BW, % Control	100%	95%	90%	82%	100%	101%	98%	93%
Δ (g)	10.7	9.4	7.8	4.9	5.2	5.5	4.9	3.8
BW gain, % initial	44%	39%	33%	21%	28%	29%	26%	20%
BW gain, % control	100%	88%	73%	46%	100%	106%	94%	73%

Wild-type mice also showed a test article related decrease in body weight gain midway through the study, at Day 92, that was more pronounced in males (74%) than females (86%), At Day 176, wild-type animals continued to have a decreased body weight (males 75%, females 86%). The data are shown in Table 19 and Figure 4.

Table 19: Mouse carcinogenicity study: Body weight analysis in wild-type mice

	Dose (mg/kg)			
	Male		Female	
	0	93.8/125.1	0	93.8/125.1
Day 1	26.1	24.9	20.5	20
Day 92	37.4	27.8	26.8	23.1
Absolute BW, % Control	100%	74%	100%	86%
Δ (g)	11.3	2.9	6.3	3.1
BW gain, % initial	43%	12%	31%	16%
BW gain, % control	100%	26%	100%	49%
Day 176	41.1	30.7	28.7	24.8
Absolute BW, % Control	100%	75%	100%	86%
Δ (g)	15	5.8	8.2	4.8
BW gain, % initial	57%	23%	40%	24%
BW gain, % control	100%	39%	100%	59%

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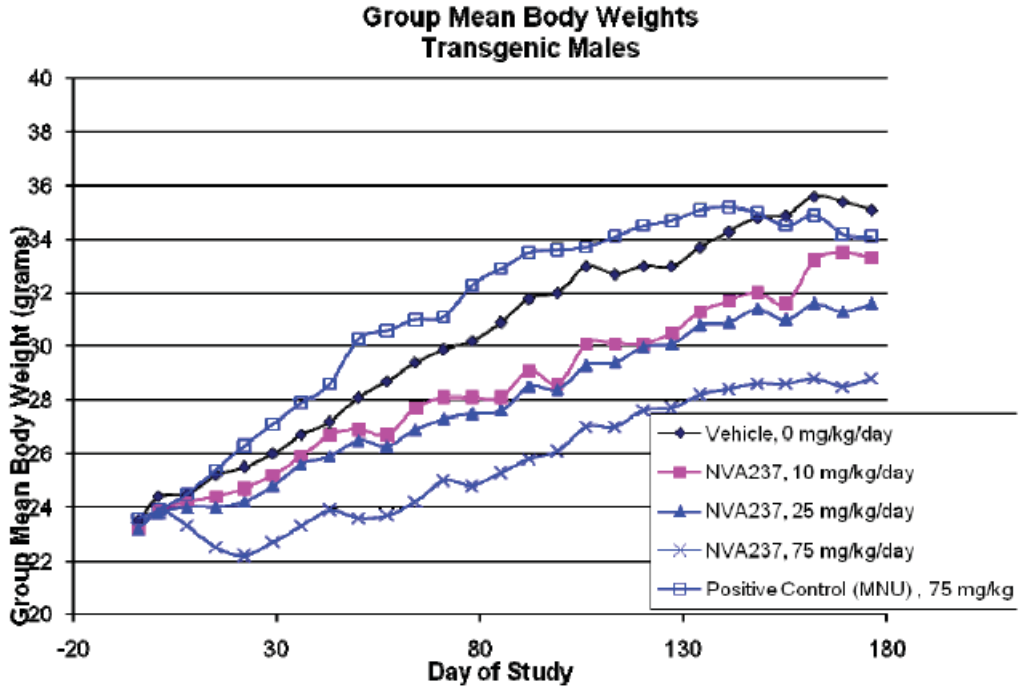
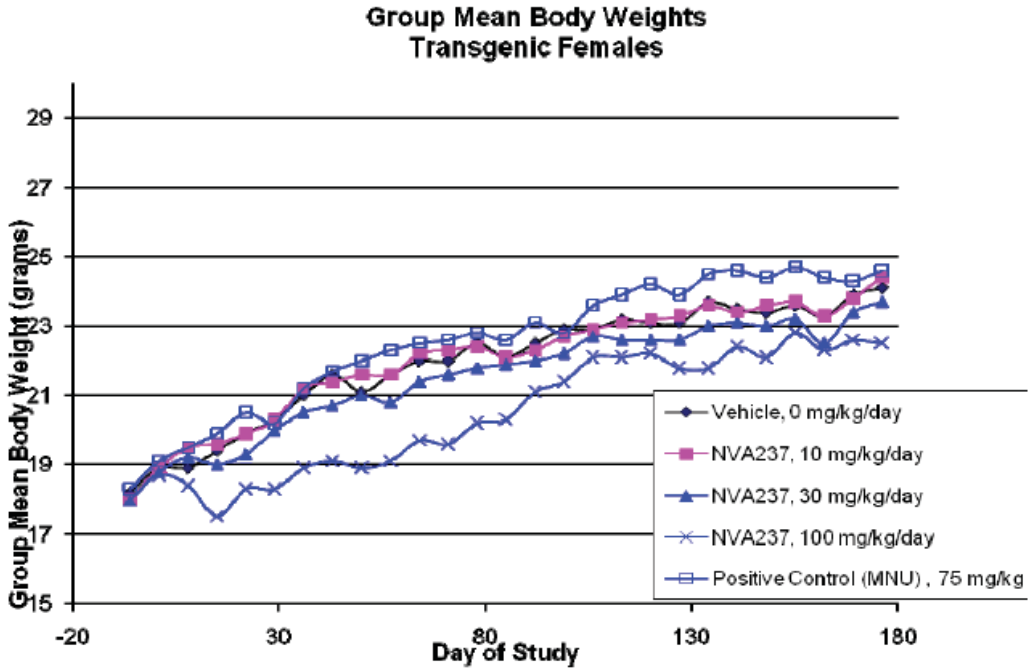
Figure 3: Mouse carcinogenicity study: Transgenic animals, body weight

