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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 22-383
Supporting document/s:	SD-30, SD-31 and SD-34
Applicant's letter date:	September 28, 2010 November 19, 2010 and December 15, 2010
CDER stamp date:	October1, 2010, November 19, 2010 and December 15, 2010
Product:	Arcapta Neohaler (Indacaterol Maleate Inhalation Powder)
Indication:	Long-term, once daily maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD) including chronic bronchitis and/or emphysema.
Applicant:	Novartis Pharmaceuticals Corporation
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products
Reviewer:	Virgil Whitehurst, Ph.D.
Supervisor/Team Leader:	Molly Topper. Ph.D.
Division Director:	Badrul Chowdhury, MD, Ph.D.
Project Manager:	Carol Hill

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Arcapta Neohaler (Indacaterol Maleate Inhalation Powder) is a once daily maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD) including bronchitis and/or emphysema. The proposed doses are 150 and 75 mcg administered daily by inhalation. The objectives of these submissions are to provide a complete response to the Division's letter dated October 16, 2010 and to provide revised labeling in compliance with the Content and Format of the Physician's Labeling Rule (21CFR201.56 and 201.57).

1.2 Brief Discussion of Nonclinical Findings

Inhalation toxicity studies were conducted in rats (up to 26 weeks) and dogs (up to 39 weeks) to delineate the target organ toxicity for indacaterol (QAB149). 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).

1.3 Recommendations

1.3.1 Approvability

Approval is recommended from the nonclinical perspective.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The proposed label for Arcapta Neohaler (Indacaterol Maleate Inhalation Powder was generally in compliance with the Content and Format of the Physician's Labeling Rule (21 CFR 201.56 and 201.57). It included appropriate sections and headings such as Sections 8.1 (Pregnancy), 8.3 (Nursing mothers), 10 (Overdosage), 12.1 (Mechanism of action), 13.1 (Carcinogenicity, Mutagenesis, impairment of Fertility) and 13.2 (Animal Toxicology and Pharmacology). Section 8.1 was amended in order to include human experience at the beginning of the teratogenic effects and to amend the nonclinical reproductive toxicology data in terms of dose multiples of animals to human. Minor changes were made in human experience (Section 8.3- Nursing Mothers). No changes were made in Sections 8.2 and 10. A minor change (word change) was made in Section 11. Section 12.1 was revised for clarification of the mechanism of action and Sections 13.1 was revised and updated to provide the animal to human dose ratios. The dose

ratios were determined for the 75 mcg dose only (refer to clinical review). Underlines and strikeouts indicate additions and deletions in the label. Section 13.2 was deleted.

From a nonclinical perspective, the nonclinical sections of the labeling for Arcapta Neohaler are approvable pending acceptance by the sponsor of the recommended changes to the label text.

2 Drug Information

2.1 Drug: Arcapta Neohaler (Indacaterol Maleate Inhalation Powder)

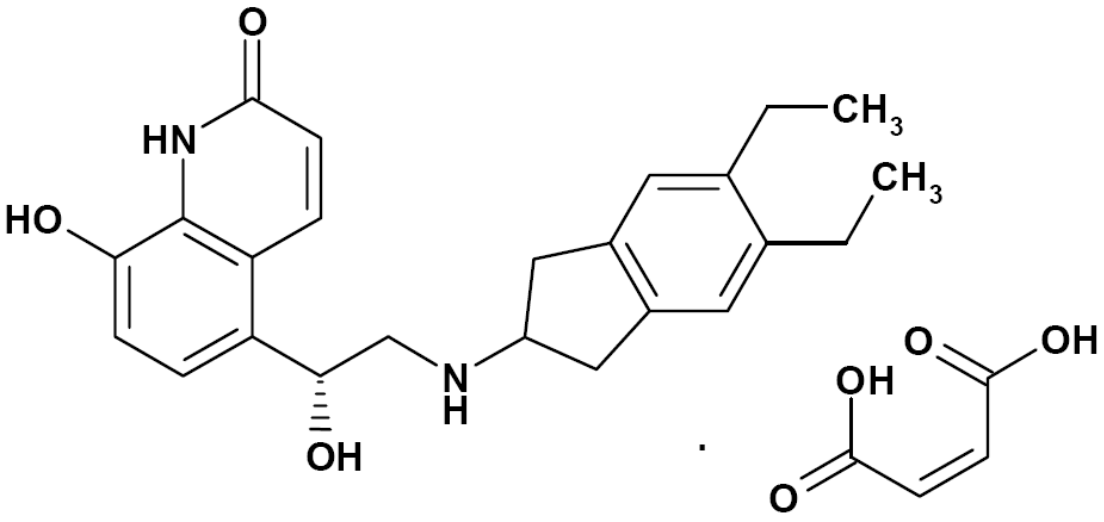
Generic Name: Indacaterol maleate inhalation powder

Code Name: QAB149

Chemical Name: (R)-5-[2-(5, 6-Diethylindan-2-ylamino)-1-hydroxyethyl]-8-hydroxy-1H-quinolin-2-one maleate

Molecular Formula/Molecular Weight: $C_{24}H_{28}N_2O_3 \bullet C_4H_4O_4$/508.76

Structure or Biochemical Description:



Pharmacologic Class: Long-acting beta 2 adrenergic agonist

2.2 Relevant INDs, NDAs, and DMFs:

The following submissions are Novartis' that are referenced in this review: IND 48, 649 (lactose), IND 66,337 (JFA), IND 69, 754 (b) (4) (b) (4) and NDA 22383 review of original submission.

2.3 Drug Formulation

(b) (4)

2.4 Comments on Novel Excipients;

There are no novel excipients in the formulation for Arcapta Neohaler

2.5 Comments on Impurities/Degradants of Concern

The proposed specifications for the heavy metals and (b) (4) in the drug substance, as well as impurities, in the drug product for Arcapta Neohaler (Indercaterol Maleate Inhalation) Powder are acceptable. The proposed limits for the inhalable particulate matter in the drug product are also acceptable (see pharmacology review dated March 12, 2009).

The sponsor agree to reassess and revise, as appropriate, the acceptance criteria for lactose impurities, once a sufficient number of batches (e.g., ≥ten) are tested using the new reporting limit of (b) (4) Propose acceptance criteria are reflective of the data obtained. The limited data for three batches of lactose provided thus far do not support the permissive limit of up to (b) (4) total impurities in lactose, with all having less than (b) (4) totals, The sponsor commits to "reassess and revise, as appropriate, the acceptance criteria for lactose impurities, once fifteen (15) additional batches of lactose monohydrate are evaluated using the new reporting limit of (b) (4) (see Chemistry review dated December 10, 2010).

2.6 Proposed Clinical Population and Dosing Regimen

Long-term, once daily maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and/or emphysema. The sponsor proposes daily doses of 75 and 150 mcg. The Division has concluded (temporarily) that the daily dose should be 75 mcg pending the decision of the Advisory Committee.

2.7 Regulatory Background

December 15, 2008, Novartis submitted a 505(b)(1) NDA application for use of Arcapta Neohaler (indacaterol maleate 150 mcg and 300 mcg dry powders for oral inhalation) for once daily maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema. The proposed dose was one inhalation of a 150 mcg once daily; with a qualifier that administration of a 300 mcg once daily has been shown to provide additional clinical benefit in some patients. A major discussion point in the review for Arcapta Neohaler was the safety of indacaterol, and the dose and dosing frequency proposed for marketing.

Novartis studied three different inhalation indacaterol products. These were the single dose dry powder inhaler (IND 48, 649), an HFA propelled inhalation aerosol (IND 66,337), and a multi-dose dry powder inhaler using the Certihaler device (IND 69,754). IND 48,649 was submitted on February 13, 2004, and IND 69,754 was submitted on April 27, 2004, both to study persistent asthma. An end-of-phase 2 meeting was held on August 1, 2005, to discuss the development of indacaterol multi-dose dry powder product for asthma and COPD. Most of the questions and ensuing discussions were on the asthma program. Novartis later suspended the development of the HFA propelled inhalation aerosol product due to technical reasons. The multi-dose dry powder inhaler using the Certihaler device was also suspended because of a Certihaler device related problem regarding possible excessive delivery of dose. With the suspension of these preferred delivery devices, which would allow multiple dose products, the single-dose dry powder product was continued. A second end-of-phase 2 meeting was held on October 10, 2006, to discuss the development of indacaterol single-dose dry powder product for COPD. There was some discussion on asthma, but most of the questions and ensuing discussions were on COPD. The application proposed a COPD study , with an adaptive design to build dose ranging assessment and determination into a pivotal efficacy and safety study. The Division cautioned that initiating such phase 3 studies was risky when using a single-dose dry powder product with limited prior information and Agency review of relevant data. On December 20, 2006, Novartis submitted the COPD study with adaptive design for Special Protocol Assessment (SPA). In a letter dated February 1, 2007, the Division expressed various concerns with the study, such as the role of the data monitoring committee (DMC), use of open-label tiotropium as an active comparator, selection of the no inferiority margin to compare to tiotropium, definition of secondary endpoint of days of COPD exacerbation, and emphasis on trough FEV1 as dose selection criterion. On
February 21, 2007, Novartis submitted questions in a Type A meeting request to seek clarification on the Division's response to SPA questions. On March 12, 2007, the

Division sent responses to Novartis's clarification question in preparation for the meeting. Upon receiving the Division's response, Novartis cancelled the Type A meeting. While several discussions occurred between the Division and Novartis on the study, there were no formal SPA agreements. There were no agreements on dose selection criteria.

As mentioned above, the sponsor submitted the original NDA application on December 15, 2008. A review of the nonclinical study data which included inhalation toxicity studies of 26 weeks in rats and 39 weeks in dogs showed that the target organs were nasal cavity and larynx and the cardiovascular system in the dog. Studies addressing genotoxicity, reproductive toxicity, and carcinogenicity did not show any findings of concern. All genotoxicity studies were negative. The drug was approvable from the nonclinical perspective. However, the clinical review of the NDA application identified 3 questions, dose selection, dosing frequency and efficacy advantage of 300 mcg over 150 mcg. The NDA application was not approved and the Division's concerns with dose and dose frequency were included in a letter to the sponsor dated October 16, 2009. The sponsor submitted a complete response to these deficiencies dated September 28, 2010.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology (Secondary Pharmacodynamics)

Effect of Beta-2 Adrenoceptor Agonist, NVP-QAB149 (Indacaterol), on the Guinea Pig Vagal Sensatory Neurons. Comparison with Formoterol**.**

Effect of Indacaterol (NVP-QAB-149) on Depolarization of Isolated Guinea Pig Vagus Nerve

Pharmacokinetics:

Evaluation of QAB149 as a Inducer of Drug Metabolizing Enzymes and Transporters in Primary Human Hepatocytes (0900287)

Assessment of QAB149 as a Inhibitor of Human Breast Cancer Resistance Protein (BCRP), P-Glycoprotein (P-gp) and Multidrug Resistance-Associated Protein 2 (MRP2)(0900394)

Assessment of QAB149 as a Inhibitor of Human Organic Cation Transporters OCT1, OCT2, MATE1 and Mate2K (0900759)

Genotoxicity studies:
Mutagenicity Test Using Salmonella typhimurium (b) (4) (0870486)

Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes (1463/235)

3.3 Previous Reviews Referenced

Pharmacology review dated August 25, 2009 and Chemistry consult review dated March 12, 2009

4 Pharmacology

4.1 Primary Pharmacology;

There were no primary pharmacology studies submitted in this NDA submission. The primary pharmacology studies were reviewed in the pharmacology reviews for NDA 22-383 dated August 25, 2009, IND 66, 337 dated May 6, 2008 and IND 48, 649 dated April 27, 2004.

4.2 Secondary Pharmacology:

The secondary pharmacology studies were reviewed in the pharmacology reviews for NDA 22-383 dated August 25, 2009, IND 66, 337 dated May 6, 2008 and IND 48, 649 dated April 27, 2004. Two additional secondary pharmacology studies were submitted in this complete response and are reviewed below.

Effect of Beta-2 Adrenoceptor Agonist, NVP-QAB149 (Indacaterol), on the Guinea Pig Vagal Sensatory Neurons. Comparison with Formoterol (RD - 2009-00344)

Inhaled indacaterol has been shown to cause a transient cough in patients. A previous pharmacology study showed that indacaterol at large concentrations, 100 μM and above stimulated a subset of C- fibers innervating lungs in guinea pigs. The purpose of this study was to investigate the possible mechanism by which indacaterol can lead to vagal C-fiber activation indacaterol and formoterol were investigated in extracellular recordings from vagal nodose sensory C-fibers projecting into guinea pig and mouse lungs. Additionally, repeated extracellular recordings of individual vagal C-fiber endings were conducted using guinea pig and mouse lungs. The trachea and lungs with intact right side extrinsic vagal innervation (including right jugular and nodose ganglia) were cut out. The lung and trachea tissues were exposed to indacaterol, formoterol and capsaicin (control) via a gravity-fed perfusion system at a rate of ~ 8 ml/min. Results of these studies show that the guinea pig and mouse vagal C-fiber models are not appropriate to determine the mechanism of action by which indacaterol induces transient cough.

Effect of Indacaterol (NVP-QAB-149) on Depolarization of Isolated Guinea Pig Vagus Nerve (RD 2009-00251)

Inhaled indacaterol has been shown to cause a transient cough in patients. A previous pharmacology study showed that indacaterol at large concentrations, 100 µM and above stimulated a subset of C- fibers innervating lungs in guinea pigs. Additionally, a recent study in the rat shows that activation of the beta 3 adrenoceptor can sensitize the cough response to other stimuli. It has also been shown that indacaterol in micromolar concentrations is able to activate the beta 3 adrenoceptor. This study conducted in the guinea pig was to investigate whether high levels of indacaterol could increase vagal sensitivity and eliminate the cough. Male, guinea pigs were sacrificed and segments of the vagus nerve caudal to the nodose ganglion were removed, the nerve was cleared of connective tissues, attached to a narrow channel and placed in a water bath. Indacaterol, formoterol and salmeterol were perfused individually into the water bath using concentration of 100 -300 µM. Indacaterol (300 µM), salmeterol (300 µM) and formoterol (100 µM) did not cause vagal sensitivity.

4.3 Safety Pharmacology:

There were no safety pharmacology studies submitted in this NDA submission. The safety pharmacology studies were reviewed in the pharmacology reviews for NDA 22-383 dated August 25, 2009, IND 66, 337 dated May 6, 2008 and IND 48, 649 dated April 27, 2004.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

PK/ADME studies were reviewed in the pharmacology reviews for NDA 22-383 dated August 25, 2009, IND 66, 337 dated July 3, 2003 and IND 48, 649 dated April 27, 2004. Three PK/ADME studies were submitted in this NDA submission and are reviewed below.

1. Evaluation of QAB149 as a Inducer of Drug Metabolizing Enzymes and Transporters in Primary Human Hepatocytes (0900287)
Indacaterol (QAB149) was examined for its potential to induce cytochrome P450 (CYP) enzymes and UDP-glucuronosyltransferase (UGT), UGT1A1 mRNA and their activities as well as mRNAs of P-glycoprotein (P-gp, ABCB1) and the multidrug resistance-associated protein-2 (MRP2,ABCC2) transporters in primary human hepatocytes. Hepatocytes were from three individual donors after 48 h of treatment. Human primary hepatocytes were prepared and treated with indacaterol concentrations of 0.0005, 0.005 and 0.05 µM. The 0.05 µM concentration is approximately 10-fold greater than the higher concentration anticipated in humans. Indacaterol was not an inducer of CYP enzymes and UGT1A1, as well as P-gp or MRP2 mRNAs *in vitro.*

2. **Assessment of QAB149 as a Inhibitor of Human Breast Cancer Resistance Protein (BCRP), P-Glycoprotein (P-gp) and Multidrug Resistance-Associated Protein 2 (MRP2)(0900394)**
The breast cancer resistance protein (BCRP), P-glycoprotein (P-gp) and the multidrug

resistance-associated protein 2 (MRP2) are ATP-dependent drug efflux transporters (ABC transporters) that are expressed in the intestine, liver and kidney. ABC transporters have the potential to influence the body distribution and elimination of the numerous therapeutic drugs they handle. Because of their broad substrate specificity, they may be a potential site of harmful drug-drug interactions . The objective of this study was to examine the potential of indacaterol (QAB149) to inhibit BCRP, P-gp and MRP2 transport activity in mammalian cells over expressing the respective transporters (BCRP, T8 cells; P-gp, MDA435 T0.3 cells; MRP2, MDCKII cells). The potential of indacaterol to inhibit BCRP- and P-gp-mediated efflux activity was assessed with flow cytometry using the fluorescent Bodipy FL prazosin (BDP) and Rhodamine 123 (Rho123) as probe substrates of BCRP and P-gp, respectively. The concentrations of indacaterol used in these studies were 0.1-50 µ.The results of these studies show that indacaterol in concentrations up to 50 µM probably will not inhibit BCRP, P-gp or MRP2.

3. Assessment of QAB149 as a Inhibitor of Human Organic Cation Transporters OCT1, OCT2, MATE1 and Mate2K (0900759).

The human orthologs of organic cation transporter 1 (hOCT1), organic cation transporter 2 (hOCT2), the multidrug and toxin extrusion transporter 1 (hMATE1) and the multidrug and toxin extrusion transporter 2K (hMATE2K) transport numerous relatively small organic compounds (<500 Da) that carry a net positive charge (i.e., organic cations). Human OCT2, hMATE1 and hMATE2K are expressed in the human kidney, whereas hOCT1 and hMATE1are expressed in the human liver. Renal tubular secretion of many organic cations involves hOCT2-mediated uptake across the basolateral membrane of tubule cells and hMATE1 and/or hMATE2K efflux across apical membranes. Hepatobiliary organic cation secretion involves hOCT1-mediated uptake across the sinusoidal membrane of hepatocytes and hMATE1 efflux across canalicular membranes. The objective of this study was to examine whether indacaterol (QAB149) can inhibit transport activity of hOCT1, hOCT2, hMATE1 and/or hMATE2K in human embryonic kidney cell strain # 293. Indacaterol concentration up to 5 µM inhibited hOCT1- and hOCT2-mediated transport by 26% and 19%, respectively. Indacaterol in concentrations 1- 50 µM inhibited hMATE1 and TE2K in a concentration related manner from 52 to 99%.

5.2 Toxicokinetics

No toxicokinetic studies were submitted in this NDA submission. The toxicokinetic studies for indacaterol were reviewed in the pharmacology reviews for NDA 22-383 dated August 25, 2009, IND 66, 337 dated May 6, 2008 and IND 48, 649 dated April 27, 2004.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicity studies were submitted in this NDA submission. The single dose toxicity studies with indacaterol were reviewed in IND 66, 337 (Pharmacology review dated May 6, 2008).

6.2 Repeat-Dose Toxicity

No repeat –dose studies were submitted in this NDA submission. The pivotal repeat-dose studies for indacaterol 26 week in the rat and 39 week in the dog were reviewed in the Pharmacology review dated August 25, 2009 and are crossed referenced for this review.

7 Genetic Toxicology

The genetic toxicology studies for indacaterol, Ames, chromosomal aberration and micronucleus test in the rat were reviewed in the Pharmacology review dated May 6, 2008 and is cross referenced for this review. Additionally, two genetic toxicology studies were submitted and are reviewed below.

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

Study title: Mutagenicity Test Using Salmonella typhimurium (b) (4) (b) (4)

Study no.:	0870486
Study report location:	Electronic
Conducting laboratory and location:	Genetic Toxicology and Safety Pharmacology and PCS Operations, Novartis Pharma AG, Basel Switzerland
Date of study initiation:	September 17, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Indacaterol with 0.33% (b) (4) (b) (4)/C0006/99%

Key Study Findings

- In this study indacaterol (QAB 149) with (b) (4) was evaluated for its mutagenic activity in an assay using Salmonella typhimurium strains TA 1535, TA97a, TA98, TA100 and TA102. QAB149 was dissolved in DMSO and used at concentrations 1.6 to 1000 mcg/plate.
- Treatment with indacaterol did not increase the revertant numbers of any of the bacterial tester strains used.

Methods

Strains:	Salmonella typhimurium strains TA1535, TA97a, TA98, TA100 and TA 102
Concentrations in definitive study:	1.6, 8, 40, 200 and 1000 µg/plate
Basis of concentration selection:	The concentrations for this study were chosen on the basis of the results from Ames Assay, Mutagenicity Test using Salmonella typhimurium (study 001808) and Range finding studies: QAB149 was dissolved in DMSO, first experiment (plate incorporation): 16-10,000 mcg/plate; second experiment (preincubation) 1250-20,000 mcg/plate and third experiment (preincubation) 625-20,000 mcg/plate
Negative control:	DMSO
Positive control:	2-aminoanthracene, Benzo(a)pyrene, sodium azide, 9-aminocridine, 2-nitrofluorene and mitomycin C
Formulation/Vehicle:	Indacaterol with (b) (4)
Incubation & sampling time:	The plates were incubated in a dark incubator, 37 ° C with 50 to 70% humidity. After 3 days, the colonies were counted and the plates were analyzed microscopically for the presence of a light background lawn of growth.

Study Validity:

The study was valid, the positive controls increased the number of revertant colonies as expected and appropriate study methodologies were used.

Results:

Dose ranging plated incorporation test was conducted using indacaterol with (b) (4) of the (b) (4) up to 1000 µg/plate. Bacteriotoxicity induced by indacaterol was noted at 1000 µg/plate with the exception of strains TA 1535, TA 100 and TA 102-S9 which showed bacteriotoxicity at 200 µg/plate. Treatment with indacaterol with the impurity did not increase the revertant number of the bacterial tester strain tested. The results, similar to the results in Ames test # 001808 (reviewed in the Pharmacology reviewed dated May 6, 2008) are shown in the tables below. The sponsor did not submit this study in the original NDA submission because the study results were similar.

Table 5-1 Experiment 1 (plate incorporation)

Strain:		TA1535		TA97a		TA98		TA100		TA102	
S9:		-	+	-	+	-	+	-	+	-	+
Concentration (µg/ plate):	0	26	18	200	198	25	39	106	119	410	402
	1.6	25	18	197	207	27	39	115	118	404	442
	8	20	13	185	187	26	35	114	134	338	438
	40	32	20	185	233	21	48	111	126	429	490
	200	22t	15	195	225	19	61	109t	113	268t	338
	1000	t	t	t	t	t	t	t	t	t	t

t: toxic

The positive controls are shown in the tables below.

Table 2 positive controls (first experiment)

Test/	Control Item	Positive K1: 2-Amino-anthra-cene	Positive K3: 2-Amino-anthra-cene	Positive K4: 9-Amino-acridine	Positive K5: Benzo-(a)pyrene	Positive K6: 2-Nitro-fluorene	Positive K7: Mitomycin C	Positive K8: Sodium azide
Conc	(µg/plate)	3	10	100	3	2	0.5	3
Strain								
TA1535 -S9	Plate 1							1083
	Plate 2							1167
	Plate 3							989
	Mean							1080
	Factor							41.54
TA1535 +S9	Plate 1	382						
	Plate 2	368						
	Plate 3	436						
	Mean	395						
	Factor	21.94						
TA97a -S9	Plate 1			2112				
	Plate 2			1378				
	Plate 3			1785				
	Mean			1758				
	Factor			8.79				
TA97a +S9	Plate 1		3853					
	Plate 2		3970					
	Plate 3		4178					
	Mean		4000					
	Factor		20.2					
TA98 -S9	Plate 1					134		
	Plate 2					112		
	Plate 3					139		
	Mean					128		
	Factor					5.12		

Test/	Control Item	Positive K1: 2-Amino-anthra-cene	Positive K3: 2-Amino-anthra-cene	Positive K4: 9-Amino-acridine	Positive K5: Benzo-(a)pyrene	Positive K6: 2-Nitro-fluorene	Positive K7: Mitomycin C	Positive K8: Sodium azide
Conc	(µg/plate)	3	10	100	3	2	0.5	3
Strain								
TA98 +S9	Plate 1	1340			148			
	Plate 2	1362			164			
	Plate 3	1352			231			
	Mean	1351			181			
	Factor	34.64			4.64			
TA100 -S9	Plate 1							902
	Plate 2							970
	Plate 3							970
	Mean							947
	Factor							8.93
TA100 +S9	Plate 1	1872						
	Plate 2	1877						
	Plate 3	1910						
	Mean	1886						
	Factor	15.85						
TA102 -S9	Plate 1						1781	
	Plate 2						1694	
	Plate 3						1930	
	Mean						1802	
	Factor						4.4	
TA102 +S9	Plate 1		1779					
	Plate 2		2667					
	Plate 3		2012					
	Mean		2153					
	Factor		5.36					

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Induction of Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

Study no.:	0870485
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 1, eoo8
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Indacaterol/C0006/99%

Key Study Findings

- The number of aberrant cells (excluding gaps) was increased at the highest concentration without S-9 (38.00 mcg/mL),at the mid concentration (45.00 mcg/mL) in the presence of S9 incubated for 3 hours in experiment 1 and at the lowest dose, 8.00 mcg/mL minus S-9 incubated for 20 hours. However, the increases in the number of aberrant cells (excluding gaps) were within the historical control range (0-3) and were not statistically significant and were not thought to have biological relevance.
- Precipitation induced by indacaterol was observed in the culture medium at 80 mcg/plate and above.
- Indacaterol did not induce chromosome aberrations in cultured human peripheral blood lymphocytes in the presence and absence of S-9.

Methods

Cell line:	Female human, non-smoking blood samples were used in this study, ages 22-30 years of age
Concentrations in definitive study:	1.0-80 mcg/mL plus and minus S9
Basis of concentration selection:	Results of the cytotoxicity range-finding experiment was used to select maximum concentrations for the main experiments . Concentrations ranged from 7.256-2000 mcg/mL in the presence and absence of S-9.
Negative control:	DMSO
Positive control:	4-Nitroquinoline 1-oxide (NQO), 2.50-5.00 mcg/mL and Cyclophosphamide (CPA), 6.26-12.5 mcg/mL
Formulation/Vehicle:	Indacaterol/DMSO
Incubation & sampling time:	The in vitro assay is designed to detect chemically induced changes in metaphase chromosomes of cells in culture. The cells were incubated with indacaterol using

concentrations of 1-60 mcg/mL in the presence and absence of S9 for 3 hours and 20 hour in the absence of S9, harvested, metaphase spreads were prepared and analyzed for presence or absence of chromosome damage.

Study Validity: The study was valid because the frequency of aberrant cells(excluding gaps) in the vehicle controls were within the laboratory vehicle controls. The positive controls induced clear unequivocal increases in structural aberrations excluding gaps.

Results: The number of aberrant cells (excluding gaps) was increased at the highest concentration without S-9 (38.00 mcg/mL),at the mid concentration (45.00 mcg/mL) in the presence of S9 incubated for 3 hours in experiment 1 and at the lowest dose, 8.00 mcg/mL minus S-9 incubated for 20 hours. However, the increases in the number of aberrant cells (excluding gaps) were within the historical control range (0-3) and were not statistically significant and were not thought to have biological relevance. Precipitation induced by indacaterol was observed in the culture medium at 80 mcg/plate and above. The results of experiments 1 and 2 are shown in the table below.

Table 1:1: Experiment 1, Results summary

Treatment	Concentration (µg/mL)	Cytotoxicity (%)	% Cells with Chromosome Aberrations (Excluding Gaps)	Historical Control Range (%) #	Statistical significance
3+17 hour -S-9	Vehicle[a]	-	1.00	0-3	-
	18.00	12	1.00		NS
	34.00	34	1.50		NS
	38.00	48	3.00		NS
	*NQO, 5.00	ND	24.40		$p \leq 0.001$
3+17 hour +S-9	Vehicle[a]	-	0.00	0-3	-
	30.00	19	0.00		NS
	45.00	30	2.50		NS
	60.00	49	1.50		NS
	*CPA, 12.5	ND	74.00		$p \leq 0.001$

[a] Vehicle control was DMSO
* Positive control
# 95th percentile of the calculated range
NS = not significant
ND = not determined

Table 1:2: Experiment 2, Results summary

Treatment	Concentration (μg/mL)	Cytotoxicity (%)	% Cells with Chromosome Aberrations (Excluding Gaps)	Historical Control Range (%) #	Statistical significance
20+0 hour -S-9	Vehicle[a]	-	0.50	0-3	-
	8.000	2	3.00		NS
	10.00	41	1.50		NS
	11.00	58	1.00		NS
	*NQO, 5.00	ND	41.24		$p \leq 0.001$
3+17 hour +S-9	Vehicle[a]	-	1.50	0-3	-
	20.00	0	1.50		NS
	30.00	16	1.50		NS
	45.00	45	1.00		NS
	50.00	59	1.50		NS
	*CPA, 12.5	ND	62.50		$p \leq 0.001$

[a] Vehicle control was DMSO
* Positive control
# 95th percentile of the calculated range
NS = not significant
ND = not determined

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

As mentioned above, no in vivo clastogenicity assay in rodents (micronucleus assay) was submitted in this NDA submission.

7.4 Other Genetic Toxicity Studies

No other genetic toxicity studies were submitted in this NDA submission.

8 Carcinogenicity:

No carcinogenicity studies were submitted in this NDA submission. Carcinogenicity studies in mice and rats were submitted and reviewed (see pharmacology studies dated August 12, 2009). Carcinogenicity was assessed in a 26 week study in C6F1/TgrasH2 hemizygous mice, and in a 24 month study in Sprague-Dawley rats. These studies showed increased incidences of uterine and endometrial stromal polyps, and ovarian leiomyomas. These tumors have been observed with other beta-2 adrenergic agonists and are known to have no human consequence. These studies were judged to be negative by the Executive CAC.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No fertility and early embryonic development studies were submitted in this NDA submission. The fertility and embryonic development studies with indacaterol were reviewed in the Pharmacology review for NDA 22, 383 dated August 25, 2009 and IND 66,337 dated May 6, 2008. The reproductive toxicity study in rats did not reveal adverse effects on male and female fertility and reproductive performance.

Embryonic Fetal Development

No embryonic fetal development studies were submitted in this NDA submission. The embryonic fetal development studies with indacaterol were reviewed in the Pharmacology review for NDA 22, 383 dated August 25, 2009 and IND 66,337 dated May 6, 2008. Embryo-fetal development studies in rats and rabbits did not show any teratogenic effects. The pregnancy category was determined to be Class C, which is the same category for many other beta-2 adrenergic agonists.

Prenatal and Postnatal Development

No prenatal and postnatal development studies were submitted in this NDA submission. The prenatal and postnatal development studies with indacaterol were reviewed in the Pharmacology review for NDA 22, 383 dated August 25, 2009 and IND 66,337 dated May 6, 2008. Indacaterol increased stillborn pups for F0 and a statistically significant decrease in the number pregnant dams for F1 observed in the high dose group. However, there were no significant treatment related effects on neurobehavioral or other reproductive parameters of the F1 offsprings or the gross appearance of the F2 pups.

10 Special Toxicology Studies

No special toxicology studies were submitted in this NDA application. However a special toxicology study was reviewed in the pharmacology review dated August 25, 2009 and shows that indacaterol at a concentration level of 250 mg/mL (topical dose of 125 mg) was not a sensitizer in the Buehler test in the Guinea Pig for Delayed Skin Sensitization Potential.

11 Integrated Summary and Safety Evaluation

Arcapta Neohaler (Indacaterol Maleate Inhalation Powder) is a long acting β_2 agonist intended for once a day treatment of COPD. The drug will be administered by inhalation. The sponsor is planning to develop2 formulations, an HFA formulation (I66, 337) and a micronized powder formulation (I 48,649).The target organ for this drug is classically the cardiovascular system, i.e., tachycardia, QT-c interval changes and myocardial necrosis. Increased cardiovascular activity with associated histopathological changes was observed in the dog. There was also glycogen deposition in the liver of the dog at all dose levels in repeat dose toxicity studies. The dose-limiting toxicity in the rat

is nasal lesions, characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium. Indacaterol has been administered to human subjects in Europe using single doses up to 2000 mcg. The nonclinical study data for Arcapta Neohaler has been previously reviewed (see Pharmacology review dated August 25, 2009). The NDA application for Arcapta Neohaler was approvable from the nonclinical perspective. However, the clinical review of this NDA application identified 3 questions, dose selection, dosing frequency and efficacy advantage of 300 mcg over 150 mcg. The NDA application was not approved and the Division's concerns with dose and dose frequency were included in a letter to the sponsor dated October 16, 2009. The sponsor submitted a complete response dated September 28, 2010. Two secondary pharmacology studies, three pharmacokinetic studies and two genetic toxicology studies were submitted in the sponsor's complete response to the Division's non approvable letter.

The objective of the secondary pharmacology studies was to determine the mechanism of action for the cough induced in patients treated with inhaled indacaterol. The studies did not delineate the mechanism of action. The pharmacokinetic studies were conducted to determine indacaterol has the potential to inducer changes in breast cancer resistance protein (BCRP), P-glycoprotein (P-gp) and the multidrug resistance-associated protein 2 (MRP2) are ATP-dependent drug efflux transporters (ABC transporters) that are expressed in the intestine, liver and kidney. Indacaterol was not an inducer of CYP enzymes and UGT1A1, as well as P-gp or MRP2 mRNAs *in vitro.* The genetic toxicology studies were an Ames assay to qualify (b) (4) found in indacaterol and a chromosomal aberration assay to determine whether indacaterol was genotoxic. Both the Ames and the chromosomal aberration assays were negative.

Suggested labeling for the nonclinical sections

5 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

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/s/

VIRGIL E WHITEHURST
02/16/2011

MOLLY E TOPPER
02/16/2011
I concur.

INTEROFFICE MEMO

TO: NDA 22-383 Complete Response Resubmission to original NDA submission
ARCAPTA Neohaler® (Indacaterol Maleate Inhalation Powder)

FROM: Molly E. Topper, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Pulmonary, Allergy and Rheumatology Products

DATE: February 28, 2010

Novartis Pharmaceutical Corporation (Novartis) submitted a Complete Response resubmission to their NDA 22-383 on October 1, 2010 for ARCAPTA NEOHALER (Indacaterol Maleate Inhalation Powder) for the long-term, once daily maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD) including chronic bronchitis and/or emphysema. The proposed inhalation doses of 75 and 150 mcg/day were included in the resubmission. The original NDA was submitted December 15, 2008 for the same indication but at inhalation doses of 150 and 300 mcg/day. The NDA was submitted for review under the 505(b)(1) pathway. A complete response action was taken for the original NDA submission on October 16, 2009 due to clinical deficiencies (adverse events including cardiovascular and cerebrovascular events and lack of dose ranging to support the clinically safe and effective doses). There were no nonclinical deficiencies in the original NDA submission.

Dr. Virgil Whitehurst was the primary reviewer for each the original NDA submission and the complete response resubmission. Dr. Timothy Robison completed the review of the carcinogenicity studies submitted in support of the NDA 22-383 application. Dr. Jean Wu was the secondary reviewed of the original submission. Per the original nonclinical reviews, NDA 22-383 was deemed approvable pending a label review from the nonclinical perspective (see nonclinical reviews dated August 25, 2009 and September 9, 2009).

In the complete response resubmission, the proposed clinical doses of 75 mcg and 150 mcg were evaluated for clinical safety and efficacy. These proposed clinical doses are lower than the originally proposed clinical doses (150 mcg and 300 mcg/day). There are no nonclinical safety concerns for the resubmission. Novartis included pharmacology, pharmacokinetic and genetic toxicity studies in their complete response. Dr. Whitehurst completed a review of these studies. Additionally, a review of the Applicant's proposed labeling for the nonclinical sections of the label was completed. The labeling review considered the 75 mcg/day dose for the purposes of labeling. However, the labeling review may be revised pending the Pulmonary Allergy Drugs Advisory Committee (PADAC) Meeting that will be held for Arcapta Neohaler on March 8, 2011. In general, I

concur with Dr. Whitehurst's review of the nonclinical studies submitted in support of NDA 22-383. There are no nonclinical deficiencies for the NDA. The labeling review completed for the complete response resubmission is acceptable with the (b) (4) . Upon secondary review of the data, no nonclinical information supports this statement and therefore, these statements should be omitted from the labeling (strikethrough in red below). Therefore, the labeling should be further edited to remove this finding as below.

Pending the outcome of the PADAC meeting to be held on March 8, 2011 and any additional revisions to the Applicant's proposed labeling, NDA 22-383 is considered approvable from the nonclinical perspective.

(b) (4)

_____
Molly E. Topper, Ph.D.
Pharmacology/Toxicology Supervisor

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/s/

MOLLY E TOPPER
02/28/2011

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology OND IO
NDA: 22-383
Submission date: December 15, 2008
Drug: Indacaterol maleate
Sponsor: Novartis Pharmaceutical Corp.
Indication: Patients with COPD
Reviewing Division: Division of Pulmonary and Allergy Products

Comments:

The pharmacology/toxicology reviewer and supervisor in the Division of Pulmonary and Allergy Products have reviewed the nonclinical information for indacaterol maleate and found it adequate to support approval from a pharm/tox perspective for the indication listed above.

Conclusions:

I read the reviews of the primary reviewer and supervisor and I concur with the Division pharm/tox recommendation that this NDA can be approved and that no additional nonclinical studies are recommended at this time. Labeling issues will be addressed at a later time when necessary.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22383	ORIG-1	NOVARTIS PHARMACEUTICALS CORP	Arcapta Neohaler

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/s/

PAUL C BROWN
10/16/2009

INTEROFFICE MEMO

TO: NDA 22-383
Sequence number/date/type of submission: S000/December 15, 2008/original

FROM: Jean Q Wu, M.D., Ph.D.
Acting Pharmacology/Toxicology Supervisor
Division of Pulmonary and Allergy Products

DATE: September 9, 2009

I concur with pharmacologist's (Dr. Virgil Whitehurst) recommendation that the pharmacology and toxicology of Arcapta (b) (4) (Indacaterol, or QAB149) have been adequately studied and the drug product should be approved pending a labeling review from a nonclinical perspective.

Indacaterol is a long-acting β_2-adrenergic agonist developed for a potential treatment of chronic obstructive pulmonary disease (COPD). It is intended for the oral inhalation route of administration with once daily dose of 150 µg or 300 µg. Indacaterol has been shown to have bronchodilatory activity in various *in vitro* and *in vivo* assays. The mechanism of action for Indacaterol involves the interaction with active sites of the receptor via the membrane lipid bilayer stimulating cyclic AMP formulation and activation of cyclic AMP-dependent protein kinase which promotes phosphorylation and smooth muscle relaxation of the airways.

The pivotal nonclinical inhalation toxicology studies were conducted in rats and dogs ranging from 2 to 39 weeks. The target organs of toxicity for Indacaterol in rats are nasal cavity (degeneration of the olfactory epithelium) and larynx (squamous metaplasia). The nasal lesion and larynx changes observed in rats are considered rat specific and of no human relevance. The target organs of toxicity in dogs are the cardiovascular system (increased heart rates, decreased blood pressure and myocardial necrosis) and the liver (periportal liver hepatocyte vacuolation due to glycogen deposition). The cardiovascular and liver findings in dogs are considered class effects of β_2-adrenergic agonists. The cardiovascular toxicities were observed in several dog studies at systemic exposure levels that are approximately 42-45 times the expected human exposure. There is an adequate margin of safety for the exposure at the proposed human dose of Indacaterol based on the AUCs at NOAELs determined in the toxicity studies (see Dr. Whitehurst's review for details). In addition, the cardiovascular findings were considered monitorable in the clinical setting. The liver hepatocyte vacuolation (periportal) was only observed in dogs and reversible. There were no associated histopathological findings. The potential liver toxicity is not considered to be a significant safety problem in humans.

The carcinogenicity potential of Indacaterol was assessed in a 26 week oral (gavage) carcinogenicity study with C6F1/TgrasH2 hemizygous mice and a 24 month inhalation oncogenicity study with Sprague-Dawley rats. The studies were judged negative by Executive CAC (See Executive CAC Meeting Minutes dated August 4, 2009). However, increased incidences of uterine endometrial stromal polyps in the 26-week oral (gavage) carcinogenicity

study with female CB6F1/TgrasH2 hemizygous mice and ovarian leiomyomas in the 24-month inhalation oncogenicity study with female Sprague-Dawley rats were statistically significant by trend test, but not significant by pairwise comparison. These tumors have been observed with other β_2-adrenergic agonists and might be attributed to treatment despite the lack of statistical significance.

Indacaterol was not genotoxic as assessed by negative results in the *in vitro* assays, Ames and chromosomal aberration (Chinese hamster cells) and in the *in vivo* assay, bone marrow micronucleus (rat).

Indacaterol was not terotogenic in the rat and rabbit embryofetal development studies. Subcutaneous and inhalation administration of Indacaterol to rats did not result in embryo-fetal toxicity, gross malformations or teratogenicity. Subcutaneous administration of Indacaterol to rabbits did not show teratogenic effects but an increase in one skeletal variation in the fetus observed in the high dose rabbits which showed a significant decrease (approximately 50%) in body weight gain. Subcutaneous administration of Indacaterol to rats did not cause significant effects in males or females related to parameters of fertility, general reproductive performance or early embryonic development. In the pre-and postnatal development study in rats subcutaneously administered with Indacaterol, there were increased stillborn pups for F_0 and a statistically significant decrease in the number pregnant dams for F_1 observed in the high dose group. However, there were no significant treatment related effects on neurobehavioral or other reproductive parameters of the F_1 offsprings or the gross appearance of the F_2 pups.

The proposed specifications for the impurities in the drug substance for Indacaterol were evaluated and considered acceptable by Dr. Whitehurst in a separate Chemistry Consultation review dated March 12, 2009.

The labeling review for the current submission is deferred to a later time when a labeling negotiation is needed.

_____

Jean Q Wu, M.D., Ph.D.
Acting Pharmacology/Toxicology Supervisor

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22383	ORIG-1	NOVARTIS PHARMACEUTICALS CORP	INDACATEROL

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/s/

JEAN Q WU
09/09/2009

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-383
SERIAL NUMBER:	001
DATE RECEIVED BY CENTER:	December 15, 2008
PRODUCT:	Arcapta (b) (4) (proposed) Indacaterol Maleate Inhalation Powder
INTENDED CLINICAL POPULATION:	Patients with COPD
SPONSOR:	Norvatis Pharmaceutical Corporation
DOCUMENTS REVIEWED:	**1**
REVIEW DIVISION:	**Division of Pulmonary and Allergy Drug Products**
PHARM/TOX REVIEWER:	Virgil Whitehurst, Ph.D.
PHARM/TOX SUPERVISOR (Acting):	Jean Wu, MD, Ph.D.
DIVISION DIRECTOR:	Badrul Chowdhury, MD, Ph.D.
PROJECT MANAGER:	Ladan Jafari
Date of review submission to DARRTS:	8/25/09

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
Approval is recommended from the nonclinical perspective

B. Recommendation for nonclinical studies
No additional non clinical studies are recommended.

C. Recommendations on labeling
The labeling will be reviewed later when a labeling negotiation is needed.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Inhalation toxicity studies were conducted in rats (up to 26 weeks) and dogs (up to 39 weeks) to delineate the target organ toxicity for indacaterol (QAB149). 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).

The carcinogenicity potential of QAB149 was assessed in a 26 week oral (gavage) carcinogenicity study with C6F1/TgrasH2 hemizygous mice and a 24 month inhalation oncogenicity study with Sprague-Dawley rats. The studies were judged to be negative by the Executive CAC (see meeting minutes dated August 4, 2009). However, there were increased incidences of uterine endometrial stromal polyps in the 26-week oral (gavage) carcinogenicity study with female CB6F1/TgrasH2 hemizygous mice, and ovarian leiomyomas in the 24-month inhalation oncogenicity study with female Sprague-Dawley rats which were statistically significant by trend test, but not significant by pairwise comparison. These tumors have been observed with other β2-adrenergic agonists and might be attributed to treatment despite the lack of statistical significance.

QAB149 was negative in a battery of genetic toxicology studies including Ames test, chromosome aberration test with V79 Chinese hamster cells and bone marrow micronucleus test in rats.

In reproduction studies in the rat, subcutaneous administration of QAB149 did not cause significant effects in males or females related to parameters of fertility, general reproductive performance or early embryonic development. In embryofetal development studies in the rat, subcutaneous and inhalation administration of QAB149 did not result in embryo-fetal toxicity, gross malformations or teratogenicity. In the embryofetal development study in rabbits, subcutaneous administration of QAB149 did not show teratogenic effects. However, there was an increase in one skeletal variation (full

supernumerary ribs) in the fetus in the high dose group whereas a significant decrease (approximately 50%) in body weight gain was observed in the high dose group. When assessed for effects on pre-and postnatal development in rats subcutaneously administered with QAB149, there were increased stillborn pups for F0 and a statistically significant decrease in the number pregnant dams for F1 observed in the high dose group. However, there were no significant treatment related effects on neurobehavioral or other reproductive parameters of the F1 offsprings or the gross appearance of the F2 pups.

Pharmacologic activity

Indacaterol (QAB 149), a long-acting beta 2 adrenoceptor agonist has been shown to have bronchodilatory activity in various in vitro and in vivo assays including the isolated guinea pig trachea, isolated human bronchus and small airways in the guinea pig. The drug has a rapid onset of action (5 minutes) with an extended duration of action (~4 1/2 hours). The mechanism of action for QAB149 involves the interaction with active sites of the receptor via the membrane lipid bilayer stimulating cyclic AMP formulation 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 β_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.

B. Nonclinical safety issues relevant to clinical use

Non clinical safety issues related to clinical use of Indacaterol include effects on the cardiovascular system, i.e. decreased blood pressure, increased heart rate and myocardial fibrosis. These toxicities occur in several dog studies at systemic exposure levels that are approximately 42-45 times the expected human exposure at maximum dose of 300 μg. However, there is an adequate margin of safety of approximately 3-5 and 11 fold for the expected human exposure based on the AUCs at NOAELs determined in the 13-week and 39-week dog toxicity studies, respectively. Furthermore, the cardiovascular findings are considered monitorable in the clinical setting.

Non-clinical safety issues related to clinical use also include liver hepatocyte vacuolation (periportal) in the dog. PAS stain confirms that the vacuolation was due to glycogen deposition. However, these findings were only observed in the dog, were reversible and there was no histopathology associated with these findings. Therefore, the potential liver toxicity is not considered to be a significant safety problem in humans.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-383
Review number: 001
Sequence number/date/type of submission: 000/December 15, 2008/original NDA**Information to sponsor**: Yes ( ) No (X)
Sponsor and/or agent: Norvatis Pharmaceutical Corporation

Manufacturer for drug substance:
Novartis Pharma AG, Lichtstrasse 35, CH-4056 Basel, Switzerland
Novartis, Ringaskiddy Limited, Ringaskiddy CO, Cork, Ireland
Reviewer name: Virgil Whitehurst, Ph.D.
Division name: Division of Pulmonary and Allergy Products

Review completion date: **August 25, 2009**

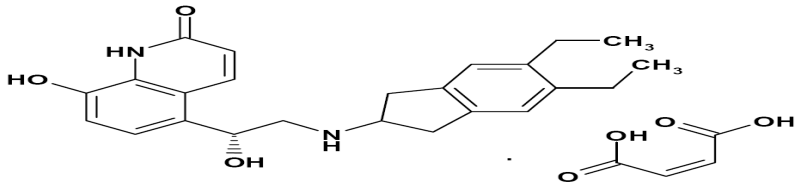
Drug:

Trade name: Arcapta (b) (4) (proposed) Indacaterol Maleate Inhalation Powder

Generic name: Indacaterol maleate inhalation powder
Code name: QAB 149

Molecular formula/molecular weight: $C_{24}H_{28}N_2O_3 \cdot C_4H_4O_4$ 508.76

Structure:



Relevant INDs/NDAs/DMFs: IND 48, 649 (lactose), IND 66, 337 (HFA) and IND 69, 754 (b) (4)

Drug class: Long –acting beta 2 adrenergic agonist

Intended clinical population: Long term, once daily, maintenance bronchodilator treatment of airflow obstruction in patients with chronic obstructive pulmonary disease (COPD)

Clinical formulation:

(b) (4)

Route of administration: Oral inhalation
Proposed clinical use: 150 and 300 µg QD

Studies reviewed within this submission:
Pharmacology studies:
RD-2002-04650-The effects of dry powder NVP-QAB149 on 5-HT-induced bronchoconstriction in the guinea pig
RD-2004-01669-Evaluation of tachyphylaxis to NVP-QAB149 on 5-HT induced bronchoconstriction in the conscious guinea pig by daily intratracheal dosing of dry powder on lactose: Comparison with formoterol and salmeterol
RD-2005-01251-In vitro binding and functional selectivity of NVP-QAB149-AF-3
RD-2006-00577-The effect of NVP-QAB149 base and its maleate and xinafoate salt forms on vagal sensory nerve fibers in guinea pig trachea.

RD 2008-01259-Comparsion of the effect of indacaterol with salbutamol, salmeterol and formoterol in the isolated, electrically- stimulated tracheal strip preparation and the isolated, electrically- stimulated left atria preparation of the guinea pig.
RD-2008-00341- Pharmacology of putative metabolites of indacaterol at the human beta-2-adrenoceptor.
RD-2008-01102-Duration of action of indacaterol and its hydroxylated metabolites in the isolated guinea pig trachea.
RD-2008-01101-Onset and duration of action for indacaterol (NVP-QAB149) and some of its analogs in the isolated guinea pig trachea.
RD-2008-1154-An investigation of the tussive effects of indacaterol in the guinea pig
RD-2008-1274- Testing of the tussive activity for indacaterol (NVP-QAB149) in the ozone-enhanced rabbit cough model.
RD-2008-01440-Effect of beta-adrenoceptor agonists on recombinant human TRPA1 function

Safety Pharmacology:

0470087-A single oral (gavage) dose general and neurobehavioral activity study in male mice.
0760652-A pharmacological assessment of the effect of QVA-149 on the central nervous system and the respiratory system of the albino rat.
0770861-Effects of QAB149, NVA237, and combination mixture QAB149 on cloned hERG potassium channels expressed in human embryonic kidney cells.
0670653- A pharmacological assessment of a single administration (inhalation) of QAB149 on the cardiovascular system in male Beagle dog using telemetry.

Pharmacokinetics studies:

Distribution studies:

ADME-R-0400060- Tissue distribution following an intravenous or oral dose of [3H]QAB149 in the rat
DMPK-R-0700884-Tissue distribution of radioactivity following a subcutaneous dose of ^{14}C]QAB149 in the pregnant rat.

Metabolism studies:

DMPK-R-0400859-Plasma concentrations, metabolism and excretion of $^{[14}$C] in the rat following a single oral dose (0.01 and 2 mg/kg)
DMPK-R-0400860-Plasma radioactivity, metabolism and excretion of [^{14}C]QAB149 in the dog following a single oral dose [3H]QAB149 in the rat
ADME-R-0301281-Oxidative metabolism of [^{3}H]QAB149 in human, rat and mouse liver microsomes.
DMPK-R-0500150- In vitro metabolism of [^{14}C]QAB149 in the rat and dog liver slices and human hepatocytes.
DMPK-R-0500025- In vitro assessment of covalent binding potential in rat and human liver microsomes and human hepatocytes and time-dependent cytochrome 450 inhibition.

Pharmacokinetic interaction studies:

DMPK-R-0500761-In vitro assessment of [^{14}C]QAB149 permeability and interactions with drug transporters across Caco-2 cell monolayers.

Toxicology studies:

Single dose studies:

0220073- Comparative single-exposure inhalation study in rats using the micronized powder and HFA formulations of QAB149
0370119- Acute oral (gavage) toxicity study in dogs
0370170- A repeat acute oral (gavage) toxicity study in dogs

Subchronic and Chronic Toxicity Studies:

0220009-13 week inhalation (dry powder aerosol) toxicity study in rats.
00220064- A 26 Week Inhalation Toxicity Study in rats with a 4 week recovery period.
0420019-13 week inhalation toxicity study in dogs.
0420020-13 week inhalation toxicity study in dogs.
0220065-39 week inhalation toxicity study in dogs with a 4 week recovery.

Reproduction Studies:

0670755-An Inhalation Embryo Fetal Development Study in Rats
0270185-A Subcutaneous Pre-and Postnatal Development Study in Rats
0270162- A Subcutaneous Neonatal and Juvenile Development Dose Range-Finding Study in Rats

Special studies:

0220082 -Buehler test in Guinea Pigs for Delayed Skin Sensitization Potential.
0320020-Potential for QAB149 to Induce Airway Obstruction or Pulmonary Eosinophilia in the guinea pig.

Carcinogenicity Studies:

The carcinogenicity studies were reviewed by Dr Tim Robison (see pharmacology review of this NDA dated August 5 and 12, 2009).

0470002-26-week oral (gavage) carcinogenicity study in CB6F1/TgrasH2 hemizygous mice
0320002-24-Month Inhalation Oncogenicity Study in Rats

Studies <u>not</u> reviewed within this submission:

<u>The following studies were reviewed in INDs 66, 337 and/or 48,649 (see attachments).</u>

Pharmacology studies:

The pharmacology studies listed below were reviewed in IND 66, 337 and IND 48, 649.

RD-1999-02232-Effects of a β2-agonist NVP on the isolated, electrically-stimulated, superfused guinea pig tracheal strip

RD-1999-03094v2-Single chamber plethysmographic measurement of airway reactivity in naïve, conscious guinea pigs

RD-1999-03095v2-Evaluation of NVP-QAB149 in guinea pigs by whole body, single chamber plethsmography

RD-2000-00233v2-Duration of action of formoterol and NVP- QAB149 in a guinea pig model of histamine–induced bronchoconstriction *in vivo*

RD-2000-00234-Determination of tachyphylaxis by forrmoterol and NVP-QAB149 using a guinea pig plethysmograph model of histamine-induced bronchoconstriction *in vivo*

RD-2000-00315-Results for PANLABS general pharmacology screen for NVP-QAB149
RD-2000-00195- Anti-Bronchoconstriction and Cardiovascular Effects of Inhaled Salmeterol in Rhesus Monkey.
RD-2000-159-Antibronchoconstrictor and cardiovascular effects of inhaled salmeterol in Rhesus monkeys.

RD-2001-03026-Activity of NVP-QAB149-AA-and its (S)-enantioner (NVP-QAB149-AA-1) in beta-1and beta –2 adrenoreceptor binding and functional assays

RD-2002-00195-Anti-broncoconstriction and cardiovascular effects of inhaled NVP-QAB149 in rhesus monkeys: comparison with formoterol

RD-2002-01685-Effects of NVP-QAB149- AA in human beta-receptor cAMP assay (ALPHA Screen) in BEAS-2B cells

RD-2002-01927-NMR studies of NVP-QAB149 in SDS micelles

RD-2002-03086- Antagonist effect of NVP-QAB149-AF-1on the α_{1D} adrenoceptor of rat aorta
RD-2003-02487-The effects of dry powder NVP-QAB149 on 5-HT-induced bronchoconstriction in the guinea pig: comparison with Salbutamol, Salmeterol and Formoterol.
RD-2004-00009: Comparison of the effect of NVP-QAB 149 in the guinea pig isolated, electrically-stimulated tracheal strip preparation and the isolated, electrically-stimulated left atrial preparation

Safety Pharmacology Studies:
This safety pharmacology study was reviewed in IND 66, 337.
0120071- Effect of QAB149 on the HERG currents recorded from stably transfected HEK239 cells.

Pharmacokinetic studies:
The pharmacokinetic studies listed below were reviewed in INDs 66, 337 and 48, 649.
Absorption studies:
DMPK-99-2113-Absorption, distribution, metabolism and excretion of [^{3}H]QAB149 in the rat following an intravenous, intratracheal, oral or subcutaneous dose.
ADME-R-030002- Relative bioavailability of oral gavage of [^{3}H]QAB149 in the mouse.
ADME-R-0101277-Absorption, distribution, metabolism and excretion of [^{3}H] QAB149 following an intravenous or, oral dose of [^{3}H]QAB149 in the mouse.
ADME-R01-0990- Absorption, distribution, metabolism and excretion following single doses of [^{3}H] QAB149 in the dog.

0220073- QAB149-Micronized powder and HFA formulations: Comparative single exposure inhalation study in rats using micronized powder and HFA formulations of QAB149.

Distribution studies:

DMPK-R-00594- In vitro binding of [^{3}H]QAB149 to red blood cells, serum and plasma proteins in the rat, dog and human.

DMPK-R-0700215-Ex vivo serum protein binding of [^{3}H] QAB149in human.

Metabolism studies:

DMPK-r-0700119-Pharmacokinetics and metabolism following an intravenous or subcutaneous dose of [^{14}C]QAB149 in the rabbit.

RD-2002-03936-Binding of NVP-ADD561-NX (the main metabolite of NVP-QAB149-AA)-to beta-1 and beta-2 adenoceptors.

DMPK-R00-397-QAB149: Comparative metabolism of [^{3}H]QAB149 in rat, dog and human liver slice culture and metabolism in human lung slice culture.

Excretion studies:

R02-0220- Excretion in Milk after a Single Subcutaneous Dose of 3[H] QAB 149 in the Rat.

Pharmacokinetic analysis studies:

DMPK-R299-2114- [3H]QAB149 synthesis of analysis. Drug metabolism and Pharmacokinetics

DMPK-R02-00490-Quantitative determination of QAB149 in rat, human, dog, rabbit and mouse serum and rabbit embryo by LC/MS/MS using liquid/liquid extraction.

DMPK-R299-2520- Quantitative determination of QAB149 in human serum by LC/MS/MS using liquid/liquid extraction.

DMPK-R00-2189- Quantitative determination of QAB149 in human urine by LC/MS/MS using liquid/liquid extraction.

Special studies:

The following special studies were reviewed in IND 66,337.

021726- QAB149: Assessment of Contact Allergenic Potential with Murine Local Lymph Node Assay (LLNA tier I).

0217031- Assessment of Contact Allergenic Potential with Murine Local Lymph Node Assay (LLNA tier I)

Toxicology Studies

Single dose studies:

The following single dose studies were reviewed in IND 66, 337.

001077-Acute oral toxicity study in rats

001078-Acute oral toxicity in mice

O170135- An acute subcutaneous toxicity study in mice

0170134-An acute subcutaneous toxicity study in rats

Subchronic Toxicity Studies:

The studies listed below were reviewed in IND 66, 337 or IND 48,649.

0220019-A 13 week inhalation (dry powder aerosol) toxicity study in mice

022007-QAB 149-2 Week Inhalation Dose-Range Finding (dry powder aerosol) Toxicity Study in Mice.
008042-Escalating dose ranging inhalation study in the rat.
002012-QAB149: 2-week inhalation toxicity study in the rat.
0670546- A two week combination inhalation study in Wistar rats with a 2 week recovery period.
0120088-QAB149: 4-week inhalation toxicity study in the rat.
311736: Preliminary inhalation toxicity study with magnesium stearate in dogs.
008043- Escalating dose ranging inhalation study in the dog.
002013-QAB149: 2-week inhalation toxicity study in the dog.
0120089- QAB149: 4-week inhalation toxicity study in the dog

Genotoxic Assays:
The genotoxic studies were reviewed in IND 66, 337.
001808- Mutagenicity Test using Salmonella Typhimurium
001834- Chromosome Aberration test With V79 Chinese Hamster cells
0212401- Subcutaneous Bone Marrow Micronucleus Test in the Rat

Reproductive Development Studies:
The reproductive development studies were reviewed in IND 66, 337.
0170145-A Subcutaneous Embryo-Fetal Development Dose Ranging-Finding Study in Rats.
0270021-A Follow-up Subcutaneous Embryo-Fetal Development Dose ranging-Finding Study in rats.
0270037-A Subcutaneous (bid) Embryo-Fetal development Study in Rats
0170146-A Subcutaneous embryo-Fetal Development Dose Range- Finding Study in Rabbits.
0270036-A Follow-up Subcutaneous Embryo-Fetal Development Dose Range-Finding Study in Rabbits.
0270038-A Subcutaneous Embryo-Fetal Development Study in Rabbit
0270074-QAB149: A Subcutaneous (bid) Fertility and Early Embryonic Development Study in Rats
0270074-QAB149: A Subcutaneous (bid) Fertility and Early Embryonic Development Study in Rats.

The studies listed below were not reviewed because QAB149 was administered via a different route of administration than is proposed for human use.

(b) (4)

The study listed below was not reviewed because drugs used in the study are salbutamol and an unidentified coded drug.

(b) (4)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Indacaterol (QAB 149), a long-acting beta 2 adrenoceptor agonist has been shown to have bronchodilatory activity in various in vitro and in vivo assays including the isolated guinea pig trachea, isolated human bronchus and small airways in the guinea pig. The drug has a rapid onset of action (5 minutes) with an extended duration of action (~4 1/2 hours).

The mechanism of action for QAB149 involves the interaction with active sites of the receptor via the membrane lipid bilayer stimulating cyclic AMP formulation 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 β_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.

The sponsor submitted numerous pharmacology studies which were reviewed in INDs 66, 337 (HFA formulation) and 48, 649 (lactose formulation) and crossed referenced for this NDA review.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

No new mechanism of action studies were submitted in this NDA submission. The studies listed in the table below were reviewed in INDs 66, 377 and 48,649.

Drug activity related to proposed indication:

Cell/Model types	Report #	Activity
The effect of NVP-QAB149 base and its maleate and xinafoate salt forms on vagal sensory nerve fibers in guinea pig trachea.	RD-2006-00577	In order to determine whether the cough phenomenon could be due to the salt form of indacaterol (maleate), a guinea pig model of vagal sensory nerve fiber was used to test the activity of QAB149 base and 2 of its salts, maleate and xinafoate). None of the compounds activated the tracheal Aδ fibers at concentrations up to 100 μM. The compounds activated the lung C-fibers at concentrations greater than 100 μM. This study model can not be used to determine whether indacateral maleate induces cough.
Comparison of the effect of indacaterol with salbutamol, salmeterol and formoterol in the isolated, electrically- stimulated tracheal strip preparation and the	RD 2008-01259	Indacaterol, salbutamol, salmeterol and formoterol inhibited electrically-stimulated contractions in the isolated guinea pig trachea strips. The inhibitions by indacaterol were concentration-dependent with a rapid

isolated, electrically- stimulated left atria preparation of the guinea pig.		onset (~28-36 minutes) and a long duration of action (~8-9 hours).
Pharmacology of putative metabolites of indacaterol at the human beta-2-adrenoceptor.	RD-2008-00341-	This study compares the activity of indacaterol and four possible diastereomers that results from benzylic hydroxylation of indacaterol (NVP-QBA088, NVP-QBA089, NVP-090 and NVP-091). The potency and affinity of indacaterol and its diastereomers was similar.
Duration of action of indatcaterol and its hydroxylated metabolites in the isolated guinea pig trachea.	RD-2008-01102	This study compares the onset and duration of action of indacaterol and 4 of its metabolites (NVP-QBA088, NVP-QBA089, NVP-090 and NVP-091) using an isolated guinea pig trachea preparation. The onset of action of indacaterol and its metabolites are similar. The duration of action of indacaterol is 4 to 7 fold greater than that of its metabolites.
Onset and duration of action for indacaterol (NVP-QAB149) and some of its analogs in the isolated guinea pig trachea.	RD-2008-01101	The objective of this study was to investigate the influence of lipophilic properties of indacaterol and its relationship to its onset and duration using a guinea pig trachea model. The results show that indacaterol has optimal lipophilic properties which results in a rapid onset (~45 minutes) and a long duration of action (~5.45 hours) when compared with Salmeterol and Salbutamol.
An investigation of the tussive effects of indacaterol in the guinea pig	RD-2008-1154	The objective of this study was to investigate the potential tussive effects of indacaterol in a guinea pig cough model. Indacaterol, at a concentration of 2 mg/mL evoked a significant cough in conscious guinea pigs. The mechanism of the cough was not delineated in this study.
Testing of the tussive activity for indacaterol (NVP-QAB149) in the ozone-enchanced rabbit cough model.	RD-2008- 1274	The objective of this study was to investigate the potential tussive effects of indacaterol in a rabbit ozone-enhanced cough model. Indacaterol, at a concentration of 1 mg/mL evoked a non- significant cough when compared with the citric acid controls. The rabbit model is not suitable to mimic the cough induced by indacaterol in humans.
Effect of beta-adrenoceptor agonists on recombinant human TRPA1 function	RD-2008-01440	TRPA1 is a non-selective channel that can be activated by a wide range of chemical mediators through Michael addition, conjugation or alkylation. Indacaterol and Salmeterol, at concentrations to 100 μM exhibited weak excitatory effects on recombinantly expressed human TRPA1.
In vitro binding and functional selectivity of NVP-QAB149-AF-3	RD-2005-01251	The purpose of this study is to investigate the biding and functional selectivity of NVP-QAB149-AF-3 and other beta 2

		adrenoceptor agonists at beta 1, beta 2 and beta3adrenoceptors expressed in CHO cells. NVP-QAB149-AF-3 was found to be a strong partial agonist at the beta 2 adrenoceptor, more selective at the beta 2 adrenoceptor than at the beta 1 or beta 3.
Evaluation of tachyphylaxis to NVP-QAB149 on 5-HT induced bronchoconstriction in the conscious guinea pig by daily intratracheal dosing of guinea pig by daily intratracheal dosing of dry powder on lactose: Comparison with formoterol and salmeterol	RD-2004-01669	The aim of this study is to determine whether repeat intratracheal dosing of NVP-QAB149 as a dry powder with lactose demonstrates tachyphylaxis in the 5-HT bronchconstricted male guinea pig (8/dose group) The QAB149 doses were 0.0006, 0.006 and 0.06% daily for 5 days. QAB149 did not induce tachyphlaxis. In the guinea pig in this study.
The effects of dry powder NVP-QAB149 on 5-HT-induced bronchoconstriction in the guinea pig	RD-2002-04650	The study was to investigate the dose-response and duration of action of intratracheal dosed NVP-QAB149 as a dry powder with lactose on 5-HT induced bronchoconstriction in male conscious guinea pigs (8/dose group). QAB149 had a 24 hour bronchodilating effect in the guinea pig.

2.6.2.3 Secondary pharmacodynamics

There were no secondary pharmacodyamic studies submitted in this NDA submission.

2.6.2.4 Safety pharmacology

(One study was reviewed in IND 66,337)

QAB149, at a concentration of 5 µg/ ml, inhibited HERG channels stably expressed in HEK293 cells. QAB149, at concentrations of 0.5 and 1.0 µg/ml did not inhibit HERG channels stably expressed in HEK293 cells. In another hERG assay, IC_{50} for QAB149 on hERG potassium current was 3.1µM. In dogs, a single inhalation pulmonary deposited dose of 0.0925 mg/kg caused increased heart rates up to 110% compared with the controls at approximately 1.5 hours post dosing, and increased heart rate ~25 % at 24 hours after dosing. The increases in heart rates were not associated with increases in QT and QTc intervals. In toxicity studies (section 2.6), transient increase in heart rate was observed in 2 to 13 weeks dog studies but slight transient increase in QTc observed in 2-week (up to pulmonary deposited dose of 0.23 mg/kg) and one of 13-week (up to pulmonary deposited dose of 0.28 mg/kg) studies was not observed in the 4-week (up to pulmonary deposit dose of 0.24 mg/kg) and the another 13-week (up to pulmonary deposited dose of 0.27 mg/kg) studies at comparable or higher exposure. A single, oral dose of 2000 mg/kg QAB149 administered to male mice had no general or neurobehavioral effects. Additionally, an oral dose of 2000 mg/kg had no kidney or GI effects. And finally, a pulmonary deposited dose of 0.0496 mg/kg administered to male rats had no effect on CNS or respiratory parameters.

Neurological effects:

A single oral (gavage) dose general and neurobehavior activity study in male mice (0470087).

The objective of this study was to investigate the effects of indacaterol (QAB149) on the general and neurobehavioral activities in 10 male mice (Crl:CD-1(ICR) Br). Indacaterol was administered as a single oral dose of 2000 mg/kg. Necropsies were performed on all the rats 24 hours after dosing. Indacaterol had no general (including renal and gastrointestinal effects) or neurobehavioral effects in male mice at a dose of 2000 mg/kg.

A pharmacological assessment of the effect of QVA-149 on the central nervous system and the respiratory system of the albino rat (0670652).

The objective of this study was to evaluate the effects of QAB 149 (indacaterol), administered by inhalation on the CNS and respiratory system of the male albino rat (Wistar-8/dose group). NVA237, an anti cholinergic drug as well as a combination NVA 237/QAB19 dose was included in the study. The study was divided into 2 parts, the first for the effects of indacaterol on the CNS and the second for the effects of indacaterol on the respiratory system. The achieved inhalation dose of indacaterol was 0.496 mg/kg (pulmonary deposited dose= 0.0496 mg/kg). Indacaterol at this dose had no effect on the CNS and respiratory system in the male rat.

Cardiovascular effects:

A pharmacological assessment of a single administration (inhalation) of QAB149 on the cardiovascular system in male Beagle dog using telemetry (0670653).

The purpose of this study was to evaluate the effects of a single inhalation dose of QAB149 (indacaterol) on the hemodynamic and electrocardiographic parameters of male Beagle dogs (n=4). Hemodynamic and electrocardiographic parameters were measured via telemetry. NVA237, an anti cholinergic drug as well as a combination NVA 237/QAB149 dose was included in the study. The achieved dose of QAB149 was 0.370 mg/kg (pulmonary deposited dose=0.0925 mg/kg). QAB149 increased heart rate by 57 to 110 %,t one and one half hours after the initiation of dosing which was still increased by 25 % compared with the controls, 24 hours post dosing. QAB149 also shortened the PR, P width, QT and QTc intervals.

Effects of QAB149, NVA237, and combination mixture QAB149 on cloned hERG potassium channels expressed in human embryonic kidney cells (0770861).

The objective of this study was to assess the in vitro effects of QAB149 (indacaterol), NVA237 and a combination mixture QVA149 on the hERG (human ether-α- go-go-related gene) channel current (a surrogate for $I_{Kr,}$ the rapidly activating, delayed rectifier cardiac potassium current). Indacaterol inhibited the hERG current at the four concentrations tested, 23% at 1 μM, 52% at 3 μM, 73% at 10 μM and 90% at 30 μM. The IC_{50} for QAB149on hERG potassium current was 3.1μM (Hill coefficient=1).

Pulmonary effects:

A pharmacological assessment of the effect of QVA-149 on the central nervous system and the respiratory system of the albino rat (0670652).

See review of this study above under Neurological Effects.

Renal effects:
A single oral (gavage) dose general and neurobehavioral activity study in male mice (0470087).
See review of this study above under Neurological Effects.

Gastrointestinal effects:
A single oral (gavage) dose general and neurobehavior activity study in male mice (0470087)
See review of this study above under Neurological Effects.
.
Abuse liability:
No abuse liability studies were submitted in this NDA submission.

Other:
QAB 149 at a concentration of 250 mg/mL (total topical dose =125 mg) was not a sensitizer in the Buehler test in guinea pig for Delayed Skin Sensitization Potential. This study was previously reviewed (see pharmacology review for IND 48, 649 dated April 27, 2004).

2.6.2.5 Pharmacodynamic drug interactions

There was no pharmacodynamic drug interaction studies submitted in this NDA submission.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

There was no pharmacology tabulated summary submitted in this NDA submission.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

QAB 149 is absorbed in the dog and the rat after oral dosing but is not highly bioavailable. The bioavailability in the dog after oral dosing is 33 % and approximately 1% in the rat and mouse. The *In vitro* metabolism of QAB149 evaluated using liver slices is similar in rats, dog and humans, involving mainly phenolic O-glucuronidation. No major active metabolites were observed. Protein binding was similar in the rat, dog and human, approximately 92, 94 and 96%. QAB149 was distributed to most tissues, except the brain, spinal column and lymph nodes. The highest concentrations of QAB149 were found in the stomach, intestines, liver and kidney. QAB149 was also distributed into the fetus in rats. QAB 149 showed no significant inhibition of P450 enzymes, CYP2C9, CYP2E1 and CYP3A4/5 when tested in concentrations up to 50 μM. Elimination of QAB149 in the rat, mouse and dog is mainly via the feces.

The Pharmacokinetics/Toxicokinetic studies cross referenced in this NDA were reviewed in INDs 66,337 and 48, 649.

2.6.4.2 Methods of Analysis

A high-performance liquid chromatographic method with mass spectrometric detection for the determination of QAB149 and its metabolites in rat mice, rabbit and dog serum. The method has a lower limit of quantitation of 0.07 00 ng/mL using a sample volume of 200 μL.

2.6.4.3 Absorption

No new absorption studies with QAB149 were submitted in this NDA submission. Absorption for QAB149 was reviewed in IND 66, 337, pharmacology review dated May 6, 2008.

2.6.4.4. Distribution

Tissue distribution following an intravenous or oral dose of [3H] QAB149 in the rat (ADME-R-0400060).

The objective of this study was to investigate the distribution of radioactivity [^{3}H]QAB149 into tissues, organs and body fluids in male, Long Evans Hooded rats (2) following a single intravenous or oral dose of 0.5 mg/kg. The tissue distribution was studied via whole-body autoradiography. Radioactivity was rapidly and widely distributed following intravenous dosing. Peak radioactivity concentrations were observed 5 minutes after dosing with the highest concentrations were found in the kidney cortex and medulla, thyroid gland and the adrenal cortex.

Tissues distribution following oral dosing was similar to distribution following intravenous dosing. Peak concentrations were noted two hours after dosing in the rat.

Tissue distribution of radioactivity following a subcutaneous dose of ^{14}C] QAB149 in the pregnant rat (DMPK-R-0700884).

The tissues distribution of radioactivity ([^{3}H]QAB149) into the tissues, organs and body fluids was studied in pregnant rats (8) and their fetuses following a subcutaneous dose of 2 mg/kg. The rats were dosed on gestational on days 12 and 17. The tissue distribution was studied via whole-body autoradiography. Results show peak radioactivity at 2 and 7 hours after dosing. The highest radioactivity was noted in the maternal kidney, spleen, liver, lung and heart. Drug-related radioactivity was distributed into the fetuses.

