

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

206307Orig1s000

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 REVIEW AND EVALUATION

Application number: 206307

Supporting document/s:

- SD 1 (original NDA submission submitted 4/25/2014)
- SD8 (Response to Information Request submitted 8/13/2014)

Applicant's letter date: April 25, 2014

CDER stamp date: April 25, 2014

Product: Finafloxacin Otic Suspension

Indication: Treatment of acute otitis externa

Applicant: Alcon Research, Ltd.

Review Division: Division of Transplant and Ophthalmology Products (DTOP), Office of Antimicrobial Products (OAP), CDER, HFD-590

Primary Reviewer for Application: Andrew J. McDougal, Ph.D., D.A.B.T., DTOP

Supervisor/Team Leader: Lori E. Kotch, Ph.D., D.A.B.T., DTOP

Division Director: Renata Albrecht, M.D., DTOP

Project Manager: Michael Puglisi

MEMORANDUM

To: The file

Cc: Renata Albrecht, M.D., Division Director, Division of Transplant and Ophthalmology Products (DTOP), Office of Antimicrobial Products (OAP)

From: Lori E. Kotch, PhD, DABT, Toxicologist/Team Leader, Division of Transplant and Ophthalmology Products (DTOP), Office of Antimicrobial Products (OAP)

NDA #: 206307

Submission Type: Original NDA application

Product: Finafloxacin Otic Suspension

Date: September 27, 2014

Recommendations: I concur with Dr. Andrew McDougal's conclusions regarding the nonclinical findings for Finafloxacin Otic Suspension, his recommendation that the submitted Application be approved for marketing, and his recommendations regarding the language for the prescribing information. A copy of Dr. McDougal's review, with supervisory sign-off (9-25-2014) has been uploaded into the DARRTs database.

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/s/

LORI E KOTCH
10/05/2014

Comments on N 206307 finafloxacin otic suspension

From : A. Jacobs, AD

Date: 9/22/14

1. I concur that there are no outstanding pharm-tox issues
2. I agree with pregnancy category C
3. I have conveyed some other comments to the reviewer- and supervisor and they will address them as appropriate

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/s/

ABIGAIL C JACOBS
10/03/2014

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1 Executive Summary

1.1 Introduction

- On April 25, 2014, Alcon Research, Ltd. submitted new drug application (NDA) #206307 for finafloxacin otic suspension for the treatment of acute otitis externa (AOE) under the 505(b)(1) pathway.
- Finafloxacin is a new molecular entity (NME). The NDA is being reviewed under the Prescription Drug User Fee Act V (PDUFA) Program.
- The clinical data to support NDA 206307 was collected under Alcon's active IND 110576.
- From a Nonclinical Pharmacology/Toxicology perspective, approval of NDA 206307 is recommended; no safety-related approvability issues were identified.

1.2 Brief Discussion of Nonclinical Findings

Finafloxacin is a member of the fluoroquinolone class of antibiotics

- Review of the Applicant's microbiology data is outside the scope of this review, and is deferred to the Clinical Microbiology discipline.
- Like other fluoroquinolones, the mechanism of action of finafloxacin is inhibition of bacterial DNA gyrase and topoisomerase IV, resulting in inhibition of bacterial cell division and bacterial cell death.

Finafloxacin was developed to retain biological activity at pH (b)(4) to treat sites of local infection

- AOE is swimmer's ear, inflammation and infection of the external ear canal. The Applicant reports that physiological pH is ~ 7.4, and the infected external ear canal has a local pH of ~6.0.
- Reportedly, ciprofloxacin and levofloxacin have reduced activity at lower pH.
- Finafloxacin is amphoteric; its activity is dependent on the pH of the surrounding medium. The Applicant reports that their data demonstrate that finafloxacin's optimal pH range is 5.8 to 6.2, with reduced activity at pH 7.0.

Topical otic safety

The safety of the AOE indication is supported by two 14-day toxicology studies in New Zealand rabbits, both conducted in compliance with good laboratory practices (GLP). Following twice-daily topical otic dosing (4 drops/dose, ~30 µl drop size):

- The first study detected minimal-to-mild local toxicity with finafloxacin hydroxide in phosphate buffer at pH 7.5. The high-dose (1.0% finafloxacin, ~ 2.18 mg/animal/day) was the no observed adverse effect level (NOAEL) for systemic toxicity. This study included clinical pathology (hematology, clinical chemistry, coagulation), and necropsy (organ weights, gross pathology and histopathology) for the ears (pinna and bulla), adrenals, brain, heart, kidney, liver, lungs, ovary, spleen and testes.

- The second study tested finafloxacin (free base). The NOAEL was the highest dose tested, 1.2% (~ 2.78 mg/animal/day). Clinical pathology was assessed, but necropsy was limited to the ears (external ear canal, tympanic membrane, bulla, middle ear ossicles, and Eustachian tube).

Patient dose	Rabbit doses	Exposure margin
0.3%, 4 drops twice daily for seven days	1.0%, 4 drops twice daily for 14 days (tolerable local effects, systemic NOAEL)	3.3-fold
	1.2% (NOAEL for local toxicity)	4-fold

Other P/T data

- Four studies were conducted to assess the toxicity of direct instillation of finafloxacin suspension into the middle ear. Local toxicity, consistent with minimal-to-mild irritation, was detected.
- In support of other indications, oral and intravenous (iv) safety pharmacology, pharmacokinetic, and general toxicity studies in rodents and dogs have been conducted.
- Finafloxacin was demonstrated to be mutagenic and clastogenic.
- Nonclinical carcinogenicity studies were not conducted, and are not warranted for this indication (topical otic dosing for 7 days, systemic exposure following topical otic dosing is minimal).
- The results of the oral nonclinical fertility and embryofetal studies are not relevant to topical otic dosing, because these studies tested a range of doses with estimated systemic exposures more than one thousand-fold higher than the highest systemic exposure detected in patients following topical otic dosing. Topical otic route or lower systemic dose studies to further investigate fertility and developmental toxicity are not warranted to support the AOE indication.
 - The rat general toxicity studies identified the male reproductive system as a sensitive target of finafloxacin. Consistent with this finding, the rat oral fertility study observed complete male infertility at 500 mg/kg/day.
 - Three oral embryofetal studies were conducted; finafloxacin was clearly teratogenic.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical Pharmacology/Toxicology perspective, finafloxacin 0.3% has been shown to be safe for the proposed indication, treatment of acute otitis externa (AOE), with or without an otowick, in pediatric (age ^{(b) (4)} and older), adult and elderly patients, with dosing by instillation into the affected ear for seven days.

1.3.2 Additional Non Clinical Recommendations

- The clinical trials to support the AOE indication for this NDA were conducted in patients with intact tympanic membranes. The Applicant's draft label does not limit the use to this patient population. One of the nonclinical middle-ear dosing studies raise potential concerns about hearing loss. P/T discussed these findings with Clinical (personal communications, McDougal/Lloyd, September 2014). Clinical will take the lead in reviewing across finafloxacin's otic investigational new drug (IND) files to determine what clinical hearing loss data are available, and if/when additional clinical evaluation would be appropriate.
- A metabolite study (report # TDOC-0016534) observed substantially more formation of AL-91591 (the β -glucuronide ester of finafloxacin) in humans compared to either dogs or rats. This difference in metabolism is not a safety issue for the AOE indication, because the amounts of AL-91591 are qualified by the oral 28-day toxicity studies. As other clinical indications are developed, the need for toxicology testing for the AL-91591 metabolite should be considered.

1.3.3 Labeling

P/T proposes the following draft labeling to the review team for consideration (please see Appendix 1 of this review for a narrative explanation):

8.1 Pregnancy Pregnancy Category C

Risk Summary

There are no adequate or well-controlled studies with [NAME] in pregnant women. Finafloxacin was shown to be teratogenic in rabbits and rats following oral administration. Neural tube defects and skeletal anomalies in both species, and limb anomalies in rabbits, were observed at exposures estimated to be 1300 ^{(b) (4)} times the maximum human systemic exposure following topical otic administration of 0.3% finafloxacin. Because animal studies are not always predictive of human responses, [NAME] should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal Data

In rabbit embryofetal studies, maternal toxicity was not observed at oral doses up to 9 mg/kg/day (estimated 8000 times the maximum human systemic exposure [0.234 ng/ml] following topical otic administration with 0.3% finafloxacin). Fetal toxicity was observed at the lowest dose tested, 1 mg/kg/day (estimated 1300 times the maximum human systemic exposure following topical otic administration with 0.3% finafloxacin), and included exencephaly, enlarged fontanel, spina bifida, phocomelia, paw hyperflexure, missing lumbar vertebra, missing lumbar arch, and sternebra fusion.

In a rat embryofetal study, no adverse maternal toxicity was observed at oral doses up to 100 mg/kg/day (estimated 60,000 times the maximum human systemic exposure following topical otic administration with 0.3% finafloxacin). The developmental no observed adverse effect level (NOAEL) was 30 mg/kg (estimated 22,000 times the maximum human systemic exposure following topical otic administration with 0.3% finafloxacin). Exencephaly was observed in one fetus at 100 mg/kg. At 500 mg/kg, additional developmental toxicities were observed including increased preimplantation loss, decreased fetal weight, decreased placental weight, increased incidence of non-ossified sternebrae, and delayed ossifications in the sternebrae, xiphisternum, sacral arches and metacarpals.

8.3 Nursing Mothers

Finafloxacin has been identified in the milk of nursing rats following oral administration. Following oral administration of 2 mg/kg finafloxacin to nursing rats, concentrations of finafloxacin in rat milk were higher (up to 6.5-fold) than that measured in maternal plasma. Finafloxacin was not detected in milk or plasma at 24 hours post dose.

The human systemic concentration of [NAME] following topical otic treatment is low [see Clinical Pharmacology (12.3)]. It is not known whether topical otic administration could result in sufficient systemic absorption to produce detectable quantities in the human milk. Caution should be exercised when finafloxacin is administered to a nursing mother.

12.1 Mechanism of Action

[Language deferred to Clinical Microbiology]

13. NONCLINICAL TOXICOLOGY**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**Carcinogenicity

Animal studies have not been conducted to determine the carcinogenic potential of finafloxacin.

Mutagenesis

Finafloxacin was shown to be genotoxic and clastogenic *in vitro*, with and without metabolic activation, and *in vivo*.

In a bacterial reverse mutation assay, finafloxacin was positive in only one strain (TA102).