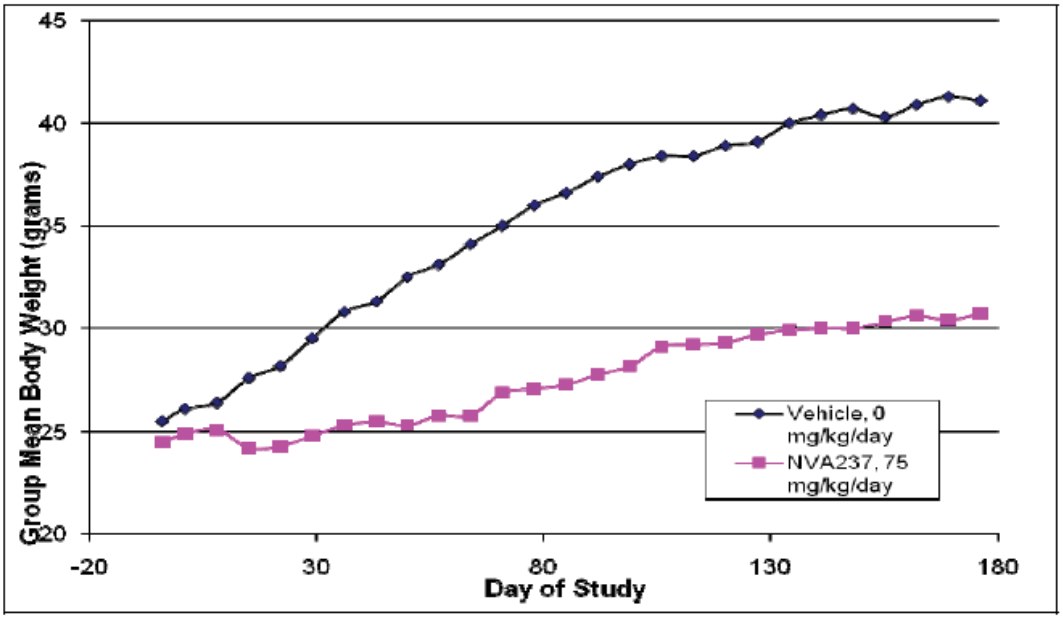




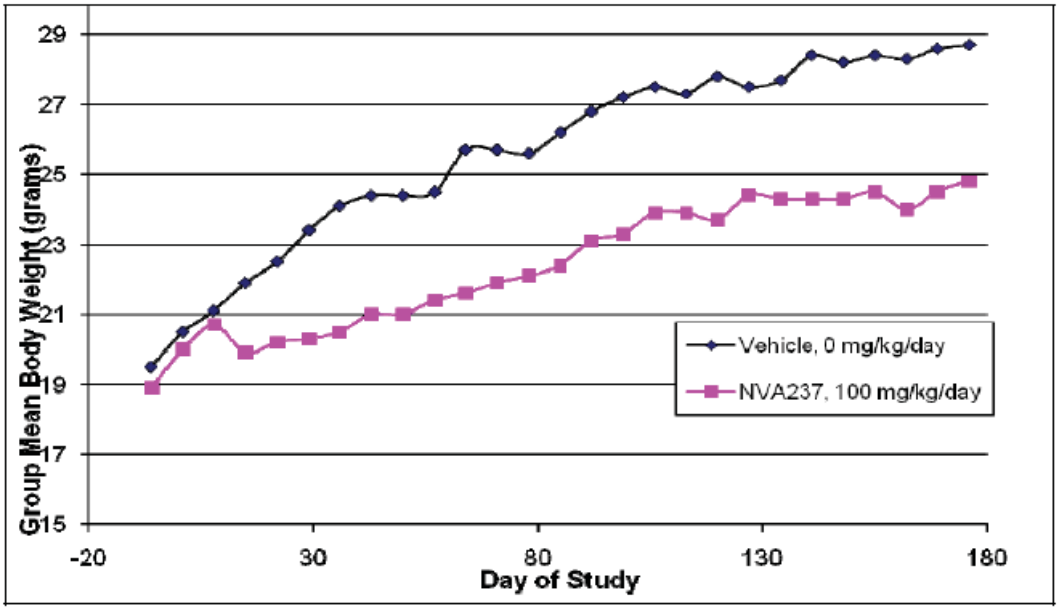
(Sponsor's figures)

Figure 4: Mouse carcinogenicity study: Wild-type animals, body weights

Males



Females



(Sponsor's figures, concentrations stated in terms of the base)

Feed Consumption

Food consumption was assessed starting on Day 1, and once weekly during the study based on difference in weight of the food container at the beginning and end of each week.

There was a decrease in food consumption in HD transgenic mice (male -13%, female -29%) and WT mice (male -8%, female -20%) after the first week (Day 8). At Day 92, HD transgenic males (-7%) and WT males (-5%) had a slight decrease in food

consumption, which was not observed in females. At the end of the study (Day 183), there were no notable decreases in food consumption.

Table 20: Mouse carcinogenicity study: Food consumption, percent change versus control

	Males					Females				
	rasH2				WT	rasH2				WT
Day	12.5	31.3/ 37.5	93.8/ 125.1	MNU	93.8/ 125.1	12.5	31.3/ 37.5	93.8/ 125.1	MNU	93.8/ 125.1
8	3%	0%	-13%	0%	-8%	12%	0%	-29%	9%	-20%
92	-2%	-2%	-7%	7%	-5%	5%	5%	5%	10%	-7%
183	-2%	0%	-4%	-2%	-7%	2%	0%	7%	10%	-4%

Gross Pathology

Animals were terminated with carbon dioxide asphyxiation. A board certified veterinary pathologist was present at necropsy. Each necropsy included examination of the external surface of the body and all orifices; the cranial, thoracic, abdominal and pelvic cavities and their contents.

