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APPLICATION NUMBER:

201820Orig1s000

PHARMACOLOGY REVIEW(S)

MEMO TO FILE, NDA 201,820

DATE: 4/30/12

TO: Carmen DeBellas, Pharm.D.
Project Manager, DAIP

FROM: Amy L. Ellis, Ph.D.
Pharmacologist, DAIP

THROUGH: Wendelyn Schmidt, Ph.D.
Supervisory Pharmacologist, DAIP

RE: Resubmission of NDA 201,820

Chiesi Pharmaceuticals Inc. has resubmitted their NDA 201,820 for CHF 1538 (Tobramycin 300 mg/4 ml Inhalation Solution). DAIP sent Chiesi a complete response letter on 8/25/11 regarding the initial submission of this NDA. The current submission provides updated data regarding drug product stability and safety. It does not contain any new nonclinical data and none was requested by the Division. The pharmacologist did not object to the approval of the original NDA submission and nothing has occurred to change this recommendation. The sponsor has not included a label in the resubmission. The original NDA submitted to the Division proposed language for label sections 8.1 Pregnancy and 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility that were based on the TOBI label. This was appropriate because both products contain the same active ingredient to be given via the same route of administration and dosing schedule.

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/s/

AMY L ELLIS
05/01/2012

WENDELYN J SCHMIDT
05/01/2012

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	201-820
Supporting document/s:	IND 72,068
Applicant's letter date:	10/22/10
CDER stamp date:	10/22/10
Product:	Tobramycin 300 mg/4 ml Inhalation Solution Trade name not yet agreed upon. Originally requested trade names, (b) (4) , were rejected by DMEPA.
Indication:	Management of cystic fibrosis patients with *P. aeruginosa*
Applicant:	Chiesi Pharmaceuticals Inc., Rockville, MD
Review Division:	Division of Anti-Infective Products
Reviewer:	Amy L. Ellis, Ph.D.
Supervisor/Team Leader:	Wendelyn Schmidt, Ph.D.
Acting Division Director:	John Farley, M.D.
Project Manager:	Carmen DeBellas, R.Ph.

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 201-820 are owned by Chiesi or are data for which Chiesi has obtained a written right of reference.
Any information or data necessary for approval of NDA 201-820 that Chiesi does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 201-820.

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1 Executive Summary

1.1 Introduction

This product is a new formulation of tobramycin for inhalation. It contains the same ingredients as the marketed product TOBI, but the concentrations of tobramycin and (b) (4) differ between the 2 products, as does the pH. The therapeutic indication for both products would be the same. The new product is approved outside the U.S. The sponsor conducted 7- and 28-day repeat dose toxicity studies in rats and dogs to bridge their new formulation for inhalational tobramycin with TOBI.

1.2 Brief Discussion of Nonclinical Findings

The inhalation toxicity studies conducted in rats and dogs showed that tobramycin inhalation solution from Chiesi does not have a toxicity profile that differs significantly from TOBI. In the rat studies, TOBI was included as a comparator. Toxicokinetic data demonstrated systemic exposure to tobramycin after it was administered to rats and dogs by inhalation. Microscopic kidney changes were observed in both species and these were not completely reversed after a 4 week recovery period; however, no clinical chemistry changes indicative of nephrotoxicity were observed. Microscopic changes in respiratory tissues were consistent with inflammation and irritation. Substantial recovery of these tissues was observed following 4 weeks without drug exposure. Accumulation of alveolar macrophages (often containing debris or pigment- possibly scavenged drug) was observed at the end of dosing and some recovery from this finding was also seen 4 weeks after drug treatment ended.

1.3 Recommendations

1.3.1 Approvability

The pharmacologist has no objection to the approval of tobramycin inhalation solution.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

The Pregnancy (8.1) and Carcinogenesis, Mutagenesis, Impairment of Fertility (13.1) sections of the label for this product will be based on information from the TOBI label. This is appropriate because both products contain the same active ingredient given by the same route of administration at the same dose using the same schedule.

2 Drug Information

2.1 Drug

CAS Registry Number: 32986-56-4

Generic Name: Tobramycin

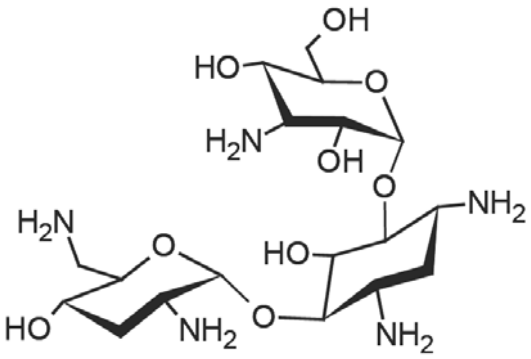
Code Name: CHF 1538

Other Names: Tobraneb, Bramitob (these are trade names used outside of the U.S.)

Chemical Name: O-3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-O-[2,6-diamino-2,3,6-trideoxy-α-D-ribo-hexopyranosyl-(1→4)]-2-deoxy-D-streptamine

Molecular Formula/Molecular Weight: $C_{18}H_{37}N_5O_9$ / 467.52

Structure or Biochemical Description



Pharmacologic Class: Aminoglycoside antimicrobial

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 72,068

2.3 Drug Formulation

From the submission:

Table 1: Unit Composition of CHF 1538

Component	Unit Formula per Single-Dose Ampule (per 4 mL)[1]	Function
Tobramycin, USP	300 mg	API
Sodium Chloride, USP	(b) (4)	(b) (4)
(b) (4) Sulfuric Acid, NF		
Sulfuric Acid, NF		pH Adjustment
Sodium Hydroxide, NF		pH Adjustment
Water for Injection, USP		(b) (4)

(b) (4)

2.4 Comments on Novel Excipients

This drug product contains no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

Nothing of concern. Impurities and residual solvents appear to have been qualified adequately or will be at levels below ICH guidance recommendations.

2.6 Proposed Clinical Population and Dosing Regimen

The clinical population that would receive this product is cystic fibrosis patients with *P. aeruginosa*, ≥ 6 years of age. The product is administered via inhalation using a nebulizer. Twice daily, patients would nebulize one ampule containing 300 mg of tobramycin in 4 ml of diluent. Treatment is chronic in cycles of 28 days on therapy and 28 days off.

2.7 Regulatory Background

This product has been marketed in the E.U. for several years as Bramitob. It is similar to TOBI which is marketed in the U.S. TOBI (300 mg tobramycin/5 ml in (b) (4) mg NaCl/ml, pH 6.0) is less concentrated and has a higher target pH than Chiesi's product (300 mg tobramycin/4 ml in (b) (4) NaCl/ml, pH 5.0). The sponsor initially sought guidance from the Division regarding U.S. development of their tobramycin solution for inhalation in 2005. On November 18 of that year, a meeting between the sponsor and the Division was held. In 2009, a series of pre-NDA questions was submitted by the sponsor and another meeting was held. At both meetings, the Division agreed that the 505 (b)(2) pathway would be appropriate for this product and did not anticipate that additional nonclinical testing would be needed. The sponsor had previously informed the Division that they had conducted 1- and 4-week inhalation studies in rats and dogs to serve as a bridge between their formulation and the approved product TOBI. Summaries of these studies were submitted in briefing documents prior to the first meeting between the sponsor and the Division.

3 Studies Submitted

3.1 Studies Reviewed

Tobraneb: Toxicity Study by Inhalation Administration to CD Rats for 1 Week (Study No. CHS 099/012798)

Tobraneb: Toxicity Study by Inhalation Administration to Beagle Dogs for 1 Week (Study No. CHS 101/012880)

Tobraneb: Toxicity Study by Inhalation Administration to CD Rats for 4 Weeks Followed by a 4 Week Recovery Period (Study No. CHS 100/013695)

Tobraneb: Toxicity Study by Inhalation Administration to Beagle Dogs for 4 Weeks Followed by a 4 Week Recovery Period (Study No. CHS 102/014192)

3.2 Studies Not Reviewed

All nonclinical pharmacology/toxicology studies submitted in the NDA were reviewed.

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Antimicrobial activity mediated by perturbation of protein synthesis. Tobramycin binds to the 30 S subunit of the ribosomal complex, causing misreading of mRNA and premature termination of translation.

4.2 Secondary Pharmacology

N/A

4.3 Safety Pharmacology

Safety pharmacology studies were not conducted with this product. Tobramycin, like other aminoglycosides, is known to cause neuromuscular blockade at high doses.

5 Pharmacokinetics/ADME/Toxicokinetics

Tobramycin is not well absorbed when given orally. Some drug is absorbed through the lung when it is administered via inhalation, but blood levels are not as great as if the same dose was given parenterally. Tobramycin is excreted unchanged by the kidney.