Finafloxacin was positive in mammalian cell culture assays: mouse lymphoma cell forward mutation assays, a mutagenicity assay in V79 Chinese hamster lung cells, and a micronucleus test in V79 cells.

Finafloxacin was clastogenic in mouse micronucleus studies.

Impairment of fertility

An oral rat fertility study detected a NOAEL for male and female fertility of 100 mg/kg/day (estimated 60,000 times the maximum human systemic exposure following topical otic administration with 0.3% finafloxacin). At 500 mg/kg/day, males were completely infertile, presumably due to low sperm count and sperm immobility.

General toxicity studies in rats have confirmed sperm toxicity following oral and intravenous dosing. Following intravenous dosing, the NOAEL for sperm toxicity was 30 mg/kg/day (150,000 times the maximum human exposure following topical otic administration with 0.3% finafloxacin).

2 Drug Information

2.1 Drug

Chemical Abstract Services Registry Number (CASRN)	209342-40-5 (for finafloxacin free base) 209342-4-6 (finafloxacin hydrochloride)
Generic name	Finafloxacin
Proposed trade name	[pending]
Code names:	<ul style="list-style-type: none"> • Finafloxacin hydrochloride • AL-60371 otic suspension 0.3% • AL-60371 (refers to finafloxacin free base) • AL-60371A (the hydrochloride salt of finafloxacin) • ██████████^{(b) (4)} (both manufacturing process code names refer to finafloxacin free base) • ██████████^{(b) (4)}, BYK 60621 (finafloxacin hydrochloride) • BAY 35-3377, BAY 14-1881 (listed in NDA module 2.6.6.1) • Gastroquinolone • BSP 1889, JJT 1660 (listed in report # PH 25561)
Chemical name (for the free base form)	(-)-8-Cyano-1-cyclopropyl-6-fluoro-7-[(4aS,7aS)-hexahydropyrrolo[3,4-b]-1,4-(oxazin-6(2H)- ^{(b) (4)}]-4-oxo-1,4-dihydroquinoline--3-carboxylic acid
Molecular formula	C ₂₀ H ₉ FN ₄ O ₄
Molecular weight	<ul style="list-style-type: none"> • 398.39 g/mole (free base) • ██████████^{(b) (4)} (hydrochloride salt)
Structure	<p>Figure 1: Structure of finafloxacin (free base)</p>
Pharmacologic class	Fluoroquinolone antibiotic

2.2 Relevant INDs, NDAs, and DMFs

Table 1: List of INDs relevant to NDA 206307

IND #	Submitter	Product	Review division	Status	Indication
	(b) (4)	Finafloxacin hydrochloride	DAIP	Active	(b) (4)
		Finafloxacin hydrochloride	DTOP	preIND	
110576	Alcon Inc.	Finafloxacin	DTOP	Active	Treatment of acute otitis externa and acute otitis media (b) (4)
(b) (4)					

- IND (b) (4) was the originator IND.
- IND 110576 is the precursor IND for this NDA (internally, the electronic document room is accessible via: <\\CDSESUB1\evsprod\IND110576\110576.enx>)
- PreIND (b) (4) has no files in DARRTS (dated to 2010)

2.3 Drug Formulation

Table 2: Drug product formulation: finafloxacin otic suspension 0.3%

Component	Concentration (% weight/volume)	Purpose	Compendial status
Finafloxacin	0.3%	Active	Non-compendial
Tyloxapol	(b) (4)	(b) (4)	USP
Hydroxyethyl cellulose (b) (4)			NF
Sodium chloride			USP
Magnesium Chloride (b) (4)			USP
Benzalkonium Chloride (BAC)		Preservative	NF
Sodium hydroxide and/or hydrochloric acid		Adjust pH to 6.0	NF
Purified water		(b) (4)	USP

Q.S. = quantum satis. USP = United States Pharmacopeia. NF = national formulary.

Alcon's (b) (4) package system" is used as the container closure system for drug delivery.

2.4 No Comments on Novel Excipients

No novel excipients are used in the drug product.

2.5 Comments on Impurities/Degradants of Concern

- No concerns identified for impurities or degradants.
- The manufacturing process for the drug substance (NDA module 2.3.S Drug Substance has):
 - (b) (4) as the starting material. (b) (4)
 - The manufacturing process uses (b) (4)
 -

2.6 Proposed Clinical Population and Dosing Regimen

The Applicant proposed:

- Finafloxacin otic suspension, 0.3% "indicated for the treatment of acute otitis externa (AOE), with or without an otowick, in (b) (4) adult and elderly patients."
- 4 drops into the affected ear, twice daily, for seven days (0.72mg/day).
- For patients needing an otowick for effective delivery, the initial daily dose is doubled (to 8 drops; 1.44mg/day), followed by 4 drops per dose, twice daily for the remainder of the 7 days (0.72mg/day).

3 Studies Submitted

3.1 Studies Reviewed

Table 3: Pharmacodynamic studies submitted (NDA module 4.2.1.1 and 4.2.1.2) and reviewed

Report # TDOC	Report title	GLP status	# of pages
Primary pharmacology			
-0014368	Evaluation of Clinical Finafloxacin (AL-60371) Suspension Formulations in a Guinea Pig Model of <i>P. aeruginosa</i> Acute Otitis Externa	No	8
-0017323	<i>In vivo</i> evaluation of AL-60371 (Finafloxacin) clinical formulation	No	43

-0017324	<i>In vivo</i> evaluation of Finafloxacin (AL-60371) formulations in a screening model of <i>P. aeruginosa</i> acute otitis externa	No	32
Secondary pharmacology			
PH-32521	<i>In vivo</i> evaluation of BAY 35-3377 in models of bacterial infection	No	53

P/T reviewed these *in vivo* studies for finafloxacin safety and toxicology. Primary review of the antimicrobial activity of finafloxacin is deferred to the Clinical Microbiology Review, by Dr. Simone Shurland.

Table 4: Pharmacokinetic (PK) studies submitted and reviewed

Report # TDOC	Report title	GLP status	# of pages
Analytical methods and validation (NDA module 4.2.2.1)			
0010319	Validation of an HPLC Tandem Mass Spectrometry (HPLC/MS/MS) Method for the Determination of Finafloxacin (AL-60371) in Rabbit K2EDTA Plasma at (b) (4)	No	190
Absorption (NDA module 4.2.2.2)			
0013950	Pharmacokinetics of Total Radioactivity in Pigmented Male Long-Evans Rats Following a Single Intravenous or Oral Dose of [¹⁴ C]AL-60371A	No	35
Distribution (NDA module 4.2.2.3)			
0015403	Whole Body Autoradiographic Tissue Distribution of Total Radioactivity in Pigmented Male Long-Evans Rats Following Repeat Oral Doses of [¹⁴ C]AL-60371A	No	79
0015407	Tissue Distribution of Total Radioactivity in Pigmented Pregnant Long-Evans Rats and Fetal Tissues Following a Single Oral Dose of [¹⁴ C]AL-60371A	No	107
0015408	Binding of [¹⁴ C]AL-60371 to Rat, Dog and Human Plasma Proteins <i>In Vitro</i>	No	41
0015409	Tissue Distribution of Total Radioactivity in Pigmented Male Long-Evans Rats Following Single or Repeat (QDx7) Oral Doses of [¹⁴ C]AL-60371A	No	181
0015411	Autoradiographic Tissue Distribution of Total Radioactivity in Male Guinea Pig Heads Following Repeated Otic Doses of [¹⁴ C]AL-60371	No	108

Metabolism (NDA module 4.2.2.4)			
013949	Chromatographic Profiles of Radioactivity in Plasma and Urine Following a Single 10 mg/kg Oral Dose of [14C]AL-60371 to Wistar and Long-Evans Rats and Beagle Dogs	No	50
0015274	Characterization of CYP450 Isozymes Involved in the <i>In Vitro</i> Metabolism of AL-60371 by Human Liver Microsomes	No	9
0015315	Inhibition of AL-60371 towards Human Hepatic Microsomal Cytochrome P450 Isozymes	No	12
0015406	Effects of AL-60371 on Hepatic Enzymes in Male and Female Long-Evans Rats Following Repeat Oral Doses of AL-60371A	No	66
0015412	Identification of Metabolites of AL-60371 in Plasma of Human, Dog and Rat Following Oral Administration of AL-60371	No	44
0016052	Chromatographic Profiles of [14C]AL-60371 Following Incubation With Rat, Rabbit, Monkey, and Human Liver Microsomes and Hepatocytes	No	76
0016434	Quantitation of AL-60371 and AL-91591 in Rat, Dog and Human Plasma Samples Following Oral Administration	No	234
Excretion (NDA module 4.2.2.5)			
0015404	Excretion and Mass Balance of Total Radioactivity in Pigmented Male Long-Evans Rats Following a Single Intravenous Dose of [14C]AL-60371A	No	57
0015405	Secretion of Total Radioactivity in Pigmented Lactating Long-Evans Rats Following a Single Oral Dose of [14C]AL-60371A	No	58

Table 5: Ototoxicity studies submitted and reviewed

Report # TDOC	Report title	GLP status	# of pages
Single-dose ototoxicity studies (NDA module 4.2.3.1)			
TDOC-0010418	Ototoxicity Screening Study with Finafloxacin (in either Zinc Chloride or Magnesium Chloride) Vehicles in Chinchillas	No	66
TODC-0011059	Exploratory Ototoxicity Dose Response Evaluation of Finafloxacin Administered via Transbullar Injection in Chinchillas	No	123
Repeat-dose ototoxicity studies (NDA module 4.2.3.2)			

TDOC-0013396	AL-60371 Otic (Finafloxacin): A One-Month Twice Daily Transbullar Middle Ear Dose Ototoxicity Study in Guinea Pigs	Yes	197
TDOC-0016033	28-Day Definitive Ototoxicity Evaluation in Guinea Pigs with AL-60371	Yes	510
TDOC-0011256	14 Day Intra-Auricular Repeated Dose Study in New Zealand White Rabbits with Finafloxacin HCl (AL-60371A) and an Ofloxacin Marketed Comparator	Yes	510
TDOC-0013365	AL-60371 (Finafloxacin): A Two-Week b.i.d. Topical Otic Toxicology Study with Two-Week Recovery Groups in New Zealand White Rabbits	Yes	342