There were no test article related gross lesions in transgenic animals that underwent scheduled or unscheduled necropsy. MNU induced multiple lesions, as shown below from scheduled necropsies, confirming its use as a positive control. MNU-treated animals that underwent unscheduled necropsy had additional MNU-related lesions (not shown). Gross lesions were not reported for WT animals.

The sponsor used the term “HDN” to describe a type of liver lesion. The meaning of “HDN” was requested in an Information Request sent on May 11, 2015, and the sponsor stated in the response submitted on May 14, 2015 that HDN indicated hepatodiaphragmatic nodule, which is a congenital finding.

Table 21: Mouse carcinogenicity study: Gross lesions associated with MNU-treatment in Tg.rasH2 mice (scheduled necropsies)

	Dose (mg/kg)									
	Males					Females				
	0	12.5	31.3/ 37.5	93.8/ 125.1	MNU	0	12.5	31.3/ 37.5	93.8/ 125.1	MNU
Observation/Number	25	25	23	22	10	25	23	23	23	20
Cavity, Thoracic										
Fluid	0	0	0	0	1	0	0	0	0	0
Kidneys										
Enlarged	0	0	0	0	2	0	0	0	0	0
Liver										
Enlarged	0	0	0	0	1	0	0	0	0	0

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Lungs										
Mass(es)	0	0	0	0	1	0	0	0	0	0
Nodule(s)	0	0	0	0	1	0	0	0	0	0
Lymph Nodes, Bronchial										
Enlarged	0	0	0	0	1	0	0	0	0	0
Lymph Nodes, Mandibular										
Enlarged	0	0	0	0	1	0	0	0	0	0
Lymph Nodes, Mediastinal										
Enlarged	0	0	0	0	1	0	0	0	0	0
Lymph Nodes, Mesenteric										
Enlarged	0	0	0	0	2	0	0	0	0	0
Lymph Nodes, Other										
Enlarged	0	0	0	0	3	0	0	0	0	0
Skin										
Mass(es)	0	0	0	0	2	0	0	0	0	4
Nodule(s)	0	0	1	0	8	0	0	0	0	14
Spleen										
Enlarged	0	0	0	0	3	0	0	0	0	1
Focus	0	0	0	0	0	0	0	0	0	1
Nodule(s)	0	0	0	0	0	0	1	0	0	1
Testes										
Enlarged	0	0	0	0	1					
Thymus										
Enlarged	0	0	0	0	3	0	0	0	0	0
Tongue										
Nodule(s)	0	0	0	0	1	0	0	0	0	0
Ovaries										
Enlarged						0	0	0	0	1
Uterus										
Cyst(s)						0	0	0	0	1
Dilatation						0	0	0	0	3
Vagina										
Prolapse						0	0	0	0	1

Histopathology

Complete necropsies were performed on main study mice that were found dead or were terminated at an unscheduled interval, as well as those for scheduled sacrifice. Blood

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and bone marrow smears were prepared on all animals sacrificed early (when possible) and on all surviving animals at scheduled necropsy. Tissues were placed in 10% neutral buffered formalin (NBF), with the exceptions of testes, epididymides, and eyes. The testes and epididymides were preserved in modified Davidson's fixative and subsequently transferred to 10% NBF. The eyes with optic nerve were fixed in Davidson's fixative and subsequently transferred to 10% NBF, per testing facility SOP. Bone marrow smears were fixed in methanol.

The following tissues were evaluated:

Animal identification[a]	Mammary gland
Adrenal glands	Ovaries (with oviduct[d])
Aorta	Pancreas
Bone with articular surface and marrow (femur)	Pituitary gland
Blood and bone marrow smear (femur)[b]	Preputial gland
Brain	Prostate gland
Cervix	Salivary gland (mandibular)
Clitoral Gland	Sciatic nerve
Epididymides	Seminal vesicles
Esophagus	Skeletal muscle (biceps femoris)
Eyes with optic nerves	Skin
Gallbladder	Spinal cord (cervical, thoracic, lumbar)
Gross lesions	Spleen
Harderian glands	Sternum
Heart	Stomach
Intestine, large (cecum, colon, rectum)	Testes
Intestine, small (duodenum, jejunum, ileum)	Thymus
Kidneys	Thyroid gland (with parathyroids, if present in routine section)[c]
Lacrimal gland	
Larynx (1 level)-longitudinal section	Tongue
Liver (median lobe and left lateral lobe)	Trachea
Lungs with bronchi	Ureters (cross-section)[d]
Lymph node (mandibular, mesenteric)	Urinary bladder
	Uterus (with cervix)
	Vagina

a. Collected but not processed.

b. Blood and bone marrow cytology smears were prepared for mice at scheduled necropsy, but were not evaluated.

c. At least one parathyroid gland was to be examined. One re-cut and/or re-check of wet tissues was to be performed to attempt to find the missing tissues.

d. Bilateral collection, unilateral histopathology

(Sponsor's table)

Peer Review: Yes. More information requested in an Information Request sent on May 11, 2015. The sponsor stated in the response submitted on May 14, 2015 that the signed peer review statement is archived on file.

Neoplastic

There were no neoplastic lesions that had a dose-dependent relationship with NVA237 in Tg.rasH2 mice. There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis in Tg.rasH2 dosed up to 93.8 and 125.1 mg/kg/day in males and females, respectively.