Toxicokinetic data from the repeat-dose inhalation toxicity studies performed in rats and dogs are presented below, in the reviews of the main studies.

6 General Toxicology

6.1 Single-Dose Toxicity

N/A

6.2 Repeat-Dose Toxicity

When administered systemically, target organs of aminoglycoside toxicity include the kidneys and inner ear (both cochlear and vestibular toxicities); drugs of this class may also cause neuromuscular blockade.

This new formulation of tobramycin for inhalation did not nebulize as efficiently as TOBI (as noted in the 7 day rat study), possibly due to its higher viscosity. However, findings in the respiratory tract appear similar for both products.

Tobraneb: Toxicity Study by Inhalation Administration to CD Rats for 1 Week

Study no.:	CHS 099/012798
Study report location:	Contract laboratory archives
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/10/01
GLP compliance:	UK, OECD, EC
QA statement:	Yes
Drug, lot #, and % purity:	Tobraneb, Batch No. 0011006, ≥95% pure by HPLC; TOBI, Batch No. 06K0B

Key Study Findings

Neither Tobraneb nor TOBI was associated with clinical signs of systemic toxicity when administered via inhalation to rats for one week during 2 hour daily dosing sessions. The mean estimated daily deposited dose of TOBI (approximately 10 mg/kg) was greater than that of the highest mean estimated daily deposited dose of Tobraneb (approximately 6 mg/kg), likely due to lower nebulization efficiency of the latter. The presence of basophilic cortical tubules and minimal single cell necrosis in the kidneys was dose-related. Despite the microscopic changes in the kidneys, none of the animals exhibited clinical chemistry changes indicative of renal toxicity. Microscopic signs of local irritation (atrophy, hyperplasia, inflammation, erosion) of respiratory tissues, especially of the respiratory epithelium, were observed in most animals treated with tobramycin. Tissue samples from the nasal passages, larynx, trachea, and lungs were examined. Most changes were graded minimal to slight, none was more than moderate. For a number of these changes, the incidence and severity were not dose related. Other signs of local irritation were dose-related, including changes in the respiratory epithelium of the nasal passages, signs of local irritation in the larynx, hyperplasia of arytenoid epithelium, and tracheal epithelial hyperplasia. The differences

between respiratory tissue findings from high dose Tobraneb rats and those exposed to TOBI were not remarkable. Both products were well tolerated by the rats.

Methods

Doses:	Rats were exposed to vehicle (0.45% saline) and low, medium, and high concentrations of Tobraneb in the test atmosphere. A reference group was exposed to TOBI at a concentration that was intended to mimic the "high" concentration of Tobraneb. The intended and achieved doses of test articles are discussed in detail below in the Results section.
Frequency of dosing:	Rats were in inhalation chambers for 2 hours daily for 7 consecutive days.
Route of administration:	Inhalation (nose-only) of nebulized solutions
Formulation/Vehicle:	The vehicle for Tobraneb was (b) (4) NaCl. This concentration of saline was also used for the vehicle control. Tobraneb contains 300 mg of tobramycin per 4 ml of vehicle. The vehicle for TOBI is a less concentrated saline ((b) (4) The concentration of tobramycin in TOBI is 300 mg/5 ml. Tobraneb has a higher viscosity than TOBI.
Species/Strain:	Sprague-Dawley Rats (Crl:CD®BR)
Number/Sex/Group:	5
Age:	8 weeks at dosing initiation
Weight:	272-337 g (males); 168-208 g (females)
Satellite groups:	5/sex/drug-treated group were designated for TK, but the results of TK analysis were not reported.
Unique study design:	Rats remained in the nose-only exposure system for 2 hours each day. They were restrained in polycarbonate tubes with the snouts projecting into the exposure chamber. Test articles were nebulized using the DeVilbiss Ultra-Neb®99. Test article concentration in the atmosphere was modulated by adjusting the air flow rates through the exposure system as well as the pump feed rate into the nebulizer. Discussion of achieved doses is presented below. Animals were sacrificed the day following the final exposure.
Deviation from study protocol:	Nothing reported

Observations and Results

Test Article Doses/Concentrations in Test Atmospheres

The administered doses of tobramycin using Tobraneb or TOBI were calculated by 2 different methods. The method used by the sponsor to calculate inhaled dose used the proportion of inhalable particles (percentage of particles (b) (4) µm aerodynamic diameter) determined to be available in the test atmosphere within the exposure chamber. The FDA generally assumes that 10% of the active drug in a test atmosphere will be deposited in the lungs of rats. During each exposure period, 3 samples were taken for measurement of tobramycin concentration in the atmosphere using a sampling trap connected to an unoccupied exposure port. The proportion of inhalable particles (respirable fraction) was determined using a May Multistage Liquid Impinger.

Actual tobramycin concentrations measured in the test atmospheres within the exposure chambers were close to the target except for the high dose Tobraneb. The investigators theorized that the increased viscosity of this formulation compared to TOBI caused it to be nebulized less efficiently. This was apparently less problematic at the lower nebulization rates needed to generate the test atmospheres of the low and mid dose groups. The concentration of tobramycin in the residual dosing liquid that remained in the nebulization cup after the rats were treated was higher in the Tobraneb groups than the TOBI group, supporting the investigator's theory that Tobraneb was nebulized less efficiently than TOBI.

Treatment Group	Tobramycin Concentration in Test Atmosphere (mg/L)		
	Target	Study Mean	% of Target
Tobraneb Low	(b) (4)	0.151	(b) (4)
Tobraneb Mid		0.468	
Tobraneb High		0.758	
TOBI		1.280	

The respirable fractions in the low, medium, and high dose Tobraneb, and TOBI groups were 0.48, 0.57, 0.76, and 0.74, respectively. The mass median aerodynamic diameter (MMAD) for each of these groups was (b) (4) µm. Geometric standard deviations for MMAD ranged from 1.57-1.70.

The equation used by the Sponsor for determining the administered dose of tobramycin was:

$$\text{Dose} = \frac{C \times RMV \times D \times F}{W}$$

Where C= concentration of tobramycin measured in test atmosphere (study mean)

RMV= respiratory minute volume, for rats the formula was $4.19 \times W\ (g)^{0.66}$

D= duration of exposure (minutes)

F= respirable fraction

W= body weight (g) across dosing period

Based on this formula, the mean daily doses of tobramycin estimated by the sponsor were 5.63, 20.90, 45.05 mg/kg of Tobraneb (low, mid and high doses) and 73.58 mg/kg for TOBI.

The FDA/CDER also uses the formula above to estimate the daily dose of tobramycin deposited in the lungs of test animals, but instead of using the respirable fraction as the value for F, 0.1 is used as the standard lung deposition value for rats.

These assumptions provide the following estimates for the mean daily dose of tobramycin deposited into the lungs of the rats (table from the study report):

Group	Target	Estimated		
		Male	Female	Combined
2 (Low dose)	(b) (4)	1.07	1.27	1.17
3 (Intermediate dose)		3.40	3.95	3.68
4 (High dose)		5.44	6.40	5.92
5 (High reference dose)		9.16	10.74	9.95

Mortality

Rats were observed at least twice daily for morbidity/mortality. There were no unscheduled deaths.

Clinical Signs

Rats were observed for clinical signs of toxicity prior to exposure (when they were being placed in the tubes), during exposure, after exposure (when being returned to the home cage), and near the end of the work day. No clinical signs of tobramycin-related toxicity were observed.

Body Weights

Body weights were measured one prior to the initiation of dosing, on Days 1, 3, and 7 or exposure, and prior to necropsy. There were no differences in body weights between the treatment groups.

Feed Consumption

Measured weekly (by cage of 5 gang-housed rats). There were no differences in food consumption between the treatment groups.

Ophthalmoscopy

Not done.

Hematology

Blood samples were drawn from fasted animals on Day 4, not at the end of the dosing period. Small reduction in reticulocyte levels between controls and all drug-treated

males and high dose Tobraneb and TOBI females was observed, but did not appear toxicologically significant in the absence of any other hematologic changes.

Clinical Chemistry

Blood samples were drawn from fasted animals on Day 4, not at the end of the dosing period. Small differences in serum protein between control and treated animals were neither dose-related nor consistent between the sexes. Thus, they were not considered to be of toxicological significance.

Urinalysis

Urine samples were collected overnight on Day 3/4, not at the end of the dosing period. Animals did not have access to food or water during the collection period. No treatment-related changes were noted.

Gross Pathology

Cecal distension was observed in all tobramycin-treated rats. This is likely secondary to antimicrobial effects on the gut flora.

Organ Weights

The following organs were weighed at necropsy: adrenals, kidneys, liver, lungs, spleen. Increased lung weights were observed in tobramycin-treated rats, but it was statistically significant only in the females.