Table 6: Genotoxicity studies submitted and reviewed

Report # TDOC	Report title	GLP status	# of pages
<i>In vitro</i> genotoxicity studies (module 4.2.3.3.1)			
198/2000	Reverse mutation assay with BYK60621 using bacteria (<i>Salmonella typhimurium</i> and <i>Escherichia coli</i>)	Yes	43
146/2001	Cell mutation assay at the thymidine kinase locus in mouse lymphoma L5178Y cells with BYK60621	Yes	34
2808/17	Mutation at the thymidine kinase (<i>tk</i>) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre ^R fluctuation technique	Yes	53
PH 30802	Bay 35-3377. V79/HPRT-test <i>in vitro</i> for the detection of induced forward mutations	Yes	34
PH-30910	Bay 35-3377. Photo-V79/HPRT-test <i>in vitro</i> for the detection of induced forward mutations	Yes	35
276/2000	Micronucleus test with V79 cells <i>in vitro</i> with BYK60621	Yes	20
<i>In vivo</i> genotoxicity studies (module 4.2.3.3.2)			
PH 25561	Bay 14-1881. Micronucleus test pilot study	No	26
70/2001	Testing of BYK6061 for mutagenicity activity in the mouse by means of the micronucleus test with oral administration	Yes	53
89/2001	Single dose toxicokinetics of BYK60621 in mice following oral administration of 500 and 2000 mg/kg BYK60621 within the scope of micronucleus test for mutagenic activity	Yes	23

2808/10	Induction of micronuclei in the bone marrow of treated mice	Yes	45
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Table 7: Local tolerance studies submitted and reviewed (NDA module 4.2.3.6)

Report # TDOC	Report title	GLP status	# of pages
289/2000	Test for sensitizing properties of BYK60621 in the guinea pig	Yes	16
419.143.2235	Local tolerance testing in the rabbit after a single intravenous, intraarterial and paravenous administration of finafloxacin versus vehicle	Yes	88

Table 8: Other toxicity studies submitted and reviewed (NDA module 4.2.3.7)

Report # TDOC	Report title	GLP status	# of pages
PH 28757	Cytotoxic, phototoxic, and convulsive potentials of gastrofluoroquinolones analogous compounds <i>in vitro</i>	No	15
PH 29356	BAY 14-1881. Study of photoreactive potential in guinea pigs	No	22
PH 30691	Cytotoxicity of BA Y 35-3377 (BYK 60621) on cartilage cells from dogs and man <i>in vitro</i>	No	15
PH 31154	Comparison of the cytotoxicity of BAY 35-3377, BAY 61-9997 and trovafloxacin in primary hepatocytes cultures of rats [sic]	No	18

3.2 Studies Not Reviewed

Table 9: Systemic route toxicity studies submitted but not reviewed herein

Report # TDOC	Report title	GLP status	# of pages
Systemic-route single-dose toxicity studies (NDA module 4.2.3.1)			
257/2000	The toxicity of BYK60621 after a single intravenous administration in the mouse	No	48
247/2000	The toxicity of BYK60621 after a single oral administration in the mouse	No	36
256/2000	The toxicity of BYK60621 after a single	No	47

	intravenous administration in the rat		
246/2000	The toxicity of BYK60621 after a single oral administration in the rat	No	36
Systemic-route repeat-dose toxicity studies (NDA module 4.2.3.2)			
45/2001	Toxicity and Toxicokinetics of BYK60621 in beagle dogs following infusion administration for 2 weeks	Yes	787
166/2000	Toxicity of BYK60621 in beagle dogs following oral administration for 4 weeks	Yes	291
176/2000	4-week toxicokinetics of BYK60621 in the dog following oral administration at three different dose levels	Yes	36
202/2009	The toxicity and toxicokinetics of BYK60621 after intravenous administration in the rat for 2 weeks	Yes	563
8227553	Finafloxacin: 14 Day Intravenous (Infusion) Administration Toxicity Study in the Rat Followed by a 10 Week (Nominal) Treatment-free Period	Yes	419
8245014	Finafloxacin: 4 Week Intravenous (Infusion) Administration Toxicity Study in the Rat Followed by an 11 Week Treatment-free Period	Yes	541
8245017	Finafloxacin: 7 Day Intravenous (Infusion) Administration Toxicokinetic study in the rat	No	116
197/2000	The toxicity and toxicokinetics of BYK60621 after oral administration in the rat for 4 weeks	Yes	782
204/2000	4-week toxicokinetics of BYK60621 in the rat following oral administration at three different dose levels	Yes	136
PH-26886	Helicobacter Quinolones ((b) (4) 34-6601, Bay 35-3377) Study for subacute oral toxicity in rats (Two-Week Application by Gavage)	No	283

Table 10: Pharmacodynamic and ADME studies submitted (NDA module 4.2.1.1.) but not reviewed herein

Report # TDOC	Report title	GLP status	# of pages
Primary pharmacology			
-0011241	<i>In vitro</i> Evaluation of Finafloxacin from MerLion Pharmaceuticals	No	18
-0017294	<i>In Vitro</i> Evaluation of Finafloxacin against	No	11

	Clinical Otic Pathogens		
Secondary pharmacology			
-207200	<i>In vitro</i> efficacy of BYK60621 on <i>Helicobacter pylori</i> and on different germs	No	14
FIN 0806	Development of Etest® finafloxacin	No	11
PH-32655	Comparative <i>in vitro</i> activity of BAY 35-3377 against defined quinolone-resistant strains	No	17
PH-32656	Antibacterial <i>in vitro</i> evaluation of BAY 35-3377	No	30
RR-2009-004	<i>In vitro</i> activity of finafloxacin against Gram negative and Gram positive pathogens and fluoroquinolone resistant strains	No	24
RR-2009-005	<i>In vitro</i> activity of finafloxacin against adherent and difficult to treat populations of <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	No	16
RR-2009-006	<i>In vitro</i> selection and characterization of finafloxacin resistant <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	No	15
RR-2009-007	<i>In vitro</i> activity of finafloxacin against community associated methicillin resistant <i>Staphylococcus aureus</i>	No	19
RR-2010-002	<i>In vitro</i> activity of finafloxacin against stationary and growth arrested Gram negative bacteria	No	21
RR-2010-004	<i>In vitro</i> antibacterial activity of finafloxacin under conditions simulating lower respiratory tract infection	No	27
Absorption			
0015232 ^a	Pharmacokinetics of AL-60371 in Male Beagle Dogs Following Intravenous and Oral Doses	No	70
015410	Pharmacokinetics of Total Radioactivity in Male Beagle Dogs Following a Single Intravenous or Oral Dose of [¹⁴ C]AL-60371A	No	70
0015608	Pharmacokinetics of AL-60371 in Pigmented Male Long-Evans Rats Following a Single Intravenous and Oral Dose of AL-60371A	No	69
ADME validation			
-0010319	Validation of an HPLC Tandem Mass Spectrometry (HPLC/MS/MS) Method for the Determination of Finafloxacin (AL-60371) in Rabbit K ₂ EDTA Plasma at (b) (4)	No	190
-0013919	Validation of PROC-0005089 for Determination of Residual Solvents – (b) (4)	No	46

	(b) (4) and DMF in (b) (4)		
	Drug Substance by Gas Chromatography		

This P/T reviewer defers primary review of the antimicrobial activity of finafloxacin to the Clinical Microbiology Reviewer, Dr. Simone Shurland.

^a A cursory examination of this absorption study (report # 0015232) found errors comparing the summary PK data to the individual animal data (i.e. C_{max} not reported correctly). Because this study is not pivotal for understanding the safety of finafloxacin, it was not reviewed further.

Table 11: Safety Pharmacology studies submitted (NDA module 4.2.1.3) but not reviewed herein

Report # TDOC	Report title	GLP status	# of pages
47/200	Investigation of BYK60621 in the guinea-pig Langendorff heart	No	6
64/2000	Influence of BYK60621 i.v. on behavior of female rats	No	6
65/2000	Influence of BYK60621 p.o. on behavior of female rats	No	5
66/2000	Influence of BYK60621 p.o. on behavior of female mice	No	6
67/2000	Influence of BYK60621 p.o. on behavior of female rats	No	5
74/2000	Continuous perfusion of the guinea-pig Langendorff heart with BYK60621	No	6
77/2000	Influence of cumulative oral administration of BYK60621 on heart rate, blood pressure and ECG in conscious dogs	No	19
83/2001	Antisecretory activity and duration of action of BYK60621 in the pentagastrin-stimulated, lumen-perfused rat stomach <i>in vivo</i> (GhoshSchild rat) after intraduodenal administration	No	8
103/2000	Influence of BYK60621 p.o. on body temperature of female mice	No	5
104/2000	Influence of BYK60621 p.o. on pupil diameter of female mice	No	5
113/2000	Effects of BYK60621 on blood pressure and heart rate after p.o. administration to conscious rats	No	8
114/2000	Effects of BYK60621 given intravenously cumulative infusion on blood pressure and heart rate in conscious rats	No	7
117/2000	Influence of BYK60621 p.o. on locomotor activity of mice in light beam cages	No	10

	[experiments at night]		
118/2000	Influence of BYK60621 p.o. on pentetrazole-induced seizures female in mice	No	5
123/2000	Influence of BYK60621 on action potential and force of contraction in guinea-pig isolated papillary muscle	No	14
125/2000	Influence of BYK60621 p.o. on gastrointestinal motility of female mice	No	5
126/2000	Influence of BYK60621 p.o. on neuromuscular function of female mice [rotarod and traction test]	No	6
148/2000	Investigation on a possible smooth muscle relaxing effect of BYK60621 in guinea-pig isolated trachea	No	6
149/2000	Continuous perfusion of the guinea-pig Langendorff heart with high concentrations of BYK60621	No	7
180/2000	Haemodynamic and respiratory effects after short-time i.v.-infusion of BYK60621 in anaesthetized cats	No	35
225/2000	Influence of BYK60621 on action potential of cardiac purkinje fibres in comparison to sparfloxacin, ofloxacin and clarithromycin	Yes	106
226/2000	Influence of BYK60621 on hERG channel in comparison to sparfloxacin, ofloxacin and clarithromycin (DFCM1004)	Yes	88
PH 30795	Effects of BAY 35-3377 and 61-9997 on hippocampal extracellular recordings	No	20

Table 12: Pharmacodynamic drug interaction studies submitted (NDA module 4.2.1.4.) but not reviewed herein

Report # TDOC	Report title	GLP status	# of pages
109/2000	Influence of BYK60621 p.o. on hexobarbital-induced loss of righting reflex in female mice	No	3
110/2000	Influence of BYK60621 p.o. on (b) (4) - induced loss of righting reflex in female mice	No	3
150/2000	Investigation on the possible inhibitory effect of BYK60621 on peripheral GABA-A receptors in guinea-pig ileum	No	6
85/2000	Investigation on the possible interaction of BYK60621 with muscarinic M ₁ - and M ₂ -receptors in rabbit isolated vas deferens	No	5

86/2000	Investigation on the possible interaction of BYK60621 with muscarinic M ₃ - and histamine H ₁ -receptors in guinea-pig isolated ileum	No	5
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3.3 Previous Reviews Referenced

- IND (b) (4):
 - Dr. Miller's 9/21/2011 P/T review of the original IND submission
 - Dr. Miller's additional P/T review (10/14/2011), which further discussed the DART findings
- IND 110576:
 - Dr. Terry Miller's 10/11/2011 P/T review of the original IND submission
 - Dr. Shukal Bala's 10/03/2011 microbiology/immunology review of the original IND submission
 - Dr. McDougal's P/T review (12/21/2013) of subsequently-submitted P/T data
- IND (b) (4): Dr. Ellis's P/T review of the preIND (11/16/2012) and original IND (11/13/2013) which included a re-review of the 28-day ototoxicity study in guinea pigs (report # 1108/012)
- IND (b) (4): two short reviews by Dr. Ellis (2/22/2013 and 11/13/2013)

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology data submitted to the NDA focuses on the antibacterial activity of finafloxacin; review of this microbiology data is outside the scope of this P/T review, and is deferred to the Clinical Microbiology reviewer. The three *in vivo* primary pharmacology studies did not report safety endpoints.