MNU-treated Tg.rasH2 animals developed malignant lymphoma (males), and neoplasias in the Harderian gland (adenoma), skin (squamous cell papilloma, carcinoma and keratoacanthoma), non-glandular stomach (squamous cell papilloma and carcinoma), and uterus (endometrial inflammatory polyp). Hemangiosarcoma was also observed in MNU-treated Tg.rasH2 animals. These neoplasias are expected effects of MNU-treatment in Tg.rasH2 mice, based on the published interlaboratory comparison of MNU-induced lesions in Tg.rasH2 mice [4]

Table 22: Mouse carcinogenicity study: MNU-treatment related neoplasias

	MNU-treated animals		Historical controls (Takaoka M, 2003)			
	Male	Female	Male		Female	
Tumor type/N examined	25	25	Range %	Mean %	Range %	Mean %
HARDERIAN GLANDS						
- Adenoma	2	5	-	0.0	0.0-7.1	3.4
SKIN						
- Papilloma, Squam.Cell:	16	13	0.0-13.3	2.2	0.0-7.1	3.4
- Keratoacanthoma	1	2	NR	NR	NR	NR
- Carcinoma, Squam.Cell	1	1	NR	NR	NR	NR
STOMACH (non-glandular)						
- Carcinoma, Squam.Cell	7	1	0.0-6.7	0.6	-	0
- Papilloma, Squam.Cell	14	13	0.0-6.7	0.6	0.0-13.3	1.7

[4] Takaoka M *et al*. Interlaboratory Comparison of Short-Term Carcinogenicity Studies Using CB6F1-rasH2 Transgenic Mice. Toxicol Pathol. 2003 Mar-Apr;31(2):191-9.

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SYSTEMIC						
- Hemangiosarcoma	3	4	#	#	#	#
- Hemangioma	1	0	#	#	#	#
- Hemangiosarcoma + Hemagioma	4	4	#	#	#	#
- Lymphoma, Malignant	14	1	*	*	*	*
UTERUS						
- Endometrial inflammatory polyp		18	NR	NR	NR	NR

NR = not reported
# not reported, but common cause of death
* common cause of death

Non Neoplastic

NVA237-related nonneoplastic lesions were identified in the fore-stomach/limiting ridge, and the liver. Findings in the forestomach were determined to be irrelevant to human because of the location in the fore-stomach/limiting ridge.

In the liver, focal and multifocal necrosis was observed at the HD (males 3/25, females 3/25, up to Grade 2) and was not observed in control animals. A clarification was requested in an Information Request sent on May 11, 2015, and the sponsor confirmed that focal and multifocal necrosis were pooled, and did not provide information to separate the findings based on distribution. The sponsor stated:

> "The term "focal necrosis" is a primary diagnostic term used to describe a very localized necrotic change that occurs randomly in one or more small patches (or foci) throughout the liver parenchyma and that is not localized to specific areas or zones (e.g. centrilobular, periportal) (Greaves P (2012), Cattley RC et al (2013)a). While the term "focal necrosis" is used as a primary diagnosis, it becomes "multi-focal" when the change is observed in several places rather than as a single entity. Thus "focal liver necrosis" can be focal (a single focus) or multifocal (two or more foci) and is still captured under the primary diagnosis of "focal necrosis".

Liver atrophy (Grade 3) was noted in 3 females (1/25 LD, 2/25 HD) and 1/25 MD male, and was not noted in control animals. The severity of the liver atrophy is a concern, and it is unclear if there is a NOAEL for this finding. Published

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historical control data do not show any incidence of liver atrophy, although the data from Japan may not be optimal[5]. Glycogen increase was noted in females (control 0/0, LD 1/25, MD 0/0, HD 3/25), but this is not considered a dose-limiting finding.

Table 23: Mouse carcinogenicity study: Nonneoplastic lesions

	Dose (mg/kg)													
	Males							Females						
	Tg.rasH2					WT		Tg.rasH2					WT	
	0	12.5	31.3	93.8	MNU	0	93.8	0	12.5	37.5	125.1	MNU	0	125.1
N	25	25	25	25	25	25	25	25	25	25	25	25	25	25
STOMACH (fore stomach/limiting ridge)														
- Epithelial Hyperplasia	0	4	3	5	12	2	3	4	9	9	10	5	4	4
Grade 1:	0	1	0	0	1	0	2	1	0	1	3	0	0	0
Grade 2 :	0	2	2	4	7	1	1	3	5	6	2	4	4	1
Grade 3 :	0	1	1	1	2	1	0	0	4	2	4	1	0	2
Grade 4:	0	0	0	0	2	0	0	0	0	0	1	0	0	1
- Hyperkeratosis	0	3	2	2	13	2	3	3	8	11	8	4	1	3
Grade 1:	0	2	0	0	2	0	3	1	1	1	1	1	1	0
Grade 2:	0	1	2	2	8	2	0	2	7	10	7	2	0	3
Grade 3 :	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Grade 4:	0	0	0	0	2	0	0	0	0	0	0	0	0	0
- Inflam.,Mixed Cell	1	3	5	7	4	1	4	7	7	11	9	2	3	2
Grade 1 :	0	2	1	2	1	0	3	3	2	2	3	1	1	1
Grade 2:	1	1	4	2	2	1	1	1	5	7	6	1	2	0
Grade 3:	0	0	0	3	1	0	0	3	0	2	0	0	0	0

[5] Kanno *et al*. Historical Background data in CB6F1-Tg-rasH2 mice and CB6F1-nonTg-rasH2 mice over a 26-week experimental period. J Toxicol Pathol 2003: 16:267-274.