Histopathology

Adequate Battery- The abbreviated battery (abnormalities*, adrenals, bronchi, kidneys*, larynx* (2 sections), liver, lungs* (all lobes and mainstem bronchi), nasal turbinates*, spleen, trachea*) was acceptable for this short study. Starred (*) tissues were examined in animals from all groups. The remaining tissues were examined only in control and high dose Tobraneb and TOBI groups.

Peer Review- Not done.

Histological Findings- Basophilic cortical tubules with karyomegaly were observed in the kidneys of all tobramycin-treated rats. In the males, there was a modest dose-related increase in severity with minimal to slight severity seen in the low and mid dose Tobraneb groups and slight to moderate severity seen in the high dose Tobraneb and TOBI groups. In the females, the change was minimal to slight in all dose groups. Mitotic figures were observed in some of these cells, but their presence was not dose-related. Minimal single cell necrosis increased in a dose related manner (Tobraneb: Low, 1/5 males and 1/5 females; Mid, 4/5 males and 1/5 females; High: 5/5 males and 2/5 females; TOBI: 5/5 males and 5/5 females).

Signs of local irritation were observed in the nasal passages of some animals from each of the tobramycin groups. None of these signs were seen in vehicle control animals. They were all graded minimal to slight, were observed in the respiratory and olfactory epithelium, and included atrophy, hyperplasia, and inflammation. Changes in the respiratory epithelium were observed slightly more frequently in animals that

received high dose Tobraneb or TOBI. Erosion in the olfactory epithelium was also seen in a few drug-treated rats, but the incidence was not dose-related.

Laryngeal tissues also showed signs of local irritation, including epithelial hyperplasia, thinning, and ulceration, squamous metaplasia, and the presence of inflammatory cells in the lamina propia. Most changes were minimal to slight and none were rated greater than moderate severity. In the Tobraneb groups, severity was generally slightly greater in the mid and high dose groups than at the low dose, but there was not much difference between the mid and high dose groups. Slight hyperplasia of arytenoid epithelium was observed in 2/5 control males and 1/5 control females. Moderate hyperplasia of this tissue was seen in rats treated with tobramycin including 3 males and 1 female from the low dose Tobraneb group, 5 males and 3 females from the mid dose Tobraneb group, 5 males and 5 females from the high dose Tobraneb group, and 4 males and 3 females from the TOBI group. The other animals in the tobramycin groups all had slight hyperplasia of the arytenoid epithelium.

Local irritation of the trachea and at the tracheal and bronchial bifurcations was not dose related. None of the microscopic changes in these tissues was graded higher than minimal to slight. Inflammatory cell infiltration of the trachea was seen in some animals from all treatment groups, especially at the low dose of Tobraneb. In contrast, epithelial hyperplasia of the trachea was observed in a few animals from the high dose Tobraneb and TOBI groups, but not in the other treatment groups. Epithelial hyperplasia at the tracheal bifurcation was seen in 3-6 animals (sexes combined) from all tobramycin groups. The loss of cilia at either bifurcation was seen in a few rats scattered among the tobramycin groups.

Minimal to slight diffuse accumulation of alveolar macrophages was observed in the lungs of most animal treated with tobramycin, regardless of dose.

Toxicokinetics

Results were not included in the study report. The TK portion of the study was said to be “preliminary” and not was not included in the study contractor’s report at the request of the sponsor. The results do not appear to have been submitted in a separate report. TK data are available from the 28-day rat study, so this omission is not crucial to study interpretation.

Dosing Solution Analysis

The concentration of tobramycin in the batch of Tobraneb used for the study was 70.40 mg/ml (94% of intended). The concentration of tobramycin in TOBI was 62.20 mg/ml (104% of intended).

Tobraneb: Toxicity Study by Inhalation Administration to CD Rats for 4 Weeks Followed by a 4 Week Recovery Period

Study no.:	CHS 100/013695
Study report location:	Contract laboratory archives
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/6/01
GLP compliance:	UK, OECD, EC
QA statement:	Yes
Drug, lot #, and % purity:	Tobraneb, Batch No. 0011006, ≥95% pure by HPLC; TOBI, Batch No. 11K0B

Key Study Findings

Neither Tobraneb nor TOBI was associated with clinical signs of systemic toxicity when administered via inhalation to rats for 28 days during 2 hour daily dosing sessions. The results of this study were consistent with the 1 week study also conducted in rats. The mean estimated daily deposited high dose of Tobraneb was 7.5 mg/kg (average of both sexes) and the mean highest estimated daily deposited dose of TOBI was approximately 5.9 mg/kg. Plasma levels of tobramycin were in the quantifiable range after treatment with either Tobraneb or TOBI indicating that systemic exposure occurred, but plasma accumulation was not observed over the course of the study. The presence of basophilic cortical and medullary tubules and minimal single cell necrosis in the kidneys was dose-related. Despite the microscopic changes in the kidneys, none of the animals exhibited clinical chemistry changes indicative of renal toxicity. Some recovery from the kidney changes was seen 4 weeks after the end of treatment, but findings were still present in the males. Microscopic signs of local irritation (atrophy, hyperplasia, metaplasia, keratinization, inflammation, erosion/ulceration) of respiratory tissues, especially of the epithelium, were observed in most animals treated with tobramycin. Tissue samples from the nasal passages, larynx, trachea, and lungs were examined. Most changes were graded minimal to slight, none was more than moderate. For a number of these changes (e.g., erosion and inflammation of olfactory epithelium, inflammatory cell infiltration of trachea and proximal bronchi), the incidence and severity were not dose related. Other signs of local irritation were dose-related, including hyperplasia of the olfactory epithelium and arytenoid process epithelium and the severity of squamous metaplasia in the larynx. Diffuse accumulation of alveolar macrophages (some containing debris) was observed in most tobramycin-treated rats; severity scores were slightly greater at higher doses. Significant recovery from the respiratory changes was observed in the high dose rats after 4 weeks without treatment. The differences between respiratory tissue findings from high dose Tobraneb rats and those exposed to TOBI were not remarkable. Both products were well tolerated by the rats.

Methods

Doses:	Rats were exposed to vehicle (0.45% saline) and low, medium, and high concentrations of Tobraneb in the test atmosphere. Reference groups were exposed to low and high doses of TOBI. The intended and achieved doses of test articles are discussed in detail below in the Results section.
Frequency of dosing:	Rats were in inhalation chambers for 2 hours daily for 28 consecutive days.
Route of administration:	Inhalation (nose-only) of nebulized solutions
Formulation/Vehicle:	The vehicle for Tobraneb was (b) (4) % NaCl. This concentration of saline was also used for the vehicle control. Tobraneb contains 300 mg of tobramycin per 4 ml of vehicle. The vehicle for TOBI is a less concentrated saline ((b) (4) %). The concentration of tobramycin in TOBI is 300 mg/5 ml. Tobraneb has a higher viscosity than TOBI.
Species/Strain:	Sprague-Dawley Rats (Crl:CD®BR)
Number/Sex/Group:	10
Age:	8 weeks at dosing initiation
Weight:	280-347 g (males); 183-226 g (females)
Satellite groups:	5/sex in the control and high dose Tobraneb and TOBI groups for 4 week recovery period. 16/sex in the low and mid dose Tobraneb and low dose TOBI groups for TK. 32/sex in the high dose Tobraneb and TOBI groups for TK.
Unique study design:	Rats remained in the nose-only exposure system for 2 hours each day. They were restrained in polycarbonate tubes with the snouts projecting into the exposure chamber. Test articles were nebulized using the DeVilbiss Ultra-Neb®99. Test article concentration in the atmosphere was modulated by adjusting the air flow rates through the exposure system as well as the pump feed rate into the nebulizer. Discussion of achieved doses is presented below. Main study animals were sacrificed the day following the final exposure and recovery groups were sacrificed 4 weeks after the end of treatment.
Deviation from study protocol:	Nothing reported

Observations and Results

Test Article Doses/Concentrations in Test Atmospheres

The administered doses of tobramycin using Tobraneb or TOBI were calculated by 2 different methods. The method used by the sponsor to calculate inhaled dose used the proportion of inhalable particles (percentage of particles (b) (4) µm aerodynamic diameter) determined to be available in the test atmosphere within the exposure chamber. The FDA generally assumes that 10% of the active drug in a test atmosphere will be deposited in the lungs of rats. During each exposure period, 3 samples were taken for measurement of tobramycin concentration in the atmosphere using a sampling trap connected to an unoccupied exposure port. The proportion of inhalable particles (respirable fraction) was determined using a May Multistage Liquid Impinger.

Actual tobramycin concentrations measured in the test atmospheres within the exposure chambers were close to the target.