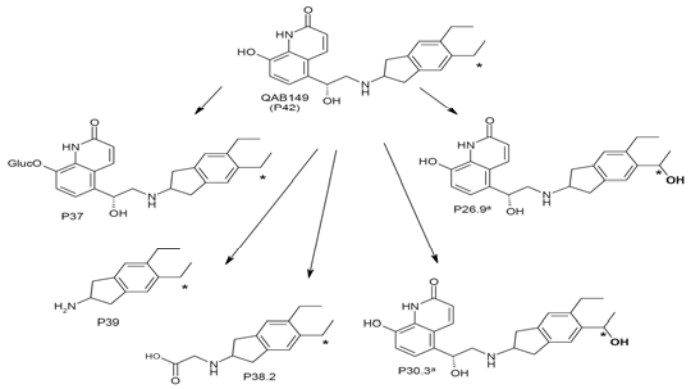
2.6.4.5 Metabolism

Plasma concentrations, metabolism and excretion of [^{14}C] QAB149 in the rat following a single oral dose (0.01 and 2 mg/kg) (DMPK-R-0400859)

The objective of this study was to investigate the metabolism of [^{14}C] QAB149 following single oral doses of 0.01 and 2 mg/kg in the Wistar rat (3/dose group). The major metabolite in plasma and urine was (P37). The major metabolic pathway of QAB149 was glucuronidation (shown below):

Best Available Copy

Metabolic pathways of QAB149 in the rat following a single oral dose of [^{14}C]QAB149



The excretion in the rat was mainly in the feces. A summary of the pharmacokinetic parameters of this study is shown below:

Summary

Species/strain/gender:	Rat/HanWistar/male	
Route/formulation:	Oral dose/0.5% carboxymethylcellulose (CMC) suspension	
Dose (mg/kg):	0.01	2
Number of animals:	3	3
Specific activity of [^{14}C](μCi/mg):	110	110
Samples collected:	Serial blood and plasma collected at (2 mg/kg dose only) 0.5, 2, 4, 8, 24, 48, 72, 96 and 168 h postdose; complete urine and feces in 24 h intervals, 0-168 h; cage wash, liver, and carcasses were collected at 168 h postdose.	
Samples analyzed:	Radioactivity was measured in pooled plasma, urine, feces, cage wash, liver, and carcass. Selected pooled plasma, pooled urine and feces samples were assayed for unchanged QAB149 and metabolites.	
Plasma [^{14}C]radioactivity		
C_{max} (ngEq/mL):	-[a]	58.0
t_{max} (h):	-[a]	2
$AUC_{0\text{-}168\ h}$ (ngEq•h/mL):	-[a]	517
Excretion in urine (% dose): radioactivity		
0-24 h:	1.93 ± 0.71	1.94 ± 0.78
0-168 h:	2.25 ± 0.74	2.21 ± 0.91
Excretion in feces (% dose): radioactivity		
0-24 h:	56.1 ± 19.5	72.4 ± 18.5
0-168 h:	82.6 ± 3.60	88.9 ± 7.21
Cage wash (% dose)	0 ± 0	0.19 ± 0.07
Carcass	3.49 ± 0.83	-[b]
Liver	1.02 ± 1.26	-[b]
Total radioactivity recovery (% dose):	89.0 ± 5.79	91.3 ± 7.75

[a] blood/plasma not collected at the 0.01 mg/kg dose level

[b] not analyzed due to high recovery in excreta

Plasma radioactivity, metabolism and excretion of [^{14}C] QAB149 in the dog following a single oral dose (DMPK-R-0400860).

The objective of this study was to investigate the metabolism of [^{14}C] QAB149 following a single oral dose of 0.3 mg/kg in the Beagle dog (2). The major metabolite in plasma and urine was (P37). The major metabolic pathway of QAB149 was glucuronidation (shown below):

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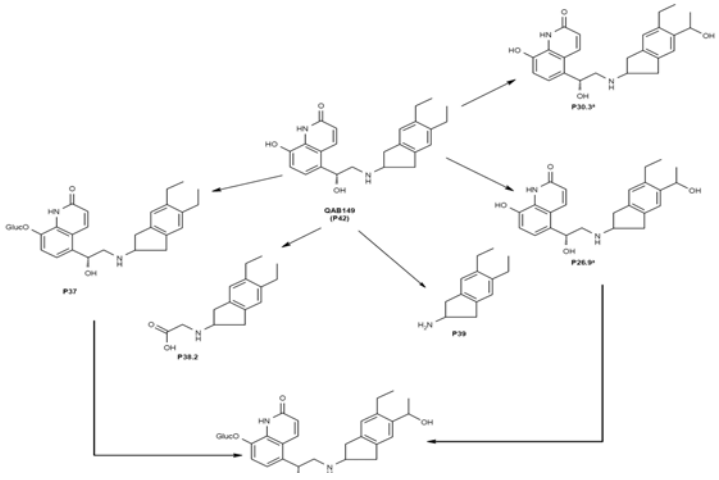
Metabolic pathways of QAB149 in the dog following a single oral dose of [^{14}C]QAB149



Summary

Species/strain/gender:	dog/beagle/male
Route/formulation:	Oral dose/ polyethylene glycol 400:0.9% sterile saline (v:v; 20:80)
Dose (mg/kg):	0.3
Number of animals:	2
Specific activity (µCi/mg)	70
Samples collected:	Serial blood and plasma collected at 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, 72, 96, and 168 h postdose; complete urine and feces in 24 h intervals, 0-168 h; cage wash collected at 168 h postdose.
Samples analyzed:	Radioactivity was measured in pooled plasma, urine, feces, cage wash, liver, and carcass. Selected pooled plasma, pooled urine and feces samples were assayed for unchanged QAB149 and metabolites.
Plasma [^{14}C]radioactivity	
C_{max} (ngEq/mL):	37.4 (28.9 – 45.8)[1]
t_{max} (h):	1.5 (1 – 2)
$AUC_{0-168\ h}$ (ngEq•h/mL):	295 (235-354)
Excretion in urine (% dose): radioactivity	
0-24 h:	4.29 (3.63 – 4.95)
0-168 h:	5.13 (4.76 – 5.50)
Excretion in feces (% dose): radioactivity	
0-24 h:	69.4 (64.4 – 74.4)
0-168 h:	79.6 (69.3 – 90.0)
Emesis	7.7 (4.50 – 10.9)
Cage wash (% dose)	1.1 (1.04 – 1.13)
Total radioactivity recovery (% dose):	93.5 (86.8 – 100)

[1] range

Excretion of QAB149 in the dog was mainly in the feces.

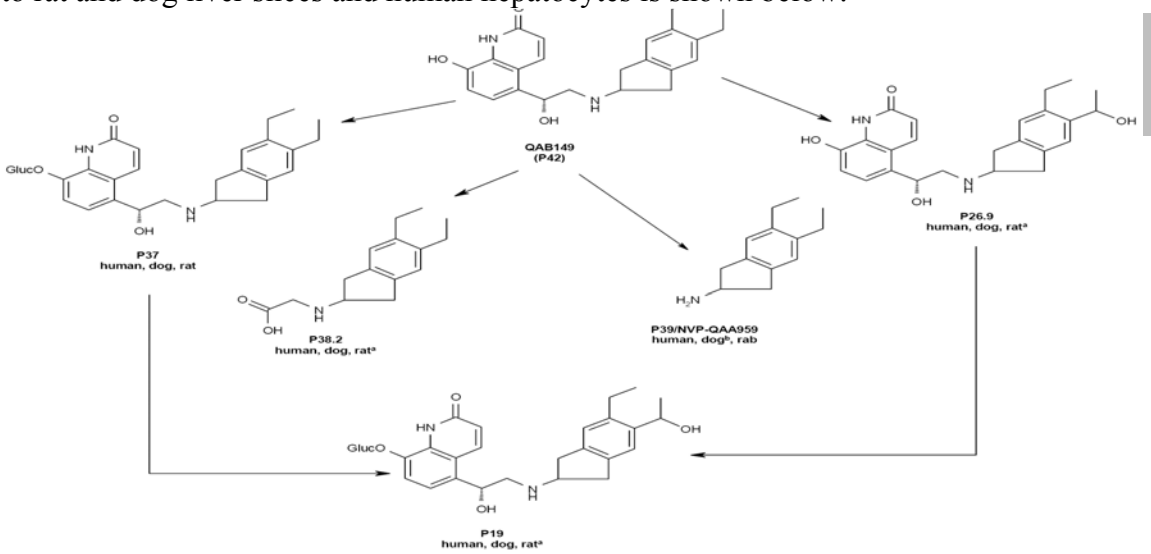
Oxidative metabolism of [^{3}H]QAB149 in human, rat and mouse liver microsomes (ADME-R-0301281).

In the presence of NADPH, [^{3}H]QAB149 was metabolized by human, rat and mouse microsomes to form qualitatively the same oxidative metabolites, P26.9, P30.3 and peak P20. These data show that that the rat and mouse dosed with QAB149 form oxidative metabolites similar to humans.

In vitro metabolism of [^{14}C] QAB149 in the rat and dog liver slices and human hepatocytes (DMPK-R-0500150).

Previous metabolism studies revealed QAB149 in humans is metabolized to QAB149 glucuronide (P37) and 4 minor metabolites, P19, P26.9, P38.2 and P39. The objective of this study was to determine whether these minor metabolites found in human serum was also present following incubation of [^{14}C] QAB149 to rat and dog liver slices and human

hepatocytes. P37 and four minor metabolites were found following incubation with rat and dog liver slices and human hepatocytes. The metabolic pathway following incubation to rat and dog liver slices and human hepatocytes is shown below:



In vitro assessment of covalent binding potential in rat and human liver microsomes and human hepatocytes and time-dependent cytochrome 450 inhibition (DMPK-R-0500025).

The objective of this study was to determine if [^{3}H]QAB149 has the potential to bind covalently to protein when incubated with rat and human liver microsomes or in human hepatocytes using non-extractable radioactivity experiments. Also, whether QAB149 has the potential to function as a time-dependent inhibitor of cytochrome 450s, CYP1A2, CYP2C9 or CYP3A4/5. In this study, QAB149 was found to have a low potential for covalent binding to rat and human liver microsomes and human hepatocytes. Also, QAB149, at a concentration of 50 μM incubated with human microsomes did not inhibit cytochrome 450s, CYP1A2, CYP2C9 or CYP3A4/5.

2.6.4.6 Excretion

No new excretion studies were submitted in this NDA submission. The excretion studies for QAB149 were reviewed in IND 48, 649, pharmacology review dated April 27, 2004.

2.6.4.7 Pharmacokinetic drug interactions

Pharmacokinetic interaction studies:

DMPK-R-0500761-In vitro assessment of [^{14}C]QAB149 permability and interactions with drug transporters across Caco-2 cell monolayers.

The in vitro permeability and transporter interaction potential of {14C] QAB149 was examined using a Caco-2 monolayer model. The results of this study show that QAB149 is a moderate permeable drug substance. These results suggest that QAB149 has the potential to be absorbed following oral dosing.

2.6.4.8 Other Pharmacokinetic Studies

No other pharmacokinetic studies were submitted in this NDA submission.

2.6.4.9 Discussion and Conclusions

QAB 149 is absorbed in the dog and the rat after oral dosing but is not highly bioavailable. The bioavailability in the dog after oral dosing is 33 % and approximately 1% in the rat and mouse. The *In vitro* metabolism of QAB149 evaluated using liver slices is similar in rats, dog and humans, involving mainly phenolic O-glucuronidation. No major active metabolites were observed. No metabolites were detected in human lung slice cultures of ^{3}[H}QAB149. Protein binding was similar in the rat, dog and human, approximately 92, 94 and 96%. QAB149 was distributed to most tissues, except the brain, spinal column and lymph nodes. The highest concentrations of QAB149 were found in the stomach, intestines, liver and kidney. QAB149 was also distributed into the fetus in rats. QAB 149 showed no significant inhibition of P450 enzymes, CYP2C9, CYP2E1 and CYP3A4/5 when tested in concentrations up to 50 µM. Elimination of QAB149 in the rat, mouse and dog is mainly via the feces.

2.6.4.10 Tables and figures to include comparative TK summary

Table 3-3 Pharmacokinetic parameters of Indacaterol in plasma or serum after a single dose in various species

Species	Route of administration	Dose (mg/kg)	t_{max} (h)	C_{max}/dose (ngEq/mL)/(mg/kg)	AUC_{last}/dose (ngEq·h/mL)/(mg/kg)	Apparent $t_{1/2}$ (h)	Bioavailability (%)
Mouse[a]	p.o.	0.5	0.5	0.32	1.12	n.a.	1.0
Rat[b]	p.o.	0.5	BLQ	BLQ	BLQ	BLQ	78
	i.t.	0.6	0.5	86.0	212	8.1	78-90
	s.c.	0.5	1.0	2.0	182	13	67
	s.c.	3.0	3.0	19.3	287	32	100
Rabbit[c]	s.c.	1.0	0.83 ± 1.0	71.5 ± 24	419 ± 150	9.7 ± 3.0	51
Dog[d]	p.o.	0.1	0.5 ± 0	86.9 ± 51.3	382 ± 209	12.0 ± 4.5	33 ± 9.5
Human[e]	p.o.	0.01‡	1.0 (1-2)†	40.3 ± 15.8‡	151 ± 55.3	n.a	n.a.
Human[f]	p.o.	0.01‡	1.5 (1-3)†	41.2 ± 19.3‡	159 ± 124	n.a	n.a.

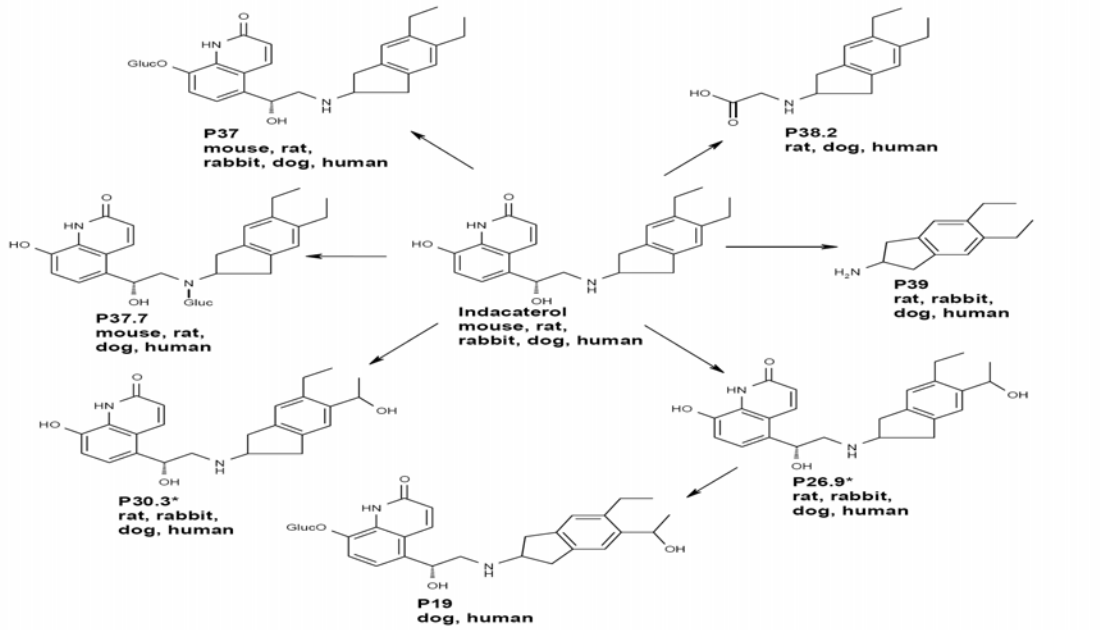
References: [a][Table 2.6.5.3A-Study R0101277]; [b][Table 2.6.5.3A-Study R99-2113]; [c][Table 2.6.5.3A-Study R070019]; [d][Table 2.6.5.3A-Study R01-0990]; [e][Table 2.6.5.3A-Study CQAB149A2214]; [f][Table 2.6.5.3A-Study CQAB149A2223]

n.a. = not applicable or not measured

† median value and range

‡ based on 800µg dose per 70 kg body weight

Figure 5-2 Metabolic pathways for indacaterol in all species



2.6.5 PHARMACOKINETICS TABULATED SUMMARY

There was no pharmacokinetics tabulated summary submitted in this NDA submission.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General:

Acute Studies: Acute studies were carried out in the mouse and the rat. QAB 149 micronized powder was administered orally and subcutaneously. Results of these studies show oral doses up to 1600 mg/kg did not induce any significant adverse events in the mouse or the rat. However, when QAB 149 was administered subcutaneously, deaths were observed at a dose of 200 mg/kg in the rat. The MTD in the rat was 100 mg/kg. Acute toxicity studies were conducted in the dog. QAB149 administered orally to dogs using a single dose of 100 mg/kg induced severe body weight losses, decreased food consumption, decreased blood pressures, abnormal electrocardiography and pathological lesions (renal tubular necrosis, dilatation and cast formation). In a repeat acute toxicity study, QAB149 administered to dogs using single oral doses of 0.1, 1 and 10 mg/kg did not induce mortality or morbidity in the dog. Dose-related clinical signs include redden skin, ears and gums, increased respiration, increased heart rates and dry mouth. QAB149 also induced abnormal electrocardiography (sinus tachycardia). The MTD in this study was 10 mg/kg.

Sub chronic Studies: Studies up to 13-weeks duration have been conducted in rats, mice and dogs.

In the rat, a two week study with micronized drug, pulmonary deposited doses were 0.21, 0.58 and 1.7 mg/kg, and resulted in an increased incidence of alveolar macrophages in the lungs of all drug treatment groups when compared to control groups. The NOAEL of 0.21 mg/kg was associated with a mean AUC of 170-289 ng.h/ml.

In a four week rat study with the HFA formulation, the pulmonary deposited doses were 0.093, 0.28 and 0.85 mg/kg. Reversible focal, olfactory epithelial degeneration characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of the epithelium in the mid and high dose groups were observed. The NOAEL was 0.093 mg/kg associated with a mean AUC of 59.3 (ng/ml).h.

The sponsor also conducted a 13 week inhalation toxicity dose–ranging study (pulmonary deposited doses: 0, 0.3, 1.01 and 3.08 mg/kg) to determine the doses for a carcinogenicity study. The results of this study again revealed changes in the nasal cavities (degeneration of the olfactory epithelium of the dorsal meatus) in the high dose rats; severity ranged from minimal to marked, with most animals showing moderate changes. The rats at the mid and high dose exhibited minimal to mild squamous metaplasia and hyperplasia of the larynx. Squamous metaplasia and hyperplasia of the larynx were also noted at 4 weeks and are considered to be rodent specific responses to inhaled particulate matter.

The sponsor also conducted a 13 week inhalation toxicity study in CD-1 mice using pulmonary deposited doses of 0, 0.024,0.074 and 0.246 mg/kg in order to determine dose for a carcinogenicity. The results of this study revealed changes in the nasal cavity in the mid and high dose groups. The lesions were characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of the epithelial and occurred on the roof of the dorsal meatus. These lesions were often accompanied by the presence of eosinophilic globules. The NOAEL in the study was 0.024 mg/kg.

The 2 week study in the dog with micronized drug included pulmonary deposited doses of 0.0025, 0.12 and 0.23 mg/kg. QAB149 induced tachycardia and myocardial fibrosis in the dogs in the mid and high dose groups. There were transient increases in heart rate with slightly increased QTc, and heart force (strength of heart beat) in all QAB 149-treated dogs. There was also dose-related periportal glycogen vacuolation in the liver of all QAB149 dosed groups. There was no NOAEL in this study.

The 4 week sub chronic inhalation study with the HFA formulation in the dog included pulmonary deposited doses of 0.0025, 0.025 and 0.24 mg/kg. QAB149 again induced tachycardia and myocardial fibrosis in the dogs in high dose group and tachycardia in the dogs in the mid dose group. The tachycardia was not associated with an increase in QTc. There was also a dose-related glycogen vacuolation of the liver of the dogs in the mid and high dose groups which was reversible. The NOAEL was 0.0025 mg/kg and associated with a mean AUC of 2.93 ng.h/ml.

In the first of two 13 inhalation toxicity studies in the dog, QAB149 was administered daily to dogs using pulmonary deposited doses of dry powder- 0, 0.005, 0.03 and 0.28

mg/kg as well as QAB149 HFA-0.26 mg/kg. Myocardial fibrosis in the papillary muscles was observed in the 0.28 mg/kg dose group QAB149 dry powder but was not observed in the HFA dose group. Significant transient increase in heart rate was observed in dry powder high dose groups and HFA dose groups, and associated with transient slightly increased QTc. Dry powder and HFA QAB149 formulations induced periportal hepatocyte glycogen vacuolation in all treated groups. The NOAEL identified for this study was 0.03 mg/kgassociated with a AUC of 37.0ng,h/mL.

In the second 13 week inhalation toxicology study in the dog, the daily doses were Certihaler dry powder- 0.005, 0.025 and 0.25 mg/kg and Aerolizer-0.27 mg/kg. QAB149 dry powder formulations induced myocardial fibrosis in the papillary muscles in the dogs in the Certihaler 0.25 and Aerolizer 0.27 mg/kg dose groups. The transient increase in heart rate without increased QTc intervals was observed in the Certihaler high dose and Aerolizer dose groups. The Certihaler and Aerolizer dry powder formulations induced periportal hepatocyte glycogen vacuolation in all QAB149-dosed groups. The NOAEL identified for this study was 0.025 mg/kg (Certihaler) associated with an AUC of 20.2 ng.h/mL.

Subchronic toxicity studies show toxicological and toxicokinetic profiles for the Indacaterol dry powder formulation and Indacaterol HFA formulation are similar.

Chronic studies:
Chronic studies were conducted in the rats (26 weeks) and the dog (39 week).

The 26 week chronic inhalation toxicity study in the rat with a HFA formulation included pulmonary deposited doses of 0.031, 0.102 and 0.314 mg/kg. Microscopic evaluations revealed that 4 out of 20 males in the high dose group had squamous metaplasia of the epithelium in the ventral larynx with U shape cartilage. Following a 4 week recovery period, these changes were no longer observed. Similar findings were observed in the 4 and 13 week inhalation toxicity studies in the rat which were considered rat specific and have no human relevance. The NOAEL in this study was 0.102 mg/kg associated with an AUC of ~38 ng.hr/ml.

The 39 week inhalation toxicity study in the dog included pulmonary deposited doses of 0.0075, 0.025 and 0.08 mg/kg. Microscopic examinations showed liver hepatocyte vacuolation (periportal) was observed in all the dogs. The cells with vacuolation were slightly enlarged and showed rarefaction of the cytoplasm. In some livers, the vacuolated cells were localized to one area while others were widespread. PAS stain confirms that the vacuolation was related to glycogen deposition. These findings were reversible following a 4 week recovery period. The NOAEL in this study was 0.08 mg/kg associated with an AUC of ~89 mg.h/ml.

Genetic toxicology:
Genotoxicity assays, i.e., Ames bacterial reverse mutation test, Mammalian Chromosomal Aberration test in the V79 Chinese hamster cell and bone marrow micronucleus test reveal that QAB149 (indacaterol) is not genotoxic under the conditions

tested (see genotoxicity assays reviews in Pharmacology review for IND 66, 337 dated May 6, 2008).

Carcinogenicity

104 week inhalation carcinogenicity study was conducted in the rat and a 26 week oral (gavage) carcinogenicty study in CB6F1/TgrasH2 hemizygous mice. The doses in the mouse were 0, (air), 100, 300 and 600 mg/kg while the doses in the rat were 0 (air), 0 (air), 0.21, 0.62 and 2.09 mg/kg. The studies were judged to be negative by the Executive CAC (see meeting minutes dated August 4, 2009). However, there were increased incidences of uterine endometrial stromal polyps in the 26-week oral (gavage) carcinogenicity study with female CB6F1/TgrasH2 hemizygous mice and ovarian leiomyomas in the 24-month inhalation oncogenicity study with female Sprague-Dawley rats, which were statistically significant by trend test, but not significant by pairwise Comparison. These tumors have been observed with other β2-adrenergic agonists and might be attributed to treatment despite the lack of statistical significance.

Reproductive toxicology:

QAB 149 was administered to male and female rats subcutaneously in a fertility and early embryonic development study. The daily doses were 0, 0.2, 0.6 and 2.0 mg/kg. QAB149 caused decreases in testes and epididymis organ weights in males at the doses of 0.2 mg/kg and above compared with the controls. However, there were no detectable effects on reproductive potential and histological evaluations in the testes or epididymis. There were no effects in males or females related to parameters of fertility, general reproductive performance or early development. The NOAEL was 2 mg/kg for fertility and early embryonic development.

QAB149 was administered to pregnant rats, days 6-17 of gestation using subcutaneous doses up to 1 mg/kg in an embryo –fetal development study. There was no evidence of teratogenicity or other reproductive effects in this study. The NOAEL was 1 mg/kg for fetal development. There were no NOAEL for local toxicity in the dams based on treatment-related injection site reactions and skin lesions at all treated dose groups. An increase in dam body weight gain was noted at all treated doses. The doses used in this study were adequate based on maternal toxicity associated with subcutaneous administration of QAB149 (see review of IND 66,337.)

Also, QAB149 was administered to pregnant rats, days 6-17 of gestation using inhalation doses up to 0.212 mg/kg in an embryo –fetal development study. There were no teratogenic or reproductive effects in this study. The NOAEL was 0.212 mg/kg for fetal and maternal toxicity.

QAB 149 was administered to pregnant rabbits, days 7-20 of gestation using subcutaneous doses up to 3 mg/kg in an embryo-fetal development study. The results of this study reveal maternal toxicity (local injection site reactions/skin lesions, and reduced body weight gain approximately 55%) and an increase in one skeletal variation (full supernumerary ribs) in fetuses in the 3 mg/kg dose group. There were no teratogenic

effects in the rabbits in this study. The NOAEL was 1 mg/kg for maternal and fetal toxicity.

In a pre-and postnatal development study, QAB149 was administered to pregnant rats, at subcutaneous doses of 0.1, 0.3 and 1.0 mg/kg. For F0, stillborn pups were greater in the high dose group than in the control group. For F1, a statistically significant decrease in the number of pregnant dams was observed in the high dose group. There were no drug-related effects on neurobehavioral and other reproductive parameters of the F1 offspring. There were no significant gross F2 findings. The NAOEL for maternal toxicity was 1.0 mg/kg and 0.3 mg/kg for fetal toxicity.

Special toxicology:
The special toxicology study was reviewed in IND 48, 649 (see pharmacology review dated April 27, 2004). QAB149 at a concentration level of 250 mg/ml (total topical dose=125 mg) was not a sensitizer in the Buehler test in the guinea pig for delayed skin sensitization potential.

2.6.6.2 Single-dose toxicity

The studies listed in the table below were reviewed in IND 66,337.

Species and (Study Number)	Administration (Batch Number)	Doses (mg/kg), base	#/animals/ group	Remarks	LD50 (mg/kg)
Rat, Wistar (001077)	Oral (0021001)	500 and 2000	4m/4f	No adverse effects	1600*
Mouse, CD-1 (001078)	Oral (0021001)	500 and 2000	4m/4f	No adverse effects	1600*
Rat, Wistar (017034)	Subcutaneous (O121002)	5, 50, 100, 200	5m/5f	Skin lesions at injection site, cold to touch, hunched posture and decreased locomotor activity. Deaths occurred at 200 mg/kg.	LD_{50} is not clearly defined. Deaths occurred at a dose of 200 mg/kg. There were No deaths at 100 mg/kg.
Mouse, CD-1 (0170135)	Subcutaneous (0121002)	5, 50, 100, 200	5m/5f	Skin lesions at the injection sites, min, cold to touch, decreased locomotor activity. Deaths occurred at 50 mg/kg in males and 200 mg/kg in the females.	LD_{50} not clearly defined. The non-lethal dose was 5 mg/kg in males and 100 mg/kg in the the females.

*The actual analytical determination of QAB149 concentrations in the test article preparations (micronized powder) showed lower than expected values by 19%. In addition, there was a variation of homogenicity by 16.3%. Therefore, the evaluation of acute toxicity was based on the analytical measured dose of 1600 mg/kg instead of the targeted dose of 2000 mg/kg.

Study Title: Comparative single-exposure inhalation study in rats using the micronized powder and HFA formulations of QAB149 (0220073).

The objective of this study was to compare the systemic exposure of a single inhalation dose of micronized powder of QAB149 (batch #0121002) with a single dose of QAB149

HFA (batch #TM-01-0046) in the male Wistar rat (10/dose group). The dose duration of exposure for both formulations was 60 minutes. The achieved dose for the micronized power formulation was 3.07 mg/kg (pulmonary deposited dose= 0.307 mg/kg) and 3.57 for the HFA formulation was 3.57 mg/kg (pulmonary deposited dose=0.357 mg/kg). One and 6 hours after dosing, blood was collected from 5 rats for toxicokinetic analysis. Twenty three hours after dosing, blood was collected from the other 5 rats for toxicokinetic analysis. The results of this study reveal all the rats were exposed to QAB149. Maximum serum concentration for QAB149, regardless of the formulation was reached 1 hour after dosing. Systemic exposure was similar when comparable doses of inhaled of QAB149 micronized powder and QAB149 HFA was administered to rats.

Study Title: Acute oral (gavage) toxicity study in dogs (0370119)
The purpose of this study was to determine the acute toxicity of QAB149 in the dog following single doses. QAB149 was administered to Beagle dogs (2/sex/dose) orally by gavage in 5% (w/v) hydroxypropylcellulose. The proposed single doses to be used in the study were 100/130mg/kg (base/salt), 500 and 1000 mg/kg. Following a single dose of 100 mg/kg, the dogs exhibited severe body weight losses, decreased food consumption, decreased blood pressures and abnormal electrocardiography. Additionally, pathological lesions (renal tubular necrosis, dilatation and cast formation) and were observed in the kidneys. The QAB149-related morbidity was so severe on day 2, the dogs were sacrificed. Based on the severe toxicity of the oral administration of QAB149, the other two doses were not given. There were no NOAEL identified in this study.

Study Title: A repeat acute oral (gavage) toxicity study in dogs (0370170)
The purpose of this study was to determine the acute toxicity of QAB149 in the dog following single doses. QAB149 was administered to Beagle dogs (2/sex/dose) orally by gavage in 5% (w/v) hydroxypropylcellulose. The proposed single doses to be used in the study were 0.1, 1 and 10 mg/kg (or 0.13, 1.2 and 13 mg/kg as the salt. There was no mortality or morbidity in the dogs in this study. Dose-related clinical signs include redden skin, ears and gums, increased respiration, increased heart rates and dry mouth. QAB149 also induced abnormal electrocardiography (sinus tachycardia). The NOAEL in this study was 10 mg/kg.

2.6.6.3 Repeat-dose toxicity
Study title: **A 13 Week Inhalation (Dry Powder Aerosol) Toxicity Study in Rats**

Key study findings:
QAB149 administered by inhalation to rats using pulmonary deposited doses of 0.03, 0. 101 and 0.309 mg/kg induced changes in the nasal cavity and larynx in the mid and high dose groups.
The changes consist of degeneration of the olfactory epithelium of the dorsal meatus in the nasal cavity and minimal to mild squamous metaplasia and squamous cell hyperplasia of the epithelium in the larynx.
The NOAEL identified in the study was 0.03 mg/kg.

Study no.: 022009

Volume # and page #: electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: April 22, 2002
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: QAB149/0121002/99.4%

Methods

Doses: Achieved doses: 0, (air control), 0.30, 1.01 and 3.09 mg/kg (pulmonary deposited doses of 0, 0.030, 0.0101 and 0.309 mg/kg)

Species/strain: Rat, Wistar Han, (Crl:WI(G1x.BRL/Han)IGSBR

Number/sex/group or time point (main study): 10/dose/sex

Route, formulation, volume, and infusion rate: Nose only inhalation/ QAB149 dry powder/~0.5 L/min

Satellite groups used for toxicokinetics or recovery:None

Age: ~5-6 weeks

Weight: Males: 115-143 g and females: 91-114 g

Sampling times:

The concentration of the total formulation in the rats' breathing zone was measured gravimetrically for groups 2, 3 and 4 throughout each exposure period. The concentration of total formulation for the vehicle control group was assessed gravimetrically toward the end of the study. Multi samples were collected for the QAB149-dosed groups; the first samples were taken as close to the start of dosing as possible.

Unique study design or methodology (if any):

The achieved doses were estimated using the following criteria:

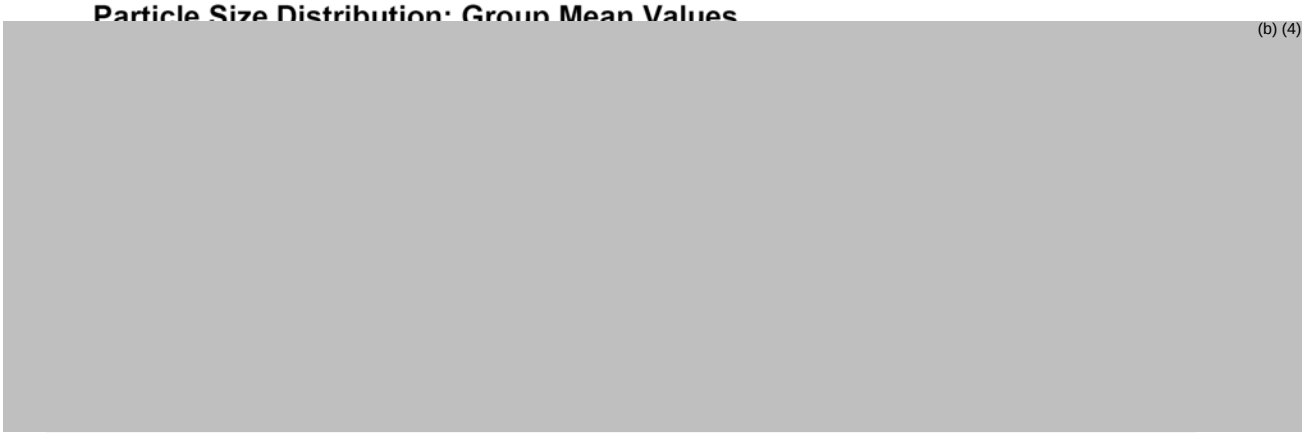
$$\text{Dose } (mg.kg^{-1}) = \frac{MV \times T \times CC}{BW}$$

$$MV = \text{Minute volume } (l.min^{-1}) = \frac{2.10 \times \text{body weight } (g)^{0.75}}{1000} \text{ [Guyton (1947)]}$$

T = Duration of exposure (min)
CC = Chamber concentration of test item ($mg.l^{-1}$)
BW = Mid week body weight (expressed in kg, means of males and females were calculated separately).

Particle size distribution group mean values are shown below:

Particle Size Distribution: Group Mean Values



Mortality: Mortality was checked twice daily (AM and PM)

Clinical signs: Clinical signs were assessed before, during and after daily exposure.

Body weights: Body weights were recorded weekly throughout the study, beginning pretrial.

Food consumption: Food consumption was recorded weekly throughout the study, beginning pretrial.

Ophthalmoscopy: The eyes of the control and high dose rats were examined pretrial and during week 13.

EKG: NA
Hematology: Hematology parameters were assessed pretrial and during week13.
Clinical chemistry: Clinical chemistry parameters were assessed pretrial and during week 13.

Urinalysis: Urinalysis parameters were assessed pretrial and during week13.

Gross pathology: Gross pathology was assessed at the end of the study, day 91.
Organ weights: The following organ weights were assesses:

Organ weight list

Adrenals*	Liver	Salivary glands*
Brain	Lung*	Spleen
Epididymides*	Ovaries*	Testes*
Heart	Pituitary	Thymus
Kidneys*	Prostate	Thyroids with parathyroids*
		Uterus

* = Paired organs weighed separately and summed for reporting and statistical evaluation

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes (x), no ( ) The larynx and nasal cavities of all rats were examined by a second pathologist as well as all gross pathologies.
The following tissues of the rats in the control and the high dose group were examined microscopically.

The following routine organs/tissues were taken from animals in Groups 1 and 4:

Abnormal tissue	Lacrimal glands (exorbital)	Spleen
Adrenals	Liver	Sternum (including bone marrow)
Aortic arch	Mammary gland	Stomach
Brain	Marrow smear	Submandibular lymph node
Caecum	Mesenteric lymph nodes	Salivary glands
Colon	Oesophagus	Testes
Duodenum	Ovaries	Thigh muscle
Epididymides	Pancreas	Thymus
Eyes with optic nerves	Pituitary	Thyroid with parathyroids
Femur (including bone marrow)	Prostate	Tongue
Harderian glands	Rectum	Urinary bladder
Heart	Sciatic nerve	Uterus
Ileum	Skin	Vagina
Implant site (microchip)	Seminal vesicles (+ coagulating gland)	
Jejunum	Spinal cord	
Kidneys		

Toxicokinetics:
Blood was collected from designated rats on days 1/2 and during week 11 at 0.5, 3, 8 and 24 hours after dosing.

Results

Mortality: There was no mortality in this study.

Clinical signs: High dose males had labored breathing on day 2 only. No other clinical signs were observed.

Body weights: Body weight gain was similar in the control and QAB149-dosed rats.

Food consumption: Food consumption was similar in the control and QAB149-dosed rats.

Ophthalmoscopy: There were no treatment-related changes in the QAB149-dosed rats when compared with the control rats.

EKG: NA

Hematology: Statistically significant decreases in platelets were observed in the male and female rats in the high dose group when compared with the controls. The decreases were 22% in males and 16% in the females.

Clinical chemistry: There were no treatment-related clinical chemistry changes observed in the rats administered QAB149.

Urinalysis: There were no treatment-related changes in urinalysis parameters in the rats administered QAB149.

Gross pathology: There was no treatment-related gross pathology in this study.

Organ weights: There were no treatment-related organ weight changes in the QAB149 treated rats when compared with the controls.

Histopathology:

> **Nasal cavity**: Degeneration of the olfactory epithelium of the dorsal meatus at Level II in the nasal cavity was described in 10/10 High dose group (Group 4) male animals and in 9/10 High dose group (Group 4) female animals. This finding was statistically significant when compared with controls. The lesion was characterised by loss of sustentacular cell cytoplasm and thinning and disorganisation of the epithelium. Similar olfactory epithelial degeneration of the dorsal meatus was also recorded at Level III in 1/10 male and 3/10 female animals in the High dose group. In a further 3/10 males and 1/10 females in the High dose group mild hyperplasia of the olfactory epithelium was recorded in the dorsal meatus at level III. This lesion was characterised by increased thickness of the epithelial layer due to proliferation and disorganisation of sustentacular and vacuolated cells. The underlying basal cells were generally uniform. Affected nasal cavities often contained increased amounts of luminal exudate.

Minimal to mild squamous metaplasia of epithelium overlying the mucous glands at the base of the epiglottis was observed in 6/10 females in the high dose group as well as 1/10 males and 1/10 females in the mid dose group. The findings in the high dose group were statistically significant. Also, minimal squamous cell hyperplasia of the epithelium overlying the U-shaped cartilage just caudal to the epiglottis was found in 3/10 females and 1/9 males in the high dose group and 1/9 females and 2/10 males in the mid dose group.

Toxicokinetics:
The toxicokinetic data show systemic exposure in all rats dosed with QAB149. There were no significant differences in systemic exposure in males and females. The systemic

exposure increased less than proportionally as the dose was increased. There was no accumulation of QAB149 in the rats. A summary of the results is shown below:

Table 2-1 TK parameters of QAB149

Sampling period	Dose of QAB149 base* (mg/kg/day)	Gender	TK parameters of QAB149				
			C_{max} (ng/mL)	t_{max}** (h)	$AUC_{(0-24h)}$ (ng.h/mL)	± SE (AUC) (ng.h/mL)	$AUC_{(0-24h)}$/dose (ng.h/mL)/(mg/kg)/day
Day 1/2	0.30 [Group 2]	Male	16.3	0.1	39.0	3.82	130
		Female	19.0	0.1	32.6	1.51	109
	1.01 [Group 3]	Male	28.2	0.33	75.6	3.72	74.9
		Female	31.0	0.33	74.1	2.21	73.4
	3.08 [Group 4]	Male	39.6	1	186	5.65	60.4
		Female	43.4	1	168	8.99	54.5
Week 11	0.30 [Group 2]	Male	17.8	0.1	30.3	2.51	101
		Female	22.1	0.1	33.3	2.12	111
	1.01 [Group 3]	Male	30.9	0.33	77.6	6.30	76.8
		Female	27.0	0.33	62.6	4.01	62.0
	3.08 [Group 4]	Male	40.1	1	169	8.03	54.9
		Female	27.0	1	129	6.38	41.9

*Overall mean achieved doses. The conversion factor for salt to free base is 0.772. Approximate inhalation times were 6 min, 20 min, and 60 min for groups 2, 3, and 4 respectively. **t_{max} is calculated from the start of inhalation.

Study title: **A 26 Week Inhalation Toxicity Study in Rats with a 4 Week recovery Period**

Key study findings:
QAB149 administered by inhalation to rats using pulmonary deposited doses of 0.031, 0.102 and 0.314 mg/kg induced increased muscle mass and decreased blood glucose in all treated dose groups (class effect for ß2 agonists).
QAB149, at the highest dose induced squamous metaplasia of the ventral larynx in 4/20 male rats. These findings were reversible after a 4 week recovery period and are thought to be rat specific and have no human relevance.
The NOAEL identified in the study was 0.102 mg/kg.

Study no.: 0220064
Volume #, and page #: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: November 7, 2002
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: QAB149HFA/YO0202, YO0902,YO841102/90%

Methods

Doses: 0, (HFA vehicle), achieved doses, 0.31, 1.02 and 3.14 mg/kg (pulmonary deposited doses, 10%- 0.031, 0.102 and 0.314 mg/kg)

Species/strain: Rat/Han Wistar (Crl: WI (Glx/BRL/Han) IGS BR
Number/sex/group or time point (main study): 20/sex/dose group
Route, formulation, volume, and infusion rate: Nose only inhalation/QAB149/HFA/*ca* 30 L min^{-1}
Satellite groups used for toxicokinetics or recovery: 10/sex/dose group for recovery and toxicokinetics
Age: 8-9 weeks on day 1 of dosing
Weight: 208-293 g (males) and 159-201 g (females)
Sampling times: The concentration of the total formulation in the rats' breathing zone was measured gravimetrically for groups 2, 3 and 4 throughout each exposure period. The concentration of total formulation for the vehicle control group was assessed gravimetrically toward the end of the study. Multi samples were collected for the QAB149-dosed groups; the first samples were taken as close to the start of dosing as possible.
Unique study design or methodology (if any):
The achieved doses were estimated using the following criteria:

$$\text{Dose } (mg.kg^{-1}) = \frac{MV \times T \times CC}{BW}$$

MV = Minute volume $(l.min^{-1}) = \frac{2.10 \times \text{body weight } (g)^{0.75}}{1000}$ [Guyton (1947)]

T = Duration of exposure (min)
CC = Chamber concentration of test item $(mg.l^{-1})$
BW = Mid week body weight (expressed in kg, means of males and females were calculated separately).

Particle size distribution group mean values are shown below:

(b) (4)

Mortality: Mortality was checked twice daily, morning and evening
Clinical signs: Clinical signs were assessed before, during and after daily exposure
Body weights: Body weights were recorded weekly throughout the study

Food consumption: Food consumption was recorded weekly throughout the study
Ophthalmoscopy: Eyes were examined pretrial, during weeks 13 and 26
EKG: NA
Hematology: Hematology parameters were assessed pretrial, during weeks 14 and 25
Clinical chemistry: Clinical chemistry parameters were assessed pretrial, during weeks 14 and 25
Urinalysis: Urinalysis parameters were assessed pretrial, during weeks 14 and 25
Gross pathology: Gross pathology was assessed at the week of the study, week 26.
Organ weight: At the end of the study, the following organs were weighed:

Adrenals*	Lungs	Submaxillary with sublingual salivary glands*
Brain	Ovaries*	Testes*
Epididymides*	Pituitary	Thymus
Heart	Prostate	Thyroids with parathyroids*
Kidneys*	Spleen	Uterus
Liver		

* = Paired organs weighed separately and summed for reporting and statistical evaluation

Histopathology: Adequate Battery: yes (x), no ( )—explain- All tissues in the control and high dose groups were evaluated microscopically. The nasal cavity and larynx tissues for groups 2 and 3 and the recovery rats were also examined microscopically.
Peer review: yes (x), no ( )

All tissues from rats 408, 409, 412, 413, 438, 439, 442 and 443 were examined by a second pathologist
Toxicokinetics: Before the start of the study, weeks 4 and 22, blood was taken from 10 rats /dose group at 0.5, 3, 8 and 24 hours after dosing.

Results

Mortality: There were no mortalities induced by the administration of QAB149. However, there were 4 unscheduled deaths in the study. Two males in the middle dose group, one with respiratory difficulties on day 32 and another with nervous behavior during the study were sacrificed. A male in the low dose group sustained a head injury and was sacrificed. A male in the recovery group had a large wound on the dorsal area and was sacrificed.

Clinical signs: Increased muscle mass was observed in the rats in the middle and high dose groups compared with the controls. Staining and loss of hair was observed in the rats in all dose groups which were thought to be due to inhalation dosing equipment and not induced by QAB149.

Body weights: Body weight gain was similar in the QAB149 –dosed rats in the low, mid and high dose groups when compared with the control group.

Food consumption: Food consumption was similar in the QAB149 –dosed rats in the low, mid and high dose groups when compared with the control group.

Ophthalmoscopy: There were no treatment-related eye changes induced by QAB149 in this study.

EKG: There were no EKG evaluation in this study.

Hematology: A significant increase in white blood cell count was found in the QAB149-treated male rats in them mid and high dose groups. The increases were 30 and 34 % in the mid and high dose groups. After a 4 week recovery period, the white blood cell counts were similar in the male rats in the control, mid and high dose groups.

Clinical chemistry: Statistically significant decreases in blood glucose levels as well as statistically significant increase in aspartame aminotransferase levels were observed in the male rats in the mid and high dose groups compared with the controls. The decreases in blood glucose levels were 15 and 23 % in the mid and high dose males while the increase in aspartate aminotransferase levels were 9 and 17%. Following a 4 week recovery period, blood glucose and aspartate aminotransferase levels were similar in control and all treated groups.

Urinalysis: There were no treatment-related urinalysis changes induced by QAB149 in this study.
Gross pathology: Macroscopic examinations revealed increases in muscle mass in QAB149 –treated rats, especially the rats in the mid and high dose groups. The muscle mass increases were still present following a 4 week recovery period. It was considered a class effect of ß 2 agonists.

Organ weights: A significant reduction in absolute and relative epididymides weights in the rats in the mid and high dose group compared with the controls. The decrease in absolute epididymide weight in the mid and high dose groups was 5 and 9% while the relative epididymide weight decreases were 8 and 11%. Following a 4 week recovery period, the absolute and relative epididymide weights were similar in the rats in the mid, high and control dose groups.
The relative liver weights were reduced in the mid and high dose males and in the high dose females when compared with the controls. The reductions in the mid and high males were 9 and 11% while the reduction in the females was 9%. Following a 4 week recovery period, these liver decreases were no longer present.

Histopathology:
Microscopic evaluations revealed that 4 out of 20 males in the high dose group had squamous metaplasia of the epithelium in the ventral larynx with U shape cartilage.

Following a 4 week recovery period, these changes were no longer evident. The similar findings were also observed in the 4 and 13 week inhalation toxicity studies in the rat. These changes are thought to be rat specific and have no human relevance.

Toxicokinetics:
Systemic exposure for inhaled QAB149 was similar in males and females following daily exposure for 26 weeks. Systemic exposure increased proportionally as the dose is increased. A summary of the toxicokinetic parameters is shown in the table (excerpted from study data provided by the sponsor) below:

Table 2-1 Mean toxicokinetic parameters of QAB149 in rat serum

Dose: 0.3 mg/kg/day							
		Days 1/2		Week 4		Week 22	
Parameter	Units	Mean Females	Mean Males	Mean Females	Mean Males	Mean Females	Mean Males
t_{max} *	H	0.6	0.6	0.6	0.1	0.1	0.6
C_{max}	ng/mL	2.59	2.72	2.22	1.92	1.89	1.34
C_{max} / dose	(ng/mL)/(mg/kg/day)	8.63	9.07	7.40	6.40	6.30	4.47
AUC(0-24h)	ng.h/mL	8.39	15.2	11.1	9.72	8.96	8.11
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	28.0	50.5	36.9	32.4	29.9	27.0
Dose: 1.0 mg/kg/day							
		Days 1/2		Week 4		Week 22	
Parameter	Units	Mean Females	Mean Males	Mean Females	Mean Males	Mean Females	Mean Males
t_{max} *	H	-	0.33	0.33	0.33	0.33	0.33
C_{max}	ng/mL	-	9.02	10.3	13.0	12.7	12.2
C_{max} / dose	(ng/mL)/(mg/kg/day)	-	9.02	10.3	13.0	12.7	12.2
AUC(0-24h)	ng.h/mL	-	28.5	39.9	36.3	39.9	37.2
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	-	28.5	39.9	36.3	39.9	37.2
Dose: 3.0 mg/kg/day							
		Days 1/2		Week 4		Week 22	
Parameter	Units	Mean Females	Mean Males	Mean Females	Mean Males	Mean Females	Mean Males
t_{max} *	H	1	1	1	1	1	1
C_{max}	ng/mL	40.2	21.6	27.6	14.4	26.6	16.6
C_{max} / dose	(ng/mL)/(mg/kg/day)	13.4	7.20	9.20	4.80	8.87	5.53
AUC(0-24h)	ng.h/mL	131	95.7	101	88.7	107	94.7
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	43.5	31.9	33.8	29.6	35.6	31.6

*: time measured from the start of inhalation
-: no results

Study title: **13 Week Inhalation Toxicity study in Dogs.**

Key study findings: QAB149 was administered by inhalation daily to dogs using pulmonary deposited doses of dry powder- 0.005, 0.03 and 0.28 mg/kg and HFA-0.26 mg/kg).

Myocardial fibrosis in the papillary muscles was observed in the 0.28 mg/kg dose group QAB149 dry powder but not observed in the HFA dose group.
Dry powder and HFA QAB149 formulations induced periportal hepatocyte glycogen vacuolation in all treated groups.
Significantly increased heart rates were observed in the high dose dry powder and HFA dose groups at 0.5-1 hour post dosing and associated with slightly increased Q-Tc values (8-17%) calculated by Friderica's formula which was returned to the values comparable to the control at 24 hours post dosing.
The systemic exposure in the dog was comparable for the dry powder and HFA formulations.
The NOAEL identified for this study was 0.03 mg/kg.
Study no.: 0420019
Volume #, and page #: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: April 26, 2004
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: QAB149/HFA/X1320304/1.6% (W/W QAB149 as a free base
QAB149MDI0.347% (w/w) QAB149 as a free base/ YO550303/101.4%

Methods

Doses: Achieved doses- 0 (air control), 0 (HFA vehicle), dry powder-0.02, 0.12 and 1.10 mg/kg. HFA- 1.02 mg/kg (pulmonary deposited doses, 25%- dry powder- 0.005, 0.03 and 0.28 mg/kg and HFA-0.26 mg/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/QAB149/HFA and QAB dry powder/ at least 5 $Lmin^{-1}$ Satellite groups used for toxicokinetics or recovery: None

Age: 11-12 months

Weight: 5.7-8.0 kg for males and 5.4-7.6 kg for females

Sampling times: Aerosol sampling were collected from a reference port in the exposure system for an appropriate time period at a sampling rate of *ca* 5.$L.min^{-1}$

Unique study design or methodology (if any):
The achieved doses were estimated using the following criteria:

$$\text{Dose (mg.kg}^{-1}) = \frac{\text{MV x T x CC}}{\text{BW}}$$

MV = Minute volume ($l.min^{-1}$) = $\frac{2.10 \times \text{body weight (g)}^{0.75}}{1000}$ [Guyton (1947)]

T = Duration of exposure (min)
CC = Chamber concentration of test item ($mg.l^{-1}$)
BW = Mid week body weight (expressed in kg, means of males and females were calculated separately).

Particle size distribution group mean values are shown below:

Particle size distribution: group mean values

(b) (4)

[redacted]

Mortality: Mortality was checked twice daily, morning and evening
Clinical signs: Clinical signs were assessed before, during and after daily exposure

Body weights: Body weights were recorded weekly throughout the study, beginning 2 weeks pretrial.

Food consumption: Food consumption was recorded weekly throughout the study

Ophthalmoscopy:
Ophthalmoscopy: Eyes were examined pretrial, during weeks 7 and 13.
EKG: Electrograph (Limb lead II) for each animal was recorded pretrial and during weeks 7 and 13. The EKGs were recorded prior to dosing, 0.5, 1, 3, 8 and 24 hours after dosing
Hematology: Hematology parameters were assessed pretrial, during weeks 7and 13.
Clinical chemistry: Clinical chemistry parameters were assessed pretrial, during weeks 7 and 13.

Urinalysis: Urinalysis parameters were assessed pretrial, during weeks7 and 13.

Gross pathology: Gross pathology was assessed at the end of the study, week 13.

Organ weights; At the end of the study, the following organs were weighed:

Adrenals*	Lungs	Submaxillary with sublingual salivary glands*
Brain	Ovaries*	Testes*
Epididymides*	Pituitary	Thymus
Heart	Prostate	Thyroids with parathyroids*
Kidneys*	Spleen	Uterus
Liver		

* = Paired organs weighed separately and summed for reporting and statistical evaluation

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )
All the dogs in all dose groups were examined microscopically.
Myocardial necrosis was evaluated using Troponin I and Mason Trichrome staining.
Liver slides were stained with Periodic Acid Schiff (PAS) to examine for the presence of glycogen.

Results

Mortality: There was no mortality in this study.

Clinical signs: Slight to moderate salivation was observed in the air and vehicle control groups and mid and high dose dry powder and high dose HFA dose groups. The dogs in the high dose dry powder and HFA dose groups also had increased heart rates (peak)~25%, 1-2 hours after dosing when compared with the air control dogs.

Body weights: Body weight gain in the males and females in mid and the high dose groups was increased when compared with the air controls. The increases were up to 25% in males and 11 % in females.

Food consumption: Food consumption in all QAB149 dosed groups was similar to that in the air control group.

Ophthalmoscopy: There were no treatment-related eye changes induced by QAB149 in the dogs in this study.

EKG: Significantly increased heart rates were observed in the high dose dry powder and HFA dose groups when compared with the vehicle controls. The increases peaked 0.5-1 hours after dosing when the increases were ~162-199% in males and 184-194 % in females. The increased heart rate was returned to the values comparable to the control values at 24 hours post dosing. The increased heart rates were associated with increased Q-Tc values calculated by Friderica's formula. The increases in QTc were 8 % in the high dose dry powder males and 17 % in the males in the HFA dose group 1 hour after dosing. The increases in QTc values were 17 % for both the females in the high dose dry powder and HFA dose groups. The QTc values for the high dose dogs and the control dogs were comparable 24 hours after dosing. The QTc changes are thought to be due to the pharmacological activity of ß2 agonists (classic effect) and can be monitored clinically.
Hematology: There were no QAB49-induced effects on the hematology parameters in the dogs in this study.

Clinical chemistry: There were increases in potassium levels in high dose dry powder and HFA males and females when compared with the air controls. The increases in high dose dry powder males were 12% and 15% in the males in the HFA high dose group. The increases in the females were 7 and 10 %. There were increases in creatine phosphokinase levels in the females in the low, mid and high dose females in the dry powder dose groups and the high dose HFA females when compared with the air controls. The increases were 39% in the low and mid dose dry powder females, 104% in the dry powder females and 96% in the high dose HFA females.

Urinalysis: There were no treatment-related urinalysis changes induced by QAB149 in this study.

Gross pathology: There were no significant gross pathology findings induced by QAB149 in the dogs in this study.

Organ weights: There were no significant effects on organ weights in the QAB149-treated dogs.

Histopathology: Microscopic evaluations revealed periportal glycogen vacuolation in the livers of males in the mid and high dose groups and female dogs in all the dose groups. The vacuolation was characterized by clumping of the cytoplasm to form glycogen lakes in the periportal areas of the liver. The glycogen was identified via periodic acid Schiff (PAS) stain. There was no histopathology associated with the vacuolation. A summary of the liver vacuolation in the dog is listed below.

HISTOLOGICAL FINDI	GROUP	GROUP TOTALS											
		Males						Females					
		Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6
	DOSE	Air Con.	Veh. Con.	Low	Inter.	High	High HFA	Air Con.	Veh. Con.	Low	Inter.	High	High HFA
LIVER		(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Glycogen vacuolation													
mild		0	0	0	1	1	0	0	0	2	1	0	1
moderate		0	0	0	1	1	1	0	0	0	1	2	0
Total Incidence		0	0	0	3	2	1	0	0	2	3	3	3
Pigment deposits		1	1	0	0	0	0	1	0	1	0	1	1
Perivasculitis, multifocal		0	0	0	0	0	0	1	0	0	0	0	0
Fibrosis, periportal		0	0	1	0	0	0	1	0	0	1	0	1

Myocardial fibrosis was observed in the left ventricular subendocardial myocardium and the papillary muscles of 1/3 males and 1/3 females in the high dose dry powder dose groups. The fibrosis was characterized by focal to multifocal areas of fibroblastic proliferation and collagen deposition and a minimum accumulation of macrophages, some of them with pigment. Myocardial fibrosis was not observed in the high dose HFA dose group.

Trivial differences in Troponon I levels were found in the dogs in the high dose dry powder and HFA dose groups compared with the control group at the end of the study. High levels of Troponin I were found in the dogs in the low (0.31 ng/mL) and mid dose dry powder groups (0.43 ng/mL) during week 13. Due to the absence of dose related effect, it was thought that the differences in Troponin I levels were due to individual variation and not an effect of QAB149 treatment.

Toxicokinetics: Dogs, in all dose groups which included dry powder and HFA formulations had systemic exposure. Systemic exposure was similar in males and females. System exposure was similar in the dogs in the dry powder and HFA formulations.
A summary of the toxicokinetic parameters in the dog is shown below (excerpted from study data submitted by the sponsor):

Table 2-1 Mean toxicokinetic parameters of QAB149 in dog serum

Dose (mg/kg/day)	0.02		0.1		1.0		1.0 HFA formulation	
Parameter	Male	Female	Male	Female	Male	Female	Male	Female
Days 1/2								
t_{max}	1.37	1.37	0.573	1.24	0.907	0.74	0.75	0.75
C_{max}	0.321	0.285	1.13	1.66	16.0	27.8	14.4	18.7
C_{max} / dose	16.0	14.3	11.3	16.6	16.0	27.8	14.4	18.7
$AUC_{(0-24h)}$	3.90	2.04	10.7	14.2	111	191	86.5	124
$AUC_{(0-24h)}$ / dose	195	102	107	142	111	191	86.5	124
Week 7								
t_{max}	1.21	0.707	2.41	1.74	2.57	1.74	1.08	0.917
C_{max}	1.39	0.904	3.42	2.74	22.5	26.8	28.2	26.3
C_{max} / dose	69.9	45.2	34.2	27.4	22.5	26.8	28.2	26.3
$AUC_{(0-24h)}$	13.9	7.79	37.5	32.6	225	247	247	267
$AUC_{(0-24h)}$ / dose	693	389	375	326	225	247	247	267
Week 13								
t_{max}	1.71	1.37	1.57	0.573	1.57	1.07	1.75	0.75
C_{max}	1.60	1.01	5.17	3.78	43.1	34.3	22.2	29.9
C_{max} / dose	80.1	50.6	51.7	37.8	43.1	34.3	22.2	29.9
$AUC_{(0-24h)}$	17.8	8.90	39.5	34.4	393	283	223	255
$AUC_{(0-24h)}$ / dose	893	444	395	344	393	283	223	255

Units: tmax: h; Cmax: ng/mL; Cmax / dose: (ng/mL)/(mg/kg/day); AUC(0-24h): ng.h/mL; AUC(0-24h) / dose: (ng.h/mL)/(mg/kg/day)

Study title: **13 Week Inhalation Toxicity Study in Dogs.**

Key study findings: QAB149 was administered by inhalation daily to dogs using pulmonary deposited doses of Certihaler dry powder- 0.005, 0.025 and 0.25 mg/kg and Aerolizer-0.27 mg/kg.

Statistically significantly increased heart rates (20-30%) were observed at 0.5-1 hour post dosing in the Certihaler mid- and high dose and Aerolizer dry powder dose groups when compared with the vehicle controls. The increased heart rate returned to the pretest value at 8-24 hours post dosing. The increased heart rates were not associated with increased Q-Tc values calculated by Van der Water's formula.

QAB149 dry powder formulations induced myocardial fibrosis in the papillary muscles in the dogs in the Certihaler 0.25 and Aerolizer 0.27 mg/kg dose groups. The Certihaler and Aerolizer dry powder formulations induced periportal hepatocyte glycogen vacuolation in all QAB149-dosed groups.

The systemic exposure (AUCs) was higher in the dogs in the Aerolizer dry powder dose group than the systemic exposure in the dogs in the Certihaler dry powder high dose group.

The NOAEL identified for this study was 0.025 mg/kg (Certihaler).

Study no.: 0420020

Volume # and page #: electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: September 7, 2004
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: QAB149 Certihaler (5.09% (w/w QAB149 as a free base) /J315/ /101.4%
QAB149 Aerolizer1.6% (w/w) QAB149 as a free base)/ x1320304/ 101.4%

Methods

Doses: Achieved doses- 0 (air control), 0 (lactose vehicle), dry powder-0.02, 0.10 and 0.98 mg/kg. Aerolizer- 1.07 mg/kg (pulmonary deposited doses, 25%- 0.005, 0.025 and 0.25 mg/kg and Aerolizer-0.27 mg/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/NA at least 5 $L.min^{-1}$

Satellite groups used for toxicokinetics or recovery: None

Age: 11-12 months

Weight: 7.6-10.5 kg for males and 5.9-9.4 kg for females

Sampling times: Aerosol sampling were collected from a reference port in the exposure system for an appropriate time period at a sampling rate of *ca* 5$L.min^{-1}$

Unique study design or methodology (if any):
The achieved doses were estimated using the following criteria:

$$\text{Dose } (mg.kg^{-1}) = \frac{MV \times T \times CC}{BW}$$

$$MV = \text{Minute volume } (l.min^{-1}) = \frac{2.10 \times \text{body weight } (g)^{0.75}}{1000} \text{ [Guyton (1947)]}$$

T = Duration of exposure (min)
CC = Chamber concentration of test item ($mg.l^{-1}$)
BW = Mid week body weight (expressed in kg, means of males and females were calculated separately).

Particle size distribution group mean values are shown below:

Particle size distribution: group mean values

(b) (4)

Mortality: Mortality was checked twice daily, morning and evening
Clinical signs: Clinical signs were assessed before, during and after daily exposure

Body weights: Body weights were recorded weekly throughout the study, beginning 2 weeks pretrial.

Food consumption: Food consumption was recorded daily throughout the study beginning 2 weeks pretrial.

Ophthalmoscopy: Eyes were examined pretrial, during weeks 7 and 13.

EKG: Electrograph (Limb lead II) for each animal was recorded pretrial and during weeks 7 and 13. The EKGs were recorded prior to dosing, 0.5, 1, 3, 8 and 24 hours after dosing

Hematology: Hematology parameters were assessed pretrial, during weeks 7 and 13.

Clinical chemistry: Clinical chemistry parameters were assessed pretrial, during weeks 7 and 13.

Urinalysis: Urinalysis parameters were assessed pretrial, during weeks 7 and 13.

Gross pathology: Gross pathology was assessed at the end of the study, week 13.

Organ weights; At the end of the study, the following organs were weighed:

Adrenals*	Lungs	Submaxillary with sublingual salivary glands*
Brain	Ovaries*	Testes*
Epididymides*	Pituitary	Thymus
Heart	Prostate	Thyroids with parathyroids*
Kidneys*	Spleen	Uterus
Liver		

* = Paired organs weighed separately and summed for reporting and statistical evaluation

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )
All the dogs in all dose groups were examined microscopically.
Myocardial necrosis was evaluated using Troponin I and Mason Trichrome staining.
Liver slides were stained with Periodic Acid Schiff (PAS) to examine for the presence of glycogen.
The following organs/tissues were taken from all animals:

Brain	Marrow smear	Submandibular lymph node
Caecum	Mesenteric lymph node	Submaxillary salivary glands
Colon	Oesophagus	Tattoo
Duodenum	Ovaries	Testes
Epididymides	Pancreas	Thymus
Eyes with optic nerves	Pituitary	Thyroids with parathyroids
Femur (including bone marrow)	Prostate	Tongue
Gall bladder	Rectum	Urinary bladder
Heart	Skeletal (thigh) muscle	Uterus, cervix and oviducts
Ileum	Sciatic nerve	Vagina
Jejunum	Skin	
Kidneys and ureter	Spinal cord	
	Spleen	

The following respiratory tract organs/tissues were taken from all animals:

Bronchial lymph node	Nasal cavity (anterior)	Retropharyngeal lymph node
Carina	Nasal cavity (posterior)	Trachea
Larynx	Pharynx	

Lungs: left anterior, middle and posterior; right anterior, middle and posterior, accessory

Abnormal tissue	Lacrimal glands	Sternum
Adrenals	Liver	Stomach
Aortic Arch	Mammary gland	Sublingual glands

Results

Mortality: There was no mortality in this study.

Clinical signs: Slight to moderate salivation was observed in the air and vehicle control groups as well as all the QAB149-treated dogs. The dogs in the high dose Certihaler dry powder and the Aerolizer dry powder dose groups also had increased heart rates (peak)~160-200%, 1-2 hours after dosing when compared with the air control dogs.

Body weights: Body weight gain in the males in the Aerolizer dry powder group was statistically significantly increased (~50%) when compared with the vehicle controls. Females in the mid and high dose Certihaler dry powder dose group as well as the females in the Aerolizer dry powder dose group had increases in body weight gain when

compared with the vehicle controls. The increases were 43 and 57% in the female dogs in the mid and high dose Certihaler dry powder dose group and 100 % in the female dogs in the Aerolizer dry powder dose group. The increases were not statistically significant.

Food consumption: There were no significant differences in food consumption between the QAB149- treated dogs and the control dogs.

Ophthalmoscopy: There were no treatment-related eye changes induced by QAB149 in the dogs in this study.

EKG: Significantly increased heart rates (up to approximately 20-30%) were observed in the mid and high dose Certihaler dry powder dose groups and the Aerolizer dry powder dose groups when compared with the vehicle controls. The increases peaked 0.5-1 hours after dosing in both males and females and returned to the pre-test levels ranged from 8 to 24 hours. The increased heart rates were not associated with increased Q-Tc values calculated by Van der Water's formula.

Hematology: There were no significant QAB49-induced effects on the hematology parameters in the dogs in this study.

Clinical chemistry: There were significant increases in potassium levels in the mid and high dose Certihaler dry powder and the Aerolizer dry powder males as well as the mid Certihaler dry powder females and the Aerolizer females when compared with the vehicle controls. The increases in the males in the mid and high dose Certihaler dry powder dose group were 8 and 15% while the increases in the males in the Aerolizer dry powder dry powder was 10%. The increases in the females dogs in the mid dose Certihaler dry powder dose group were 16% and the increases in the females in the Aerolizer dry powder dose group were 24% This is a class effect for beta 2 adrenergic agonists. Increases in potassium levels have the potential to lead to EKG changes, e.g., ventricular fibrillation.

Urinalysis: There were no treatment-related urinalysis changes induced by QAB149 in this study.

Gross pathology: There were no significant gross pathology findings induced by QAB149 in the dogs in this study.

Organ weights: There were no significant effects on organ weights in the QAB149-treated dogs.

Histopathology: Microscopic evaluations revealed periportal glycogen vacuolation in the livers of males in the mid and high dose groups and female dogs in all the treated groups. The vacuolation was characterized by clumping of the cytoplasm to form glycogen lakes in the periportal areas of the liver. The glycogen was identified via periodic acid Schiff (PAS) stain. There was no histopathology findings associated with the vacuolation. A summary of the liver vacuolation in the dog is listed below.

Best Available Copy

Summary of histological findings (continued)

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS Males Grp 1 Air Con.	Grp 2 Veh. Con.	Grp 3 Low	Grp 4 Inter.	Grp 5 High	Grp 6 Aero. Form.	Females Grp 1 Air Con.	Grp 2 Veh. Con.	Grp 3 Low	Grp 4 Inter.	Grp 5 High	Grp 6 Aero. Form.
ALIMENTARY SYSTEM													
INTESTINES											(1)		
Haemorrhage, mucosal											1		
LIVER		(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
No abnormality detected		2	0	1	1	0	0	1	3	0	0	0	0
Periportal hepatocyte vacuolation													
minimal		0	0	1	1	3	1	0	0	2	3	1	2
mild		0	0	0	0	0	1	0	0	0	0	2	1
Total Incidence		0	0	1	1	3	2	0	0	2	3	3	3
Periodic acid Schiff with diastase stain examined		3	3	3	3	3	3	3	3	3	3	3	3
Oval cell hyperplasia, with inflammation, focal		0	1	0	0	0	0	0	0	0	0	0	0
Focal necrosis		0	0	0	0	0	0	0	0	1	0	2	0
Inflammatory cell foci		1	2	1	2	0	1	2	0	1	3	0	2

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

Pathology File Ref.: PLAFOR_664966_MICRO_ROCKEEP1.SPL

Myocardial fibrosis was observed in the left ventricular subendocardial myocardium and the papillary muscles of 2/3 males in the high dose Certihaler dry powder dose group as well as all of the males in the Aerolizer dry powder dose The fibrosis was characterized by focal to multifocal areas of fibroblastic proliferation and collagen deposition and a minimum accumulation of macrophages, some of them with pigment. A summary of the cardiovascular findings are shown below:

HISTOLOGICAL FINDINGS	GROUP DOSE	GROUP TOTALS Males Grp 1 Air Con.	Grp 2 Veh. Con.	Grp 3 Low	Grp 4 Inter.	Grp 5 High	Grp 6 Aero. Form.	Females Grp 1 Air Con.	Grp 2 Veh. Con.	Grp 3 Low	Grp 4 Inter.	Grp 5 High	Grp 6 Aero. Form.
CARDIOVASCULAR SYSTEM													
HEART		(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
No abnormality detected		2	2	2	2	1	0	2	3	1	3	3	1
Mesothelial hyperplasia, atrial													
minimal		1	0	1	0	0	0	1	0	0	0	0	0
mild		0	0	0	1	0	1	0	0	1	0	0	0
Total Incidence		1	0	1	1	0	1	1	0	1	0	0	0
Myocardial fibrosis, papillary muscle													
minimal		0	0	0	0	2	1	0	0	0	0	0	1
mild		0	0	0	0	0	1	0	0	0	0	0	0
moderate		0	0	0	0	0	1	0	0	0	0	0	1
Total Incidence		0	0	0	0	2	3	0	0	0	0	0	2
Inflammatory cell foci, atrial		0	0	0	0	0	0	0	0	1	0	0	0
No abnormality detected with trichrome stain		3	3	3	3	2	0	3	3	3	3	3	1

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

The Troponon I levels were slightly increased in one female in the Certihaler dry powder high dose group and one female in the Aerolizer dry powder dose group. Troponin I levels were not detected in the dogs in the low and mid Certihaler dry powder dose group or in the males in the Aerolizer dry powder dose group.

Toxicokinetics: Dogs, in all dose groups which included Certihaler and Aerolizer dry powder formulations had systemic exposure. Systemic exposure was slightly higher in males than females. System exposure (AUCs) was higher in the dogs treated with the Aerolizer dry powder formulation than the systemic exposure in the dogs in the Certihaler dry powder formulation.
A summary of the toxicokinetic parameters in the dog is shown below:

Table 2-1 Mean toxicokinetic parameters of QAB149 in dog serum

Dose (mg/kg/day)	0.02		0.1		1.0		1.0 (Aeroliser formulation)	
Parameter	Male	Female	Male	Female	Male	Female	Male	Female
Days 1/2								
t_{max}	0.55	1.38	0.877	0.71	0.967	0.8	1.3	1.13
C_{max}	0.223	0.184	4.39	1.95	22.6	25.4	38.6	26.6
C_{max} / dose	11.2	9.23	43.9	19.5	22.6	25.4	38.6	26.6
$AUC_{(0-24h)}$	2.58	1.23	36.4	17.6	187	141	320	221
$AUC_{(0-24h)}$ / dose	129	61.4	364	176	187	141	320	221
Week 7								
t_{max}	0.55	1.38	1.71	1.38	2.63	0.633	2.63	1.97
C_{max}	0.300	1.09	2.11	1.65	17.8	32.0	34.7	36.5
C_{max} / dose	15.0	54.8	21.1	16.5	17.8	32.0	34.7	36.5
$AUC_{(0-24h)}$	4.37	12.7	21.2	18.6	231	264	381	369
$AUC_{(0-24h)}$ / dose	218	635	212	186	231	264	381	369
Week 13								
t_{max}	2.22	1.38	2.38	2.38	2.3	0.967	1.3	1.97
C_{max}	0.723	0.380	1.96	1.80	23.7	40.0	50.3	39.0
C_{max} / dose	36.2	19.0	19.6	18.0	23.7	40.0	50.3	39.0
$AUC_{(0-24h)}$	9.00	5.18	19.2	21.2	297	342	503	396
$AUC_{(0-24h)}$ / dose	450	259	192	212	297	342	503	396

t_{max}: time measured from the start of inhalation
Units: t_{max}: h; C_{max}: ng/mL; C_{max} / dose: (ng/mL)/ (mg/kg/day); $AUC_{(0-24h)}$: ng.h/mL;

Study title: **39 Week Inhalation Toxicity study in Dogs with a 4 Week Recovery Period.**

Key study findings: QAB149 administered to dogs daily by inhalation for 39 weeks using pulmonary deposited doses up to 0.08 mg/kg induced periportal liver hepatocyte vacuolation in all drug –treated dogs. This finding was due to the deposition of glycogen in the liver and was reversible after a 4 week recovery period.

QAB149 also induced increase muscle mass/ body weight gain, particularly at the highest dose (class effect) which has no human relevance.

QAB149 induced myocardial inflammatory cell infiltration in 2 male dogs in the high dose group as well as one female dog in the control group. Myocardial fibrosis observed

in the 4-week and 13-week dog studies was not detected in this study. The reason may be due to the fact that the highest pulmonary deposited dose in this study were lower than the pulmonary deposited doses of 0.24 and above in the 4 and 13 week dog inhalation toxicity studies at which myocardial fibrosis was observed.

The NOAEL was 0.08 mg/kg.

Study no.: 0220065
Volume #, and page #: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: November 7, 2002
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: QAB149-1.6% (w/w) QAB149 as a free base) /YO120202, YO790902, YO831002 andYO841102/ 101.4%

Methods

Doses: Achieved doses-0 (HFA vehicle), 0.03, 0.10 and 0.31 mg/kg (pulmonary deposited doses, 25%= 0.0075, 0.025 and 0.08 mg/kg)

Species/strain: Dog, Beagle

Number/sex/group or time point (main study): 4/sex/dose group

Route, formulation, volume, and infusion rate: Face mask inhalation/ QAB149/HFA/ at least 5L.min^{-1}

Satellite groups used for toxicokinetics or recovery: 2/sex/dose group for control and high dose

Age: 10-11 months

Weight: 6.8-9.9 kg for males and 6.2-8.5 kg for females

Sampling times: Aerosol sampling were collected from a reference port in the exposure system for an appropriate time period at a sampling rate of *ca* 5.1.min^{-1}

Unique study design or methodology (if any):
The achieved doses were estimated using the following criteria:

$$\text{Dose (mg.kg}^{-1}) = \frac{\text{MV x T x CC}}{\text{BW}}$$

$$\text{MV} = \text{Minute volume (l.min}^{-1}) = \frac{2.10 \times \text{body weight (g)}^{0.75}}{1000} \text{ [Guyton (1947)]}$$

T = Duration of exposure (min)
CC = Chamber concentration of test item (mg.l^{-1})
BW = Mid week body weight (expressed in kg, means of males and females were calculated separately).

Particle size distribution group mean values are shown below:

Particle Size Distribution: Group Mean Value

(b) (4)

Mortality: Mortality was checked twice daily, morning and evening
Clinical signs: Clinical signs were assessed before, during and after daily exposure

Body weights: Body weights were recorded weekly throughout the study, beginning 2 weeks pretrial.

Food consumption: Food consumption was recorded weekly throughout the study
:
Ophthalmoscopy: Eyes were examined pretrial, during weeks 13, 39 and at the end of the recovery period.

EKG: Electrograph (Limb lead II) for each animal was recorded pretrial and during weeks 13, 39 and after the recovery period. The EKGs were recorded prior to dosing, 0.5, 1, 3, 8 and 24 hours after dosing
Hematology: Hematology parameters were assessed pretrial, during weeks 13, 39 and after the recovery period.
Clinical chemistry: Clinical chemistry parameters were assessed pretrial, during weeks 13, 39 and after the recovery period.