Grade 4:	0	0	0	0	0	0	0	0	0	0	0	0	0	1
LIVER														
- Necrosis, Focal/Multifocal	0	1	0	3	6	0	0	0	1	0	3	1	1	0
Grade 1 :	0	1	0	2	4	0	0	0	1	0	2	1	1	0
Grade 2:	0	0	0	1	2	0	0	0	0	0	1	0	0	0
- Atrophy,Hepatocel.	0	0	1	0	2	0	0	0	1	0	2	2	0	0
Grade 3 :	0	0	1	0	2	0	0	0	1	0	2	1	0	0
Grade 4 :	0	0	0	0	0	0	0	0	0	0	0	1	0	0
- Glycogen Increase	0	0	0	0	0	0	0	0	1	0	3	0	0	1
Grade 2 :	0	0	0	0	0	0	0	0	1	0	2	0	0	1
Grade 3 :	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Toxicokinetics

At Week 22, 3 animals per sex/group were bled at 0.5, 1, 3, 7, and 24 hours post-dose from groups 2, 3, 4, and 7, and two animals per sex/group at 0.5 hour post-dose from groups 1 and 6 (controls). Approximately 0.5 mL of whole blood was collected by percutaneous cardiocentesis under CO_2/O_2 anesthesia. The toxicokinetic animals were terminated by CO_2 inhalation and with no further evaluation.

Exposure to NVA237 was not consistently shown in transgenic animals. At specific time points, NVA237 was not detectable, or was only detectable in 2 of 3 animals, as shown in Table 24. In contrast, NVA237 was detected in WT animals that received 93.8 or 125.1 mg/kg of NVA237, although only in 2/3 animals/sex at 24 hrs post dose. The toxicokinetic parameters are shown in Table 25, but are difficult to interpret due to the lack of consistent NVA237 plasma concentration data. NVA237 has previously been shown to have low bioavailability in rats (study 1000618, 0.82%).

Due to the inconsistent exposure data in Tg.rasH2 animals, the metabolite CJL603 was examined.

Table 24: Mouse carcinogenicity study: NVA237 mean plasma concentrations (ng/mL)

	Dose (mg/kg)							
	Males				Females			
	Tg.rasH2			WT	Tg.rasH2			WT
Time (h)	12.5	31.3	93.8	93.8	12.5	37.5	125.1	125.1
0.5	0	55.9	12.0	4.39	0.0367	0	16.2	0.951
1	11.1	0	17.5	0.954	0	0.993	1.36	0.763
3	0	4.26	0.896	22.9	0.0810	0	0.448	0.824
7	0.0390	0	0.152*	0.283	0	0	1.29	0.878
24	0	0	0.105*	0.0615*	0	0.166*	0	0.247*

* n = 2

Table 25: Mouse carcinogenicity study: NVA237, available toxicokinetic parameters

	Dose (mg/kg)			
	Males		Females	
	Tg.rasH2	WT	Tg.rasH2	WT
	93.8	93.8	125.1	125.1
AUC (0-24hr) (ng*hrs/mL)	33.1	75.6	24.7	15.3
Cmax (ng/mL)	17.5	22.9	16.2	0.951
Tmax (hr)	1	3	0.5	0.5

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Mice had notably higher concentrations of CJL603 compared to NVA237 (1800 to 13000-fold increase), and plasma concentrations were detected more consistently. At the following time points, CJL603 was detected in only 2 of 3 animals: 1 hr (male HD Tg.rasH2, female MD Tg.rasH2), 7 hrs (male HD Tg.rasH2), 24 hrs (male MD/HD Tg.rasH2, WT; female LD/MD Tg.rasH2, WT).

With regard to CJL603, systemic exposure (AUC) for CJL603 increased in a roughly dose proportional manner. The mean for Tmax was 7 hours post dose for CJL603. However, there were no time points between 7 and 24 hrs post dose and it was possible that the Tmax may be during this non-sampling period. CJL603 remained detectable at 24 hrs post dose.

Table 26: Mouse carcinogenicity study: CJL603 toxicokinetic parameters

	Dose (mg/kg)							
	Males				Females			
	Tg.rasH2			WT	Tg.rasH2			WT
Parameter	12.5	31.3	93.8	93.8	12.5	30	125.1	125.1
AUC (0-24hr) (ng*hrs/mL)	12100	18200	70300	96900	18300	35300	142000	137000
Cmax (ng/mL)	804	1130	4320	6310	1210	2470	9670	9160
Tmax (hr)	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00

Dosing Solution Analysis

The test article was prepared weekly and stored at 2 to 8°C. Formulations were tested by HPLC using a Symmetry Shield RP18 3.5 micron (150 x 3.0 mm) column. Stability testing was conducted on test article stored under the same conditions for 2 weeks, but not reported in the study report: "Stability was previously determined for concentrations and storage conditions used in this study ([redacted] (b) (4), Project Nos. [redacted] (b) (4))." These studies were requested in an Information Request sent on May 11, 2015. In the sponsor response submitted on May 14, 2015, the sponsor provided of the cited study reports. Homogeneity was tested at Week 1. Concentrations were tested at Weeks 4, 13, and 22.

Stability testing (study [redacted] (b) (4)) initially showed that the test article at 300 mg/mL (duplicate samples) failed 14-day stability testing, with a bias of [redacted] (b) (4)%. An "out-of-specification" investigation was initiated by the sponsor, and the same samples were measured on Day 15, resulting in a bias of [redacted] (b) (4)%. The sponsor averaged the measurements on Day 14 and Day 15 in the study report, which resulted in a bias of -14%. Although this is not optimal, it is reasonably acceptable.

Testing for homogeneity showed that all five concentrations of formulation taken from the top, middle and bottom of the preparation vessel and dosing containers were within 15% of the target concentration.

Reference ID: 3788692

All four control samples had no detectable test article. Concentrations of test article formulations were within 15% of the target concentration, with the exception of the 6 mg/mL Week 13 sample which tested at 84% of the target. The backup 6 mg/mL Week 13 sample, however, tested within 97% of the target. This is acceptable.

5 Integrated Summary and Safety Evaluation

Novartis has submitted a 505 (b) (1) application for Seebri Neohaler glycopyrrolate (GP; NVA237) 15.6 mcg BID under NDA 207923 for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema. Seebri Neohaler is a dry powder inhaler that delivers a dry powder of GP in magnesium stearate and lactose monohydrate.