Treatment Group	Tobramycin Concentration in Test Atmosphere (mg/L)		
	Target	Study Mean	% of Target
TOBI Low	(b) (4)	0.127	(b) (4)
Tobraneb Low		0.089	
Tobraneb Mid		0.348	
Tobraneb High		1.013	
TOBI High		0.803	

The respirable fractions in the low, medium, and high dose Tobraneb, and low and high TOBI groups were 0.57, 0.54, 0.73, 0.70, and 0.72, respectively.

The equation used by the Sponsor for determining the administered dose of tobramycin was:

$$\text{Dose} = \frac{C \times RMV \times D \times F}{W}$$

Where C= concentration of tobramycin measured in test atmosphere (study mean)

RMV= respiratory minute volume, for rats the formula was $4.19 \times W\ (g)^{0.66}$
D= duration of exposure (minutes)
F= respirable fraction
W= body weight (g) across dosing period

Based on this formula, the mean daily doses of tobramycin estimated by the sponsor were 3.74, 13.60, 54.31 mg/kg of Tobraneb (low, mid and high doses) and 6.46, 42.26 mg/kg for TOBI.

The FDA/CDER also uses the formula above to estimate the dose of tobramycin deposited in the lungs of test animals, but instead of using the respirable fraction as the value for F, 0.1 is used as the standard lung deposition value for rats.
These assumptions provide the following estimates for the mean daily dose of tobramycin deposited into the lungs of the rats (table below from the study report). Note that "reference" refers to TOBI, the other dose estimates are for Tobraneb.

Mean Daily Inhaled Dose (mg Tobramycin/kg)				
Group	Target	Estimated[1]		
		Male	Female	Combined
2 (Low reference)	(b) (4)	0.86	1.01	0.9
3 (Low dose)		0.62	0.71	0.7
4 (Intermediate dose)		2.36	2.73	2.5
5 (High dose)		6.95	8.10	7.5
6 (High reference)		5.50	6.37	5.9

[1] Data for C used in estimated dose calculations were the group mean C values for Days 1 - 28

There were some significant differences in estimated exposure on Days 1 and 28. This table from the report is included to present them:

Daily Inhaled Dose (mg Tobramycin/kg)					
Group	Target	Estimated			
		Day 1		Day 28[1]	
		Male	Female	Male	Female
2 (Low reference)	(b) (4)	1.58	1.83	0.94	1.10
3 (Low dose)		2.09	2.38	0.79	0.91
4 (Intermediate dose)		0.27	0.31	0.70	0.81
5 (High dose)		2.91	3.35	1.94	2.26
6 (High reference)		1.75	2.00	6.35	7.36

[1] Data for C used in estimated dose calculations were values for Day 1 and Day 28

Mortality

Rats were observed at least twice daily for morbidity/mortality. There were no unscheduled deaths.

Clinical Signs

During the treatment period, rats were observed for clinical signs of toxicity prior to exposure (when they were being placed in the tubes), during exposure, after exposure (when being returned to the home cage), and near the end of the work day. During recovery, animals were observed once daily. No clinical signs of tobramycin-related toxicity were observed. Loose stools observed in the high dose groups during the

treatment period likely resulted from the antimicrobial effects on tobramycin on the gut flora.

Body Weights

Body weights were measured one week prior to the initiation of dosing, weekly thereafter, and prior to necropsy. The tobramycin-treated males did not gain as much weight as controls over the course of the dosing period, but the reduction in body weight gain was not dose-related. Mean body weight gain of the male controls was 92 g and mean body weight gains in the tobramycin groups ranged from 66-84 g. The high dose TOBI females gained less weight than controls during the treatment period (38 g vs. 28 g).

Feed Consumption

Measured weekly (by cage of 4-5 gang-housed rats). Group mean cumulative food consumption was lower in the tobramycin-treated males than in controls and in the high dose TOBI females compared to controls. These reductions were modest, although they were statistically significant.

Ophthalmoscopy

Performed on all rats from the main study and recovery groups prior to the initiation of dosing, then during week 4 for rats from the control and high dose Tobraneb and TOBI groups. There were no findings that appeared to be treatment-related.

Hematology

Blood samples were drawn from fasted main study and recovery animals during week 4. Total WBCs in the low dose TOBI females and the high dose Tobraneb and TOBI groups of both sexes were less than controls. Although these reductions were statistically significant, they were modest and did not appear to be of toxicological significance.

Clinical Chemistry

Blood samples were drawn from fasted main study and recovery animals during week 4. There were no changes that appeared to be of toxicological significance.

Urinalysis

Urine samples were collected overnight from main study and recovery animals during week 4. Animals did not have access to food or water during the collection period. No treatment-related changes were noted.

Gross Pathology

Distended cecum (likely related to the antimicrobial effect of tobramycin on the gut flora) was observed in several drug treated animals and was somewhat more prevalent in the high dose groups. There were no other gross observations that appeared to be treatment-related.

Organ Weights

Mean absolute and relative (to body weight) lung weights were slightly higher than controls in the female rats treated with Tobraneb or high dose TOBI (statistically significant) and the males treated with high dose TOBI (not statistically significant). Liver weights of the drug-treated rats tended to be lower than controls, but it the absence of any microscopic changes in the liver or serum chemistry changes, this did not appear to be of toxicological significance. Organ weights at the end of the recovery period were similar between the control and high dose tobramycin groups.

Histopathology

Adequate Battery- A standard battery of tissues was collected and preserved, including an expanded panel of respiratory-associated tissues. All tissues from control and high dose animals were examined. The kidneys and respiratory-associated tissues from all animals in all dose groups were examined.

Peer Review- Not done.

Histological Findings- Treatment with the mid and high doses of Tobraneb or the high dose of TOBI was associated with kidney changes, as seen in the table below (copied from the study report). These included an increase in basophilic cortical tubules and the presence of single cell necrosis in these tubules as well as an increase in basophilic medullary tubules.

		Males						Females					
Group		**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Treatment		**V**	**T**	**Tobraneb**			**T**	**V**	**T**	**Tobraneb**			**T**
Dosage (mg/kg/day)		**0**	**6.5**	**3.7**	**13.6**	**54.3**	**42.3**	**0**	**6.5**	**3.7**	**13.6**	**54.3**	**42.3**
Basophilic cortical tubules	Total	8	10	10	10	10	10	7	5	7	9	10	7
	Minimal	8	9	9	7	3	7	7	5	7	9	5	2
	Slight	0	1	1	3	7	3	0	0	0	0	4	5
	Moderate	0	0	0	0	0	0	0	0	0	0	1	0
Single cell necrosis in basophilic cortical tubules	Total	0	0	0	3	6a	3	0	0	0	0	1	0
	Minimal	0	0	0	3	6	3	0	0	0	0	1	0
Mitotic figures in basophilic cortical tubules	Total	0	0	0	0	1	0	0	0	0	0	0	0
	Minimal	0	0	0	0	1	0	0	0	0	0	0	0
Basophilic medullary tubules	Total	2	1	1	4	4	4	0	0	0	3	3	6a
	Minimal	2	1	1	3	3	3	0	0	0	3	2	4
	Slight	0	0	0	1	1	1	0	0	0	0	1	2
Number of kidneys examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

a – $p < 0.05$ with Fisher's Exact Test, on total incidences only
V = Control (vehicle), T = Tobi

Consistent with the findings in the 7 day rat study with Tobraneb and TOBI, signs of local irritation were seen in the nasal passages, laryngeal tissues, trachea and at the tracheal and bronchial bifurcations. Almost all findings were graded as "minimal" or "slight".

In the nasal passages, erosion of olfactory epithelium and inflammation of olfactory epithelium were related to treatment with Tobraneb or TOBI, but were not dose-related. The incidence and severity of hyperplasia in the olfactory epithelium was related to the dose of the drug that was administered to the rats. Epithelial atrophy or disorganization was seen in a few more rats from the high dose groups than at the lower doses and it was also marginally more severe.
Epithelial hyperplasia of the arytenoid processes, as well as the ventral and lateral regions of the larynx, was observed in most rats that were treated with tobramycin. The severity of arytenoid epithelial hyperplasia was greater at the high doses of Tobraneb and TOBI than at the lower doses (more grades of “moderate”, as compared to “slight” and “minimal”); however, the severity of epithelial hyperplasia of the other regions was not related to dose. Arytenoid epithelial keratinization was observed in around half of the animals in each treatment group. A few rats exhibited ulceration of the arytenoid epithelium (including the rostral portion) or thinning or ulceration of the lateral region of the larynx, but the incidence of these findings was not related to dose. Metaplasia of the squamous epithelium in the ventrolateral region of the larynx was observed in most of the tobramycin-treated rats, with more animals in the mid and high dose Tobraneb groups and high dose TOBI group having grades of “slight” as opposed to “minimal” in the lower dose groups.