Urinalysis: Urinalysis parameters were assessed pretrial, during weeks 13, 39 and after the recovery period

Gross pathology: Gross pathology was assessed at the week of the study, week 39 and after the recovery period.

Organ weights; At the end of the study, the following organs were weighed:

Adrenals*	Lungs	Submaxillary with sublingual salivary glands*
Brain	Ovaries*	Testes*
Epididymides*	Pituitary	Thymus
Heart	Prostate	Thyroids with parathyroids*
Kidneys*	Spleen	Uterus
Liver		

* = Paired organs weighed separately and summed for reporting and statistical evaluation

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )
All the dogs in all dose groups were examined microscopically.
Myocardial necrosis was evaluated using Troponin I and Mason Trichrome staining.
Liver slides were stained with Periodic Acid Schiff (PAS) to examine for the presence of glycogen.

Results

Mortality: There was no mortality in this study.

Clinical signs: There were no significant clinical symptoms in the dogs induced by QAB149 in this study.

Body weights: Body weight gain in the males and females in the high dose group was increased when compared with the controls. The statistically significant increase in males was 12 % while the increase in females was 5%. After a 4 week recovery period, body weight gain was similar in the males and remained increased by 5% in females when compared with the controls. QAB149 induced increase in muscle mass in rats and dogs as a class effect, but this effect has no human relevance.

Food consumption: Food consumption was similar in the QAB149 –dosed dogs in the low, mid and high dose groups when compared with the control group.

Ophthalmoscopy: There were no treatment-related eye changes induced by QAB149 in he dogs in this study.

EKG: Compared with the predose value, an increase ~5-5% in the heart rate was observed in the high dose group at the 39 week evaluations. The increase was observed only ½- 1 hour after dosing. The increase in heart rate was associated with a slight increase (~5%) of QTc values (calculated by Bazitt's and Friderica's formula). The heart rate increase was not observed after a 4 week recovery period.

Hematology: There were no QAb149-induced effects on the hematology parameters in the dogs in this study.

Clinical chemistry: Creatinine levels were statistically significant increased in the dogs in all dose groups when compared with the controls. In the males, the increases were24, 22 and 31 %, respectively in the low, mid and high dose groups while the increases in the females were 9, 17 and 14%, respectively. The creatinine levels remained increased after a 4 week recovery period, 25% in the high dose males and 20 % in the high dose females.

Urinalysis: There were no treatment-related urinalysis changes induced by QAB149 in this study.

Gross pathology: There were no gross pathology findings induced by QAB149 in the dogs in this study.

Organ weights: There were no effects on organ weights in the QAB149-treated dogs.

Histopathology: Liver hepatocyte vacuolation (periportal) was observed in all the male and female dogs in the high dose group, all the males in the mid dose and ¾ males in the low dose group, and 2/4 females in the mid dose and ¾ females in the low dose group. The vacuolation was graded as minimal to mild. The cells with vacuolation were slightly enlarged and showed rarefaction of the cytoplasm. In some livers, the vacuolated cells were localized to one area while others were widespread. PAS stain confirms that the vacuolation was related to glycogen deposition. These findings were reversible following a 4 week recovery period.

A summary of the findings in the liver is shown below:

LIVER	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Hepatocyte vacuolation, widespread								
minimal	0	3	4*	4*	0	2	1	2
mild	0	0	0	0	0	1	1	2
Total Incidence	0	3	4*	4*	0	3	2	4*
Centrilobular hepatocyte vacuolation	0	0	0	0	1	0	1	0
Inflammatory cell foci, scattered	4	3	1	1	3	2	3	3
Periodic acid Schiff stain examined	4	4	4	4	4	4	4	4
Oil red O stain positive for fat	1	0	0	0	2	1	0	2

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

Pathology File Ref.: PLAFOR_MICMAIN_SD_17NOV03_KEEP.SPL

A small foci of minimal inflammation was observed in the myocardium of 2 males in the high dose group as well as one female in the control group. A summary of the findings in the heart is shown below. Since the myocardial inflammation occurred at low incidence with minimal severity and also observed in one control animal, it is not considered a significant toxicity finding. Myocardial fibrosis observed in the 4-week and 13-week dog studies was not detected in this study. The reason may be due to the fact that the highest pulmonary deposited dose in this study were lower than the pulmonary deposited doses of 0.24 and above in the 4 and 13 week dog inhalation toxicity studies at which myocardial fibrosis was observed.

Trace amounts of Troponin I (from 0.18 to 1.53 ng/mL) were found in the samples from the dogs in the control and high dose groups. There were no differences in the samples from control and high dose dogs. Troponin I was not detectable in the samples from the dogs in the other dose groups.

HISTOLOGICAL FINDINGS	GROUP TOTALS							
	Males				Females			
GROUP DOSE	Grp 1 Veh. Con.	Grp 2 Low	Grp 3 Inter.	Grp 4 High	Grp 1 Veh. Con.	Grp 2 Low	Grp 3 Inter.	Grp 4 High
HEART	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
No abnormality detected	3	4	4	2	3	3	4	4
Inflammatory cell infiltration	0	0	0	2	1	0	0	0
Inflammatory cell foci, epicardial	0	0	0	0	0	1	0	0
Valvular haematocyst	1	0	0	0	0	0	0	0
Masson Trichrome stain examined	4	4	4	4	4	4	4	4

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

Toxicokinetics: Systemic exposure for inhaled QAB149 was similar in males and females following daily exposure for 39 weeks. Systemic exposure increased proportionally as the dose is increased. A summary of the toxicokinetic parameters is shown in the table below:

Table 2-2 Mean toxicokinetic parameters of QAB149 in dog

Dose (mg/kg/day)		0.03		0.1		0.3	
Parameter	Units	Male	Female	Male	Female	Male	Female
Weeks 1/2							
t_{max}	h	0.375	1.25	1.13	1.75	1.42	0.583
C_{max}	ng/mL	0.594	0.572	1.73	1.58	3.79	5.10
C_{max} / dose	(ng/mL)/(mg/kg/day)	19.8	19.1	17.3	15.8	12.6	17.0
AUC(0-24h)	ng.h/mL	4.37	4.44	16.9	17.7	40.5	56.1
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	146	148	169	177	135	187
Week 13							
t_{max}	h	0.5	1.13	0.625	1.38	3.08	1
C_{max}	ng/mL	0.487	0.877	1.23	1.76	5.11	4.62
C_{max} / dose	(ng/mL)/(mg/kg/day)	16.3	29.2	12.3	17.6	17.0	15.4
AUC(0-24h)	ng.h/mL	4.14	7.53	13.9	17.5	69.8	42.7
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	138	251	139	175	233	142
Week 39							
t_{max}	h	0.5	1.75	0.5	0.75	0.583	0.417
C_{max}	ng/mL	0.641	0.43	2.14	1.90	9.79	9.24
C_{max} / dose	(ng/mL)/(mg/kg/day)	21.4	14.3	21.4	19.0	32.6	30.8
AUC(0-24h)	ng.h/mL	5.39	3.60	18.4	16.2	90.1	87.2
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	179	120	184	162	301	290

2.6.6.4 Genetic toxicology

Genetic assays for QAB149 were reviewed in IND 66, 337, (see pharmacology review dated May 6, 2008) and are cross-referenced for this NDA review. Genetic toxicology assays, i.e., Ames bacterial reverse mutation test, Mammalian Chromosomal Aberration test in the V79 Chinese hamster cell and bone marrow micronucleus test (rat) reveal that QAB149 (indacaterol) is not genotoxic under the conditions tested.

2.6.6.5 Carcinogenicity

Carcinogenicity studies were conducted in the mouse and the rat. The doses in the mouse were 0, (air), 100, 300 and 600 mg/kg while the doses in the rat were 0 (air), 0 (air), 0.21, 0.62 and 2.09 mg/kg. The studies were judged to be negative by the Executive CAC (see meeting minutes dated August 4, 2009). However, there were increased incidences of uterine endometrial stromal polyps in the 26-week oral (gavage) carcinogenicity study with female CB6F1/TgrasH2 hemizygous mice and ovarian leiomyomas in the 24-month inhalation oncogenicity study with female Sprague-Dawley rats which were statistically significant by trend test, but not significant by pairwise comparison. These tumors have been observed with other β2-adrenergic agonists and might be attributed to treatment despite the lack of statistical significance.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

The fertility and early embryonic development studies were reviewed in IND 48,649 (see pharmacology review dated April 27, 2004) and are cross referenced for this NDA review.

Embryofetal development

Embryofetal development studies were reviewed in IND 66, 337 (see pharmacology review dated May 6, 2008) and are cross referenced for this NDA review. An additional inhalation embryofetal development study was submitted in this NDA and is reviewed below.

Study title: **An Inhalation Embryo Fetal Development Study in Rats**

Key study findings:
QAB149 was administered by inhalation to pregnancy rats on days 6-17 of gestation using pulmonary deposited doses of 0.021, 0.064, 0.212 and 0.270 mg/kg.
QAB149 at the high dose increased body weight gain by 38%.
There were no QAB149 effects on fetal weight, major malformation, minor external, visceral or skeletal anomalies or other skeletal variants up to a dose of 0.270 mg/kg.
The NOAEL for the dams was 0.212 mg/kg and for fetuses 0.270 mg/kg.
The doses in this study induced very limited maternal toxicity, and it is approximately 20 fold greater for maternal toxicity than the doses proposed for clinical use on an AUC basis.

Study no.: 0670755
Volume #, and page #: Electronic transmission
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: April 17, 2007
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: QAB149 (19.2%QAB149-free base), 1 % magnesium stearate, 92.9% lactose/T0050107/101.4%
NVA237 64mg/g powder inhalation (6.4% NVA237 free base), 1% magnesium stearate, 79.8% lactose/T0040107/98.0%

Methods

Doses: QAB149 (achieved doses- 0 (air), 0 (lactose and magnesium stearate), 0.21, 0.64, 2.12 and 2.70 mg/kg (pulmonary deposited doses: 0, 0, 0.021, 0.064, 0.212 and 0.270 mg/kg- NVA237 (achieved doses- NVA237-0.07, 0.21, 0.71 and 0.62 mg/kg (pulmonary deposited doses-0, 0, 0.007, 0.021, 0.0071 and 0.062 mg/kg).

Species/strain: Rats, Wistar Hannover Crl WI (Glx/BRL/Han) IGS BR

Number/sex/group: 22 mated females/dose group/

Route, formulation, volume, and infusion rate: Nose only inhalation/ QAB149(19.2% QAB149-free base), 1 % magnesium stearate, 92.9% lactose/ NVA237 64mg/g powder inhalation(6.4% NVA237 free base), 1% magnesium stearate, 79.8% lactose/95% of the target concentration (t_{95})/40-50 liters /minutes

Satellite groups used for toxicokinetics: 3 mated females for toxicokinetics

Study design: All rats were administered QB149 by nose only inhalation daily for up to 150 minutes, days 6-17 of gestation. The achieved dose was calculated as follows:

Achieved total dose of active test article[1] (mg/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 (mg/L)	=	chamber concentration of active test material determined by chemical analysis
T (min)	=	treatment time
BW (kg)	=	mean body weight 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.

Parameters and endpoints evaluated:

The following parameters were evaluated: clinical signs, body weights, food consumption, toxicokinetic evaluation, gross observations at necropsy, uterine findings, fetal weight and external, visceral and skeletal findings.

Results

Mortality (dams): The dams were examined twice daily. There was no mortality in this study.

Clinical signs (dams): The dams were examined twice daily. There were no significant clinical signs in this study.

Body weight (dams): Body weight was measured on days 0, 3, 6, 9, 12, 15, 17, 18 and 21 of gestation. On day 21 of gestation bodyweight gain was similar in the treated and the air and placebo dose groups.

Food consumption (dams): Food consumption was measured on days 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18 and 18 to 21 of gestation. Food consumption was significantly increased in the QAB-treated dams when compared with placebo controls. The increases ranged from 11-16%.

Toxicokinetics:

- 0 hour (immediately after the completion of inhalation), 30 minutes, 1 hour, 3 hours and 8 hours after the completion of inhalation and 24 hours after the initiation of inhalation on an individual animal basis in dose groups 3 to 7.
- 0 hour (immediately after the completion of inhalation) and 30 minutes after the completion of inhalation on an individual animal basis in the air and vehicle control in groups 1 and 2.

Systemic exposure was observed in all QAB149 –treated rats. Systemic exposure of QAB149 was not proportionally increased as the dose was increased. A summary of the toxicokinetic parameters is shown below:

Table 4-3 Mean toxicokinetic results in rat plasma on gestation day 17

Parameter	Units	QAB149	NVA237
	Group 3: QVA149 low		
Mean achieved dose	mg/kg/day of QAB149 base or quaternary cation of NVA237	0.23	0.07
t_{max}	h	0*	0*
C_{max}	ng/mL	21.9	5.14
$AUC_{(0-24h)}$	ng.h/mL	25.4	10.2
* end of inhalation			

Parameter	Units	QAB149	NVA237
	Group 4: QVA149 mid		
Mean achieved dose	mg/kg/day of QAB149 base or quaternary cation of NVA237	0.59	0.21
t_{max}	h	0*	0*
C_{max}	ng/mL	24.4	7.77
$AUC_{(0-24h)}$	ng.h/mL	50.9	19.6
* end of inhalation			

Parameter	Units	QAB149	NVA237
	Group 5: QVA149 high		
Mean achieved dose	mg/kg/day of QAB149 base or quaternary cation of NVA237	2.31	0.70
t_{max}	h	0*	0*
C_{max}	ng/mL	32.1	21.1
$AUC_{(0-24h)}$	ng.h/mL	160	71.5
* end of inhalation			

Parameter	Units	QAB149	NVA237
	Group 6: NVA237 high		
Mean achieved dose	mg/kg/day of QAB149 base or quaternary cation of NVA237	0	0.66
t_{max}	h	-	0*
C_{max}	ng/mL	-	10.8
$AUC_{(0-24h)}$	ng.h/mL	-	55.0
* end of inhalation			

Parameter	Units	QAB149	NVA237
	Group 7: QAB high		
Mean achieved dose	mg/kg/day of QAB149 base or quaternary cation of NVA237	2.48	-
t_{max}	h	0*	-
C_{max}	ng/mL	74.4	-
$AUC_{(0-24h)}$	ng.h/mL	267	-

* end of inhalation

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): Pregnancy rate was 96% for all dose groups. QAB149 had no effect on corpora lutea, implantation rate, live fetuses, dead fetuses, resorptions, sex ratios, pre- implantation and post- implantation loses.

Offspring (malformations, variations, etc.):
There were no QAB149 effects on fetal weight, major malformation, minor external, visceral or skeletal anomalies or other skeletal variants.

Prenatal and postnatal development

Study title: **A Subcutaneous Pre-and Postnatal Development Study in Rats**

Key study findings: Maternal body weight gain (~12% for both dose groups) was statistically significant increased in the mid and high dose groups.
For F0, stillborn pups were greater (14.3%) in the high dose group than in the control group (4.9%).
For F1, there was a statistical significant, drug related decrease in number of pregnant dam in the high dose compared with the controls. There were no drug-related effects on neurobehavioral and other reproductive parameters of the F_1 offspring.
There were no significant treatment related effects on F2 observations.
The NAOEL for maternal toxicity was 1 mg/kg and for fetal toxicity was 0.3 mg/kg.

Study no.: 0270185
Volume # and page #: electronic transmission
Conducting laboratory and location: Norvatis Pharmaceuticals Corporation, East Hanover, New Jersey
Date of study initiation: December 22, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: QAB149 maleate micronized (base)/ 0121002/99.1%

Methods

Doses: 0 (PEG400, 0.9%sodium chloride), 0.1, 0.3 and 1.0 mg/kg

Species/strain: Rat, Wistar Hannover, Crl: WI (GIx BRL/Han)GS BR
Number/sex/group: 24 females/sex groups
Route, formulation, volume, and infusion rate: Subcutaneous/2 mL/kg
Satellite groups used for toxicokinetics: None
Study design: QAB149 was administered sc. The duration of dosing was implantation of the embryo to the end of lactation for F_0 females. Postpartum day 4 through 20 for F_1 offspring.
Parameters and endpoints evaluated: Female rats (F0): in –life observations, body weight, food consumption, parturition and necropsy; offspring (F1): in –life observations, body weight, plasma drug concentrations and skeletal (sternum and cranium) examinations of weanings and toxicokinetics of F1 offspring.

Results

F_0 in-life:
Mortality: Observed twice daily and once on the weekends- There were no QAB149-related deaths in this study.
Clinical signs: Assessed once daily during predose phase and twice daily during the dosing phase.
Body weight: Body weight recorded days 0, 3, 6, 9, 12, 15, 18 and 20. There were slight body weight gain in the dams in the mid and high dose groups. The increases, statistically significant in the high dose group, was approximately 3%
Food consumption: Food consumption was calculated on days 3, 6, 12, 15, 18 and 20. Food consumption was similar in the QAB149-treated dams and the control females during the gestation and the lactation periods.
Delivery observations:
F_0 females were observed day 20 of gestation until the delivery was completed, day 25. The percent of pregnant females was similar in control and QAB149-treated dose groups~90%. There was statistically significant increases in the duration of gestation compared with the controls (22.2 days) at the low (22.6 days) and mid (22.7 days) dose group as well as an non-statistically significant increase in duration of gestation in the high dose females (22.5 days) group. As the differences in duration of gestation were in small magnitude and not in a dose-dependent manner, it was not considered toxicologically significant.
F_0 necropsy:
There were no drug-related necropsy findings in the dams in this study.
F_1 physical development:
F_1 Offspring Number, Viability and in –Life Observations:

3.6.1 Pre-weaning evaluations

Test or measurement	Pups; age (days post partum)
Viability, mortality, clinical observations	All pups; days 0 to 21
Sex ratio	All pups; day 0
Righting reflex	All pups; days 0-2
Pinna detached	All pups; days 2-5
Eye opening	All pups; days 14-17

3.6.2 Post-weaning evaluations

On weaning at day 21 post partum the litters were standardized to two per sex whenever possible according to a randomization provided by L Fortin, MSc, CTBR.

Test or measurement	Animals; age (days post partum)
Mortality	All remaining animals, twice daily (am and pm) on weekdays and once daily on weekends, holidays and plant emergencies/closings.
Clinical observations	All remaining animals; at least once daily
Acoustic startle	All remaining animals, day 28
Pupillary reflex	All remaining animals, day 35
Vaginal opening	All remaining females, days 32-40
Preputial separation	All remaining males; days 42-48
Open field motor activity	All remaining animals; day 56-58 days
Passive avoidance – learning/acquisition	One per sex per litter if possible; day 63 ± 2 days
Passive avoidance – retention/memory	One per sex per litter if possible; day 70 ± 2 days
M-maze – learning/acquisition	One per sex per litter if possible; day 63 ± 2 days
M-maze – retention/memory	One per sex per litter if possible; day 70 ± 2 days
F_1 mating	One per sex per litter if possible; at least 10 weeks of age
Individual body weights	Males: Once weekly (days 28, 35, 42, etc.) until necropsy
	Females: once weekly until necropsy and, if selected for fertility, gestation days 0, 3, 6, 9, 13

The number of still born pups was greater in the high dose group when compared with the controls (14.3 % in the high dose and 4.9 % in the control).

F_1 Offspring Body weight:

At the end of the observation period, day 70, body weight gain was similar in the pups in the QAB149 treated dose groups and the controls.

F_1 Offspring Morphology:

There was no significant differences in developmental parameters in the pups in the QAB149 treated dose groups and the pups in the control group.

F_1 behavioral evaluation:

There were no significant differences in the behavior of the pups in the QAB149 treated dose groups and the pups in the control group.

F_1 reproduction:

There was no drug-related effect on mating but a statistically significant decrease in the number of pregnant dams in the high dose group compared with the controls. There was no effect on corpora lutea, number of implantations sites, pre-/post implantation loss, total/early resorptions and number of viable fetuses.

F_2 findings:
There were no significant drug-related findings for F2 off spring.

Study title: **A Subcutaneous Neonatal and Juvenile Development Dose Range-Finding Study in Rats**

Key study findings: The objective of this study was to obtain information for the selection of doses for a subsequent definite study to determine potential adverse effects of QAB149 on the postnatal development of the neonatal and juvenile rat, day 4-20 post partum. Results of the study reveal no drug-related effects on mortality, clinical signs or necropsy findings. The NOEL was identified as the high dose, 1.3 mg/kg.

Study no.: 0270162
Volume # and page #: electronic transmission
Conducting laboratory and location: Norvatis Pharmaceuticals Corporation, East Hanover, NJ
Date of study initiation September 27, 2002
GLP compliance: No
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: QAB149 maleate micronized/ 012100299.4%

Methods

Doses: (base), 0. 0.13, 0.39 and 1.3 mg/kg
Species/strain: Rat, Wistar Hannover, Crl: WI (GIx BRL/Han)GS BR

Number/sex/group: 0, (8/sex/dose group), 0.13 mg/kg, (18 males and 24 females), 0.39 mg/kg (24 males and 18 females) and 1, 3 mg/kg (24 males and 24 females)
Route, formulation, volume, and infusion rate: Subcutaneous/QAB149, PEG400, 0.9% sodium chloride/2 mL/kg/NA
Satellite groups used for toxicokinetics: None
Study design: On post partum day 4, litters were assigned to dose groups; sc dosing was initiated on day4 and continued through day 28. On day 28, the study animals were sacrificed and a gross evaluation of the major viscera was conducted.
Parameters and endpoints evaluated: Mortality, clinical signs, body weight, post-mortem examinations and gross examinations

Results

F_0 in-life:
Mortality: Once daily, there was no mortality in this study.

Clinical signs: Once daily before dosing- there were no significant drug-related clinical signs in this study,

Body weight: Daily post partum days 4-28 – On day 19, body weight gain increases were 11, 11 and 5%, respectively in the low, mid and high dose males and 19, 11 and 6 %, respectively in the females.

F_0 necropsy: Day 21 for maternal animals and day 28 for the pups, there were no drug-related findings in the maternal rats or pups in this study.
F_1 physical development: Not evaluated

F_1 behavioral evaluation: Not evaluated

F_1 reproduction: not evaluated

F_2 findings: Not evaluated

2.6.6.7 Local tolerance
Local tolerance studies conducted with QAB149 were reviewed in the pharmacology review of IND 48, 649 dated April 27, 2004.

2.6.6.8 Special toxicology studies

Study title: **Buehler test in Guinea Pigs for Delayed Skin Sensitization Potential (Study No. 0220082)**

The study was reviewed in the pharmacology review of IND 48, 649 dated April 27, 2004. A topical dose of 250 mg/mL of QAB149 was used to determine whether the drug was a sensitizer in the guinea pig. QAB149 was not a sensitizer in the female guinea pig under the conditions tested.

Study title: **Potential for QAB149 to Induce Airway Obstruction or Pulmonary Eosinophilia in the Guinea pig**

Key study findings:
BAL fluid from guinea pigs sensitized with QAB149 and then challenged with QAB149 did not have increases in eosinophilia when compared with naive guinea pigs challenged with QAB149.

Study no.: 0320020
Volume #, and page #: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: April 17, 2003
GLP compliance: Yes

QA reports: yes (x) no ( )
Drug, lot #, and % purity: QAB149 /223004/100%
Formulation/vehicle: QAB149, 20% PEG400 in saline/20PEG400 in saline
Methods

Doses: 400 µg/mL

Study design:

Ten groups of male guinea-pigs (10/group) were administered ovalbumin (OA; 0.5 mL of 20 µg/mL), QAB149 (0.5 mL of 400 µg/mL base; batch no. 0223004) or vehicle (20% PEG 400 in saline; 0.5 mL) subcutaneously (s.c.) once weekly for 3 weeks in an attempt to induce a sensitization response. The study was divided into 2 procedures measuring different end points in response to a single intratracheal challenge dose (100 µL of either OA (1 or 300 µg/mL), QAB149 (0.5 mg/mL, base) or vehicle) administered using a Penn Century dosing device: assessment of lung function and assessment of eosinophilia using bronchoalveolar lavage (BAL)-fluid. Each guinea-pig received a single intratracheal dose.

Assessment of lung function

Group 1	Naïve, OA challenged
Group 2	OA sensitized, OA challenged
Group 3	Naïve, QAB149 challenged
Group 4	QAB149 sensitized, QAB149 challenged
Group 5	Vehicle sensitized, vehicle challenged

Guinea-pigs were anaesthetized with hypnorm and hypnovel, paralyzed with gallamine and ventilated *via* a tracheal cannula with a mixture of air and oxygen for measurement of lung function (airways resistance (R_L) and dynamic lung compliance (C_{dyn})). Blood pressure was recorded from the carotid artery and heart rate was derived from the blood pressure signal. After a minimum of 10 min equilibration, QAB149, vehicle or OA was administered intratracheally and R_L, C_{dyn}, mean arterial blood pressure and heart rate were monitored for 30 min following administration. Changes in the various measured parameters were evaluated relative to time zero [t(0)].

Assessment of eosinophilia

Group 6	Naïve, OA challenged
Group 7	OA sensitized, OA challenged
Group 8	Naïve, QAB149 challenged
Group 9	QAB149 sensitized, QAB149 challenged
Group 10	Vehicle sensitized, vehicle challenged

Guinea-pigs were anaesthetized and intubated. OA, QAB149 or vehicle was instilled into the trachea using a Penn Century dosing device. Twenty-four hours later animals were killed and the lungs were lavaged *via* a tracheal cannula using 3 aliquots (10 mL) of Hank's solution. Recovered cells were pooled and the total volume of recovered fluid adjusted to 30 mL by addition of Hank's solution. Total cells were counted and smears made by diluting recovered fluid (to approximately 10^6 cells/mL) and pipetting an aliquot into a centrifuge. Smears were air dried, fixed and stained in order to differentiate leukocytes. A total of approximately 500 cells per smear were counted by light microscopy under oil immersion (x1000).

Results:
BAL fluid from guinea pigs sensitized with QAB149 and then challenged with QAB149 did not have increases in eosinophilia when compared with naive guinea pigs challenged with QAB149.

2.6.6.9 Discussion and Conclusions
Nonclinical inhalation toxicology studies were conducted with the active agent, Indcaterol (QAB149) in the rat and the dog to delineate the toxicity profile for this drug. The inhalation studies ranged from 2 to 26 weeks in the rat and 2 to 39 weeks in the dog. The target organs of toxicity for QAB149 in the rat are nasal lesions, e.g., degeneration of

the olfactory epithelium and larynx, e.g., squamous metaplasia. The target organs of toxicity in the dog are the cardiovascular system, e.g., increased heart rates, decreased blood pressure and myocardial necrosis (class effects) and the liver, e.g., periportal liver hepatocyte vacuolation due to glycogen deposition. The cardiovascular toxicities occur in several dog studies at systemic exposure levels that are approximately 42-45 times the expected human exposure. However, there is an adequate margin of safety (approximately 3-5 and 11 fold in the 13 and 39 week dog toxicity studies) for the expected human exposure based the NOAELs determined in the toxicity studies. Non-clinical safety issues related to clinical use also include liver hepatocyte vacuolation (periportal) in the dog. However, these findings were only observed in the dog, are reversible and there is no histopathology associated with these findings. Therefore, potential liver toxicity is not considered to be a significant safety problem in humans. The nasal lesion and larynx changes observed in the rat are not considered to be relevance in human.

2.6.6.10 Tables and Figures

Table 5-2 Repeated dose toxicity studies

Species strain	Duration weeks	Route of administration	No. of animals/group	Dose per day (mg/kg)	Study number
Rat (Wist)	Group 1: 1 day per dose level	Inhalation	4M 4F	4.4-19.5	[008042] Non-GLP
	Group 2: 4 days		5M 5F	21.1	
	2	Inhalation	10M 10F TK: 6M 6F	0-2.1-5.8-17.0	[002012] GLP
	4	Inhalation	10M 10F R:5m 5f	0-0.93-2.77-8.46	[0120088] GLP
	13	Inhalation	10M 10F	0-0.3-1.01-3.09	[0220009] GLP
	26	Inhalation	20M 20F R:10M 10F	0-0.31-1.02-3.14	[0220064] GLP
	2	Intravenous	Sighting study: 2M 10M 10F	Sighting study: 0.1-1 single dose 0-0.1-0.3-1	[0520035] GLP
	1	Subcutaneous	5F	0-1-10-100	[0170165] Non-GLP
Dog (Beagle)	Group 1: 1 day per dose level	Inhalation	1M 1F	M: 0.09-0.33-0.86-1.91 F: 0.09-0.4-1.2-1.36	[008043] Non-GLP
	Group 2: 4 days		1M 1F	0.94/0.87	
	2	Inhalation	3M 3F	0-0.01-0.47-0.93	[002013] GLP
	4	Inhalation	3M 3F R: 2M 2F	0-0.01-0.1-0.97	[0120089] GLP
	13	Inhalation	3M 3F	Aerolizer® dry powder formulation: 0-0.02-0.12-1.10 HFA aerosol formulation: 1.02	[0420019] GLP
	13	Inhalation	3M 3F	Certihaler® dry powder formulation: 0-0.02-0.1-0.98 Aerolizer® dry powder formulation: 1.07	[0420020] GLP
	39	Inhalation	4M 4F R: 2M 2F	0-0.03-0.1-0.31	[0220065] GLP
	2	Intravenous	3M 3F	0-0.1-0.3-1	[0520036] GLP

2.6.7 TOXICOLOGY TABULATED SUMMARY

The AUC comparison between animals and humans was listed below in the table submitted by the sponsor (excerpted from Nonclinical Overview in Module 2). The human AUC0-24 hr at dose of 300 µg was 8.137 ng.h/mL .

Table 5-9 Indacaterol exposure multiples in toxicity studies

Species/ Study number	NOAEL (mg/kg)	Sex	$AUC_{(0-24h)}$[c] (ng·h/mL)	C_{max}[c] (ng/mL)	Exposure multiples[a/aa] Based on $AUC_{(0-24h)}$ 150 µg[a]	300 µg[aa]	Based on C_{max} 150 µg[a]	300 µg[aa]
2-week rat [002012]	2.05	male	303.90	27.93	78.30	37.30	63.70	32.5
		female	179.03	14.44	46.12	22.00	32.92	16.82
4-week rat [0120088]	0.93	male	50.91	12.26	13.11	6.26	27.95	14.28
		female	67.73	14.96	17.45	8.32	34.11	17.42
13-week rat [0220009]	0.30	male	39.00	16.30	10.05	4.79	37.16	18.98
		female	32.60	19.00	8.40	4.01	43.32	22.13
26-week rat [0220064]	1.02	male	37.20	12.20	9.58	4.57	27.82	14.21
		female	39.90	12.70	10.28	4.90	28.96	14.79
2-week dog [002013]	0.01	male	0.57	0.16	0.15	0.07	0.36	0.19
		female	below LOQ	below LOQ	-	-	-	-
4-week dog [0120089]	0.01[b]	male	3.93	0.36	1.01	0.48	0.82	0.42
		female	1.94	0.14	0.50	0.24	0.32	0.16
13-week dog [0420019]	0.12	male	39.5	5.17	10.18	4.85	11,79	6.02
		female	34.4	3.78	8.86	4.23	8.62	4.40
[0420020]	0.10	male	19.2	1.96				
		female	21.2	1.80	4.95	2.36	4.47	2.28
					5.46	2.61	4.10	2.10
39-week dog [0220065]	0.31	male	90.1	9.79	23.21	11.07	22.32	11.40
		female	87.2	9.24	22.46	10.72	21.07	10.76
Rat embryo-fetal development [0270037]	1[#]	female	345	26.1	88.87	42.40	59.51	30.40
Rabbit embryo-fetal development [0270038]	1[#]	female	795	168	204.79	97.70	383.04	195.67
Mouse carcinogenicity [0470002]	600	male	399	47.7	102.78	49.04	108.76	55.56
		female	862	71.0	222.05	105.94	161.88	82.69
Rat carcinogenicity [0320002]	2.09	male	114[d]	38.2[d]	29.37	14.01	87.10	44.49
	0.62	female	55.6[d]	31.4[d]	14.32	6.83	71.59	36.57

[a] based on 150 µg, multiple dose [Study CQAB149B2339], C_{max} = 0.4386 ng/mL, $AUC_{(0-24h)}$ = 3.882 ng·h/mL; [aa] based on 300 µg, multiple dose [Study CQAB149B2339], C_{max} = 0.8586 ng/mL, $AUC_{(0-24h)}$ = 8.137 ng·h/mL; NOAEL = No-Observed-Adverse-Effect-Level; [b] No effect level; [c] Values at the end of the stated treatment-period; [d] week 52; [#] NOAEL for effects on the embryo-fetus; LOQ = limit of quantification

Sources: [Table 2.6.7.7C-Study 002012], [Table 2.6.7.7D-Study 0120088], [Table 2.6.7.7E-Study 0220009], [Table 2.6.7.7F-Study 0220064], [Table 2.6.7.7H-Study 002013], [Table 2.6.7.7I-Study 0120089], [Table 2.6.7.7J-Study 0420019], [Table 2.6.7.7K-Study 0420020], [Table 2.6.7.7L-Study 0220065], [Table 2.6.7.13A-Study 0270037], [Table 2.6.7.13B-Study 0270038], [Table 2.6.7.10A-Study 0470002], [Table 2.6.7.10B-Study 0320002]

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Indacaterol maleate Inhalation Powder (QAB149) is a long acting β_2 agonist intended for once a day treatment of asthma/COPD. The drug will be administered by inhalation and the daily proposed doses are 150 and 300 mcg/day. The sponsor is planning to develop 3 formulations, an HFA formulation (IND 66,337), and a micronized powder formulation with lactose (IND 48,649) and a (b) (4) formulation (IND 69,754).

In vitro and *in vivo* studies show that QAB149 is a β_2 adrenergic agonist with bronchodilatory activity. The mechanism of action for QAB149 involves the interaction with active sites of the receptor via the membrane lipid bilayer stimulating cyclic AMP formulation and activation of cyclic AMP-dependent protein kinase which promotes phosphorylation and smooth muscle relaxation of the airways.

QAB 149 is absorbed and distributed in the dog and the rat following inhalation administration but is not highly bioavailable. Metabolism is extensive and qualitatively similar across species. Excretion is mainly via the feces.

The toxicity profile for QAB149 was delineated in the rat and the dog. The inhalation toxicity studies were up to 26 weeks in rats and up to 39 weeks in dogs.

The target organs of toxicity for QAB149 in the rat are nasal lesions, e.g., degeneration of the olfactory epithelium and larynx, e.g., squamous metaplasia. These changes observed in the rat are not considered to have relevance to human. The target organs of toxicity in the dog are the cardiovascular system and liver. The cardiovascular toxicities including increased heart rates, decreased blood pressure and myocardial necrosis (class effects). These toxicities occur in several dog studies at systemic exposure levels that are approximately 42-45 times the expected human exposure at maximum dose of 300 μg. However, there is an adequate margin of safety of approximately 3-5 and 11 fold for the expected human exposure based on the AUCs at NOAELs determined in the 13-week and 39-week dog toxicity studies, respectively. Furthermore, the cardiovascular findings are considered monitorable in the clinical setting.

The liver findings included periportal liver hepatocyte vacuolation due to glycogen deposition. However, these findings were only observed in the dog, were reversible and there was no histopathology associated with these findings. Therefore, the potential liver toxicity is not considered to be a significant safety problem in humans.

The carcinogenic potential of QAB149 was assessed in a 24-month inhalation carcinogenicity study in Sprague-Dawley rats and a 26-week oral (gavage) carcinogenicity study with CB6F1/TgrasH2 hemizygous mice. The studies were judged to be negative by the Executive CAC (see meeting minutes dated August 4, 2009). However, there were increased incidences of uterine endometrial stromal polyps in the 26-week oral (gavage) carcinogenicity study with female CB6F1/TgrasH2 hemizygous mice and ovarian leiomyomas in the 24-month inhalation oncogenicity study with female Sprague-Dawley rats which were statistically significant by trend test, but not significant by pairwise comparison. These tumors have been observed with other β2-adrenergic agonists and might be attributed to treatment despite the lack of statistical significance.

Genotoxicity assays including Ames bacterial reverse mutation test, Mammalian Chromosomal Aberration test in the V79 Chinese hamster cell and bone marrow micronucleus test reveal that QAB149 is not genotoxic under the conditions tested.

In reproduction studies in the rat, subcutaneous administration of QAB149 did not cause significant effects in males or females related to parameters of fertility, general reproductive performance or early embryonic development. In embryofetal development studies in the rat, subcutaneous and inhalation administration of QAB149 did not result in embryo-fetal toxicity, gross malformations or teratogenicity. In the embryofetal development study in rabbits, subcutaneous administration of QAB149 did not show teratogenic effects. However, there was an increase in one skeletal variation (full supernumerary ribs) in the fetus in the high dose group whereas a significant decrease (approximately 50%) in body weight gain was observed in the high dose group. When assessed for effects on pre-and postnatal development in rats subcutaneously administered with QAB149, there were increased stillborn pups for F0 and a statistically significant decrease in the number pregnant dams for F1 observed in the high dose group. However there were no significant treatment related effects on neurobehavioral or other reproduction of the F1 offspring or the gross appearance of the F2 pups.

The proposed specifications for the impurities in the drug substance for Arcapta (b) (4) have been reviewed and considered acceptable in a separate Chemistry Consultation review dated March 12, 2009.

Recommendation:
Approval is recommended for the NDA from a nonclinical perspective.

Suggested labeling:
The labeling will be reviewed later when a labeling negotiation is needed.

APPENDIX/ATTACHMENTS:

IND 66,337 (Pharmacology review dated May 6, 2008, July 2, and December 22, 2003)
IND 48,649 Pharmacology reviews dated April 27, 2004

PHARMACOLOGY/TOXICOLOGY COVER SHEET

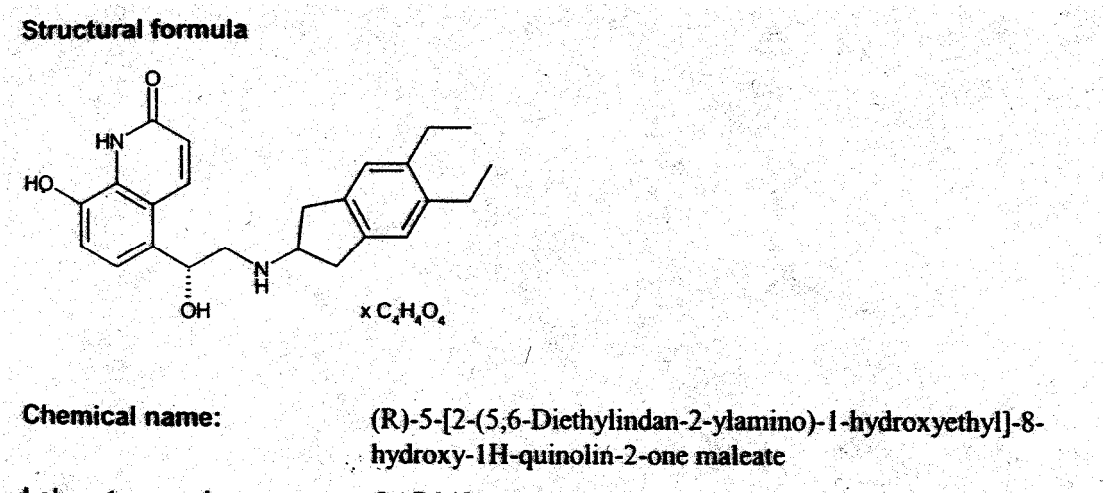
IND number: IND 66, 337
Review number: 001
Sequence number/date/type of submission: 000/ dated December 6, 2002/Original IND

Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Norvartis Pharma AG
Manufacturer for drug substance: Norvartis Pharma AG

Reviewer name: VE Whitehurst
Division name: Division of Pulmonary and Allergy Products
HFD #: HFD 570
Review completion date: May 6, 2008

Drug:

Trade name: NA
Generic name (list alphabetically): NA
Code name: QAB149 maleate
Chemical name: (R)-5-[2-(5, 6-diethylindan-2-ylamino)-1-hydroethyl]-8-hydroxy-1H-quinolin-2-one maleate
CAS registry number: 435273-74-8
Mole file number: NA
Molecular formula/molecular weight: $C_{24}H_{28}N_2O_3 \bullet C_4H_4O_4$/508.56
Structure:



Relevant INDs/NDAs/DMFs: IND 48, 649 (QAB 149 lactose); IND 69,754 (QAB149 with (b) (4))

Drug class: Long acting β_2 agonist

Indication: Treatment of asthma/CODP

Clinical formulation:

Table 1 Declared content per QAB149 50 µg / actuation (metered dose)[1]:

Ingredient	Theoretical amount per actuation (metered dose)		Function	Reference to standards
	[% w/w]	[mg]		
QAB149 maleate	0.11[2]	0.065[3]	Drug substance	Novartis Monograph
Sorbitan trioleate	0.02	0.012	Surfactant	USP
Dehydrated alcohol	10.00	5.830	Co-solvent	USP
1,1,1,2-tetrafluoroethane (propellant HFA-134a)	89.87	52.393	Propellant	IPACT-I[4] DMF 9859 DuPont DMF 9654
Total weight	100.00%	58.300		

1 50 µg / actuation (metered dose) correspond to 35 µg / actuation (emitted dose)

2 corresponds to 0.09 % w/w of QAB149 free base

3 corresponds to 0.050 mg of QAB149 free base

4 International Pharmaceutical Aerosol Consortium for Toxicology Testing of HFA-134a

Route of administration: Inhalation

Proposed clinical protocol:
The sponsor is proposing a double-blind, placebo-controlled, four-period, cross-over study to compare the magnitude, duration and onset of action of single inhaled doses of 100 µg and 400 µg of QAB 149 delivered by HFApMDI device. The study will include a single dose of 50 mcg of salmeterol as a positive control. The study will include 32 patients with persistent asthma and a predicted FEV1 of 60 to 90%. The ages of the patient will be 18-65 years.

Previous clinical experience:
QAB149 has been used to treat asthmatic and COPD patients. Single inhaler doses of 25-2000 mcg have been used to treat asthmatics in phase 1 studies. In phase 2 studies, doses of 200-800 mcg have been used. Repeated doses of 800 mcg have been used in the treatment of COPD patients. These studies were conducted in Europe.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: QAB149 is a long acting β_2 agonist intended for once a day treatment of asthma/COPD. The sponsor is planning to develop 3 formulations, an HFA formulation (IND 66,337) and 2 micronized powder formulations (IND 48,649 and 69,754). The sponsor has conducted 2 and 4 week inhalation studies in the Wistar rat and the Beagle dog. The two week studies utilized micronized drug formulation and the 4 week studies utilized the HFA formulation. In order to show that the 2 formulations are similar (kinetics and toxicity profile) the sponsor is planning to conduct a 90 day subchronic inhalation bridging study in the dog. The target organ for this drug is classically the cardiovascular system, i.e., tachycardia, QTc interval changes and myocardial necrosis. Increased cardiovascular activity with associated histopathological changes was observed in the dog. There was also glycogen deposition in the

liver of the dog at all dose levels. The dose-limiting toxicity in the rat is nasal lesions, characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium.

Studies reviewed within this submission:

Pharmacology reports:

RD-1999-02232-Effects of a β2-agonist NVP on the isolated, electrically-stimulated, superfused guinea pig tracheal strip Volume 2, page 8-125

RD-1999-03094v2-Single chamber plethysmographic measurement of airway reactivity in naïve, conscious guinea pigs Volume 2, page 8-133

RD-1999-03095v2-Evaluation of NVP-QAB149 in guinea pigs by whole body, single chamber plethsmography Volume 2, page 8-144

RD-2000-00233v2-Duration of action of formoterol and NVP- QAB149 in a guinea pig model of histamine–induced bronchoconstriction *in vivo* Volume 2, page 8-150

RD-2000-00234-Determination of tachyphylaxis by formoterol and NVP-QAB149 using a guinea pig plethysmograph model of histamine-induced bronchoconstriction *in vivo* Volume 2, page 8-158

RD-2000-00315-Results for PANLABS general pharmacology screen for NVP-QAB149 Volume 2, page 8-167

RD-2001-03026-Activity of NVP-QAB149-AA-and its (S)-enantioner (NVP-QAB149-AA-1) in beta-1and beta –2 adrenoreceptor binding and functional assays Volume 2, page 8-293

RD-2002-00195-Anti-broncoconstriction and cardiovascular effects of inhaled NVP-QAB149in rhesus monkeys: comparison with formoterol Volume 2, page 8-300

RD-2002-01685-Effects of NVP-QAB149- AA in human beta-receptor cAMP assay (ALPHA Screen) in BEAS-2B cells Volume 2, page 8-322

RD-2002-01927-NMR studies of NVP-QAB149in SDS micelles Volume 2, page 8-331

RD-2002-03086- Antagonist effect of NVP-QAB149-AF-1on the α_{1D} - adrenoceptor of rat aorta Volume 2, page 8-368

RD-2002-03936-Binding of NVP-ADD561-NX (the main metabolite of NVP-QAB149-AA)-to beta-1 and beta-2 adrenoceptors. Volume 3, page 8-200

Safety Pharmacology:

0120071-Effect of QAB149 on the HERG currents recorded from stably transfected HEK293 cells Volume 10, page 8-116

Pharmacokinetic Reports:

DMPK-R299-2114- [^{3}H]QAB149 synthesis of analysis. Drug metabolism and Pharmacokinetics Volume 3, page 8-1

DMPK-R02-00490-Quantitative determination of QAB149 in rat, human, dog, rabbit and mouse serum and rabbit embryo by LC/MS/MS using liquid/liquid extraction. Volume 3, page 8-12

DMPK-R299-2520- Quantitative determination of QAB149 in human serum by LC/MS/MS using liquid/liquid extraction. Volume 3, page 8-73

DMPK-R00-2189- Quantitative determination of QAB149 in human urine by LC/MS/MS using liquid/liquid extraction. Volume 3, page 8-95

Toxicology reports:

Acute Toxicology:

Repeated Dose Studies:

Genotoxic Assays

Reproductive Development Studies:

Studies <u>not</u> reviewed within this submission:

Pharmacokinetics Reports:

(b) (4)

Special Studies: 021726- QAB149: Assessment of Contact Allergenic Potential with Murine Local Lymph Node Assay (LLNA tier I).
Volume 10, page 160
0217031- Assessment of Contact Allergenic Potential with Murine Local Lymph Node Assay (LLNA tier I) Volume 10, page 183

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: The K-I values for displacement of CGP12177 from recombinant human ß$_1$ and ß$_2$- receptors (nM) in comparison with formoterol and salmeterol are summarized below (data from study # RD-2001-03026):

Compound	β_1 K_i (nM)	β_2 K_i (nM)	Selectivity
QAB149	180 ± 22 (n=5)	15.9 ± 1.7 (n=5)	11
QAB495*	1568 ± 1802 (n=2)	190 ± 208 (n=2)	8.4
Salmeterol	1035 ± 413 (n=3)	4.0 ± 1.2 (n=3)	259
Formoterol	646 ± 95 (n=3)	9.6 ± 0.5 (n=3)	68

QAB495 is the *(S)*-enantiomer of QAB149

These data were obtained comparing the displacement of radiolabeled –CGP12177 (a non-specific β-adrenergic receptor agonist) from membrane preparations expressing human β_1 and ß$_2$ adrenergic receptors. The table above compares QAB149's affinity for the β_1 vs β_2 adrenergic receptors. These data show that QAB 149 has a greater affinity for the ß2 adrenergic receptor than salmeterol and formoterol. The KI was larger or the β_1 receptor but was relatively smaller in comparison to formoterol and salmeterol. QAB495 is the S enantiomer of QAB149. β_1 adrenergic activity is thought to play a major role in tachycardia, a classic side-effect associated with adrenergic agonists. B_2 adrenergic activity also contributes to the increases in heart rate, however, its major role is thought to be bronchodilation of smooth muscle.

The results of this assay confirm that QAB149 AA-1 has a greater affinity for the beta 2 adrenergic agonist than QAB495-AA-1 and salmeterol and formoterol. Additionally, QAB149–AA-1 induced greater concentrations of cAMP than QAB494-AA_1, salmeterol or formoterol.

Study # RD-2002-01685 describes the effect of formoterol, salmeterol and QAB 149 in an *in vitro* assay of intracellular cAMP production in BEAS-2B cells. The BEAS-2B cell endogenously expresses the human ß$_2$ adrenoreceptor (40-70 fmol/mg) allowing detection of partial agonism. Formoterol and salmeterol stimulated cAMP production with a p[A] ~ 9.5 while QAB 149 had a p[A] of 8.1. In the study, QAB 149 was found to be a full agonist with respect to formoterol. The functional β_2 adrenergic receptor activity of QAB149 was further determined by measuring cAMP creation in CHO-KI cells. The bronchodilation activity of β_2 adrenergic agonists including QAB149 is mediated via cAMP. The results from a single experiment for QAB149 along with the comparator drugs are shown below:

Compound	EC_{50} for cAMP (nM)
QAB149	0.23
QAB495	11.14
Salmeterol	0.03
Formoterol	0.05

QAB 149 induced greater cAMP creation which should potentially result in greater bronchodilation than that induced by Salmeterol and Formoterol.

In study # RD-2000-00315, QAB 149 was tested in Panlabs Spectrumscreen® to assess its specificity of action. At 10 μM, QAB 149 was active at a wide variety of receptors. QAB 149 inhibited the expected target by 80 % and 15 other receptors by 50-80 %. To ascertain the significance of these observations binding constants (Ki) or dose response curves (IC_{50}) were determined. The results show QAB 149 bound effectively to its target receptors, beta-2 adrenoceptors, Ki 12 nM, as well as to alpha adrenoceptors $á_{1D}$ (Ki 201 nM) and $á_{1A}$ (Ki 454 nM).

Mechanism of action: QAB149 interacts with active sites of the receptor via the membrane lipid bilayer causing relaxation of smooth muscle. 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 interreaction between the drug and the receptor membrane lipid bilayer.

Study # RD-2002-01927 was carried out to get insight into the interaction of QAB 149 with membranes. The duration of action of long-acting beta agonists is thought to correlate with the association of the drug with membranes. In this study, QAB 149 was studied in sodium dodecyl sulphate (SDS) micelles in aqueous solution by high-resolution NMR spectroscopy. Although the SDS micelle is not a membrane, it is thought to be a relevant system to study the conformational properties of QAB 149. The results of this study show that QAB 149 is almost exclusively bound to the micelle in an extended conformation. The NMR data suggest that the carbostyril head group is oriented toward the surface of micelle. The data indicate interaction of the negatively charged sulphate of SDS with the positive charged ethanolamine moiety of QAB 149.

Drug activity related to proposed indication: QAB149 is thought to be an effective bronchodilator with an extended duration of action which will be used once daily in the treatment of patients with reversible bronchoconstriction of the pulmonary airways.

In an effort to determine potency, maximum effect (T_{max}) and duration of action, the ability of QAB149 to inhibit electrically stimulated contractions of isolated guinea pig trachea was investigated (study # RD-1999-02232) . The results of this study show a 100 nM concentration of QAB149 inhibited contractions of isolated guinea pig trachea by approximately 62% at T_{max}. T_{max} occurred at approximately 34 minutes and the duration was approximately 400 minutes. Salmeterol at the same concentration showed greater potency as it inhibited contractions by 79%. T_{max} occurred at 85 minutes and the duration of action was 500 minutes.

In study # RD-1999-03094 v 2 a plethysmographic method was used in an *in vivo* assay of bronchorelaxant drugs to determine the duration of action of formoterol. Conscious guinea pigs (Dunkin Hartley) were exposed to histamine (25-1000 mcg/ml) for 1 minute and the degree of bronchoconstriction was measured using whole body plethysmography. The effects of formoterol (1-1000 mcg/ml aerosolized for 10 minutes) on histamine-induced bronchoconstriction was determined at 2, 4, 6 and 24 hours following challenge. At 2 hours, formoterol at concentrations of 1, 10, 100 and 1000 mcg/ml significantly reduced histamine-induced bronchoconstriction by 54.7, 84.9, 75.0 and 86.3%, respectively. At 4 hours, formoterol at concentrations of 10, 100 and

1000 mcg/ml significantly reduced histamine-induced bronchoconstriction by 59.7, 40.0 and 86.7%, respectively. At 24 hours, formoterol at concentrations of 1-1000 mcg/ml was not effective in inhibiting histamine-induced bromchoconstriction. The study was carried out to demonstrate that this technique can be used to determine duration of action of a known drug, formoterol. Formoterol was used because QAB 149 and formoterol have similar structures.

In study # RD-1999-03095 v2 the plethysmographic method with conscious guinea pigs was used to determine the duration of action for QAB 149. Again, as in the previous study, the guinea pigs were exposed to aerosol histamine (25-200 mcg/ml) for 1 minute. QAB 149 at a concentration of 1 mg/ml, administered by inhalation for 10 minutes, significantly reduced histamine-induced bronchconstriction at 2, 4 and 6 hours by 92.8, 83.1 and 66.3%, respectively. QAB149 was not effective at inhibiting histamine-induced bronchoconstriction at the 24 hour challenge. Formoterol was used as the positive control in this study.

In study # RD-2000-00233 v2, the plethysmographic method with conscious guinea pigs was again used to determine the duration of action for QAB 149. The guinea pigs were exposed to histamine-induced bronchoconstriction (25-200 mcg/ml) for 1 minute. The following day the conscious guinea pigs were administered aerosolized QAB 149, 1 mg/ml for 10 minutes. At 4, 8 and 12 hours, the guinea pigs were exposed to 200 mcg/ml of histamine for 1 minute. QAB 149 significantly inhibited histamine-induced bronchoconstriction at 4, 8 and 12 hours by 80.7, 64.2 and 54.7%, respectively. Formoterol at a concentration of 0.1 mg/kg inhibited bronchoconstriction at 4 and 8 hours but had no effect at 12 hours.

In study # RD-2000-234 v2 guinea pigs were exposed to 200 mcg/ml of histamine to measure their degree of bronchoconstriction using whole body plethysmography. The guinea pigs were then dosed with aerosolized 1 mg/ml of QAB 149 or 1 mg/ml of formoterol for 10 minutes every 12 hours for 5 days. Two hours after the final dose, the guinea pigs were exposed to 200 mcg/ml of histamine for 1 minute. QAB 149 inhibited histamine-induced bronchoconstriction by 63% while formoterol inhibited the bronchoconstriction by 22%.

Secondary pharmacodynamics:

The systemic side-effect profile for QAB149 was determined in spontaneously breathing anesthetized Rhesus monkeys using a methacholine–induced model *in vivo* (study # RD-2002-00195). Inhalation doses of 0.4, 2.5 and 11.8 µg/kg were administered for 10 minutes. Heart rate increased by 13% at the highest dose and was still present at the 280 minute evaluations (more accurate results can be obtained using non-anesthetized monkeys) There were no significant effects on blood pressure, serum potassium and respiratory rate in the monkeys in this study. The maximum onset of action was within 5 minutes and at the highest dose, QAB 149 inhibited methacholine-induced bronchoconstriction by 75%. The duration of action at this dose was 225 minutes.
In study # RD-2002-03086 a broad radioligand receptor binding selectivity screen was carried out using male, Brown-Norway rat thoracic aortas. The tissues were incubated in QAB 149 at a concentration of 10^{-6} M for 30 minutes then the tissues were contracted with phenylephrine (3.16^{-6} M) or 5-hydroxytryptamine (3.10^{-5} M). The study was carried out with and without propranolol (10^{-6} M) to eliminate the possible interference of the beta 2 activity of QAB 149. The results of

this study reveal a shift to the right in the phenylephrine dose response curve in the presence of QAB 149 with and without propranolol. The KB (equilibrium dissociation constant) values were 155 and 320 nM, respectively. QAB 149 had no effect on the hydroxytryptamine dose response.

Binding of NVP-ADD561-NX (The Main Metabolite of NVP-QAB 149 to Beta-1 and Beta-2 Adrenergic Receptors (Study # RD-2002-03936)

The purpose of this study is to determine the affinity of the glucuronide of NVP-QAB 149 (NVP-ADD561-NX) to bind to beta-1 and beta-2 adrenergic receptors in cardiovascular tissues. This study was carried out to determine whether NVP-ADD561-NX has the potential to induce tachycardia, a major side effect of beta 2 agonists. NVP-ADD 561 showed no binding activity to either beta-1 or beta -2 adrenergic agonists.

Pharmacology conclusions*:* QAB149 has demonstrated binding activity with both β_1 and β_2 receptors with greater affinity for the β_2-adrenergic receptor. *In vitro* and *in vivo* studies with guinea pigs 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 (approximately 225 minutes).

II. SAFETY PHARMACOLOGY:

Cardiovascular effects: The effect of QAB149 on the tail current recorded from HEK293 cells stably transfected with HERG cDNA was investigated (Study # 0120071). 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 by approximately 25%. QAB 149 did not inhibit the HERG tail at the 2 lower concentrations. 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.

Safety pharmacology conclusions: Only an in vitro cardiovascular safety pharmacology study was conducted. QAB149, at a concentration of 5 μg/ml, inhibited the HERG tail current by 25%. QAB149, at concentrations of 0.5 and 1.0 μg/ml, did not inhibit HERG channels.

Data from repeat dose inhalation toxicology studies demonstrated potential cardiovascular effects of QAB 149. The QTc values (normalized for heart rate) were increased in the dogs in the mid (0.12 mg/kg) and the high (0.23 mg/kg) groups in the 2 week inhalation study. On day 1, the 24 hour increases were 13% and 16%, respectively. On day 14, 8 hour increases were 11% and 3%, respectively. The Q-Tc value was similar at the 24 hour evaluations on day 14. A 4-week study at pulmonary deposited doses up to 0.24 mg/kg did not demonstrate any effects on QTc values of the dogs.

Studies to assess CNS and respiratory effects were not conducted. These studies should be conducted by the sponsor in an expeditious fashion. This comment was sent to the sponsor in a letter regarding IND 48,649 dated May 21, 2004.

III. PHARMACOKINETICS/TOXICOKINETICS:

The synthesis and analysis of [^{3}H]QAB149 is discussed in study # R99-2114. Prior to tritiation, brominated QAB 149 was synthesized by mixing QAB 149 at room temperature with bromine and acetic acid. In the presence of 10% Pd/C and tritium gas, the bromine in this compound was then exchanged by tritium to [^{3}H]QAB149.

Quantitative determination of QAB 149 in rat, human, dog, rabbit and mouse serum and rabbit embryo by LC/MS/MS using liquid/liquid extraction is discussed (study # R02-00490). The study presents a sensitive method for the quantitative determination of QAB 149 in serum. The lower limit of quantitation (LLOQ) in this study was 0.07 ng/mL using a serum volume of 100 of μL.

In study # R99-2520 quantitative determination of QAB 149 in human serum by LC/MS/MS using liquid/liquid extraction is discussed. For the determination of QAB 149 in human serum, an HPLC-MS method has been developed. The LLOQ in this study was 0.25 ng/mL using a serum volume of 200 of μL.

In study #R00-2189 quantitative determination of QAB 149 in human urine by LC/MS/MS is discussed. The method consists of liquid/liquid extraction, evaporation of the organic layer to dryness, and the analyses of the constituted sample by liquid chromatography/ tandem mass spectrometry (HPLC-MS/MS) in selected reaction monitoring mode using APCI as an interface. The LLOQ in this study was 0.25 ng/mL using a sample volume of 1 mL urine.

Absorption, Distribution, Metabolism and Excretion of ^{3}[H]QAB149 in the Rat Following an Intraveneous, Intratracheal, Oral and Substaneous Dose (DMPK(US0 R99-2113)

The objective of the study was to examine absorption, distribution, metabolism and excretion of QAB149 following a intravenous (iv), subcutaneous (sc), intratracheal and oral dose of [^{3}H]QAB149 in the male, Wistar rat. The doses were 0.65 mg/kg QAB149 maleate iv, 0.65 and 3.75 mg/kg sc, 0.65 mg/kg PO and 0.78 mg/kg intratracheally.

Absorption:

Following oral administration, 20-34% was absorbed in the rat and QAB149 was not highly bioavailable. Sc and intratracheal dosing resulted in approximately 100 and 78-90% absorption and 100 and 78% bioavailability, respectively.

Distribution:

The tissue distribution of [^{3}H]QAB149 was studied by qualitative whole-body autoradiography after iv, oral and intratracheal administration. Radioactive material was distributed to all tissues except the brain, spinal column, testis and lymph nodes. The greatest concentrations were found in the stomach, intestines, liver and kidneys. Five minutes after intratracheal dosing, most of the

radioactive material was present in the lungs. At subsequent times, the radioactivity distribution resembled that which was observed following iv dosing.

Metabolism:

Metabolism was similar after iv, sc and intratracheal dosing. After oral dosing, QAB149 was subjected to a higher first pass metabolism which resulted in a higher metabolite to QAB149 ratio. The main metabolic pathway was glucuronidation. There were 2 main components in the rat plasma, unchanged QAB 149 and O-glucuronide of QAB149 regardless of the route of administration. In the rat following sc administration of QAB 149 in a pharmacokinetic study (PK), there were 2 metabolites, N and O-glucuronides in the plasma, approximately 10-20%. Similar results were observed in the mouse following oral dosing. In the dog following oral dosing of QAB 149, the main component in circulating plasma was unchanged QAB 149. There were 2 minor metabolites, N and O glucuronides. The metabolites represented 8-16 %. The mouse and dog metabolism data were obtained from toxicity studies.

In vivo studies in rats demonstrated 2 major components, unchanged QAB149 and the phenolic O-glucuronide. After oral dosing, QAB149 was subject to first pass metabolism which resulted in plasma concentrations of QAB149 below the limit of detection of quantitation of the LC-MS/MS method.

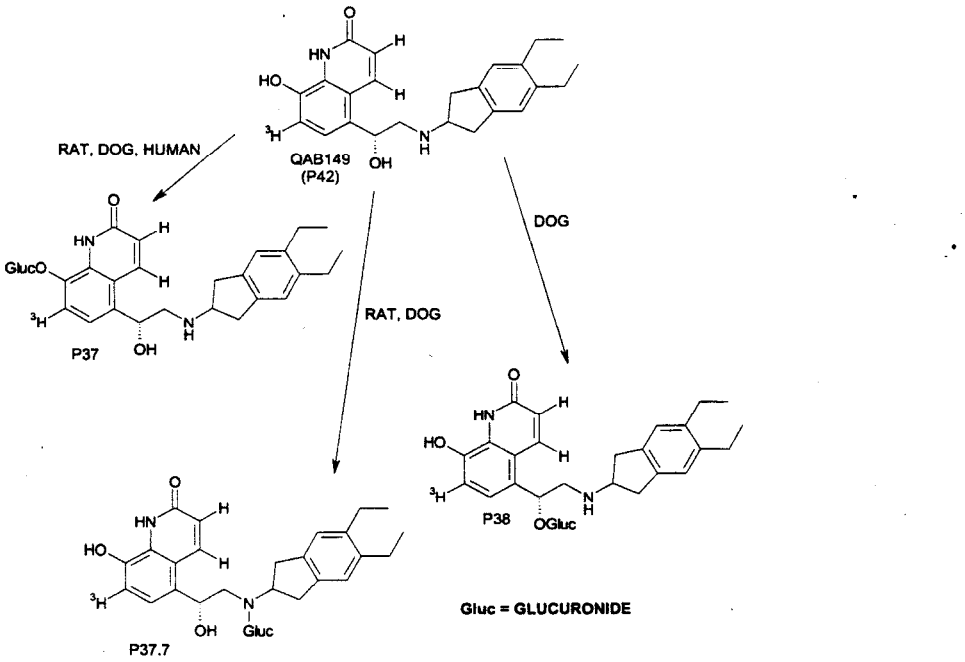
Comparative Metabolism of [^{3}H]QAB149 in Rat, Dog and Human Liver Slice Culture and Metabolism in Human Lung Slice Culture (Study # R00-397)

Incubates (24 hours) of [^{3}H] QAB149 from rat, dog and human liver slices were analyzed in order to determine the *in vitro* metabolism and metabolic pathways of the QAB149. Human lung slices were also analyzed. The liver and lung extract incubation media was examined by high pressure liquid chromatography. Following a pre-incubation period, tissue slices, fresh media [^{3}H] QAB149 and 0.1% DMSO was added to give the following concentrations for the rat, dog, and human:

Species	Low concentration µM	High concentration µM
Rat	1.3	13.3
Dog	1.1	9.8
Human	1	7.5

In vitro metabolism of QAB149 in the liver is similar in rats, dogs and humans, involving mainly phenolic O-glucuronidation. After incubation, the O-glucuronide accounted for 39% of radioactivity in rat liver slices, 16% in the dog and 14% of radioactivity in human liver slices; QAB149 accounted for 33%, 60% and 69%, respectively. The O-glucuronide is not pharmacologically active. No metabolism of QAB149 was found in the human lung (lung slices and microsomal preparations). The metabolic pathway of QAB149 in the rat, dog and human liver slices is shown below.

Figure 3-3 Metabolic pathways of QAB149 in rat, dog and human liver slices



Distribution:

***In Vitro* Binding of ^{3}H-Labeled QAB149 to Red Blood Cells, Serum and Plasma Proteins in the Rat, Dog and Human (Study # R00-594)**

The objective of this study was to investigate the binding of radiolabeled QAB 149 to serum, proteins and red blood cells of the rat, dog and human in vitro over a concentration of 1-2000 ng/mL. For the binding studies, heparinized blood was obtained from 3 male Wistar-Hanover rats (250-300 g), 3 Beagle dogs (8-12 kg) and 3 human volunteers. 3 mg of QAB 149 was introduced into the pooled blood of the rat, dog and human, respectively. Distribution of [^{3}H] QAB 149 and plasma, serum and red cells was determined at concentrations of 1, 10, 100, 500 and 2000 ng/mL. Over the concentrations of 1-2000 ng/mL, QAB 149 showed a higher affinity to red blood cells than plasma in all species evaluated. The binding to the red blood cell was highest in the rat, followed by the dog and then the human. The binding was independent of concentration. QAB 149 was relatively highly bound to plasma proteins; the plasma protein binding of QAB149 in the rat, dog and human using ultracentrifugation was approximately, 92, 94 and 96% in the rat, dog and human, respectively.

Fraction of [^{3}H]QAB 149 distributed to red blood cells (f_{BC}) at 37 ˚C is shown below:

Table 2: Fraction of [^{3}H]QAB149 distributed to red blood cells (f_{BC}) at 37°C

Blood concentration, ng/mL	f_{BC} (mean ± SD)[a]					
	Rat[b,c]	Dog[b,c]	Human Subject 1[d]	Human Subject 2[d]	Human Subject 3[d]	Human Average[e]
1	0.742 ± 0.003	0.570 ± 0.042	0.578	0.482	f	0.530
10	0.735 ± 0.010	0.608 ± 0.026	0.696	0.563	0.493	0.584 ± 0.10
100	0.717 ± 0.008	0.573 ± 0.004	0.590	0.488	0.530	0.536 ± 0.05
500	0.705 ± 0.006	0.561± 0.006	0.537	0.459	0.512	0.503 ± 0.04
2,000	0.688 ± 0.015	0.525 ± 0.014	0.580	0.452	0.465	0.499 ± 0.07

[a]hematocrit value was 0.43, 0.48 and 0.44, in rat, dog and human, respectively.

[b]data were obtained from triplicate analyses

[c]pooled blood (n ≥ 3)

[d]data were obtained from single analysis

[e]mean data were obtained from the three individual human subjects

[f]sample was contaminated

Excretion:

The biliary pathway was the predominant elimination pathway regardless of the route of administration in the rat. The recovery of the radioactive material was nearly complete within 96 hours and ranged from 77.3-88.9 % in the rats. The half-life for elimination of total drug-related material was 21-46 hours at a dose of 0.5 mg/kg with either IV, SC or intratracheal dosing. The predominant route of elimination in the rat is the feces regardless of route as levels reached up to 86% over 96 hours; urinary levels were < 10%.

The summary of the ADME parameters in the rat is shown below:

1 Summary

Species/strain/gender:	rat/HanWistar/male				
Route/formulation:	Intravenous solution	Intratracheal solution	Oral gavage solution	Subcutaneous solution	
Dose (mg/kg):	0.5	0.6	0.5	0.5	3
Specific activity (mCi/mg):	1.87	1.87	1.87	1.93	0.400
Samples collected:	Serial blood and plasma at various times from 0-96 h postdose from 3 (iv, po, sc) or 4 (intratracheal) rats; serial plasma collected from additional animals for metabolism analysis; complete urine and feces in 24 h intervals 0-96 h; cage wash and carcasses were collected at 96 h postdose. Complete urine, feces and bile was collected from three bile-duct cannulated animals for 72 h following an iv dose. Five animals were sacrificed at designated time points for tissue radioactivity analysis following iv and intratracheal doses.				
Samples analyzed:	Radioactivity was measured in all plasma, blood, excreta, carcasses and cage wash samples; radioactivity was assessed qualitatively in tissues following iv and intratracheal doses. Pooled plasma and selected, pooled urine and feces samples were assayed for unchanged QAB149 and metabolites. Selected urine samples were assayed for tritiated water.				
Plasma QAB149:					
C_{max} (ng/mL):	113[a]	51.6	-	16.7	57.8
t_{max} (h):	0.083[a]	0.5	-	1.0	3.0
$AUC_{0-96\ h}$ (ng•h/mL):	136	127	-	90.8	860
$t_{1/2,\ z}$ (h):	7.9	8.1	-	13	32
CL (L/h/kg):	3.7	-	-	-	-
V_{ss} (L/kg):	26	-	-	-	-
Blood [^{3}H]radioactivity					
C_{max} (ngEq/mL):	264 ± 3.06[a]	87.1 ± 64.5	18.8 ± 3.82	54.6 ± 5.43	157 ± 37.0
t_{max} (h):	0.083 ± 0[a]	1.1 ± 1.3	2.0 ± 0	1.5 ± 0.9	3.0 ± 1.0
$AUC_{0-48\ h}$ (ngEq•h/mL):	688 ± 58.1	410 ± 204	141 ± 23.6	666 ± 55.5	4120 ± 284
$t_{1/2,\ z}$ (h):	21	22	120	51	82
Plasma [^{3}H]radioactivity					
C_{max} (ngEq/mL):	191 ± 6.56[a]	46.0 ± 23.5	22.2 ± 8.19	30.2 ± 1.80	104 ± 26.5
t_{max} (h):	0.083 ± 0[a]	1.0 ± 1.3	2.3 ± 0.6	0.8 ± 0.3	5.0 ± 2.6
$AUC_{0-48\ h}$ (ngEq•h/mL):	559 ± 65.6	308 ± 105	142 ± 50.7	546 ± 41.9	3990 ± 662
$t_{1/2,\ z}$ (h):	46	21	43	44	60
Excretion in urine (%dose): Radioactivity,					
0-24 h:	4.64 ± 0.75	4.75 ± 1.84	2.06 ± 0.61	6.53 ± 0.84	6.66 ± 0.99
0-96 h:	6.69 ± 0.36	6.00 ± 2.43	2.29 ± 0.61	8.55 ± 0.88	8.98 ± 0.98
QAB149, 0-24 h:	1.7[b]	1.6	0.6	3.5	5.3
Excretion in feces (% dose): Radioactivity,					
0-24 h:	50.4 ± 5.34	59.5 ± 4.51	76.6 ± 2.97	44.4 ± 5.908	15.1 ± 22.2
0-96 h:	71.0 ± 2.17	79.2 ± 7.44	84.4 ± 3.70	66.0 ± 1.60	74.7 ± 3.14
QAB149, 0-48 h:	58[b]	66	75	56	70

[a]concentration at first sampling time, 0.083 h postdose
[b]in bile duct-cannulated animals, QAB149 was 4.5% of the dose in urine and only 0.52% of the dose in bile

[^{3}H]QAB149: Metabolic Profile in Human Liver Microsomes and Potential to Inhibit Cytochrome P450-Mediated Reactions (Study # R01-994)

The objective of this study was to explore the biotransformation pathways of QAB149 in human liver microsomes to confirm the predominant metabolism by glucuronidation observed in the liver slice cultures. The goals of this study were to examine the roles of specific human UDP-glucuronyl tranferases and cytochrome 450s in the metabolism of QAB149 and to investigate the potential of QAB149 to function as an *in vitro* inhibitor of cytochrome 450-mediated reactions. Another objective was to determine if QAB149 could be metabolized in human lung by

examination of QAB 149 biotransformation in human pulmonary microsomes. The results of this study show that QAB 149 (10 μM) is metabolized in human liver microsomes in the presence of NADPH and UDPGA to the phenolic O-glucuronide (P37) followed by formation of minor mono-oxygenation products, P26.9 and 30.3 (2.1 and 92.1 $nmol.mg^{-1}.hr$). QAB 149 showed no significant inhibition of P450 enzymes, CYP2C9, CYP2E1 and CYP3A4/5 when tested in concentrations up to 100 μM. Relatively weak inhibition of P450 enzymes, CYP1A2, CYP2C8, CYP2C19 and CYP2D6 was noted. QAB149 metabolism by human pulmonary microsomes reveals no biotransformation. This is confirmed by the lack of biotransformation of QAB149 in the human lung tissues slice cultures.

PK/TK conclusions: SC and intratracheal dosing resulted in approximately 100 and 78-90% absorption and 100 and 78% bioavailability, respectively, in the rat. The in *vitro* metabolism of QAB149 evaluated using liver slices is similar in rats, dog and humans, involving mainly phenolic O-glucuronidation. No major active metabolites were observed. QAB 149 was not metabolized in the human lung. In vivo metabolic studies in the rat with various route of administration also determined that O-glucuronidation was a primary metabolic pathway; N-glucuronidation was also noted following SC administration. Protein binding was similar in the rat, dog and human, approximately 92, 94 and 96%. QAB149 was distributed to most tissues, except the brain, spinal column and lymph nodes. The highest concentrations of QAB149 were found in the stomach, intestines, liver and kidney. QAB 149 showed no significant inhibition of P450 enzymes CYP2C9, CYP2E1 and CYP3A4/5 when tested in concentrations up to 100 μM. Elimination of QAB149 in the rat, mouse and dog is mainly via the feces with minimal urinary excretion.

The pharmacokinetic parameters for QAB 149 in the rat and the dog from 4 week inhalation toxicity studies are shown below:

Animal Species	**Pulmonary Deposited Dose (mg/kg)**	**AUC 0-24 (ng/ml)*h****	**Cmax (ng/ml)****	**Tmax (h)****
Rat	0.093	59	13	0.6
	0.28	122	25	1.3
	0.85	330	48	3.8
Dog	0.0025	3	0.24	3
	0.025	23	2.1	0.3
	0.24	300	29	1-3

** Mean value for males and females
Pharmacokinetic parameters were evaluated on day 27/28 of the studies.

There were no significant differences in pharmacokinetic parameters in the female and male rats and dogs. QAB 149 was found in all the control samples in the rat study. AUCs increased linearly on day 27/28. At comparable pulmonary deposited doses (~ 0.25 mg/kg), systemic exposure was almost 2-fold greater in dogs than in rats.