Title	Evaluation of Clinical Finafloxacin (AL-60371) Suspension Formulations in a Guinea Pig Model of <i>P. aeruginosa</i> Acute Otitis Externa
Report #	TDOC-0014368
Report date:	August 22, 2011
Key findings	<ul style="list-style-type: none"> • No safety information reported
Methods	<ul style="list-style-type: none"> • Guinea pigs (4 ears/dose group) • Ear canals "were slightly abraded" • 100 µl of ~ 10⁸ colony forming units (CFU) of <i>Pseudomonas aeruginosa</i> were instilled into each ear • 16 hours later, ears were dosed with 100 µl of 0 or 0.3%

	finafloxacin, or with a positive control. <ul style="list-style-type: none"> • 4 hours later, ears were lavaged with saline to culture bacteria • Antimicrobial activity was assessed.
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Title	<i>In vivo</i> evaluation of AL-60371 (Finafloxacin) clinical formulation
Report #	TDOC-0017323
Report date:	October 8, 2013
Key findings	No safety information reported
Methods	<ul style="list-style-type: none"> • Ears of guinea pigs were infected with strains of <i>P. aeruginosa</i> [no abrasion mentioned] • Same dosing as above: 16 hours later, two guinea pigs (4 ears) received 100 µl 0.3% finafloxacin [multiple control groups] • 24 and 48 hours after infection, ears were lavaged with saline to culture bacteria. • Antimicrobial activity was assessed.

Title	<i>In vivo</i> evaluation of Finafloxacin (AL-60371) formulations in a screening model of <i>P.aeruginosa</i> acute otitis externa
Report #	TDOC-0017324
Report date:	October 8, 2013
Key findings	No safety information reported
Methods	<ul style="list-style-type: none"> • Ears of guinea pigs were infected with one strain of <i>P. aeruginosa</i> • Beginning 14 hours later, groups of 2 guinea pigs (4 ears/group) received 0. 0.003%, 0.03%, or 0.3% finafloxacin [volume not reported] • Single dose groups; groups dosed at 14, 16, 18 and 20 hours • 24 hours after infection, ears were lavaged with saline to culture bacteria. • Antimicrobial activity was assessed.

4.2 Secondary Pharmacology

The secondary pharmacology study reports submitted to the NDA are limited to evaluations of antibacterial activity. No assessment of potential off-target pharmacology was submitted.

As with the primary pharmacology results related to microbiological activity, review of the Applicant's secondary pharmacology data related to microbiology activity is outside the scope of this P/T review, and is deferred to the Clinical Microbiology reviewer.

One *in vivo* secondary pharmacology study submitted to the NDA was reviewed for safety:

Title	<i>In vivo</i> evaluation of BAY 35-3377 in models of bacterial infection
Report #	PH-32521
Report date	December 18, 2002
Summary	<ul style="list-style-type: none"> • No safety concerns were identified • Highest doses tested were 50 mg/kg/day orally for 5 days, and 25 mg/kg/day intravenously (iv) for 5 days • Oral finafloxacin was tested in several mouse and rat models of infection. The only endpoints reported were survival and bacterial load (CFU). Finafloxacin-treated rodents survived longer than control-treated infected rodents • Single oral-dose PK study in mice observed a linear dose response for C_{max}
Disease model notes	<ul style="list-style-type: none"> • Groups of 5 female CFW-1 mice (18-20 grams) • Groups of 5 female Wistar rats (150-200 grams) • Multiple disease models (with different types of bacteria): systemic bacteremia; cyclophosphamide-induced neutropenia; pneumonia; pyelonephritis (bacteria injected into the right kidney); bladder infection; subcutaneous abscess/pouch generation and infection; subcutaneous implantation of biofilms; intramuscular injection of bacteria; gastrointestinal and intra-abdominal infection; oral infection; sepsis model
PK study	<ul style="list-style-type: none"> • A single-dose oral PK study in CFW-1 mice was conducted (3 mice per time point) • Following 1 mg/kg of finafloxacin: <ul style="list-style-type: none"> ○ $AUC = 0.571 \text{ kg}\cdot\text{h/L}$ ○ $C_{max} = 0.364 \text{ kg/L}$ ○ Elimination $t_{1/2} = 1.52 \text{ hours}$ • C_{max} was linear over 3 dose levels (25, 75, 225 mg/kg)

4.3 Safety Pharmacology

Safety Pharmacology studies were submitted (NDA module 4.2.1.3), but were not reviewed.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 Absorption

The NDA included four non-GLP absorption studies, each conducted by Alcon Research Limited to compare oral versus intravenous dosing in rats and dogs, with consistent results. One is reviewed fully below:

Title	Pharmacokinetics of Total Radioactivity in Pigmented Male Long-Evans Rats Following a Single Intravenous or Oral Dose of [¹⁴C]AL-60371A
Report #	TDOC-0013950
Key findings	<ul style="list-style-type: none"> • Oral absorption was 57.1% • Slight differences were noted comparing blood PK and plasma PK. This reviewer concludes that plasma TK is acceptable for the toxicology studies. <ul style="list-style-type: none"> ○ C_{max} was slightly higher for plasma than blood following oral (1.1x) and iv (1.2x) dosing ○ Elimination t_{1/2} for oral dosing found a slight difference (1.1x longer for plasma versus blood), but no difference was apparent for iv dosing
Method notes	<ul style="list-style-type: none"> • Male Long-Evans rats (pigmented) were used (44 total for oral dosing, 48 total for iv dosing) • Rats were fasted from 16 hours pre-dose to 4 hours post-dose • Dosing: <ul style="list-style-type: none"> ○ Single oral dose of 1.82 mg/kg of finafloxacin ○ Single iv dose of 1.78 mg/kg of finafloxacin • Test article is radiolabeled [¹⁴C]finafloxacin hydrochloride, lot # CFQ41156 (same as used for the other radiolabel studies) • Blood and plasma collected (4 rats/group) for iv group only at 5 minutes, and for other groups at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours • Lower limits of quantitation (LLOQ): 7.2 to 7.5 ng/g blood, 3.2 to 3.3 ng/g plasma
Results	<ul style="list-style-type: none"> • Comparison of iv and oral dosing allowed the author to calculate that fasted rats absorbed 57.1% of the orally-administered dose. • Following iv dosing, the elimination half-life in blood and plasma were the same (1.2 hours)
Table 13: Male rat PK following single 2 mg/kg oral or iv dose (report # 0013950)	

Parameter (unit) (Mean N=4)	Oral / Blood	Oral / Plasma	Intravenous / Blood	Intravenous / Plasma
C_{max} / C_0 ($\mu\text{g eq/g}$)	0.344 ± 0.0696	0.380 ± 0.0676	1.89	2.19
T_{max} (h)	0.500	0.500	-	-
T_{last} (h)	10	12	6	8
$t_{1/2}$ (h)	2.15	2.47	1.17	1.15
AUC_{0-4h} ($\mu\text{g eq}^*\text{h/g}$)	0.625 ± 0.0528	0.737 ± 0.0622	1.39 ± 0.0888	1.56 ± 0.0979
AUC_{0-6h} ($\mu\text{g eq}^*\text{h/g}$)	0.725 ± 0.0603	0.850 ± 0.0699	1.46 ± 0.0972	1.65 ± 0.107
AUC_{0-last} ($\mu\text{g eq}^*\text{h/g}$)	0.806 ± 0.0626	0.960 ± 0.0726	1.46 ± 0.0972	1.68 ± 0.110
AUC_{0-inf} ($\mu\text{g eq}^*\text{h/g}$)	0.827	0.986	1.50	1.69
Percent Absorbed (%)	-	57.1	-	-

C_{max} = maximum observed concentration after oral dose
 C_0 = maximum extrapolated concentration after intravenous dose

Report #	Description	Summary
TDOC-0015232	Male Beagle dogs, single iv dose (1 mg/kg) or oral dose (2 mg/kg), PK for 24 hours	<ul style="list-style-type: none"> Fasted oral bioavailability reported as 92%
TDOC-0015410	Male Beagle dogs, single iv dose (1 mg/kg) or oral dose (2 mg/kg), PK for 48 hours	<ul style="list-style-type: none"> Fasted oral bioavailability reported as 73% IV $t_{1/2}$ = 7.26 hours Oral $t_{1/2}$ = 5.7 hours
TDOC-0015608	Male Long-Evans rats, single iv or oral dose (2 mg/kg), 24 hour PK	<ul style="list-style-type: none"> Fasted oral bioavailability reported as 48.9% IV $t_{1/2}$ = 0.8 hours Oral $t_{1/2}$ = 2.5 hours

5.2 Distribution

Title:	Binding of [¹⁴C]AL-60371 to rat, dog and human plasma proteins <i>in vitro</i>
Report #	TDOC-0015408
Key findings	<ul style="list-style-type: none"> Finafloxacin bound plasma proteins: rat < human < dog. This reviewer concludes that the differences are unlikely to cause a significant species difference in pharmacology or toxicology.