Inflammatory cell infiltration of the trachea and the proximal region of the bronchi was observed in only a few control rats, but it was seen in the majority of the tobramycin-treated rats (not dose-related). The infiltration was primarily submucosal, but mucosal infiltration was occasionally observed as well. Epithelial hyperplasia and/or loss of cilia were observed at the tracheal bifurcation of several tobramycin-treated rats from all dose groups. These findings were not seen as frequently at the bronchial bifurcation, but they were present in a few animals that had been treated with Tobraneb or TOBI.

Diffuse accumulation of alveolar macrophages was observed in the lungs of all tobramycin-treated animals. Sometimes the macrophages contained debris. Almost all of the rats from the low dose Tobraneb and TOBI groups had a “minimal” severity rating for this finding, while some in the higher dose groups had ratings of “slight”, and in one case, “moderate”. Hyperplasia of bronchiolar goblet cells was observed in a few rats from each tobramycin group. Epithelial hyperplasia at a bronchiolar bifurcation was seen in 2 males. Neither finding of hyperplasia was related to dose.

After a one month recovery period, the degree to which basophilic cortical tubules were present in the kidneys of male rats treated with the high doses of Tobraneb and TOBI was greater than that of controls (“slight” vs. “minimal”). Additionally, basophilic medullary tubules were also present in these tobramycin-treated male rats and they were not observed in controls. The kidneys of the female rats that had been treated with tobramycin did not differ from the control group. The investigators considered that some recovery had occurred.

The high dose Tobraneb and TOBI rats showed significant recovery from the inflammatory changes that had been present in the nasal passages, larynx, and trachea

(including tracheal and bronchial bifurcations). The inflammatory changes were not reversed completely in all of the animals, but the incidence and severity were lower in the recovery animals compared to those who were sacrificed at the end of treatment. In addition, although alveolar macrophages (some containing debris) were still observed in the lungs of most tobramycin-treated rats, the degree of accumulation was lower than it had been at the end of the dosing period. Some of the changes in the respiratory tissues that had been present at the end of treatment (especially those seen in only a few animals) were not observed at all in the recovery groups. These included hyperplasia of olfactory epithelium in the nasal passages, squamous metaplasia in the larynx, hyperplasia of bronchiolar goblet cells in the lungs, and loss of cilia at the tracheal and bronchial bifurcations.

Toxicokinetics

Blood samples for TK were drawn from 4 rats per time point prior to dosing, immediately after dosing was finished, and 2 and 4 hours afterward. For the low and mid dose rats, TK was performed only on Day 28. It was done on Days 1 and 28 for the 2 high dose groups. Rats were sacrificed after blood draws. Plasma was collected, frozen, and shipped to (b) (4) for analysis using a validated HPLC method with tandem mass spectrometric detection.

There were significant differences between the intended doses and the actual doses administered to the rats on Days 1 and 28. Thus, the pharmacokinetic parameters were scaled to an administered dose of 1 mg/kg/day before comparisons were made between the treatment groups by the authors of the study report. Additionally, at some time points, there was considerable variability between the plasma levels of rats within the same dose group. Due to this variability, the reviewer hesitates to draw anything other than very broad conclusions from the TK data. Systemic exposure to tobramycin was observed following its administration via inhalation. Plasma accumulation was not observed over the 28 day course of treatment. Systemic exposure in females tended to be slightly higher than that in males, even after dose scaling.

This table from the study report provides values for TK parameters (not scaled):

Pharmacokinetic parameters of tobramycin on Days 1 and 28 of 4 weeks of daily inhalation exposure of rats to Tobraneb or Tobi (reference formulation)

Day 1

Males

Formulation	Nominal dose level (mg/kg/day)	C_{max} (ng/ml)	T_{max} (hours)	AUC_6 (ng.h/ml)	k ($hours^{-1}$)	t½ (hours)
Tobraneb	45	2296.17	End	5817	a	-
Tobi	45	2393.44	End	6561	0.5486	1.3

Females

Formulation	Nominal dose level (mg/kg/day)	C_{max} (ng/ml)	T_{max} (hours)	AUC_6 (ng.h/ml)	k ($hours^{-1}$)	t½ (hours)
Tobraneb	45	3536.64	End	8547	a	-
Tobi	45	2722.13	End	8440	0.4556	1.5

Day 28

Males

Formulation	Nominal dose level (mg/kg/day)	C_{max} (ng/ml)	T_{max} (hours)	AUC_6 (ng.h/ml)	k ($hours^{-1}$)	t½ (hours)
Tobi	5	576.48	End	1756	a	-
Tobraneb	5	689.29	End	2024	a	-
Tobraneb	15	769.50	End	2144	0.5966	1.2
Tobraneb	45	799.42	End	2691	a	-
Tobi	45	2007.11	End	5685	0.5919	1.2

Females

Formulation	Nominal dose level (mg/kg/day)	C_{max} (ng/ml)	T_{max} (hours)	AUC_6 (ng.h/ml)	k ($hours^{-1}$)	t½ (hours)
Tobi	5	1904.39	End	5077	0.6021	1.2
Tobraneb	5	847.23	End	2724	0.4193	1.7
Tobraneb	15	709.66	End	1940	0.5111	1.4
Tobraneb	45	1586.59	End	4384	0.6202	1.1
Tobi	45	3228.97	End	9001	0.6520	1.1

End End of 2 hour exposure period
a Could not be estimated in accordance with acceptance criteria (see Data Processing)

This table from the study report provides AUC values (for 0-6 hours after the initiation of dosing) scaled for a dose of 1 mg/kg/day:

		Nominal dose (mg/kg/day)	AUC_6 (ng.h/ml)/(mg/kg) Males	Females
Tobraneb	Day 1	45	273	351
	Day 28	5	471	539
		15	581	447
		45	194	275
Tobi	Day 1	45	522	580
	Day 28	5	273	676
		45	127	170

Dosing Solution Analysis

The concentration of tobramycin in the batch of Tobraneb used for the study was 70.40 mg/ml (94% of intended). The concentration of tobramycin in TOBI was 62.20 mg/ml (104% of intended).

Tobraneb: Toxicity Study by Inhalation Administration to Beagle Dogs for 1 Week

Study no.:	CHS 101/012880
Study report location:	Contract laboratory archives
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/10/01
GLP compliance:	UK, OECD, EC
QA statement:	Yes
Drug, lot #, and % purity:	Tobraneb, Batch No. 0011006, ≥95% pure by HPLC

Key Study Findings

The dogs tolerated one week of daily dosing with Tobraneb without exhibiting clinical signs of toxicity. The highest mean deposited dose of tobramycin in this study was about 16 mg/kg/day given during a 3 hour exposure session. There was no NOAEL in this study and the lowest mean deposited dose of tobramycin was about 4 mg/kg/day. All of the dogs exposed to Tobraneb had microscopic signs of local irritation in their nasal passages including hyperplasia, thinning, necrosis, and ulceration of squamous epithelium, erosion and hyperplasia of respiratory epithelium, and necrosis, hyperplasia, and inflammation of olfactory epithelium. The majority of these changes were minimal to slight and none was more than moderate.

Methods

Doses:	Dogs were exposed to vehicle (0.45% saline) and low, medium, and high concentrations of Tobraneb in the test atmosphere. The intended and achieved doses of test articles are discussed in detail below in the Results section.
Frequency of dosing:	Dogs breathed the test atmosphere 3 hours daily for 7 consecutive days.
Route of administration:	Inhalation (nose-only) of nebulized solutions using a face mask.
Formulation/Vehicle:	The vehicle for Tobraneb was 0.45% NaCl. This concentration of saline was also used for the vehicle control.
Species/Strain:	Beagle dogs
Number/Sex/Group:	1
Age:	Approximately 12 months
Weight:	11.4-12.7 (males) and 11.1-12.4 (females)
Unique study design:	Dogs were exposed to the test atmospheres for 3 hours each day. They were restrained in slings and the face masks placed over their noses and mouths were held in place by a muzzle. Face masks were connected to an aerosol expansion chamber fed by a nebulizer. Test articles were nebulized using the DeVilbiss Ultra-Neb®99. Test article concentration in the atmosphere was modulated by adjusting the air flow rate and the pump feed rate into the nebulizer. Discussion of achieved doses is presented below. Blood samples for TK were drawn 1,3, and 7 hour after dosing on Day 1. Animals were sacrificed the day following the final exposure.
Deviation from study protocol:	None reported

Observations and Results

Test Article Doses/Concentrations in Test Atmospheres

The administered doses of tobramycin were calculated by 2 different methods. The method used by the sponsor to calculate inhaled dose used the proportion of inhalable particles (percentage of particles (b) (4) µm aerodynamic diameter) determined to be available in the test atmosphere within the exposure chamber. The FDA generally assumes that 25% of the active drug in a test atmosphere will be deposited in the lungs of dogs. Samples were taken for measurement of tobramycin concentration in the atmosphere from an exposure port 5 minutes after the generation of the test atmosphere began (just before the masks were placed on the dogs) and 5 minutes after the masks were removed from the animals. The proportion of inhalable particles

(respirable fraction) was determined using a May Multistage Liquid Impinger. The particle size distribution sample was taken after exposure of the animals.