IV. GENERAL TOXICOLOGY:

Acute Toxicity Studies:

Species (Study Number)	Administration (Batch Number)	Doses (mg/kg), base	#/animals /group	Remarks	Maximum Non-Lethal Dose (mg/kg)	Minimum lethal dose (mg/kg)
Rat, Wistar (001077)	Oral (0021001)	500 and 2000	4m/4f	No adverse effects	1600*	> 1600
Mouse, CD-1 (001078)	Oral (0021001)	500 and 2000	4m/4f	No adverse effects	1600*	> 1600
Rat, Wistar (017034)	Subcutaneous (O121002)	5, 50, 100, 200	5m/5f	Skin lesions at injection site, cold to touch, hunched posture and decreased locomotor activity. Deaths occurred at 200 mg/kg.	100	200
Mouse, CD-1 (0170135)	Subcutaneous (0121002)	5, 50, 100, 200	5m/5f	Skin lesions at the injection sites, min, cold to touch, decreased locomotor activity. Deaths occurred at 50 mg/kg in males and 200 mg/kg in the females.	M: 5 F: 100	M: 50 F: 200

*The actual analytical determination of QAB149 concentrations in the test article preparations (micronized powder) showed lower than expected values by 19%. In addition, there was a variation of homogeneity by 16.3%. Therefore, the evaluation of acute toxicity was based on the analytical measured dose of 1600 mg/kg instead of the targeted dose of 2000 mg/kg.

Summary of Acute Toxicity:

Acute toxicity studies were carried in the rat and the mouse using micronized drug. QAB149 was administered orally and subcutaneously (sc). Oral doses in rats and mice were 500 and 2000 mg/kg (actually 1600 mg/kg). There were no clinical signs reported in these animal models and the minimum lethal oral dose was > 1600 mg/kg. The sc doses in the acute studies were lower than those given orally (5-200 mg/kg) and produced more severe effects. The clinical signs in the mouse include abdominal distention, labored respiration and decreased locomotor activity. One male mouse died in the 50 mg/kg group (1/10), 2 males in the 100 mg/kg group died (2/10) and 4 males and 4 females died in the 200 mg/kg dose group (8/10). The deaths occurred on days 2-3. The maximum non-lethal dose was 5 mg/kg in the males and 100 mg/kg in the females. The minimum-lethal dose was 50 mg/kg in males and 200 mg/kg in females. The clinical signs in the rat include swelling, scabs, bruising, thickened skin, cold to touch, reduced locomotor activity and discharge from the nose. There were 6/10 deaths in the males and females in the 200 mg/kg dose group. The maximum non-lethal dose was 100 mg/kg and the minimum lethal dose was 200 mg/kg.

Subchronic Toxicity Studies:

Study title: Escalating Dose Range Finding Inhalation Study in the Rat

Key study findings: A nominal inhaled dose of QAB149, 20 mg/kg daily (pulmonary deposited dose = 2 mg/kg) administered for 4 days to rats resulted in respiratory epithelial erosion, squamous metaplasia and regeneration and secondary inflammation in the nasal cavity as well as an increased numbers of globule leukocytes and a low incidence of subacute inflammation in the trachea. This was a dose finding study and the MTD was considered to be approximately 2.0 mg/kg (pulmonary deposited dose). No findings of note were reported following single pulmonary deposited doses of 0.5 or 2 mg/kg.

Study no: 008042
Volume #, and page #: Volume 4, page 1
Conducting laboratory and location: (b) (4)
Date of study initiation: April 24, 2000
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: Micronized QAB149 maleate batch # KL-5994/12.4/98.9%

Methods (unique aspects)**:**
Dosing:

Species/strain: Han Wistar Crl:WI (Gix/BRL/Han)BR VAF/PLUS
#/sex/group or time point (main study): 5 males and 5 females/dose group; There was also 5 females and 5 males/ dose group in the initial study.
Satellite groups used for toxicokinetics or recovery: None
Age: 7-9 weeks
Weight: 273-325 g males and 193-218 g females
Doses in administered units: 20 mg/kg for 4 days (main study) (pulmonary deposited dose= 2 mg/kg); 5 mg/kg, day 1 (pulmonary deposited dose=0.5 mg/kg) and 20 mg/kg day 2 (initial study), pulmonary deposited dose= 2 mg/kg). Dose levels were calculated as base. There was no control group in this study.
Route, form, volume, and infusion rate: nose only, micronized QAB149, chamber airflow rate was 20-30 liters/minute.
The dose was calculated as follows:

Table 3.3-1. Dose level estimation

$$\text{Dose (mg.kg}^{-1}) = \frac{MV \times T \times CC}{BW}$$

MV = Respiratory volume/minute[1] (litres) = $2.10 \times \text{weight}^{0.75}$
T = Duration of exposure (minutes)
CC = Chamber concentration of active drug = mg/litre^{-1}
BW = Body weight (expressed in kg - males and females were calculated separately)

(1) Guyton, Measurement of Respiratory Volumes of Laboratory Animals, 1947.

Observations and times:

Clinical signs: The rats were examined 1 hour before treatment, continuous during treatment and 1 hour after the treatment.

Body weights: The body weights were recorded weekly pretest and daily during the study.

Food consumption: Food consumption was recorded weekly pretest and during the study.

Ophthalmoscopy: NA

EKG: NA

Hematology: Hematology parameters were analyzed once during the study period.

Clinical chemistry: Clinical chemistry parameters were analyzed once the study period.

Urinalysis: NA

Gross pathology: Gross pathology was carried out at the end of the study.

Organs weighed: NA

Histopathology: A full histopathology including tissues from all dose groups was analyzed microscopically.

Toxicokinetics: Blood was obtained from the tail vein of 1 male and 1 female in the 20 mg/kg dose group. The blood was collected at 0 (predose), 1, 2, 4, 8 and 24 hours after dosing.

Results:

Particles Size Characteristics: (b) (4)

Mortality: There were no mortalities in this study.

Clinical signs: There were no clinical signs in the 0.5 mg/kg dose group receiving QAB149 for 1 day. The rats in the 2.0 mg/kg dose group for 1 day and 4 days had irregular breathing and subdued behavior.

Body weights: Body weight gain was similar in all dose groups except the males in the 2.0 mg/kg administered QAB149 daily for 4 days that had approximately a 5% decrease in body weight gain when body weight gain on day 4 of the study was compared with body weight on day-7 prior to the initiation of dosing.

Food consumption: Food consumption was decreased in males and females (2.0 mg/kg) in the 1 and 4 days studies. The decreases in the males and females in the 1 day study were 32 and 19%, respectively compared with the food consumption of the males and females prior to dosing. In the 4 day study, the decreases in the males and the females were 43 and 21%, respectively.

Ophthalmoscopy:NA

Electrocardiography:NA

Hematology: The hematology parameters were similar in males and females in the 1, 2 and 4 day studies when these parameters were compared control values..

Clinical chemistry: The clinical chemistry parameters were similar in the males and the females in the 1, 2 and 4 day studies when these parameters were compared to control values.

Urinalysis: NA

Organ weights: NA

Gross pathology: One female in the 2.0 mg/kg dose group (1 day) and 1 female in the 2.0 mg/kg dose group (4 days) had froth in the trachea. This finding was not noted microscopically because proper infusion of the lungs with formalin removed trachea contents.

Histopathology: The lining of the nasal cavities of all rats in this study was damaged. Minimal to slight areas of erosions were observed in the olfactory and respiratory epithelium. Minimum to marked areas of squamous metaplasia were present in the respiratory epithelium of all dosed animals. There was regeneration and secondary inflammation in the nasal cavity as well as an increased numbers of globule leukocytes and a low incidence of subacute inflammation in the trachea of these rats. There was minimum to slight regeneration of olfactory epithelium which was disorganized and thinner than normal olfactory epithelium. No drug-related systemic lesions were reported.

Toxicokinetics: Rats administered 2 mg/kg had measurable serum drug concentration. The serum drug concentrations were similar in males and females. The PK parameters for the QAB149 in these rats dosed for 4 days are shown below:

PK parameters	**Male (n=1)**	**Female (n=1)**	**Mean**
AUC(0-24hours) ng.h/mL	538	471	504
Cmax (ng/mL)	67.12	90.52	78.82
Tmax (h)	1.0	-	0.5

Summary of individual study findings:
QAB149 was administered to rats by inhalation for 1- 4 days. The nominal doses were 5-20 mg/kg (0.5-2.0 mg/kg deposited doses). A pulmonary deposited dose of 2 mg/kg resulted in epithelial erosion, regeneration and secondary inflammation in the nasal cavity as well as an increased numbers of globule leukocytes and a low incidence of subacute inflammation in the trachea. Minimum to marked areas of squamous metaplasia was also present in the respiratory epithelium of all dosed animals. This was a dose finding study and the MTD was considered to be 2.0 mg/kg.

Study title: A Subcutaneous 1- Week Toxicity Study in Rats

Key study findings: QAB149 was administered subcutaneously to female Wistar rats using 1, 10 and 100 mg/kg doses daily for 7 days. The results of this study show significant increase in body weight gain in the rats in the 1, 10 and 100 mg/kg dose group on days 1- 5 of dosing and in the rats in the 100 mg/kg dose group on days 1- 8. QAB149-related discoloration of the injection sites and the underlying muscles was observed. The dose of 1 mg/kg was considered to be the maximum tolerated dose.

Study no: 0170165
Volume #, and page #: Volume 7, page 338
Conducting laboratory and location: Novartis Pharmaceuticals
Date of study initiation: December 13, 2001
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: Micronized QAB149 maleate/#0021002/99.5%
Formulation/vehicle: Micronized QAB149 maleate, PEG 400 and saline

Methods (unique aspects)**:**

Dosing:

Species/strain: Rat, Wistar Hannover, CRL:WI (GLx/BRL/HAN)IGS BR
#/sex/group or time point (main study): 5 females
Satellite groups used for toxicokinetics or recovery: NA
Age: 7-8 weeks
Weight: 141-174
Doses in administered units: 0, 1, 10 and 100 mg/kg
Route, form, volume, and infusion rate: Subcutaneously/solution/dose volumes were based on body weight/5mL/kg.

Observations and times:

Clinical signs: Clinical signs were recorded twice daily pretest and daily during the study
Body weights: Body weight was recorded once pretest and on days 1, 5 and 8 of the study.
Food consumption: Food consumption was collected on days 1, 5 and 8.
Ophthalmoscopy: NA
EKG: NA
Hematology: NA
Clinical chemistry: NA
Urinalysis: NA
Gross pathology: Gross pathology was performed on all animals at the end of the study.
Organs weighed: NA
Histopathology: There was no histopathology in this study.
Toxicokinetics: NA

Results:

Mortality: There were no mortalities in this study.
Clinical signs: There were discoloration and scabs/wounds at the injection sites and underlying muscle areas in the rats in the 10 and 100 mg/kg dose group. One rat in the 1 mg/kg had treatment-related wound/scab.
Body weights: There were statistically significant increases in body weight gain vs control rats in the treated rats in the 1, 10 and 100 mg/kg dose groups, days 1-5 by 58, 53 and 50%, respectively. There was also statistically significant increases in body weight gain in the rats in the 100 mg/kg dose group, day 1-8 by 50%.
Food consumption: There were statistically significant increases in food consumption in the rats in all dose groups when compared to control values, days 5-8 by 12-18%.
Ophthalmoscopy: NA
Electrocardiography: NA
Hematology: NA
Clinical chemistry: NA
Urinalysis: NA
Organ weights: NA
Gross pathology: All the rats in the 10 and 100 mg/kg dose groups had dose-related discoloration and wounds/scabs at the injection sites and underlying muscles.
Histopathology: NA
Toxicokinetics: NA

Summary of Individual Study Findings: QAB149 was administered subcutaneously to female Wistar rats using 1, 10 and 100 mg/kg doses daily for 7 days. The results of this study show significant increase in body weight gain in the rats in the 1, 10 and 100 mg/kg dose group on days 1- 5 of dosing and in the rats in the 100 mg/kg dose group on days 1- 8. QAB149-related discoloration of the injection sites and the underlying muscles was observed. The dose of 1 mg/kg was considered to be the maximum tolerated dose.

Study title: 2-Week Inhalation Toxicity Study in Rats

Key study findings: Accumulations of alveolar macrophages at the broncho-alveolar junction of the lung, primarily at the high dose (1.7 mg/kg pulmonary deposited dose) and atrophy or loss of olfactory epithelium in the nasal cavity at the two highest doses (0.58 and 1.7 mg/kg pulmonary deposited doses). There were no accompanying inflammatory cells. There were no gender differences in toxicokinetics although no dose dependency was observed in regard to systemic exposure. QAB149 was found in all the control samples. The source of the contamination is unknown. The NOAEL was the low dose of 0.21 mg/kg based on the pulmonary and nasal lesions at the two higher doses.

Study no: 002012
Volume #, and page #: Volume 4, page 8
Conducting laboratory and location: (b) (4)
Date of study initiation: June 22, 2000
GLP compliance: Yes
QA report: Yes (x) no ( )
Drug, lot #, radiolabel, and % purity: White maleate micronized, batch 0021001, NA, 94.3%
Control rats were exposed to air only.

Methods (unique aspects):
Dosing:

Species/strain: Han Wistar (Crl:WI (GLx.BRL/Han) IGSBR VAF/PLUS
#/sex/group or time point (main study): 10 M/10 F
Satellite groups used for toxicokinetics or recovery: 5 m & 5f/dose group
Age: About 7-8 weeks at the start of the study
Weight: 196-246 g (males) and 146-174 g (females)
Doses in administered units:

Dose group	**Achieved doses-(mg/kg)/ Pulmonary Deposited doses (mg/kg)***
Control	0- air control
Low	2.05/ 0.21
Intermediate	5.81/0.58
High	17/ 1.7

Dose levels were calculated as the base (conversion factor 1.296)
* 10% pulmonary deposition assumed

Route, form, volume, and infusion rate: Inhalation-the white micronized powder drug was administered nose only daily, the low, intermediate and high dose animals were dosed for

16, 45 and 150 minutes, respectively. The control rats were exposed to air for 150 minutes daily. A target aerosol concentration of 0.3 mg/liter was used for this study. The achieved doses were estimated using the following criteria:
The achieved doses were estimated using the following criteria:

Table 3.3-1. Dose level estimation

$$\text{Dose (mg.kg}^{-1}) = \frac{MV \times T \times CC}{BW}$$

MV = Respiratory volume/minute[1] (litres) = $2.10 \times \text{weight}^{0.75}$
T = Duration of exposure (minutes)
CC = Chamber concentration of active drug = mg/litre^{-1}
BW = Body weight (expressed in kg - males and females were calculated separately)

(1) Guyton, Measurement of Respiratory Volumes of Laboratory Animals, 1947.

(b) (4)

Observations and times:
Mortality: daily
Clinical signs: twice daily
Body weights: weekly
Food consumption: weekly
Ophthalmoscopy: pretrial and day 13
EKG: NA
Hematology: day 14
Clinical chemistry: day 14
Urinalysis: day 14
Gross pathology: terminal
Organs weighed: terminal
Histopathology: terminal, all tissues from groups 1 and 4 as well as nasal cavity, larynx and lungs from the rats in groups 2 and 3.
Toxicokinetics: days 13/14, animals were bled at 0.5, 1, 2.5, 8 hours after the treatment period and at 24 hours right before the next inhalation treatment period.

Results:
Mortality: There were no mortalities in this study.
Clinical signs: There were increased respiration rates, reduced reactions to sudden sound and vocalization in 2/20 rats in the high dose group on day 1. No other clinical signs were observed.

Body weights: The mean body weight gain was reduced by 20% in the high dose males vs control rats. Body weight gain was increased in the low, mid and high dose females by 42, 76 and 65%, respectively.

Food consumption: Food consumption was similar in all dose groups.

Ophthalmoscopy: There were no treatment-related eye changes.

Electrocardiography: NA

Hematology: There were significant treatment-related increases in the neutrophil and eosinophil counts in female and males rats in all dose groups. The increases in neutrophil counts in the males in the low, mid and high dose groups ranged from 44, 39 and 72%, respectively. In the females, the increase in neutrophil counts ranged from 39-69%. The eosinophil increases versus control values were 29, 43 and 57% in the males and 13, 20 and 50% in the females in the low, mid and high dose groups.

The hematology group mean values (neutrophil and eosinophils) on day 14 are shown below:

Parameters	**Pulmonary Deposited Doses (mg/kg)**	**Males**	**Percent Increase**	**Females**	**Percent Increase**
Neutrophil counts	Air control	0.82	-	0.70	-
	0.21	1.18	44	0.97	39
	0.58	1.14	39	0.98	40
	1.17	1.41	72	1.18	69
Eosinophils	Air control	0.07	-	0.08	-
	0.21	0.09	29	0.09	13
	0.58	0.10	43	0.10	20
	1.7	0.11	57	0.12	50

Clinical chemistry: There were no significant treatment-related clinical chemistry changes.

Urinalysis: The urine pH was increased significantly in the males and females in the high dose groups.

pH values for the males and females is show below:

Parameters	**Pulmonary Deposited Doses**	**Males**	**Females**
pH	Air control	8.2	7.9
	0.21	8.3	7.7
	0.58	8.5	7.9
	1.7	8.9	9.0

Organ weights: There were no treatment-related changes in organ weights.

Gross pathology: There were no treatment-related changes in gross pathology.

Histopathology: 10/10 male and 9/10 female rats in the high dose group had accumulations of alveolar macrophages with abundant foamy cytoplasm in the centroacinar area of the lungs (broncho-alveolar junction). There was no accompanying inflammatory cell infiltration or damage to the alveolar epithelium. 1/10 males and 2/10 females in the control

group, 3/10 males and 2/10 females in the low dose group as well as 3/10 males in the mid dose and 1/10 females in the high dose group also had minimal focal macrophage accumulation. Loss of olfactory epithelium/atrophy was observed in the nasal cavity in 6/10 males and 5/10 females in the high dose group as well as 2/10 males and 1/10 females in the mid dose group. The dorsal meatus in level II was the main site for the lesion.

Toxicokinetics:

The TK data below were collected on days 13/14 of the 2 week study.

Table 2-1: TK parameters for QAB149 in rat serum

Dose mg/kg/day	*Sex*	*AUC* (ng/ml)*h	C_{max} ng/ml	T_{max} (h)	*AUC/Dose* (ng/ml)*h / mg/kg/day	C_{max}/*Dose* (ng/ml) / mg/kg/day
2.05	M	288.75	27.93	24.26	140.85	13.62
	F	170.57	14.44	24.26	83.20	7.04
5.80	M	121.48	17.63	1.75	20.9	3.04
	F	100.65	12.09	3.25	17.4	2.08
17.00	M	236.71	33.43	5.00	13.92	1.97
	F	222.42	24.40	5.00	13.08	1.44

For TK calculations, the inhalation time was added to each sampling time point (0.26, 0.75 and 2.5 h for the 2.05, 5.80 and 17.00 mg/kg/day dose groups, respectively).

Elevated levels (24 hours) compared with the mid and high dose groups were observed in the rats in the low dose group rats. There were no significant gender differences in this study. The reason for the increased drug concentrations at the lowest dose is unknown. Measurable QAB149 was found in some the control samples. The levels in the control rats were much lower than the levels in the QAB149-dosed rats. The serum concentrations (ng/mL) of QAB149 in the individual control rats are displayed in the table below. The source of the contamination is unknown.

Table 5.1-1: Serum concentration (ng/mL) of QAB149 in control animals

Animal No.	Sex	Serum concentration (ng/mL) 0.5 h	1 h	2.5 h	8 h	24 h
101	Male	2.26*				
102			1.65*			
103				0.55*		
104					BLQ*	
105						BLQ*
125	Female	2.80**				
126			BLQ*			
127				1.32*		
128					BLQ*	
129						2.18*

BLQ: Below limit of quantitation (0.5 ng/mL, dilution factor is 2).
*: Sample was repeated. The repeat analysis confirmed the presence of drug in some of the control animal samples. The original value is reported.
**: There was not enough sample left to repeat the analysis. The original value is reported.

Summary of Individual Study Findings: Micronized QAB149 powder was administered by inhalation to Wistar rats using daily pulmonary deposited doses of 0, 0.21, 0.58 and 1.7 mg/kg

for 2 weeks. There were accumulations of alveolar macrophages at the broncho-alveolar junction of the lung, primarily at the high dose, 1.7 mg/kg pulmonary deposited dose, and atrophy or loss of olfactory epithelium in the nasal cavity at the two highest doses 0.58 and 1.7 mg/kg pulmonary deposited doses). There were no accompanying inflammatory cells. The NOAEL was the low dose of 0.21 mg/kg. Kinetic measurements showed a lack of dose-dependency, especially at the low dose.

Study title: 4 Week Inhalation Study in the Rat

Key study findings: Increases in body weight gain in all drug treated animal groups, and reversible focal, olfactory epithelial degeneration, characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of epithelium in the mid and high dose groups were observed following administration of QAB149 in an HFA formulation. Reversible increases in white blood cells were observed in most treatment groups. The NOAEL in this study was 0.093 mg/kg and was associated with an AUC of 50-68 (ng/ml).hr on day 27/28.

Study no: 0120088
Volume #, and page #: Volume 5, page 8
Conducting laboratory and location: (b) (4)
Date of study initiation: November 7, 2001
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: QAB149 (batch number TM-01-00046) supplied in metered dose inhalers, 94.3%
Formulation/vehicle: QAB149-HFA (batch number TM-01-00045)

Methods (unique aspects):
Dosing:

Species/strain: Rat,Wistar (Crl:WI (GLx.BRL/Han) IGS BR
#/sex/group or time point (main study): 10/sex/group
Satellite groups used for toxicokinetics or recovery: 6/sex/group satellite, 5/sex/group recovery animals.
Age: 8 weeks
Weight: 191-266 g males, 146-198 g females
Doses in administered units:

Dose Group	**Achieved Dose (mg/kg)/Pulmonary Deposited doses (mg/kg)**
Vehicle control	0
Low dose	0.93/0.093
Mid dose	2.77/0.28
High dose	8.46/0.85

The target doses refer to the free base of QAB149 and the conversion factor for salt to base is 0.7718.

Route, form, volume, and infusion rate: Inhalation, QAB 149/HFA metered dose inhaler, nose only, exposure durations were approximately 27, 78 and 230 minutes in the low, mid and high dose groups, respectively. The control rats were exposed to air for 230 minutes. The achieved dose levels were estimated using the following criteria:

$$\text{Dose } (mg.kg^{-1}) = \frac{MV \times T \times CC}{BW}$$

MV = minute volume (l) = $\frac{2.10 \times \text{body weight } (g)^{0.75}}{1000}$ [Guyton AC (1947)]

T = Duration of exposure (min)

CC = Chamber concentration of active drug = $mg.l^{-1}$

BW = Body weight (expressed in kg – the mid-week mean for males and females was calculated separately)

(b) (4)

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Clinical signs: Once daily
Body weights: Once weekly
Food consumption: Once weekly
Ophthalmoscopy: End of the study, 4 weeks
EKG: NA
Hematology: 4 weeks and at the end of the recovery period
Clinical chemistry: 4 weeks and at the end of the recovery period
Urinalysis: End of the study, 4 weeks
Gross pathology: Terminal
Organs weighed: Terminal, Organ weighed include adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, submaxillary salivary glands, testes, thymus, thyroids with parathyroids and uterus.
Histopathology: Terminal, groups 1 and 4 as well as respiratory tissues from groups 2 and 3.
Toxicokinetics: Days 1 and 2 and 29/30 at 0.5, 1.0, 3.0, 8.0 hours and 24 hours, just prior to the next dosing period.

Results:

Mortality: There was no unscheduled mortality in this study.
Clinical signs: There were no treatment-related clinical symptoms observed in this study.
Body weights: There were significant, treatment-related increases in body weight gain in all QAB149 treated rats vs the control rats in this study. The mean increases were approximately 22, 23 and 17% in the males in the low, mid and high dose groups, respectively. The mean

increases in the females were 23, 40 and 43%. Following a 15 day recovery period body weight gains were similar in the control and high dose group.

Food consumption: There were no treatment-related changes in food consumption.

Ophthalmoscopy: There were no treatment-related eye effects.

Electrocardiography: NA

Hematology: Treatment-related increases in white blood cells, neutrophils, lymphocytes and monocytes were observed in all QAB149 treated groups; increases were not dose-dependent in males. In males, the increases ranged from 36-46% for lymphocytes, 41-69% for neutrophils, 30-49% for lymphocytes and 33-67% for monocytes. In females, the increases ranged from 14-47% for lymphocytes, 1-58% for neutrophils, 17-43% for lymphocytes and 20-120% for monocytes. Following a 15-day recovery period, the hematology parameters were similar in the control and the high dose rats. Eosinophil counts were increased (50-75%) at the two highest doses) and platelet counts were decreased (12-17% in all treatment groups) in females. The hematology group means values are shown below on day 29.

Haematology: Main Study (Day 29): Group Mean Values: Females

Dose Group/Treatment		Hb	RBC	Hct	MCH	MCV	MCHC	RDW	HDW	Reti	WBC	Neut	Lymp	Mono	Eos	Baso	LUC	Plat	PT	APTT
1	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9
Vehicle Control	Mean	14.0	7.55	0.389	18.5	51.5	35.9	13.4	2.40	2.7	4.39	0.78	3.45	0.05	0.08	0.01	0.03	831	14	41.4
	SD	0.5	0.27	0.015	0.6	1.4	0.6	1.2	0.08	0.4	1.22	0.29	1.00	0.02	0.04	0.01	0.02	66	1	5.2
2	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	4
Low Dose	Mean	13.8	7.38	0.388	18.7	52.6	35.6	14.2	2.46	2.9	5.02	0.79	4.03	0.06	0.09	0.01	0.04	733	14	42.0
	SD	0.3	0.24	0.008	0.6	1.4	0.4	2.8	0.10	0.5	1.36	0.32	1.06	0.03	0.03	0.01	0.02	73	1	3.5
	Prob.																	**		
3	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	6
Intermediate Dose	Mean	13.6	7.20	0.377	18.8	52.3	36.0	14.1	2.49	2.9	5.65	1.06	4.30	0.09	0.12	0.01	0.05	708	15	40.3
	SD	0.4	0.24	0.015	0.7	1.8	0.9	1.4	0.09	0.5	1.71	0.28	1.61	0.04	0.03	0.01	0.02	68	1	4.2
	Prob.		**									*		**	*		**	***		
4	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	3
High Dose	Mean	13.9	7.48	0.390	18.6	52.2	35.7	13.0	2.51	2.6	6.46	1.23	4.92	0.11	0.14	0.01	0.05	693	15	45.0
	SD	0.4	0.26	0.015	0.4	1.0	0.6	1.4	0.12	0.4	1.89	0.40	1.73	0.04	0.05	0.01	0.02	67	1	3.5
	Prob.										**	**		***	**		**	***	**	

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Haematology: Main Study (Day 29): Group Mean Values: Males

Dose Group/ Treatment		Hb	RBC	Hct	MCH	MCV	MCHC	RDW	HDW	Reti	WBC	Neut	Lymp	Mono	Eos	Baso	LUC	Plat	PT	APTT
1	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9
Vehicle Control	Mean	15.1	8.28	0.428	18.3	51.7	35.4	14.9	2.71	2.3	5.32	0.90	4.20	0.06	0.11	0.01	0.04	796	15	46.0
	SD	0.5	0.39	0.019	0.7	1.7	0.6	3.3	0.15	0.3	1.06	0.28	0.90	0.02	0.04	0.01	0.02	55	1	3.2
2	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	7
Low Dose	Mean	14.8	8.03	0.418	18.4	52.1	35.3	15.5	2.85	2.8	7.78	1.27	6.24	0.08	0.13	0.02	0.05	756	15	42.7
	SD	0.6	0.33	0.020	0.4	1.1	0.8	3.0	0.07	0.4	1.75	0.39	1.75	0.02	0.04	0.02	0.02	85	0	3.4
	Prob.								**	**	***	*	***	*						
3	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	6
Intermediate Dose	Mean	14.6	8.07	0.416	18.1	51.6	35.0	14.0	2.86	2.9	7.49	1.52	5.66	0.10	0.15	0.02	0.04	759	15	45.0
	SD	0.6	0.27	0.014	0.9	2.0	0.8	1.7	0.11	0.4	1.24	0.39	1.11	0.01	0.07	0.01	0.02	81	1	3.2
	Prob.								**	**	***	***	*	***						
4	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	3
High Dose	Mean	14.8	8.24	0.426	18.0	51.9	34.7	13.9	2.82	2.8	7.25	1.48	5.47	0.09	0.15	0.02	0.04	656	17	48.0
	SD	0.4	0.16	0.010	0.4	1.2	0.5	1.5	0.09	0.4	1.14	0.37	0.89	0.02	0.08	0.01	0.02	205	1	5.3
	Prob.								*	**	**	**	*	***					***	

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Clinical chemistry: Total bilirubin was similarly increased versus control values in all treated groups. The increases were approximately 50% in males and females. The total bilirubin was similar in control and the high dose rats following a 15-day recovery period. The total bilirubin values for the males and female is shown below on day 29:

Parameters	Pulmonary Deposited Doses	Males	Females
Total Bilirubin	Vehicle control	0.8	0.8
	0.09	1.2	1.4
	0.28	1.1	1.4
	0.85	1.2	1.2

Urinalysis: There were no treatment-related findings.

Organ weights: There were no treatment-related findings.

Gross pathology: There were no treatment-related findings.

Histopathology: Reversible (15 days recovery period), focal, olfactory epithelial degeneration, characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of epithelium was observed in 5/10 M, 6/10 F in the high dose group as well as 1/10 M in the mid dose group (minimal). The changes were found along the roof of the dorasal meatus. Reversible (2 weeks recovery period), focal squamous metaplasia was seen in the epithelium lining of the ventral floor of the larynx at the base of the epiglottis, cranial to the ventral laryngeal diverticulum in 8/10 males and 8/10 females in the high dose group. The laryngeal metaplasia is considered a rodent specific response to inhaled particulates. There was no reported macrophage accumulation in the dosed rats.

Toxicokinetics:

Table 2-1 Mean TK parameters of QAB149 in rat

Day 1/2								
Dose* (mg/kg/day)	Sex	AUC (ng/mL)*h	SE	Tmax (h)	Cmax (ng/mL)	AUC/Dose (ng/mL)*h/(mg/kg/day)	SE/Dose	Cmax/dose (ng/mL)/(mg/kg/day)
0.93	M	411.19	136.47	3.43	90.10	442.14	146.74	96.88
0.93	F	168.46	17.88	3.43	33.89	181.14	19.23	36.44
2.77	M	571.70	310.74	4.28	108.81	206.39	112.18	39.28
2.77	F	381.74	114.86	4.28	43.73	137.81	41.47	15.79
8.46	M	365.52	106.05	3.75	48.01	43.21	12.54	5.67
8.46	F	287.11	20.06	3.75	64.07	33.94	2.37	7.57
Day 27/28								
Dose (mg/kg/day)	Sex	AUC (ng/mL)*h	SE	Tmax (h)	Cmax (ng/mL)	AUC/Dose (ng/mL)*h/(mg/kg/day)	SE/Dose	Cmax/dose (ng/mL)/(mg/kg/day)
0.93	M	50.91	5.82	0.93	12.26	54.74	6.26	13.18
0.93	F	67.73	5.72	0.43	14.96	72.83	6.15	16.09
2.77	M	127.62	5.40	1.28	29.50	46.07	1.95	10.65
2.77	F	118.32	12.25	1.28	19.41	42.71	4.42	7.01
8.46	M	324.92	40.14	3.75	33.00	38.41	4.74	3.90
8.46	F	336.67	38.04	3.75	65.50	39.80	4.50	7.74

* Dose reported as the free base. Inhalation time was 0.43 h, 1.28 h and 3.75 h for the 0.93, 2.77, and 8.46 mg/kg/day dose groups respectively.

QAB149 was found in all the control samples. The plasma concentrations were less than those concentrations observed in the low dose group (see individual concentrations for control rats in

table below). The source of the contamination is unknown. There were no significant differences in the systemic exposure of QAB149 in male and female rats. The AUC values for the days 1/2 analyses were extremely high compared with days 27/28 in both males and females, but especially in the males, in the low and mid-dose groups. Tmax was between 1 and 4 hours. AUCs increased linearly on days 27/28.

Table 4-1 Concentration of QAB149 on day 1/2 group 1 control animals

Animal sex/No	Time (h)[x]					
	Immediate post dose	0.5	1	3	8	24
m 111	0.61*			0.45**		
m 112	0.43			2.08**		
m 113		1.25*			0.80	
m 114		0.72*			0.72*	
m 115			0.13			0.51*
m 116			0.40			0.23
f 132	0.30			0.43*		
f 133	0.32			0.88		
f 134		0.22			1.10*	
f 135		0.16			0.64*	
f 136			0.80			0.14
f 137			0.86*[b]			0.48**

[x] The time points given are post dose (end of inhalation) with the exception of the 24 h time point, which is measured from the start of inhalation. The vehicle inhalation time was 3.75 h for this dose group.

Summary of Individual Study Findings: QAB149 in an HFA formulation was administered by inhalation to Wistar daily using pulmonary deposited doses of 0, 0.093, 0.28 and 0.85 mg/kg for 4 weeks. There were increases in body weight gain in all treated animal groups. Reversible focal, olfactory epithelial degeneration, characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of epithelium was observed in the mid and high dose groups. Reversible increases in white blood cells were observed in most treatment groups. The NOAEL in this study was 0.093 mg/kg and was associated with and AUC of 51-68 ng/ml.hr.

Study title: Escalating Dose Ranging Finding Inhalation Study in Dogs

Key Individual Study Findings: QAB149 administered to Beagle dogs as single doses and multi doses, 1-4 days, using inhalation doses of 0.1-3.0 mg/kg (pulmonary deposited doses of 0.25-0.75 mg/kg) resulted in clinical pathology alterations (decreased RBC, hematocrits, hemoglobins as well as increased CPK and ALT levels) in some or all dogs. Histopathology analyses show changes in liver, heart and nasal passages in some or all dogs. Increases in heart rate were also noted. Liver changes include periportal hepatocellular vacuolation. The heart changes include slight to moderate hemorrhage, myofiber degeneration and mineralization of the papillary muscles of the left ventricle. The nasal changes included focal areas of submucosal edema of the nasoturbinates. The MTD was approximately 0.25 mg/kg (pulmonary deposited dose).

Study no:659977
Volume #, and page #: Volume 5, page 319
Conducting laboratory and location: (b) (4)
Date of study initiation: April 10, 2000
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: QAB149 maleate/#KL-5994/12.4/98.9%

Methods (unique aspects)**:**
Dosing:

Species/strain: Dog, Beagle
#/sex/group or time point (main study): see below

Dose group	Number of Males/ Females	Day of Treatment*	Target Dose (mg/kg)	Pulmonary Deposited Dose (mg/kg)
1	1/3	1 2 3 7	0.1 0.3 1.0 3.0	0.025 0.075 0.25 0.75
2	2/4	1-4	1.0	0.25

*Dogs in group 1 were not treated days 4-6. Dogs in group 1 were treated with different doses on different days.

Satellite groups used for toxicokinetics or recovery: None
Age: 6 months
Weight: 11-11.6 kg
Doses in administered units: 0.1, 0.3, 1.0 and 3.0 mg/kg (single doses) and 1.0 mg/kg for the 4 day study. Pulmonary deposited doses listed in table above. The achieved doses were estimated using the following criteria:

Table 3.3-1. Dose level estimation

$$\text{Dose (mg.kg}^{-1}) = \frac{MV \times T \times CC}{BW}$$

MV = Respiratory volume/minute[1] (litres) = $2.10 \times \text{weight}^{0.75}$
T = Duration of exposure (minutes)
CC = Chamber concentration of active drug = mg/litre^{-1}
BW = Body weight (expressed in kg - males and females were calculated separately)

(1) Guyton, Measurement of Respiratory Volumes of Laboratory Animals, 1947.

(b) (4)

Observations and times:

Clinical signs: Clinical signs were observed daily, approximately 1 hour after dosing.

Body weights: Daily

Food consumption: Food consumption was recorded daily

Ophthalmoscopy: NA

EKG: EKGs were conducted predose, 10 minutes, 30 minutes, 1,2, 3,4,5, and 6 hours after dosing.

Hematology: Predose and at the end of dosing

Clinical chemistry: Predose and at the end of dosing.

Urinalysis: NA

Gross pathology: At the end of the dosing period.

Organs weighed: NA

Histopathology: The following tissues from all dose groups were analyzed: bronchial lymph node, liver, kidney, heart, lung, nasal passages, larynx, jejunum, trachea and macroscopic lesions.

Toxicokinetics: Blood was collected from 2 dogs in 4 day study at 0, 1, 2, 4, 8 and 24 hours, day 4.

Results:

Mortality: There were no mortalities.

Clinical signs: All the dogs had reddened ears, gum and abdomen and increased heart force and rate.

Body weights: The body weight gain was similar in the dogs in this study.

Food consumption: The food consumption was similar in all dogs in this study.

Ophthalmoscopy: NA

Electrocardiography: The heart rate in the dogs in group 1 was markedly increased following pulmonary deposited doses of 0.25 (day 3) and 0.75 mg/kg (day 8). The heart rates were also increased in the dogs administered deposited doses of 0.25 mg/kg (day 3) (group 2). There were no reported Q-Tc changes.

Hematology: Treatment-related hematology changes (day 9) include decreases in hematocrits, hemoglobin and red blood cells in the dogs in group 1. The decreases were 27, 25 and 24% for hematocrit, hemoglobin and red blood cells in the males (1 dog values), respectively, and 31, 31 and 27%, respectively, in the females. Dogs in group 2 also had decreases in hematocrits, hemoglobin and red blood cells on day 5. The decreases were 17, 18 and 19%, respectively, in the males and 15, 18 and 17%, respectively, in the females.

Clinical chemistry: There were treatment-related clinical chemistry value increases in the dogs in group 2 (0.25 mg/kg) on day 5. The increases in CPK parameters were 446% in males and 53% in the females and in ALTs (43% in the males and 92% in the females). There were no other clinical chemistry changes.

Urinalysis: NA

Organ weights: NA

Gross pathology: There were treatment-related dark purple foci on the papillary muscle of the heart and pale discoloration areas in the liver in the male in group 1.

Histopathology: There were histopathologic changes in the heart, lungs, liver and nasal passages. Small areas of hemorrhage were observed in the myocardium or subendocardial regions of the papillary muscles of the left ventricles in both dogs examined in group 1, i.e, 1/1 males and 1/3 females. Moderate to marked periportal cytoplasmic vacuolation of the liver was observed in all dogs in both dose groups. Submucosal edema was observed in the nasoturbinates of females in group 2 and 1 male and 1 female in group 1. Multifocal subacute to chronic

inflammatory infiltrates were noted in the lungs of all dogs in both dose groups. These infiltrates were characterized by mononuclear cells, neutrophils and fibrocytes.

Toxicokinetics: Both animals analyzed for drug concentrations had measurable drug concentrations. The drug concentrations were similar in the male and female dogs. The PK parameters for the dogs administered 0.25 mg/kg daily (pulmonary deposited dose) for 4 days are shown below:

PK parameters	**Male (n=1)**	**Female (n=1)**	**Mean**
AUC **(**0-24 hours (ng.h/mL)	280	148	214
Cmax (ng.mL)	28.05	24.67	26.36
Tmax (h)	1.0	2.0	1.5

Summary of individual study findings

QAB149 administered to Beagle dogs as single doses and multi doses, 1-4 days, using inhalation doses of 0.1-3.0 mg/kg (pulmonary deposited doses up to 0.75 mg/kg) resulted in clinical pathology alterations (decreased RBC, hematocrits, hemoglobins as well as increased CPK and ALT levels) in some or all dogs. Increases in heart rate were also observed. Histopathology analyses show changes in liver, heart and nasal passages in some or all dogs. Liver changes include periportal hepatocellular vacuolation. The heart changes include slight to moderate hemorrhage, myofiber degeneration and mineralization of the papillary muscles of the left ventricle. The nasal changes included focal areas of submucosal edema of the nasoturbinates. The MTD was approximately 0.25 mg/kg (pulmonary deposited dose).

Study title: 2 Week Inhalation Toxicity Study in Dogs

Key study findings: Tachycardia and myocardial necrosis was present in the dogs in the mid and high dose groups after dosing with a dry powder formulation. The tachycardia was most severe on day 1. There were increases in heart rate and heart force (strength of heart beat) in all QAB 149-treated dogs. These dogs also had increased respiratory rates on day 1. Also noted was corresponding reductions in interval data, primarily Q-T. Assessment of the Q-Tc (i.e. normalization of Q-t interval for heart rate, based on Bazett's and Friderica's formulas) showed a trend toward increased values. The increase was consistent with the heart rate. There was dose-related periportal glycogen vacuolation in the liver of all QAB149 dosed groups. There was no NOAEL in this study (< 0.0025 mg/kg) based on the liver lesions.

Study no: 002013
Volume #, and page #: Volume 6, page 1
Conducting laboratory and location: (b) (4)
Date of study initiation: June 29/30, 2000
GLP compliance:Yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: QAB149 Maleate micronized (batch # 0021001), 94.3% purity
Formulation/vehicle: QAB 149 micronized powder, conversion factor salt/base 1.296

Methods (unique aspects):
Dosing:

Species/strain: Beagle dog
#/sex/group or time point (main study): 3M and 3F/ dose group
Satellite groups used for toxicokinetics or recovery: None
Age: 7-71/2 months
Weight: 6.7-8.6 kg for males and 5.9- 7.8 kg for females
Doses in administered units:

Dose Groups	**Achieved Dose (mg/kg)/Pulmonary Deposited dose (mg/kg)**
Control	0
Low dose	0.01/0.0025
Mid dose	0.47/0.12
High dose	0.93/0.23

Route, form, volume, and infusion rate: The dogs were dosed daily by dry powder inhalation for 14 days using a closed face mask. Dose duration was approximately 5-8, 1.75-2.25, 3-3.5 5.5-7.5 minutes for the control, low, mid and high dose groups, respectively. Aerosol generation was undertaken using a rotating generation device (RBC 1000, Palas, Germany). (b) (4) The resultant aerosol was delivered to the dose chamber from which the dog breathed via an oro-pharyngeal tube enclosed in a close fitting face mask. The aerosol flow rate to each mask was at least 5 1.min^{-1} with a balanced input and exhaust. The mean analytical aerosol concentrations of QAB149 ranged from 0.002-0.013 mg.l^{-1} for the low dose, 0.144-0.289 mg.l^{-1} for mid dose and 0.174-0.279 mg.l^{-1} for the high dose. (b) (4)

The achieved doses were estimated using the following criteria:

$$\text{Dose (mg.kg}^{-1}) = \frac{RV \times T \times CC}{BW}$$

RV = Nominal volume of 5 litre.min^{-1}
T = Duration of exposure (minutes)
CC = Chamber concentration of active drug = mg.litre^{-1}
BW = Mid-week body weight (expressed in kg)

Assuming a 25% deposition in the dog, the pulmonary deposited doses in this study are 0.0025, 0.12 and 0.23 mg/kg

Observations and times:

Morality: Twice daily
Clinical signs: Once before, once during the treatment and once after treatment
Body weights: Recorded weekly

Food consumption: Recorded daily

Ophthalmoscopy: Evaluated pretest and on week 13

EKG: Measurements were taken pretest and on days 1 and 14 at dosing (0), at 0.5, 1, 2.5, 8 and 24 hours after dosing.

Hematology: Blood samples were collected pretest and on day 14

Clinical chemistry: Blood samples were collected pretest and on day 14

Urinalysis: Samples were collected pretest and on days 13 and 14.

Gross pathology: Examinations were carried out at termination, days 14 and 15.

Organs weighed: Organ weighed included adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, submaxillary salivary glands, testes, thymus, thyroids with parathyroids and uterus. Organs were weighed on days 14 and 15.

Histopathology: Full histopathology examinations were carried out on all the dogs in each dose group.

Toxicokinetics: Blood samples were obtained from all the dogs on days 1 and 14 at 0.5, 1, 2.5, 8 and 24 hours.

Results:

Mortality: There were no mortalities in this study.

Clinical signs: The dogs in all dose groups had reddened ears and or gums. All the treated dogs had increases in heart rates and heart force (strength of heart beat) which varied in severity for each animal (6/6 in all dose groups). Carotid pulse was noted in the dogs in the mid and high dose groups (6/6 dogs in each dose group).

Body weights: Body weight gain was similar in all dose groups except the female dogs in the control group. The control dogs had a 5% decrease in body weight gain, comparing body weight gain at the end of the study with body weight gain at pretrial.

Food consumption: There were no treatment–related effects on food consumption in this study.

Ophthalmoscopy: There were no treatment-related effects on the eyes of the dogs in this study.

Electrocardiography: Tachycardia was noted in the male and female dogs in the mid and high dose groups. The maximum increase in heart rates was noted 1 hour after dosing on day 1 (approximately 140-160%). On day 14, the maximum heart rates were noted 2.5 hours after dosing (approximately 40-60%). On day 1, the 8 hour evaluations reveal heart rate increases of 37 and 49% for the males in the mid and high dose groups while the increases were 78 and 60% in the mid and high dose females. After 24 hours, tachycardia was reduced but higher than predose. The 24 hour evaluations reveal 19 and 24% increases in the males and 44 and 40% in the females. On day 14, the 8 hour and 24 evaluations show heart rates were similar in the males and females except for lower heart rates in the females in the high dose group at the 8 hours evaluations (25%) and the males in the mid dose group (25%). The increase in heart rate is reflected by reduction in interval data, primarily the Q-T interval. The reduction in Q-T is expected with an increase in heart rate. Assessment of the Q-Tc (normalization of the Q-T interval for heart rate) by the Bazett's formula showed increased Q-Tc values for the dogs in the mid and high dose groups for up to 24 hours on day 1 and up to 8 hours on day 14. Q-Tc values calculated by Fridericia's formula showed similar trends. The increases on day 1 as calculated by Bazett's formula were 17 and 7% in the mid and high dose males and 23 and 41 mid and high dose female. The increases on day 1 as calculated by Fridericia's formula were 11 and 7% in the males in the mid and high dose groups while the increases in the females were 13 and 16%. On

day 14, the increases on day 1 as calculated by Bazett's formula were 11 and 3% in the in the males in the mid and high dose groups and 15 and 3% in the females. The increases on day 14 as calculated by Fridericia's formula were 5 and 1% in both the males and females.

Hematology: There were no treatment–related changes in hematology parameters.

Clinical chemistry: There were no treatment–related changes in clinical chemistry parameters.

Urinalysis: There were no treatment-related changes in urinalysis parameters.

Organ weights: Covariance analyses show a statistically significant increase in relative liver weight of the males in the mid dose group by 42%. These data also show a statistically significant decrease in the relative salivary gland weight of the female dogs in the low and mid dose groups, 25 and 20%, respectively. These changes are not considered to be drug-related. The relative salivary glands weight was reduced in the females in the high dose group by 15 % (decreases were not significant). There were no findings in the lungs.

Gross pathology: There were no significant, drug-related lesions found at necropsy.

Histopathology: Minimal to moderate focal myocardial necrosis and mild to marked fibrosis was observed in 1/3 males and 2/3 females in the high dose group and 1/3 females in the mid dose group. The lesions were found mainly in the papillary muscles but individual incidences were noted in the septum and left ventricle wall. Focal myocardial necrosis was characterized by clear vacuolation, increased eosinophilia, granularity or fragmentation of the cytoplasm and clear pyknosis. In all of the groups exposed to QAB149, there was dose–related periportal glycogen vacuolation of the liver; severity of lesion generally increased with increasing dose. The periportal hepatocytes of the affected dogs contained irregular intracytoplasmic clear spaces consistent with the presence of glycogen. No local pulmonary findings were noted. The incidence and severity of periacinar glycogen vacuolation in the liver are shown below:

Table 4.4-1. Incidence and severity of periacinar glycogen vacuolation in the liver

	Sex/Group/Dose Level ($mg.kg^{-1}.day^{-1}$)/Group Totals							
	Males				Females			
	1 (0)	2 (0.01)	3 (0.46)	4 (0.92)	1 (0)	2 (0.01)	3 (0.47)	4 (0.93)
LIVER	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Glycogen vacuolation, periportal								
Mild	0	1	0	2	0	2	0	0
Moderate	0	0	3	1	0	0	3	2
Marked	0	0	0	0	0	0	0	1
Total Incidence	0	1	3	3	0	2	3	3

Toxicokinetics: Drug plasma levels were measurable in the dogs in the mid and high dose groups and in the low dose males. Drug exposure in the females in the low dose group was below detection. Systemic exposure increased with dose but dose proportionality could not be assessed because of the small numbers of dogs in each dose group and the high inter-animal variability. Systemic exposures appeared to increase significantly, from day 1 to day 14, especially in the males in the mid and high dose groups. Tmax ranged from 0.5-2.5 hours. The TK parameters for QAB149 in dog serum are shown below:

Table 2-1: TK parameters for QAB149 in dog serum

TK Parameter	Day 1				Day 14			
	Male		Female		Male		Female	
Dose = 0.01 mg/kg/day	Mean*	SD	mean*	SD	mean*	SD	mean*	SD
AUC(0-24h) (ng*h/mL)	0.23	0.21	0.00	0.00	0.57	0.99	0.00	0.00
Cmax (ng/mL)	0.20	0.18	0.00	0.00	0.16	0.28	0.00	0.00
AUC(0-24h)/dose	22.83	21.06	0.00	0.00	57.17	99.02	0.00	0.00
Cmax/dose	20.00	17.58	0.00	0.00	16.33	28.29	0.00	0.00
tmax (h)	0.75	-	-	-	-	-	-	-
Dose = 0.47 mg/kg/day	Mean*	SD	mean*	SD	mean*	SD	mean*	SD
AUC(0-24h) (ng*h/mL)	19.77	15.41	17.96	8.01	29.38	-	61.40	26.26
Cmax (ng/mL)	8.20	8.63	2.04	0.99	4.41	3.17	6.69	4.19
AUC(0-24h)/dose	42.05	32.79	38.22	17.04	62.50	-	130.63	55.88
Cmax/dose	17.44	18.37	4.35	2.12	9.38	6.73	14.23	8.91
tmax (h)	1.00	-	0.50	-	1.00	-	1.00	-
Dose = 0.93 mg/kg/day	Mean*	SD	mean*	SD	mean*	SD	mean*	SD
AUC(0-24h) (ng*h/mL)	24.49	14.30	43.63	31.80	155.65	82.95	96.20	46.00
Cmax (ng/mL)	4.41	2.26	4.17	2.47	32.67	23.82	14.72	7.64
AUC(0-24h)/dose	26.34	15.37	46.91	34.19	167.36	89.19	103.44	49.46
Cmax/dose	4.75	2.43	4.49	2.66	35.13	25.61	15.83	8.21
tmax (h)	1.00	-	0.50	-	2.50	-	1.00	-

*** median value is given for the tmax**

Summary of Individual Study Findings: QAB 149 was administered to Beagle dogs daily for 2 weeks using pulmonary deposited doses of 0.0025, 0.12 and 0.23 mg/kg. Tachycardia and myocardial necrosis was present in the dogs in the mid and high dose groups. The tachycardia was most severe on day 1. There were increases in heart rate and heart force (strength of heart beat) in all QAB 149-treated dogs. These dogs also had increased respiratory rates on day 1. Also noted was corresponding reductions in interval data, primarily Q-T. Assessment of the Q-Tc (i.e. normalization of Q-t interval for heart rate, based on Bazett's and Friderica's formulas) showed a trend toward increased values. The increase was consistent with the heart rate. There was also dose-related periportal glycogen vacuolation in the liver of all QAB149 dosed groups. There was no NOAEL (< 0.0025 mg/kg) in this study based on the liver lesions at all doses.

Study title: 4 Week Inhalation Toxicity Study in Dogs

Key study findings: Tachycardia and myocardial necrosis were present in the dogs in the high dose groups administered QAB 149 in a HFA formulation. Tachycardia was also present in the dogs in the mid dose. There was dose-related, reversible, periportal glycogen vacuolation in the liver of the dogs in the mid and high dose groups. No local pulmonary effects were noted. The NOAEL in this study is 0.0025 mg/kg and it was associated with a mean AUC of 2.93 ng.h/mL.

Study no: 0120089
Volume #, and page #: Volume 7, page 1
Conducting laboratory and location: (b) (4)
Date of study initiation: October 30, 2001

GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: QAB149, lot# TM-01-00046
Formulation/vehicle: 250 µg of QAB149-HFA maleate salt per actuation (100 actuation/MDI)/HFA vehicle (lot # TM-01-00045)

Methods (unique aspects):
Dosing:

Species/strain: Dog, Beagle
#/sex/group or time point (main study): 3/sex/dose group
Satellite groups used for toxicokinetics or recovery: Recovery period included 2 dogs/sex in the control and high dose groups.
Age: 4-5 1/2
Weight: 7.8-9.7 kg for males and 6.4-8.1 kg for females
Doses in administered units:

Dose Groups	Achieved Dose (mg/kg)/ Pulmonary deposited doses (mg/kg)
Control, vehicle	0
Low dose	0.01/0.0025
Mid dose	0.1/0.025
High dose	0.97/0.24

The achieved doses refer to the free base of QAD149 and the conversion factor for salt to base is 0.7718.
Route, form, volume, and infusion rate: QAB149/HFA inhalation solution was administered by inhalation daily for 28 days using a closed mask system. The aerosol flow rate was at least 5 $1.min^{-1}$ with balanced input and exhaust. Dose duration was 19-29.5 minutes for the males and 15-23.5 minutes for females in the control group; 1.07-2.57 minutes for males and 0.70-3.28 minutes for females in the low dose; 1.58-4.00 for males and 0.45-3.17 minutes for females in the mid dose and 20.0-41.5 minutes for males and 15.0-31.5 minutes for females in the high dose. The test aerosol was generated using an in-house MDI actuating system which accommodated up to 6 MDIs. The weekly mean analytical concentrations of QAB149 ranged from 0.015-0.023 mg^{-1} for the low dose; 0.070-0.097 mg^{-1} for the mid dose and 0.071-0.094 mg^{-1} for the high dose. (b) (4)

Achieved dose levels were estimated using the following criteria:

$$\text{Dose}\ (\text{mg.kg}^{-1}) = \frac{\text{MV x T x CC}}{\text{BW}}$$

MV = Minute volume (nominal value of 5 l.min^{-1} used based on in-house laboratory data)
T = Duration of exposure (min)
CC = Chamber concentration of active drug = mg.l^{-1}
BW = Mid-week body weight (expressed in kg)

Assuming 25% deposition in the dog, the deposited doses in this study are 0.0025, 0.025 and 0.24 mg/kg.

Observations and times:

Mortality: Evaluated daily

Clinical signs: Evaluated before dosing, during the dosing period and after dosing.

Body weights: Evaluated weekly

Food consumption: Recorded daily

Ophthalmoscopy: Evaluate pretest, week4 and at the end of the recovery period.

EKG: Evaluated prior to pretest, 0.5, 1, 3 and 8 hours after dosing.

Hematology: Blood samples were collected pretest, day 28 and at the end of the recovery period.

Clinical chemistry: Blood samples were collected pretest, day 28 and at the end of the recovery period.

Urinalysis: Urine samples were collected pretest, days 25/26 and at the end of the recovery period.

Gross pathology: Evaluations were carried on day 29 and at the end of the recovery period.

Organs weighed: The following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, submaxillary salivary glands, testes, thymus, thyroids with parathyroids and uterus. The organs were weighed on day 29.

Histopathology: Full histopathology was carried out on all the dogs in each dose group.

Toxicokinetics: Blood samples were obtained from all animals at the end of the inhalation period, at 0.5, 1, 3 and 8 hours after the inhalation period and 24 hours after the beginning of the inhalation (just prior to the next dose) on days1/2 and 27/28.

Results:

Mortality: There were no mortalities in this study.

Clinical signs: The heart rates were increased in the dogs in the low dose group during the last week of the study. In the mid dose, heart rates and heart force (strength of heart beat) were increased during weeks 3 and 4. Redness, increased heart rates and heart force and strong carotid pulse were observed in the dogs in the high dose group. These clinical signs were observed 1 hour after dosing and lasted for 3 hours after dosing.

Body weights: Body weight gain was comparable in all dose groups.

Food consumption: Food consumption was reduced in the males in the high dose group by 6 % during the first week of the study. Food consumption was statistically significantly reduced in the females in the mid and high dose groups in the second week by 11 and 12 %,

respectively. Food consumption was similar in all dose groups throughout the remainder of the study except for the third week of the recovery period. The females in the high dose group had greater food consumption than the females in the control group, however, in the third week for only 3 days.

Ophthalmoscopy: There were no treatment-related eye changes in this study.

Electrocardiography: On days 1 and 2 of the study, tachycardia was observed for 8 hours after dosing in the males and females in the high dose groups as well as in the males and females in the mid and high dose groups at the 3 hour evaluations. At the 3 hour evaluations, heart rate increases in the male dogs were 34% in the mid dose group and 61% in the high dose group. The heart rate increases in the males were 39% in the high dose group at the 8 hour evaluations. In the females, heart rate increases were 71% in the high dose group at 3 hours and 39% in the females in the 8 hours evaluations. The heart rates were similar in all dose groups 24 hours after dosing. There were reductions in the Q-T interval in the high dose dogs, mainly the female dogs. The reduction in the females was 16% at 3 hours and 12% at 8 hours. The Q-T intervals were similar in the dogs in all dose groups 24 hours after dosing. At the end of the study and following the 17 day recovery period, heart rates and Q-T intervals were similar in all dose groups as assessed by both Bazett's and Fridericia's formulas.

Hematology: Hematology and hematocrit values were statistically significantly reduced in the males and females in the high dose group at the end of the study. The reductions were 14 and 15% in the males and 7 and 12% in the females. The males in the high dose group had reductions in red blood cell counts by 14%. These changes were not observed after the recovery period (17 days).

Clinical chemistry: There were increases in the aspartate aminotransferase values in the males in the low dose group as well as the potassium values. The increases were 45% for aspirate aminotransferase and 14% for potassium; no changes were noted at higher doses. The creatine phosphate values were increased in a non-dose-dependent fashion in the female and males in all dose groups. The increases were 21, 25 and 19% in the males in the low, mid and high dose groups, respectively. The increases were 6, 14 and 3% in the females in the low, mid and high dose groups, respectively. These changes were not drug-related and were not observed following the recovery period.

Urinalysis: There were statistically significant increases in pH in the males. There were also statistically significant increases in pH in the females low, mid and high dose groups, respectively. Following the recovery period, the pHs were similar in all dose groups. pH values for the dogs in this study are shown below:

Parameter	Dose/ Pulmonary deposited doses	Males	Females
pH	Vehicle control	5.3	5.3
	0.01/ 0.0025	7.0	7.2
	0.1/0.025	6.8	7.2
	0.97/0.24	8.2	7.5

Organ weights: The prostate weight, absolute and relative, was decreased in the mid and high dose dogs. The absolute weight decreases were 21 and 52% while the relative weight decreases were 12 and 53%. The absolute prostate weight was increased in the high dose dogs following the recovery period by 90%. The prostate weight was not evaluated in the dogs in the mid dose group after the recovery period. The absolute and relative weights of the adrenal glands and uteri

were increased in the females in the low and mid dose groups. The absolute weight increases in the adrenal glands were 17 and 17% in the low and mid dose groups while the uteri increases were 30 and 64%.The relative weight increases 22 and 23% for the adrenal glands in the low and mid dose groups and 50 and 72% for the uteri in the low and mid dose groups. The absolute weight for adrenal glands in the females was decreased in the high dose group by 9% and the uteri were decreased in the females in the high dose group by 36%. After the recovery period, the adrenal glands and the uteri organ weight were still decreased by 17 and 37%, respectively. There were no associated histopathologic findings in these organs.

Gross pathology: There were no gross pathology findings in this study.

Histopathology: Myocardial fibrosis with and without mineralization was present in the papillary muscles in 2 of the 3 male dogs in the high dose group. Myocardial fibrosis was also present in 1 out of 3 male dogs in the vehicle control group (unexplained). After the 17 day recovery period, 2/2 male and 1/2 female dogs in the high dose group had myocardial fibrosis in the papillary muscles. In all of the dogs in the high dose group (severity=5/6 mild and 1/6 minimal) and 5/6 dogs (severity = minimal) in the mid dose group, there was hepatocyte vacuolation in the periportal area. Oil–red-O staining was positive in only one dog so the sponsor feels that the vacuolation was not due to fat deposition. Periodic acid Schiff stains examined from the main study dogs in the control, mid and high dose groups indicated a clear difference in the pattern of glycogen deposition. In the dogs in the mid and high dose groups, the glycogen deposition was in the periportal area while in the control dogs, the glycogen deposition was concentrated in the centrilobular area. The glycogen deposition was not present after a 17 day recovery period. There were no microscopic findings in the lung.

Toxicokinetics: The toxicokinetic report reveals that dogs in all dose groups were exposed to QAB149. After multiple dosing, exposure to QAB149 was dose proportional in the female but supra-proportional in the males. Exposures tended to be greater in males than females and drug appeared to accumulate with increasing dose duration. T_{max} ranged from 0.5 -3 hours. The mean TK parameters of QAB149 in the dog are shown below:

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Table 2-1 Mean TK parameters of QAB149 in Dog

	Day 1/2				Day 27/28			
Dose 0.01 mg/kg/day	**Male**		**Female**		**Male**		**Female**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Cmax (ng/mL)	0.21	0.20	0.13	0.04	0.36	0.04	0.14	0.03
Tmax (h)[A]	3.00		0.50		3.00		3.00	
AUC (ng/mL)*h	1.05	0.70	0.34	0.26	3.93	0.38	1.94	0.32
AUC/Dose (ng/mL)*h/(mg/kg/day)	105.47	70.27	33.73	26.47	393.09	37.87	194.11	31.97
Cmax / Dose (ng/mL) /(mg/kg/day)	20.67	19.50	13.00	4.36	36.33	3.81	14.00	2.65
Dose 0.1 mg/kg/day	**Male**		**Female**		**Male**		**Female**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Cmax (ng/mL)	1.99	0.47	0.81	0.38	2.14	1.44	2.09	0.29
Tmax (h)[A]	1.00		1.00		3.00		0.50	
AUC (ng/mL)*h	16.06	1.50	6.84	3.54	24.84	11.52	21.36	6.24
AUC/Dose (ng/mL)*h/(mg/kg/day)	160.63	15.04	68.37	35.46	248.44	115.18	213.55	62.42
Cmax / Dose (ng/mL) /(mg/kg/day)	19.93	4.74	8.13	3.82	21.43	14.38	20.90	2.89
Dose 0.97 mg/kg/day	**Male**		**Female**		**Male**		**Female**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Cmax (ng/mL)	35.62	26.81	17.09	11.92	36.23	10.06	22.66	7.12
Tmax (h)[A]	0.50		0.50		1.00		3.00	
AUC (ng/mL)*h	227.27	120.95	142.47	82.62	360.02	63.70	239.77	77.97
AUC/Dose (ng/mL)*h/(mg/kg/day)	234.30	124.69	146.88	85.18	371.15	65.67	247.18	80.38
Cmax / Dose (ng/mL) /(mg/kg/day)	36.62	27.64	17.62	12.29	37.35	10.37	23.26	7.34

[A] Median Value reported for T_{max}.

Summary of Individual Study Findings: QAB 149 was given by inhalation to Beagle dogs daily for 4 weeks using pulmonary deposited doses of 0, 0.0025, 0.025 and 0.24. Tachycardia and myocardial necrosis were present in the dogs in the high dose groups. Tachycardia was present in the dogs in the mid dose group. There was dose-related periportal glycogen vacuolation in the liver of the dogs in the mid and high dose groups. There was no glycogen vacuolation of the liver in these dogs following a 17 day recovery period. The NOAEL in this study is 0.0025 mg/kg and it was associated with a mean AUC of 2.93 (ng/ml).hr.

Toxicology summary: Acute studies were carried out in the mouse and the rat. QAB 149 micronized power was administered orally and subcutaneously. Results of these studies show oral doses up to 1600 mg/kg did not induce any adverse events in the mouse or the rat. However, when QAB 149 was administered subcutaneously, deaths were observed at a dose of 200 mg/kg in the rat. The maximum non-lethal dose was 100 mg/kg in the rat. In the mouse, deaths were observed at 50 mg/kg in the males and 200 mg/kg in the females. The maximum non-lethal dose was 5 mg/kg in the male mouse and 100 mg/kg in the females. The cause of deaths in these rodents was thought to be cardiovascular related.

Subchronic inhalation dose-ranging studies were conducted in the rat and the dog with the objective of identifying the inhalation doses to be used in 2 week studies with micronized drug and 4 week subchronic studies with an HFA formulation. In the two week study in the rat with micronized drug, pulmonary deposited doses were 0.21, 0.58 and 1.7 mg/kg. Adverse findings were primarily pulmonary-related; alveolar macrophages with foamy cytoplasm were observed in lungs of the rats including the control group. The degree of macrophage infiltration was dose related. Other findings included loss of olfactory epithelium/atrophy in the nasal cavity. Toxicokinetic parameters show QAB149 exposure in all dosed rats, however, exposure was not dose-dependent; QAB149 was also found in all control samples. The NOAEL was 0.21 mg/kg and was associated with a mean AUC of 170-289 (ng/ml).h. Similar results were observed in the four week study with the HFA formulation in which the pulmonary deposited doses were 0.093, 0.28 and 0.85 mg/kg. Reversible focal, olfactory epithelial degeneration characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of the epithelium in the mid and high dose groups were observed. Reversible increases in white blood cells were observed in most treatment groups. The study included toxicokinetics, however QAB149 was again found in all control samples. The NOAEL was 0.093 mg/kg associated with a mean AUC of 59.3 (ng/ml).h.

The 2 week study in the dog with micronized drug included pulmonary deposited doses of 0.0025, 0.12 and 0.23 mg/kg. QAB149 induced tachycardia and myocardial necrosis in the dogs in the mid and high dose groups. There were increases in heart rate and heart force (strength of heart beat) in all QAB 149-treated dogs. These dogs also had increased respiratory rates on day 1. Also noted was corresponding reductions in cardiac cycle interval data, primarily Q-T. Assessment of the Q-Tc (i.e. normalization of Q-T interval for heart rate, based on Bazett's and Friderica's formulas) showed a trend toward increased values. The increase was consistent with the heart rate. There was also dose-related periportal glycogen vacuolation in the liver of all QAB149 dosed groups. There was no NOAEL in this study (< 0.0025 mg/kg). Toxicokinetic parameters reveal drug plasma levels in all the treated dogs. The 4 week subchronic inhalation study with the HFA formulation includes pulmonary deposited doses of 0.0025, 0.025 and 0.24 mg/kg. As in the 2-week study, QAB149 induced tachycardia and myocardial necrosis in the

dogs in high dose group and tachycardia in the dogs in the mid dose group. There was also dose-related glycogen vacuolation of the liver of the dogs in all dose groups that was reversible following a 17 day recovery period. In contrast to the rat studies, no pulmonary-related findings were observed. Toxicokinetic parameters reveal QAB149 exposures in all dosed dogs. Exposures were greater in males than in female. The NOAEL was 0.0025 mg/kg associated with a mean AUC of 2.93 (ng/ml).h. The incidence and severity of the vacuolation of the liver in the dog was not increased with duration.

A comparison of the micronized drug vs the HFA formulation in 2 and 4 week inhalation studies in the rat and the dog reveal that the formulations produce a similar toxicity profile in the rat and the dog. In the rat studies toxicity in the 2 week study was microphage accumulation in the lungs which was related to drug particles in the lungs and epithelial degeneration of the nasal cavity. The toxicity in the 4 week study was epithelial degeneration of the nasal tissues. Thus, both formulations suggest local toxicity (irritation) of the tissues in the rat. In the 2 and 4 week studies in the dog, toxicity included tachycardia, myocardial necrosis and fibrosis and glycogen vacuolation of the liver.

A comparison of the toxicokinetics of the two drug formulations in the rat and the dog was conducted and resulted in opposing results. An inhaled dose of 2 mg/kg of the micronized drug on day 14 resulted in a systemic exposure that was ~ 50-100% greater than an inhaled dose of 2.77 mg/kg of the HFA formulation on day 27/28. In dogs at inhalation doses of ~ 0.9 mg/kg, systemic exposure was increased with the HFA formulation on day 27/28 versus the micronized drug formulation on day 14; this increase may be due to the change in formulation or drug accumulation with increased dosing duration.

V. GENETIC TOXICOLOGY:

Study title: **Mutagenicity Test using Salmonella Typhimurium**

Key findings: In this study QAB 149 was evaluated for its mutagenic activity in an assay using Salmonella typhimurium strains TA 1535, TA97a, TA98, TA100 and TA102. QAB149 was dissolved in DMSO and used at concentrations 1.6 to 1000 mcg/plate. Dosing was limited due to cytotoxicity. Treatment with QAB149 did not increase the revertant numbers of any of the bacterial tester strains used. Thus, QAB149 did not show evidence of a mutagenic activity under the experimental conditions used and applying standard mutagenicity criteria.

Study no: 001808
Study type (if not reflected in title): Ames
Volume #, and page #: Volume 10, page 1
Conducting laboratory and location:Toxicology/Pathology Department, Novartis Pharma AG, Basel, Switzerland
Date of study initiation: May 26, 2000
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: Micronized QAB149/#0021001/99.4%

Formulation/vehicle: Micronized QAB149/DMSO

Methods:

Strains/species/cell line: Salmonella typhimurium strains TA1535, TA97a, TA98, TA100 and TA102

Dose selection criteria:

Basis of dose selection: The highest dose was based on the concentration of QAB149 that was slightly bacteriotoxic. A change in the background lawn was taken as evidence of the bacteriotoxic activity. The highest dose and the 4 lower doses were chosen from previous experiments with QAB149. The data from these experiments are not included in this submission.

Range finding studies: QAB149 was dissolved in DMSO, first experiment (plate incorporation): 16-10,000 mcg/plate; second experiment (preincubation) 1250-20,000 mcg/plate and third experiment (preincubation) 625-20,000 mcg/plate

Test agent stability: QAB149 was stable in DMSO (10 mg/ml) and it was stable for 24 hours at ambient light and room temperature.

Metabolic activation system: The liver homogenate was prepared from 5 male CRL:WL (GLX/BRL/HAN) IGS BR rats , 7-9 week old. The liver homogenate consists of:

Supernatant: 0.1 ml
NADP: 4μmol
Gkucose-6-phosphate 5 μmol
Na/K phosphate buffer pH 7.4 100 μmol
Mg-asparate 8 μmol
KCL 33 μmol

Controls:

Vehicle: DMSO

Negative controls: DMSO

Positive controls: 2-aminoanthracene, Benzo(a)pyrene, sodium azide, 9-aminoacridine, 2-nitrofluorene and mitomycin C

Comments: Positive experiments were included in each experiment. They were carried out with both direct and indirect acting mutagens in order to demonstrate that the S-9 mix and the bacteria tester strains were functional. Adequate positive controls, with and without metabolic activation, were used with each strain.

Exposure conditions:

Incubation and sampling times: The plates were incubated in a dark incubator, 37 ˚ C with 50 to 70% humidity. After 3 days, the colonies were counted and the plates were analyzed microscopically for the presence of a light background lawn of growth.

Doses used in definitive study: Experiment 1 (plate incorporation): 1.6, 8, 40, 200 and 1000 mcg/plate, experiment 2 (preincubation): 46.88, 93.75, 187.5, 375, 750 mcg/plate, experiment 3 (preincubation): 23.44, 46.88, 93.75 187.5, 375 and 750 mcg/plate. In experiment 2, an unusually high bacteriotoxicity was observed in all strains. The reason for this toxicity could not be explained. As a result, experiment 3 was conducted which confirmed the cytotoxicity observed in experiment 1.

Study design: Histidine auxotrophic strains of Salmonella typhimurium were plated on minimum agar together with QAB149, both in the presence and absence of rat-liver homogenate for metabolic activation, with and without a preincubation period in the liquid phase. There was a trace of histidine in the medium which allows some cell division. After the trace of histidine is exhausted, only the mutant bacteria have the potential to grow. A clear increase in mutant bacteria indicates mutagenic potential of the test article.

Analysis:

No. of replicates: For each concentration of QAB149, the solvent control group and each positive control, three plates were used.

Counting method: Colony counting was done with a DOMINO image analyzer, unless this was impossible due to precipitation or other technical difficulties.

Criteria for positive results: A test article is considered to be mutagenic if it produces, in a least one concentration and one strain, a response equal to twice or more the control incidence. The exception is strain TA102 which has a relative high spontaneous revertant number where an increase by a factor 1.5 above the control level is considered an indication of mutagenic activity.

Summary of individual study findings:

Study validity: The study was valid as shown by the dose selection. QAB 149 was soluble in DMSO and there was bacteriotoxicity at 1000 µg/plate in experiment 1 with and without S9 except for strain TA 100, bacteriotoxicity was observed at 200 µg/plate without S9. However, in experiment 2, bacteriotoxicity without S9 was observed at 46.88 µg/plate. The reason for the toxicity is unknown and as a consequence the third experiment was conducted which showed toxicity at 750 µg/plate. At 1000 µg/plate concentration there was no background growth. QAB149 did not precipitate on the tester plates up to the highest concentrations. The positive controls produced the expected results. Appropriate study methodologies were used.

Study outcome: Treatment of QAB149 did not increase the revertant number of any bacteria tester strain. Cytotoxicity was observed at 1000 µg/plate with and without S9 in experiment 1. In experiment 2, cytotoxicity was observed at 46.88 µg/plate without S9. As a result of the data in experiment 2, an additional experiment (3) was conducted which confirmed the cytotoxicity results obtained in experiment 1. Thus, under the conditions of the study, QAB 149 was not mutagenic.

Study title: Chromosome Aberration test With V79 Chinese Hamster cells

Key findings: QAB149 did not induce chromosome aberrations in cultured V79 Chinese hamster cells under the conditions of the assay. There was no increase frequency of polyploid cells with and without activation.

Study no: 001834
Study type (if not reflected in title)**:**
Volume #, and page #: Volume 10, page 37
Conducting laboratory and location: Norvartis Pharma AG, Basel, Switzerland

Date of study initiation: May 22, 2000
GLP compliance: Yes
QA reports: yes (x) no ( )

Drug, lot #, radiolabel, and % purity: QAB149/#0021001/99.4%

Formulation/vehicle: QAB 149 dissolved in DMSO and diluted 100 times.

Methods:

Strains/species/cell line: V79 Chinese hamster cells

Dose selection criteria:

Basis of dose selection: The selection of biologically relevant concentrations was done by examination of the cell counts during the chromosome aberration test. The highest concentration was selected because it reduced the cell number by 50%. Other concentrations were selected based on data concerning the reductions of cell counts.

Range finding studies: Concentrations used in experiment A (20 h treatment minus S9, 3 h treatment, 17 h recovery period, + S9) and B (3 h treatment, 17 h recovery minus S9) were: 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/ml; experiment B (3 h treatment, 17 h recovery + S9): 80, 93, 107, 124, 144, 167, 193, 224, 259 and 300 µg/ml; experiment C, the concentrations were as follows: 1.0, 1.5, 2.4, 3.7, 5.7, 8.8, 13.6, 21.0, 32.4, 50.0 µg/plate (3h treatment, 17 h recovery minus S9) or 50, 58, 68, 79, 93, 108, 126, 147, 171 and 200 µg/ml; experiment D, (20 h treatment minus S9): 10, 12, 14, 17, 20, 24, 29, 35, 42 and 50 µg/ml.

Test agent stability: QAB149 was dissolved in DMSO and was stable as a 10 mg/ml solution at room temperature and ambient light for 24 hours.

Metabolic activation system: S9 fraction from liver homogenate from male Crl:Wister Han rats.

Controls:

Vehicle: DMSO, 1%

Negative controls: DMSO

Positive controls: Ethyl methanesulphonate (EMS) and Cyclophosphamide (CP)

Comments: Vehicle controls experiments were carried out both in the presence and in the absence of an S9 mix. Appropriate controls were included in this assay.