	<ul style="list-style-type: none"> Plasma proteins were not saturated by the concentrations of finafloxacin tested (up to 1000 ng/ml) 						
Report date:	September 17, 2012						
Report location	NDA module 4.2.2.3 (Pharmacokinetics – Distribution)						
Study laboratory	(b) (4)						
Test article	<ul style="list-style-type: none"> [14C] AL-60371A Lot # CFQ41156 Purity 97.5% Specific activity: 126 µCi/mg Dissolved in water: (b) (4) 						
Method	<ul style="list-style-type: none"> Finafloxacin was incubated with pooled plasma from male Sprague Dawley rats, beagle dogs, or human volunteers at 37°C in dialysis cells Transfer of the radiolabel from the plasma cell through the dialysis filter to the buffer cell was used to calculate protein binding 						
Preliminary equilibration experiment	<ul style="list-style-type: none"> Human plasma samples were incubated with 1000 ng/ml finafloxacin for 1, 3, 5, 6, or 7 hours Equilibration reached by 5 hours 						
pH stability experiment	<ul style="list-style-type: none"> 1000 ng/ml of finafloxacin was added to acidified rat, dog and human plasma for 6 hours [pH not reported] Recovery of radiolabel was 95% for all three species. The authors conclude that finafloxacin is stable for at least 6 hours in acidified plasma. 						
Dose-response experiment	<ul style="list-style-type: none"> No apparent relationship between concentration and % binding 10, 100, 500 and 1000 ng/ml of finafloxacin were incubated in rat, dog and human plasma for 6 hours Results: <table border="0"> <tr> <td>Binding in rat plasma:</td> <td>44.1 to 55.3%</td> </tr> <tr> <td>Binding in human plasma:</td> <td>70.4 to 79%</td> </tr> <tr> <td>Binding in dog plasma:</td> <td>82.1 to 84.3%</td> </tr> </table> 	Binding in rat plasma:	44.1 to 55.3%	Binding in human plasma:	70.4 to 79%	Binding in dog plasma:	82.1 to 84.3%
Binding in rat plasma:	44.1 to 55.3%						
Binding in human plasma:	70.4 to 79%						
Binding in dog plasma:	82.1 to 84.3%						

Title	Whole Body Autoradiographic Tissue Distribution of Total Radioactivity in Pigmented Male Long-Evans Rats Following Repeat Oral Doses of [14C]AL-60371A
Report #	TDOC-0015403
Key finding:	After 7 daily oral doses of 2 mg/kg finafloxacin hydrochloride, whole body radiography in in male rats found rapid absorption and wide distribution (46 of the 56 tissues evaluated)

	<ul style="list-style-type: none"> • Distribution: urine > bile > eye uveal tract > kidney cortex > kidney (whole) > kidney medulla > liver > cartilage > epiphyseal line > small intestine > periosteum > arterial wall > other tissues 	
Remarks on biological relevance	<ul style="list-style-type: none"> • The observed distribution to cartilage, epiphyseal line, and periosteum is consistent with the class chondrotoxicity associated with fluoroquinolones • The distribution to the eye uveal tract is notable, but the toxicological significance is unclear (given the relatively low distribution to other eye tissues) <ul style="list-style-type: none"> ○ The lack of detectable finafloxacin in the vitreous humors suggests that finafloxacin does not pass through the blood-retina barrier • CNS toxicity is a class potential concern for fluoroquinolones. Finafloxacin was not detected in brain tissues, except for the choroid plexus and meninges, the site of the brain-cerebrospinal fluid barrier. <ul style="list-style-type: none"> ○ The lack of detectable finafloxacin in the cerebrum, cerebellum, medulla, olfactory lobe suggest that finafloxacin does not pass through the blood-brain barrier ○ Similarly, the lack of detectable finafloxacin in the spinal cord suggests that finafloxacin does not pass through the blood-cerebrospinal fluid barrier • Finafloxacin was detected in the epididymis and testis (at relatively low levels) 	
Report details	Report date	October 23, 2012
	Study laboratory	(b) (4)
	GLP	No
	Test article	<ul style="list-style-type: none"> • Radiolabeled [¹⁴C]-finafloxacin hydrochloride (AL-60371A) • Lot # CFQ41156 • specific activity of 59 mCi/mmol (126 µCi/mg) • purity 97.5%
Methods	Formulation	Aqueous suspension: 8.029 mg/ml magnesium chloride, 13.04 mg/ml glycerin, pH 8.0 ± 0.1
	Species	Male Long-Evans rats
	Dosing	<ul style="list-style-type: none"> • One group of 6 rats • 2 mg/kg (calculated as free base), volume 3.64 ml/kg • Seven daily oral gavage doses <ul style="list-style-type: none"> ○ The first 6 doses were 24 ± 1 hour apart ○ The last doses was 24 ± 4 hours after the 6th dose [reason for the delay not clear] • For the last dose only, animals were fasted (6 hours before until 4 hours after)

	<p>Autoradiography</p>	<ul style="list-style-type: none"> • One rat per time point was prepared for whole body autoradiography at 1, 2, 4, 8, 12 and 24 hours after the last dose • Animals were sacrificed with anesthesia and exsanguinated, then immediately frozen in a dry ice/hexane bath. • Carcasses were embedded into blocks frozen, sectioned, and sections were phosphorimaged.
<p>Results</p>	<ul style="list-style-type: none"> • Quantifiable radioactivity was detected in 49 of the 56 tissues evaluated • T_{max} was 1 hour for 26 tissues, 2 hours for 18 tissues, 8 hours for 1 tissue (uveal tract of the eye), and 12 hours for 1 tissue (meninges) 	
<p>From the report:</p> <p>Table 14: Results from the rat oral-dose autoradiography study (report # 0015403)</p>		

Tissue	Concentration (ng Equivalents [¹⁴ C]AL-60371/g)					
	Animal Number (Time Point Hours After 7 th Daily Dose)					
	B30610 (1 Hour)	B30611 (2 Hours)	B30612 (4 Hours)	B30613 (8 Hours)	B30614 (12 Hours)	B30615 (24 Hours)
Adrenal gland(s)	131	175	30.3	ND	ND	ND
Arterial wall	292	295	ND	ND	ND	ND
Bile	7470	6630	585	384	192	ND
Blood	190	183	22.7	BLQ	ND	ND
Bone	27.0	24.4	ND	ND	ND	ND
Bone marrow	170	143	BLQ	BLQ	ND	ND
Brain cerebellum	BLQ	BLQ	ND	ND	ND	ND
Brain cerebrum	BLQ	BLQ	ND	ND	ND	ND
Choroid plexus	84.8	47.5	ND	ND	ND	ND
Brain medulla	BLQ	BLQ	ND	ND	ND	ND
Brain olfactory lobe	BLQ	BLQ	ND	ND	ND	ND
Bulbo-urethral gland	223	250	57.6	BLQ	ND	ND
Cartilage	1150	1120	1030	129	75.6	71.0
Cecum	279	240	ND ^a	287	334	BLQ
Diaphragm	208	197	35.7	BLQ	ND	ND
Epididymis	92.4 ^b	67.6 ^b	BLQ ^b	BLQ	ND	ND
Epiphyseal line	954	876	308	107	67.1	98.2
Esophagus	243	335	26.7	ND	ND	ND
Exorbital lacrimal gland	133	172	27.8	ND	ND	ND
Eye lens	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Eye uveal tract	2600	3870	3680	4080	2430	3910
Eye vitreous humor	BLQ	36.5	BLQ	BLQ	BLQ	27.3
Eye(s), whole	354	620	435	371	351	505
Fat (abdominal)	BLQ	BLQ	ND	ND	ND	ND
Fat (brown)	161	101	ND	BLQ	ND	ND
Harderian gland	78.2	73.8	ND	ND	ND	ND
Intra-orbital lacrimal gland	216	174	25.1	ND	ND	ND