Actual tobramycin concentrations measured in the test atmospheres within the exposure chambers were close to the target for the low and high doses, but were greater than intended at the mid dose.

Treatment Group	Tobramycin Concentration in Test Atmosphere (mg/L)		
	Target	Study Mean	% of Target
Tobraneb Low	(b) (4)	0.289	(b) (4)
Tobraneb Mid		0.981	
Tobraneb High		1.154	

The respirable fractions in the low, medium, and high dose groups were 0.84, 0.80, and 0.73, respectively. The mass median aerodynamic diameter (MMAD) for each of these groups was (b) (4) µm. Geometric standard deviations for MMAD ranged from 1.48-1.73.

The equation used by the Sponsor for determining the administered dose of tobramycin was:

$$\text{Dose} = \frac{C \times RMV \times D \times F}{W}$$

Where C= concentration of tobramycin measured in test atmosphere (study mean)

RMV= respiratory minute volume, for dogs the formula was $4.99 \times W\ (kg)^{0.809}$

D= duration of exposure (minutes)

F= respirable fraction

W= body weight (kg) across dosing period

Based on this formula, the mean daily doses of tobramycin estimated by the sponsor were 13.71, 43.48, and 47.36 mg/kg.

The FDA/CDER also uses the formula above to estimate the dose of tobramycin deposited in the lungs of test animals, but instead of using the respirable fraction as the value for F, 0.25 is used as the standard lung deposition value for dogs.

These assumptions provide the following estimates for the mean daily dose of tobramycin deposited into the lungs of the dogs (table from the study report):

Group	Mean Daily Inhaled Dose (mg Tobramycin/kg)			
	Target	Estimated		
		Male	Female	Combined
2 (Low dose)	(b) (4)	4.06	4.10	4.08
3 (Intermediate dose)		13.56	13.64	13.60
4 (High dose)		16.04	16.38	16.21

Mortality

Dogs were observed at least twice daily for morbidity/mortality. There were no unscheduled deaths.

Clinical Signs

During the treatment period, dogs were observed for clinical signs of toxicity prior to exposure, 0.5-2 hours after exposure, and near the end of the work day. No clinical signs of tobramycin-related toxicity were observed.

Body Weights

Recorded weekly prior to the initiation of dosing and daily during the dosing period. No treatment-related effects were observed.

Feed Consumption

Recorded weekly, beginning 2 weeks before dosing was initiated. No treatment-related effects were observed.

Ophthalmoscopy

Not done.

ECG

Not done.

Hematology/Clinical Chemistry

Blood samples were drawn from fasted animals before the start of dosing and during week 1 (day not specified, not necessarily at the end of the study). No treatment-related effects on these parameters were observed.

Urinalysis

Urine was collected overnight while water was withheld before the start of dosing and during week 1 (day not specified, not necessarily at the end of the study). No treatment-related effects on urinary parameters were observed.

Gross Pathology

No treatment-related observations.

Organ Weights

These organs were weighed: adrenals, kidneys, liver, lungs. No treatment-related differences in organ weights was noted.

Histopathology

Adequate Battery: The abbreviated battery (abnormalities, adrenals, bronchi, kidneys, larynx, liver, lungs, nasal turbinates, trachea) was acceptable for this short study.

Peer Review: Not done.

Histological Findings: Microscopic changes indicative of local irritation were seen in the nasal passages of all dogs that were treated with Tobraneb, as described in the table below:

		Males				Females			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dosage (mg/kg/day)		**Veh**	**13.71**	**43.48**	**47.36**	**Veh**	**13.71**	**43.48**	**47.36**
Nasal vestibule									
Squamous epithelial ulceration	Total	0	0	1	0	0	0	1	1
	Minimal	0	0	1	0	0	0	1	0
	Slight	0	0	0	0	0	0	0	1
Squamous epithelial necrosis and inflammation	Total	0	1	0	0	0	0	0	0
	Minimal	0	1	0	0	0	0	0	0
Squamous epithelial thinning and inflammation	Total	0	0	1	0	0	1	0	0
	Slight	0	0	1	0	0	1	0	0
Squamous epithelial hyperplasia	Total	0	1	1	1	0	1	1	1
	Slight	0	1	1	1	0	1	1	0
	Moderate	0	0	0	0	0	0	0	1
Number of nasal passages examined		**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

		Males				Females			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dosage (mg/kg/day)		**Veh**	**13.71**	**43.48**	**47.36**	**Veh**	**13.71**	**43.48**	**47.36**
Respiratory epithelium									
Erosion	Total	0	0	0	0	0	0	1	0
	Minimal	0	0	0	0	0	0	1	0
Hyperplasia	Total	0	1	1	1	0	1	1	1
	Minimal	0	0	1	1	0	1	0	0
	Slight	0	1	0	0	0	0	1	1
Olfactory epithelium									
Necrosis	Total	0	1	1	1	0	1	1	1
	Minimal	0	0	0	1	0	1	0	0
	Slight	0	1	1	0	0	0	1	0
	Moderate	0	0	0	0	0	0	0	1
Hyperplasia	Total	0	0	0	0	0	0	1	0
	Minimal	0	0	0	0	0	0	1	0
Inflammatory cells in lamina propria	Total	0	1	0	1	0	1	1	1
	Minimal	0	1	0	1	0	1	1	1
Number of nasal passages examined		**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

None of the findings in the other tissues examined microscopically appeared to be related to Tobraneb.

Toxicokinetics

Results were not included in the study report. The TK portion of the study was said to be "preliminary" and not was not included in the study contractor's report at the request of the sponsor. The results do not appear to have been submitted in a separate report. TK data are available from the 28-day dog study, so this omission is not crucial to study interpretation.

Dosing Solution Analysis

The concentration of tobramycin in the batch of Tobraneb used for the study was 70.40 mg/ml (94% of intended).

Tobraneb: Toxicity Study by Inhalation Administration to Beagle Dogs for 4 Weeks Followed by a 4 Week Recovery Period

Study no.:	CHS 102/014192
Study report location:	Contract laboratory archives
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/12/01
GLP compliance:	UK, OECD, EC
QA statement:	Yes
Drug, lot #, and % purity:	Tobraneb, Batch No. 0011006, ≥95% pure by HPLC

Key Study Findings

Tobraneb was not associated with clinical signs of systemic toxicity when administered via inhalation to dogs for 28 days during 150 minute daily dosing sessions. The mean estimated daily deposited high dose of Tobraneb was 17 mg/kg (average of both sexes). Plasma levels of tobramycin were in the quantifiable range after treatment and the observation that plasma samples taken prior to dosing on Day 28 contained measurable quantities of tobramycin suggested that accumulation occurred over the course of the study. Dose-related microscopic findings in the kidneys included basophilic cortical tubules and minimal single cell necrosis, among others. Despite the microscopic changes in the kidneys, none of the animals exhibited clinical chemistry changes indicative of renal toxicity. Kidney changes were still seen 4 weeks after the end of treatment in the male dog assigned to the recovery group, but severity of the renal findings was less in the female. Microscopic signs of local irritation (hyperplasia, metaplasia, parakeratosis, inflammation, erosion/ulceration) of respiratory tissues, especially of the epithelium, were observed in most animals treated with Tobraneb. Tissue samples from the nasal passages, larynx, trachea, and lungs were examined. Findings in the respiratory tissues were consistent with those in the 1-week dog study. Most changes were graded minimal to slight, none was more than moderate. For a number of these changes (e.g., epithelial hyperplasia and inflammation in the larynx and trachea; necrosis and erosion of olfactory epithelium of the nasal turbinates), the incidence and severity were not dose-related. Other signs of local irritation appeared

dose-related, including hyperplasia of the respiratory and olfactory epithelium of the nasal turbinates and metaplasia of the olfactory epithelium. Accumulation of alveolar macrophages containing debris was observed in the mid and high dose tobramycin-treated dogs; severity scores were slightly greater at the high dose. Significant recovery from the respiratory changes was observed in the high dose dogs after 4 weeks without treatment. Tobraneb was relatively well tolerated by the dogs.