Under NDA 207930, Novartis has submitted a 505 (b) (1) application for 15.6 mcg GP/27.5 mcg Indacaterol for inhalation via the Neohaler BID for the long-term, maintenance treatment of airflow obstruction in patients with COPD, including chronic bronchitis and/or emphysema.

Two-year rat carcinogenicity and 26-week Tg.rasH2 transgenic mouse carcinogenicity studies assessing NVA237 are included in this review. Special protocol assessments were conducted under IND 48655 for the rat carcinogenicity protocol (ECAC minutes October 5, 2006), and the mouse carcinogenicity protocol (ECAC minutes February 24, 2010).

NVA237 was negative in genetic toxicology testing based on results from the *in vitro* bacterial reverse mutation, the human peripheral lymphocyte chromosomal aberration assay, and the *in vivo* rat micronucleus assay.

The sponsor conducted a 2-year Wistar rat bioassay rats (50 animals/sex/group). Animals received NVA237 by inhalation (nose-only) at doses of 0 (air, on loading rack in restraint tubes in separate room), 0 (air, rotated on a flow through chamber), 0 (vehicle: 1% magnesium stearate and 99% lactose monohydrate), 0.07, 0.21, or 0.56 mg/kg/day through 60 minute exposures (estimated achieved doses, quaternary ammonium salt), consistent with doses recommended by the ECAC. Treatment with NVA237 was not associated with a dose-dependent increase in mortality. Males developed a dose dependent decrease in body weight (LD 95%, MD 93%, HD 85%), compared to vehicle controls. HD females had decreased body weight (91%), compared to vehicle controls. There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis. Thus, NVA237 was determined to not be tumorigenic in male or female Wistar rats.

Test article related non-neoplastic findings in rats were identified in the nasal cavity/sinuses, larynx, lungs, and tracheobronchial lymph nodes. Findings in the nasal cavity/sinuses were not deemed clinically relevant as rats are obligate nose breathers, and humans will be dosed via oral inhalation. Squamous metaplasia in the larynx was a result of irritation, and not considered a clinically relevant finding because of rat-specific

Reference ID: 3788692

anatomy/physiology. In the lung, epithelial hypertrophy was noted at the bronchioloalveolar junction, with a dose dependent increase in incidence and severity. Hypertrophic epithelia were noted to have eosinophilic inclusions. Macrophage accumulation (all types) was increased with a dose dependent increase in incidence in females, and a dose dependent increase in severity in both sexes. Pigment deposits were noted in the tracheobronchial lymph nodes in HD males and MD/HD females. A dose dependent increase in anterior cortical subcapsular lens opacities was observed in both male and female rats. These findings should be listed in the product label in Section 13.2. Dose dependent pupil dilation was noted in males; mydriasis has been observed with other muscarinic acetylcholine receptor antagonists. Systemic exposure (AUC) to NVA237 was similar in both genders at all dose groups, with slight accumulation at Week 52. Exposure was dose proportional between the LD and MD, but was slightly less than dose proportional between the MD and HD. Based on dose dependent epithelial hypertrophy at all doses, no NOAEL was established. However, extensive clinical data is available from Phase 3 clinical trials and suggests that lung findings in rats are not predictive of human response.

In the 26-week oral gavage carcinogenicity study in Tg.rasH2 mice (25 animals/sex for NVA237 dosed groups), males received NVA237at 0 (vehicle: Deionized water), 12.5, 31.3, and 93.8 mg/kg/day and females received NVA237 at 0 (vehicle), 12.5, 37.5, and 125.1 mg/kg/day, based on the concentration of the quaternary ammonium salt. Positive control mice were administered N-methyl-N-nitrosourea (MNU; 75 mg/kg) by a single intraperitoneal injection on Day 1. There were no effects on survival in NVA237 treated animals, based on a lack of dose response and overall low incidences of mortality. MNU-treated Tg.rasH2 animals had increased mortality (males 15/25, females 5/25), compared to control Tg.rasH2 animals (males 0/25, females 0/25). Clinical signs in males and females included hunched posture. Lethargy was noted in HD females, and 2-3 males at all doses. Tg.rasH2 mice treated with NVA237 showed a dose dependent decrease in body weight (males: LD 95%, MD 90%, HD 82%; females: MD 98%, HD 93%). Wild-type mice treated with NVA237 also developed decreased body weight (males 75%, females 86%). Transient decreases in food consumption were noted in HD Tg.rasH2 and WT animals. There were no neoplastic lesions that had a clear dose-dependent relationship with NVA237 in Tg.rasH2 mice. There were no treatment-related neoplastic findings based on the lack of statistical significance for both trend and pair-wise statistical analysis in Tg.rasH2 dosed up to 93.8 and 125.1 mg/kg/day in males and females, respectively.

NVA237-related nonneoplastic lesions in mice were identified in the fore-stomach/limiting ridge, and the liver. Findings in the fore-stomach were determined to be not relevant to human. In the liver, focal and multifocal necrosis was observed at the HD (males 3/25, females 3/25, up to Grade 2) and was not observed in control animals. A clarification was requested in an Information Request sent on May 11, 2015, and the sponsor confirmed that focal and multifocal necrosis were pooled, and did not provide information to separate the findings based on distribution. Liver atrophy (Grade 3) was noted in 3 females (1/25 LD, 2/25 HD) and 1/25 MD male, and was not noted in control animals. The severity of the liver atrophy is a concern, and it is unclear if there is a

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NOAEL for this finding. Published historical control data do not show any incidence of liver atrophy, although the data from Japan may not be optimal[6]. The clinical significance of these low incidence findings is unclear. Glycogen increase was noted in females (control 0/0, LD 1/25, MD 0/0, HD 3/25), but this is not considered a dose-limiting finding. Exposure to NVA237 was not consistently shown in transgenic animals. NVA237 has previously been shown to have low bioavailability in rats (study 1000618, 0.82%). Due to the inconsistent exposure data in Tg.rasH2 animals, the metabolite CJL603 was examined. Mice had notably higher concentrations of CJL603 compared to NVA237 (1800 to 13000-fold increase). At several points, CJL603 was not detected in all tested animals. Systemic exposure (AUC) for CJL603 increased in a roughly dose proportional manner. CJL603 remained detectable at 24 hrs post dose.