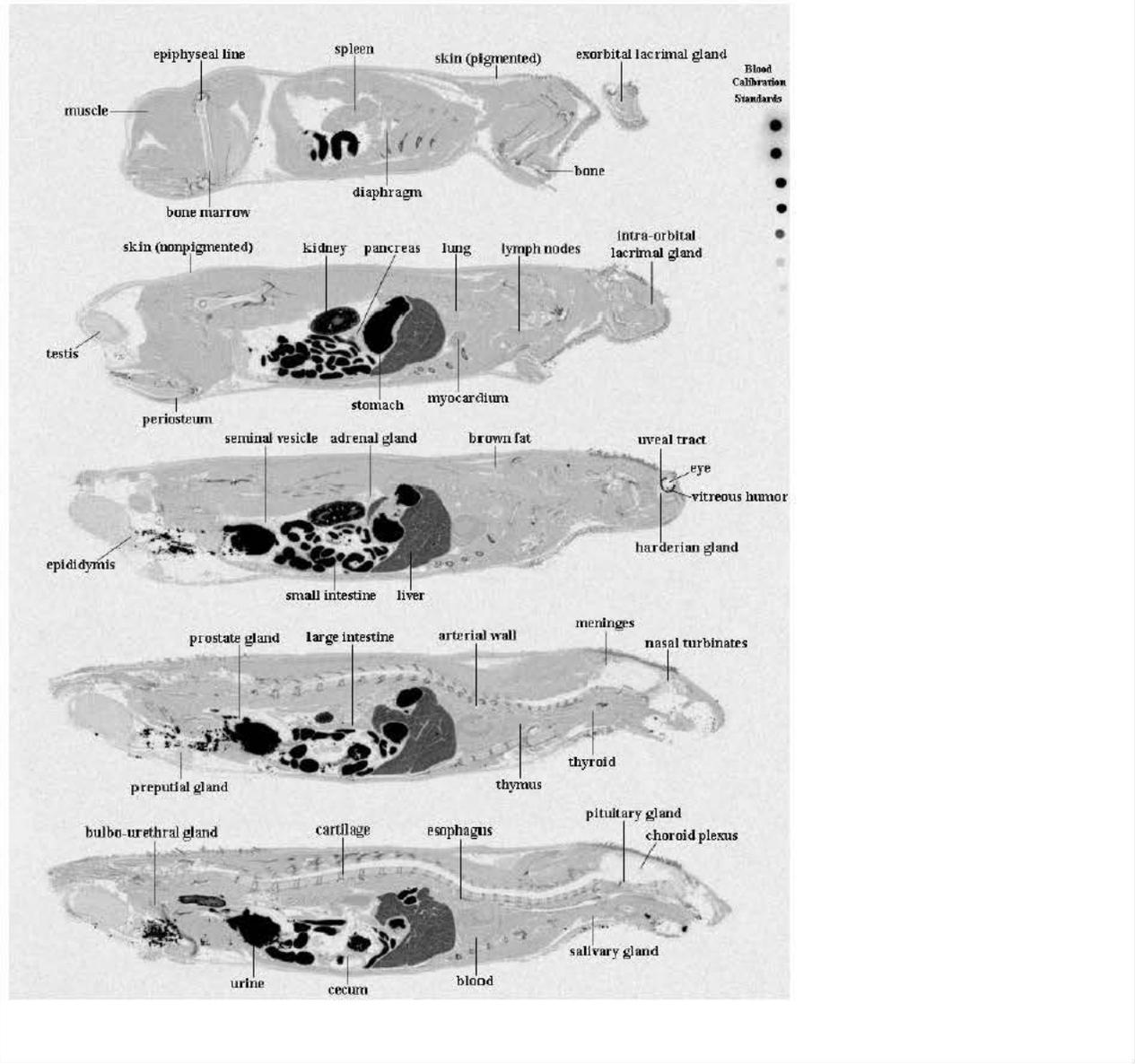
Kidney cortex	2400	2800	438	122	58.6	ND
Kidney medulla	2040	2220	329	98.7	45.4	ND
Kidney(s), whole	2330	2580	379	110	56.1	ND
Large intestine	241	186	87.5	22.9	85.6	44.2
Liver	1280	1180	136	61.2	23.8	ND
Lung(s)	214	187	34.7	ND	ND	ND
Lymph node(s)	230	212	28.0	BLQ	BLQ	ND
Meninges	156	258	76.0	238	622	271
Muscle	172	156	23.9	ND	ND	ND
Myocardium	287	258	31.7	ND	ND	ND
Nasal turbinates	77.0	90.8	BLQ	28.1	BLQ	ND
Pancreas	163	175	24.2	BLQ	ND	ND
Periosteum	374	369	147	72.8	43.8	77.5
Pituitary gland	175	194	30.5	ND	ND	ND
Preputial gland	49.3 ^b	77.1 ^b	BLQ	BLQ ^b	BLQ	ND
Prostate gland	72.6	187	BLQ	ND	ND	ND
Salivary gland(s)	194	178	23.2	ND	ND	ND
Seminal vesicle(s)	30.7	34.2	ND	ND	ND	ND
Skin (nonpigmented)	171	122	27.0	BLQ	ND	ND
Skin (pigmented)	179	127	144	85.5	169	127
Small intestine	458	326	82.0	40.5	41.4	ND
Spinal cord	BLQ	BLQ	ND	ND	ND	ND
Spleen	235	192	27.8	BLQ	ND	ND
Stomach	198	182	22.1	27.6	BLQ	ND
Testis(es)	66.6	73.9	BLQ	BLQ	ND	ND
Thymus	223	203	23.4	ND	ND	ND
Thyroid	161	151	24.1	BLQ	ND	ND
Urinary bladder	ND ^c	ND ^c	470	56.1	BLQ	ND
Urine	96400	154000	49800	616	401	91.1

Subscripts:

BLQ: discernable image on radiography, but below the limit of quantitation (21 ng finafloxacin/g tissue)

- a Cecum was not detectable at 4 hours due to flare from gastrointestinal contents
- b Epididymis tissue and preputial gland tissue were permeated with fat
- c Urinary bladder tissue was not detectable at 1 and 2 hours due to flare from the urine.

Figure 2: Rat whole-body autoradiography 1 hour after the last oral dose of [¹⁴C]-finafloxacin hydrochloride (report # 0015403)



Title	Tissue Distribution of Total Radioactivity in Pigmented Male Long-Evans Rats Following Single or Repeat (QDx7) Oral Doses of [14C]AL-60371A	
Report #	TDOC-0015409	
Key finding:	<ul style="list-style-type: none"> • Following a single or 7 daily oral doses given to male rats, finafloxacin distributed: small intestine > urinary bladder > cecum > kidneys > liver > stomach • Low but detectable levels of finafloxacin were detected in the brain and testes • Eyes showed prolonged retention of finafloxacin (46% at 120 hours compared to T_{max}), which the authors attribute to melanin binding 	
Report details	Report date	November 12, 2012
	Study laboratory	(b) (4)
	GLP status	No
	Test article	<ul style="list-style-type: none"> • Radiolabeled [¹⁴C]-finafloxacin hydrochloride (AL-60371A) • Lot # CFQ41156 • specific activity of 59 mCi/mmol (126 µCi/mg) purity 97.5%
Method notes	Formulation	Aqueous suspension: 8.029 mg/ml magnesium chloride, 13.04 mg/ml glycerin, pH 8.0 ± 0.1
	Species	Male Long-Evans rats
	Dose	<ul style="list-style-type: none"> • 2 mg/kg (calculated as equivalent to the free base) • Volume 3.64 ml/kg
	Dose groups	<ul style="list-style-type: none"> • 44 rats received a single oral dose • 44 rats received once-daily oral dosing
	Sample collection	<ul style="list-style-type: none"> • 4 males/time point were harvested • After the single/last dose at: 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96 and 120 hours • In addition to blood and plasma, 25 tissues were collected for analysis
Results	<ul style="list-style-type: none"> • T_{max} was the first time point measured (0.5 hr), except for: <ul style="list-style-type: none"> ○ 1 hour: muscle, testes, urinary bladder ○ 2 hours: pancreas, eyes, abdominal fat, urinary bladder ○ 4 hours: cecum 	
Table 15: Repeat-dose distribution data for the rat oral 7-day experiment (report # 0015409)		

Tissue	$C_{max} \pm SD$ (ng eq/g)	T_{max} (h)	$AUC_{0-last} \pm SD$ (ng eq*h/g)	T_{last} (h)	Initial $t_{1/2}$ (h)	Late $t_{1/2}$ (h)
Adrenal glands	321 ± 67.2	0.5	591 ± 50.8	8	3.19	ND
Blood	707 ± 86.6	0.5	1110 ± 57.8	12	2.12	ND
Bone	333 ± 43.4	0.5	1710 ± 55.8	120	3.91	109
Brain	26.2 ± 4.05	0.5	36.5 ± 2.27	2	ND	ND
Cecum	7950 ± 5880	4	59300 ± 12000	24	2.53	ND
Eyes	289 ± 50.9	8	25400 ± 1410	120	ND	151
Fat (abdominal)	49.6 ± 11.4	0.5	97.1 ± 7.22	8	2.19	ND
Heart	625 ± 95.7	0.5	1070 ± 84.0	8	2.18	ND
Kidneys	6350 ± 635	0.5	11000 ± 563	24	2.91	ND
Liver	3290 ± 346	0.5	5230 ± 272	12	2.02	ND
Lungs	472 ± 58.4	0.5	845 ± 54.7	8	2.22	ND
Lymph nodes	1080 ± 117	0.5	1930 ± 326	12	1.92	ND
Muscle	451 ± 150	1	893 ± 70.9	8	2.04	ND
Pancreas	808 ± 852	2	1900 ± 648	4	ND	ND
Plasma	782 ± 113	0.5	1260 ± 69.5	12	2.00	ND
Salivary glands	434 ± 63.7	0.5	781 ± 40.0	48	1.96	ND
Sciatic nerve	260 ± 262	1	515 ± 113	8	2.24	ND
Skin (dorsal shaved)	414 ± 46.1	0.5	3650 ± 179	96	3.99	97.8
Small intestine	23600 ± 14100	0.5	59700 ± 9320	12	1.22	ND
Spleen	586 ± 83.9	0.5	1050 ± 86.6	8	2.52	ND
Stomach	3090 ± 1710	0.5	3030 ± 485	12	3.11	ND
Testes	173 ± 26.1	1	392 ± 19.8	8	1.91	ND
Thymus	442 ± 54.4	0.5	867 ± 39.9	96	2.11	60.8
Thyroid	310 ± 61.6	0.5	510 ± 21.0	4	0.995	ND
Trachea	569 ± 367	0.5	1410 ± 164	12	2.17	ND
Urinary bladder	14100 ± 15800	1	15800 ± 6100	120	2.54	ND

ND: not determined

Title:	Tissue Distribution of Total Radioactivity in Pigmented Pregnant Long-Evans Rats and Fetal Tissues Following a Single Oral Dose of [14C]AL-60371A
Report #	TDOC-0015407
Key findings:	<ul style="list-style-type: none"> • Following maternal exposure in pregnant rats (GD12 or GD18), finafloxacin was detected in the whole fetus, fetal tissues, amniotic fluid and placenta <ul style="list-style-type: none"> ○ Maternal: Kidney > liver > plasma > blood > heart > lungs > uterus > placenta > ovaries > mammary > brain ○ Fetal: liver > heart > lungs > blood > brain • Fetal exposures were lower than the maternal plasma concentration • Comparing maternal and fetal tissues, the concentration was lower in fetal tissues, except for brain

		<ul style="list-style-type: none"> ○ The relatively higher concentration in fetal brain compared to maternal brain suggests that the blood:brain barrier may not be as strong a barrier in fetuses compared to adults ● Note: this study was a focused distribution study, and did not evaluate the complete panel of tissues
Report details	Report date	November 26, 2012
	Study laboratory	(b) (4)
	GLP status	No
	Test article	<ul style="list-style-type: none"> ● Radiolabeled [¹⁴C]-finafloxacin hydrochloride (AL-60371A) ● Lot # CFQ41156 ● specific activity of 59 mCi/mmol (126 μCi/mg) ● purity 97.5%
Methods	Formulation	Aqueous suspension: 8.029 mg/ml magnesium chloride, 13.04 mg/ml glycerin, pH 8.0 ± 0.1
	Dose	Single oral gavage dose of 2 mg/kg of finafloxacin (dose calculated as finafloxacin free base)
	Dosing	<ul style="list-style-type: none"> ● 64 timed-pregnant Long-Evans rats were used ● One group of 32 was dosed on “approximately” gestation day 12 (GD12) ● The other group of 32 was dosed on “approximately” gestation day 18 (GD18)
	PK	<ul style="list-style-type: none"> ● 4 females/group were sacrificed at 0.5, 1, 2, 4, 8, 12, 24 and 48 hours after dosing ● For both groups, tissues collected were: blood, plasma, whole fetus, placenta, amniotic fluid, brain, heart, liver, lungs, kidneys, mammary gland, uterus, and ovaries ● For GD18 only, fetal blood, fetal brain, fetal heart, fetal liver and fetal lungs were also collected ● Note: two fetuses/female were evaluated for “whole fetus”, and GD18 fetal tissue samples were collected from two other fetuses. The other fetuses/litter were not assessed.
	No reproductive endpoints	<ul style="list-style-type: none"> ● Body weight was measured only pre-dose (to calculate the appropriate dose), but not after (e.g. no assessment of finafloxacin-induced weight loss) ● No reproductive parameters were assessed. ● Based on the dedicated embryofetal toxicity