Methods

Doses:	Dogs were exposed to vehicle (0.45% saline) and low, medium, and high concentrations of Tobraneb in the test atmosphere. The intended and achieved doses of test articles are discussed in detail below in the Results section.
Frequency of dosing:	Dogs breathed the test atmosphere 150 minutes daily for 28 consecutive days.
Route of administration:	Inhalation (nose-only) of nebulized solutions using a face mask.
Formulation/Vehicle:	The vehicle for Tobraneb was 0.45% NaCl. This concentration of saline was also used for the vehicle control.
Species/Strain:	Beagle dogs
Number/Sex/Group:	3
Age:	Approximately 7 months
Weight:	6.7-11.0 kg
Satellite groups:	1/sex in the vehicle control and high dose groups for recovery.
Unique study design:	Dogs were exposed to the test atmospheres for 150 minutes each day. They were restrained in slings and the face masks placed over their noses and mouths were held in place by a muzzle. Face masks were connected to an aerosol expansion chamber fed by a nebulizer. Test articles were nebulized using the DeVilbiss Ultra-Neb®99. Test article concentration in the atmosphere was modulated by adjusting the air flow rate and the pump feed rate into the nebulizer. Discussion of achieved doses is presented below. Blood samples for TK were drawn prior to dosing, and 0, 2, 4, and 6 hours after dosing on Days 1 and 28. Animals in the main study groups were sacrificed the day following the final exposure. Recovery animals were sacrificed after 4 weeks without treatment.
Deviation from study protocol:	None reported

Observations and Results

Test Article Doses/Concentrations in Test Atmospheres

The administered doses of tobramycin were calculated by 2 different methods. The method used by the sponsor to calculate inhaled dose used the proportion of inhalable particles (percentage of particles (b) (4) µm aerodynamic diameter) determined to be available in the test atmosphere within the exposure chamber. The FDA generally assumes that 25% of the active drug in a test atmosphere will be deposited in the lungs of dogs. Samples for measurement of tobramycin concentration in the test atmosphere were taken immediately after exposure of the low and mid dose groups or from an unoccupied exposure port while test article was administered to the high dose dogs. The proportion of inhalable particles (respirable fraction) was determined using a May Multistage Liquid Impinger. Samples for determining the particle size distribution in the test atmosphere were taken immediately before or immediately after exposure of the low and mid dose dogs or from an unoccupied exposure port during exposure of the high dose animals.

Actual mean tobramycin concentrations measured in the test atmospheres within the exposure chambers were relatively close to the targets. The sponsor presented the data for the high dose Tobraneb test atmosphere by sex; it was not clear why since the concentrations were close to one another.

Treatment Group	Tobramycin Concentration in Test Atmosphere (mg/L)		
	Target (b) (4)	Study Mean	% of Target (b) (4)
Tobraneb Low		0.189	
Tobraneb Mid		0.542	
Tobraneb High		1.350 (M); 1.441 (F)	

The respirable fractions in the low, medium, and high dose groups were 0.84, 0.89, and 0.79, respectively.

The equation used by the Sponsor for determining the administered dose of tobramycin was:

$$\text{Dose} = \frac{C \times RMV \times D \times F}{W}$$

Where C= concentration of tobramycin measured in test atmosphere (study mean)

RMV= respiratory minute volume, for dogs the formula was $4.99 \times W\,(kg)^{0.809}$

D= duration of exposure (minutes)

F= respirable fraction

W= body weight (kg) across dosing period

Based on this formula, the mean daily doses of tobramycin estimated by the sponsor were 7.7, 23.5, and 53.9 mg/kg.
The FDA/CDER also uses the formula above to estimate the dose of tobramycin deposited in the lungs of test animals, but instead of using the respirable fraction as the value for F, 0.25 is used as the standard lung deposition value for dogs.

These assumptions provide the following estimates for the mean daily dose of tobramycin deposited into the lungs of the dogs (table from the study report):

Group	Mean Daily Inhaled Dose (mg Tobramycin/kg)			
	Target	Estimated[1]		
		Male	Female	Combined
2 (Low dose)	(b) (4)	2.28	2.32	2.30
3 (Intermediate dose)		6.46	6.74	6.60
4 (High dose)		16.09	17.91	17.00

[1] Calculated using the mean C (concentration of tobramycin in air) for Days 1 – 28

There were significant differences in estimated exposure on Days 1 and 28 (especially at the low and mid doses). This table from the report is included to present them:

Daily Inhaled Dose (mg Tobramycin/kg)					
Group	Target	Estimated			
		Day 1[1]		Day 28[2]	
		Male	Female	Male	Female
2 (Low dose)	(b) (4)	0.35	0.36	4.59	4.68
3 (Intermediate dose)		9.79	10.21	5.28	5.51
4 (High dose)		23.40	15.79	14.45	20.87

[1] Calculated using the C for Day 1
[2] Calculated using the C for Day 28

Mortality

Dogs were observed at least twice daily (in the morning and at the end of the work day) for morbidity/mortality. There were no unscheduled deaths.

Clinical Signs

During the treatment period, dogs were observed for clinical signs of toxicity prior to exposure, 0.5-2 hours after exposure, and near the end of the work day. In addition, detailed clinical observations were recorded daily during Week 1 of treatment, twice weekly during Weeks 2-4, and weekly during recovery. Occasionally, the animals treated with Tobraneb vomited or had liquid feces. These signs are more likely due to the anti-microbial effect of tobramycin rather than compound specific toxicity.

Body Weights

Recorded at the initiation of dosing, twice weekly during the study, and at necropsy. There were no treatment-related effects.

Feed Consumption

Recorded weekly. There were no treatment-related effects.

Ophthalmoscopy

Conducted prior to the initiation of dosing and during Week 4. No changes related to drug treatment were observed.

ECG

Not done.

Hematology

Blood samples were drawn from fasted main study and recovery animals during week 4. There were no treatment-related effects.

Clinical Chemistry

Blood samples were drawn from fasted main study and recovery animals during week 4. There were no treatment-related effects.

Urinalysis

Urine samples were collected overnight from main study and recovery animals during week 4. Animals did not have access to food or water during the collection period. No treatment-related changes were noted.

Gross Pathology

Enlarged kidneys were seen in 2/6 high dose dogs (one per sex). Pale areas were observed on the lungs of 2/3 high dose males and 1 female each in the mid and high dose groups. The high dose recovery male also had pale areas on its lungs. There were no other gross findings that appeared treatment-related.

Organ Weights

A modest increase in mean absolute kidney weight was observed in the high dose groups, but was only statistically significant in the males ($p \leq 0.05$). Absolute lung weights were greater in the drug-treated dogs than controls, but the increase was not statistically significant.

Histopathology

Adequate Battery- A standard battery of tissues was collected and preserved, including an expanded panel of respiratory-associated tissues. All tissues from the main study and recovery animals were examined.

Peer Review- Not done.

Histological Findings- Changes in the kidneys were observed more frequently in the tobramycin-treated dogs than controls and the incidence and severity were drug related. Findings were most frequently graded minimal in the low dose group and ranged from slight to moderate at the higher doses. Kidney changes included basophilic cortical tubules, eosinophilic granular cytoplasm of the cortical tubules, cortical tubular single cell necrosis, and cortical interstitial inflammatory cell infiltrate. Medullary inflammatory cell infiltrate was also observed in 1 mid and 1 high dose male and 2 high dose females. One high dose male exhibited dilated Bowman's capsules. The majority of kidney changes observed in the high dose animals sacrificed at the end of treatment were still observed in the high dose male sacrificed after the 4-week recovery period. The high dose recovery female showed only minimal basophilic cortical tubules and dilated tubules.

Microscopic findings consistent with irritation and inflammation were observed in tissues from the respiratory tract, similar to other studies with inhaled tobramycin.

In the larynx (including the vestibular and vocal folds, lateral ventricles, laryngopharynx, and areas overlying the arytenoid cartilage), minimal to moderate epithelial hyperplasia was seen in some dogs from all Tobraneb groups. In some cases, this finding was accompanied by minimal to moderate epithelial and subepithelial inflammation. Parakeratosis (minimal to slight) in the vocal folds was also more frequently observed in Tobraneb-treated dogs compared to controls. The laryngeal findings did not appear dose-related.

Hyperplasia of the respiratory and olfactory epithelium of the nasal turbinates was observed in dogs treated with Tobraneb. The incidence and severity of this finding were related to dose. At the low dose, the epithelial hyperplasia was graded minimal to slight; grades up to moderate were seen at the high dose. In the respiratory epithelium of the nasal turbinates, minimal to slight erosion and epithelial vacuolation were observed in several Tobraneb-treated dogs (not dose-related). Control dogs all exhibited minimal inflammatory cells in the lamina propia of the respiratory epithelium, but this finding was more prominent (grade of slight) in several of the dogs treated with tobramycin. In the olfactory epithelium, minimal to slight respiratory metaplasia was observed in dogs from the Tobraneb groups and it was seen more frequently in the mid and high dose animals. Minimal to slight necrosis and erosion (sometimes accompanied by regenerative hyperplasia) were observed in the olfactory epithelium of the dogs that received Tobraneb, but these findings did not appear related to dose. Inflammatory cells were seen in the lamina propia of the olfactory epithelium in a few dogs from each Tobraneb group.