In conclusion, NVA237 was determined to not be tumorigenic based on evaluation in Wistar rats and Tg.rasH2 mice.

Exposure ratios for clinical exposures of 15.6 mcg/day BID of NVA237 are shown in the table below. Exposure margins achieved, based on the high-dose in the rat carcinogenicity study, provide safety margins greater than 300-fold over clinical exposure in COPD patients. It should be noted that there was no NOAEL in the rat for nonneoplastic lung findings, but clinical date is available from Phase 3 trials to address human lung findings. Target organs identified in the rat (lung, eye) and mouse (liver) studies were discussed with the clinical team of Drs. Erika Torjusen and Banu Karimi-Shah.

Table 27: Exposure ratios for clinical exposure to glycopyrrolate

		Proposed clinical dose (15.6 mcg BID)
Achieved dose (mg/kg; salt)	AUC (ng*h/mL)	AUC = 0.111 ng*h/mL #
0.07	8.19	73
0.21	22.15	200
0.56	36.45	330

based on study # CQVA149A2107, discussion with Clinical Pharmacologist Dr. Lei He on May 15, 2015

6 Appendix/Attachments

1) ECAC minutes October 5, 2006, rat carcinogenicity protocol
2) ECAC minutes February 24, 2010, mouse carcinogenicity protocol

[6] Kanno *et al*. Historical Background data in CB6F1-Tg-rasH2 mice and CB6F1-nonTg-rasH2 mice over a 26-week experimental period. J Toxicol Pathol 2003: 16:267-274.

Reference ID: 3788692

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: October 5, 2006

To: Bispla Ashiru	**From:** Adele Seifried
Company: Novartis	OND IO
Fax number: (973) 781-2565	**Fax number:** 301-796-9855
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Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 48,655

Total no. of pages including cover: 4

Comments:

Document to be mailed: ❑ YES ☑ NO

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Executive CAC
Date of Meeting: Oct. 3, 2006

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Joseph Contrera, Ph.D., OPS, Member
Abby Jacobs, Ph.D., OND IO, Member
Dan Mellon, Ph.D., DAARP, Alternate Member
Team Leader: C. Joseph Sun, Ph.D., HFD-570

Presenting Reviewer: Lawrence F. Sancilio, Ph.D.

Author of Minutes: Lawrence F. Sancilio, Ph.D.

IND No.: 48,655
Drug Names: Glypyrronium bromide, glycopyrrolate, USP
Sponsor: Novartis Pharmaceutical Corporation

Background

Glycopyrronium bromide has long been marketed as an injectable muscarinic receptor antagonist as a premedication during anesthesia, topically for hyperhidrosis and orally for the treatment of peptic ulcers. It is being developed as an inhalation product in view of its ability to antagonize cholinergic bronchoconstriction and mucus secretion. One of the excipients, magnesium stearate in the drug product, has been only approved for oral use. In the dose selection inhalation toxicity study in rats, the excipient (magnesium stearate) and drug product (and glycopyrronium bromide plus magnesium stearate) were tested.

Rat Carcinogenicity Study Protocol and Dose Selection

For dose selection, a 13-week rat inhalation toxicity study was submitted. The formulation consisted of 8% glycopyrronium bromide, 1% magnesium stearate and 91% lactose. The inhalation doses administered were: 0 (air), 0 (magnesium stearate plus lactose) 0.1 (LD), 0.6 (MD) and 4.0 (HD) mg/kg of glycopyrronium bromide. In the vehicle control group, the dose of magnesium stearate was 0.5 mg/kg, equivalent to the dose in the HD glycopyrronium bromide group. There was a dose related decrease in body weight gained at the MD (male, 15%; female, 15%) and HD (male, 32%; female, 27%). Histopathology was observed in the nasal cavity/sinuses involving the respiratory and olfactory epithelium and goblet cells, the Hardarian gland and larynx. No changes were seen in the lower respiratory tract, and the vehicle, magnesium stearate, was not toxic. From this study, the sponsor proposed inhalation doses of (b) (4) mg/kg for the rat carcinogenicity assay.

Executive CAC Recommendations and Conclusions:

The Committee concluded that the inhalation maximum tolerated dose (MTD) in the 13-week inhalation study in rats was 0.6 mg/kg based on decreased body weight gained. The recommended inhalation doses in the 2-year carcinogenicity are 0.06, 0.2 and 0.6 mg/kg.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\

/Division File, DPAP
/Team leader, Joseph C. Sun, Ph.D., DPAP
/Reviewer, Lawrence F. Sancilio, Ph.D. DPAP
/CSO/PM, M. Raggio, DPAP
/ASeifried, OND IO

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/s/

David Jacobson-Kram
10/5/2006 11:33:03 AM

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: February 24, 2010

To: Arlene McLeer	**From:** Adele Seifried
Company: Novartis	OND IO
Fax number: (973) 781-2565	**Fax number:** 301-796-9855
Phone number: (862) 778-1159	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 48,655

Total no. of pages including cover: 5

Comments:

Document to be mailed: ❑ YES ☑ NO

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Executive CAC
Date of Meeting: February 23, 2010

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Molly Shea, Ph.D., DPAP, Supervisor
Lawrence Sancilio, Ph.D., DPAP, Presenting Reviewer

Author of Draft: Lawrence Sancilio, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the carcinogen bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND # 48,655
Drug Name: Glycopyrronium Bromide (NVA237)
Sponsor: Novartis

Background: Glycopyrronium bromide is a quaternary ammonium antimuscarinic compound that is being developed for the treatment of COPD administered by the inhaled route. The proposed daily inhalation dose is [redacted] (b) (4)).