	<p>studies (rat and rabbit), the lack of these data is not a study limitation</p>
<p>Results</p>	<ul style="list-style-type: none"> • Following single oral gavage doses of 2 mg/kg: <ul style="list-style-type: none"> ○ The GD12 plasma C_{max} = 622 ng/g and the blood C_{max} = 533 ng/g. ○ The GD18 plasma C_{max} = 809 ng/g and the blood C_{max} = 724 ng/g. • Maternal T_{max} was the first time point measured (0.5 hours), except for: <ul style="list-style-type: none"> ○ Amniotic fluid (T_{max} = 1 hour at GD12, T_{max} = 2 hours at GD18) ○ Kidneys (T_{max} = 1 hour on GD18) • Fetal T_{max} was 1 hour on both GD12 and GD18, consistent with the route of exposure (maternal oral dosing) • Maternal distribution (for the tissues measured) was highest in kidneys > liver > plasma, consistent with the male distribution study data • Maternal tissue concentrations were 1.1x to 7.4x higher than fetal tissue concentrations, except for the brain. <ul style="list-style-type: none"> ○ Fetal:maternal brain C_{max} on GD18 was 3.5x. ○ This suggests that the blood:brain barrier is weaker in fetuses compared to adults ○ Note: this data <i>per se</i> does not suggest the fetal brain is a target tissue (because higher concentrations were observed in other fetal tissues compared to fetal brain)
<p>Table 16: Maternal and fetal rat PK data following maternal oral dosing on GD12 or GD18 (report # 0015407)</p>	

Group	Tissue	C _{max} (ng eq/g)		T _{max} (h)	t _{1/2} (h)	AUC _{last} (ng eq*h/g)		T _{last} (h)
		Mean	Stdev			Mean	Stdev	
1 [¹⁴ C]AL-60371 Gestation Day 12	Amniotic fluid	81.9	35.2	1	1.25	321	31.4	8
	Blood	533	150	0.5	1.23	1370	104	12
	Brain	24.3	7.26	0.5	1.93	51.8	4.73	4
	Whole fetus	121	46.2	1	NC	177	16.2	2
	Heart	472	23.1	0.5	1.10	1210	115	8
	Kidneys	4100	1400	0.5	14.2	11300	1070	48
	Liver	2010	309	0.5	5.86	4270	281	24
	Lungs	416	92.3	0.5	1.12	972	90.7	8
	Mammary glands	232	24.9	0.5	NC	431	41.6	4
	Ovaries	266	42.9	0.5	1.25	677	59.2	8
	Placenta	336	59.2	0.5	1.96	754	79.4	4
	Plasma	622	170	0.5	1.17	1630	125	12
	Uterus	390	4.51	0.5	1.27	1150	99.6	8
2 [¹⁴ C]AL-60371 Gestation Day 18	Amniotic fluid	44.3	12.5	2	4.04	373	36.7	24
	Blood	724	168	0.5	1.70	1570	165	12
	Brain	34.6	8.56	0.5	1.30	71.7	6.64	4
	Fetal blood	185	36.6	1	2.07	511	32.4	12
	Fetal brain	120	32.3	1	1.51	440	38.4	48
	Fetal heart	302	95.5	1	NC	720	48.6	4
	Fetal liver	312	71.0	1	1.81	880	63.3	12
	Fetal lungs	256	68.2	1	1.45	653	38.8	12
	Whole fetus	250	62.4	1	2.77	660	41.3	12
	Heart	781	209	0.5	1.22	1450	121	8
	Kidneys	4870	140	1	13.4	11900	386	48
	Liver	1940	467	0.5	4.55	4190	281	24
	Lungs	613	173	0.5	1.88	1180	95.2	12
	Mammary glands	371	91.1	0.5	2.94	797	74.0	12
	Ovaries	337	70.4	0.5	2.16	735	54.8	12
	Placenta	327	78.2	0.5	2.72	856	69.3	12
Plasma	809	150	0.5	1.68	1740	178	12	
Uterus	606	165	0.5	2.28	1550	110	12	

NC: Not calculated due to limited time points.

Title	Autoradiographic Tissue Distribution of Total Radioactivity in Male Guinea Pig Heads Following Repeated Otic Doses of [¹⁴C]AL-60371																							
Report #	TDOC-0015411																							
Key findings:	<p>Following otic dosing, fluroxacin distributed into the middle and inner ear (malleus and cochlea)</p> <ul style="list-style-type: none"> On D1 (after 2 otic doses), distribution was tympanic membrane and external ear canal > tympanic bulla wall On D7 (after 14 total otic doses), distribution was malleus > external ear canal > tympanic membrane > tympanic bulla wall > cochlea 																							
Report date:	May 1, 2013																							
Report location:	NDA module 4.2.2.3 (Pharmacokinetics – Distribution)																							
Study laboratory:	(b) (4)																							
Test article:	<ul style="list-style-type: none"> [¹⁴C]AL-60371 Lot # CFQ41569 Specific activity 59 mCi/mmol (149 µCi/mg) 0.3% fluroxacin 																							
Formulation	<table border="1"> <thead> <tr> <th>Component</th> <th>Formulation for this study</th> <th>Clinical formulation (for comparison)</th> </tr> </thead> <tbody> <tr> <td>Fluroxacin</td> <td>0.3%</td> <td>0.3%</td> </tr> <tr> <td>pH</td> <td colspan="2">(b) (4)</td> </tr> <tr> <td>Magnesium chloride hexahydrate</td> <td colspan="2">(b) (4)</td> </tr> <tr> <td>Hydroxyethyl cellulose</td> <td colspan="2">(b) (4)</td> </tr> <tr> <td>BAC</td> <td colspan="2">(b) (4)</td> </tr> <tr> <td>Sodium chloride</td> <td colspan="2">(b) (4)</td> </tr> </tbody> </table>			Component	Formulation for this study	Clinical formulation (for comparison)	Fluroxacin	0.3%	0.3%	pH	(b) (4)		Magnesium chloride hexahydrate	(b) (4)		Hydroxyethyl cellulose	(b) (4)		BAC	(b) (4)		Sodium chloride	(b) (4)	
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Hydroxyethyl cellulose	(b) (4)																							
BAC	(b) (4)																							
Sodium chloride	(b) (4)																							
Species	<p>Male Hartley albino guinea pigs</p> <ul style="list-style-type: none"> 3 months of age weight range 725 to 787 g single-housed 																							
Dosing:	<ul style="list-style-type: none"> One 60 µl drop every 12 hours, either 2 or 14 doses total (~ 0.2 mg/kg per dose) Right ear only dosing, with restraint and gauze over the ear Animals were placed in an intermediate cage for < 30 minutes, which was wiped afterward to measure any discharge of radioactivity from the ear (i.e. due to head shaking) 																							
Analysis:	<ul style="list-style-type: none"> Groups of 3 guinea pigs were sacrificed at 4 and 24 hours after the second dose, or 4 and 24 hours after the last dose on D7. Blood was collected. 																							

	<ul style="list-style-type: none"> • The treated external auditory canal was flushed repeatedly with saline; the flushes were analyzed for radioactivity • The heads were then shaved and frozen intact, embedded in carboxymethylcellulose, and processed • Quantitative autoradiography performed on head sections for blood, the brain, and additionally for dosed and nondosed ear: <ul style="list-style-type: none"> ○ Cochlea ○ External ear canal ○ Malleus ○ Temporal bone ○ Tympanic bulla wall ○ Tympanic membrane ○ Vestibular apparatus • Lower limit of quantitation (LLOQ) was 36.4 ng/gram of tissue 														
Clinical signs	<ul style="list-style-type: none"> • No signs of toxicity were noted for any animal • Animals were observed twice daily for mortality and signs of pain or distress 														
Dose recovered outside of tissues	<ul style="list-style-type: none"> • Less than 1% of administered dose was recovered in the gauze during restraint (during and after dosing) • ≤ 0.4% of administered dose was recovered in the cage after dosing (i.e. from head shaking) • Flushing the ear canal recovered 2 to 11% of administered dose at sacrifice (i.e. prior to autoradiography) <ul style="list-style-type: none"> ○ 11% after the second dose at 4 hours post-dose ○ 6% after the second dose, at 24 hours post-dose ○ 2 to 3% after the last dose (at 4 or 24 hours post-dose) 														
Day 1 results	<table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th rowspan="2"></th> <th colspan="2">Concentration (ng finafloxacin/gram tissue)</th> </tr> <tr> <th>4 hours</th> <th>24 hours</th> </tr> </thead> <tbody> <tr> <td>Tympanic membrane</td> <td>730</td> <td>300</td> </tr> <tr> <td>External ear canal</td> <td>560</td> <td>1280</td> </tr> <tr> <td>Tympanic bulla wall</td> <td>235</td> <td>230</td> </tr> </tbody> </table> <p style="text-align: center;">Below LLOQ for other tissues</p>		Concentration (ng finafloxacin/gram tissue)		4 hours	24 hours	Tympanic membrane	730	300	External ear canal	560	1280	Tympanic bulla wall	235	230
	Concentration (ng finafloxacin/gram tissue)														
	4 hours	24 hours													
Tympanic membrane	730	300													
External ear canal	560	1280													
Tympanic bulla wall	235	230													
Day 7 results	<table border="1" style="margin-left: auto; margin-right: auto;"> <thead> <tr> <th rowspan="2"></th> <th colspan="2">Concentration (ng finafloxacin/gram tissue)</th> </tr> <tr> <th>4 hours</th> <th>24 hours</th> </tr> </thead> <tbody> <tr> <td>Malleus</td> <td>5400</td> <td>Below quantitation</td> </tr> <tr> <td>External ear canal</td> <td>3600</td> <td>1530</td> </tr> </tbody> </table>		Concentration (ng finafloxacin/gram tissue)		4 hours	24 hours	Malleus	5400	Below quantitation	External ear canal	3600	1530			
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	4 hours	24 hours													
Malleus	5400	Below quantitation													
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		Tympanic membrane	950	480
		Tympanic bulla wall	220	140
		Cochlea	57	Below quantitation
	Below LLOQ for other tissues			