Exposure conditions:

Incubation and sampling times: The cells were treated with the dilutions of QAB149 in 5 ml culture medium or S9-mix for 3 hours at 37 ˚ C and 5 % CO_2. The dishes were then washed with PBS (5 ml) and further incubated with 10 ml of culture medium for 17 hours. At the end of the incubation period, the dishes were examined for cell density, morphological appearance of the cells and cell debris using a phase-contrast microscope. Cell growth was estimated relative to controls: +++, normal cell density; ++, <100-75% cell density of the control; +, <50-25% and --, <25%. Two hours before the end of the incubation period, 0.15 µg/ml colcemid was added to the dishes. The cells were trypsinized with 2 ml Trysin/EDTA PBS 1:250 for 5 to 10 minutes.

Doses used in definitive study: Without metabolic activation: 30, 20, 10 µg/ml (3 h treatment, 17 h recovery, experiment B); 32.4, 21.0, 13.6 (3 h treatment, 17 h

recovery, experiment C); 30, 20, 10 µg/ml; (20 h treatment continuous treatment, experiment D). With metabolic activation: 100, 60, 30 µg/ml (3 h treatment, 17 hour recovery, experiment A); 144, 124, 107, 93, 80 µg/ml (3 h treatment, 17 h recovery, experiment B); 171, 126, 108 µg/ml (3 h treatment, 17 h recovery, experiment C).

Study design:

Analysis:

No. of replicates: One slide from each concentration was used as well as 2 vehicle control cultures. One slide from a single positive control was also included.

Counting method: The slides were analyzed for the presence of chromosomal aberrations according to well known publications [Dean BJ, Danford N 1985] [Savage JRK 1976] [Scott D et al 1983] using the (b) (4) on the (b) (4) system.

Criteria for positive results: The following biological factors were considered for evaluation: Statistically significant increases in the frequency of metaphases with aberrant chromosomes are observed at one or more concentrations. Increases exceed the historical negative control range of this laboratory. Increases are reproducible between replicate cultures and between tests. The increases are not associated with significant changes in pH or osmolarity of the treatment medium or extreme toxicity. Evidence of dose-response relationship or increases at both sampling times will considered to be evidence of clastogenicity. Statistical analysis was performed only with results exceeding the historical control range.

Summary of individual study findings:

Study validity: The study was considered valid by the sponsor if there are increased numbers of cells carrying chromosome aberrations after treatment with cyclophosphamide (+S9) or ethyl hamsters cells under test conditions. The study appears to be valid as appropriate methodologies were used, dose selection was appropriate and positive controls produced the expected responses.

Study outcome: QAB 149 was dissolved in DMSO and diluted 100 times in the treatment medium. In the preliminary solubility experiment QAB149 precipitated at a concentration of ≥ 240.4 mg/ml (base form) in the treatment medium. In experiment B in the presence of S9, no precipitation was found up to the highest concentration tested, 300 µg/ml, at the end of culture incubation and prior to colcemid addition. QAB149 induced a concentration dependent decrease in cell counts after 20 hour treatment without S9 and at 3 hour treatment with and without S9. QAB 149 induced a steep dose-effect curve in the experiments without metabolism activation preventing the target of approximately 50% cytotoxicity. In experiment C, QAB 149 without S9, 3 hour treatment an analyzable dose of 32.4 µg/ml, the relative cell count was 71.7%. The next higher dose, 40 µg/ml could not be analyzed because of cytotoxicity (5.8% relative cell count). In experiment A, 20 hour treatment without S9 the relative cell count was 96.6 % at the highest analyzable dose (30 µg/ml. However, the mitotic index at the next 2 lower doses, 20 and 10 µg/ml showed toxicity. In experiment D, 20 hour treatment without S9, relative cell count for the highest analyzable dose (12 µg/ml) was 76.1%. In the presence of S9 a reduction to 50% relative cell counts was achieved in experiment C at the concentration of 171 µg/ml. With metabolic activation no significant increase in the percentages of structural chromosomal aberrations was observed. All values were within the historical controls except for two low

concentrations, 30 mcg/ml in experiment A and 107 mcg/ml in experimental B. The differences were not statistically different. No increases in frequency of polyploidy were observed with and without metabolic activation.

Study title: Subcutaneous Bone Marrow Micronucleus Test in the Rat

Key findings: QAB149 did not induce micronuclei in the bone marrow cells of rats when administered subcutaneously using doses of 200-2000 mg/kg. These data show that QAB149 has no clastogenic and/or aneugenic potential under the conditions tested.

Study no: 0212401
Study type (if not reflected in title)**:** NA
Volume #, and page #: Volume 10, page 89
Conducting laboratory and location: Novartis Pharma AG, Basel, Switzerland
Date of study initiation: February 15, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: QAB149 maleate/0121002/99.5 %

Formulation/vehicle: QAB149/PEG400/NaCl

Methods:

Strains/species/cell line: Rats, CRL:WI (GLX/BRL/HAN)IGS BR
Dose selection criteria:

Basis of dose selection: Dose-finding was done according to Mackay and Elliot "Dose-Ranging and Dose –Setting for In Vivo Toxicology Studies". Mutat. Res. 1992; 271: 97-99. The dose selection was based on the results of a previous dose ranging study described below.
Range finding studies: Starting dose of 500 mg/kg sc using 1 male and 1 female. The high dose of 2000 mg/kg was administered to 4 males and 4 females. Each rat was administered 2 doses of QAB149 with an interval of 24 hours between doses. These doses induced decreased locomotor activity as well as swelling and necrosis at injection site. A slight decrease in body weight was noted, approximately 0.4-33%. On the basis of the results, doses of 200, 630 and 2000 mg/kg were selected for the bone marrow micronucleus assay.

Test agent stability: QAB149 was stable as a 150 mg/ml suspension of PEG 400/ NaCl 0.9 % 920/80, v/v) for 24 hours.
Controls:

Vehicle: PEG 400
Negative controls: PEG400/NaCl
Positive controls: Cyclophosphamide (CP; Endoxan-Lyophilisate)

Doses used in definitive study: 200, 630 and 2000 mg/kg

Study design: QAB149 was administered twice sc with a 24 hour interval between administered at doses of 200, 630 and 2000 mg/kg. There were 7 males in each dose

group. There were 2 additional rats included in the high dose group. There were 5 males in the positive control group. There were 7 males in the negative control group.

Analysis:
Criteria for positive results: A test article is considered to be mutagenic in the rat micronucleus test if it induced a micronucleus frequency which is statistically significantly above the control level. A test compound producing no dose-related increase in the number of micronucleated polychromated erythrocytes is considered non-clastogenic in this assay.

Summary of individual study findings:

Study validity: The study was considered valid if the proportion of polychromatic erythrocytes with micronuclei in the positive control group is significantly higher than the negative control group. The methodology and dose used in this study were reasonable. The positive control produced the expected response.
Study outcome: QAB149 did not induce micronuclei in the bone marrow cells of rats up to the highest dose tested (2000 mg/kg). These results indicate that QAB149 has no clastogenic and/or aneugenic potential *in vivo* under the conditions tested. The positive control, cyclophophosamide, induced mean percentage micronucleated polychromatic erythrocytes of 2.38 ±1.02 which was clearly higher than the negative control value (0.16±0.0650). The clinical signs for the rats in this study were not submitted.

Genetic toxicology summary: The sponsor conducted *in vitro* assays, Ames bacterial reverse mutation test and Mammalian Chromosome Aberration test in the V79 Chinese hamster cell, and an *in vivo* micronucleus study in order to evaluate the genotoxic potential of QAB149. In the Ames assay, Salmonella typhimurium strains TA 1535, TA97a, TA98, TA100 and TA102 were used. QAB149 at concentrations 1.6 to 1000 mcg/plate did not increase the revertant numbers of any of the bacterial tester strains used. QAB149 did not show evidence of clastogenic activity under the experimental conditions in the chromosomal assay with V79 hamsters. QAB149 was used at doses of 10-32.4 mcg/ml without metabolic activation and 30- 171 mcg/ml with metabolic activation. There was no increased frequency of polyploid cells with and without activation. In the rat bone marrow micronucleus test, QAB149 was administered using sc doses of 200-2000 mg/kg. QAB149 did not induce micronuclei in the bone marrow cells of rats up to the highest dose tested (2000 mg/kg). These results indicate that QAB149 has no clastogenic and/or aneugenic potential *in vivo* under the conditions tested.

Genetic toxicology conclusions: A review of the genotoxicity assays, i.e., Ames bacterial reverse mutation test, Mammalian Chromosomal Aberration test in the V79 Chinese hamster cell and bone marrow micronucleus test reveal that QAB149 is not genotoxic under the conditions tested.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: A Subcutaneous Embryo-Fetal Development Dose Range-Finding Study rats

Key study findings: No maternal NOAEL was established in this study. Discolorations, wounds/scabs and blackened hardened regions were observed at the injection sites of the rats causing premature sacrifices. Skin lesions were noted on 2/6, 1/6, 4/6, 5/6 and 6/6 rats in the vehicle control, 3, 10, 30 and 100 mg/kg dose groups. When the lesions became extensive, the rats were sacrificed moribund. There were also swollen areas and dark gelatinous areas in these rats attributable to the test article/ vehicle in the rats in the 3 and 10 mg/kg dose groups. There were no effects on the reproduction parameters in the 3 mg/kg dose group. Due to early sacrifices, the reproduction parameters could not be well evaluated in the rats in the 10 mg/kg dose groups and higher. Since dosing appears to be limited due to the local injection site toxicity, the use of the formulation via the subcutaneous route is questionable.

Study no.: 0170145
Volume #, and page #: Volume 8, page 1
Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover, NJ
Date of study initiation: November 13, 2001
GLP compliance: No
QA reports: yes () no (x)
Drug, lot #, radiolabel, and % purity: QAB 149, lot # 0021002, purity 99.5 %
Formulation/vehicle: QAB 149/ vehicle 2-hydroxypropyl-beta-cyclodextrin solution, 50 wt-% in sterile water for injection.

Methods:

Species/strain: Rat/Wistar Hannover, Crl: WI (GLxBRL/HAN) IGS BR
Doses employed: 0, 3.9/3, 13.0/10, 39.0/30 and 130/100* mg/kg (*salt/base); salt/base ratio for QAB 149 is 1.30
Route of administration: Subcutaneous injection
Study design: Timed –pregnant female rats were dosed with QAB 149 once daily on gestation days 6-17. Dams were sacrificed on day 21.
Number/sex/group: 6F/group, age 10-11 weeks, body weight range 179-211 g
Parameters and endpoints evaluated: Body weight and food consumption was measured at specific times points in gestation, clinical signs were recorded daily pretest and twice daily during gestation, surviving females were sacrificed on gestation day 21 and a gross necropsy examination, uterine weight and site description, # of corpora lutea. Live fetuses were weighed and given a gross examination.

Results:

Mortality: There were no mortalities but 1/6 animals at 3 mg/kg (sacrificed day 12), 4/6 at 10 mg/kg (sacrificed day 12), 5/6 at 30 mg/kg (sacrificed days 3, 6 and 12) and 6/6 at 100 mg/kg (sacrificed day 6) were sacrificed moribund. A total of 2/6 of the control rats (vehicle) were also sacrificed in moribund condition (day 12).

Clinical signs: The clinical signs include skin discoloration, wounds/scabs at 3 mg/kg and above and black hardened regions at 10 mg/kg and higher. Since control animals were also sacrificed in moribund condition, it is assumed that the vehicle played a role in the severity of the skin lesions.
Body weight: Body weight gain in the 3 and 10 mg/kg dose group was statistically significantly increased in the rats compared with the controls, days 6-9. The increases were approximately 40 and 50%, respectively. The rats in the 30 and 100 mg/kg were apparently in or close to moribund condition and were sacrificed early so no body weight gain data are available after day 6.
Food consumption: Food consumption was statistically significantly increased in the dams in the 3 mg/kg dose group, days 9-21 of gestation. The increases ranged from 27% on days 6-9 and 8% on days 19-21. The food consumption was also increased in the dams in the 10 mg/kg dose group, days 15-21. The increases were 33%. The rats in the 30 and 100 mg/kg were apparently in or close to moribund condition and were sacrificed early so no food consumption data are available after day 6.

Toxicokinetics: NA

Terminal and necroscopic evaluations:
Dams: Examinations of necropsy revealed test article-related discolorations and or/ gelatinous areas in the sc injection areas in the rats in the 3mg/kg dose group and above and discoloration areas of the muscle in the rats in the 10 mg/kg dose group and above.
Offspring: There were no external variations in this study. A single malformation (omphalocele) was noted in 1 fetus in the 3 mg/kg dose group. At 10 mg/kg, there were statistically significant increases in the total resorptions and early resorptions
as well as statistically significant decreases in the number of viable fetuses relative to the control group. These data are probably not relevant because 4/6 rats in the 10 mg/kg dose group had to be sacrificed early and only data from 2 dams and their offspring are available for evaluation. The potential effects of QAB149 on reproduction parameters in the rats in the 30 and 100 mg/kg dose groups are not available because the animals were sacrificed early.

Summary of individual study findings: QAB149 was administered subcutaneously to pregnant rats, days 6-17 using doses of 3-100 mg/kg. Discolorations, wounds/scabs and blackened hardened regions were observed at the injection sites of the rats causing premature sacrifices in all groups including controls. There were no effects on the reproduction parameters in the 3 mg/kg dose group, the only group in which a reasonable assessment could be conducted. Due to early sacrifices in the dose ranging study, the reproduction parameters could not be evaluated in the rats in the 10 mg/kg dose groups and higher. The results indicate that the subcutaneous route with the formulation used is not appropriate for reproductive toxicity assessment.

Study title: A Follow-Up Subcutaneous Embryo-Fetal Development Dose Range –Finding Study in Rats

Key study findings: Pregnant rats were dosed subcutaneously using daily doses of 0.1, (single dose), 1 (single dose) and 1 (bid dose) mg/kg, days 6-17 of gestation. There were no effects on the embryo/fetus. Increased body weight gain on days 6-9 was observed in the dams in the 1

mg/kg (single dose) and 1 mg/kg (bid) dose groups compared with the controls. There was local injection site toxicity in the dams in the 1 mg/kg dose groups, single and bid. There was no systemic toxicity in the dams. There was increased preimplantation loss at 1 mg/kg (single dose) relative to concurrent controls that did not result in notable differences in the number of implantation sites/litter to the concurrent controls.
The NOAEL was 0.1 mg/kg for local injection site maternal toxicity but was greater than the high dose of 1 mg/kg for systemic effects. The NOAEL was 0.1 mg/kg for developmental toxicity based on the increased pre-implantation loss at 1 mg/kg. The study did not produce adequate maternal systemic toxicity.

Study no.: 0270021
Volume #, and page #: Volume 8, page 116
Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover, NJ
Date of study initiation: January 20, 2002
GLP compliance: No
QA reports: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: Micronized QAB149 maleate/0121002/99.5%
Formulation/vehicle: Micronized QAB149/ PEG 400 and NaCl 20:80 (v/v)

Methods:

Species/strain: Pregnant rats, Hannover, Crl :WI 9Glx/BRL/Han) IGS BR
Doses employed: Salt/base 0, 0.13/0.1; 1.3/1 (single dose) and 1.3/1 mg/kg (0.5 mg/kg bid, total dose, 0.1 mg/kg base mg/kg or 1.3 mg/kg salt).
Route of administration: Subcutaneously (sc)
Study design: The pregnant rats were injected daily sc with QAB149 days 6-17 of gestation. The bid dose group was injected twice daily, there were approximately 6 hours between doses. The rats were sacrificed on day 21 and dams and offspring evaluated for effects induced by QAB149.
Number/sex/group: 6 F/group
Parameters and endpoints evaluated: Clinical signs and mortality, body weight, food consumption, gross evaluations of dams, and toxicokinetics. Reproduction parameters include gravid uterine weight, uterine site description and number of corpora lutea.

Results:

Mortality: There were no drug-related mortalities in this study.
Clinical signs: There were no clinical symptoms except for minor scabs at the injection sites in all dose groups. There were no signs suggestive of systemic toxicity.
Body weight: There was a statistically significant increase in body weight gain vs control in the 0.1 mg/kg dose group, days 6-9, the increase was approximately 133%. The sponsor believes that this change was insignificant because the value for the control rats was considered to be low. There were also statistically significant, drug-related increases in the rats in the 1 mg/kg (single dose) and 1 mg/kg (bid dose), days 6-9 of the study. The mean increases were approximately 217 and 183%, respectively.
Food consumption: There were statistically significant increases in the food consumption of the rats in the 1 mg/kg (single dose) group on gestation days 9-12 and 15-19. The

increases were approximately 12% in comparison to control values and were thought not to be drug-related but due to a low control value.
Toxicokinetics: Blood was collected from the Dams on day 17 at time points 0.5, 1.5, 3, 8 and 24 hours for the control and 0.1 and 1mg/kg single dose groups. Blood was collected on the last day of dosing after the first dose for the rats in the 1 mg/kg bid dose group.

Toxicokinetics Parameters for QAB149 in the Pregnant Rat

Parameters*	**0.1 mg/kg**	**1 mg/kg (QD dose)**	**1 mg/kg (bid dosing)**
Number	5	6	4
$AUC_{(0\text{-}24h)}$, ng.h/mL	40.7	299	598
$C_{(max)}$ ng/mL	8.06	37.9	42.3
$T_{(max)}$, h	0.5	1.5	8.00

*Dose is in terms of the base. The salt/base ratio for QAB149 is 1.296

The animals in all dose groups were exposed to QAB149. There was no QAB149 in the control samples. The AUC (ng.h/mL) in the 1 mg/kg (bid) was much higher than the AUC in the 1 mg/kg single dose. After multiple daily single-dosing using 1 mg/kg, QAB149 exposure was less than dose proportional.

Terminal and necroscopic evaluations:
Dams: QAB149 –related dark discoloration of the injection areas was observed in 4/6 rats in the 1 mg/kg dose groups single dose and 2/6 rats in the 1 mg/kg bid dose group. Red discoloration of the injection areas of the rats in the 0.1 mg/kg dose group was not considered to be drug-related. There were no other gross findings.

Offspring: There was increased preimplantation loss at 1 mg/kg single dose relative to concurrent controls. The percentage losses were 9.0, 10.9, 19.9 and 11.6% in the controls, 0.1 mg/kg, 1 mg/kg (single dose) and 1 mg/kg (bid dose). The increased loss did not result in notable differences in the number of implantation sites/litter to the concurrent controls. There were no effects on fetal weight or reproductive parameters including dams with live fetuses, corpora lutea, implantation sites, fetal body weight or gravid uterine weight. No drug-related external malformations or variations were noted.

Summary of individual study findings: Pregnant rats were dosed subcutaneously using daily doses of 0.1, (single dose), 1 (single dose) and (1 bid dose) mg/kg, day 6-17 of gestation. There were no effects on the embryo/fetus. Increased body weight gain vs controls was observed in the dams in the 1 mg/kg (single dose) and 1 (bid) mg/kg dose groups. There was local injection site toxicity in the dams in the 1 mg/kg dose groups, single and bid. There was increased preimplantation loss in the dams in the 1 mg/kg, single dose group compared with the concurrent controls.
The NOAEL was 0.1 mg/kg for local maternal toxicity based on local injection site toxicity but the NOAEL for systemic effects was greater than the high dose of 1 mg/kg. The NOAEL is greater than 1.0 mg/kg for the embryo-fetal developmental effects.

Study title: A Subcutaneous (bid) Embryo-Fetal development Study in Rats

Key study findings: QAB149 was administered to pregnant rats using sc total daily doses of 0.1, 0.3 and 1 mg/kg. The doses were administered bid. The results of this study reveal local injection site maternal toxicity at 0.1 mg/kg and above due to skin lesions as well as increased body weight gain and food consumption. Increased body weight gain was observed in all dose groups. There was no evidence of teratogenicity or other reproductive effects in this study. Toxicokinetic parameters show QAB149 exposure was dose proportional in this study. The NOAEL for the embryo-fetal development was the high dose of 1 mg/kg. There was no NOAEL for local toxicity in the pregnant rat due to skin lesions in the dams in all dose groups; systemic effects included increased body weight gain.

Study no.:0270037
Volume #, and page #: Volume 8, page 220
Conducting laboratory and location: Novartis Pharmaceuticals, East Hanover, NJ
Date of study initiation: March 31, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: Micronized QAB149 maleate/#0121002/99.5 and 99.4 %
Formulation/vehicle: Micronized QAB149 maleate/PEG400/ NaCl 20:80 (v/v)

Methods:

Species/strain: Pregnant rats, Hannover, Crl :WI 9Glx/BRL/Han) IGS BR
Doses employed: Salt/base: 0, 0.13/0.1, 0.39/0.3 and 1.3/1 mg/kg, doses were based on the dose –ranging study (0270021). Total daily doses were 0. 0.1, 0.3 and 1.0 mg/kg, administered bid, approximately 6 hours apart. Daily total of 2 ml/kg or 1 ml/kg per injection. Dose concentrations ranged from 0.065-0.65 mg/ml.
Route of administration: Subcutaneously
Study design: QAB149 was administered sc to pregnant rats (25/group) daily from day 6-17 of gestation. The drug was given bid daily. There were satellite rats (n = 3-5) included in this study for toxicokinetic analysis. Dams were sacrificed on day 21 of gestation.
Number/sex/group: 25 F/group
Parameters and endpoints evaluated: Mortality, clinical signs, body weight, food consumption, toxicokinetics, and gross evaluation of the dams. Reproductive parameters include gravid uterine weight, uterine site description (uterine implant site, live fetus, dead fetus, early and late resorption), number of corpora lutea (pregnant –full term: left and right ovarian count), fetal examination (visceral and skeletal examinations), fetal processing and fetal pathology.

Results:

Mortality: There were no mortalities in this study.
Clinical signs: There was local toxicity, i.e., skin lesions at the injection sites in the rats in the 1 mg/kg dose group (10/25).

Body weight: There were statistically significant increases in body weight gain compared with control values in all QAB149–dosed rats. In the 0.1 mg/kg dosed rats, increased body weight gain was observed day 6-9, approximately 71%. In the 0.3 mg/kg dose group, increased body weight gain ranged from approximately 26-100% on days 6-12. In the 1 mg/kg dose group, increased body weight gain ranged from 38-128% on days 6-15. The body weight gain was similar in all dose groups on days 17-21.
Food consumption: There were statistically significant increases in the food consumption of the rats in the 0.3 and 1 mg/kg dose groups. The increases in the 0.3 mg/kg were approximately 9% on days 12-18 and in the 1 mg/kg, the increases were also approximately 9% on days 9-21.
Toxicokinetics: Blood samples were collected on day 17, at 0.5, 1.5, 3, 8 and 24 hours after the first dose.

Toxicokinetic Parameters of QAB149 in the Pregnant Rat

Parameters*	0.1 mg/kg	0.3 mg/kg	1 mg/kg
$AUC_{(0\text{-}24h)}$, ng.h/mL	37.5	114	345
$C_{(max)}$, ng/mL	3.89	9.00	26.1
$T_{(max)}$, h	0.500	0.900	0.833

* The dose concentrations refer to free base of QAB149

There were measurable serum concentrations of QAB149 in all dosed rats up to 24 hours after dosing. There were no measurable serum concentrations of QAB149 in the control rats. QAB149 exposure increased dose proportionally in this study.

Terminal and necroscopic evaluations:

Dams: There was treatment-related discoloration of the injection sites in 1/25, 2/25 and 6/25 rats in the 0.1, 0.3 and 1 mg/kg dose groups, respectively. There was no discoloration at the injection sites in the control dams. There were no effects on reproductive parameters, ie, number of fetuses, number of dead fetuses, resorptions etc.

Offspring: There were no drug-related external malformations or variations in the rats in this study. The sole external malformation, gastroschisis, was observed in 1 fetus in the 0.1 mg/kg dose group and 1 fetus in the 1 mg/kg dose group. There were no visceral malformations or variations. There were no drug-related skeletal malformations or variations. A statistically significant decrease in the total incidence of skeletal malformations in the fetuses in the 0.3 mg/kg dose group was considered incidental. There was a statistically significant increase in the fetal and litter incidences of incomplete ossification of the squamosal bone in the high dose group (fetal incidence of 6/104 and litter incidence of 5/21 vs 0/120 and 0/23 in controls) which was also considered to be incidental due to the lack of any other effect on bones or the fetal skeleton. A statistically significant decrease in the incidence of short supernumerary ribs and in the total incidence of skeletal variations at 1 mg/kg was also considered to be incidental.

Summary of individual study findings: QAB149 was administered to pregnant rats using sc total daily doses of 0.1, 0.3 and 1 mg/kg. The doses were administered bid. The results of this

study reveal local toxicity at 0.1 mg/kg and higher, i.e., wounds and discoloration of the skin at the injection sites, as well as increased body weight gain and food consumption. There was also increased incidence of skin lesions in the dams in the 1 mg/kg dose group (10/25). There was no significant evidence of teratogenicity or other developmental effects in this study. Toxicokinetic parameters show QAB149 exposure was dose proportional in pregnant dams. The NOAEL for the fetal development was 1 mg/kg. There was no NOAEL for local toxicity in the dams based on treatment-related discoloration of the injection sites and skin lesions in all dose groups; increase in dam body weight gain were noted at all doses. The doses used in this reproductive study were adequate based on maternal toxicity associated with sc administration of QAB 149.

Study title: A Subcutaneous embryo-Fetal Development Dose Range- Finding Study in Rabbits

Key study findings: QAB149 was administered subcutaneously to pregnant rabbits using daily doses of 0, 3, 10, 30 and 100 mg/kg on days 7-20 of gestation. All animals in the upper two dose groups and 1 of 3 in the 10 mg/kg group were sacrificed early due to skin lesions at the injection site. The local toxicity included discoloration of the injection sites, swelling, scabs and wounds at 3 mg/kg and higher. There were marked reductions in body weight and food consumption of the rabbits in the control group that began on day 7 and lasted until the end of the study. There were similar decreases in body weight gain and food consumption in the rabbits in the 10 mg/kg dose group (30 and 100 mg/kg not evaluated due to early sacrifices). There was no NOAEL for local maternal toxicity in this study due to wound/scabs at the injection sites and associated drug-related skin lesions; however, no maternal systemic toxicity was noted. The NOAEL for fetal development was the highest evaluated dose of 10 mg/kg. The results of this study show that the use of this formulation (2-hydroxypropyl–beta-cyclodextrin solution 50 wt-% in sterile water) and route of administration may not be appropriate in the rabbit due to the dose limiting local toxicity.

Study no.: 0170146
Volume #, and page #: Volume 9, page 1
Conducting laboratory and location: Novartis Pharmaceuticals, East Hanover, NJ
Date of study initiation: November 20, 2001
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: QAB149 maleate/#0021002/99.5%
Formulation/vehicle:QAB149, 2-hydroxypropyl –beta-cyclodextrin solution 50 wt-% in sterile water/ 2-hydroxypropyl–beta-cyclodextrin solution 50 wt-% in sterile water.

Methods:

Species/strain: rabbit. New Zealand white
Doses employed: Salt/base: 0, 3.9/3, 13.0/10, 39.0/30 and 130/100 mg/kg; dose volumes of 5.2, 0.16, 0.52, 1.56 and 5.2 ml/kg, respectively.
Route of administration: Subcutaneous (sc)
Study design: Pregnant rabbits were dosed with QAB 149 once daily, days 7-20 of the gestation period. On the odd-numbered days, the anterior region of the back was injected

and on even-numbered days the posterior region of the back was injected. Dams were sacrificed on day 29.
Number/sex/group: 3F/dose group
Parameters and endpoints evaluated: Mortality, clinical signs, body weight, food consumption, gross evaluation of dams, histopathology, reproductive parameters include gravid uterine weight, uterine site description (uterine implant site, live fetus, dead fetus, early and late resorption), and number of corpora lutea(pregnant –full term: left and right ovarian count).

Results:

Mortality: 1/3 rabbits in the 10 mg/kg dose group had to be sacrificed (day 18) because of moribund condition (skin lesions). All rabbits in the 30 and 100 mg/kg dose groups had to be sacrificed on day 8 because of the severe nature of the skin lesions.

Clinical signs: Control animals demonstrated discoloration, swelling, staining, and wounds in the skin/fur that were considered to be due to the vehicle/injection volume. Similar lesions were also observed in all dosed rabbits. At 3 and 10 mg/kg, the skin lesions consisted of discoloration of the injection sites and wound/scabs. On day 20, one rabbit in the 10 mg/kg dose group aborted. It is unclear but the abortion may be drug-related.

Body weight: Control animals lost weight from days 7-17 and days 21-24 with limited weight gain from days 17-21; similar findings were noted in the 10 mg/kg dose group. The dams in these dose groups gained weight from days 0-29 but gained less weight than the dams in the 3 mg/kg dose group. Body weight was increased in the 3 mg/kg dose group by 14%; these animals gained 335 g from days 7-21 while animals in the control and 10 mg/kg groups lost 365 g and 78 g, respectively. The effect on body weight appears to be primarily related to the vehicle and injection volume. The rabbits in the 30 and 100 mg/kg dose groups were sacrificed on day 8 because of toxicity, therefore there no body weight gain data on these animals.

Food consumption: Food consumption was increased by 2- to 6-fold in the rabbits in the 3 mg/kg dose group days 7-12 in comparison to the control animals. Food consumption in the controls was reduced by up to 8-fold in comparison to pre-dosing values. Values in the 10 mg/kg dose group were similar to control values indicating that the effect was primarily due to vehicle and dose volume.

Toxicokinetics: NA

Terminal and necroscopic evaluations:
Dams: Subcutaneous gelatinous masses were noted in all rabbits in the 30 mg/kg dose group and higher. Subcutaneous yellowish areas were noted in 2/3 rabbits in the 3 and 10 mg/kg dose groups. These findings were observed in the injection areas and were drug-related. The dams in the 30 and 100 mg/kg dose group were sacrificed because of the severity of the skin lesions. There were subcutaneous gelatinous masses in all the dams in the 30 and 100 mg/kg dose groups.

Offspring: The number of corpora lutea or implantation sites was similar in the control, 3 and 10 mg/kg dose groups. There were no dead fetuses in this study. There was a statistically significant decrease in total resorptions in the 3 and 10 mg/kg dose groups and early resorptions in the 3 mg/kg dose group. The differences were due to early resorption of one entire litter in the control group. There were no remarkable differences in fetal weight in the control, 3 and 10 mg/kg dose groups. The effects of QAB149 on the reproductive parameters and fetal weight in the 30 and 100 mg/kg dose groups could not be determined because of early sacrifices. Post implantation losses in these groups resulted in a decreased number of viable fetuses. A definitive conclusion cannot be made concerning a potential effect of the vehicle since there were only 3 control litters available for evaluation and there were no litters to be evaluated at 100 mg/kg that was administered a similar dose volume of the vehicle.

Summary of individual study findings: QAB149 was administered subcutaneously to rabbits using daily doses of 0, 3, 10, 30 and 100 mg/kg on days 7-20 of gestation. There was local toxicity, i.e., discoloration of the injection sites, swelling, scabs and wounds at 3 mg/kg and higher. The rabbits in the 30 mg/kg dose group and above had to be sacrificed on day 8 due to nature of the skin lesions; 1 of 3 in the 10 mg/kg group were sacrificed early. There was a marked reduction in body weight and food consumption of the rabbits in the control group that began on day 7 and lasted until the end of the study. There were similar decreases in body weight gain and food consumption in the rabbits in the 10 mg/kg dose group. There was no NOAEL for local maternal toxicity in this study due to wound/scabs at the injection sites and associated drug-related skin lesions; however, no systemic maternal toxicity was observed.. The systemic toxicity for the dams in the 30 and 100 mg/kg dose groups could not be determined because of early sacrifices. The NOAEL for fetal development was 10 mg/kg which was the highest evaluable dose. The results of this study show that the use of this formulation by this route of administration is not appropriate in the rabbit since local toxicity limited the ability to produce maternal systemic toxicity.

Study title: A Follow-up Subcutaneous Embryo-Fetal Development Dose Range-Finding Study in Rabbits

Key study findings: QAB149 was administered subcutaneously daily to pregnant rabbits days 7-20 of gestation. The doses were 0.1, 1, 1 (bid) and 3 mg/kg. There was wound/scabs in the injection site areas in 1/5, 1/5 and 4/5 rabbits in the 1 (single dose), 1 (bid) and 3 mg/kg, respectively. There was one humane sacrifice in the 3 mg/kg dose group on gestation 14. There was no embryo-fetal toxicity or gross malformations. The NOAEL for embryo fetal development was 3 mg/kg. The NOAEL for local maternal toxicity was 0.1 mg/kg due to drug-related injection site toxicity, i.e., wounds/scabs and hair loss. There was no significant systemic toxicity in the dams. The results of this study show that the use of this formulation by this route of administration may not be appropriate in the rabbit since local toxicity limited the ability to produce sufficient maternal systemic effects.

Study no.: 0270036

Volume #, and page #: Volume 9, page 84
Conducting laboratory and location: Novartis Pharmaceuticals, East Hanover, NJ
Date of study initiation: February 19, 2002
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: Micronized QAB149 maleate/#0121002/99.5%
Formulation/vehicle: QAB149/2-hydroxypropyl-beta-cyclodextrin solution 5 wt-% in sterile water.

Methods:
Species/strain: Rabbits, New Zealand, White Hra: (NZW)SPF
Doses employed: Vehicle control (single dose), vehicle (administered bid), 0.1, 1 (single dose), 1 (administered bid, 0.5 mg/kg each injection) and 3 mg/kg; 0.065-1.95 mg/ml
Route of administration: Subcutaneously; Groups 1, 3, 4 and 6 – 1 injection of 2 ml/kg; Groups 2 and 5 – 2 injections of 1 ml/kg each
Study design: Pregnant rabbits were injected subcutaneously with QAB149 days 7-20 of gestation. Groups 1, 3, 4, and 5 were injected sc (2 ml/kg) once daily; groups 2 and 5 were injected with 1 ml/kg twice daily, 6 hours apart. The dams were sacrificed on day 29.
Number/sex/group: 5 pregnant rabbits/dose
Parameters and endpoints evaluated: Mortality, clinical signs, body weight, food consumption, gross evaluation of dams, toxicokinetics, reproductive parameters include gravid uterine weight, uterine site description (uterine implant site, live fetus, dead fetus, early and late resorption) and number of corpora lutea (pregnant –full term: left and right ovarian count). Live fetuses were weighed individually and given a gross examination.

Results:
Mortality: There were no mortalities in this study; however, one dam in the 3 mg/kg dose group was sacrificed on day 14 of the study due to extensive test-article related dermal lesions.

Clinical signs: There was one humane sacrifice in the 3 mg/kg dose group on gestation day 14. There were wounds/scabs in the injection site areas in 1/5, 1/5 and 4/5 rabbits in the 1 (single dose), 1 (bid) and 3 mg/kg, respectively. There was drug-related hair loss in the injection site of the rabbits in the 1 and 3 mg/kg single dose groups. There were no clinical signs reported for the dams in the control group.

Body weight: Body weight gain was similar in the rabbits in all dose groups.

Food consumption: Food consumption was similar in the rabbits in all dose groups,

Toxicokinetics: Blood samples were taken from each animal on day 20 of gestation at 0.5, 1.5. 3, 8 and 24 hours after the last dose from the single dose vehicle, 0.1, 1 (single dose), 1 (bid) and 3 mg/kg (single dose).

Toxicokinetic Parameters of QAB 149 in the Pregnant Rabbit

Parameters	0.1 mg/kg	1 mg/kg (single dose)	1 mg/kg (bid)	3 mg/kg
N	5	5	5	5
$AUC_{(0\text{-}24h)}$, ng.h/mL	109	994	941	2830
$C_{(max)}$, ng/mL	18.2	179	101	468
$T_{(max)}$,h	0.5	0.5	0.5	0.5

Dose is in terms of base. The salt/base ratio for QAB149 is 1.296

All dosed rabbits were exposed to QAB149. There were no significant differences in exposure (AUCs) in animals in the 1 mg/kg dose group (single dose) and the animals in the 1 mg/kg dose group (bid). After multi-dosing, exposure in the rabbits increased proportionally with dose. There was no QAB149 in the control samples.

Terminal and necroscopic evaluations:
Dams: There was wound/scabs in the injection site areas in 1/5, 1/5 and 4/5 rabbits in the 1 (single dose), 1 (bid) and 3 mg/kg, respectively. There was one humane sacrifice in the 3 mg/kg dose group on gestation 14 due to test-article-related extensive skin lesion. There was drug-related hair loss in the injection site of the rabbits in the 1 and 3 mg/kg single dose. No other gross findings were observed at necropsy.

Offspring: There were no drug-related effects on the reproductive parameters or fetal weights. There were no significant differences in the results for the two control groups. There was a statistically significant decrease in total resorptions and early resorptions at 1 mg/kg (single dose) and 1 mg/kg (bid) dose groups. The decrease was due to the absence of any resorptions in these dose groups and was considered to be incidental to the administration of the drug. There was a statistically significant difference (decrease) in the total number of viable fetuses at 1 mg/kg (single dose) and 1 mg/kg (bid) relative to values for the concurrent single control dose. Since there were no resorptions and 100% of the fetuses were viable in these groups, these differences were considered to be incidental to the administration of QAB149. There were no drug-related malformations in this study.

Summary of individual study findings: QAB149 was administered subcutaneously daily to pregnant rabbits days 7-20 of gestation. The doses were vehicle, vehicle (bid), 0.1, 1, 1 (bid) and 3 mg/kg. There were wounds/scabs in the injection site areas in 1/5, 1/5 and 4/5 rabbits in the 1 (single dose), 1 (bid) and 3 mg/kg, respectively. There was one humane sacrifice in the 3 mg/kg dose group on gestation 14. There was no embryo-fetal toxicity or gross malformations. The NOAEL for embryo-fetal toxicity was the high dose of 3 mg/kg. The NOAEL for local maternal toxicity was 0.1 mg/kg due to injection site effects, i.e., wounds/scabs and skin lesions. There was, however, no systemic toxicity in the dams (NOAEL > 3 mg/kg) as dosing was limited by the local effects. There were no remarkable differences in the results between the single and bid dose vehicle control groups. The results of this study show that the use of this formulation by this route of administration may not be appropriate in the rabbit since local toxicity limited the ability to produce sufficient maternal systemic toxicity.

Study title: A Subcutaneous Embryo-Fetal Development Study in Rabbits

Key study findings: QAB149 was administered subcutaneously to pregnant rabbits on days 7-20 of gestation. The doses were 0, 0.1, 1 and 3 mg/kg. Results of this study show an increased incidence of cuts/scratches/ scabs/ wounds in the rabbits in the 3 mg/kg dose group when compared to control animals. These rabbits also had body weight gain decreases (days 17-20; ~55%) as well as decreases in food consumption (days 15-20) of ~26%. There were no mortalities or morbidity and gross or microscopic pathology in this study. As mentioned, there was local maternal toxicity and an increase in one skeletal variation in the fetuses in the 3 mg/kg dose group. There were no teratogenic effects noted in this study. The NOAEL in this study was 1 mg/kg for maternal systemic toxicity, maternal local toxicity and fetal toxicity.

Study no.: 0270038
Volume #, and page #: Volume 9, page 217
Conducting laboratory and location: Novartis Pharmaceuticals, East Hanover, NJ
Date of study initiation: April 15 and 16, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: Micronized QAB149 maleate/0121002/99.5%
Formulation/vehicle: QAB149/2-hydroxypropyl-beta-cyclodextrin solution 25 wt-% diluted to 5 %w/v with 0.9 % sodium chloride.

Methods:
Species/strain: Rabbits, New Zealand, White Hra: (NZW)SPF
Doses employed: Salt/base: 0 (vehicle), 0.13/0.1, 1.3/1, 3.9/3 mg/kg
Route of administration: Subcutaneously
Study design: Pregnant rabbits were administered QAB 149 subcutaneously daily duration gestation, days 7-20. The doses were 0 (vehicle), 0.1, 1 and 3 mg/kg. The dams were sacrificed on day 29. Dose concentrations ranged from 0.065-1.95 mg/ml; dose volume was 2 ml/kg.
Number/sex/group: 20 pregnant females in main study; 3 control and 5 drug dosed animals included for toxicokinetics.
Parameters and endpoints evaluated: Mortality, clinical signs, body weight, food consumption, gross evaluation of dams, toxicokinetics; reproductive parameters include gravid uterine weight, uterine site description (live or dead fetuses, early or late resorption) and number of corpora lutea (left and right ovarian count), fetal exam included weights, gross internal and external exam, and pathological exam.

Results:

Mortality: There were no mortalities in this study.

Clinical signs: There were increased numbers of drug -related cuts/scratches/ scabs/ wounds in the rabbits in the 3 mg/kg dose group. There were wounds/scabs in 0/20, 1/20, 2/20 and 8/20 in the control and 0.1, 1.0 and 3 mg/kg dose groups, respectively. There

was a premature delivery on gestation day 29 in the 1 mg/kg dose group and one on day 28 in the 3 mg/kg dose group.

Body weight: There were statistically significant increases in body weight gain versus control on day 14-21 of gestation in the 0.1 (96%) and in the 1 mg/kg dose group on days 10-14 by 100%. There was a statistically significant decrease in body weight gain, days 17-21, in the 3 mg/kg dose group. The decrease was approximately 55%.

Food consumption: There were statistically significant decreases in food consumption versus control values on days 7-10 and 15-21 in the 3 mg/kg dose group. The decreases were 22 and 26%, respectively.

Toxicokinetics: Blood samples were taken from each animal on day 20 at 0.5, 1.5, 3, 8 and 24 hours after the last dosing.

Toxicokinetic Parameters of QAB149 in Pregnant Rabbit and Fetus

Parameters	**0.1 mg/kg**	**1 mg/kg**	**3 mg/kg**
Pregnant rabbit AUC $_{(0\text{-}24h)}$, ng.h/mL	151	795	2010
C_{max}, (ng/mL)	42.7	168	450
T_{max}, (h)	0.5	0.5	0.5
Fetal tissues Concentration, ng/g	0.0	0.0	1.51

The concentrations and doses refer to free base of QAB149. The conversion factor for salt to free base is 0.772

There was measurable QAB149 (0-24 h) exposure in the rabbits dosed with QAB149 for 24 hours. From 0.1 to 3 mg/kg there was an overall sub-proportional increase in exposure with increasing dose. QAB149 was not observed in the control samples. Small levels of QAB149 were detected in the tissues of the fetuses of the rabbits treated with 3 mg/kg.

Terminal and necroscopic evaluations:

Dams: There were increased numbers of drug -related cuts/scratches/ scabs/ wounds in the rabbits in the 3 mg/kg dose group. There were wounds/scabs in 1/20, 2/20 and 8/20 in the 0.1, 1.0 and 3 mg/kg dose groups, respectively. There was an enlarged gallbladder in 1/20 dams in the 3 mg/kg dose group. There were no other gross or histopathology findings. There was a premature delivery on gestation day 29 in the 1 mg/kg dose group and one on day 28 in the 3 mg/kg dose group. There was no local or systemic toxicity in the control dams.

No drug-related effects were noted in regard to uterine weight or any of the assessed reproductive parameters.

Offspring: There were no external malformations or variations, visceral malformations or variations, or skeletal malformations noted in the fetuses in this study. There was a statistically

significant increase in the fetal incidence of full supernumerary ribs at 0.1 and 3 mg/kg dose groups (fetal incidence of 51 and 60%, respectively) versus control (fetal incidence of 38.8%). The 0.1 increase was considered to be incidental due to the lack of dose association at the mid-dose and comparability to the MARTA 1996 database. The fetal incidence at 1 mg/kg was similar to the fetal incidence in the controls. The increase in the 3 mg/kg dose group is considered drug-related.

There were statistically significant increases in the fetal incidence of incomplete ossification of the parietal at 1 mg/kg compared with the controls (6/171 in the 1 mg/kg dose group and 0/147 in the control group) and the litter incidence of detached short supernumerary ribs at 1 mg/kg (13/18 vs 6/17). There were statistically significant decreases in the fetal incidence of incomplete ossification of the metacarpal at 0.1 mg/kg (69%) and fetal incidence of incomplete ossification of forepaw phalanx at 1 mg/kg (80%). The findings were thought to be incidental due to the lack of a dose association.

Summary of individual study findings: QAB149 was administered subcutaneously to pregnant rabbits on days 7-20 of gestation. The doses were 0 (vehicle), 0.1, 1 and 3 mg/kg. Results of this study show an increased incidence of cuts/scratches/ scabs/ wounds in the rabbits in the 3 mg/kg dose group. There were no mortalities or morbidity in this study, however body weight gain was decreased in the rabbits, days 17-20 ~55%. There was an increase in one skeletal variation (full supernumerary ribs) in the fetuses in the 3 mg/kg dose group. There was no teratogenicity observed in this study. The NOAEL in this study was 1 mg/kg for local and systemic maternal toxicity; the NOAEL for fetal toxicity was 1 mg/kg.

Summary of the Reproduction and Development Studies: Embryo-fetal developmental studies were conducted in rats and rabbits. Dose-ranging studies were carried out to determine the doses to be used in the definitive studies. In the dose-ranging studies in the Wistar rat, QAB149 was administered subcutaneously, days 6-17 using doses 3-100 mg/kg in 2-hydroxypropryl-beta-cyclodextrin and water. Discolorations, wounds/scabs and blackened hardened regions were observed at the injection sites of the rats causing premature sacrifices. There were no effects on the reproduction parameters in the 3 mg/kg dose group. Due to early sacrifices in the 30 and 100 mg/kg dose groups and loss of litters in the control group, the reproduction parameters were not evaluated in those rats. No local maternal NOAEL was established in this study but no systemic toxicity was observed. In a second dose-ranging study, pregnant rats were dosed subcutaneously using daily doses of 0.1, (single dose), 1 (single dose) and 1 (bid dose) mg/kg with PEG 400 and sodium chloride, day 6-17 of gestation. There were no effects on the embryo/fetus. Increased body weight gain was observed in the dams in the 1 mg/kg (single dose) and 1 (bid) mg/kg dose groups compared with the controls on gestation days 6-9. There was local dermal toxicity in the dams in the 1 mg/kg dose groups, single and bid dose groups. There was no systemic toxicity in the dams. The NOAEL was 0.1 mg/kg for local maternal toxicity, 1 mg/kg for systemic maternal toxicity and 1.0 mg/kg for developmental toxicity. In the definitive study in the rat, QAB149 with PEG 400 and sodium chloride was administered to pregnant rats using sc doses of 0.1, 0.3 and 1 mg/kg. The doses were administered bid. The results of this study reveal drug-related local toxicity at 0.1 mg/kg (wounds/scabs, discoloration) and above. There was no evidence of teratogenicity or other

developmental effects in this study. The NOAEL for the fetal development was 1 mg/kg. There was no local NOAEL for the pregnant rat based on the injection site toxicity.

In the initial dose ranging study in the New Zealand rabbit, QAB149 in a 2-hydroxypropyl –beta-cyclodextrin solution with sterile water was administered subcutaneously to pregnant rabbits using daily doses of 0, 3, 10, 30 and 100 mg/kg on days 7-20 of gestation. There was local toxicity, i.e., discoloration of the injection sites, swelling, scabs and wounds at 3 mg/kg and higher. The rabbits in the 30 mg/kg dose group and above had to be sacrificed on day 8 due to nature of the skin lesions. There was no NOAEL for local maternal toxicity in this study due to drug-related local toxicity, i.e., wound/scabs at the injection sites and associated drug-related skin lesions, however, no systemic toxicity was observed. The NOAEL for fetal development was the highest evaluated dose of 10 mg/kg. The results of this study show that the doses and/or formulation used are not appropriate for subcutaneous injection into the rabbit since local toxicity impeded the ability to produce systemic maternal toxicity. In the follow-up dose ranging study, QAB149 in a 2-hydroxypropyl-beta-cyclodextrin solution in sterile water was administered subcutaneously daily to pregnant rabbits days 7-20 of gestation at doses of 0.1, 1, 1 (bid) and 3 mg/kg. There were wounds/scabs in the injection site areas in 1/5, 1/5 and 4/5 rabbits in the 1 (single dose), 1 (bid) and 3 mg/kg, respectively. There was one humane sacrifice in the 3 mg/kg dose group on gestation 14 due to skin lesions. There was no embryo-fetal toxicity or gross malformations. The NOAEL for embryo-fetal developmental was 3 mg/kg. The NOAEL for local maternal toxicity was 0.1 mg/kg due to drug-related injection site wounds/scabs and hair loss. There was no systemic toxicity in the dams (NOAEL = 3 mg/kg). In the definitive study in the rabbit, QAB149 in 2-hydroxypropyl-beta-cyclodextrin solution diluted with 0.9% sodium chloride was administered subcutaneously to pregnant rabbits on days 7-20 of gestation at doses of 0, 0.1, 1 and 3 mg/kg. Results of this study show cuts/scratches/ scabs/ wounds in the rabbits in the 3 mg/kg dose group. There were no mortalities or morbidity in this study. There was local maternal toxicity and an increase in one skeletal variation (full supernumerary ribs) in the fetuses in the 3 mg/kg dose group. High dose animals also demonstrated reduced body weight gain and food consumption. There was no teratogenicity in this study. The NOAEL in this study was 1 mg/kg for local and systemic maternal toxicity and fetal developmental effects.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

QAB149 is a long acting β_2 intended for once a day treatment of asthma/COPD. The drug will be administered by inhalation. The sponsor is planning to develop 3 formulations, an HFA formulation (I66,337) and a micronized powder formulation with lactose (I 48,649) and a (b) (4) (I 69,754) . The target organ for this drug is classically the cardiovascular system, i.e., tachycardia, QTc interval changes and myocardial necrosis. Increased cardiovascular activity with associated histopathological changes was observed in the dog. There was also glycogen deposition in the liver of the dog at all dose levels. The dose-limiting toxicity in the rat is nasal lesions, characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium. QAB 149 has been administered to human subjects in Europe using single doses up to 2000 mcg.

The sponsor is proposing a double-blind, placebo-controlled, four-period, cross-over study to compare the magnitude, duration and onset of action of single inhaled doses of 100 µg and 400 µg of QAB 149 delivered by an HFApMDI device. The study will include a single dose of 50 mcg of salmeterol as a positive control. The study will include 32 patients with persistent asthma and a predicted FEV1 of 60 to 90 %. The ages of the patient will be 18-65 years.

Pharmacology*: In vitro* and *in vivo* 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 (approximately 225 minutes).

Safety Pharmacology: QAB149, at a concentration of 5 µg/ml, inhibited HERG channels stably expressed in HEK293 cells. QAB149, at concentrations of 0.5 and 1.0 µg/ml did not inhibit HERG channels stably expressed in HEK293 cells. Respiratory and CNS effects have not been specifically studies but no obvious effects have been noted in toxicology studies. The sponsor has been informed that the safety pharmacology battery should be completed.

Pharmacokinetic/Toxicokinetics: QAB 149 is absorbed in the dog and the rat after oral dosing but is not highly bioavailable. The bioavailability in the dog after oral dosing is 33% and 0 in the rat. *In vitro* metabolism of QAB149 evaluated using liver slices is similar in rats, dog and humans, involving mainly phenolic O-glucuronidation. No major active metabolites were observed. QAB 149 was not metabolized in the human lung. Protein binding was similar in the rat, dog and human, approximately 92, 94 and 96%. QAB149 was distributed to most tissues, except the brain, spinal column and lymph nodes. The highest concentrations of QAB149 were found in the stomach, intestines, liver and kidney. QAB 149 showed no significant inhibition of P450 enzymes, CYP2C9, CYP2E1 and CYP3A4/5 when tested in concentrations up to 100 µM. Elimination of QAB149 in the rat, mouse and dog is mainly via the feces.

General Toxicity:

Acute Studies: Acute studies were carried out in the mouse and the rat. QAB 149 micronized power was administered orally and subcutaneously. Results of these studies show oral doses up to 1600 mg/kg did not induce any adverse events in the mouse or the rat. However, when QAB 149 was administered subcutaneously, deaths were observed at a dose of 200 mg/kg in the rat. The maximum non-lethal dose was 100 mg/kg in the rat. In the mouse, deaths were observed at 50 mg/kg in the males and 200 mg/kg in the females. The maximum non-lethal dose was 5 mg/kg in the male mouse and 100 mg/kg in the females.

Subchronic Studies: In the two week study in the rat with micronized drug, pulmonary deposited doses were 0.21, 0.58 and 1.7 mg/kg were used. Alveolar macrophages were observed in lungs of the rats including the control group. The NOAEL was 0.21 mg/kg associated with a mean AUC of 112 ng.h/mL. In the four week study in the rat with the HFA formulation, the pulmonary deposited doses were 0.093, 0.28 and 0.85 mg/kg. Reversible focal, olfactory epithelial degeneration characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of the epithelium in the mid and high dose groups were observed. The NOAEL was 0.093 mg/kg associated with a mean AUC of 59.3 ng.h/mL. The 2 week study in the dog with micronized drug included pulmonary deposited doses of 0.0025, 0.12 and 0.23 mg/kg. QAB149 induced tachycardia and myocardial necrosis in the dogs in the mid and high

dose groups. There were increases in heart rate and heart force (strength of heart beat) in all QAB 149-treated dogs. There was dose-related periportal glycogen vacuolation in the liver of all QAB149 dosed groups. There was no NOAEL in this study. The 4 week subchronic inhalation study with the HFA formulation in the dog included pulmonary deposited doses of 0.0025, 0.025 and 0.24 mg/kg. QAB149 induced tachycardia and myocardial necrosis in the dogs in high dose group and tachycardia in the dogs in the mid dose group. There was also dose-related glycogen vacuolation of the liver of the dogs in all dose groups. This finding was reversible following a 17 day recovery period. The NOAEL was 0.0025 mg/kg associated with a mean AUC of 2.93 ng.h/mL.

Genetic Toxicity: : Genotoxic assays, i.e., Ames bacterial reverse mutation test, Mammalian Chromosomal Aberration test in the V79 Chinese hamster cell and bone marrow micronucleus test reveal that QAB149 is not genotoxic under the conditions tested.

Reproductive developmental Studies: Studies on the fertility and early embryonic developmental effects of QAB149 were submitted and reviewed under IND 48,649 (see review #1). Rats were administered 0.2, 0.6 and 2 mg/kg, SC. Decreased reproductive organ weights were observed in the males of all drug-treatment groups. There were no detectable effects on reproductive potential and histological evaluations found no article-related change in the testes or epididymis. There was drug-related local toxicity, i.e., discoloration at the injection sites in the male rats at 0.6 mg/kg and hair loss, scabs/wounds/cuts in the males and females in the 2 mg/kg dose group. Additionally, there were skin lesions in the rats in the 0.6 and 2.0 mg/kg dose group. One of the male rats in this dose group was sacrificed because of skin lesions. Systemic effects include increases in body weight parameters and food consumption in the males in all treated dose groups. There were also increases in the body weight parameters and food consumption in the females in all dose groups during premating; body weights remained increased during the gestation period. There were no effects in males or females related to parameters of fertility, general reproductive performance or early development. The NOAEL was 2 mg/kg for fertility and early embryonic development. There was no NOAEL (< 0.2 mg/kg) for male and maternal toxicity because of local, i.e., cuts, scabs/skin lesions at the injection sites, and systemic toxicity, i.e., increased body weight parameters and food consumption, at all doses.

Dose-ranging reproduction and development studies were carried out in the rat and rabbit in an attempt to determine the doses to be used in the definite studies. QAB149 was not teratogenic in rats. In the dose-ranging studies in the Wistar rat, QAB149 was administered subcutaneously to pregnant rats, days 6-17 using doses 3-100 mg/kg. Discolorations, wounds/scabs and blackened hardened regions were observed at the injection sites of the rats causing premature sacrifices. No NOAEL was established in this study. In a second dose-ranging study, pregnant rats were dosed subcutaneously using daily doses of 0.1, (single dose), 1 (single dose) and 1(bid dose) mg/kg, day 6-17 of gestation. The NOAEL was 0.1 mg/kg for local maternal toxicity and 1.0 mg/kg for developmental toxicity. In the definite study in the rat, QAB149 was administered to pregnant rats using sc doses of 0.1, 0.3 and 1 mg/kg. The doses were administered bid. The results of this study reveal drug-related local toxicity at 0.1 mg/kg (wounds/scabs) and above, there were drug-related skin lesions in the dams in the 3 mg/kg dose group. There was no evidence of teratogenicity in this study. The NOAEL for the fetal development was 1 mg/kg. There was no NOAEL for the pregnant rat due to injection site toxicity and body weight changes.

In the dose ranging studies in the New Zealand rabbit QAB149 was administered subcutaneously to pregnant rabbits using daily doses of 0 (vehicle – 2-hydroxypropyl-beta-cyclodextrin solution 25 wt-% diluted to 5 %w/v with 0.9 % sodium chloride), 3, 10, 30 and 100 mg/kg on days 7-20 of gestation. There was local toxicity, i.e., discoloration of the injection sites, swelling, scabs and wounds at 3 mg/kg and higher. There was no NOAEL for local maternal toxicity in this study due to drug-related local toxicity, i.e., wound/scabs at the injection sites and associated drug-related skin lesions. There was a statistically significant decrease in total resorptions at the 3 and 10 mg/kg as well as early resorptions at 3 mg/kg. These differences were due to an elevated postimplantation loss in the controls. The increase was due to the early resorption of one entire litter and approximately half of a second litter. In the follow-up dose ranging study, QAB149 was administered subcutaneously daily to pregnant rabbits days 7-20 of gestation. The doses were 0.1, 1, 1 (bid) and 3 mg/kg. There was wound/scabs in the injection site areas in 1/5, 1/5 and 4/5 rabbits in the 1 (single dose), 1 (bid) and 3 mg/kg, respectively. There was one humane sacrifice in the 3 mg/kg dose group on gestation 14. There was no embryo-fetal toxicity or gross malformations. The NOAEL for embryo-fetal developmental was 3 mg/kg. The NOAEL for maternal toxicity was 0.1 mg/kg due to drug-related local toxicity, i.e., wounds/scabs and hair loss. There was no systemic toxicity in the dams. In the definitive study in the rabbit, QAB149 was administered subcutaneously to pregnant rabbits days 7-20 of gestation. The doses were 0 (2-hydroxypropyl-beta-cyclodextrin solution 25 wt-% diluted to 5 %w/v with 0.9 % sodium chloride), 0.1, 1 and 3 mg/kg. Results of this study show cuts/scratches/ scabs/ wounds in the rabbits in the 3 mg/kg dose group. There were no mortalities or morbidity in this study. There was local maternal toxicity and an increase in one skeletal variation in the fetuses in the 3 mg/kg dose group. High dose animals demonstrated reduced body weight gain and food consumption. There was no teratogenicity in this study. The NOAEL in this study was 1 mg/kg for maternal systemic toxicity, maternal local toxicity and fetal toxicity.

Conclusions: The proposed clinical trial (single inhaled doses of 100 and 400 µg QAB 149) may be initiated. There is an adequate margin of safety from the rat toxicity studies on an AUC basis to support the proposed doses in the clinical trial (see table below). In the 2 week inhalation study the NOAEL is 0.21 mcg/kg with an AUC of 112 ng.h/mL while the NOAEL in the 4 week inhalation study is 0.093 with an AUC of 59.3 ng.h/mL. Using AUC as the basis for calculation and using the pulmonary deposited dose and noting that the that the high dose in the clinical trial is 8 mcg/kg, the 2 week study NOAEL provides a 12-fold margin of safety and the 4 week study provides a 7-fold margin of safety.

Duration	Doses Achieved (mg/kg)	Doses Deposited (mg/kg)	Rat AUC @ NOAEL (ng.h/mL)	Human AUC @ 400 µg dose (ng.h/mL)	Margin of safety (AUC basis)
2 weeks	2.05 5.81 17.0	0.21* 0.58 1.7	112	10.3	12x
4 weeks	0.93 2.77 8.46	0.093* 0.28 0.85	59.3	10.3	7x

*=NOAEL

There was no margin of safety provided by the dog studies as is shown below:

Duration	Doses Achieved (mg/kg)	Doses Deposited (mg/kg)	Dog AUC @ NOAEL (ng.h/mL)	Human AUC 400 µg Dose (ng.h/mL)	Margin of safety (AUC basis)
2 weeks	0.01 0.47 0.93	0.0025 0.12 0.23	-	10.3	None
4 weeks	0.01 0.1 0.97	0.0025* 0.025 0.24	3	10.3	0.33

*=NOAEL

Even though there was not an adequate margin of safety in the dog, the study was allowed to proceed because of previous human data, i.e., human subjects administered single doses up to 2000 mcg without any reported significant adverse events. The lack of a margin of safety in the dog was due to the drug-related periportal glycogen vacuolation in the liver of the dog. Recovery studies reveal that the vacuolation of the liver is reversible in the dog. Additionally, in the proposed clinical trial, the subjects will receive a single dose of QAB149. Therefore, the potential liver toxicity was not considered to be a significant safety problem for this clinical trial. And finally, as part of our 30 day safety review for IND 48,649 (QAB149 micronized drug), it was our conclusion, that after reviewing the 2 weeks subchronic studies in the rat and the dog, there was a margin of safety in these animal species. The margins of safety supported a single dose of 3000 mcg in human clinical trials.

General Toxicology Issues: Glycogen vacuolation in the liver and tachycardia and myocardial necrosis were observed in dogs and should be monitored in clinical trials. The sponsor should address the relevance of the liver findings in humans.

Recommendation: The proposed clinical trial is considered safe to proceed based on previous human experience.

External comments (to sponsor): None

Linked Applications	Sponsor Name	Drug Name
IND 66337	NOVARTIS PHARMACEUTICALS CORP	QAB149

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
05/07/2008

TIMOTHY J MCGOVERN
05/08/2008

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: IND 66,337
Review number: 005
Sequence number/date/type of submission: N-007-it- dated March 4, 2003, IN-009/SX dated June 6, 2003

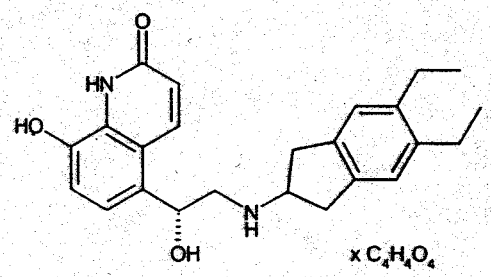
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Norvartis Pharmaceuticals Corporation
Manufacturer for drug substance : Norvartis

Reviewer name: V Whitehurst, PhD
Division name: Division of Pulmonary and Allergy Drug Products
HFD #: HFD 570
Review completion date: July 2, 2003

Drug:

Trade name:QAB149 (long-acting β_2 agonist) maleate
Generic name (list alphabetically):NA
Code name: QAB149
Chemical name: (R)-5-[2-(5,6-diethyllindan-2-ylamino)-1-hydroethyl]- 8-hydroxy-1H- quinolin-2- one maleate
CAS registry number:435273-74-8
Mole file number: NA
Molecular formula/molecular weight: $C_{24}H_{28}N_2O_3$ $C_4H_4O_4$

Structural formula



Chemical name: **(R)-5-[2-(5,6-Diethylindan-2-ylamino)-1-hydroxyethyl]-8-hydroxy-1H-quinolin-2-one maleate**

Relevant INDs/NDAs/DMFs: None

Drug class: Long-acting β_2 agonist

Indication:Treatment of asthma/COPD

Clinical formulation:

Table 1 Declared content per QAB149 50 µg / actuation (metered dose)[1]:

Ingredient	Theoretical amount per actuation (metered dose)		Function	Reference to standards
	[% w/w]	[mg]		
QAB149 maleate	0.11[2]	0.065[3]	Drug substance	Novartis Monograph
Sorbitan trioleate	0.02	0.012	Surfactant	USP
Dehydrated alcohol	10.00	5.830	Co-solvent	USP
1,1,1,2-tetrafluoroethane (propellant HFA-134a)	89.87	52.393	Propellant	IPACT-I[4] DMF 9859 DuPont DMF 9654
Total weight	100.00%	58.300		

1 50 µg / actuation (metered dose) correspond to 35 µg / actuation (emitted dose)

2 corresponds to 0.09 % w/w of QAB149 free base

3 corresponds to 0.050 mg of QAB149 free base

4 International Pharmaceutical Aerosol Consortium for Toxicology Testing of HFA-134a

Route of administration: Inhalation

Proposed clinical protocol: NA

Previous clinical experience: A total of 29 asthmamatic patients have been treated with single doses up to 2000 µg of inhaled QAB149. QAB149 induced drug-related tachycardia, QTC interval changes as well as decreases in serum potassium.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: QAB149 is a long acting β_2 agonists intended for once a day treatment of asthma. The target organ for this class of drug is classically the cardiovascular system, i.e., tachycardia, QTc interval changes and myocardial necrosis. The dose-limiting toxicity in the rat is nasal lesions, characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium.

Studies reviewed within this submission :
13-Week Inhalation (Dry Powder Aerosol) Toxicity Study in Mice- Volume 2
1-Week Oral (gavage) Feasibility Study in Mice-Volume 3

Studies not reviewed within this submission:None

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PHARMACOLOGY/TOXICOLOGY REVIEW

IV. GENERAL TOXICOLOGY:

Study title: 13- Week Inhalation (Dry Powder Aerosol) Toxicity Study in Mice

Key study findings: Dose-limiting toxicity nasal lesions, characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium.

Study no: 0220019
Volume #, and page #: Volume #1, page # 1
Conducting laboratory and location: (b) (4)
Date of study initiation: May 14, 2002
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: Drug product, white to off white maleate salt, batch # 0121002, purity 99.4%,
Formulation/vehicle: Formulation, salt to free base, 0.7718

Methods (unique aspects)**:**
Dosing:

Species/strain: Mouse/CD1
#/sex/group or time point (main study):10/sex/dose group
Satellite groups used for toxicokinetics or recovery:10/sex/dose group
Age: Approximately 5 weeks
Weight: Males-about 21.5-27.8 g; females-about 18.5-24.1g

Route, form, volume, and infusion rate: Inhalation, flow rate was about 0.5 L/minute

Table 3-2 Study design, animal allocation and test item doses

Dose Group/ Treatment	Target Aerosol Concentration (mg QAB149.l^{-1})	Target Dose (mg QAB149.kg^{-1}.day^{-1})	Animal Designation	Animal Number	
				Males	Females
1 – Air Control	0	0	Main Study	101-110	131-140
			Satellite	111-120	141-150
				121-130	151-160
2 – Low Dose	0.1	0.5	Main Study	201-210[a]	231-240[c]
			Satellite	211-220	241-250
				221-230[b]	251-260
3 – Intermediate Dose	0.1	1.5	Main Study	301-310[d]	331-340[e]
			Satellite	311-320	341-350
				321-330	351-360
4 – High Dose	0.1	5	Main Study	401-410	431-440[f]
			Satellite	411-420	441-450
				421-430	451-460

The target aerosol concentrations as well as the target doses refer to the free base of QAB149 and the conversion factor for salt to free base is 0.7718.

Satellite animals were used for toxicokinetic blood sampling . The first set of Satellite animals were designated to Days 1/2 toxicokinetic blood sampling and the second set designated to Week 11 toxicokinetic blood sampling.

Spare animals were numbered as: Males 500-504; females 505-509

Replacement

As a result of findings noted at the pretrial ophthalmic examination, the following animal replacements were made:

a = Animal 208 replaced by Animal 501
b = Animal 227 replaced by Animal 502
c = Animal 232 replaced by Animal 505, Animal 233 swapped with 241, Animal 234 replaced by Animal 506
d = Animal 302 replaced by Animal 503
e = Animal 331 replaced by Animal 507
f = Animal 432 replaced by Animal 508, Animal 439 replaced by Animal 509

* Estimated achieved doses are not deposited doses. A deposition factor of 0.05 should be applied to the estimated achieved doses.

Observations and times:

Clinical signs: Daily
Body weights: Weekly
Food consumption: Weekly
Water consumption: Daily, visual analyses
Ophthalmoscopy: Pretrial, week 13, groups 1 and 4
EKG: NA
Hematology: Week 13
Clinical chemistry: Week 13
Urinalysis: Not performed
Gross pathology: Terminal
Organs weighed: Terminal
Histopathology: Terminal, groups 1 and 4, respiratory tract tissues from all the mice in dose groups 2 and 3.
Toxicokinetics: Days ½ and week 11, animals were bled 0.5, 3.0, 8.0 and 24 hours after drug inhalation.

Results:

Mortality: One female mouse (1.47 mg/kg dose group) was found dead on day 75, the death was due to a dosing error.

Clinical signs: There were no clinical signs induced by QAB149 in the treated mice.

Body weights: The QAB 149 treated males in the low and mid dose groups gained more weight than the males in the control group, approximately 6 and 20%. However, the males in the high dose group, 4.91 mg/kg gained less than the males in the control group, approximately 4%. The QAB149 treated females gained more weight than the females in the control group, approximately 2, 17, and 15%, respectively.

Food consumption: Food consumption was increased in the QAB149 treated males, approximately 4, 15 and 9 %, respectively in the low, mid and high dose group. Food consumption was also increased in the QAB149- treated females, approximately 5, 8 and 2 % in the low, mid and high dose groups.

Water consumption: Water consumption was comparable in all dose groups.

Ophthalmoscopy: There were no eye changes due to treatment with QAB149.

Electrocardiography: NA

Hematology: There were no hematological changes due to QAB 149.

Clinical chemistry: The clinical chemistries were comparable in the mice in all dose groups in this study.

Urinalysis: Not performed

Organ weights: Some statistically significantly differences (absolute and covariance) were observed in the organ weights of the heart, lung, kidney and spleen in the QAB 149 treated males when compared with the control group. The increases in heart and lung weight (absolute and relative) were approximately 19 % in the males in the mid and high dose groups. The absolute kidney weight increases were approximately 19 % while the relative kidney weight increases were approximately 12 % in the mid and high dose groups. The absolute and relative increases in spleen weight were approximately 24 % in both the mid and high dose groups. When body weight differences were taken into account, the only statistically differences in organ weights noted were in the heart weights of the males in the high dose group (approximately 20 %). Organ weights were comparable in the females in all dose groups in this study.

Gross pathology: There were no macroscopic findings that could be attributable to QAB149.

Histopathology: Microscopic analyses of the respiratory tissues revealed statistical significant minimal to mild respiratory epithelial atrophy in 14/20 mice in the high dose group and 1/20 mice in the mid dose group. Additionally, 4/20 animals in the high dose group and 3/20 animals in the mid dose group had olfactory epithelial atrophy. The lesions were characterized by loss of sustentacular cell cytoplasm , epithelial disorganization and thinning of the epithelial and occurred on the roof of the dorsal meatus. These lesions were often accompanied by the presence of eosinophilic globules. In some males in low dose group, there were eosinophilic globules in the respiratory epithelial but no other findings. Summary of the treatment-related findings are shown below:

Table 3-1 Summary of principal treatment-related findings (minimal/mild) following exposure of mice to QAB149 for 13 weeks

Group / Dosage (mg/kg/day)	Group 1 Air control	Group 2 0.48	Group 3 1.47	Group 4 4.91	Group 1 Air control	Group 2 0.48	Group 3 1.47	Group 4 4.91
	Males				Females			
Number of animals examined	9	10	10	10	10	10	10	10
NASAL CAVITY								
No abnormality detected	8	7	6	2	10	10	6	3
Respiratory epithelial atrophy, level I	0	0	1	8	0	0	0	6
Olfactory epithelial atrophy, level II	0	0	2	2	0	0	1	2
Eosinophilic globules, level I	1	0	1	5	0	0	1	4
Eosinophilic globules, level II	0	2	0	2	0	0	1	3
Eosinophilic globules, level III	0	0	2	0	0	0	2	1

Toxicokinetics:

2 Summary of results

Table 2-1 TK parameters of QAB149

Sampling period	Dose of QAB149 base* (mg/kg/day)	Gender	TK parameters of QAB149				
			C_{max} (ng/mL)	t_{max}** (h)	$AUC_{(0-24h)}$ (ng.h/mL)	± SE (AUC) (ng.h/mL)	$AUC_{(0-24h)}$/dose (ng.h/mL)/(mg/kg)/day
Day 1/2	0.48 [Group 2]	Male	43.2	0.1	68.0	26.2	142
	0.48 [Group 2]	Female	5.35	0.1	11.8	0.465	24.6
	1.47 [Group 3]	Male	43.6	0.33	83.3	8.71	56.7
	1.47 [Group 3]	Female	32.1	0.33	63.6	6.45	43.3
	4.91 [Group 4]	Male	83.7	1	235	15.2	47.9
	4.91 [Group 4]	Female	81.9	1	172	36.1	35.0
Week 11	0.48 [Group 2]	Male	12.9	0.1	21.0	2.31	43.8
	0.48 [Group 2]	Female	8.44	0.1	16.0	3.11	33.3
	1.47 [Group 3]	Male	42.7	0.33	83.7	4.16	56.9
	1.47 [Group 3]	Female	79.8	0.33	152	9.12	103
	4.91 [Group 4]	Male	84.7	1	236	30.8	48.1
	4.91 [Group 4]	Female	146	1	419	38.5	85.3

*Overall mean achieved doses. The conversion factor for salt to free base is 0.772. Approximate inhalation times were 6 min, 20 min, and 60 min for groups 2, 3, and 4 respectively. **t_{max} is calculated from the start of inhalation.

The results of the toxicokinetic study shows that the animals were exposed to DAB149. There were no gender differences, however, exposure was increased in females after multi dosing.

Histopathology Inventory for IND

Study 0220019				
Species Mice/CD1				
Adrenals	X			
Aorta	X			
Bone Marrow smear	X			
Bone (femur)	X			
Brain	X			
Cecum	X			
Cervix	-			
Colon	X			
Duodenum	X			
Epididymis	x			
Esophagus	-			
Eye	X			
Fallopian tube	-			
Gall bladder	X			
Gross lesions	X			
Harderian gland	x			
Heart	X			
Ileum	X			
Injection site	-			
Jejunum	X			
Kidneys	X			
Lachrymal gland	X			
Larynx	X			
Liver	X			
Lungs	X			
Lymph nodes, cervical	X			
Lymph nodes mandibular	-			
Lymph nodes, mesenteric	X			
Mammary Gland	X			
Nasal cavity	X			
Optic nerves	X			
Ovaries	X			
Pancreas	X			
Parathyroid	X			
Peripheral nerve	-			
Pharynx	X			
Pituitary	X			
Prostate	X			

Rectum	X			
Salivary gland	X			
Sciatic nerve	-			
Seminal vesicles	X			
Skeletal muscle	-			
Skin	X			
Spinal cord	X			
Spleen	X			
Sternum	X			
Stomach	X			
Testes	X			
Thymus	X			
Thyroid	X			
Tongue	X			
Trachea	x			
Urinary bladder	X			
Uterus	X			
Vagina	X			
Zymbal gland	X			
Standard List				

X, histopathology performed
*, organ weight obtained

Toxicology summary:
A 13-week inhalation toxicology study was carried out in CD-1 mice.The estimated achieved doses were 0, 0.48, 1.47 and 4.91 mg/kg. These doses are not the deposited dose (approximately 5% in the mouse) and therefore, may be overestimated. Toxicokinetic data reveal that the mice were exposed to QAB149 as evidenced by the presence of the drug in serum up to 24 hours post dosing. Body weight gain was increased in the males in the low (approximately 6%) and the mid (20%) dose groups. However, body weight gain in the males in the high dose groups was less than body weight gain in the control group, approximately 4 %. The increased body weight gain in the QAB149 treated females was approximately 2, 17 and 15 % in the low, mid and high dose groups, respectively. Perhaps, one of the factors in the decreased body weight gain observed in the males in the high dose group can be explained by the decreased food consumption in the high dose group when compared with the control group (approximately 15 %). Microscopic analyses of the tissues revealed changes in the nasal cavity. These changes were characterized by statistical significant minimal to mild respiratory epithelial atrophy in 14/20 mice in the high dose group and 1/20 mice in the mid dose group. Additionally, 4/20 animals in the high dose group and 3/20 animals in the mid dose group had olfactory epithelial atrophy. The lesions were characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of the epithelial and occurred on the roof of the dorsal meatus. These lesions were often accompanied by the presence of eosinophilic globules. Urinalysis parameters were not included in this study.The NOAEL in this study was determined to be 0.48 mg/kg. The MTD was the high dose, 4.91 mg/kg.