Minimal to slight epithelial hyperplasia of the trachea or at the tracheal bifurcation was observed more frequently in tobramycin-treated dogs than controls, but neither its incidence nor its severity appeared dose-related. Epithelial erosion and inflammation was seen in one of the low dose males. Minimal epithelial hyperplasia was also

observed in the female control recovery dog, demonstrating that this finding may not be related to specific treatment.
Alveolar macrophages containing pigment or debris were seen in the lungs of the mid and high dose dogs. This finding was minimal in most of the mid dose dogs and the high dose dogs all received scores of slight. In the high dose group, 4/6 dogs also had granulomas with macrophages that contained pigment or debris.

Substantial recovery from the respiratory findings was apparent in the high dose dogs after the 4-week period without treatment. Tobramycin-related changes were not evident in the larynx and there were fewer findings in the nasal turbinates. Findings were present only in the olfactory epithelium and they were of reduced severity. They included minimal hyperplasia, minimal atrophy/disorganization, and minimal to slight respiratory metaplasia. In the lungs, alveolar macrophages containing pigment or debris were still present, but the finding was minimal.

Toxicokinetics

Plasma collected from the dogs for tobramycin TK determination was frozen and shipped to (b) (4) for analysis using a validated HPLC method with tandem mass spectrometric detection.

There were significant differences between the intended doses and the actual doses administered to the dogs on Days 1 and 28. Thus, the pharmacokinetic parameters were scaled to an administered dose of 1 mg/kg/day before comparisons were made between the treatment groups by the authors of the study report. Sporadically, tobramycin was detected in plasma samples from control dogs, but most values were near the limit of quantification (5 ng/ml).

This table from the study report provides the sponsor's intended nominal dose and the exposures that they estimated as having been achieved on Days 1 and 28 according to their methodology (see above):

Nominal dose level (mg/kg/day)	Estimated exposure(mg/kg/day) Day 1		Day 28	
	Males	Females	Males	Females
5	1.181	1.212	15.288	15.615
15	34.864	36.365	18.667	19.356
45	75.923	49.337	46.359	64.566

The systemic exposure of the dogs to tobramycin increased in a roughly dose-proportional manner. In most animals, Cmax occurred at the end of the exposure period. Systemic exposure was generally lower in female dogs than males. At the end of the dosing period, all of the dogs treated with Tobraneb had measurable plasma

concentrations of tobramycin, increasing with dose. This suggests accumulation after repeated dosing.
This table from the study report provides values for Cmax and $AUC_{0\text{-}8.5\ h}$ on Days 1 and 28 of administration(not scaled):

Nominal dose level (mg/kg/day)	C_{max} (ng/ml) Day 1 Males	Day 1 Females	Day 28 Males	Day 28 Females	$AUC_{8.5}$ (ng.h/ml) Day 1 Males	Day 1 Females	Day 28 Males	Day 28 Females
5	256.46	124.51	4747.00	4206.87	897	535[a]	18526	15640
	(133.77)	(63.64)	(349.30)	(1339.32)	(419)	(-)	(-)	(4153)
15	6720.93	6517.58	6366.64	5345.87	23622	18991	21743	16518
	(842.22)	(1487.83)	(726.34)	(492.06)	(3654)	(-)	(2072)	(1794)
45	9515.59	10779.84	22310.40	15935.37	42039	37286	131476	52962
	(-)	(2655.03)	(-)	(2490.14)	(-)	(11763)	(-)	(11574)

[a] Calculated from one animal only

This table from the study report provides AUC values (for 0-8.5 hours after the initiation of dosing) scaled for a dose of 1 mg/kg/day:

	Nominal dose (mg/kg/day)	$AUC_{8.5}$ (ng.h/ml)/(mg/kg) Males	Females
Day 1	5	760	441
	15	678	522
	45	554	756
Day 28	5	1212	1002
	15	1165	853
	45	2836	820

Dosing Solution Analysis

The concentration of tobramycin in the batch of Tobraneb used for the study was 70.40 mg/ml (94% of intended).

7 Genetic Toxicology

From the TOBI® label: “TOBI® has been evaluated for genotoxicity in a battery of in-vitro and in-vivo tests. The Ames bacterial reversion test, conducted with 5 tester strains, failed to show a significant increase in revertants with or without metabolic activation in all strains. Tobramycin was negative in the mouse lymphoma forward mutation assay, did not induce chromosomal aberrations in Chinese hamster ovary cells, and was negative in the mouse micronucleus test.”

8 Carcinogenicity

From the TOBI® label: "A two-year rat inhalation toxicology study to assess carcinogenic potential of TOBI® has been completed. Rats were exposed to TOBI® for up to 1.5 hours per day for 95 weeks. The clinical formulation of the drug was used for this carcinogenicity study. Serum levels of tobramycin of up to 35 mcg/mL were measured in rats, in contrast to the average 1 mcg/mL levels observed in cystic fibrosis patients in clinical trials. There was no drug-related increase in the incidence of any variety of tumor."

9 Reproductive and Developmental Toxicology

Aminoglycoside antimicrobials have been assigned to Pregnancy Category D, based on clinical data. From the TOBI® label: "Subcutaneous administration of up to 100 mg/kg of tobramycin did not affect mating behavior or cause impairment of fertility in male or female rats. Subcutaneous administration of tobramycin at doses of 100 or 20 mg/kg/day during organogenesis was not teratogenic in rats or rabbits, respectively. Doses of tobramycin ≥40 mg/kg/day were severely maternally toxic to rabbits and precluded the evaluation of teratogenicity. Aminoglycosides can cause fetal harm (e.g., congenital deafness) when administered to a pregnant woman. Ototoxicity was not evaluated in offspring during nonclinical reproduction toxicity studies with tobramycin."

10 Special Toxicology Studies

None were performed with tobramycin inhalation solution.

11 Integrated Summary and Safety Evaluation

This new formulation of tobramycin for inhalation (300 mg tobramycin/4 ml (b) (4) % NaCl) submitted by Chiesi Pharmaceuticals does not appear produce substantially different toxic effects compared to the approved product TOBI (300 mg tobramycin/5 ml (b) (4) % NaCl) based on repeat dose toxicity studies in rats.

Chiesi's formulation of tobramycin for inhalation was tested in both rats and dogs in repeat dose toxicity studies. These were 1 week and 28 days in length. The rat studies used Chiesi's tobramycin for inhalation and included TOBI as a comparator. The dog studies used only Chiesi's product.

Toxicokinetic analysis demonstrated systemic exposure to tobramycin in the rats and dogs after administration via inhalation. However, no clinical signs of systemic toxicity were observed in any of the studies. In the 28-day dog study, plasma levels of tobramycin were measurable before tobramycin was administered on the final day of dosing, suggesting that some accumulation occurred in these animals over the course of the study. Accumulation was not apparent in the rats.

Microscopic kidney changes were observed in both species at the end of tobramycin treatment (regardless of formulation), consistent with what would be expected following exposure to an aminoglycoside. No clinical chemistry changes indicative of renal toxicity were seen. Complete recovery of the kidneys was not observed after a 4 week drug-free period. In both rats and dogs, it appeared that the females experienced greater recovery from the drug's effects on the kidneys.

Microscopic changes in respiratory tissues were observed in rats and dogs following either 7 or 28 days of treatment with inhaled tobramycin. In rats, where both TOBI and the Chiesi product were tested in the same studies, there did not appear to be a substantial difference in findings between either formulation when dose was taken into account. Changes in the respiratory tissues were consistent with irritation and inflammation. Most were graded minimal or slight and none was more than moderate. Some changes (e.g., hyperplasia of the respiratory and olfactory epithelium of the nasal turbinates) were dose-related in both rats and dogs, but a number of other microscopic changes in respiratory tissues were not related to the tobramycin dose (particularly inflammatory changes). Substantial recovery of the respiratory tissues was observed following 4 weeks without drug treatment. Accumulation of alveolar macrophages (most containing debris or pigment- possibly scavenged drug) was observed in the rats and dogs treated with tobramycin. The higher doses of drug were associated with increased severity of this finding. Although alveolar macrophages were still evident in the lungs after the recovery period, the severity of the finding was reduced.

The data from the repeat dose toxicity studies submitted in this application suggest that the toxicology profile of Chiesi's formulation of tobramycin for inhalation is comparable to the approved product TOBI. Thus, the pharmacologist has no objection to the approval of this NDA.

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/s/

AMY L ELLIS
07/19/2011

WENDELYN J SCHMIDT
07/19/2011