There are no ADME data in mice. Glycopyrronium bromide protein binding capacity was low: humans, 27-49%; dogs, 25-29%; rats, 27-30%. In humans, the oral and inhalation bioavailability was low, ≤ 7% and approximately 10%, respectively. No oral absorption studies were reported in animals although the quaternary ammonium structure of glycopyrronium bromide makes the systemic absorption from the gastrointestinal tract slow and variable. In mice, glycopyrronium bromide is metabolized via hydroxylation and oxidation. In rats, it was excreted predominantly (95.8%) unchanged in the feces, and in humans, glycopyrronium bromide was excreted virtually unchanged in the urine.

Glycopyrronium bromide was negative in the in vitro bacterial reverse mutation, human Pperipheral lymphocyte chromosomal aberration assay and in the in vivo rat micronucleus assay.

The sponsor sought concurrence for dose selection for the TgRasH2 mouse assay.

Mouse Carcinogenicity Study Protocol and Dose Selection

The sponsor proposed a 26-Week TgRasH2 assay in CByB6F1-Tg(HRAS)2Jic and wild type CB6F1/TgrasH2 by oral gavage at doses of 0 (vehicle-deionized water), 5, 15, and 50 mg/kg/day. A positive control, N-methyl-N-nitrosourea (MNU) will be administered as a single intraperitoneal dose of 75 mg/kg. Additionally, the sponsor wants to compare the activity of the 50 mg/kg dose in the CByB6F1-Tg(HRAS)2Jic and wild-type CB6F1/Tgras H2 mice

The proposed oral doses of up to (b) (4) mg/kg/day were based on two 4-week oral toxicity studies in wild-type CB6F1 mice. In the first study, no deaths, no clinical signs and no drug-related histopathology were observed at doses of 30, 75, and 150 mg/kg, in both sexes. The lack of toxicity at 150 mg/kg prompted the sponsor to conduct a second study in which the initial oral doses were 300 and 750 mg/kg in both sexes. Lethality was observed at all doses tested (≥ 225 mg/kg) in wild-type CB6F1 mice. After a single dose of 750 mg/kg, severe toxicity occurred on day 2 and deaths occurred within 2-5 days; survivors were euthanized on day 7. A week after the initial dosing, oral doses of 500 mg/kg were administered to females and 225 mg/kg to males. After 5 daily doses to females, severe toxicity and deaths occurred; dosing was stopped and the animals were sacrificed on day 6. These animals showed decreased activity, prostration, labored and shallow breathing, hunched posture, rigidity upon handling, little or no feces, urine stain, apparent hypothermia, dehydration, and ptosis. During the 4-weeks, 3 males died in the 225 mg/kg (males) and 2 females in the 300 mg/kg groups. The survivors were dosed daily until termination of the study on day 29. The males (225 and 300 mg/kg) showed decreased fecal output and ptosis while the females (300 mg/kg) showed no clinical signs, apparently due to the higher exposure in males.

The sponsor selected the high dose of (b) (4) mg/kg as the MTD for both sexes based on the steep dose response for mortality. The sponsor believed that survival over 26-weeks administration was doubtful at higher doses.

The MTD from the second 4-week study was exceeded for males at 225 mg/kg and females at 300 mg/kg/day as deaths were observed at these doses.

Executive CAC Recommendations and Conclusions:

- The Committee recommended doses of 0, 10, 25, and 75 mg/kg/day in males and 0, 10, 30, and 100 mg/kg/day in females, by oral gavage, based on MTD (mortality at 225 mg/kg/day in males and 300 mg/kg/day in females).
- The Committee noted that it is not necessary to measure organ weights at the end of the study since organ weights are not required in a carcinogenicity study.
- For transgenic mouse studies, the sponsor should conduct histopathological examination in all dose groups.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
IND 48655/Division File, DPAP
SheaM/Team leader, DPAP
SancilioL/Reviewer, DPAP
RaggioM/PM, DPAP
/SeifriedA, OND IO

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-48655	ORIG-1	NOVARTIS PHARMACEUTICALS CORP	NVA237 GLYCOPYRRONIUM BROMIDE

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/s/

DAVID JACOBSON KRAM
02/24/2010

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/s/

JANE J SOHN
07/07/2015

TIMOTHY W ROBISON
07/07/2015
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 207930 **Applicant:** Novartis **Stamp Date:** Dec 29, 2014

Drug Name: Glycopyrrolate/Indacaterol Inhalation Powder Hard Capsules **NDA Type:** 505(b)(1)

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		Yes. Studies are submitted in eCTD format.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		Yes.
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		Yes.
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		Yes.
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		The proposed clinical formulation and the formulation used in the pivotal 13 week dog inhalation study, and rat embryofetal development study are similar. The excipients are lactose and magnesium stearate. Animals were not administered the API using the capsule shell used in the clinical device.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		Yes. All pivotal studies are via inhalation.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		Yes. The sponsor stated in their written summary that all pivotal toxicology studies were carried out in compliance with GLP regulations.

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		No additional studies were recommended based on minutes from the pre-NDA meeting.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		The proposal labeling is in the PLR format. The labeling is not in the PLLR format.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			To be determined in consultation with the reviewing chemist.
11	Has the applicant addressed any abuse potential issues in the submission?			There appear to be no issues regarding abuse potential.
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.
None.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.
None.

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

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/s/

JANE J SOHN
02/19/2015

TIMOTHY W ROBISON
02/19/2015
I concur