Study title: 1-Week Oral (Gavage) Feasibility Study in Mice
Key study findings: None

Study no: 0370021
Volume #, and page #: Volume 3, page# 4
Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover, NJ
Date of study initiation: January 15, 2003
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: QAB149 maleate micronized, batch # 0121002, 99.1%.
Formulation/vehicle: QAB 149 suspensed in 0.5 % (w/v) hydropropyl cellulose, NF(Klucel), aqueous solution

Dosing:
Species/strain: Mouse (Crl:CD-1 (ICR)BR)
#/sex/group or time point (main study): 10 animals/sex/dose group
Satellite groups used for toxicokinetics or recovery: 2 animals/sex/group
Age: 7-8 weeks
Weight: 21.7 to 36.2 g
Doses in administered units: 0, 100, 250 and 500 mg/kg
Route, form, volume, and infusion rate: Oral, aqeous solution, via gavage, 10 mL/kg dose volume, dosed daily for 8 days.

Observations and times:
Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Pretest, days, 1, 4 and 7
Food consumption:days 1, 4 and 7
Ophthalmoscopy: NA
EKG:NA
Hematology: NA
Clinical chemistry: NA
Urinalysis: NA
Gross pathology: Six animals/ sex/dose group
Organs weighed:NA
Histopathology: Six animal/sex/ dose group; the following tissues were evaluated microscopically: cecum, colon, duodenum, esophagus, ileum, jejunum, liver, rectum, stomach
Toxicokinetics: Blood samples were collected from the orbital sinus at 0.5, 1, 3, 8 and 24 hours after dosing, days ½ and 8/9.

Results:
Mortality: There were no drug-related deaths in this study.
Clinical signs: There were no test article-related clinical signs in this study.

Best Available Copy

Body weights: Increases in body weight gain were noted in the males and females at all dose levels (increases ranged from 200 to 600 %).

Table 1.3
Novartis Pharmaceuticals Corporation
Toxicology Department
Mouse/CD-1

Mean Animal Absolute Weight Gains in (g)
Study number: 0370021
Absolute weight gains referenced to treatment phase (Day 1)
Study start date: 15-Jan-03

Printed: 06-Mar-03
Page: 1
preclinical study/

Group(s)		Day of Phase 4	Day of Phase 7
		Male Animals	
1	(N)	10	10
	Means	0.8	0.3
	Sdevs	0.60	0.56
2	(N)	10	10
	Means	1.1	1.3
	Sdevs	0.62	1.11
3	(N)	10	10
	Means	1.1	1.1
	Sdevs	0.55	1.07
4	(N)	10	10
	Means	1.0	2.3+
	Sdevs	0.87	1.28
		Female Animals	
1	(N)	10	10
	Means	0.6	0.0
	Sdevs	0.46	0.61
2	(N)	10	10
	Means	1.1	1.1+
	Sdevs	0.89	0.55
3	(N)	10	10
	Means	1.1	1.5+
	Sdevs	0.72	0.66
4	(N)	10	10
	Means	1.5+	2.4+
	Sdevs	0.85	1.01

Note: Data for treatment phase
*(+) = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
%($) = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Food consumption: There were statistical significant increases in food consumption in the males in all dose groups.

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology: NA

Clinical chemistry: NA

Urinalysis: NA

Organ weights NA:

Gross pathology: There was no drug-related gross pathology.

Histopathology: There were no microscopic findings that were considered to be drug-related.

Toxicokinetics:

Table 4-1 TK parameters of QAB149 in male and female mice

Dose		[a]$AUC_{(0-24h)}$ (ng.h/mL)		[a]C_{max} (ng/mL)		[b]T_{max} (h)	
(mg/kg/day)	Group	Day 1/2	Day 8/9	Day 1/2	Day 8/9	Day 1/2	Day 8/9
Males							
100	2	446 ± 44.3	946 ± 33.7	73.9	300	1	1
250	3	1250 ± 534	2010 ± 551	80.0	279	8	1
500	4	1360 ± 387	3010 ± 611	111	557	3	1
Females							
100	2	670 ± 195	1100 ± 338	69.6	207	1	1
250	3	1910 ± 964	1390 ± 205	148	218	8	1
500	4	1700 ± 208	2580 ± 890	173	425	3	1

[a]Values are mean and, if indicated, ± SE.
[b]Values are median.

The data show that QAB 149 is absorbed in mice after oral multi dosing. QAB149 exposure increased in non-proportional manner in male and female mice between the low and high dose groups, however, systemic exposure was decreased in the females when AUCs were compared on days ½ and 8/9. There are no other gender differences

Toxicology summary: It was recommended by the CAC (attached, CAC meeting notes dated March 4, 2003) to conduct the second carcinogenicity study by the systemic route.

A 1- week oral toxicology study was carried out in the mouse to determine absorption, to assess toxicokinetic parameters, to provide information on the suitability of oral route and dose selection for 2- year and 26 weeks carcinogenic studies and to provide data for dose selection for subsequent potential GLP toxicity studies. The doses used were 0, 100, 250 and 500 mg/kg. The doses were given once daily for 8 days. This was not a GLP study. It is difficult to assess the results because clinical chemistries, hematological and urinalysis parameters were not included in this study and complete histopathology was not submitted. However, the results show increased body weight gain in the males and females in all dose groups. There were no drug-related microscopic findings in the tissues (only selected tissues were evaluated) of the mice treated with QAB149. The cardiovascular tissues were not evaluated microscopically, this is unusual since one of the major target organ for beta agonists is the cardiovascular system. Previous studies in dogs and humans show that inhaled QAB149 causes increases in heart rate, tachycardia, heart lesions (dogs only), decreases in serum potassium and changes in QTc interval. The NOAEL and the MTD could not be determined in this study. The sponsor submitted proposals for both studies. However, only one of these studies will be carried out. The sponsor proposed a high dose of 50 mg/kg for the 2-year and 500 mg/kg for the 26 week TgHras2 carcinogenicity studies based on approximately 70 and 420 times, respectively, the anticipated daily maximum human AUC of 400 mcg. Pharmacokinetic endpoints can be used as a basis for dose selection for 2 year oral carcinogenicity studies provided that the oral formula is not genotoxic. Considering that QAB149 will be marketed as an inhalation drug, a toxicity endpoint rather than PK endpoint should be the basis for the dose selection for a 2 year oral carcinogenicity study. Furthermore, pharmacokinetic endpoints can not be used as a basis for dose selection for the 26 week TgHras2

carcinogenicity study. Dose range finding studies, durations of 4 and 13 weeks are appropriate to determine the dose selections for 26 week TgHras2 and 104 week carcinogenicity studies. Because only 1-week toxicity study data was submitted, the dose selections proposed for the 26 week TgHras2 and 104 week carcinogenicity studies in the mouse can not be substantiated.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

No dose selections can be determined from the limited 1-week oral toxicity study in mice. The sponsor should conduct 4-week and 13-week oral dose range finding studies which can be used as the basis for dose selection for the 26 week TgHras2 and 104 carcinogenicity studies, respectively. This recommendation will be presented to the CAC for concurrence.

Reviewer signature: Virgil Whitehurst

Supervisor signature: Concurrence -

Non-Concurrence -
(see memo attached)

cc: list:
HFD-570/VWhitehurst
HFD-570/JSun
HFD-570/AGreen

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Virgil Whitehurst
7/9/03 01:55:02 PM
PHARMACOLOGIST

Joseph Sun
7/9/03 02:13:48 PM
PHARMACOLOGIST
I concur.

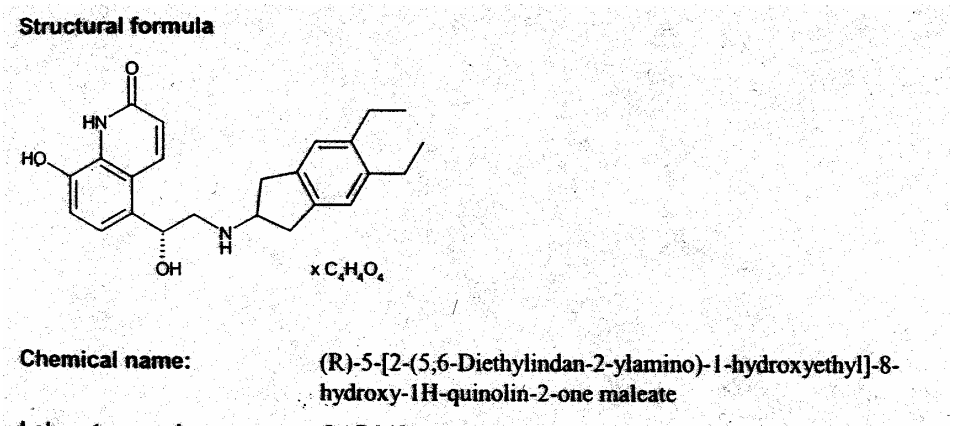
PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: IND 48, 649
Review number: 001
Sequence number/date/type of submission: 000/February 18, 2004/Original IND
001/March 4, 2004/IT
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Norvartis Pharma AG
Manufacturer for drug substance : Norvartis Pharma AG

Reviewer name: Virgil Whitehurst
Division name: Division of Pulmonary and Allergy Drug Products
HFD #: HFD 570
Review completion date: April 27, 2004

Drug:

Trade name: QAB 149 (long-acting ß$_2$ agonist) Inhalation Powder Hard capsules
Generic name (list alphabetically): NA
Code name: QAB149
CAS registry number: 435273-74-8
Mole file number: NA
Molecular formula/molecular weight: $C_{24}H_{28}N_2O_3$ ● $C_4H_4O_4$/508.56
Structure:



Relevant INDs/NDAs/DMFs: IND 66,337 (QAB 149 HFA formulation)

Drug class: Long-acting beta 2 adrenergic agonist

Indication: Treatment of asthma and COPD

Clinical formulation:

QAB149- 0.1, 0.2 and 0.4 mg Inhalation Powder Hard capsules

Ingredients	0.1 mg	0.2 mg	0.4 mg
QAB 149	(b) (4)		
Lactose Monohydrate			
Capsule shell Approximate weight (gelatin, (b) (4)			
Total weight			

Route of administration: Inhalation

Proposed clinical protocol: Protocol # CQAB149A2211: An open-labeled, dose escalation study to assess the safety and tolerability of incremental doses of QAB149 delivered via a single-dose, dry powder inhaler to adults (18-65 years old, n = 20) with persistent asthma is proposed. The proposed doses are 400, 1000, 2000, 3000 and 4000 mcg. Each patient will receive each of these single doses followed by a washout period of 7 days.

Previous clinical experience: QAB149 has been used to treat asthmatic and COPD patients. Single inhaler doses of 25-2000 mcg have been used to treat asthmatics in phase 1 studies. In phase 2 studies, doses of 200-800 mcg have been used. Repeated doses of 800 mcg have been used in the treatment of COPD patients. The clinical trials were conducted in Europe.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: QAB149 is a long acting β_2 intended for once a day treatment of asthma/COPD. The sponsor is developing 2 formulations, a HFA formulation (I66,337) and a micronized drug with lactose formulation (I 48,649). The sponsor has conducted 2 and 4 week inhalation studies in the Wistar rat and the Beagle dog that were reviewed in IND 66, 337 (see original IND review). The two week studies ultilized the micronized drug and the 4 week studies ultilized the HFA formulation. In order to show that the 2 formulations (micronized QAB 149 drug with lactose and QAB 149/HFA) are similar (kinetics and toxicity profile), the sponsor is planning to conduct a 90 day subchronic inhalation bridging study in the dog. A protocol for this bridging study was submitted in March 2004 (SN 001). The target organ for this drug is classically the cardiovascular system, i.e., tachycardia, QTc interval changes and myocardial necrosis. Increased cardiovascular activity with associated histopathological changes was observed in the dog. There was also glycogen deposition in the liver of the dog at all dose levels. The dose-limiting toxicity in the rat is nasal lesions, characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium.
In the pre-IND meeting the Division informed the sponsor that until complete and detailed comparative chemistry data regarding the HFA and lactose formulations, manufacturing, purity, dose performance characteristics and stability of the dry powder and HFA formulations are

available, toxicology studies should be performed using the lactose formulation. The Division further stated that there is no margin of safety in the dog for the initially proposed up to 600 mcg inhalation doses in the phase 2 clinical trial (the actual submitted protocol proposes doses up to 4000 mcg). This conclusion was based on cardiovascular and liver toxicities observed in the dog studies. The sponsor should demonstrate that the liver toxicity is glycogen mediated prior to phase 2 studies.

During a teleconference dated January 29, 2003, the Division informed the sponsor that until the 13-week nonclinical bridging study is completed, the sponsor can initiate the proposed clinical trial using the micronized powder with lactose using the data from 2 week animal studies with micronized drug. This conclusion is based on the fact that glycogen deposition observed in the liver of the dog is mild and reversible. The Division agreed with Novartis that tachycardia may be used in clinical trials to monitor for myocardial toxicity. However, the Division does not agree with Novartis that tachycardia can be used as a biomarker for the deposition of glycogen into the liver. The bridging study should be conducted in the dog.

Studies reviewed within this submission:
Pharmacology Studies:
RD-2000-00195- Anti-Bronchoconstriction and Cardiovascular Effects of Inhaled Salmeterol in Rhesus Monkey. Volume 2, page 170
RD-2003-02487-The Effects of Dry Powder NVP-QAB 149 on 5-HT-Induced Bronchoconstriction in the Guinea Pig: Comparison with Salbutamol, Salmeterol and Formoterol. Volume 2, page 187
Pharmacokinetic/Toxicokinetic Studies:
R0101277- Absorption, Distribution, Metabolism and Excretion Following an Intravenous or Oral Gavage Dose of 3[H] QAB149 in the Mouse. Volume 3, page 323
R030002- Relative Bioavailability of Oral Gavage Doses of 3[H]QAB 149 in the Mouse. Volume 3, page 356
R02-0220- Excretion in Milk After a Single Subcutaneous Dose of 3[H] QAB 149 in the Rat. Volume 3, page 435
R01-0990-Absorption, Distribution, Metabolism and Excretion Following a Single Dose of ^{3}[H]QAB 149 in the Dog. Volume 3, Page 454.
0220073-QAB149-Micronized Powder and HFA Formulation:Comparative Single-Exposure Inhalation Study in Rats using Micronized Powder and HFA Formulation of QAB149. Volume 3, page 605
Toxicology Studies:
0420019- 13 Week Inhalation Toxicity Study in Dog (Proposed Bridging Study) Submission 001.
0370021- 1-Week Inhalation Dose-range Finding (Dry Powder Aerosol) Toxicity Study in Mice. Volume 8, page 957
022007-QAB 149:2 Week Inhalation Dose-Range Finding (dry powder aerosol) Toxicity Study in Mice. Volume 5, page 1063

Reproductive Development Studies:
0270074-QAB149: A Subcutaneous (bid) Fertility and Early Embryonic Development Study in Rats. Volume 7-8, page 1541

Special Studies:
0220082- Buehler Test in Guinea Pig for Delayed Skin Sensitization Potential. Volume 8, page 2197

Studies <u>not</u> reviewed within this submission: The following studies have been reviewed in IND 66, 337.
Pharmacology reports:
RD-1999-02232-Effects of a β2-agonist NVP on the isolated, electrically-stimulated, superfused guinea pig tracheal strip
RD-1999-03094v2-Single chamber plethysmographic measurement of airway reactivity in naïve, conscious guinea pigs
RD-1999-03095v2-Evaluation of NVP-QAB149 in guinea pigs by whole body, single chamber plethysmography
RD-2000-00233v2-Duration of action of formoterol and NVP- QAB149 in a guinea pig model of histamine–induced bronchoconstriction *in vivo*
RD-2000-00234-Determination of tachyphylaxis by forrmoterol and NVP-QAB149 using a guinea pig plethysmograph modelof histamine-induced bronchoconstriction *in vivo*
RD-2000-00315-Results for PANLABS general pharmacology screen for NVP-QAB149
RD-2001-03026-Activity of NVP-QAB149-AA-and its (S)-enantiomer (NVP-QAB149-AA-1) in beta-1and beta –2 adrenoreceptor binding and functional assays
RD-2002-00195-Anti-broncoconstriction and cardiovascular effects of inhaled NVP-QAB149in rhesus monkeys: comparison with formoterol
RD-2002-01685-Effects of NVP-QAB149- AA in human beta-receptor cAMP assay (ALPHA Screen) in BEAS-2B cells
RD-2002-01927-NMR studies of NVP-QAB149in SDS micelles
RD-2002-03086- Antagonist effect of NVP-QAB149-AF-1on the α_{1D} adrenoceptor of rat aorta.
RD-2000-00159- Anti-Bronchoconstriction and cardiovascular Effects of Inhaled salmeterol in Rhesus Monkeys. Volume 2, page 170.
RD-2003-02487- The Effects of Dry Powder NVP-QAB149 on 5-HT-Induced Bronchoconstriction in the Guinea Pig: Comparison with Salbutamol, Salmeterol and Formoterol. Volume 2, page 187.
RD-2002-04880: A method to determine the effects of isoprenaline and formoterol in isolated, electrically stimulated guinea-pig left atria. Volume 2, page 8-159 (this study was not reviewed since it does not assess effects of QAB 149)
Safety Pharmacology:
0120071-Effect of QAB149 on the HERG currents recorded from stably transfected HEK293 cells
Pharmacokinetic Reports:
DMPK-R299-2114- [^{3}H]QAB149 synthesis of analysis. Drug metabolism and Pharmacokinetics
MPK-R02-00490-Quantitative determination of QAB149 in rat, human, dog, rabbit and mouse serum and rabbit embryo by LC/MS/MS using liquid/liquid extraction.
DMPK-R299-2520- Quantitative determination of QAB149 in human serum by LC/MS/MS using liquid/liquid extraction.
DMPK-R00-2189- Quantitative determination of QAB149 in human urine by LC/MS/MS using liquid/liquid extraction.
DMPK-R99-2113-QAB149: Absorption, distribution, metabolism and excretion of [^{3}H]QAB149 in the rat following an intravenous, intratracheal oral or subcutaneous dose.

DMPH-R00-594- In vitro binding of 3H-labeled QAB149 to red cells, serum and plasma proteins in the rat, dog and human.
RD-2002-03936-Binding of NVP-ADD561-NX (the main metabolite of NVP-QAB149-AA)-to beta-1 and beta-2 adernoceptors.
DMPK-R00-397-QAB149: Comparative metabolism of [^{3}H]QAB149 in rat, dog and human liver slice culture and metabolism in human lung slice culture.
DMPK-R01-994-Metabolic profiles in human liver microsomes and potential to inhibit cytochrome P450-mediated reactions.

Toxicology reports:

Acute Toxicology:

0170135-An acute subcutaneous toxicity in the mice.
001077-QAB149: Acute oral toxicity study in rats.
001078- QAB149: Acute oral toxicity study in mice.
An acute subcutaneous toxicity study in rats.

Repeated Dose Studies:

008042-Escalating dose ranging inhalation study in the rat.
0170165-QAB149: A subcutaneous 1-week toxicity study in rats.
002012-QAB149: 2-week inhalation toxicity study in the rat.
0120088-QAB149: 4-week inhalation toxicity study in the rat.
008043- Escalating dose ranging inhalation study in the dog.
002013-QAB149: 2-week inhalation toxicity study in the dog.
0120089- QAB149: 4-week inhalation toxicity study in the dog.
022009-QAB 149: A 13-Week Inhalation (dry powder aerosol) Toxicity Study in Rats.
0220019- QAB149: A 13-Week Inhalation (dry powder aerosol) Toxicity Study in Mice.

Genotoxic Assays

001808- Mutagenicity Test using Salmonella Typhimurium
001834- Chromosome Aberration test With V79 Chinese Hamster cells
0212401- Subcutaneous Bone Marrow Micronucleus Test in the Rat

Reproductive Development Studies:

0170145-A Subcutaneous Embryo-Fetal Development Dose Ranging-Finding Study in Rats.
0270021-A Follow-up Subcutaneous Embryo-Fetal Development Dose ranging-Finding Study in rats.
0270037-A Subcutaneous (bid) Embryo-Fetal development Study in Rats
0170146-A Subcutaneous embryo-Fetal Development Dose Range- Finding Study in Rabbits
0270036-A Follow-up Subcutaneous Embryo-Fetal Development Dose Range-Finding Study in Rabbits
0270038-A Subcutaneous Embryo-Fetal Development Study in Rabbit

Clinical Pharmacokinetics Reports:

CQAB149A2101-Single Center, Double-Blind, Placebo Controlled, Randomized, Time-lagged, Ascending Alternating, Inhaled Dose (25, 100, 300, 600, 1200 and 2000 μg) Study of the Safety, Tolerability, Pharmacodynamics and Pharmacokinetics of QAB149in Mild Asthmatic Patients.

Special Studies:

021726- QAB149: Assessment of Contact Allergenic Potential with Murine Local Lymph Node Assay (LLNA tier I).
0217031- Assessment of Contact Allergenic Potential with Murine Local Lymph Node Assay (LLNA tier I)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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Pharmacology studies cross referenced in this IND were reviewed in IND 66,337. A summary of the pharmacology studies are shown below. Additionally, two additional studies are reviewed below.

New studies:

In study # RD-2000-00195 the action of salmeterol, a long-acting beta 2 agonist, to suppress methacholine –induced bronchoconstriction was studied in spontaneously breathing, anesthetized rhesus monkeys. Airway resistance was used as an index for pulmonary function. Salmeterol was administered to the monkeys using inhalation doses of 1.4, 5.5, 30 and 54 mcg/kg, respectively. Salmeterol, 5.5, 30 and 54 mcg/kg, inhibited bronchoconstriction in the monkeys for 105-165 minutes. Salmeterol, at these doses increased heart rate by 13-43 % and reduced blood pressure. Salmeterol, at the two highest doses decreased serum potassium levels.

In study # RD-2003-02487 the potency and duration of action QAB 149 was compared with marketed compounds formoterol, salmeterol and salbuterol. The study ultilized, conscious, unrestrained guinea pigs and used plethysmographic techniques. The effects of QAB149 and the other beta 2 agonists on 5-hydroxytryptamine- induced bronchoconstriction was investigated 2, 4, 6, 9, 12, 18, 24, 36 and 48 hours after inhalation dosing. The inhaled concentrations for QAB149 were 0.006, 0.006 and 0.06 or 0.6 % (or approximately 0.017. 1.7 and 17 mg/kg). Similar doses were used for the other beta 2 agonists. The duration of action was ranked as follows: QAB 149 ≥ salmeterol ≥formoterol>salbutamol. QAB149 had a duration of action up to 24 hours.

Pharmacology Summary:

Primary pharmacodynamics: The $ß_1$ and β_2 adrenoceptor receptor binding and functional activity of QAB149 were evaluated. The binding assays were performed against human $ß_1$ and β_2 adrenoceptor receptors overexpressed in insect cell membranes to evaluate potency and selectivity. The ability to stimulate cAMP production via human $ß_1$ and β_2 adrenergic receptors overexpressed in CHO-K1 cells were used to evaluate functional activity and selectivity of adrenergic receptors. The data are shown below:

Ki for various compounds at human beta1 and beta 2 adrenergic receptors

Compounds	Beta 1± SEM (nM)	Beta 2 ± SEM (nM)	Selectivity
Formoterol	646	9.6	68
Salmeterol	1035	4.0	259
QAB 149	180	15.9	11

These data show that the selectivity of QAB149 for human $ß_2$ adrenergic receptors compared to β1 receptors is greater than the affinity of Salmeterol and Formoterol for human $ß_2$ adrenergic agonists. Selectivity was calculated by dividing selectivity of the compound for beta 1 by the selectivity of beta 2.

The functional β_2 adrenergic receptor activity of QAB149 was determined by measuring cAMP creation. The EC_{50} for formoterol , salmeterol and QAB 149 are shown below:

EC 50 for CAMP Production from Human Beta 1 and Beta 2 Receptors Expressed in CHO-K1 Cells

Compounds	Beta 1± SEM (nM)	Beta 2 ± SEM (nM)	Selectivity
Formoterol	0.75	0.047	16
Salmeterol	28.3	0.025	1132
QAB 149	1.25	0.23	5.43

QAB 149 induced greater cAMP creation which should potentially result in greater bronchodilation than that induced by Salmeterol and Formoterol.

QAB 149 was demonstrated to be a bronchodilator with an extended duration of action which is proposed to be used once daily in the treatment of reversible bronchodilation of the pulmonary airway. Multiple studies demonstrated airway relaxant effects in guinea pigs following airway challenge.

Therefore, *in vivo* 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. One of the major side-effects with QAB149 is tachycardia. The mechanism of action for QAB149 involves the interaction with active sites of the receptor via the membrane lipid bilayer causing relaxation of smooth muscle. The lipid bilayer acts as a depot for B_2 adregergic 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 intereraction between the drug and the receptor membrane lipid bilayer.

II. SAFETY PHARMACOLOGY:

Safety pharmacology studies cross referenced in this IND were reviewed in IND 66,337. The summary of safety pharmacology is shown below:

Cardiovascular effects: 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, by approximately 25 %. The two lower concentrations did not inhibit HERG tail current. The frequency-dependence of QAB149 inhibition of HERG current was also 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.

Safety pharmacology conclusions: In an effort to determine whether QAB149 has the potential to prolong the QT interval in man, the effect of QAB149 on tail current from HEK293 cells stably transfected with HERG cDNA was assessed. QAB149, at a concentration of 5 μg/ml, inhibited HERG channels stably expressed in HEK293 cells. QAB149, at concentrations of 0.5 and 1.0 μg/ml did not inhibit HERG channels stably expressed in HEK293 cells. The Q-Tc values (normalized for heart rate) were increased in the dogs in the mid (0.12 mg/kg pulmonary deposited dose) and the high (0.23 mg/kg pulmonary deposited dose) doses in the 2 week inhalation toxicology study with micronized drug. On day 1, the 24 hour increases were 13 %

and 16%, respectively. On day 14, 8 hour increases were 11 % and 3%, respectively. The Q-Tc value was similar at the 24 hour evaluations on day 14. Tachycardia was observed at the mid and high doses also. The Q-Tc values of the dogs in the 4 week inhalation studies were similar. The effects of QAB 149 on the CNS and respiratory parameters were not investigated although no notable effects were observed in toxicology studies in rats and dogs of up to 28 days duration or in previously conducted clinical trials. The sponsor should be informed that the battery of safety pharmacology studies as described in ICH Guidance for Industry S7A should be completed.

III. PHARMACOKINETICS/TOXICOKINETICS:

The Pharmacokinetics/Toxicokinetic studies cross referenced in this IND were reviewed in IND 66,337. The summary of pharmacokinetics/toxicokinetics is shown below. Additionally, five additional studies are reviewed below.

New ADME Studies:

In study # RO101277 the absorption, distribution, metabolism and excretion of radiolabeled QAB149 were investigated in the CD/1 mouse. [^{3}H]QAB149 maleate was prepared as a solution in PEG 400:0.9% sterile saline and administered iv and orally. The single doses were 0.65 mg/kg. Results show absorption of QAB149 following oral dosing was 53-58% and the bioavailability was 1%. After iv dosing the elimination half-life of QAB 149 was 5.7 hours while radioactivity elimination half-life in plasma was 431 hours. QAB 149 clearance was 9.4 L/h/kg. There were 2 major circulating metabolites in the plasma, O and N –glucuronides of QAB149. The same metabolites were observed in the urine. In feces, 37-50 % of the collected material was unchanged QAB149 regardless of the route of administration. The amount of unchanged QAB 149 in urine was not reported. The excretion of QAB 149 in the feces after oral dosing was 63.1 %, after iv dosing was 68.9%. Urinary excretion after oral dosing was 17.4 % and 11.9 % after iv dosing.

In study # R030002 the metabolic parameters of QAB 149 in the CD-1 mouse was investigated. [^{3}H]QAB 149 was administered as a solution (PEG 400). The single doses were 10 or 100 mg/kg. The result of this study was similar to the results observed in study # RO101277, namely 2 circulating metabolites (O- and N-glucuronides) in mouse plasma. There was no change in metabolite pattern over the dose range used. The $AUC_{0\text{-}24h}$ after the 10 mg/kg was 23000 ngEq.h/mL and 216000 ngEq.h/mL after the 100 mg/kg dose. There was no apparent saturation of first pass metabolism.

In study # RO2-0220 the excretion of QAB149 in milk was studied in the pregnant HAN Wistar rat. [^{3}H}QAB 149 was administered as a solution (PEG 400). The single dose was 650 mg/kg QAB149 maleate was administered sc to the dams 8-9 days post partum. Plasma and milk samples were collected at 1, 2, 4, 8 and 24 hours after dosing. QAB149 was found in the milk, however at lower concentrations than in the plasma. The $AUC_{0\text{-}24\ h}$ in the milk was 502 ng.h/mL while the AUC in the plasma was 1440 ng.h/mL. The metabolites were similar in plasma and the milk. The metabolites include O-glucuronide and N-glucuronide in addition to unchanged QAB 149.

In study # R01-0990 the absorption, distribution, metabolism and excretion was investigated in the Beagle dog. [^{3}H]QAB 149 was administered in a saline solution iv and orally, single doses. Doses of 0.1 and 0.133 mg/kg (iv) and 0.1 mg/kg (oral) were included. Blood was collected at

time points from 0-96 hours, complete urine and feces was collected in 24 hour intervals up to 96 hours. The absorption in the dog after oral dosing was 72 % while bioavailability was approximately 33 %. The distribution of QAB 149 was highest in the kidney, liver and muscle. In the feces, unchanged QAB 149 was the major component after iv and oral dosing. The metabolites in the dog were O and N-glucuronides. QAB was eliminated slowly with renal and hepatic pathways playing a significant role. A total of 10 % radioactivity was found in the urine and 50-60 % in the feces regardless of the dose route.

Study title: QAB 149 Micronized Powder and HFA Formation: Comparative Single-Exposure Inhalation Study in Rats Using Micronized Powder and HFA Formulation of QAB 149

Key study findings: The objective of this single dose study was to compare the systemic exposure of inhaled micronized powder with QAB 149 HFA. The inhaled achieved doses were 3.07 mg/kg micronized powder and 3.57 mg/kg HFA formulation. The pulmonary deposited doses were 0.31 and 0.36 mg/kg, respectively. The results of the study show similar plasma drug concentrations and systemic bioavailablility.

Study no: 020073
Volume #, and page #: Volume 3, page 605
Conducting laboratory and location: Novartis Pharmaceuticals, East Hanover, NJ
Date of study initiation: October 1, 2002
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: QAB149 maleate/# 0121002/99.4% and QAB 149HFA/#TM-01-00046/99.4%

Methods (unique aspects)**:**
Dosing:

Species/strain: Rat, Wistar
#/sex/group or time point (main study): 10 males/dose group
Satellite groups used for toxicokinetics or recovery: None
Age: 8 weeks
Weight: 293-331 g
Doses in administered units: QAB149 powder 3.07 mg/kg or 0.31 mg/kg pulmonary deposited dose; QAB149 HFA-3.57 mg/kg or 0.36 mg/kg pulmonary deposited dose
Route, form, volume, and infusion rate: Inhalation, nose only/ 0.51.min^{-1} / based on analytical estimation, 75.8 % (group 1 and 51.5% (group 2) was less than 4.2 μM. The MMAD (±GSD) was 2.06 μM (±2.007) and 2.50 μM (±1.802) dose group 1 and 2, respectively.

Observations and times:

Clinical signs:Daily
Body weights: Weekly
Food consumption: NA
Ophthalmoscopy: NA

EKG: NA
Hematology: NA
Clinical chemistry: NA
Urinalysis: NA
Gross pathology: NA
Organs weighed: Lungs were collected at sacrificed and weighted.
Histopathology: NA
Toxicokinetics: Blood was collected at 1 and 6 hours after dosing from 5 rats and at 3 and 23 hours from the second 5 rats.

Results:

Mortality: There were no mortalities in this study.
Clinical signs: There were no clinical signs observed in this study.
Body weights: Body weights were similar in the two dose groups.
Food consumption:
Ophthalmoscopy:NA
Electrocardiography:NA
Hematology: NA
Clinical chemistry: NA
Urinalysis: NA
Organ weights:Mean lung weights sampled at 6 and 23 hours after dosing were 1.372 and 1.574 g for the micronized powder dosed rats and 1.329 and 1.479 g for the HFA formulation dosed rats.
Gross pathology: NA
Histopathology: NA
Toxicokinetics: Toxicokinetic results show that all rats were exposed to QAB 149. C max was reached within 1 hour with both formulations. The AUC for the micronized drug was 99.7 ng.h/ml while the AUC for the HFA formulation was 78 ng.h/ml. At all time points except one, the mean serum concentration was similar. Thus, there was no real difference in the systemic exposure values between the two formulations.

Summary of Pharmacokinetic/Toxicokinetics:

Absorption:

Following oral administration, 20-34% was absorbed in the rat and QAB149 was not bioavailability. Sc and intratracheal dosing resulted in approximately 100 and 78-90% absorption and 100 and 78 % bioavailability. In the dog, the absorption was 72 % while bioavailability was approximately 33% after oral dosing. In the mouse after oral and iv dosing, absorption was 53-58 % and the bioavailability was 1%.

Distribution:

Radioactive materials were distributed into most issues after iv and oral dosing except for the brain, spinal column, testes and lymph nodes. Five minutes after intratracheal dosing, most of the radioactivity material was present in the lungs. The highest concentrations were found in the stomach, kidney and liver in the rat and kidney and liver in the dog. Plasma protein binding ranged from 90.6% to 96.2% with greatest binding observed in rats.

Metabolism:

Metabolism was similar after iv, sc, oral and intratracheal dosing in the rat, mouse and dog. After oral dosing, QAB149 was subjected to a higher first pass metabolism which resulted in a higher metabolite to QAB149 ratio. There were 2 main metabolites in the rat, mouse, and dog plasma (O- and N-glucuronide), and O-glucuronide of QAB149 was the primary metabolite regardless of the route of administration. The main metabolic pathway was O-glucuronidation. In vitro studies with liver slices demonstrated a similar metabolic profile in rats, dogs and humans. No unique human metabolites were identified and no metabolism was identified with human lung slices.

Excretion:
The renal and hepatic pathways were the predominant elimination pathways regardless of the route of administration or animal species. Unchanged QAB149 was the main component in the feces. The recovery of the radioactivity material was nearly complete within 96 hours and ranged from 77.3-88.9 % in the rats. The summary of the ADME parameters in the rat is shown below:

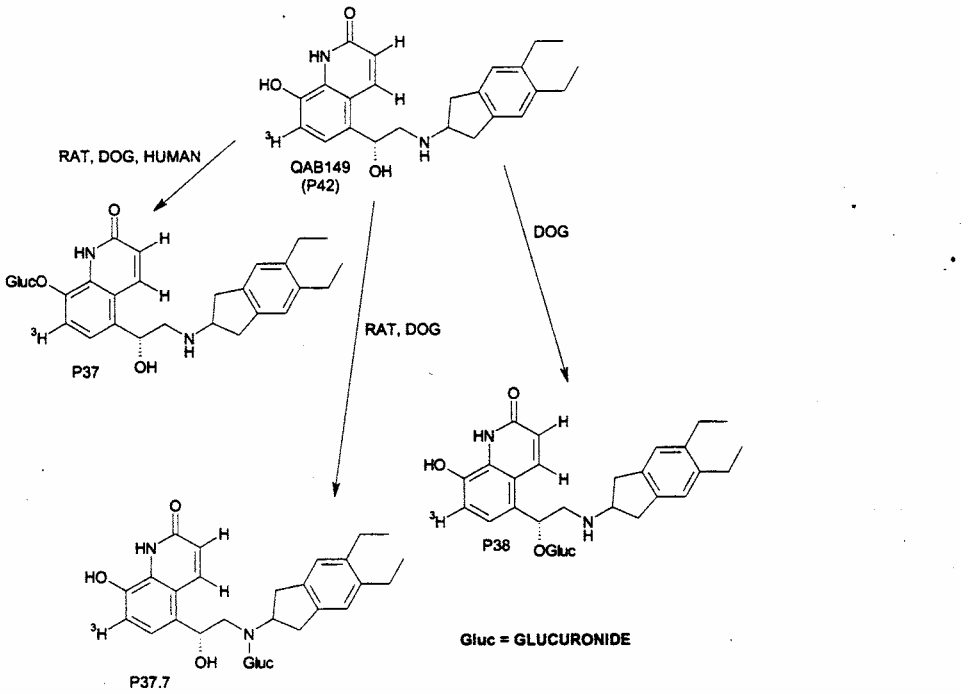
1 Summary

Species/strain/gender:	rat/HanWistar/male				
Route/formulation:	Intravenous solution	Intratracheal solution	Oral gavage solution	Subcutaneous solution	
Dose (mg/kg):	0.5	0.6	0.5	0.5	3
Specific activity (mCi/mg):	1.87	1.87	1.87	1.93	0.400
Samples collected:	Serial blood and plasma at various times from 0-96 h postdose from 3 (iv, po, sc) or 4 (intratracheal) rats; serial plasma collected from additional animals for metabolism analysis; complete urine and feces in 24 h intervals 0-96 h; cage wash and carcasses were collected at 96 h postdose. Complete urine, feces and bile was collected from three bile-duct cannulated animals for 72 h following an iv dose. Five animals were sacrificed at designated time points for tissue radioactivity analysis following iv and intratracheal doses.				
Samples analyzed:	Radioactivity was measured in all plasma, blood, excreta, carcasses and cage wash samples; radioactivity was assessed qualitatively in tissues following iv and intratracheal doses. Pooled plasma and selected, pooled urine and feces samples were assayed for unchanged QAB149 and metabolites. Selected urine samples were assayed for tritiated water.				
Plasma QAB149:					
C_{max} (ng/mL):	113[a]	51.6	-	16.7	57.8
t_{max} (h):	0.083[a]	0.5	-	1.0	3.0
$AUC_{0\text{-}96\ h}$ (ng•h/mL):	136	127	-	90.8	860
$t_{1/2,\ z}$ (h):	7.9	8.1	-	13	32
CL (L/h/kg):	3.7	-	-	-	-
V_{ss} (L/kg):	26	-	-	-	-
Blood [^{3}H]radioactivity					
C_{max} (ngEq/mL):	264 ± 3.06[a]	87.1 ± 64.5	18.8 ± 3.82	54.6 ± 5.43	157 ± 37.0
t_{max} (h):	0.083 ± 0[a]	1.1 ± 1.3	2.0 ± 0	1.5 ± 0.9	3.0 ± 1.0
$AUC_{0\text{-}48\ h}$ (ngEq•h/mL):	688 ± 58.1	410 ± 204	141 ± 23.6	666 ± 55.5	4120 ± 284
$t_{1/2,\ z}$ (h):	21	22	120	51	82
Plasma [^{3}H]radioactivity					
C_{max} (ngEq/mL):	191 ± 6.56[a]	46.0 ± 23.5	22.2 ± 8.19	30.2 ± 1.80	104 ± 26.5
t_{max} (h):	0.083 ± 0[a]	1.0 ± 1.3	2.3 ± 0.6	0.8 ± 0.3	5.0 ± 2.6
$AUC_{0\text{-}48\ h}$ (ngEq•h/mL):	559 ± 65.6	308 ± 105	142 ± 50.7	546 ± 41.9	3990 ± 662
$t_{1/2,\ z}$ (h):	46	21	43	44	60
Excretion in urine (%dose):					
Radioactivity,					
0-24 h:	4.64 ± 0.75	4.75 ± 1.84	2.06 ± 0.61	6.53 ± 0.84	6.66 ± 0.99
0-96 h:	6.69 ± 0.36	6.00 ± 2.43	2.29 ± 0.61	8.55 ± 0.88	8.98 ± 0.98
QAB149, 0-24 h:	1.7[b]	1.6	0.6	3.5	5.3
Excretion in feces (% dose):					
Radioactivity,					
0-24 h:	50.4 ± 5.34	59.5 ± 4.51	76.6 ± 2.97	44.4 ± 5.908	15.1 ± 22.2
0-96 h:	71.0 ± 2.17	79.2 ± 7.44	84.4 ± 3.70	66.0 ± 1.60	74.7 ± 3.14
QAB149, 0-48 h:	58[b]	66	75	56	70

[a]concentration at first sampling time, 0.083 h postdose
[b]in bile duct-cannulated animals, QAB149 was 4.5% of the dose in urine and only 0.52% of the dose in bile

After oral dosing, QAB149 was subject to first pass metabolism which resulted in plasma concentrations of QAB149 below the limit of detection of quantitation of the LC-MS/MS method in the rat. The metabolic pathway of QAB149 in the rat, dog and human liver slices is shown below.

Figure 3-3 Metabolic pathways of QAB149 in rat, dog and human liver slices



The primary route of excretion in all animal species including humans is the feces.

PK/TK conclusions: The *In vitro* metabolism of QAB149, evaluated using liver slices, is similar in rats, dog and humans, involving mainly phenolic O-glucuronidation. No major active metabolites were observed. These findings were confirmed by *in vivo* studies in the mouse, rat and dog. QAB 149 was not metabolized in lung tissues in studies in the human. Protein binding was similar in the rat, dog and human. QAB149 was distributed to most tissues, except the brain, spinal column and lymph nodes. The highest concentrations of QAB149 were found in the stomach, intestines, liver and kidney. Elimination of QAB149 in the rat, mouse and dog is mainly via the feces. In a single dose study in the rat to compare the systemic exposure of inhaled micronized powder QAB 149 with QAB 149 HFA inhaled doses, similar lung concentrations and systemic bioavailability were observed.

IV. GENERAL TOXICOLOGY:

The acute and subchronic studies referenced in this IND are reviewed in IND 66, 337. In this IND, there are three additional items, two subchronic studies in the mouse and a proposed 13 week bridging study in the dog.

Study title: 13 Week Inhalation Toxicity Study in Dogs (Proposed Bridging Study Protocol)

Key study findings: NA

Study no: 04200019
Volume #, and page #: NA
Conducting laboratory and location: (b) (4)
Date of study initiation: End of April, 2004
GLP compliance:NA
QA report: yes () no ( )
Drug, lot #, radiolabel, and % purity: HFA formulation vs micronized drug with lactose/NA formulation
Formulation/vehicle: Dry powder QAB 149 with lactose vs QAB 149/HFA

Methods (unique aspects)**:**
Dosing:

Species/strain:Dog, Beagle
#/sex/group or time point (main study): 3 F and 3 M
Satellite groups used for toxicokinetics or recovery: NA
Age: 9-10 months
Weight: Males: 7-9 kg and females: 6-8 kg
Doses in administered units:

Dose Group/Treatment	Target inhaled dose (mg/kg)*
Air control	0
Vehicle control	0
Low dose - lactose formulation	0.02
Intermediate dose - lactose formulation	0.1
High dose - lactose formulation	0.5
High dose (HFA formulation)	0.5

* The target doses refer to the free base of QAB 149 and the conversion factor for salt to base is 0.7718

Route, form, volume, and infusion rate: Inhalation via closed face mask fitted with a mouth tube, HFA and micronized drug with lactose/5 L.min-1

Observations and times:

Clinical signs: Evaluations for clinical signs will be made several times daily.
Body weights: Body weights will be recorded weekly
Food consumption: Food consumption will be recorded daily
Ophthalmoscopy: Examinations will be pretrial, weeks 7 and 13 of treatment
EKG: EKGs will be recorded pretrial and during 1, 7 and 13 weeks prior to dosing 0.5, 1, 3, 8 hours and 24 hours after dosing.

Hematology: Evaluations will be made pretrial, weeks 7 and 13
Clinical chemistry: Evaluations will be made pretrial, weeks 7 and 13
Urinalysis: Evaluations will be made pretrial, weeks 7 and 13
Gross pathology: Terminal
Organs weighed: Terminal
Histopathology: Full histopathology will be performed on all dogs in this study.
Toxicokinetics: Blood samples will be collected pretrial, weeks 7 and 13 at 0.5, 1, 3, 8 and 24 hours.
Other: The sponsor is planning to moderate the pharmacological effects of QAB149 of dose groups 5 (0.5 mg/kg micronized with lactose) and group 6 (QAB149/HFA) by administering an inhalation dose of 0.1 mg/kg for the first 4 days of the study. Only then will the dog be administered the proposed dose of 0.5 mg/kg. Blood samples will be collected for troponin analyses. The blood samples will be collected predose, weeks 2, 7 and 13.

Evaluation of protocol:
(1)It is recommended that the high dose in the study should be increased to 1.0 mg/kg (approximate pulmonary deposited dose of 0.25 mg/kg). QAB149 dry powder at an inhalation dose of 0.47 mg/kg induced cardiotoxicity (tachycardia and necrosis) in 1/6 dogs in a previous 2-week study with micronized drug. QAB 149 HFA at an inhalation dose of 0.97 mg/kg induced cardiotoxicity in 2/6 dogs in a 4-week study. The data in the escalating dose study in the dog also show cardiotoxicity in the one dog administered 1.0 mg/kg. In order to fully compare the toxicity profiles between the two formulations, doses should be selected to induce previously observed cardiac histopathology. Should a lower dose be selected, the bridging study will only provide support for clinical development of the lactose formulation up to the high dose of the bridging study. (2) Secondly, blood samples for troponin analyses should be collect at C_{max}, if possible or 3-6 hours after dosing in addition to predose. Troponin is rapidly metabolized and may be below the level of quantification if collected only at predose. The blood collection for troponin analysis should be during week 1 and, if possible, after the initial dose. (3) EKGs should also be recorded at C_{max}. (4) The doses in the high dose groups, i.e., micronized drug with lactose and drug with HFA, should not be gradually increased. The dogs should be administered the targeted high dose beginning on day 1 of the study. And finally, (5) the final clinical formulation of micronized powder drug and lactose should be used in the study.

Study title: One Week Feasibility (gavage) Study in Mice

Key study findings: The objective of the study was to determine absorption after oral administration, to estimate the toxicokinetic profile, to assess the suitability of oral route for subsequent studies and to provide date for dose selection for potential subsequent GLP studies. QAB149 was administered to CD-1 mice orally using doses of 0, 100, 250 and 500 mg/k kg base or 130, 324 and 648 mg/kg salt daily for 8 days. Increased body weight gain vs controls was observed in the female mice in the 500 mg/kg dose group on day 4. On day 7, increased body weight gain was observed in the males in the 500 mg/kg dose group and the females in the 100 mg/kg dose group. Food consumption was decreased in the males in the 100 mg/kg dose group vs controls, days 4-7. The effects were statistically significant. Microscopic analyses of the esophagus show foci of inflammation or fibrosis of treated and control animals, mainly involving the muscularis. The lesions may be due to mechanical trauma during the gavage procedure but

did increase in incidence compared to controls in the mid and high dose groups. A NOAEL was not identified due to the nature of the study.

Study no: 0370021
Volume #, and page #: Volume 5, page 957
Conducting laboratory and location: Novartis Pharmaceuticals, East Hanover, NJ

Date of study initiation: January 15, 2003
GLP compliance: No
QA report: yes (x) no ( ), signed
Drug, lot #, radiolabel, and % purity: QAB 149 maleate/#0121002/99.1%
Formulation/vehicle: QAB 149/ 0.5 % (w/v) hydroxypropylcellulose

Methods (unique aspects)**:** The mice were administered drug for 1 or 7 days.
Dosing:

Species/strain: Mouse (Crl:CD-1® (ICb)(BR)
#/sex/group or time point (main study):10/sex/dose group
Satellite groups used for toxicokinetics or recovery: None
Age: 7-8 weeks
Weight: 21.7-36.2
Doses in administered units:

Dose groups	Number of Doses	Doses (mg/kg)
Control	Single dose	0
Control	Multiple doses	0
Low	Single dose	100
Low	Multiple	100
Mid	Single	250
Mid	Multiple dose	250
High	Single	500
High	Multiple dose	500

Route, form, volume, and infusion rate: Oral, gavage

Observations and times:

Clinical signs: Twice daily
Body weights: Pretrial, days 1, 4 and 7
Food consumption: Days 1, 4 and 7
Ophthalmoscopy: NA
EKG: NA
Hematology: NA
Clinical chemistry: NA
Urinalysis: NA
Gross pathology: at termination
Organs weighed: At termination
Histopathology: The following tissues were collected for histology: cecum, colon, duodenum, esophagus, ileum, jejunum, liver, rectum and stomach.

Toxicokinetics: Blood samples were collected at 0.5, 1, 3, 8 and 24 hours after the first dose from 2/sex/group and on day 8after dosing at the same time points.

Results:

Mortality: There were no mortalities in this study.

Clinical signs: There were no drug-related clinical signs in this study.

Body weights: The female mice in the 500 mg/kg dose group (multiple doses) gained 50 % more body weight than the control mice on day 4. The increases were statistically significant and drug-related. There was increased body weight gain in the males in the 500 mg/kg dose and the females in the 100 mg/kg dose group on day 7 compared with the controls. The increases were 9 and 60%, respectively and were statistically significant and drug-related.

Food consumption: There was statistically significant, drug-related decreases in food consumption in the males in the 500 mg/kg dose group compared with the control group, day 1-4. The decrease was approximately 12 %. There were also decreases in the food consumption in the males in the 100 and 250 mg/kg dose groups compared to controls, days 1-4. The non-statistically significant decreases were 5 and 9 %, respectively. There were statistically significant increases in the males in 100, 250 and 500 mg/kg dose groups compared with controls on days 4-7. The increases were 15, 15 and 25 %, respectively. Food consumption was similar in the females in all dose groups in this study.

Ophthalmoscopy: NA

Electrocardiography: NA

Hematology:NA

Clinical chemistry: NA

Urinalysis: NA

Organ weights: NA

Gross pathology: There were no drug-related macroscopic observations at necropsy.

Histopathology: There were no microscopic findings that were considered to be definitively related to QAB 149. Foci of inflammation or fibrosis occurred in the esophagus in 2/20 controls, 4/20 in the 250 mg/kg dose group and 7/20 mg/kg in the 500 mg/kg. The sponsor states that the lesions were considered to be related to local mechanical trauma sustained during gavage and were not drug-related. However, there seems to be some drug-related effect at the mid and high doses since the incidence increased with dose when compared to control levels. If the inflammation was due solely to gavage trauma, the numbers of animals in each dose group should be similar and involve all dose groups.

Toxicokinetics: The PK parameters of QAB149 in male and female mice are shown below:

Dose (mg/kg/day)	Group	[a]$AUC_{(0-24h)}$ (ng.h/mL) Day 1/2	[a]$AUC_{(0-24h)}$ (ng.h/mL) Day 8/9	[a]C_{max} (ng/mL) Day 1/2	[a]C_{max} (ng/mL) Day 8/9	[b]T_{max} (h) Day 1/2	[b]T_{max} (h) Day 8/9
Males							
100	2	446 ± 44.3	946 ± 33.7	73.9	300	1	1
250	3	1250 ± 534	2010 ± 551	80.0	279	8	1
500	4	1360 ± 387	3010 ± 611	111	557	3	1
Females							
100	2	670 ± 195	1100 ± 338	69.6	207	1	1
250	3	1910 ± 964	1390 ± 205	148	218	8	1
500	4	1700 ± 208	2580 ± 890	173	425	3	1

[a]Values are mean and, if indicated, ± SE.

[b]Values are median.

Exposure increased with multiple dosing compared to day 1/2 with the exception of the mid dose females. QAB 149 exposure increased in a sub- proportional manner in both males and females. QAB149 was also found in 6/40 control samples. The detected concentrations varied from 0.0879 ng/ml to 6.80 ng/ml. The reason for the contamination is unknown. We noted that QAB 149 has been found in control samples of toxicokinetic analyses in several toxicology studies. The sponsor should identify the source of the drug contamination in control samples in this and other toxicology studies.

Summary of individual study findings: QAB 149 was administered orally to CD-1 mice daily using dosing of 0, 100, 250 and 500 mg/kg for 8 days. Some other mice receive single doses of 100, 250 and 500 mg/kg. Relevant data concerning the mice in the single dose groups were not provided. In the mice that received multi doses there was increased body weight gain vs controls in the female mice in the 500 mg/kg dose group on day 4. On day 7, increased body weight gain was observed in the males in the 500 mg/kg dose group and the females in the 100 mg/kg dose group. Food consumption was decreased in the males in the 100 mg/kg dose group vs controls, days 4-7. Heart tissues were not included in the histopathology analyses. In the tissues examined microscopically, there were foci of inflammation in the esophagus, possibly drug-related, in the mice in the control, mid and high dose groups. The number of mice affected increased with dose, however, the mice in the 100 mg/kg dose group had no findings in their esophagus. In toxicokinetic studies QAB 149 exposure increased in a sub- proportional manner in both males and females. Exposures were generally greater on days 8/9 than on days 1/2. QAB149 was found in 6/40 control plasma samples. The detected concentrations varied from 0.0879 ng/ml to 6.80 ng/ml. The reason for the contamination is unknown but should be clarified by the sponsor.

Study title: 2 Week Inhalation Dose Ranging Finding (Dry Powder Aerosol Toxicity Study in Mice

Key study findings: The objective of the study was to determine doses for subsequent toxicity studies. CD-1 mouse were administered QAB 149 by inhalation daily, nose only for 14 days. The doses were 0, 1.07, 3.23, 9.91 and 30.76 mg/kg achieved doses or 0.1, 0.3, 1.0 and 3.1 mg/kg pulmonary deposited doses. The results of this study reveal lethality at the highest dose, and drug-related decreases in body weight gain and increases in lung weight. Microscopic evaluations revealed changes in the lungs and the nasal cavity in the 3.1 mg/kg mice as well as nasal changes in the mice in the 1.0 mg/kg dose group. The changes consisted of hydrotrophic alveolar macrophages in the lungs and focal olfactory epithelium atrophy, squamous metaplasia of the epithelium in the ventral epiglottis. The NOAEL could not be determined due to limited histopathology assessment; the MTD appears to be ~ 1 mg/kg (pulmonary deposited dose).

Study no: 022007
Volume #, and page #: Volume5, page 1063
Conducting laboratory and location: Novartis Pharmaceuticals, East Hanover, NJ
Date of study initiation: march 15, 2002
GLP compliance: No
QA report: yes (x) no ( ), signed
Drug, lot #, radiolabel, and % purity: QAB 149 maleate/0121002/99.5
Formulation/vehicle: QAB149 dry powder

Methods (unique aspects)**:**
Dosing:

Species/strain: Mouse, CD-1
#/sex/group or time point (main study): 5/sex/dose group
Satellite groups used for toxicokinetics or recovery: 10/sex/dose group
Age: 7 weeks
Weight: Males-25.8-33.7g: females; 17.8-30.9g
Doses in administered units:

Dose Groups	Achieved Doses/Pulmonary Deposited Doses (mg/kg)
Air control	0
Low	1.07/0.1
Mid	3.23/0.3
Mid	9.91/1.0
High	30.76/3.1

(b) (4)

Achieved doses were estimated using the following criteria:

$$\text{Dose (mg/kg)} = \frac{MV \times T \times CC}{BW}$$

MV= Minute volume (1) = $\frac{2.10 \times \text{body weight (g)}^{0.75}}{}$ [Guyton AC (1947)]

$$\frac{T \times CC \times BW}{1000}$$

T = Duration of exposure in minutes
CC = Chamber concentration of actual drug ($mg.1^{-1}$)
BW = mid-week body weight (expressed in kg-mean of males and females was calculated separately).

Observations and times:

Clinical signs: Once daily
Body weights: Once weekly
Food consumption: Once weekly
Ophthalmoscopy: NA
EKG: NA
Hematology: Day 15/ 2/sex/dose group
Clinical chemistry: Day 15/2/sex/dose group
Urinalysis:NA
Gross pathology: Terminal

Organs weighed: The following organs were weighted at termination: adrenals, brain, epididymis, heart, kidneys, liver, gall bladder, lungs, ovaries, prostate, spleen, submaxillary salivary glands, testes, thymus and uterus.

Histopathology: The following tissues were collected from all mice at termination for microscopic analyses: abnormal tissues, heart, kidney, liver, gall bladder, bronchial lymph, cervical lymph node, larynx, lungs, nasal cavity, pharynx, and trachea, (anterior and posterior). All tissues were microscopically evaluated in the control and high dose group. The respiratory tissues from all dose groups were evaluated microscopically.

Toxicokinetics: Blood was collected on days 14/15 at 0.5, 1, 3, 8 and 24 hours after dosing.

Results:

Mortality: There was 1 death; one male mouse in the high dose group was found dead on day 10 due to respiratory difficulties.

Clinical signs: There was labored respiration, staggering behavior and unsteady gait in the mice in the high dose group (10/10).

Body weights: Body weight gain deceases were observed in the mice in the high dose group compared with the controls. The decreases were 93% in the males and 98% in the females. The decreases were statistically significant in the males. Body weight gain in the control and 0.1, 0.3 and 1.0 mg/kg dose groups was similar.

Food consumption: Food consumption was decreased in all dose groups including the controls. In the males, the decreases were 45, 23, 29, 24, and 32% comparing pretrial food consumption with 14 day food consumption. In the females, the decreases were 15, 12, 4, 15 and 20 %. There does not appear to be a significant drug-relatedness to the decrease but there may be an effect of treatment.

Ophthalmoscopy: NA
Electrocardiography: NA
Hematology: There were no drug-related changes in hematology parameters.
Clinical chemistry: There were no drug-related changes in clinical chemistry parameters.
Urinalysis: NA

Organ weights: There were increased relative lung weights in the males in the 0.3 and 3.1 mg/kg males compared with controls. The increases were 11 and 20 % in the 0.3 and 3.1 mg/kg dose groups. The increase was statistically significant in the high dose males. There were also increased relative lung weights in the females in all dose groups compared with the controls. The increases were 20, 13, 20 and 33 % in the 0.1, 0.3, 1.0 and 3.1 mg/kg dose groups. The increases were statistically significant. The absolute and relative weight of the thymus was increased in the females in the 0.1, 0.3 and 1.0 mg/kg dose groups compared with the controls. The increases were 20, 60 and 46% for the absolute and 19, 58 and 42 % for the relative thymus weights. These increases were significant. There were also statistically significant relative and absolute increases in the uterus weights. The increases were 133, 177, 122 and 56 % for both relative and absolute weights in the 0.1, 0.3, 1.0 and 3.1 mg/kg dose groups.

Gross pathology: There was no remarkable gross pathology in this study.

Histopathology:

Nasal tissues: marked focal olfactory epithelial atrophy was found in 2/5 males in the 1.0 mg/kg dose group and 10/10 mice in the 3.1 mg/kg dose group. The change was characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of the epithelium.

Lungs: Multifocal minimal accumulation of alveolar macrophages was seen in 4/5 males and 3/5 females in the high dose group. The macrophages were in the broncho-alveolar junction (centroacinar regions) and were not associated with anti inflammatory response. This finding was statistically significant in the males with a p value of less than 0.05.

Larynx: Focal minimal squamous metaplasia was observed in the lining of the ventral floor at the base of the epiglottis, cranial to the ventral laryngeal diverticulum. This finding was present in 4/4 males and 5/5 females in the 1.0 mg/kg dose group as well as 3/5 males and 5/5 females in the high dose group. This finding was statistically significant in the females.

Toxicokinetics: the mean TK parameters of QAB149 in the mouse are shown below.

Dose Pulmonary *Deposited Dose	AUC ng.h/ml	C max ng/ml	Tmax (h)
0.1	59 (M) 59.5 (F)	22.4 (M) 12 (F)	0.1 (M0 0.1 (F)
0.3	195 (M 132 (F)	58.0 (M) 39.1 (F)	0.32 (M) 1.32 (F)
1.0	311 (M) 388 (F)	120 (M) 85.9 (F)	0.96 (M) 0.96 (F)
3.1	-** 546 (F)	450 (M) 166 (F)	0.98 (M) 1.48 (F)

* Dose reported as the free base

** AUC not determined

QAB 149 was found in the control samples (slightly above quantification) in 2 extra animals prior to the beginning of the study. There was no contamination in the control samples in the actual study. The source of the contamination is unknown. Measurable concentrations of QAB 149 were found in the plasma of mice for 24 hours. Exposures in the mouse were approximately linear up to a dose of 1 mg/kg but were sub-proportional at doses greater than 1 mg/kg. T_{max} was within 0.1-1.5 hours.

Summary of individual study findings: The objective of the study was to determine doses for subsequent toxicity studies. CD-1 mouse were administered QAB 149 by inhalation daily, nose only for 14 days. The doses were 0, 1.07, 3.23, 9.91 mg/kg and 30.76 achieved doses or 0.1, 0.3, 1.0 and 3.1 mg/kg pulmonary deposited doses. One male mouse died due to respiratory difficulties in the high dose group. There were also decreases in body weight gain in the mice in the high dose group. Microscopic evaluations revealed changes in the lungs and the nasal cavity in the 3.1 mg/kg mice as well as nasal changes in the mice in the 1.0 mg/kg dose group. The changes consisted of hydrotrophic alveolar macrophages in the lungs and focal olfactory epithelium atrophy, and squamous metaplasia of the epithelium in the ventral epiglottis. An associated increase in lung weight was also noted. The MTD appears to be 1.0 mg/kg; a NOAEL could not be identified since a complete histologic evaluation was not conducted. The sponsor suggests that the NOAEL is 3.1 mg/kg based on a conclusion that the lung and nasal cavity findings are expected in inhalation studies in rodents.

Toxicology summary: There were two subchronic studies carried out in the CD-1 mouse. In a one week study, QAB 149 was administered daily orally for 8 days. The objective of the study was to assess the suitability of oral route for subsequent studies and to provide date for dose selection for potential subsequent GLP studies. The doses were 0, 100, 250 and 500 mg/ kg base or 130, 324 and 648 mg/kg salt daily for 8 days. Increased body weight gain vs controls was observed in the mice in the 100 mg/kg (females) and 500 mg/kg dose groups. Males demonstrated a decrease in food consumption vs controls. A dose-related increase in esophageal inflammation was observed but may be due to the gavage procedure. In a 14 day inhalation study in the mouse, pulmonary deposited doses were 0, 0.1, 0.3, 1.0 and 3.1 mg/kg. The results reveal 1 dead male mouse due to respiratory difficulties in the high dose group. There were also decreases in body weight gain in the mice in the high dose group. Limited microscopic evaluations revealed changes in the lungs and the nasal cavity in the 3.1 mg/kg mice as well as nasal changes in the mice in the 1.0 mg/kg dose group. The changes consisted of hydrotrophic alveolar macrophages in the lungs and focal olfactory epithelium atrophy, squamous metaplasia of the epithelium in the ventral epiglottis. The MTD appears to be ~ 1 mg/kg (pulmonary deposited dose); a NOAEL could not be determined due to limited histologic evaluations.

The sponsor also submitted a 13-week inhalation study protocol in dogs to support their bridging program for the lactose formulation to the HFA formulation. This bridging program was a topic of pre-IND discussions. Overall, the protocol is adequate in terms of methodology although the sponsor is recommended to increase the administered doses, alter the timing of EKG and troponin assessments, to delete the gradual dose-escalation scheme for the high-dose group and to use the final clinical formulation.

V. GENETIC TOXICOLOGY:

The genotoxic studies referenced in this IND were received in IND 66, 337. A summary of these studies is shown below:

Genetic toxicology summary**:** The sponsor conducted *in vitro* assays, Ames bacterial reverse mutation test and Mammalian Chromosome Aberration test in the V79 Chinese hamster cell and an *in vivo* micronucleus study in order to evaluate the genotoxic potential of QAB149. In the Ames assay, Salmonella typhimurium strains TA 1535, TA97a, TA98, TA100 and TA102 were used. QAB149 was dissolved in DMSO and used at concentrations 1.6 to 1000 mcg/plate. Treatment with QAB149 did not increase the revertant numbers of any of the bacterial tester strains used. QAB149 did not show evidence of mutagenic activity under the experimental conditions in the chromosomal assay with V79 hamsters. QAB149 was used at doses of 10-32.4 mcg/ml without metabolic activation and 30- 171 mcg/ml with metabolic activation. There was no increase in frequency of polyploid cells with and without activation. QAB149 did not induce chromosome aberrations in cultured V79 Chinese hamster cells. In the rat bone marrow micronucleus test, QAB149 was administered using sc doses of 200 -2000 mg/kg. QAB149 did not induce micronuclei in the bone marrow cells of rats up to the highest dose tested (2000 mg/kg). These results indicate that QAB149 has no clastogenic and/or aneugenic potential *in vivo* under the conditions tested. Thus, QAB149 demonstrated no genotoxic activity in a standard battery of assays under the conditions tested.

VI. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Reproductive development studies referenced in this IND were reviewed in IND 66, 337. A fertility and early embryonic development study was submitted in this IND and is reviewed below:

Study title: A subcutaneous (bid) Fertility and Early Embryonic Development Study in Rats

Key study findings: QAB149 dissolved in PEG 400 was administered to male and female Wistar rats prior to mating, during the mating period and through gestation day 6 or until termination (males). Results of this study reveal minor decreases in the relative weight of testes and decrease in the relative and absolute weight of epididymis weight in the males in the 0.2 mg/kg dose group and higher. There were no detectable effects on reproductive potential and histological evaluations found no article-related change in the testes or epididymis. There was drug-related local toxicity, i.e., discoloration at the injection sites in the male rats at 0.6 mg/kg and hair loss, scabs/wounds/cuts in the males and females in the 2 mg/kg dose group. Additionally, there were skin lesions in the rats in the 0.6 and 2.0 mg/kg dose group. One of the male rats in this dose group was sacrificed because of skin lesions. Systemic effects include increases in body weight parameters and food consumption in the males in all treated dose groups. There were also increases in the body weight parameters and food consumption in the females in all dose groups during premating; body weights remained increased during the gestation period. There were no effects in males or females related to parameters of fertility, general reproductive performance or early development. The NOAEL was 2 mg/kg for fertility and early embryonic development. There was no NOAEL (< 0.2 mg/kg) for male and maternal toxicity because of local, i.e., cuts, scabs/skin lesions at the injection sites, and systemic toxicity, i.e., increased body weight parameters and food consumption, at all doses.

Study no.: 0270074

Volume #, and page #: Volume 7, page 1
Conducting laboratory and location: Novartis Pharmaceuticals, East Hanover, NJ
Date of study initiation: Males-May 7, 2002 and females-May 20, 2002
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: QAD 149 maleate/# 0121002/99.4%
Formulation/vehicle: QAB149 was dissolved in PEG 400/0.95 sodium chloride 20:80 (v/v)/ PEG 400

Methods:

Species/strain: Rat, Wistar Hannover Crl:WI (Glx/BRL/HAN)IGS BR; ~ 11 weeks old
Doses employed:

Group	**Doses (mg/kg/day) Salt/base**
Control	0
Low	0.26/0.2
Mid	0.78/0.6
High	2.59/2.0

Route of administration: Subcutaneous
Study design: The rats in the study were injected twice daily, approximately 6 hours apart. The total daily dose volume was 4 ml/kg administered as two injections per day, 2 mL/kg/injection. The report states that the subcutaneous route should provide an exposure equivalent to inhalation, the anticipated clinical route. The rats were injected in the anterior portion of the back one day and the posterior portion of the back on the next. The males were administered QAB 149 for at least 28 days prior to mating, during the two week mating period and until terminal necropsy. The females were administered QAB 149 for 2 week before mating, during mating period and until day 6 of gestation. There was 1 male and 1 female in each cage. Once mating occurred, the female was removed. Males were sacrificed following completion of mating and sperm positive females were sacrificed on day 13 of gestation.
Number/sex/group: 25/sex/dose group
Parameters and endpoints evaluated: Mortality, clinical signs, body weight, food consumption, vaginal cytology, estrous cycle, gross and histolopathology (testes and epididymis from control and high dose), toxicokinetics after at least 2 weeks dosing and prior to cohousing. Reproductive parameters include: males- right and left epididymis and testes were weighed, examined histologically and % motile sperm assessed; females- # of corpora lutea recorded, uterine site (live fetus or early resorption).

Results:

Mortality: There were no unscheduled deaths in this study. However, one male rat in the 2 mg/kg dose group was sacrificed early on day 39 due to severe skin lesions.
Clinical signs: The male rats in the 2.0 mg/kg dose group as well as the females in the 0.6 and 2.0 mg/kg had drug-related discolorations, scabs/cuts/wounds in the region of the injection sites.

Body weight: Mean body weight was increased in all male dose groups when compared to control values. The increases were significantly increased at the low and mid doses from day 8 onward and day 50 in the high dose group. On day 50, the increases were 8%, 11% and 5% in the low, mid and high dose groups, respectively. There were also increases in body weight gain in the males in all dose groups compared with the controls, day 52. The increases were 77, 100 and 25% in the 0.2, 0.6 and 2.0 mg/kg, respectively. The primary increases were observed through the first 22 days of dosing with significant increase versus control values noted in all dose groups.

Summary of mean body weight for the male rats is shown below.

Day of Study	**Controls**	**0.2 mg/kg**	**0.6 mg/kg**	**2.0 mg/kg**
Day 1	345	346	345	344
Day 8	358	376	375	355
Day 15	371	398	402	380
Day 22	381	414	418	394
Day 29	391	423	429	400
Day 36	397	428	435	409
Day 43	404	440	447	419
Day 50	405	439	450	426
Day 52	393	431	441	404

Before mating, there was a dose-related, statistically significant increase in body weight gain in females in all dose groups compared with the controls. The increases were 73, 84 and 100 %. The summary of the mean body weight for females before mating is shown below.

Day of Study	**Controls**	**0.2 mg/kg**	**0.6 mg/kg**	**2.0 mg/kg**
Day 1	206	207	206	206
Day 8	216	255	227	228
Day 15	219	231	234	237

During gestation days 0-13, overall body weight gain was comparable among groups (47-51 g) although mean body weights continued to be increased in the drug treatment groups. The increases in absolute body weight versus the control value were 4, 5 and 8% in the 0.2, 0.6 and 2.0 mg/kg dose groups, respectively, on day 13. The summary of mean body weight in females during gestation is shown below:

Day of Study	**Controls**	**0.2 mg/kg**	**0.6 mg/kg**	**2.0 mg/kg**
Day 1	226	239	243	251
Day 3	241	257	258	263
Day 6	252	266	268	274
Day 9	259	274	277	282
Day 13	277	289	292	298

Food consumption: Food consumption for the males was calculated on treatment days 1, 8, 15 and 29 and in the females, treatment days, 1, 8 and 15 and gestation days 0, 3, 6, 9 and 13. There were drug-related increases in food consumption in the males in all dose groups compared with the control males except days 1-8 at 0.6 and 2.0 mg/kg. On days 1-

8, the food consumption in the males in the 0.6 mg/kg dose group and the control was similar. On days 1-8, food consumption was decreased in the males in the 2.0 mg/kg dose group. The statistically significant decrease was 17%. On the days 8-29, the increases were 8, 12 and 8 % in the low, mid and high dose males, respectively. Premating, there were drug related and statistically significant increases in food consumption in all the females in this study compared with the controls, days 8-15. The increases were 11, 11, and 16 % 0.2, 0.6 and 2.0 mg/kg dose groups, respectively. During gestation, food consumption increases were observed in the females in all dose groups, days 0-13. The increases were 4, 4 and 8% (1-3 g/animal/day) in the low, mid and high dose females.

Gross pathology: Only local effects were noted and included an increased incidence of discoloration of the subcutaneous aspect of the skin in the region of the injection and underlying muscle in mid dose (males only) and high-dose animals.

Toxicokinetics:
Fourteen days toxicokinetic parameters of QAB149 in male and female rats are shown below :

Parameters	**Doses (mg/kg)***		
Males	**0.2**	**0.6**	**2.0**
$AUC_{(0-24),}$ ng.h/ml	135 ± 4.70	315 ± 11.4	921 ± 56.9
C_{max}, ng/ml	10.6	22.9	60.0
T_{max}, h	7	7	7
Females			
$AUC_{(0-24),}$ ng.h/ml	96.4 ± 3.78	258 ± 18.1	694 ±32.2
C_{max}, ng/ml	7.72	17.5	45.9
T_{max}, h	7	7	7

* Dose is in terms of base.
QAB 149 was measurable in the plasma of the treated rats for 24 hours after dosing. The $AUCs_{(0-24h)}$ were 22-40% higher in the males than in the females. Exposure in the males and females was slightly sub-proportional with increasing dose. There was no QAB 149 detected in the control samples.

For fertility studies:

Vaginal cytology and mating indices: There were no drug-related effects on estrus cycle based on the evaluation of vaginal cytology or parameters of reproduction potential investigated including precoitus interval, mating index, pregnancy rate/fecundity and fertility (number of females pregnant/number of females co-housed.

Male reproductive parameters: There were no drug-related effects on sperm counts or percent mobility. In addition, there were no drug effects on the parameters of reproduction potential, shared by males and females, namely, precoitus interval, mating index and pregnancy rate. Relative to concurrent controls there were statistically significant though mild decreases in relative weights of testes and the relative and absolute weights of the epididymis in the males in all dose groups. The decreases in the testes were 7, 11 and 8 % in the low, mid and high dose males. The deceases in relative epididymis weights were 6, 5 and 11% in the low, mid and high dose males, respectively, while the decrease in absolute epididymis weights were 14% in all

groups. There were no significant histological findings in these organs. Based on the absence of drug-related histological alternations, effects on sperm count or percent motility and/or effect on parameters of reproductive potential, the decreases in testes and epididymis weights has no toxicological significance.

Female reproductive parameters: There was no QAB 149-related effect on the number of corpora lutea, preimplantation loss or implantation sites. There was a statistically significant increase in the number of corpora lutea per dam at 0.6 mg/kg (12.9 vs 11.1 in controls). This finding was not considered to be drug-related because of a lack of dose association. There was also an increase in the number of early and total resorptions at 0.2 mg/kg and above. The increases in early and total resorptions versus control values were 183, 216 and 125 % in the low, mid and high dose groups. These findings were not considered to be drug-related because there were no remarkable differences in viable percent fetuses or mean number of fetuses per litter.

Summary of individual study findings:
QAB 149 was administered to male and female rats subcutaneously in this fertility and early embryonic development study. The daily doses were 0, 0.2, 0.6 and 2.0 mg/kg. The males were administered QAB 149 for at least 28 days prior to mating, during the two week mating period and until terminal necropsy. The female were administered QAB 149 for 2 week before mating, during mating until day of gestation. Results of this study reveal mild decreases in the relative weight of testes and decrease in the relative and absolute weight of epididymis weight in the males in the 0.2 mg/kg dose group and higher. There were no detectable effects on reproductive potential and histological evaluations found no article-related change in the testes or epididymis. There was drug-related local toxicity, i.e., discoloration at the injection sites in the male rats at 0.6 mg/kg and hair loss, scabs/wounds/cuts in the males and females in the 2 mg/kg dose group. Additionally, there were skin lesions in the male rats in the 2.0 mg/kg dose group. One of the male rats in this dose group was sacrificed early because of skin lesions. Systemic effects include increases in body weight parameters and food consumption in the males in all treated dose groups. There were also increases in the body weight parameters and food consumption in the females in all dose groups during premating; body weights continued to be increased during the gestation period. There were no effects in males or females related to parameters of fertility, general reproductive performance or early development. The NOAEL was 2 mg/kg for fertility and early embryonic development. There was no NOAEL for male and maternal toxicity because of local, i.e., cuts/scabs/skin lesions and systemic toxicity, i.e., increased body weight parameter changes and increased food consumption.

VII. SPECIAL TOXICOLOGY STUDIES:

Study title: Buehler Test in Guinea Pig for Delayed Skin Sensitization Potential

Key study findings: Topical QAB 149 was applied to the back of shaved guinea pigs via a patch covered with aluminum foil for 6 hours. The dose was 250 mg.ml-1 free base. Each guinea pig received 3 consecutive weekly applications. The animals were then challenged with a topical application of QAB 149. QAB 149 was not a sensitizer in the guinea pig.

Study no: 0220082
Volume #, and page #: Volume 8, page 2197
Conducting laboratory and location: (b) (4)
Date of study initiation: November 7, 2002
GLP compliance: Yes
QA reports: yes (x) no ( ):
Drug, lot #, radiolabel, and % purity: QAB 149 maleate/0121002/99.1%
Formulation/vehicle: QAB 149/corn oil

Methods:
Dosing: QAB 149 dissolved in corn oil was applied topically to the back of shaved Dunkin-Hartley guinea pigs via a patch covered with aluminum foil for 6 hours. The dose was 250 mg.ml-1 free base. The volume was 0.5 ml (125 mg total dose). Each guinea pig received 3 consecutive weekly applications. Two weeks later, the animals were then challenged with a topical application of QAB 149.

Observations and times: The guinea pigs were checked twice daily, morning and afternoon for duration of the study. The guinea pigs were evaluated for clinical signs, the test sites were checked for skin reactions. The body weight of the guinea pigs was evaluated at the beginning and at the end of the study. The strain of guinea pig was checked for sensitivity using a mild/moderate sensitizer, hexylcinnamicaldehyde (HCA) at 6 month intervals. The vehicle for the positive control was acetone:PEG 400 (70:30, v/v).

Results: There were no clinical signs in the study. Body weight gain was similar and satisfactory in the control and treatment group. Following the challenge, no positive responses were observed in the test and control groups. QAB149, at a concentration of 250 mg/ml was not a sensitizer in the guinea pig. Following challenge with HCA, test guinea pigs reacted positively.

Summary of individual study findings: Topical QAB 149 was applied to the back of shaved guinea pigs via a patch covered with aluminum foil for 6 hours. The concentration was 250 mg.ml-1 free base. Each guinea pig received 3 consecutive weekly applications. The animals were then challenged with a topical application of QAB 149. QAB149 was not a sensitizer in the guinea pig under the conditions of the study.

VIII. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: QAB149 is a long acting β_2 agonist intended for once a day treatment of asthma/COPD. The drug will be administered by inhalation. The sponsor is planning to develop 2 formulations, an HFA formulation (I66, 337) and a micronized powder formulation (I 48,649). The target organ for this drug is classically the cardiovascular system, i.e., tachycardia, QT-c interval changes and myocardial necrosis. Increased cardiovascular activity with associated histopathological changes was observed in the dog. There was also glycogen deposition in the liver of the dog at all dose levels in repeat dose toxicity studies. The dose-limiting toxicity in the rat is nasal lesions, characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium. QAB 149 has been administered to human subjects in Europe using single doses up to 2000 mcg.

Pharmacology*: In vitro* and *in vivo* studies show that QAB149 is a β_2 adrenergic agonist with bronchodilatory activity. The drug has a fairly rapid onset (approximately 5 minutes) with an extended duration of action (approximately 225 minutes).

Safety Pharmacology: QAB149, at a concentration of 5 μg/ml, inhibited HERG channels stably expressed in HEK293 cells (EC_{25} > 5 μg/ml). QAB149, at concentrations of 0.5 and 1.0 μg/ml did not inhibit HERG channels stably expressed in HEK293 cells. In the 2 week inhalation study in the Beagle dog in which the pulmonary deposited doses were 0.0025, 0.12 and 0.23 mg/kg changes in QTc were noted. The changes were reductions in interval data, primarily Q-T. Assessment of the Q-Tc (ie normalization of Q-t interval for heart rate, based on Bazett's and Friderica's formulas) showed a trend toward increased values. The increase was consistent with increases in the heart rate. In a 4 week inhalation study in the beagle dog in which the pulmonary deposited doses were 0.0025, 0.025 and 0.24 mg/kg, no QTc changes were found. Safety pharmacology studies assessing CNS and respiratory effects have not been conducted although no findings of concern were noted in repeat dose animal studies. The sponsor should complete the battery of studies during drug development.

Pharmacokinetic/Toxicokinetics: QAB 149 is absorbed in the dog and the rat after oral dosing but is not highly bioavailable. The bioavailability in the dog after oral dosing is 33 % and 0 in the rat. *In vitro* metabolism of QAB149 evaluated using liver slices is similar in rats, dog and humans, involving mainly phenolic O-glucuronidation. No major active metabolites were observed. QAB 149 was not metabolized *in vitro* using human lung slices. Protein binding was similar in the rat, dog and human, approximately 92, 94 and 96%. QAB149 was distributed to most tissues, except the brain, spinal column and lymph nodes. The highest concentrations of QAB149 were found in the stomach, intestines, liver and kidney. QAB 149 showed no significant inhibition of P450 enzymes, CYP2C9, CYP2E1 and CYP3A4/5 when tested in concentrations up to 100 μM. Elimination of QAB149 in the rat, mouse and dog is mainly via the feces.

General Toxicity:
Acute Studies: Acute studies were carried out in the mouse and the rat. QAB 149 micronized powder was administered orally and subcutaneously. Results of these studies show oral doses up to 1600 mg/kg did not induce any adverse events in the mouse or the rat. However, when QAB 149 was administered subcutaneously, deaths were observed at a dose of 200 mg/kg in the rat.

The maximum non-lethal dose was 100 mg/kg in the rat. In the mouse, deaths were observed at 50 mg/kg in the males and 200 mg/kg in the females. The maximum non-lethal dose was 5 mg/kg in the male mouse and 100 mg/kg in the females.

Sub chronic Studies: Studies up to 13-weeks duration have been conducted in rats and mice and up to 4 weeks in dogs. In the rat, a two week study with micronized drug, pulmonary deposited doses were 0.21, 0.58 and 1.7 mg/kg, resulted in an increased incidence of alveolar macrophages in the lungs of all drug treatment groups when compared to control groups. Additionally, loss of olfactory epithelium/atrophy was noted in the nasal cavity. The NOAEL of 0.21 mg/kg was associated with a mean AUC of 170-289 ng.h/mL. In a four week study with the HFA formulation, the pulmonary deposited doses were 0.093, 0.28 and 0.85 mg/kg. Reversible focal, olfactory epithelial degeneration characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of the epithelium in the mid and high dose groups were observed. The NOAEL was 0.093 mg/kg associated with a mean AUC of 59.3 ng.h/mL. Of note, kinetic data was highly variable and confounded by the presence of drug in control group samples. The sponsor also conducted a 13 week inhalation toxicity dose–ranging study (0, 0.3, 1.01 and 3.08 mg/kg) to determine the doses for a carcinogenicity study. The results of this study again revealed changes in the nasal cavities (degeneration of the olfactory epithelium of the dorsal meatus) in 19/20 high dose rats; severity ranged from minimal to marked, with most animals showing moderate changes. The lesion was characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium, and the changes were described as dose limiting. The rats at the mid and high dose exhibited minimal to mild squamous metaplasia and hyperplasia of the larynx. The ExCAC concurred with the sponsors proposed doses for the 2-year carcinogenicity study of 0.2, 0.6 and 2 mg/kg based on MTD due to findings in the nasal cavity. Squamous metaplasia and hyperplasia of the larynx were also noted at 4 weeks and is considered to be a rodent specific response to inhaled particulate matter.

The 2 week study in the dog with micronized drug included pulmonary deposited doses of 0.0025, 0.12 and 0.23 mg/kg. QAB149 induced tachycardia and myocardial necrosis in the dogs in the mid and high dose groups. There were increases in heart rate and heart force (strength of heart beat) in all QAB 149-treated dogs. There was also dose-related periportal glycogen vacuolation in the liver of all QAB149 dosed groups. There was no NOAEL in this study. The 4 week sub chronic inhalation study with the HFA formulation in the dog included pulmonary deposited doses of 0.0025, 0.025 and 0.24 mg/kg. QAB149 again induced tachycardia and myocardial necrosis in the dogs in high dose group and tachycardia in the dogs in the mid dose group. There was also a dose-related glycogen vacuolation of the liver of the dogs in the mid and high dose groups which was reversible. The NOAEL was 0.0025 mg/kg and associated with a mean AUC of 2.93 ng.h/mL. The sponsor is planning a 13 week inhalation bridging study in the Beagle dog. The objective of the study is to compare the toxicological and toxicokinetic profile of 2 different formations of QAB 149, a micronized powder with lactose and a HFA formulation. The sponsor submitted the protocol for our comments which are in the recommendations section of this review.

There were three subchronic studies carried out in the CD-1 mouse. In the one week oral study, QAB 149 was administered daily for 8 days to assess the suitability of oral route for subsequent studies and to provide date for dose selection for potential subsequent GLP studies. The doses were 0, 100, 250 and 500 mg/ kg base or 130, 324 and 648 mg/kg salt daily for 8 days. Effects

included increased body weight gain vs controls in the mid and high dose females and high dose males. Food consumption was also decreased in the males in the 100 mg/kg dose group vs controls, days 4-7. In the 14 day inhalation study in the mouse, pulmonary deposited doses were 0, 0.1, 0.3, 1.0 and 3.1 mg/kg. One high dose male mouse died due to respiratory difficulties. There were also decreases in body weight gain in the high dose group and food consumption was decreased in all the treated and control mice in this study. Microscopic evaluations revealed changes in the lungs and the nasal cavity in the 3.1 mg/kg mice as well as nasal changes in the mice in the 1.0 mg/kg dose group. The changes consisted of hydrotrophic alveolar macrophages in the lungs and focal olfactory epithelium atrophy, squamous metaplasia of the epithelium in the ventral epiglottis. The MTD appears to be 1.0 m/kg; a NOAEL could not be identified due to limited histologic evaluations. In a 13 week inhalation dose-ranging toxicity study, mice were administered 0, 0.48, 1.47 and 4.91 mg/kg (nominal dose). One mid-dose male died due to a dosing error. Increased heart and lung weight (absolute and relative) were noted in the mid and high dose males. The absolute kidney weight increases were approximately 19 % while the relative kidney weight increases were approximately 12 % in the mid and high dose groups. The absolute and relative increases in spleen weight were approximately 24 % in both the mid and high dose groups. Microscopic analyses of the respiratory tissues revealed minimal to mild respiratory epithelial atrophy in 14/20 mice in the high dose group and 1/20 mice in the mid dose group. Additionally, 4/20 animals in the high dose group and 3/20 animals in the mid dose group had olfactory epithelial atrophy. The lesions were characterized by loss of sustentacular cell cytoplasm, epithelial disorganization and thinning of the epithelial and occurred on the roof of the dorsal meatus and were often accompanied by the presence of eosinophilic globules. The NOAEL was 0.48 mg/kg and the MTD was 4.91 mg/kg. Of note, the lethality observed in the 14-day study may not have been drug-related based on the findings of the 13-week study.

The sponsor conducted 1 and 8 week dose–ranging oral studies to determine the doses for a 26-week TgHras carcinogenicity study. The sponsor initially proposed doses of 0, 50, 250 and 500 mg/kg based on the results of the 1-week oral feasibility study in the [CRL: CD-1(ICR) BR] mouse described earlier. No apparent dose limiting toxic effects were reported; however, the non-GLP study did not include clinical chemistry, hematology, urology parameters or complete histopathology. The ExCAC could not concur with the doses proposed for the 26 week TgHras2 carcinogenicity study since the doses proposed by the sponsor were based on the pharmacokinetic (rodent/human AUC ratio) endpoint and the CB6F-1 strain mouse should be used for TgHras2 carcinogenicity study dose selection. In a subsequent submission, the sponsor proposed oral (gavage) doses of 0, 30, 100 and 300 mg/kg in 0.5% hydroxypropylcellulose in the CB6F-1 TgHras2 heterozygous mouse based on the results of an 8-week oral toxicity study in which the MTD was estimated by the sponsor to be 300 mg/kg. The doses in the study were 0, 100, 300, 1000 and 4123-3000 mg/kg. There were deaths in the mice in the 2 highest dose groups. The dose limiting toxicity was the kidney (basophilic tubules, tubular degeneration, casts and increases in BUN) that were most prominent in the two highest dose groups. There were also gastrointestinal tract changes (erosion, decreased goblet cell) in the mice in the 100 mg/kg dose group and higher. The ExCAC recommended doses of 100, 300 and 600 mg/kg in the TgHras assay.

Overall, the results of the subchronic toxicity studies show that the toxicological and toxicokinetic profile for the micronized powder drug and HFA formations are similar.

Genetic Toxicity: Genotoxicity assays, i.e., Ames bacterial reverse mutation test, Mammalian Chromosomal Aberration test in the V79 Chinese hamster cell and bone marrow micronucleus test reveal that QAB149 is not genotoxic under the conditions tested.

Reproductive toxicity Studies: QAB 149 was administered to male and female rats subcutaneously in a fertility and early embryonic development study. The daily doses were 0, 0.2, 0.6 and 2.0 mg/kg. Results of this study reveal decreases in the relative weight of testes and decreases in the relative and absolute weight of epididymis weight in the males in the 0.2 mg/kg dose group and higher. There were no detectable effects on reproductive potential and histological evaluations in the testes or epididymis. There was drug-related local toxicity, i.e., discoloration at the injection sites in the male rats at 0.6 mg/kg and hair loss, scabs/wounds/cuts in the males and females in the 2 mg/kg dose group. Additionally, there were skin lesions in the male rats in the 2.0 mg/kg dose group. One of the male rats in this dose group was sacrificed early because of skin lesions. Systemic effects included increases in body weight parameters and food consumption in the males in all treated dose groups. There were also increases in the body weight parameters and food consumption in the females in all dose groups premating and during the gestation. There were no effects in males or females related to parameters of fertility, general reproductive performance or early development. The NOAEL was 2 mg/kg for fertility and early embryonic development. There was no NOAEL for male and maternal toxicity because of local, i.e., cuts/scabs/skin lesions and systemic toxicity. i.e., increased body weight parameter changes and increased food consumption. The dose-ranging reproduction and embryo-development studies in the rat and the rabbit were reviewed in IND 66, 337 (see review # 1).

Special Studies: QAB149 at a concentration level of 250 mg/ml (total topical dose was 125 mg) was not a sensitizer in the Buehler Test in Guinea Pig for Delayed Skin Sensitization Potential.

The proposed clinical study may be initiated; however, single doses should be limited to 3000 mcg. Also, the age of the subjects in this study should be no greater than 45 years of age. This conclusion is based on preclinical data which show that older animals are more sensitive to beta agonists than younger animals (Rona, G et al, 1959. The Effect of Breed, Age and Sex on Myocardial Necrosis Produced by Isoproterenol in the Rat. *J Gerontol. 14, 169-173*; Wexler, BC, 1978. Myocardial Infarction in Young and Old Rats: Pathophysiology Changes. *Am Heart J. 96: 70-80* and Hanig, JP and Herman EH, 1991, In *Casarett and Doull's Toxicology, 4th ed,* Mary O Amdur (ed); Toxic responses of the Heart and Vascular System. New York, McGraw-Hill, 1991, p 430-462). The sponsor has agreed to these recommendations. The clinical trial initiation is based on the following data obtained from 2 week inhalation toxicity studies in the rat and dog with the micronized drug substance. The margin of safety in rats is based on a mg/kg basis while the margin of safety in the dog is based on pharmacokinetic data, i.e, AUCs. This is due to questions related to the validity of the rat kinetic data.

The NOAEL in the rat study was 0.21 mg/kg. The NOAEL dose of 0.21 mg/kg will not support the proposed maximum clinical dose of 4000 mcg (2.6 x safety margin). However, analyses of the toxicity observed in the rat reveals that the macrophage accumulation was minimal, was also present in some rats in the control group and was not observed in the dog in the 2 week study. Based on these data and the fact that this is a single dose study followed by a washout period, it is our conclusion that the high dose in the rat study can be used to calculate the margin of safety

for the proposed study. Using the high dose (1.7mg/kg), there is a 21 fold margin of safety in the rat for the proposed 4000 mcg clinical dose.

There was no NOAEL in the 2-week dog study based on glycogen vacuolation in the liver. Glycogen vacuolation of the liver is not considered a significant safety concern in the proposed study because this finding has been shown to be reversible and the patients in this study will receive single doses followed by a 7 day washout period. The Division has also agreed that tachycardia can be used to monitor for myocardial necrosis. Based on this conclusion, we concurred that the high dose in the dog study can be used to calculate the margin of safety in the dog. The high dose, 0.23 mg/kg has an AUC range of 24-44 ng.h/mL in the dog on day 1. The AUC of the human subjects administered an inhalation dose of 2000 mcg of QAB149 is approximately 15 ng.h/mL. The pharmacokinetists have concluded that the pharmacokinetics for QAB149 is linear at doses of 800-2000 mcg. They project that human subjects administered a single inhalation dose of 3000 mcg of QAB149 will have an AUC that ranges from 20-25 ng.h/mL. Using the high dose AUC data there is an adequate margin of safety to support a 3000 mcg dose in human subjects. Additionally, the high dose in the dog provides a 5-fold margin of safety for pulmonary toxicities on the basis of lung mass comparison.

General Toxicology Issues: Glycogen vacuolation in the liver and tachycardia and myocardial necrosis was observed in dogs and should be monitored in clinical trials. The sponsor should address the relevance of the liver findings in humans. The local toxicity observed in the rats should be considered as repeat dose clinical trials are proposed.

Recommendations:
The proposed clinical trial at doses up to 3000 mcg is considered safe to proceed based on margins of safety in the rat and the dog.

The sponsor should identify the source of the measurable levels of active drug in control samples from various rat and mouse studies and the relevance of this finding in the overall interpretation of the study results.

The complete battery of safety pharmacology studies should be completed.

The sponsor's proposed 13-week bridging toxicity study protocol is generally acceptable. Specific comments, noted below, should be forwarded to the sponsor.

Draft comments to the sponsor:

1. Several repeat dose studies in rats and mice demonstrated measurable levels of QAB149 in the plasma samples of control animals. Identify the source of the contamination and the relevance of the findings in terms of interpretation of study results.

2. Complete the battery of safety pharmacology studies described in ICH Guidance for Industry S7A. It is noted that only an assessment of cardiovascular effects has currently been submitted.

3. The final draft of your 13 week inhalation study protocol submitted March 4, 2004 (serial # 001) in the dog has been reviewed and the following comments are provided:
   a) It is recommended that the high dose in the study should be increased to 1.0 mg/kg (approximate pulmonary deposited dose of 0.25 mg/kg) based on the findings in previously conducted studies. In order to fully compare the toxicity profiles of the two drug formulations, doses should be selected to induce previously observed cardiac-associated histopathology. Should a nominal dose lower than 1 mg/kg be selected, the bridging study will only provide support for clinical development of the lactose formulation up to the high dose of the bridging study.

   b) Blood samples for troponin analysis should be collected at C_{max}, if possible, or 3-6 hours after dosing since troponin is rapidly metabolized and may be below the level of quantification if collected only at predose. The blood collection for troponin analysis should be during week 1 and, if possible, after the initial dose to determine whether QAB149 induces myocardial necrosis after a single dose.

   c) EKGs should be recorded at C_{max}.

   d) Dosing in the high dose groups, i.e., micronized drug with lactose and HFA formulations, should not be gradually escalated. The dogs should be administered the targeted high dose beginning on day 1 of the study.

   e) The final clinical formulation of micronized drug and lactose should be used in the study.

Reviewer signature: Virgil Whitehurst

Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Virgil Whitehurst
4/29/04 11:27:31 AM
PHARMACOLOGIST

Timothy McGovern
4/29/04 11:56:51 AM
PHARMACOLOGIST
I concur.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22383	ORIG 1	NOVARTIS PHARMACEUTICALS CORP	INDACATEROL

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
08/25/2009

JEAN Q WU
08/25/2009

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

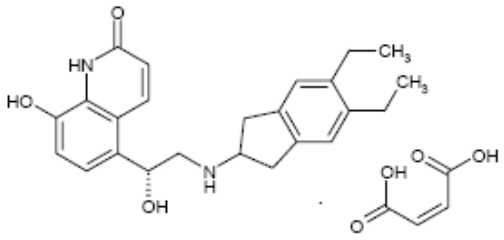
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-383
Review number: #02
Sequence number/date/type of submission:
Submissions dated December 15, 2009 and June 26, 2009
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Novartis Pharmaceuticals Corporation
Drug Regulatory Affairs
One Health Plaza
East Hanover, NJ 07936-1080
Manufacturer for drug substance: Novartis Pharmaceuticals Corporation

Reviewer name: Timothy W. Robison, Ph.D., D.A.B.T.
Division name: Pulmonary and Allergy Products
HFD #: 570
Review completion date: August 12, 2009

Drug:

Trade name: Arcapta™ (b) (4) r™
Generic name: Indacaterol maleate inhalation powder
Code name: QAB-149
Chemical name: (R)-5-[2-(5,6-diethylindan-2-ylamino)-1-hydroxyethyl]-8-hydroxy-1H-quinolin-2-one maleate
Molecular formula/molecular weight: $C_{24}H_{28}N_2O_3 \cdot C_4H_4O_4$
392.49 g/mole (free base); 508.56 g/mole (maleate salt)
Structure:



Relevant INDs/NDAs/DMFs:
INDs 48,649, 69,754, and 66,337 from Novartis

Drug class: Long-acting β_2 agonist intended for once-daily maintenance therapy

Intended clinical population: Chronic obstructive pulmonary disease (COPD)

Clinical formulation: Arcapta (b) (4) for the treatment of COPD will be marketed as 2 inhalation dosage strengths, 150 mcg and 300 mcg administered once-daily by a single-dose dry powder inhaler, (b) (4)

Route of administration: Inhalation

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

Studies reviewed within this submission:
1. 26-week oral (gavage) carcinogenicity study in CB6F1/TgrasH2 hemizygous mice
2. 24-Month Inhalation Oncogenicity Study in Rats

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2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Carcinogenicity:

Mice:
QAB149 was administered by oral gavage to male and female CB6F1/Jic-TgrasH2@Tac hemizygous mice at doses of 0, 100, 300 and 600 mg/kg/day of base and to male and female CB6F1 wild-type mice at doses of 0 and 600 mg/kg/day of base for at least 26 weeks. An additional group of CB6F1/Jic-TgrasH2@Tac hemizygous mice received 75 mg/kg N-methyl-N-nitrosourea, as an intraperitoneal injection on day 1 only, and served as a positive control. The sponsor used doses of QAB149 recommended by the ECAC (see meeting minutes dated December 17, 2003). The duration of treatment was at least 26 weeks, which is acceptable.

Deaths or moribund sacrifices of 1 transgenic female in the 300 mg/kg/day group and 1 transgenic male and 3 transgenic females in the 600 mg/kg/day group were potentially treatment-related. Moribund sacrifices of 1 wild-type male and 1 wild-type female in the 600 mg/kg/day group were potentially treatment-related. Other deaths and moribund sacrifices were attributed to oral gavage errors.

Based upon examination of body weight curves, body weight gains appeared to be lower for the three transgenic male QAB149 treatment groups; however, body weight gains were unaffected for the three transgenic female QAB149 treatment groups.

Deaths at 300 and 600 mg/kg/day as well as decreased body weights for males at all doses suggest that a MTD was achieved and possibly exceeded in the study.

QAB149 treatment-related histopathological findings were primarily evident in the stomach and kidneys.

Uterine endometrial stromal polyps were observed for 3 of 25 females in the 600 mg/kg/day group. This tumor finding was statistically significant by trend test, but not significant by pairwise comparison (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). It is noted that in a 2-year carcinogenicity study with mice that received another β2-adrenergic agonist, uterine endometrial stromal polyps were observed at a much higher incidence.

There were neoplastic findings for MNU-treated mice in several tissues.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 4, 2009).

Rats: Rats in the control-1, control-2, low dose, mid dose, and high dose groups were exposed to achieved inhalation doses of 0, 0, 0.21, 0.62, and 2.09 mg/kg/day, respectively. The route was the same as that used in the clinical setting. The duration of treatment was at least 104 weeks, which is acceptable.

There were no treatment-related effects on survival. Absolute body weights of males in the high dose group on days 546 and 728 were decreased to 88.26 and 86.15% of the pooled control, respectively. Decreased absolute body weight for males in the high dose group appears to indicate that a MTD was achieved for males.

Potential treatment-related non-neoplastic findings were observed in the heart, nasal cavity, lung, larynx, thymus, ovaries, testes, epididymides, pancreas, and eye. Non-neoplastic findings were also observed in the eye that might be attributed to animal housing conditions. Findings in the heart and ovaries appear to be characteristic of β_2-adrenergic agonists. Findings in the testes and epididymides may also be characteristic of β_2-adrenergic agonists. Findings in the nasal cavity, larynx, and lung might be related to irritation associated with nose-only administration of QAB149.

Potential treatment-related neoplastic findings were evident in the pituitary gland and ovary.

In the pituitary gland, combined incidences of adenoma and carcinoma were increased for all male treatment groups and females in the high dose group. For males, the combined incidences of adenoma and carcinoma were statistically significant by pairwise comparison for the mid and high dose groups (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). For females, the combined incidence of adenoma and carcinoma was statistically significant by trend test and statistically significant by pairwise comparison for the high dose group. The historical control mean and range of pituitary adenoma in male and female Wistar rats were reported to 27.74% (18.0-58.3%) and 54.89% (42.0-68.0%), respectively (Fundamental and Applied Toxicology 22: 65-72, 1994). From (b) (4) the mean incidences of pituitary adenoma and carcinoma in male Wistar rats were 31.89% (21.82-50.91%) and 0.54% (0.00-3.63%), respectively. From (b) (4) (2003), the mean incidence of pituitary adenoma in female Wistar rats was 46.90% (1.67-61.82%). The findings in the present study appear to be within the published historical control range.

In the ovaries, leiomyoma was observed for 2 of 49 females in the high dose group. There were no findings in the low and mid dose groups. This tumor finding was statistically significant by trend test, but negative by pairwise comparison (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). It is noted that ovarian leiomyomas have been previously reported for other beta-adrenergic agonist drugs at much higher incidences. The relevance of this tumor finding to human use is unknown.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 4, 2009.

2.6.6.5 Carcinogenicity

Study title: **26-week oral (gavage) carcinogenicity study in CB6F1/TgrasH2 hemizygous mice**

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:

● QAB149 was administered by oral gavage to male and female CB6F1/Jic-TgrasH2@Tac hemizygous mice at doses of 0, 100, 300 and 600 mg/kg/day of base and to male and female CB6F1 wild-type mice at doses of 0 and 600 mg/kg/day of base for at least 26 weeks. An additional group of CB6F1/Jic-TgrasH2@Tac hemizygous mice received 75 mg/kg N-methyl-N-nitrosourea, as an intraperitoneal injection on day 1 only, and served as a positive control. The sponsor used doses of QAB149 recommended by the ECAC (see meeting minutes dated December 17, 2003). The duration of treatment was at least 26 weeks, which is acceptable.

● Deaths or moribund sacrifices of 1 transgenic female in the 300 mg/kg/day group and 1 transgenic male and 3 transgenic females in the 600 mg/kg/day group were potentially treatment-related. Moribund sacrifices of 1 wild-type male and 1 wild-type female in the 600 mg/kg/day group were potentially treatment-related. Other deaths and moribund sacrifices were attributed to oral gavage errors.

● Based upon examination of body weight curves, body weight gains appeared to be lower for the three transgenic male QAB149 treatment groups; however, body weight gains were unaffected for the three transgenic female QAB149 treatment groups.

● Deaths at 300 and 600 mg/kg/day as well as decreased body weights for males at all doses suggest that a MTD was achieved and possibly exceeded in the study.

● QAB149 treatment-related histopathological findings were primarily evident in the stomach and kidneys.

Evaluation of tumor findings:

● Uterine endometrial stromal polyps were observed for 3 of 25 females in the 600 mg/kg/day group. This tumor finding was statistically significant by trend test, but negative by pairwise comparison (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). It is noted that in a 2-year carcinogenicity study with mice that received another β2-adrenergic agonist, uterine endometrial stromal polyps were observed at a much higher incidence.

● There were neoplastic findings for MNU-treated mice in several tissues.

● The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 4, 2009.

Study no.: 0470002
Volume #, and page #: Electronic Document, Pages 1 to 642
Conducting laboratory and location: Novartis Pharmaceuticals Corporation
Safety Profiling and Assessment
East Hanover, New Jersey
Date of study initiation: April 5, 2004 (start of treatment)
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity:
QAB149, batch number 0223004 (99.6% as assayed by HPLC)
N-methyl-N-nitrosourea (MNU), Lot number V4E1668

CAC concurrence: Yes. The ECAC discussed the sponsor's 8-week oral dose range finding toxicology study and dose selection proposal on December 17, 2003. Doses in the 8-week study were 0, 100, 300, 1000, and 4123/3000 mg/kg/day. There were deaths of mice in the 2 highest dose groups. The dose limiting toxicity was the kidney (basophilic tubules, tubular degeneration, casts and increases in BUN). These changes were most prominent in the mice in the two highest dose groups. There were also gastrointestinal tract changes (erosion, decreased goblet cell) in the mice in the 100 mg/kg dose group and higher. The sponsor proposed oral (gavage) doses of 0, 30, 100 and 300 mg/kg/day. The Committee recommended a high dose of 600 mg/kg for the TgHras mouse assay due to the observed lethality and significant renal toxicity at doses of 1000 mg/kg or greater in the 8-week dose ranging study. The committee did not feel that the dose of 300 mg/kg achieved the MTD in that study. The Committee recommended doses of 100 and 300 mg/kg for the low and mid dose groups, respectively.

Methods
Doses: QAB149 was administered by oral gavage to male and female CB6F1/Jic-TgrasH2@Tac hemizygous mice at doses of 0, 100, 300 and 600 mg/kg/day of base and to male and female CB6F1 wild-type mice at doses of 0 and 600 mg/kg/day of base for at least 26 weeks. An additional group of CB6F1/Jic-TgrasH2@Tac hemizygous mice received 75 mg/kg N-methyl-N-nitrosourea, as an intraperitoneal injection on day 1 only, and served as a positive control.

Table 3-1 Study design, animal allocation and test article doses (0470002) CB6F1/TgrasH2 (hemizygous mice)

Group	Number/sex	Animal numbers Males	Animal numbers Females	Dose Base/Salt* (mg/kg/day)	Conc. Salt* (mg/ml)
1 Control	25 2 toxicokinetic	1001-25 1026-27	1501-25 1526-27	0	0
2 Low	25 10 toxicokinetic	2001-25 2026-35	2501-25 2526-35	100/129.6	12.96
3 Mid	25 10 toxicokinetic	3001-25 3026-35	3501-25 3526-35	300/388.8	38.88
4 High	25 10 toxicokinetic	4001-25 4026-35	4501-25 4526-35	600/777.6	77.76
5 MNU (Positive control)	25	5001-25	5501-25	75 (dosed IP on day 1 only)	7.5

*Salt/base ratio for QAB149 is 1.296.

Table 3-2 Study design, animal allocation and test article doses (0470002w) CB6F1/TgrasH2 (wild-type mice)

Group	Number/sex	Animal numbers Males	Animal numbers Females	Dose Base/Salt* (mg/kg/day)	Conc. Salt* (mg/ml)
6 Control	25 2 toxicokinetic	6001-25 6026-27	6501-25 6526-27	0	0
7 High	25 10 toxicokinetic	7001-25 7026-35	7501-25 7526-35	600/777.6	77.76

*Salt/base ratio for QAB149 is 1.296.

In order to facilitate on-line data collection, CB6F1/TgrasH2 hemizygous mice were assigned to Xybion study number 0470002 and wild-type mice were assigned to the Xybion study number 0470002w.

Basis of dose selection (MTD, MFD, AUC etc.): MTD
Species/strain: CB6F1/Jic-TgrasH2@Tac hemizygous mice and CB6F1 wild-type mice were obtained from Taconic Farms, Germantown, NY.
Number/sex/group (main study): 25 mice/sex/group (Transgenic and wild-type)
Route, formulation, volume: Vehicle (0.5% (w/v) hydroxypropylcellulose (Klucel) aqueous solution) and solutions of QAB149 were administered by oral gavage using a dose volume of 10 mL/kg.
Frequency of dosing: Once daily
Satellite groups used for toxicokinetics or special groups: 25 mice/sex/group (Transgenic) were used for a positive control group that received MNU. For toxicokinetic sampling, 2 mice/sex/group (Transgenic and wild-type) were included in Groups 1 and 6 and 10 mice/sex/group (Transgenic and wild-type) were included in Groups 2, 3, 4, and 7.
Age: Mice were approximately 9-10 weeks of age at the start of dosing. Body weight ranges in the main study were 19.1 to 32.2 g for males and 16.2 to 24.7 g for females.
Animal housing: Animals were housed singly in open bottom cages.
Restriction paradigm for dietary restriction studies: No

Drug stability/homogeneity: QAB149 suspensions in 0.5% Klucel, 0.0130 to 376 mg/mL, were found to be stable for at least 13 days at 6°C and at least 4 hr stirring at room temperature. The 0.5% Klucel was found to be suitable for use for at least 4 hr stirring at room temperature, at least 24 hr stored at room temperature, and at least 12 days stored at 6°C. Samples of dosing formulations from weeks 1, 4, 12, 20 and 26 were analyzed for uniformity. The concentrations were 95% to 114% of targets.
Dual controls employed: No
Interim sacrifices: No
Deviations from original study protocol: Study deviations were minor and had no impact on the study. On 02-Jun-2004, toxicokinetic animal nos. 7026, 7027 and 7028 (600 mg/kg/day) were inadvertently dosed with the group 6 control formulation. On 07-Jul-2004, animal nos. 7016 and 7017 were inadvertently dosed twice (received 1200 instead of 600 mg/kg). The animals were checked immediately after dosing and approximately 3 hours post-dose and no significant observations were noted. On 05-Aug-2004, animal no. 5012 had palpable mass data (palpated, no masses) inadvertently entered although the animal was found dead at the time of the mass exams.

Observation times
Mortality: Mice were checked twice daily for moribundity/mortality.
Clinical signs: Mice were observed at least once daily at 2-4 hr postdose for clinical signs of toxicity. Palpable mass examinations were conducted on all main study animals beginning week 2 and every 2 weeks thereafter.
Body weights: Body weights were measured weekly.
Food consumption: Food consumption was measured weekly.
Gross necropsy: Main study animals were fasted overnight (approximately 18 hr) prior to terminal necropsy. Complete necropsies were performed on all main study animals with a recording of macroscopic observations for all protocol tissues. Blood and bone marrow smears were collected/prepared on all animals sacrificed early and on all surviving animals at scheduled necropsy. Fixation and storage of specimens were in 10% neutral-buffered formalin, except for the initial fixation of testes and epididymides (Bouin's) and eyes and Harderian glands (3% Sorensen's buffered glutaraldehyde).
Organ Weights: Absolute and relative organ weights were measured for the brain, heart, kidneys, liver, ovary, and testis.
Histopathology: The tissues specified in the list were processed to hematoxylin and eosin-stained tissue sections for all main study animals. Additional lung and heart tissue from animal numbers 1514 and 4506, respectively, was processed for immunohistochemical evaluation with Chromogranin (Chr-A) and Macrophage (F4/80) stains. Toluidine blue stain was used for Mast Cell tumor confirmation in animal number 7516.
Toxicokinetics: Blood was obtained from all scheduled surviving toxicokinetic animals during week 22. Two animals per sex were bled 0.5, 1, 3, 8 and 24 ht postdose from groups 2, 3, 4 and 7, and 1 hr postdose from groups 1 and 6 (controls). Plasma concentrations of QAB149 were measured using a HPLC-MS/MS method.

Results

Mortality: Deaths or moribund sacrifices of 1 transgenic female in the 300 mg/kg/day group and 1 transgenic male and 3 transgenic females in the 600 mg/kg/day group were potentially treatment-related. Moribund sacrifices of 1 wild-type male and 1 wild-type female in the 600 mg/kg/day group were potentially treatment-related. Other deaths and moribund sacrifices were attributed to oral gavage errors.

Transgenic female #3514 in the 300 mg/kg/day group was found dead on day 128. Blood was observed in the abdominal cavity. A hemangiosarcoma in the spleen (soft, dark, red mass, 10 x 5 x 5 mm) was judged to be the cause of death.

Transgenic male #4017 in the 600 mg/kg/day group was sacrificed on day 170. The moribund sacrifice was attributed to a squamous cell carcinoma in the oral cavity (gingiva).

Transgenic female #4511 in the 600 mg/kg/day was found dead on day 186. A hemangiosarcoma in the spleen (soft, red and black, mottled mass, 12 x 5 x 3 mm) was judged to be the cause of death.

Transgenic female #4518 in the 600 mg/kg/day group was sacrificed on day 163. The moribund sacrifice was attributed to a skin squamous cell carcinoma. Metastatic sites were evident in the rectum and vagina.

Transgenic female #4522 in the 600 mg/kg/day group was sacrificed on day 25. The cause of the moribund sacrifice was undetermined. Histopathological findings were evident in the adrenal gland, kidney, liver, muscle, spleen, and thymus. A hemangioma was evident in the liver. Minimal subcapsular cell hyperplasia was observed in the adrenal gland. Findings in the kidneys consisted of marked basophilic tubules in the cortex, minimal fibrosis of the cortex, minimal interstitial lymphocytic inflammation, minimal pigmented macrophages located in the cortex, and moderate cellular casts. Findings in muscle include slight myofiber nuclear proliferation, minimal mixed cell interstitial inflammation, minimal myofiber hyalinization, and minimal myofiber atrophy. Moderate lymphoid depletion was evident in the spleen. Moderate atrophy was evident in the thymus.

Wild-type male #7014 in the 600 mg/kg/day group was sacrificed on day 22. The moribund sacrifice was attributed to hindlimb paralysis. Histopathological findings considered the cause of the moribund sacrifice were observed in the spinal cord. Findings included moderate, focal vacuolation with white matter; minimal, focal hemorrhage with white matter; slight, focal axonal degeneration; and minimal, focal necrosis with gray matter. The relationship of this death to treatment to treatment was somewhat questionable.

Wild-type female #7501 in the 600 mg/kg/day was sacrificed on day 113. The moribund sacrifice was attributed to findings in the kidneys that included severe glomerular

amyloidosis, moderate basophilic tubules in the cortex, and moderate proteinaceous casts.

Transgenic males #3021 (300 mg/kg/day, Day 3) and #4005 (600 mg/kg/day, Day 98) and females #1507 (Control, Day 161), #1514 (Control, Day 145), and #4506 (600 mg/kg/day, Day 173) were found dead due to gavage errors. Wild type females #7502 (600 mg/kg/day, Day 9) and #7521 (600 mg/kg/day, Day 26) were found dead or sacrificed in a moribund condition due to gavage errors.

In the MNU group, 19 males and 17 females were found dead or sacrificed in a moribund condition.

Table 4-1 Summary of survival ratios with % survival (n=25) at termination

Group Dose (mg/kg/day)	Survival (%) Males	Survival (%) Females
1 (0)	25/25 (100%)	23/23 (100%)*
2 (100)	25/25 (100%)	25/25 (100%)
3 (300)	24/24 (100%)*	24/25 (96%)
4 (600)	23/24 (96%)*	21/24 (88%)*
5 (MNU @75)	6/25 (24%)	8/25 (32%)
6 (0 wild -type)	25/25 (100%)	25/25 (100%)
7 (600 wild-type)	24/25 (96%)	22/23 (96%)*

*= survival adjusted to exclude early sacrifices/deaths attributable to gavage accidents.

Clinical signs: A number of clinical signs were evident for MNU-treated male and female CB6F1/TgrasH2 mice that included pale appearance, cold to touch, thin, dehydration, abdominal distention, hunched posture, unkempt coat, decreased locomotor activity, ataxia, abnormal gait, and recumbency. These clinical signs were also evident at low incidences (1 or 2 mice/sex/group) for male and female CB6F1/TgrasH2 and wild-type CB6F1 mice treated with QAB149 at 600 mg/kg/day.

Body weights: Based upon examination of body weight curves, body weight gains appeared to be lower for the three transgenic male QAB149 treatment groups; however, body weight gains were unaffected for the three transgenic female QAB149 treatment groups. Examination of absolute body weights on day 183 suggests modest effects (i.e., up to an 11.7% decrease) on absolute body weights for males treated with QAB149 at doses up to 600 mg/kg/day.

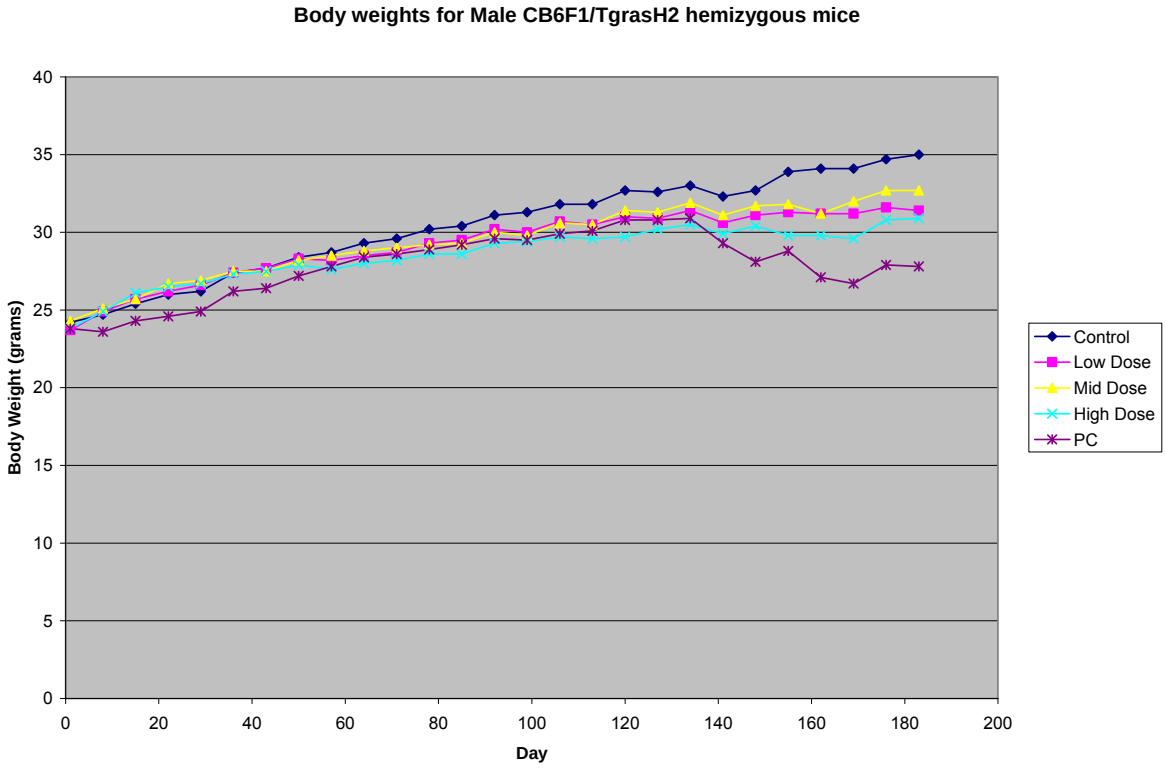
Absolute body weights and body weight gains were unaffected for wild-type male and female mice treated with QAB149 at a dose of 600 mg/kg/day.

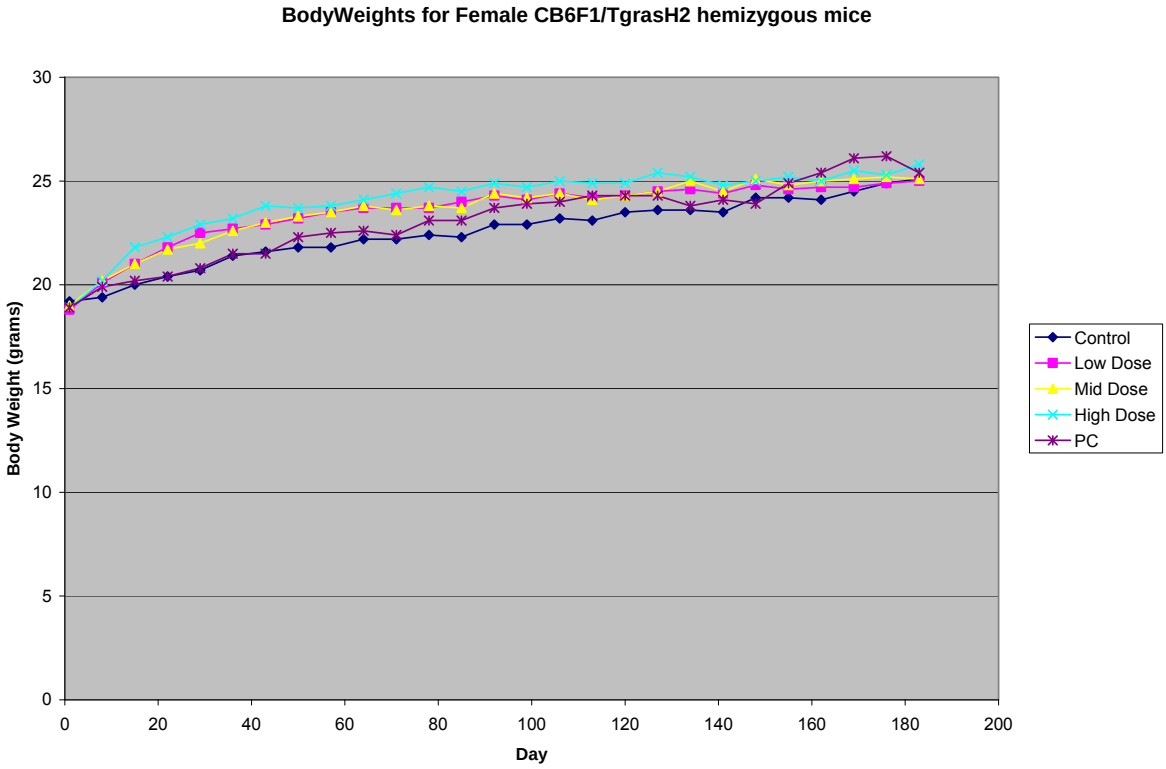
Absolute body weights and body weight gain were decreased for MNU-treated male mice; however, MNU-treated female mice were unaffected.

Absolute body weights and body weight gains for CB6F1/Jic-TgrasH2@Tac hemizygous mice

Parameter	Males					Females				
	0	100	300	600	PC	0	100	300	600	PC
BW, day 1	24.2	23.7	24.3	23.8	23.8	19.2	18.8	19.0	18.9	18.9
BW, day 183	35.0	31.4	32.7	30.9	27.8	25.1	25.1	25.1	25.8	25.4
% Control (BW, day 183)	100.0	89.7	93.4	88.3	79.4	100.0	100.0	100.0	102.8	101.2
Δ (BW Gain)	10.8	7.7	8.4	7.1	4.0	5.9	6.3	6.1	6.9	6.5
% Initial (BW Gain/BW day 1)	44.63	32.49	34.57	29.83	16.81	30.73	33.51	32.11	36.51	34.39
% Control (N. BW Gain)	100.0	72.8	77.5	66.8	37.7	100.0	104.5	104.5	118.8	111.9

Parameters are percent of absolute control body weight, body weight gain (Δ), body weight gain expressed as a percentage of initial body weight on day 1 (normalized), and normalized body weight gain expressed as a percentage of the control.





Absolute body weights and body weight gains for CB6F1 wild-type mice

Parameter	Males		Females	
	0	600	0	600
BW, day 1	28.1	27.6	20.8	20.8
BW, day 183	42.2	41.0	31.3	30.3
% Control (BW, day 183)	100.0	97.2	100.0	96.8
Δ (BW Gain)	14.1	13.4	10.5	9.5
% Initial (BW Gain/BW day 1)	50.18	48.55	50.48	45.67
% Control (N. BW Gain)	100.0	96.8	100.0	90.0

Parameters are percent of absolute control body weight, body weight gain (Δ), body weight gain expressed as a percentage of initial body weight on day 1 (normalized), and normalized body weight gain expressed as a percentage of the control.

Food consumption: Food consumption for male mice in the low, mid, and high dose groups were 104.1, 106.7, and 97.8% of the control, respectively. Food consumption for female mice in the low, mid, and high dose groups were 118.5, 129.6, and 127.8% of the control, respectively. These increases or slight decreases of food consumption appear to have no toxicological significance. Increased food consumption is an expected pharmacological effect of β_2-adrenergic agonists.

Gross pathology:
Treatment with QAB149 at 600 mg/kg/day produced a rough or pitted surface to the kidneys in 1/25 male and 9/25 female CB6F1-TgrasH2 mice and 3/25 male and 11/25 female CB6F1 wild-type mice. This lesion was typically bilateral and related to variably prominent cortical tubular degeneration.

For MNU-treated mice, macroscopic changes of organ enlargement and pale or mottled discoloration was related to multicentric lymphoma, occurring in lymph nodes, thymus, spleen, liver, and kidney. Additional MNU-related macroscopic findings were observed for the skin (nodules, masses) and non-glandular stomach (foci, discoloration, nodules, masses) and reflected squamous cell papillomas or carcinomas.

Organ weights: QAB149-treatment resulted in increased relative heart weights (%BW) for both male and female CB6F1-TgrasH2 mice at all doses. Increased heart, kidney and liver weights (absolute and relative to body and brain weights) also occurred in MNU-treated female mice. QAB149 treatment increased ovarian weights in the CB6F1 wild-type mice. There were no corresponding histopathological changes to these organ weight changes.

Histopathology:

Non-neoplastic: QAB149 treatment-related histopathological findings were primarily evident in the stomach and kidneys. Histopathological findings were also evident in the preputial gland, epididymides, muscle, thymus, lung, and Harderian gland. MNU treatment-related histopathological findings were evident in the preputial gland, epididymides, muscle, bone, spleen, pancreas, lung, and Harderian gland. This review is primarily focused on QAB149 treatment-related histopathological findings.

In the stomach, treatment of male and female CB6F1/TgrasH2 mice with QAB149 produced increased incidences of dyskeratosis at the limiting ridge, mucous neck cell eosinophilia, glandular erosion, hyperkeratosis at the limiting ridge, epithelial hyperplasia at the limiting ridge, mucous neck cell hyperplasia, and leukocytic infiltrate in the submucosa. Dose-response relationships were not always present.

Treatment with QAB149 produced histopathological changes in the kidneys primarily for female CB6F1/TgrasH2 mice and wild-type CB6F1 male and female mice. Basophilic tubules in the cortex, cellular casts, and interstitial lymphocytic inflammation were observed for male CB6F1/TgrasH2 mice at 600 mg/kg/day, female CB6F1/TgrasH2 mice at 300 and 600 mg/kg/day, and wild-type male and female CB6F1 mice at 600 mg/kg/day. Proteinaceous casts were observed for female CB6F1/TgrasH2 mice at 300 and 600 mg/kg/day and wild-type CB6F1 male and female mice at 600 mg/kg/day. Tubular atrophy and pigmented macrophages in the cortex were observed for female CB6F1/TgrasH2 mice at 600 mg/kg/day and wild-type CB6F1 male and female mice at 600 mg/kg/day. Fibrosis in the cortex and interstitial mixed cell inflammation were observed for female CB6F1/TgrasH2 and wild-type CB6F1 mice at 600 mg/kg/day.

For the preputial gland, the incidence of lymphocytic inflammation was increased for wild-type male CB6F1 mice at 600 mg/kg/day. Incidences of glandular atrophy and ectasia were increased for MNU-treated male CB6F1/TgrasH2 mice.

In the epididymides, the incidence of congestion was increased for wild-type male CB6F1 mice at 600 mg/kg/day. The incidence of mixed cell inflammation in the adventitia was increased for MNU-treated male CB6F1/TgrasH2 mice.

In muscle, incidences of myofiber atrophy and myofiber fragmentation were increased for male CB6F1/TgrasH2 mice treated with QAB149. The incidence of myofiber atrophy was also increased for MNU-treated male mice. Incidences of myofiber atrophy were high for all female CB6F1/TgrasH2 groups with no relation to treatment with QAB149. Incidences of myofiber fragmentation were high for the female CB6F1/TgrasH2 control and low dose groups.

Incidences of cysts in the medulla of the thymus were slightly increased for all male and female CB6F1/TgrasH2 and wild-type CB6F1 mice.

Incidences of retinal atrophy, increased myeloid in the bone marrow, and increased zymogen in the pancreas were increased for male and female mice treated with MNU. The incidence of extramedullary hematopoiesis in the spleen was increased for female mice treated with MNU.

Incidences of interstitial mixed cell inflammation and focal type II pneumocyte hyperplasia in the lung were increased for female CB6F1/TgrasH2 mice treated with QAB149 at 600 mg/kg/day or MNU. The incidence of interstitial lymphocytic inflammation in the lung was increased for male CB6F1/TgrasH2 mice treated with QAB149 at 600 mg/kg/day.

Incidences of interstitial lymphocytic inflammation in the Harderian gland were increased for male CB6F1/TgrasH2 mice treated with QAB149 at 600 mg/kg/day or MNU.

Non-neoplastic findings in mice treated with QAB-149 for up to 26 weeks

Organ/Tissue	Males							Females						
	CB6F1/TgrasH2 mice					CB6F1 mice		CB6F1/TgrasH2 mice					CB6F1 mice	
	0	LD	MD	HD	PC	0	HD	0	LD	MD	HD	PC	0	HD
Stomach														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-dyskeratosis, limiting ridge, minimal-slight	1	6	10	7	1	1	0	2	9	5	8	1	1	2
-eosinophilia, mucous neck cell, minimal-slight	4	18	19	21	3	2	3	9	14	15	13	5	6	10
-glandular erosion, minimal-slight	0	2	5	11	6	0	2	2	5	7	8	3	0	2
-hyperkeratosis, limiting ridge, minimal-slight	0	7	10	8	1	1	0	2	9	5	5	0	0	1
-hyperplasia, epithelium, limiting ridge, minimal-slight	0	4	9	7	1	1	0	2	10	2	5	1	0	1
-hyperplasia, mucous neck cells, minimal-slight	0	12	9	8	1	0	0	2	10	5	7	1	0	2
-infiltrate, leukocytic, submucosa, minimal-slight	6	18	17	20	5	2	3	4	13	7	8	4	0	2
Kidneys														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-atrophy, cortex, tubular, minimal-slight	0	1	0	1	0	0	5	0	0	0	15	0	0	19
-basophilic tubules, cortex, minimal-marked	1	3	2	6	0	3	17	2	1	10	20	2	2	24
-cast, cellular, minimal-moderate	0	0	0	2	0	0	2	0	0	3	14	0	0	20
-cast, proteinaceous, minimal-slight	3	2	4	4	0	0	19	0	1	7	20	0	1	22
-fibrosis, cortex, minimal-slight	1	1	0	0	0	0	0	0	0	0	7	0	0	9
-inflammation, lymphocytic, interstitial, minimal-moderate	2	1	2	5	0	1	12	0	1	3	14	6	0	20
-inflammation, mixed cell, interstitial, minimal-moderate	0	0	0	0	0	0	0	0	0	1	4	0	0	2
-pigment, macrophage, cortex, minimal-slight	0	0	0	1	0	0	3	0	0	0	12	0	0	17
-inflammation, mixed cell, pelvis, minimal	0	0	0	0	0	0	1	0	0	0	0	0	0	3
Preputial gland														
-NE	24	25	25	24	25	25	25							
-atrophy, glandular	0	0	0	0	8	0	1							
-ectasia	3	1	1	1	11	2	1							
-inflammation, lymphocytic	0	0	2	0	1	2	12							
Epididymis														
-NE	25	25	25	25	25	25	25							
-congestion	0	0	1	1	0	1	4							
-inflammation, mixed cell, adventitia	0	0	0	0	5	0	0							
Muscle														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-atrophy, myofiber	2	7	11	7	12	0	0	18	13	19	18	11	0	0
-fragmentation, myofiber	0	1	7	6	0	0	0	12	12	1	1	0	0	0
Thymus														
-NE	25	24	24	24	23	25	25	24	25	24	24	25	25	24
-cyst, medulla	1	3	3	4	4	3	2	2	4	6	6	0	4	9
Eye														
-NE	25	25	25	25	24	25	25	25	25	25	25	25	25	25
-atrophy, retina	0	0	0	0	19	0	0	0	0	0	0	15	0	0
Bone marrow														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-increase myeloid	0	0	0	0	3	0	0	0	0	0	0	5	0	0
Spleen														

Organ/Tissue	Males							Females						
	CB6F1/TgrasH2 mice					CB6F1 mice		CB6F1/TgrasH2 mice					CB6F1 mice	
	0	LD	MD	HD	PC	0	HD	0	LD	MD	HD	PC	0	HD
-extramedullary hematopoiesis	250	250	250	250	250	250	250	251	253	250	250	2514	250	250
Pancreas														
-NE	253	253	258	255	2516	259	252	2515	258	256	256	2523	258	258
-zymogen increased														
Lung														
-NE	240	251	250	240	250	250	250	250	250	250	251	254	250	250
-hyperplasia, pneumocytes, type II, focal														
-inflammation, mixed cell, interstitial	1	1	0	2	2	0	3	0	0	2	5	8	0	5
-inflammation, lymphocytic, interstitial	0	2	0	4	1	0	0	3	2	0	4	0	1	0
Harderian gland														
-NE	250	252	252	255	248	252	251	252	253	250	253	253	252	252
-inflammation, lymphocytic, interstitial														

Neoplastic:

Uterine endometrial stromal polyps were observed for 3 of 25 females in the 600 mg/kg/day group. This tumor finding was statistically significant by trend test, but negative by pairwise comparison (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). This tumor was observed for 1 wild-type control female. It is noted that in a 2-year carcinogenicity study with mice that received another β2-adrenergic agonist, uterine endometrial stromal polyps were observed at a much higher incidence.

The occurrence of squamous cell carcinoma of the stomach in 1 female CB6F1-TgrasH2 mouse at 100 mg/kg/day, the skin of 1 female CB6F1-TgrasH2 mouse at 100 mg/kg/day, the skin of two females at 600 mg/kg/day, and the gingiva of a single male CB6F1-TgrasH2 mouse at 600 mg/kg/day were considered incidental and not test article-related. Mitsumori et al. (Toxicologic Pathology 26: 520-531, 1998) reported that the spontaneous incidences of squamous cell carcinoma in the stomach for male and female TgrasH2 mice were 0.56% (1/180) and 0% (0/174), respectively.

The combined incidence of hemangioma and hemangiosarcoma without regard to site displayed no relationship to treatment.

There were neoplastic findings for MNU-treated mice in several tissues. Multicentric lymphoma was observed in 18/25 and 14/25 for MNU-treated male and female, transgenic mice, respectively. The most common sites of lymphoma distribution in the MNU-treated mice were lymph nodes, thymus, spleen, liver, kidneys, and lung. The MNU positive reference material produced an increased incidence of neoplastic changes in a variety of tissues in both males and females including non-glandular stomach (squamous cell papilloma, squamous cell carcinoma), skin (squamous cell papilloma, squamous cell carcinoma), uterus (adenoma, endometrial stromal polyp), and urethra (transitional cell papilloma), and thymus (thyoma).

Neoplastic findings in mice treated with QAB-149 for up to 26 weeks

Organ/Tissue	Males							Females						
	CB6F1/TgrasH2 mice					CB6F1 mice		CB6F1/TgrasH2 mice					CB6F1 mice	
	0	LD	MD	HD	PC	0	HD	0	LD	MD	HD	PC	0	HD
Uterus														
-NE								25	25	25	25	25	24	25
-adenoma [B]								0	0	0	0	3	0	0
-endometrial stromal polyp [B]								0	0	0	3	14	1	0
-hemangioma [B]								0	0	0	0	1	0	0
Systemic/Any Site														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-lymphoma [M]	0	0	0	0	18	0	0	0	0	0	0	14	0	0
-leukemia [M], myeloid	0	0	0	0	0	0	0	0	0	0	0	2	0	0
-hemangioma	0	1	0	0	0	0	0	0	0	0	2	2	0	0
-hemangiosarcoma	2	5	1	0	3	0	0	4	0	1	2	5	0	0
-total	2	6	1	0	0	0	0	4	0	1	4	7	0	0
-squamous cell carcinoma (skin, stomach, or oral cavity as primary site)	0	0	0	1	11	0	0	0	2	0	2	7	0	0
Stomach														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-papilloma [B], squamous cell	0	0	0	0	10	0	0	0	1	0	0	23	0	0
-squamous cell, carcinoma [M]	0	0	0	0	2	0	0	0	1	0	0	3	0	0
Skin														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-papilloma [B], squamous cell	0	0	0	0	7	0	0	0	0	0	0	16	0	0
-squamous cell carcinoma [M]	0	0	0	0	1	0	0	0	1	0	2	4	0	0
-mast cell tumor [B]	0	0	0	0	0	0	0	0	0	0	0	0	0	1
-sebaceous adenoma [B]	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Oral cavity														
-NE	0	0	0	1	0	0	0	0	0	0	0	2	0	0
-squamous cell carcinoma [M], gingiva				1								0		
Lung														
-NE	24	25	25	24	25	25	25	25	25	25	25	25	25	25
-adenoma [B], bronchiolo-alveolar	3	2	1	2	6	1	0	0	2	0	1	1	0	0
Urethra														
-NE	0	0	0	0	2	0	0	0	0	1	0	6	0	2
-transitional cell papilloma [B]					0					1		6		2
Spleen														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-hemangiosarcoma	2	4	1	0	2	0	0	3	0	1	2	3	0	0
Thymus														
-NE	25	24	24	24	23	25	25	24	25	24	24	25	25	24
-thyoma [B]	0	0	0	0	0	0	0	0	0	0	1	3	0	0
Harderian Gland														
-NE	25	25	25	25	25	25	25	25	25	25	25	25	25	25
-adenoma [B]	0	0	1	0	0	0	0	0	0	0	0	2	0	0
-carcinoma [M]	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Adrenal														
-NE	23	25	25	25	25	25	25	25	25	25	25	25	25	25
-cortical adenoma [B]	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Toxicokinetics: Plasma C_{max} and AUC values for QAB149 in male transgenic mice did not increase with elevating doses as C_{max} and AUC values at 300 mg/kg/day were lower than those observed at 100 mg/kg/day and the C_{max} value at 600 mg/kg/day was

approximately equivalent to that observed at 100 mg/kg/day. The C_{max} value at 600 mg/kg/day for wild-type male mice was lower than the C_{max} value for male transgenic mice at 100 mg/kg/day. The AUC value for male wild-type mice at 600 mg/kg/day was approximately 1.25-fold higher as compared to male transgenic mice at 600 mg/kg/day.

Plasma C_{max} and AUC values for QAB149 in female transgenic mice generally increased with elevating doses although increases were less than dose proportional. Further, the C_{max} at 300 mg/kg/day was greater than that observed at 600 mg/kg/day. C_{max} and AUC values for QAB149 in wild-type females at 600 mg/kg/day were slightly greater than values in transgenic females at 600 mg/kg/day.

Plasma C_{max} and AUC values in female transgenic and wild-type mice were greater than values observed in comparable males.

Table 2-1 Toxicokinetic parameters of QAB149 in mouse serum (week 22)

Parameter	Units	Mean Males	Mean Females
Dose: 100 mg/kg/day		**Hemizygous mice**	
t_{max}	h	1	1
C_{max}	ng/mL	49.1	59.8
C_{max} / dose	(ng/mL)/(mg/kg/day)	0.491	0.598
$AUC_{(0\text{-}24h)}$	ng.h/mL	245	376
$AUC_{(0\text{-}24h)}$ / dose	(ng.h/mL)/(mg/kg/day)	2.45	3.76
Dose: 300 mg/kg/day		**Hemizygous mice**	
t_{max}	h	1	1
C_{max}	ng/mL	23	85
C_{max} / dose	(ng/mL)/(mg/kg/day)	0.0767	0.283
$AUC_{(0\text{-}24h)}$	ng.h/mL	221	552
$AUC_{(0\text{-}24h)}$ / dose	(ng.h/mL)/(mg/kg/day)	0.737	1.84
Dose: 600 mg/kg/day		**Hemizygous mice**	
t_{max}	h	3	3
C_{max}	ng/mL	47.7	71
C_{max} / dose	(ng/mL)/(mg/kg/day)	0.0795	0.118
$AUC_{(0\text{-}24h)}$	ng.h/mL	399	862
$AUC_{(0\text{-}24h)}$ / dose	(ng.h/mL)/(mg/kg/day)	0.665	1.44
Dose: 600mg/kg/day		**Wild-type mice**	
t_{max}	h	0.5	1
C_{max}	ng/mL	33.2	94.2
C_{max} / dose	(ng/mL)/(mg/kg/day)	0.0553	0.157
$AUC_{(0\text{-}24h)}$	ng.h/mL	499	954
$AUC_{(0\text{-}24h)}$ / dose	(ng.h/mL)/(mg/kg/day)	0.831	1.59

Study title: **24-Month Inhalation Oncogenicity Study in Rats**

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:
● Rats in the control-1, control-2, low dose, mid dose, and high dose groups were exposed to achieved inhalation doses of 0, 0, 0.21, 0.62, and 2.09 mg/kg/day, respectively. The route was the same as that used in the clinical setting. The duration of treatment was at least 104 weeks, which is acceptable.

● There were no treatment-related effects on survival. Absolute body weights of males in the high dose group on days 546 and 728 were decreased to 88.26 and 86.15% of the pooled control, respectively. Decreased absolute body weight for males in the high dose group appears to indicate that a MTD was achieved for males.

● Potential treatment-related non-neoplastic findings were observed in the heart, nasal cavity, lung, larynx, thymus, ovaries, testes, epididymides, pancreas, and eye. Non-neoplastic findings were also observed in the eye that might be attributed to animal housing conditions. Findings in the heart and ovaries appear to be characteristic of β_2-adrenergic agonists. Findings in the testes and epididymides may also be characteristic of β_2-adrenergic agonists. Findings in the nasal cavity, larynx, and lung might be related to irritation associated with nose-only administration of QAB149.

Evaluation of tumor findings:
● Potential treatment-related neoplastic findings were evident in the pituitary gland and ovary.

● In the pituitary gland, combined incidences of adenoma and carcinoma were increased for all male treatment groups and females in the high dose group. For males, the combined incidences of adenoma and carcinoma were statistically significant by pairwise comparison for the mid and high dose groups. For females, the combined incidence of adenoma and carcinoma was statistically significant by trend test and statistically significant by pairwise comparison for the high dose group (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). The historical control mean and range of pituitary adenoma in male and female Wistar rats were reported to 27.74% (18.0-58.3%) and 54.89% (42.0-68.0%), respectively (Fundamental and Applied Toxicology 22: 65-72, 1994). From (b) (4) the mean incidences of pituitary adenoma and carcinoma in male Wistar rats were 31.89% (21.82-50.91%) and 0.54% (0.00-3.63%), respectively. From (b) (4) ((b) (4) the mean incidence of pituitary adenoma in female Wistar rats was 46.90% (1.67-61.82%). The findings in the present study appear to be within the published historical control range.

● In the ovaries, leiomyoma was observed for 2 of 49 females in the high dose group. There were no findings in the low and mid dose groups. This tumor finding was statistically significant by trend test, but negative by pairwise comparison (See

Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). It is noted that ovarian leiomyomas have been previously reported for other beta-adrenergic agonist drugs at much higher incidences. The relevance of this tumor finding to human use is unknown.

● The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 4, 2009.

Study no.: 0320002
Volume #, and page #: Electronic Submission, Pages 1-2377
Conducting laboratory and location: (b) (4)

Date of study initiation: March 13, 2003
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: QAB149, batch number 0223004 (99.6% as assayed by HPLC)
CAC concurrence: Yes. The ECAC discussed the sponsor's 13-week inhalation range finding toxicology study and dose selection proposal on March 4, 2003. Doses in the 13-week study were 0, 0.2, 1.01, and 3.08 mg/kg/day. Minimal to marked degeneration of the olfactory epithelium of the dorsal meatus in the nasal cavities was observed for 19/20 high dose rats. Rats in the mid and high dose groups exhibited minimal to mild squamous metaplasia and hyperplasia of the larynx. The Committee concurred with the sponsor's proposed doses of 0, 0.2, 0.6, and 2.0 mg/kg/day based on MTD (degeneration of the olfactory epithelium of the dorsal meatus in the nasal cavity).

Methods
Doses: Rats in the control-1, control-2, low dose, mid dose, and high dose groups were exposed to achieved doses of 0, 0, 0.21, 0.62, and 2.09 mg/kg/day, respectively. The two control groups were exposed to air only for 32-47 min per day. The low, intermediate, and high dose groups were exposed to QAB149 once daily at mean aerosol concentrations of 0.106, 0.107 and 0.107 mg/L for 3-5 min, 9-14 min and 32-47 min, respectively. The duration of treatment was at least 104 weeks.

Dose Group/ Treatment	Target Aerosol Concentration mg QAB149/L	Target Dose[1] mg QAB149 /kg/day	Achieved Dose mg QAB149 /kg/day	Rats/sex/ group	Animal numbers [2, 3]	
1 – Air Control	0	0	0	50	100-119, 602, 121-149	150-195, 620, 197-199
2 – Air Control	0	0	0	50	200-217, 601, 219-249	250-265, 615, 267-271, 616,

						617, 274-299
3 - Low Dose	0.1	0.2	0.21	50	300-349	350-391, 618, 393-399
4 - Mid Dose	0.1	0.6	0.62	50	400-422, 603, 424-439, 604, 441-449	450-499
5 - High Dose	0.1	2.0	2.09	50	500-549	550-556, 619, 614, 559-599

*Group 2 Air Control was housed and dosed in a separate room to allow the animals being housed and dosed in a fully controlled and clean environment for comparison

1 = The target aerosol concentrations, as well as the target doses, refer to the free base of QAB149 and the conversion factor for salt to free base is 0.7718

2 = The following replacements were made before Day 1 of the study
Group 1 602M replaced 120M due to eye injury caused by tubing procedure
620F replaced 196F due to wet staining on perigenital area
Group 2 601M replaced 218M due to only one testicle being present
615F replaced 266F due to unusual ophthalmic findings
616F replaced 272F due to eye injury caused by tubing procedure
617F replaced 273F due to eye injury caused by tubing procedure
Group 3 618F replaced 392F due to unusual ophthalmic findings
Group 4 603M replaced 423M due to unusual ophthalmic findings
604M replaced 440M due to protruding penis
Group 5 619F replaced 557F due to eye injury caused by tubing procedure

3 = The following replacement was undertaken on Day 6 of the study
Group 5 614F replaced 558F due to a damaged tail

Animals in Groups 3, 4 and 5 were dosed at a target aerosol concentration of 0.10 mg/L of QAB149 using the same modular stainless steel flow post chamber system. The duration of inhalation exposure was adjusted based on animal body weight profile in order to achieve the targeted doses.

Achieved dose levels were estimated using the following criteria:

Dose (mg/kg/day) = (MV x T x CC)/BW

MV = Minute volume (L/minute) = (2.10 x body weight (g)$^{0.75}$)/1000

T = Duration of exposure (minutes)

CC = Chamber concentration of actual drug (mg/L)

BW = Body weight (expressed in kg) - mean of males and females was calculated separately using mid-week body weight (Weeks 1-13) or mid-term body weight (measured every 4 weeks between Week 13 and 77 or every 2 weeks between Week 77 and 105).

Basis of dose selection (MTD, MFD, AUC etc.): MTD
Species/strain: Han Wistar Crl:WI (Glx/BRL/Han) IGS BR rats were obtained from (b) (4)
Number/sex/group (main study): 50 rats/sex/group
Route, formulation, volume: Rats were exposed by nose-only inhalation. Each animal was restrained in a polycarbonate restraint tube with adjustable back-stop. The animal's snout protruded through the anterior end of the restraint tube which was connected to the exposure chamber by way of a push-fit through a rubber "o" ring in the chamber wall. This exposure technique minimized concurrent exposure by the oral and dermal routes. The animals were not allowed access to food or water during the exposure period.

The exposure to aerosols of QAB149 or air only were performed using modular stainless steel flow past chamber systems, which allowed snout only inhalation.

The animals' positions on the chamber were rotated weekly to eliminate exposure variation.

Separate exposure chambers were used for the Control and test groups. The same chamber was used for dosing Groups 3, 4 and 5. Each exposure chamber was located in an extract booth (for the protection of personnel and to prevent cross-group contamination) and were operated to sustain a dynamic air flow sufficient to ensure an evenly distributed exposure atmosphere. The chamber air flow rate was 40 L/min. Flow rates were monitored continuously using calibrated flow meters.

Chamber air flow rates, temperature and humidity were monitored and recorded at appropriate intervals during each exposure period, the ranges are presented in the following table:

Group	Chamber Air Flow (L/min)	Temperature (°C)	Humidity (%)
Group 1 – Air Control	40	15.2-24.0	6.2-50.4
Group 2 – Air Control*	40	17.4-25.8	9.7-60.0
Groups 3/4/5 – Low/Intermediate/High Dose	40	15.8-24.2	0.3-51.4

* = Group 2 was housed and dosed in a separate room

Aerosol generation
Test aerosols of QAB149 were generated using a rotating brush generator device. In this electrically operated device a canister of test item was slowly advanced towards a high speed rotating brush which scraped the powder into a compressed air stream. The piston advance rate was determined during preliminary characterization investigations.

The generator ran continuously over the exposure duration and the QAB149 delivery rate adjusted as necessary to achieve the target aerosol concentration. Prior to commencement of the animal dosing phase of the study, aerosol characterization investigations were undertaken in a separate study to investigate particle size distribution and to demonstrate spatial and temporal stability of the test aerosols within the exposure system. (b) (4)

Aerosol concentration

The aerosol concentration of the test formulation in the exposure system was measured gravimetrically for Groups 3, 4 and 5 throughout each exposure period. Filter samples were collected daily. Multiple samples were taken for Group 5 and the first sample was taken as close as possible to the start of exposure. A filter sample was also collected for Control Groups 1 and 2 throughout each exposure period. During each exposure period chamber air was sampled using glass fiber filters ((b) (4) placed in an open faced conical filter holder in-line with a sampling system comprising a vacuum pump, flow meter, and gas meter. The nominal sampling flow rate was *ca* 0.5 L/min. Filter samples were taken over an appropriate time period to ensure that there was no overloading of the filter. The filters were weighed before and after sampling and the aerosol concentration calculated using the weight of total particulate collected and volume of air sampled. The in-line gas meter recorded the total air volume sampled to allow calculation of the aerosol concentrations. Filter samples were collected from a reference sampling port at the animals' exposure level of the chamber (i.e., middle of chamber). The filters were retained in amber glass jars, stored at 2-8°C (apart from Group 1 samples, which were kept at ambient conditions to avoid cross contamination with QAB149 from samples collected from treated groups) and batch assayed weekly. Chemical analysis was performed on all retained filters to determine the aerosol concentration of QAB149. Dose levels were estimated based on the analytically determined aerosol concentration of QAB149.

Particle size distribution

(b) (4)

The substrate collection plates and back-up filters were weighed before and after sampling in order to determine the amount of total particulate collected in each size

range. The plates and back-up filters for Groups 3, 4 and 5 were stored in amber glass jars at 2-8°C until submission for chemical analysis to determine the amount of QAB149 present. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the aerosols were derived.

Particle size distribution: group mean values

Chemical analysis

The aerosol filter samples and particle size samples were subjected to chemical analysis using a high performance liquid chromatography (HPLC) method supplied by the Sponsor and validated at (b) (4)

Frequency of dosing: Animals were exposed once daily for a period of 104 weeks.
Satellite groups used for toxicokinetics or special groups:
Age: Animals were 6 to 8 weeks old at the start of treatment. Body weight ranges were 127-212 g for males and 91-151 g for females at start of treatment.
Animal housing: The inhalation exposures for Groups 1, 3, 4 and 5 were conducted in (b) (4) within the (b) (4) This room was adjacent to (b) (4) where these animals were housed. Inhalation exposures for Group 2 were conducted in (b) (4), which was also where these animals were housed. The animals were housed 5 per cage by sex and dose group. Suspended polypropylene cages with stainless steel mesh tops (including internal food hopper) and bottoms were used (cage size 61 x 42.5 x 24 cm). Beneath each cage was a suspended tray containing absorbent paper. Each cage was supplied with a plastic water bottle. Cages, racks, trays, absorbent paper and water bottles were changed when necessary throughout the course of the study.

Restriction paradigm for dietary restriction studies: No
Drug stability/homogeneity: A micronized powder of QAB149 was used in the inhalation study.
Dual controls employed: Yes
Interim sacrifices: No
Deviations from original study protocol: Deviations in particle size determination are discussed.

Observation times
Mortality: Animals were checked at least twice daily for viability.
Clinical signs: Animals were observed for clinical signs at pre-dose, intermittently during the inhalation exposure, immediately on completion of exposure and at approximately 1 hr after exposure. Once each week all animals received a detailed clinical examination and palpation including appearance, movement and behavior patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. The size, appearance, position and duration of any masses detected were recorded.
Body weights: Body weights were recorded once weekly commencing one week before the start of treatment until the end of the first 13 weeks of the study. Thereafter, body weights were recorded once every 4 weeks until Week 77 and then once every 2 weeks until the end of the study.
Food consumption: Food consumption was recorded weekly for the first 13 weeks of the dosing period followed by one weekly measurement during the following 3 weeks. After this the quantity of food consumed was recorded over one week in every 4 weeks until the end of the study. Water consumption was qualitatively monitored by visual inspection, commencing one week pretrial and continuing to the end of the study.
Hematology: Blood samples for measurement of hematology parameters were collected from all surviving animals, during week 104.
Necropsy: All surviving animals were necropsied after completion of at least 104 weeks inhalation treatment (i.e., Days 729-730 and 735-743). Any animals killed prematurely or found dead were subjected to a detailed macroscopic examination as soon as possible after death. Duplicate femoral bone marrow smears were taken from each animal at necropsy and stained using Romanowsky stain; however, smears were not evaluated.
Histopathology: Tissue sections from all animals were cut 4-6 µm thick, stained with hematoxylin and eosin, and evaluated by the Study Pathologist. All tissues from all animals were examined by the Study Pathologist. All tissues from animals 110, 112, 205, 213, 214, 305, 306, 307, 308, 312, 313, 408, 409, 411, 412, 505, 506, 509, 513, 514, 515, 516, 517, 518, 519, 151, 153, 160, 162, 164, 252, 254, 260, 262, 263, 352, 353, 360, 361, 364, 451, 453, 460, 461, 463, 551, 553, 554, 562 and 564 and tumors and pre-neoplastic lesions from all animals were examined by a second pathologist. Slides for tissues with suspected treatment-related findings were also reviewed by the sponsor's pathologists during the draft report phase of the study. The data in the report reflected the consensus view of the Study Pathologist, (b) (4) Reviewing Pathologist and the Sponsor's Reviewing Pathologists.
Toxicokinetics: Blood samples (1.0 mL) were collected on days 1/2 and during week 52 from the first 10 animals of each group at the end of the inhalation period, at 0.5, 3, and

8 hr after the end of the inhalation period and 24 hr after the beginning of the inhalation period (just prior to the next dose). Samples were shipped to the Principal Investigator, Bioanalytics and Pharmacokinetics Novartis Pharma S.A.S., France. All samples collected from designated animals in both the QAB149 treated groups and Air Control groups were analyzed for QAB149 using a HPLC-MS/MS method. The LLOQ was 0.1 ng/mL using 100 µL serum.

Results

Mortality: There were no treatment-related effects on survival.

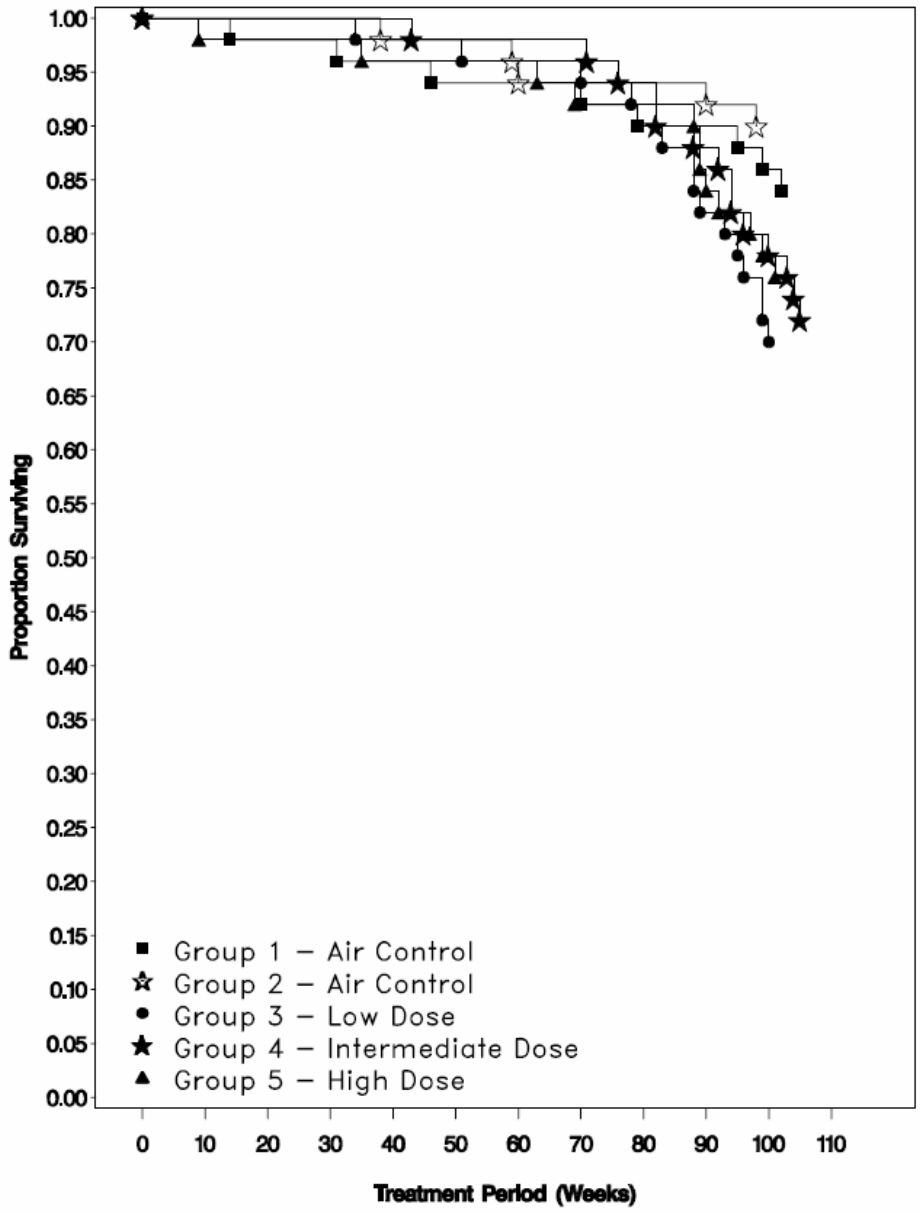
In total 128 animals were killed *in extremis* or died during the minimum 104 week treatment period. Approximately 10% of these animals were found dead in the cage; 15% were due to accidents or injuries while the remaining 75% were killed *in extremis*. Animals that were killed *in extremis* during the necropsy period were classified as terminal kill.

Table 4-1 The incidence of premature deaths in each group

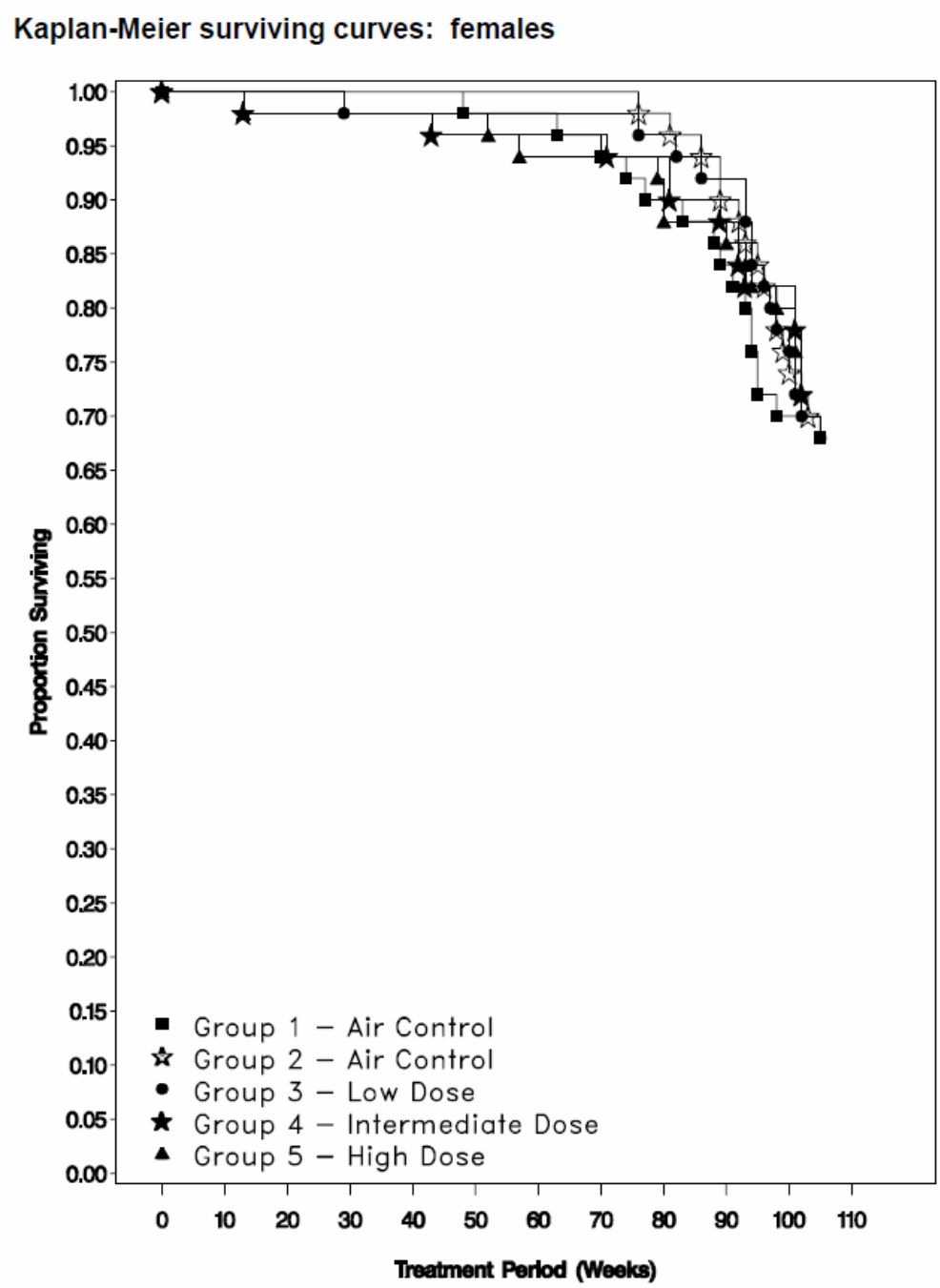
	Group No./Sex									
	Males					Females				
	1	2*	3	4	5	1	2*	3	4	5
Animals died/killed due to accidents or injuries	2	0	2	3	5	2	0	2	2	1
Animals found dead	1	0	0	2	0	1	1	3	1	4
Animals killed *in extremis*	5	5	13	10	7	13	14	11	11	7
Total	8	5	15	15	12	16	15	16	14	12
As % of Total in Group	16	10	30	30	24	32	30	32	28	24

* = Group 2 was housed and dosed in a separate room

Kaplan-Meier surviving curves: males



Note: Group 2 housed and dosed In a separate room



Note: Group 2 housed and dosed in a separate room

Clinical signs: Increased muscle mass was observed in all male and female treatment groups (Groups 3-5) with a dose-related pattern. The mean time to onset also displayed a dose-related pattern. This was attributed to a known pharmacological effect of β_2-adrenergic agonists.

Evaluation of palpable mass data showed no differences in the number of animals bearing palpable masses or in the mean time to the onset of palpable masses and no significant trend in the type of masses observed which could be attributed to treatment with QAB149.

Table 4-3 Number of animals with increased muscle mass and mean time of onset

Group No./ Treatment	Number of Animals in Group		Number of Animals with Muscle Mass Increased		Mean Time of Onset (Days)#	
	Male	Female	Male	Female	Male	Female
1 - Air Control	50	50	0	1	-	521
2 - Air Control*	50	50	0	0	-	-
3 - Low Dose	50	50	5	9	338	274
4 - Intermediate Dose	50	50	22	28	269	266
5 - High Dose	50	50	49	49	98	94

* = Group 2 was housed and dosed in a separate room
# = Means of individual mean onset time

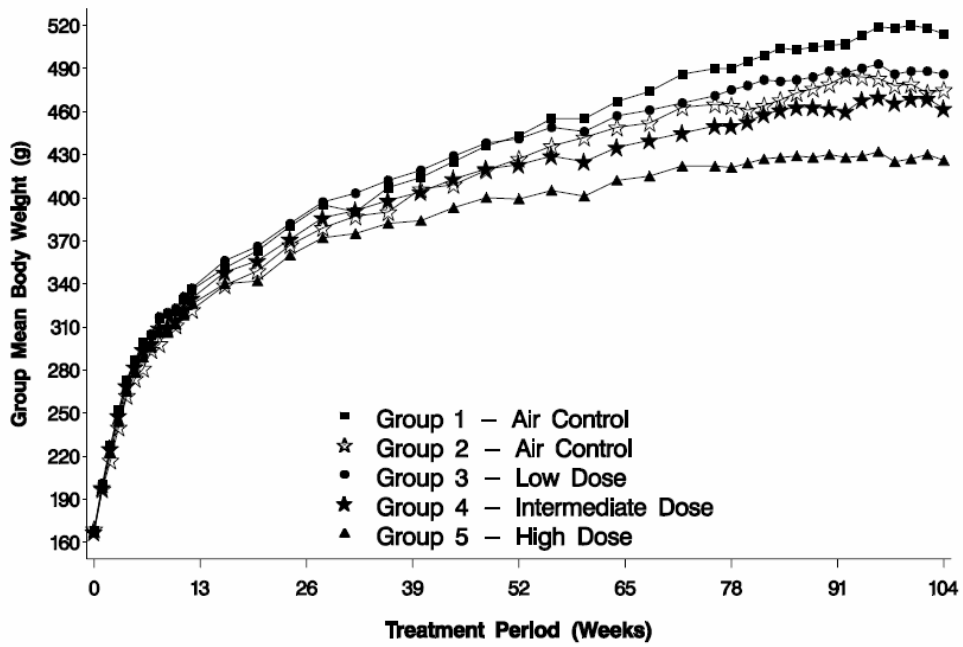
Body weights: Absolute body weights of males in the high dose group on days 546 and 728 were decreased to 88.26 and 86.15% of the pooled control, respectively. Absolute body weights of female treatment groups were unaffected. Decreased absolute body weight for males in the high dose group appears to indicate that a MTD was achieved for males.

Absolute body weights and body weight gain

	Males						Females				
	0-1	0-2	0.2	0.6	2		0-1	0-2	0.2	0.6	2
Day 0	168	169	168	167	167	Day 0	127	127	128	127	126
Day 196	395	379	397	386	372	Day 196	236	237	240	249	243
%Cont			102.584	99.7416	96.12403	%Cont			101.4799	105.2854	102.7484
Δ	227	210	229	219	205	Δ	109	110	112	122	117
%Init	135.119	124.2604	136.3095	131.1377	122.7545	%Init	85.82677	86.61417	87.5	96.06299	92.85714
%Cont			105.1044	101.1165	94.65246	%Cont			101.484	111.4155	107.6973
Day 364	443	427	441	423	399	Day 364	249	258	256	266	256
%Cont			101.3793	97.24138	91.72414	%Cont			100.9862	104.931	100.9862
Δ	275	258	273	256	232	Δ	122	131	128	139	130
%Init	163.6905	152.6627	162.5	153.2934	138.9222	%Init	96.06299	103.1496	100	109.4488	103.1746
%Cont			102.7333	96.91283	87.82725	%Cont			100.3953	109 8814	103.5824
Day 546	490	464	475	450	421	Day 546	278	284	277	283	275
%Cont			99.58071	94.33962	88.25996	%Cont			98.57651	100.7117	97.86477
Δ	322	295	307	283	254	Δ	151	157	149	156	149
%Init	191.6667	174.5562	182.7381	169.4611	152.0958	%Init	118.8976	123.622	116.4063	122.8346	118.254
%Cont			99.79611	92.54533	83.06188	%Cont			95.99736	101.2987	97.52113
Day 728	514	475	486	462	426	Day 728	301	300	293	288	290
%Cont			98.28109	93.4277	86.14762	%Cont			97.50416	95.84027	96.50582
Δ	346	306	318	295	259	Δ	174	173	165	161	164
%Init	205.9524	181.0651	189.2857	176.6467	155.0898	%Init	137.0079	136.2205	128.9063	126.7717	130.1587
%Cont			97.81766	91.28617	80.14616	%Cont			94.35789	92.79539	95.27469

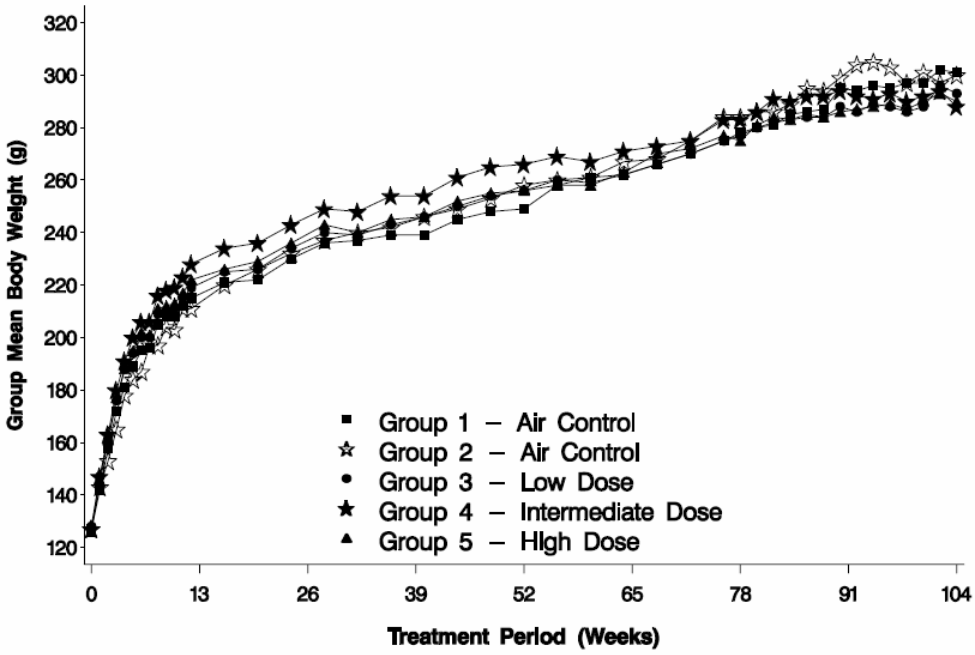
Parameters are percent of absolute control body weight, body weight gain (Δ), body weight gain expressed as a percentage of initial body weight on day 0 (normalized), and normalized body weight gain expressed as a percentage of the control.

Body weight curves: males



Note: Group 2 housed and dosed in a separate room

Body weight curves: females



Note: Group 2 housed and dosed in a separate room

Food consumption: Food consumption measurements over the 104-week exposure period were slightly increased for male (up to 5%) and female (up to 7%) treatment groups as compared to pooled controls. Increased food consumption is an expected effect with β_2-adrenergic agonists.

Hematology: White blood cell counts at week 104 were increased for all male treatment groups. These increases were primarily attributed to elevations of neutrophil counts. Slight elevations of lymphocyte and monocyte counts were also evident for male treatment groups. There were slight decreases of hemoglobin content, hematocrit, and red blood cell counts for all male and female treatment groups although the toxicological significance appeared to be minimal.

Hematology parameters, Week 104

Parameter	Males					Females				
	0-1	0.2	0.21	0.62	2.09	0-1	0-2	0.21	0.62	2.09
White blood cells x 10^9/L	4.34	4.03	4.91 (113%)	5.25 (121%)	5.01* (115%)	4.75	3.02*	4.08	4.58	4.01
Neutrophils x 10^9/L	1.21	1.17	1.49 (123%)	1.79* (148%)	1.53* (126%)	2.35	1.16	1.82	1.98	1.39
Lymphocytes x 10^9/L	2.86	2.57	3.11 (109%)	3.10 (108%)	3.16 (110%)	2.08	1.64*	1.97	2.28	2.34
Monocytes x 10^9/L	0.14	0.14	0.16 (114%)	0.20* (143%)	0.316 (114%)	0.19	0.12	0.16	0.19	0.15
Hemoglobin g/dL	15.6	15.4	15.1*	15.1*	15.2*	14.9	14.8	14.7	14.3*	14.3*
Hematocrit L/L	0.453	0.447	0.428*	0.437*	0.440*	0.428	0.428	0.425	0.413	0.414
Red blood cells x10^{12}/L	8.34	8.24	8.20	8.12	8.21	7.60	7.56	7.55	7.34	7.39

Gross pathology: Potential treatment-related gross pathological findings were observed in the pituitary gland, skeletal muscle, and stomach. Enlarged pituitary gland may correlate with findings of pituitary adenoma and carcinoma. Findings for skeletal muscle of enlarged and increased muscle mass were dose-related and a known pharmacological effect of β_2 adrenergic agonists. Findings of reddened stomach might correlate with histopathological findings of squamous cell hyperplasia at the limiting ridge.

Gross necropsy findings

Organ/Tissue	Males					Females				
	0-1	0-2	0.21	0.62	2.09	0-1	0-2	0.21	0.62	2.09
N =	50	50	50	50	50	50	50	50	50	50
Pituitary gland -enlarged	1	2	5	6	6	9	13	12	9	20
Skeletal muscle -enlarged	0	0	0	0	2	0	0	0	0	0
-increased muscle mass	0	0	0	4	15	0	0	4	12	21
Stomach -reddened	2	1	1	1	5	3	3	0	2	0

Histopathology:

Non-neoplastic: Potential treatment-related non-neoplastic findings were observed in the heart, nasal cavity, lung, larynx, thymus, ovaries, testes, epididymides, pancreas, and eye. Non-neoplastic findings were also observed in the eye that might be attributed to animal housing conditions.

An increased incidence and severity of progressive cardiomyopathy in the heart was observed for high dose females when compared to Control Groups 1 and 2. Increased incidences of progressive cardiomyopathy were also observed for all male treatment groups and females in the low and mid dose groups. The incidences of progressive cardiomyopathy were high for males in Control Groups 1 and 2 and females in Control Group 2, which might place some doubts on the significance of findings for male treatment groups and the low dose female group. Murine progressive cardiomyopathy is a rodent-specific condition which is characterized by fibrosis, minor inflammatory cell infiltrates, and myocardial degeneration or necrosis. It increases in incidence and severity with age, although severity remains relatively low in Wistar rats. This finding is attributed to treatment with QAB149.

In the nasal cavity, there were significant increases in the incidences of olfactory epithelial atrophy in males and females from the high dose group when compared with Control Groups 1 or 2. The lesion was present in the dorsal meatus of levels II and III of the nasal cavity and the severity ranged from minimal to moderate. Olfactory epithelial atrophy is an indicator of irritation in the nasal cavity and was attributed to treatment with QAB149. Significant increases in the incidences of rhinitis were observed for males and females in the mid and high dose groups when compared with both Control Groups 1 and 2. The lesion was present in the lateral meatus of level I of the nasal cavity and the severity ranged from minimal to moderate. Rhinitis was an indication of irritation in the nasal cavity and was attributed to treatment with QAB149.

In the lung, incidences of foamy alveolar macrophage accumulation were increased for females in the mid and high dose groups although the significance was questionable based upon high incidences observed for females in Control Groups 1 and 2. The incidence of inflammatory cell infiltration was increased for females in the high dose group.

In the larynx, incidences of mineralization of ventral diverticulum were increased for male and female treatment groups although dose-response relationships were not present.

In the thymus, incidences of tubular cystic hyperplasia were increased for all male treatment groups and females in the mid and high dose groups. However, the high incidences observed in males and females from Control Groups 1 and 2 places some doubt on the relationship of this finding to treatment with QAB149.

In the ovaries, the incidence of focal epithelial hyperplasia was increased for all female treatment groups although a dose-response relationship was not present. Incidences and severities of focal smooth muscle hyperplasia were increased for females in the mid and high dose groups. These finding might be a class effect of β_2-adrenergic agonists.

The incidence of squamous cell hyperplasia of the limiting ridge in the stomach was increased for males in the high dose groups. Incidences were comparable for female control and treatment groups.

In the testes, increased incidences of tubular mineralization were increased for males in the mid and high dose groups although a dose-response relationship was not present. Incidences of sperm accumulation were slightly increased for male treatment groups although a dose-response relationship was not present.

In the epididymides, incidences of oligospermia were increased for male treatment groups although a dose-response relationship was not present.

In the pancreas, incidences of acinar cell atrophy and interstitial inflammation were increased for females in the high dose group.

In the eye, incidences of retinal atrophy were increased for males and females in Control Group 2, which might be attributed to housing in a different room as compared to Control Group 1 and treatment groups. Incidences of cataracts and keratitis were increased for females in the high dose group.

Non-neoplastic lesions: All Animals

Organs/Tissues	Males					Females				
	0-1	0-2	0.2	0.6	2.0	0-1	0-2	0.2	0.6	2.0
Heart										
N=	50	50	50	50	50	50	50	50	50	50
-progressive cardiomyopathy										
minimal	20	24	28	26	31	5	14	14	20	20
mild	5	2	5	6	5	1	0	1	2	6
moderate	0	0	1	0	0	0	0	0	0	0
Total	25	26	34	32	36	6	14	15	22	26
Nasal cavity										
N=	50	50	50	50	50	50	50	50	50	50
-olfactory epithelial atrophy										
minimal	0	0	0	0	3	0	0	0	0	6
mild	0	0	0	0	1	0	0	0	0	6
moderate	0	0	0	0	0	0	0	0	0	2
Total	0	0	0	0	4	0	0	0	0	14
-rhinitis										
minimal	0	1	1	4	13	1	0	0	2	7
mild	0	0	0	3	3	0	0	0	2	1
moderate	0	0	1	0	0	0	0	0	1	0
Total	0	1	2	7	16	1	0	0	5	8
Lung										
N=	50	50	50	50	50	50	50	50	50	50

Organs/Tissues	Males					Females				
	0-1	0-2	0.2	0.6	2.0	0-1	0-2	0.2	0.6	2.0
-alveolar foamy macrophage accumulation										
minimal	17	14	19	19	22	13	18	10	21	24
mild	5	3	2	3	0	5	3	3	4	3
moderate	0	0	1	0	0	1	0	0	1	0
Total	22	17	22	22	22	19	21	13	26	27
-inflammatory cell infiltration	0	1	0	0	0	0	1	0	0	4
Larynx										
N=	50	50	49	50	50	50	50	48	50	50
-mineralization, ventral, diverticulum	4	2	5	11	8	0	0	4	1	5
Thymus										
N=	49	49	50	46	50	49	47	48	49	50
-tubular cystic hyperplasia										
minimal	3	12	14	10	16	12	8	10	7	15
mild	6	4	5	8	7	10	13	10	16	12
moderate	0	0	0	2	2	2	3	3	8	4
marked	0	0	0	0	0	0	0	1	0	1
Total	9	16	19	20	25	24	24	24	31	32
Ovary										
N=						50	50	50	50	49
-focal hyperplasia, epithelial						2	1	5	4	8
-focal hyperplasia, smooth muscle										
minimal						1	0	0	0	5
mild						0	1	0	1	4
moderate						0	0	0	1	1
Total						1	1	0	2	10
Stomach										
N=	50	50	50	50	50	49	50	50	50	49
-squamous cell hyperplasia, limiting ridge	2	1	2	3	10	5	4	3	6	6
Testis										
N=	50	50	50	50	50					
-tubular mineralization	5	9	9	16*	12					
-sperm accumulation, rete testis	0	0	2	1	2					
Epididymis										
N=	50	50	50	50	50					
-oligospermia	5	3	14	12	7					
Pancreas (Exocrine)										
N=	50	50	50	50	50	50	49	50	50	50
-acinar cell atrophy	13	10	12	13	14	8	6	7	4	14
-inflammation, interstitial	1	4	2	3	3	1	1	1	1	6
Eye										
N=	50	50	50	50	50	49	50	49	50	49
-retinal atrophy	4	**13**	4	1	4	11	**42**	6	8	6
-cataract	1	0	0	3	2	0	1	0	0	5
-keratitis	0	0	0	0	1	0	0	0	0	2

Neoplastic: Potential treatment-related neoplastic findings were evident in the pituitary gland and ovary. Other tumor findings were negative in both the trend test and pairwise comparison.

In the pituitary gland, combined incidences of adenoma and carcinoma were increased for all male treatment groups and females in the high dose group. For males, the combined incidences of adenoma and carcinoma were statistically significant by pairwise comparison for the mid and high dose groups. For females, the combined incidence of adenoma and carcinoma was statistically significant by trend test and statistically significant by pairwise comparison for the high dose group (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). The historical control mean and range of pituitary adenoma in male and female Wistar rats were reported to 27.74% (18.0-58.3%) and 54.89% (42.0-68.0%), respectively (Fundamental and Applied Toxicology 22: 65-72, 1994). From (b) (4) the mean incidences of pituitary adenoma and carcinoma in male Wistar rats were 31.89% (21.82-50.91%) and 0.54% (0.00-3.63%), respectively. From (b) (4) ((b) (4) the mean incidence of pituitary adenoma in female Wistar rats was 46.90% (1.67-61.82%). The findings in the present study appear to be within the published historical control range.

In the ovaries, leiomyoma was observed for 2 of 49 females in the high dose group. There were no findings in the low and mid dose groups. This tumor finding was statistically significant by trend test, but negative by pairwise comparison (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). Ovarian leiomyomas are a known class effect of β_2-adrenergic agonists. These tumor findings might be attributed to treatment with QAB-149 although the incidence was low as compared to other β_2 agonists. The induction of leiomyomas following chronic administration of a β_2-adrenergic agonist demonstrates that the exaggerated pharmacological effects of a drug can lead to an increased incidence of a specific tumor type. Ovarian leiomyomas are extremely rare spontaneous tumors in rats. Proliferation of the mesovarian smooth muscle is considered an adaptive physiologic response to prolonged stimulation of the β_2-receptor, which has the effect of causing muscle relaxation. Tumor induction has been shown to be a function of adrenergic stimulation, as concurrent administration of the adrenergic blocker, propanolol, prevents their development. Leiomyomas of ovarian tissue are rare in women, and no increase in incidence has been reported despite the long history of use of β_2-stimulants in the treatment of bronchial asthma.

In the brain, benign granular cell tumors were observed for 1 male and 1 female in the high dose group. The mean and range of granular cell tumor for males was reported to be 0.44% (0.0-2.0%) (Fundamental and Applied Toxicology 22: 65-72, 1994).

In the femur, osteosarcomas were observed for 1 male in each of the mid and high dose groups. Osteosarcoma has been reported to occur in 1 or 2 control male Wistar rats (Fundamental and Applied Toxicology 22: 65-72, 1994).

In the vertebrae 2, an osteosarcoma was observed for 1 male in the high dose group. Osteosarcoma has been reported to occur in 1 or 2 control male Wistar rats (Fundamental and Applied Toxicology 22: 65-72, 1994).

In the mammary gland, the incidence of fibroadenomas was increased for females in the mid dose group; however, the incidence was unaffected in the high dose group. The mean and range of mammary fibroadenoma in female Wistar rats was reported to be 25.26% (12.9-34.0%). The findings in the present study appear to be within the published historical control range (Fundamental and Applied Toxicology 22: 65-72, 1994).

In the skin and subcutis, incidences of keratoaconthomas were increased for male treatment groups and females in the high dose group. The mean and range of keratoacanthoma in male and female rats was reported to be 0.73% (0.0-2.0%) and 0.15% (0.0-2.0%), respectively (Fundamental and Applied Toxicology 22: 65-72, 1994).

Neoplastic lesions: All Animals that received the vehicle or dosing solutions for up to 104 weeks

Organs/Tissues	Males					Females				
	0-1	0-2	0.2	0.6	2.0	0-1	0-2	0.2	0.6	2.0
Pituitary gland										
N=	50	47	50	50	49	50	48	50	49	50
-carcinoma, anterior lobe [M]	0	0	0	0	1	1	0	1	0	0
-adenoma, anterior lobe [B]	3	4	7	11	11	12	19	16	18	28
-adenoma, intermediate lobe [B]	0	1	2	1	0	1	1	0	1	0
Adenoma + Carcinoma (Total)	3	5	9	12	12	14	20	17	19	28
Ovary						50	50	50	50	49
N=										
-leiomyoma [B]						0	0	0	0	2
Brain										
N=	50	50	50	50	50	50	50	50	50	49
-granular cell tumor [B]	0	0	0	0	1	0	0	0	0	1
Femur										
N=	50	49	48	50	50	49	50	50	50	49
-osteosarcoma [M]	0	0	0	1	1	0	0	0	0	0
Vertebrae2										
N=	2	1	0	1	3	3	5	1	5	3
-osteosarcoma [M]	0	0	0	0	1	0	0	0	0	0
Mammary gland										
N=										
-fibroadenoma						1	3	4	8	1
Skin and Subcutis	50	50	50	50	50	50	50	50	50	50
N=										
-keratoaconthoma [B]	2	0	3	4	4	1	1	1	0	3

Toxicokinetics: Plasma AUC values for QAB149 on days 1/2 and week 52 increased in an approximately dose proportional manner. AUC values on days 1/2 and week 52 were generally comparable for the low and mid dose groups; however, AUC values for the high dose group were lower during week 52 as compared to day 1/2. There were no significant differences of AUC values between male and female rats.

All concentrations of QAB149 in samples from control groups were measured below the LLOQ (0.100 ng/mL) except in three samples on days 1/2 at 24 hr after the beginning of the inhalation period as follows: female rat #158 (Control Group 1 in the same room as the treated groups) and from the male rat #208 and the female rat #259 (Control Group 2, in a separate room). These concentrations were confirmed by re-analysis. The concentrations measured were close to the LLOQ (0.233, 0.126, and 0.126 ng/mL), especially in the samples from the control group located in a separate room (0.126 ng/mL). In the low dose group, the concentration measured at the end of inhalation was around 7 ng/mL. QAB149 was not detected in control plasma samples during week 52.

Table 2-1 Toxicokinetic parameters of QAB149 in rat serum

Dose: 0.2 mg/kg/day					
		Days 1/2		Week 52	
Parameter	Units	Males	Females	Males	Females
t_{max}	h	0.07 (a)	0.07 (a)	0.07 (a)	0.07 (a)
C_{max}	ng/mL	7.1	6.86	4.97	5.51
C_{max} / dose	(ng/mL)/(mg/kg/day)	35.5	34.3	24.9	27.6
AUC(0-24h)	ng.h/mL	20.4	18.7	18.8	22.5
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	102	93.3	94.1	112
Dose: 0.6 mg/kg/day					
		Days 1/2		Week 52	
Parameter	Units	Males	Females	Males	Females
t_{max}	h	0.2 (a)	0.2 (a)	0.2 (a)	0.2 (a)
C_{max}	ng/mL	23.8	29.4	20.4	31.4
C_{max} / dose	(ng/mL)/(mg/kg/day)	39.7	49.0	34.0	52.3
AUC(0-24h)	ng.h/mL	48.9	50.5	51.2	55.6
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	81.5	84.2	85.3	92.6
Dose: 2.0 mg/kg/day					
		Days 1/2		Week 52	
Parameter	Units	Males	Females	Males	Females
t_{max}	h	0.67 (a)	0.67 (a)	0.67 (a)	0.67 (a)
C_{max}	ng/mL	46.1	54.6	38.2	48.2
C_{max} / dose	(ng/mL)/(mg/kg/day)	23.1	27.3	19.1	24.1
AUC(0-24h)	ng.h/mL	138	161	114	118
AUC(0-24h) / dose	(ng.h/mL)/(mg/kg/day)	69.1	80.6	57.2	59.0

(a): first time-point taken at the end of the inhalation period

Histopathology inventory (optional)

Study	26-week carcinogenicity study	2-year carcinogenicity study
Species	TgrasH2@Tac hemizygous mice	Rats
Adrenals	X	X

Aorta	X	X
Blood smear	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X*	X
Cecum	X	X
Cervix	X	X
Clitoral gland	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X (w/Optic nerve)
Fallopian tube		
Gall bladder	X	
Gross lesions	X	X
Harderian gland	X	X
Heart	X*	X
Ileum	X	X
Implant		X
Injection site		
Jejunum	X	X
Kidneys	X*	X
Lachrymal gland	X	X
Larynx		X
Liver	X*	X
Lungs	X	X
Lymph nodes, bronchial	X	X
Lymph nodes, cervical		X
Lymph nodes mandibular	X	
Lymph nodes, mesenteric	X	X
Lymph nodes, submandibular		X
Mammary Gland	X	X
Nasal cavity	X	X
Optic nerves		X (w/Eyes)
Ovaries	X*	X
Oviducts		X
Pancreas	X	X
Parathyroid	X	X (w/Thyroid)
Peripheral nerve		Sciatic
Pharynx		X
Pituitary	X	X
Preputial gland	X	X
Prostate	X	X
Rectum	X	X
Rib		X
Salivary gland	X	X
Salivary gland - parotid		X
Sciatic nerve	X	X

Seminal vesicles	X	X (w/coagulating gland)
Skeletal muscle	X	X (thigh)
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 (w/PT)
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		X

X, histopathology performed

*, organ weight obtained

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

QAB149 is a long-acting β_2-adrenergic agonist intended for once a day treatment of COPD. QAB149 was not genotoxic as assessed by negative results in the *in vitro* assays, Ames and chromosomal aberration (Chinese hamster cells) and in the *in vivo* assay, bone marrow micronucleus (rat). The carcinogenic potential of QAB149 was assessed in a 26-week oral (gavage) carcinogenicity study with CB6F1/TgrasH2 hemizygous mice and a 24-month inhalation oncogenicity study with Sprague-Dawley rats.

26-week oral (gavage) carcinogenicity study with CB6F1/TgrasH2 hemizygous mice:

QAB149 was administered by oral gavage to male and female CB6F1/Jic-TgrasH2@Tac hemizygous mice at doses of 0, 100, 300 and 600 mg/kg/day of base and to male and female CB6F1 wild-type mice at doses of 0 and 600 mg/kg/day of base for at least 26 weeks. An additional group of CB6F1/Jic-TgrasH2@Tac hemizygous mice received 75 mg/kg N-methyl-N-nitrosourea, as an intraperitoneal injection on day 1 only, and served as a positive control. The sponsor used doses of QAB149 recommended by the ECAC (see meeting minutes dated December 17, 2003). The duration of treatment was at least 26 weeks, which is acceptable.

Deaths or moribund sacrifices of 1 transgenic female in the 300 mg/kg/day group and 1 transgenic male and 3 transgenic females in the 600 mg/kg/day group were potentially treatment-related. Moribund sacrifices of 1 wild-type male and 1 wild-type female in the 600 mg/kg/day group were potentially treatment-related. Other deaths and moribund sacrifices were attributed to oral gavage errors.

Based upon examination of body weight curves, body weight gains appeared to be lower for the three transgenic male QAB149 treatment groups; however, body weight gains were unaffected for the three transgenic female QAB149 treatment groups.

Deaths at 300 and 600 mg/kg/day as well as decreased body weights for males at all doses suggest that a MTD was achieved and possibly exceeded in the study.

QAB149 treatment-related histopathological findings were primarily evident in the stomach and kidneys.

Uterine endometrial stromal polyps were observed for 3 of 25 females in the 600 mg/kg/day group. This tumor finding was statistically significant by trend test, but negative by pairwise comparison (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). It is noted that in a 2-year carcinogenicity study with mice that received another β2-adrenergic agonist, uterine endometrial stromal polyps were observed at a much higher incidence.

There were neoplastic findings for MNU-treated mice in several tissues.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 4, 2009.

24-month inhalation oncogenicity study in rats:
Rats in the control-1, control-2, low dose, mid dose, and high dose groups were exposed to achieved inhalation doses of 0, 0, 0.21, 0.62, and 2.09 mg/kg/day, respectively. The route was the same as that used in the clinical setting. The duration of treatment was at least 104 weeks, which is acceptable.

There were no treatment-related effects on survival. Absolute body weights of males in the high dose group on days 546 and 728 were decreased to 88.26 and 86.15% of the pooled control, respectively. Decreased absolute body weight for males in the high dose group appears to indicate that a MTD was achieved for males.

Potential treatment-related non-neoplastic findings were observed in the heart, nasal cavity, lung, larynx, thymus, ovaries, testes, epididymides, pancreas, and eye. Non-neoplastic findings were also observed in the eye that might be attributed to animal housing conditions. Findings in the heart and ovaries appear to be characteristic of β_2-adrenergic agonists. Findings in the testes and epididymides may also be characteristic of β_2-adrenergic agonists. Findings in the nasal cavity, larynx, and lung might be related to irritation associated with nose-only administration of QAB149.

Potential treatment-related neoplastic findings were evident in the pituitary gland and ovary.

In the pituitary gland, combined incidences of adenoma and carcinoma were increased for all male treatment groups and females in the high dose group. For males, the combined incidences of adenoma and carcinoma were statistically significant by pairwise comparison for the mid and high dose groups (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). For females, the combined incidence of adenoma and carcinoma was statistically significant by trend test and statistically significant by pairwise comparison for the high dose group. The historical control mean and range of pituitary adenoma in male and female Wistar rats were reported to 27.74% (18.0-58.3%) and 54.89% (42.0-68.0%), respectively (Fundamental and Applied Toxicology 22: 65-72, 1994). From (b) (4) the mean incidences of pituitary adenoma and carcinoma in male Wistar rats were 31.89% (21.82-50.91%) and 0.54% (0.00-3.63%), respectively. From (b) (4) ((b) (4) the mean incidence of pituitary adenoma in female Wistar rats was 46.90% (1.67-61.82%). The findings in the present study appear to be within the published historical control range.

In the ovaries, leiomyoma was observed for 2 of 49 females in the high dose group. There were no findings in the low and mid dose groups. This tumor finding was statistically significant by trend test, but negative by pairwise comparison (See Statistical Review by Dr. Mohammad Atiar Rahman dated August 10, 2009). Ovarian leiomyomas are a known class effect of β_2-adrenergic agonists. These tumor findings might be attributed to treatment with QAB-149 although the incidence was low as compared to other β_2 agonists. The induction of leiomyomas following chronic administration of a β_2-adrenergic agonist demonstrates that the exaggerated pharmacological effects of a drug can lead to an increased incidence of a specific tumor type. Ovarian leiomyomas are extremely rare spontaneous tumors in rats. Proliferation of the mesovarian smooth muscle is considered an adaptive physiologic response to prolonged stimulation of the β_2-receptor, which has the effect of causing muscle relaxation. Tumor induction has been shown to be a function of adrenergic stimulation, as concurrent administration of the adrenergic blocker, propanolol, prevents their development. Leiomyomas of ovarian tissue are rare in women, and no increase in incidence has been reported despite the long history of use of β_2-stimulants in the treatment of bronchial asthma.

The study was judged to be negative by the Executive CAC (See Meeting Minutes dated August 4, 2009.

Conclusions:
Increased incidences of uterine endometrial stromal polyps in the 26-week oral (gavage) carcinogenicity study with female CB6F1/TgrasH2 hemizygous mice and ovarian leiomyomas in the 24-month inhalation oncogenicity study with female Sprague-Dawley rats were statistically significant by trend test, but not significant by pairwise comparison (See Executive CAC Meeting Minutes dated August 4, 2009). These tumors have been observed with other β_2-adrenergic agonists and might be attributed to treatment despite the lack of statistical significance.

Recommendations: None.

Reviewer Signature _____
Timothy W. Robison, Ph.D.

Supervisor Signature _____
Jean Wu, M.D., Ph.D.
Concurrence Yes ___ No ___

cc: list:
NDA 22-383, HFD-570
HillC, HFD-570
WhitehurstV, HFD-570
WuJ, HFD-570
RobisonT, HFD-570

APPENDIX/ATTACHMENTS

Appendix 1. ECAC Meeting Minutes Dated March 4, 2003
Appendix 2. ECAC Meeting Minutes dated July 15, 2003
Appendix 3. ECAC Meeting Minutes dated December 17, 2003
Appendix 4. ECAC Meeting Minutes dated August 4, 2009

Appendix 1. ECAC Meeting Minutes Dated March 4, 2003

Executive CAC
Date of the Meeting: March 4, 2003
Committee: Joseph Contrera, Ph.D., HFD- 901, Acting Chair
Barry Rosloff, Ph.D., HFD-120, Alternate Member
Jeri El-Hage, Ph.D, HFD-510, Alternate Member
Robin Huff, Ph.D, HFD-570, Team Leader
Virgil Whitehurst, Ph.D., Presenting Reviewer

Author of the Draft: Virgil Whitehurst, Ph.D.
The following information reflects a brief summary of the committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity 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 section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND 66,337
Drug Name: QAB149
Sponsor: Novartis Pharmaceutical Corporation

Background: QAB149 is a long- acting β_2 adrenergic receptor agonist intended for once a day treatment of asthma/COPD. The target organ for this class of drugs is the cardiovascular system, i.e., tachycardia, QTc interval changes and myocardial necrosis. The dose-limiting toxicity in the rat is nasal lesions, characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium. QAB149 was not genotoxic as assessed by negative results in the *in vitro* assays, Ames and chromosomal aberration (Chinese hamster cells) and in the *in vivo* assay, bone marrow micronucleus (rat).

Rat Carcinogenicity Protocol and Dose Selection: The sponsor is planning to conduct a two-year carcinogenicity study in the Wistar rat using inhalation doses of 0, 0.2, 0.6 and 2.0 mg/kg. The dose selection was based on the results of 2, 4 and 13-week inhalation toxicity studies in the rat. The doses in the 13-week study were 0. 0.3, 1.01 and 3.08 mg/kg. The results of this study revealed changes in the nasal cavities (degeneration of the olfactory epithelium of the dorsal meatus) in 19/20 rats high dose rats; severity ranged from minimal to marked, with most animals showing moderate changes. The lesion was characterized by loss of sustentacular cell cytoplasm and thinning and disorganization of the epithelium, and the changes were described as dose limiting. The rats at the mid and high dose exhibited minimal to mild squamous metaplasia and hyperplasia of the larynx. The results from the 2 and 4-week studies also showed changes in the nasal cavity. Based on the nasal changes in the rats at doses of 2.77 mg/kg and above, the sponsor selected 2.0 mg/kg was selected as the high dose for the carcinogenicity study.

Executive CAC Recommendations and Conclusions:

1. The Committee concurred with the sponsor's proposed doses of 0, 0.2, 0.6, and 2.0 mg/kg based on MTD (degeneration of olfactory epithelium of dorsal meatus in nasal cavity).

2. The Committee concurred with the sponsor's plan to use the micronized powder of QAB149.

3. The Committee concurred with the sponsor's plan to administer the drug by inhalation, provided that the second carcinogenicity study is designed to optimally assess systemic consequences of drug treatment.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD 570
/Team leader, HFD-570/RHuff
/Reviewer, HFD-570/VWhitehurst
/CSO/PM, HFD-570/COstroff
/ASeifried, HFD-024

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/s/

Joe Contrera

3/10/03 11:25:24 AM

Appendix 2. ECAC Meeting Minutes dated July 15, 2003

Executive CAC
Date of the Meeting: July15, 2003
Committee: Joseph Contrera, Ph.D., HFD- 901, Acting Chair
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Jeri El-Hage, Ph.D., HFD-510, Alternate Member
Joseph Sun, Ph.D., HFD-570, Team leader
Virgil Whitehurst, Ph.D., Presenting Reviewer

Author of the Draft: Virgil Whitehurst, Ph.D.

The following information reflects a brief summary of the committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr 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 section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND: IND 66,337
Drug Name: QAB149
Sponsor: Novartis Pharmaceutical Corporation

Background: QAB149 is a long- acting β_2 adrenergic receptor agonist intended for once a day treatment of asthma/COPD. The target organ for this class of drugs is the cardiovascular system, i.e., tachycardia, QTc interval changes and myocardial necrosis. QAB149 was not mutagenic as assessed by negative results in the *in vitro* assays, Ames and chromosomal aberration (Chinese hamster cells) and in the *in vivo* assay, bone marrow micronucleus (rat). With CAC concurrence (see meeting minutes dated March 24, 2003), a 2-year inhalation carcinogenicity study in rats has been initiated. The doses were 0, 0.2, 0.6 and 2.0 mg/kg. Furthermore, the CAC recommended that the second carcinogenicity study be designed to optimally assess the carcinogenic potential of QAB149 following systemic exposure. The sponsor is proposing 2 protocols (26-week TgHras2 and 104-week carcinogenicity studies). Only one of them will be performed.

26-Week Oral Mouse TgHras2 Carcinogenicity Study Protocol and Dose Selection:

The sponsor proposed doses of 0, 50, 250 and 500 mg/kg in the CB6F-1 mouse. The dose selection for this study was based on the results of a 1-week oral feasibility in the [CRL: CD-1(ICR) BR] mouse. The doses in the study were 0,

100, 250 and 500 mg/kg. No apparent dose limiting toxic effects were reported, however, the non-GLP study did not include clinical chemistry, hematology, urology parameters or complete histopathology. The doses proposed by the sponsor for the 26-week TgHras2 carcinogenicity study were based on the pharmacokinetic (rodent/human AUC ratio) endpoint. It is a policy that the pharmacokinetic endpoint cannot be used as a basis for dose selection for transgenic models such as the 26-week TgHras2 carcinogenicity study. Furthermore, the CB6F-1 strain mouse should be used for TgHras2 carcinogenicity study dose selection. The CB6F-1 strain mouse is the base strain used for TgHras2 mouse studies. For these reasons the committee could not concur with the doses proposed for the 26week TgHras2 carcinogenicity study.

104-Week Oral Mouse Carcinogenicity Study Protocol and Dose Selection

The sponsor proposed doses of 0, 10, 25 and 50 mg/kg in the CD-1 mouse based on the results of the 1-week oral feasibility in the [CRL: CD-1(ICR) BR] mouse and an extrapolated >25 fold rodent/human AUC ratio. The doses in the 1week oral study were 0, 100, 250 and 500 mg/kg. The non-GLP study did not include clinical chemistry, hematology, urology parameters or complete histopathology. Although there was no apparent dose limiting toxicity in the 1week study the committee had serious concerns about the potential consequences of longer term exposure at the high systemic exposure multiples attained in the oral study. For this reason the committee was reluctant to accept the sponsor's proposed doses especially based on the limited results of a 1week non-GLP study. The systemic exposure of the drug at 100 mg/kg/day in the oral 1week study (M/F: 946/1100 ng.h/ml) was several multiples of the exposure attained at the maximum dose (5mg/kg/day) in a13 week inhalation study (M/F: 236/419 ng.h/ml) in mice.

Executive CAC Conclusions and Recommendations

26-Week Oral TgHras2 Carcinogenicity Study

The committee could not concur with the sponsor's dose selection. There did not appear to be any dose limiting effects reported in the 1week dose ranging study. However, the non-GLP study did not include clinical chemistry, hematology, urology parameters or complete histopathology and therefore it is possible that toxic effects may be present and not noted. The doses proposed by the sponsor for the 26-week TgHras2 carcinogenicity study were based on the pharmacokinetic (rodent/human AUC ratio) endpoint. It is a policy that the pharmacokinetic endpoint cannot be used as a basis for dose selection for transgenic models such as the 26-week TgHras2 carcinogenicity study.

It is recommended that the sponsor conduct a 4-week oral dose range finding study in CB6F-1 mouse to determine the doses for the 26-week oral TgHras2 carcinogenicity study using either the MTD or maximum feasible dose as an

endpoint.

104-Week Oral Carcinogenicity Study

Due to the short duration of the 1week study, lack of detailed histopathology data and concerns about potential toxicity that may be associated with the relatively high systemic exposures attained at the doses proposed by the sponsor, the committee could not concur on the dose selection for a 104-week mouse oral carcinogenicity study.

It is recommended that a 13-week dose ranging study be conducted in the same strain of mouse to determine the dose for the 104-week oral mouse carcinogenicity study. Dose selection based on the PK (AUC ratio) endpoint will be applicable for the current inhalation drug product at the current proposed human inhalation dose but may not be applicable to an oral drug product due to higher doses and systemic exposure that can be attained by the oral route.

Other Recommendations

26-Week Oral TgHras2 Carcinogenicity Study

The sponsor should consider conducting histopathological examination in all dose groups. The sponsor is reminded that if they plan to conduct the histopathological evaluation of tissues from only the control and high dose animals, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances: a) any macroscopic findings in the low or mid dose groups for that particular tissue or organ, b) an increase in the incidence of tumors (rare or common) observed in the high dose animals for a particular tissue or organ even if the increase is not statistically significant, c) any increase in tumors that should be analyzed across tissue sites as well as by tissue site (e.g. hemangiosarcoma, lymphoma; see McConnell et al., JNCI 76:283, 1986) will necessitate that all relevant tissues from that dose level and the next lower dose level(s) be examined, or d) an excessive decrease in body weight or survival in the examined dose group. We note that given the limited experience with transgenic mouse models, the types of tumors that may need to be combined may not be adequately or completely described in the recommendations by McConnell et al.

104-Week Oral Carcinogenicity Study

If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:
(a) For any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups
(b) For an increase in the incidence of tumors (rare or common) in the high dose

group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group
(c) For an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,
(d) For an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD 570
/Team leader, HFD-570/JSun
/Reviewer, HFD-570/VWhitehurst
/CSO/PM, HFD-570/AGreen
/ASeifried, HFD-024

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/s/

Joe Contrera

7/24/03 01:45:29 PM

Appendix 3. ECAC Meeting Minutes dated December 17, 2003

Executive CAC
Date of the Meeting: December 16, 2003

Committee: David Jacobson-Kram, Ph.D., HFD- 024, Chair
Joe Contrera, Ph.D, HFD-901, Member
Abby Jacobs, Ph.D., HFD-540, Member
Robert Osterberg, Ph.D., HFD-510, Alternate Member
Tim McGovern, Ph.D., HFD-570, Team leader
Virgil Whitehurst, Ph.D., Presenting Reviewer

Author of Draft: Virgil Whitehurst, Ph.D.

The following information reflects a brief summary of the committee discussion and its recommendations. Detailed study information can be found in the individual review.

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 section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND#: IND 66,337
Drug Name: QAB149
Sponsor: Novartis Pharmaceutical Corporation

Background: QAB149 is a long- acting β_2 adrenergic receptor agonist intended for once a day treatment of asthma/COPD. The target organ for this class of drugs is the cardiovascular system, i.e., tachycardia, QTc interval changes and myocardial necrosis. QAB149 was not mutagenic as assessed by negative results in the *in vitro* assays, Ames and chromosomal aberration (Chinese hamster cells) and in the *in vivo* assay, bone marrow micronucleus (rat). With CAC concurrence (see meeting minutes dated March 24, 2003), a 2-year inhalation carcinogenicity study in rats has been initiated. Furthermore, the CAC recommended that the second carcinogenicity study in mice be designed to optimally assess the carcinogenic potential of QAB149 following systemic exposure. Previously, the sponsor submitted protocols with dose selection for a 104 week carcinogenicity and a 26 week TgHras2 carcinogenicity in the CD-1 mouse (see meeting minutes dated July 15, 2003). The sponsor planned to carry out only one of the studies. The dose selection was based on pharmacokinetic data from a 1 week oral study and a 13 inhalation study in the CD-1 mouse. The CAC did not concur with the dose selections for the studies because of the duration of the oral study and because the doses were based on PK data. The committee recommended that the doses for a 104 carcinogenicity study should be based on a 13 week dose ranging study and doses for the 26 week TgHras2 should be based on the MTD in at least a 4 week oral dose ranging study. The sponsor is currently proposing doses for a 26-week TgHras2 week carcinogenicity study in the CB6F-1 mouse based on the MTD in an 8 week oral toxicity study.

26-Week Oral Mouse TgHras2 Carcinogenicity Study Protocol and Dose Selection:

The sponsor proposed oral (gavage) doses of 0, 30, 100 and 300 mg/kg in 0.5% hydroxypropylcellulose in the CB6F-1 TgHras2 heterozygous mouse (n = 25/sex/group). The dose selection for this study was based on the results of an 8-week oral toxicity study in the CB6F-1 mouse in which the maximum tolerated dose (MTD) was estimated by the sponsor to be 300 mg/kg. The doses in the study were 0, 100, 300, 1000 and 4123-3000 mg/kg (animals were initially administered a higher than anticipated dose). There were deaths in the mice in the 2 highest dose groups. The dose limiting toxicity was the kidney (basophilic tubules, tubular degeneration, casts and increases in BUN). These changes were most prominent in the mice in the two highest dose groups. There were also gastrointestinal tract changes (erosion, decreased goblet cell) in the mice in the 100 mg/kg dose group and higher. A positive control group (MNU, single ip dose of 75 mg/kg) is to be included. In addition, 25 wild type mice/sex will be tested at doses of 0 and 300 mg/kg. Ten mice/sex/QAB149 dose group and 2/sex for the vehicle control will be included for kinetic assessment. Palpable mass examinations and tissue pathology is to be conducted on all main study animals.

Executive CAC Conclusions and Recommendations

1. The Committee recommended a high dose of 600 mg/kg for the TgHras mouse assay due to the observed lethality and significant renal toxicity at doses of 1000 mg/kg or greater in the 8-week dose ranging study. The committee did not feel that the dose of 300 mg/kg achieved the MTD in that study.

2. The Committee recommended doses of 100 and 300 mg/kg for the low and mid dose groups, respectively.

David Jacobson-Kram, Ph.D.,
Chair, Executive CAC

cc:
/Division File, HFD 570
/Team leader, HFD-570/TMcGovern
/Reviewer, HFD-570/VWhitehurst
/CSO/PM, HFD-570/AGreen
/ASeifried, HFD-024

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/s/

David Jacobson-Kram

12/17/03 02:58:04 PM

Appendix 4. ECAC Meeting Minutes dated August 4, 2009

Executive CAC
Date of Meeting: August 4, 2009

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Paul Brown, Ph.D., OND IO, Member
Todd Bourcier, Ph.D., DMEP, Alternate Member
Jean Wu, M.D., Ph.D., DPAP, Team Leader
Tim Robison, Ph.D., DPAP, Presenting Reviewer

Author of Draft: Tim Robison, Ph.D., DPAP

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 22-383
Drug Name:
Trade name: Arcapta™ (b) (4) I (b) (4)
Generic name: Indacaterol maleate inhalation powder
Code name: QAB-149
Sponsor: Novartis Pharmaceuticals Corporation

Background:
QAB149 was not genotoxic as assessed by negative results in the *in vitro* assays, Ames and chromosomal aberration (Chinese hamster cells) and in the *in vivo* assay, bone marrow micronucleus (rat). The carcinogenic potential of QAB149 was assessed in a 24-month inhalation oncogenicity study in Sprague-Dawley rats and a 26-week oral (gavage) carcinogenicity study with CB6F1/TgrasH2 hemizygous mice.

Rat Carcinogenicity Study
Rats in the control-1, control-2, low dose, mid dose, and high dose groups were exposed to achieved inhalation doses of 0, 0, 0.21, 0.62, and 2.09 mg/kg/day, respectively. The route was the same as that used in the clinical setting. The duration of treatment was at least 104 weeks, which is acceptable.

There were no treatment-related effects on survival. Absolute body weights of males in the high dose group on days 546 and 728 were decreased to 88.26 and 86.15% of the pooled control, respectively. Decreased absolute body weight for males in the high dose group appears to indicate that a MTD was achieved for males.

Potential treatment-related non-neoplastic findings were observed in the heart, nasal cavity, lung, larynx, thymus, ovaries, testes, epididymides, pancreas, and eye. Non-neoplastic findings were also observed in the eye that might be attributed to animal housing conditions. Findings in the heart and ovaries appear to be characteristic of β_2-adrenergic agonists. Findings in the testes and epididymides may also be characteristic of β_2-adrenergic agonists. Findings in the nasal cavity,

larynx, and lung might be related to irritation associated with nose-only administration of QAB149.

Potential treatment-related neoplastic findings were evident in the pituitary gland and ovary.

In the pituitary gland, combined incidences of adenoma and carcinoma were increased for all male treatment groups and females in the high dose group. For males, the combined incidences of adenoma and carcinoma were statistically significant by pairwise comparison for the mid and high dose groups. For females, the combined incidence of adenoma and carcinoma was statistically significant by trend test and statistically significant by pairwise comparison for the high dose group. The historical control mean and range of pituitary adenoma in male and female Wistar rats were reported to 27.74% (18.0-58.3%) and 54.89% (42.0-68.0%), respectively (Fundamental and Applied Toxicology 22: 65-72, 1994). From (b) (4) ((b) (4) the mean incidences of pituitary adenoma and carcinoma in male Wistar rats were 31.89% (21.82-50.91%) and 0.54% (0.00-3.63%), respectively. From (b) (4) ((b) (4) the mean incidence of pituitary adenoma in female Wistar rats was 46.90% (1.67-61.82%). The findings in the present study appear to be within the published historical control range.

In the ovaries, leiomyoma was observed for 2 of 49 females in the high dose group. There were no findings in the low and mid dose groups. This tumor finding was statistically significant by trend test, but negative by pairwise comparison. It was noted that ovarian leiomyomas have been previously reported for other beta-adrenergic agonist drugs at much higher incidences, and are considered of limited relevance to human risk.

Tg.rasH2 Mouse Carcinogenicity Study

QAB149 was administered by oral gavage to male and female CB6F1/Jic-TgrasH2@Tac hemizygous mice at doses of 0, 100, 300 and 600 mg/kg/day of base and to male and female CB6F1 wild-type mice at doses of 0 and 600 mg/kg/day of base for at least 26 weeks. An additional group of CB6F1/Jic-TgrasH2@Tac hemizygous mice received 75 mg/kg N-methyl-N-nitrosourea, as an intraperitoneal injection on day 1 only, and served as a positive control. The sponsor used doses of QAB149 recommended by the ECAC (see meeting minutes dated December 17, 2003). The duration of treatment was at least 26 weeks, which is acceptable.

Deaths or moribund sacrifices of 1 transgenic female in the 300 mg/kg/day group and 1 transgenic male and 3 transgenic females in the 600 mg/kg/day group were potentially treatment-related. Moribund sacrifices of 1 wild-type male and 1 wild-type female in the 600 mg/kg/day group were potentially treatment-related. Other deaths and moribund sacrifices were attributed to oral gavage errors.

Based upon examination of body weight curves, body weight gains appeared to be lower for the three transgenic male QAB149 treatment groups; however, body weight gains were unaffected for the three transgenic female QAB149 treatment groups.

Deaths at 300 and 600 mg/kg/day as well as decreased body weights for males at all doses suggest that a MTD was achieved and possibly exceeded in the study.

QAB149 treatment-related histopathological findings were primarily evident in the stomach and kidneys.

Uterine endometrial stromal polyps were observed for 3 of 25 females in the 600 mg/kg/day group. This tumor finding was statistically significant by trend test, but negative by pairwise comparison. It was noted that in a 2-year carcinogenicity study with mice that received another β2-adrenergic agonist, uterine endometrial stromal polyps were observed at a much higher incidence.

There were neoplastic findings for MNU-treated mice in several tissues.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee agreed that the study was adequate, noting prior Exec CAC protocol concurrence.

- The Committee found that the study was negative for statistically significant increases in neoplasms, although there was an increased incidence of ovarian leiomyomas in high dose females. It was noted that this is a rare tumor in rats and has been found with other β2-adrenergic agonists at much higher incidences (i.e., class effect), and is considered of limited relevance to human risk. The increased incidence of ovarian leiomyomas found in the present study did not reach the level of statistical significance.

- Pituitary tumors found in this study were statistically significant; however, the incidence was found to be within the historical control range and thus, considered to be unrelated to treatment.

Mouse:

- The Committee agreed that the study was adequate, noting prior Exec CAC protocol concurrence.

- The Committee found that the study was negative for statistically significant increases in neoplasms, although the study did show a positive trend in females for uterine endometrial stromal polyps. It was noted that this tumor has been observed before in mice treated with another β2-adrenergic agonist.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/NDA 22-383Division File, DPAP
/JWu, DPAP
/VWhitehurst, DPAP
/TRobison, DPAP
/CHill, DPAP
/ASeifried, OND IO

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22383	ORIG 1		INDACATEROL

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/s/

ADELE S SEIFRIED
08/05/2009

DAVID JACOBSON KRAM
08/05/2009

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22383	ORIG 1		INDACATEROL
NDA 22383	ORIG 1		INDACATEROL

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/s/

TIMOTHY W ROBISON
08/12/2009

JEAN Q WU
08/12/2009

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW CHEMISTRY CONSULT

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: NDA 22-383
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent:

Novartis Pharmaceuticals Corporation
Drug Regulatory Affairs
One Health Plaza
East Hanover, NJ 07936-1080

Reviewer name: Virgil Whitehurst, Ph.D.
Division name: Division of Pulmonary and Allergy Products
Manufacturer for drug substance:

The manufacture, quality control of QAB149 maleate is performed by:

Site/Address	CFN	Manufacturing	Quality control	Stability
Novartis Pharma AG Lichtstrasse 35 CH-4056 Basel Switzerland	9611204	QAB149-E6 QAB149-E7 QAB149-E15	X	-
Novartis Pharma Schweizerhalle AG Rothausweg CH-4133 Pratteln Switzerland	9692042	QAB149-E15	X	-
Novartis Pharma Stein AG Schaffhauserstrasse CH-4332 Stein Switzerland	9692043	QAB149-E14 QAB149-E16	-	-
Novartis Ringaskiddy Limited Ringaskiddy Co. Cork Ireland	9612715	QAB149-E6 QAB149-E7 QAB149-E9 QAB149-E12 QAB149-E13	X	-
Novartis International Pharmaceutical Ltd. Branch Ireland Ringaskiddy Co. Cork Ireland	9612715	-	X	X
Solvias AG Klybeckstrasse 191 CH-4058 Basel Switzerland	9617363		X	

Review completion date: March 12, 2009

Drug:

Trade name:

Arcapta™ (b) (4) (Proposed)
Indacaterol Maleate Inhalation Powder

Code name: QAB149 maleate

Chemical name:

(R)-5-[2-(5,6-Diethylindan-2-ylamino)-1-hydroxyethyl]-8-hydroxy-1H-quinolin-2-one maleate

CAS registry number:

753498-25-8

Free base: 312753-06-3

Maleic acid: 110-16-7

Molecular formula/molecular weight:

3 Molecular formula

Maleate salt: $C_{24}H_{28}N_2O_3 \bullet C_4H_4O_4$

Free base: $C_{24}H_{28}N_2O_3$

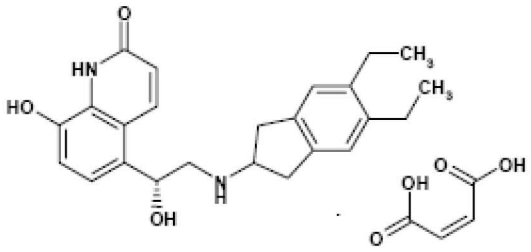
4 Relative molecular mass

Maleate salt: 392.49 + 116.07 = 508.56

Free base: 392.49

Salt/base ratio: 1.296

Structure:



Relevant INDs/NDAs/DMFs: INDs 66,337; 48, 169 and IND 69, 754

Drug class: Long-acting beta 2 adrenergic agonist

Intended clinical population: Treatment of COPD

Clinical formulation:

(b) (4)

(b) (4)

Route of administration: Oral inhalation

Proposed use: The sponsor is proposing a single daily dose of 150 or 300 mcg.

Introduction and history:
This review is in response to the chemistry consult from Dr Craig Bertha for NDA 22-383 dated December 17, 2008 to evaluate the acceptability of the proposed specifications of the impurities in the drug substance and the drug substance of Arcapta (b) (4) (proposed) Indercaterol Maleate Inhalation Powder.

(b) (4)

(b) (4)

Very little information concerning the toxicity of the identified impurities is available.

The level of the residual solvent, (b) (4) in the150 and 300 mcg dosage formulations is less than the drug substance qualification threshold of (b) (4) Therefore, the proposed specification for (b) (4) is acceptable.

The maximum daily doses of (b) (4) exceed the drug substance qualification threshold of (b) (4) but are less than the drug product qualification threshold of (b) (4) It is noted that patients take the drug product not the drug substance. Furthermore, none of the impurities contain structure alerts for either irritancy or mutagenicity/carcinogenicity. Therefore, the proposed specifications for (b) (4) are acceptable.

Heavy Metals:

Heavy metals	Proposed specification/capsules	Maximum daily human exposure 300 mcg capsule	Maximum daily human exposure 150 mcg capsule	Qualified

(b) (4)

The proposed specifications of NMT (b) (4) each for (b) (4) is less than the drug substance qualification threshold of (b) (4). The proposed specification for NMT (b) (4) total for (b) (4)) is less than the drug substance qualification threshold of (b) (4) Therefore, proposed specifications for (b) (4) at (b) (4) each and total (b) (4) at NMT (b) (4) are acceptable.

(b) (4)

In regard to the limit for the inhalable particulate matter with particles< (b) (4) of NMT (b) (4) per capsule Dr Bertha states

(b) (4)

The daily exposure of fine particles (b) (4) is small (b) (4) and is well below the EPA recommended limit of 300 µg/day (b) (4) The limit of NMT (b) (4) for particles per capsule of less than (b) (4) is acceptable.

Daily exposure of fine particles (b) (4) and greater (b) (4) are small and are not likely to be deposited into the lung. Fine particles (b) (4) are deposited into the human lung. The larger particles, (b) (4) and greater, are most likely deposited in the oropharyngeal region and therefore are not a safety concern. The limits of NMT (b) (4) per capsule for particles (b) (4) and NMT (b) (4) per capsule for particles (b) (4) are acceptable.

Conclusion

The proposed specifications for the heavy metals and (b) (4) in the drug substance as well as impurities, (b) (4) in the drug product for Arcapta (b) (4) (proposed) Indercaterol Maleate Inhalation Powder are acceptable. The proposed limits for the inhalable particulate matter in the drug product are also acceptable.

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/s/

Virgil Whitehurst
3/17/2009 09:28:46 AM
PHARMACOLOGIST

Timothy Robison
3/19/2009 04:05:45 PM
PHARMACOLOGIST
I concur

NDA Pharmacology Fileability Check List

NDA No: 22-383

Date of submission: December 17, 2008

Date of Fileability meeting: February 6, 2009

Information to Sponsor Yes ( ) No (X)

Date of check list: February 12, 2009

(1) On its face, is the Pharm/Tox section of the NDA organized in a manner to allow substantive review? Yes (X) No ( ) NA ( )

(2) On its face, is the Pharm/Tox section of the NDA legible for review?
Yes (X) No ( ) NA ( )

(3) Are final reports of all required and requested preclinical studies submitted in this NDA? Yes (X) No ( ) NA ( )

	Yes	No	NA
Pharmacology	(X)	( )	( )
ADME	(X)	( )	( )
Toxicology (duration, route of administration and species specified)			
acute	(X)	( )	( )
subchronic and chronic studies	(X)	( )	( )
reproductive studies	(X)	( )	( )
carcinogenicity studies	(X)	( )	( )
mutagenicity studies	(X)	( )	( )
special studies	(X)	( )	( )
others	(X)	( )	( )

(4) If the formulation to be marketed is different from the formulation used in the toxicology studies, is repeating or bridging the studies necessary? Yes ( ) No (X) NA ( )

If no, state why not?
The to be marketed formulation was used in toxicology studies in the rat and the dog.

If yes, has the applicant made an appropriate effort to repeat the studies using the to be marketed product, to bridge the studies or to explain why such repetition or bridging should not be required? Yes () No ( ) NA (x)

(5) Are the proposed preclinical labeling sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage) appropriate (including

human dose multiples expressed in either mg/m^2 or comparative systemic exposure levels) and in accordance with 201.57? Yes (X) No ( ).

(6) Has the applicant submitted all special studies/data requested by the Division prior to the submission including but not limited to pre-NDA discussion? Yes (X) No ( ) NA ( )

(7) On its face, does the route of administration used in the pivotal toxicity studies appear to be the same as the intended clinical route? Yes (X) No ( ) NA ( )

If not, has the applicant submitted a rationale to justify the alternative route?
Yes ( ) No (x) NA

(8) Has the applicant submitted a statement(s) that all of the toxicity studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? Yes (x) No ( ) NA ()

(9) Has the applicant submitted any studies or data to address any impurity or extractable issues (if any)? Yes (X) No ( ) NA ( )

(10) Are there any outstanding preclinical issues? Yes ( ) No (X)
If yes, identify those below.

(11) From a preclinical perspective, is this NDA fileable? Yes (X) No ( )

If no, state below why it is not.

(12) Should any additional information/data be requested? Yes (X) No ( )

In consultation with the Chemistry reviewer, additional information may need to be requested for qualifications of impurities, extractables, and leachables.

NDA Planning Timeline

NDA No.: 22-383

Date of planning timeline: February 12, 2009

PDUFA Due Date: October 18, 2009

Projected review completion date: August 1, 2009

	Milestone Dates
Pharmacology and ADME	7-10-09
Toxicology	
General toxicity studies	7-18-09
Carcinogenicity studies and mutagenicity studies	7-25-09
a. Statistical consult request for CA studies	3-1-09
b. Submission of CA studies for CAC concurrence	Completed
Reproductive studies	Completed
Special studies and Others	7-20-09
Labeling	7-27-09

Signatures (optional):

Reviewer Signature _____
Virgil Whitehurst, Ph.D.
Supervisor Signature _____
Timothy W. Robison, Ph.D.

Concurrence Yes ___ No ___

cc:
NDA 22-383, 570 Division Files
WuL, 570
Prasad P, Division 1
RobisonT, 570
Whitehurst V, 570

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Virgil Whitehurst
2/12/2009 04:06:31 PM

Timothy Robison
2/12/2009 04:09:18 PM