5.3 Metabolism

Title	Characterization of CYP450 Isozymes Involved in the <i>In Vitro</i> Metabolism of AL-60371 by Human Liver Microsomes																	
Report #	TDOC-0015274																	
Methods	<ul style="list-style-type: none"> 1 μM (0.4 $\mu\text{g/ml}$) finafloxacin hydrochloride was incubated with six recombinant human CYP enzymes for 30 minutes, with cofactors The amount of finafloxacin remaining after 30 minutes was measured 																	
Results	<table border="1"> <thead> <tr> <th>Enzyme</th> <th>% metabolized in 30 minutes</th> </tr> </thead> <tbody> <tr> <td>Control</td> <td>0 %</td> </tr> <tr> <td>CYP1A2</td> <td>45 %</td> </tr> <tr> <td>CYP2B6</td> <td>18 %</td> </tr> <tr> <td>CYP2C9</td> <td>4 %</td> </tr> <tr> <td>CYP2C19</td> <td>27 %</td> </tr> <tr> <td>CYP2D6</td> <td>29 %</td> </tr> <tr> <td>CYP3A4</td> <td>23 %</td> </tr> </tbody> </table>		Enzyme	% metabolized in 30 minutes	Control	0 %	CYP1A2	45 %	CYP2B6	18 %	CYP2C9	4 %	CYP2C19	27 %	CYP2D6	29 %	CYP3A4	23 %
Enzyme	% metabolized in 30 minutes																	
Control	0 %																	
CYP1A2	45 %																	
CYP2B6	18 %																	
CYP2C9	4 %																	
CYP2C19	27 %																	
CYP2D6	29 %																	
CYP3A4	23 %																	

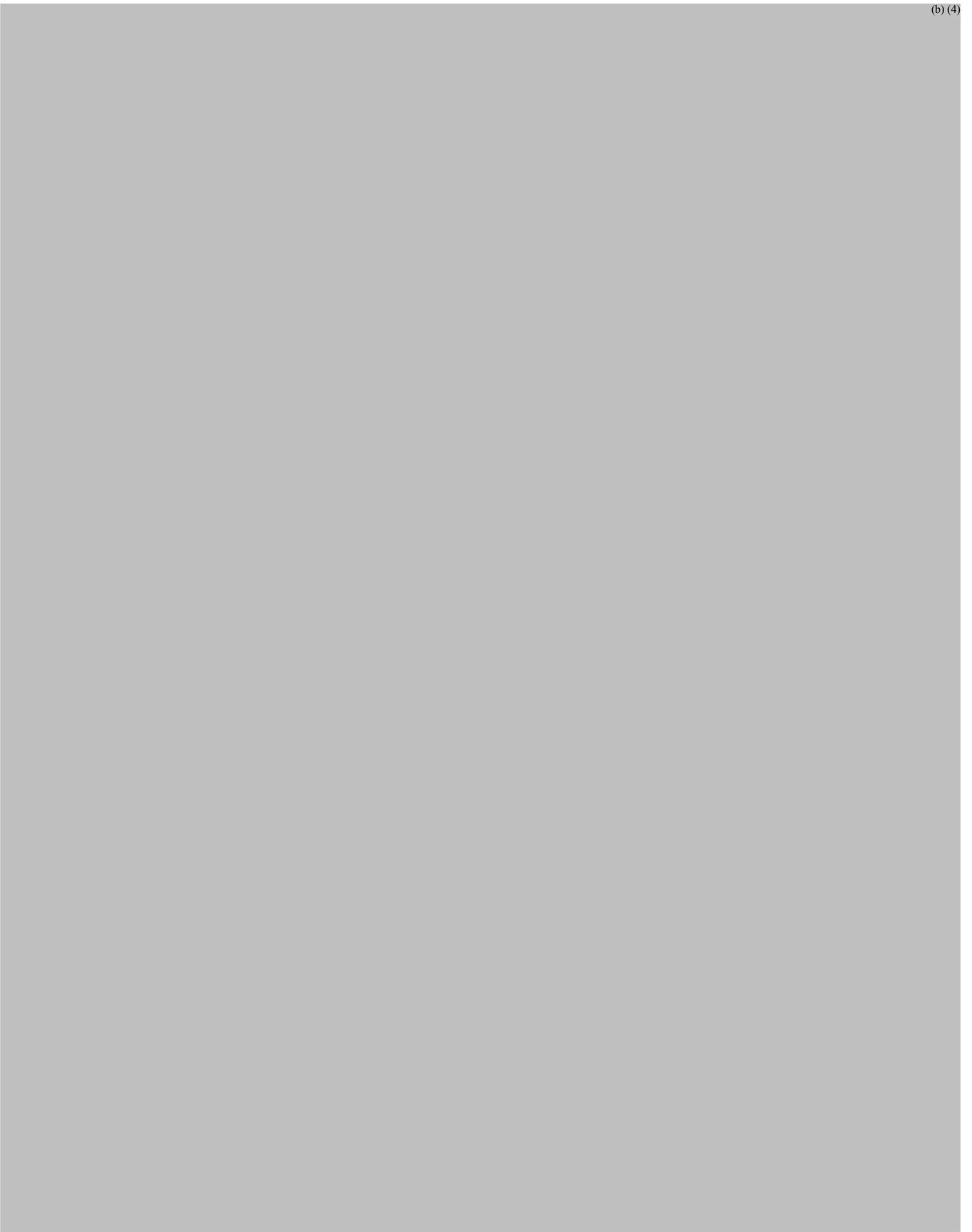
Title	Inhibition of AL-60371 towards Human Hepatic Microsomal Cytochrome P450
Report #	TDOC-0015315
Key finding	Finafloxacin did not inhibit the human cytochrome P450 isoforms tested
Methods	<ul style="list-style-type: none"> The goal of the study was to determine the inhibition (IC_{50} values). Eight dilutions were tested (0.047 to 100 μM finafloxacin) Microsomes from human liver samples were used (source not reported) Inhibition of CYPs 1A2, 2D6, 2C9, 2C19 and 3A4 were tested (using appropriate substrates, and with positive controls for inhibition) Incubation times were specific for each enzyme and substrate combination (5 to 40 minutes)
Results	No inhibition of any of the enzymes by finafloxacin was detected.

Title	Effects of AL-60371 on hepatic enzymes in male and female Long-Evans rats following repeat oral doses of AL-60371A	
Report #	TDOC-0015406	
Key findings	2 mg/kg of finafloxacin induced only minor changes in the enzymes measured: <ul style="list-style-type: none"> • Males exhibited a 21% increase in glucuronosyltransferase (UDPGT) activity • Females exhibited a decrease (to 58% of control) in CYP2B 	
Report details	Study laboratory	(b) (4)
	GLP status	No
	Test article	0.6% solution of finafloxacin hydrochloride (batch # 11-65082), purity not reported
Method notes	Vehicle	0.8% magnesium chloride, pH 8
	Species	Male and female Long Evans rats
	Dosing	0 or 2 mg/kg/day of finafloxacin (volume 3.67 ml/kg) for 14 days
	Positive control	Phenobarbital
	Enzymes measured	Hepatic microsomal fractions were prepared, and assayed for: <ul style="list-style-type: none"> • Total protein • Total cytochrome P450 content • CYP1A • CYP2B • CYP3A/CYP4A • Glucuronosyltransferase (UDPGT)
	Other endpoints	Body weight, liver weight
Results	No treatment-related changes in body weight or liver weight	
	<ul style="list-style-type: none"> • No biologically-significant changes in enzyme activity • Two minor treatment-related changes in enzyme activity were detected: <ul style="list-style-type: none"> ○ Males exhibited a 21% increase in glucuronosyltransferase activity compared to controls ○ Females exhibited a decrease (to 58% of control) in CYP2B 	

Title	Chromatographic Profiles of Radioactivity in Plasma and Urine Following a Single 10 mg/kg Oral Dose of [¹⁴C]AL-60371 to Wistar and Long-Evans Rats and Beagle Dogs	
Report #	TDOC-0013949	
Key findings	<ul style="list-style-type: none"> • Four hours after oral dosing, the majority of finafloxacin in plasma was unmetabolized • The primary metabolite detected in plasma was AL-91591, the β-glucuronide ester (conjugated at the carboxylic acid of finafloxacin), at < 10% of finafloxacin • The major urinary metabolite is the N-sulfate conjugate 	
	<p>Because the toxicology studies used Wistar rats, and the ADME studies used Long-Evans rats, the authors compared to metabolite profiles (in plasma and urine) for the two rat strains versus beagle dogs</p> <ul style="list-style-type: none"> • Slight differences between the rat strains were apparent • Absorption was slower in dogs, with plasma AL-91591 levels BLLOQ 	
Report details	Report date	July 31, 2013
	GLP status	Not GLP
	Study laboratory	Alcon Research Ltd. 6201 S. Freeway, Fort Worth, Texas 76134
	Report location	NDA module 4.2.2.4 metabolism
Test article: finafloxacin hydrochloride	[¹⁴ C]AL-60371	<ul style="list-style-type: none"> • Lot # 17620 • Purity 99.6%
	Formulation	Water <i>quantum satis</i> (q.s.) (b) (4) magnesium chloride (b) (4) pH 8.0
Methods	Dose	<ul style="list-style-type: none"> • Single oral dose of 10 mg/kg [¹⁴C]finafloxacin hydrochloride • Fasting 16 hours pre-dose to 4 hours post-dose
	Rats	<ul style="list-style-type: none"> • A total of 10 male Wistar rats and 10 male Long-Evans rats were dosed • 2 rats/strain were harvested at 0.5, 1, 2 and 4 hours post-dose to collect plasma • 2 rats/strain were kept in metabolism cages for 12 hours, to collect urine
	Dog	<ul style="list-style-type: none"> • 2 male Beagle dogs were dosed • Plasma was collected at 0.5, 1, 2 and 4 hours • Urine was collected for 12 hours post-dose
Results	<ul style="list-style-type: none"> • AL-91591 was the only metabolite identified in plasma within 4 hours post-dose (see Figure 3) • The N-sulfate conjugate of AL-60371 (not given a code number by the authors) was the major urinary metabolite (see Figure 3) • Two other minor urinary metabolites were also detected (see 	

Figure 4 and Figure 5), < 0.5% of total radioactivity			
Table 17: Plasma concentrations of finafloxacin and the metabolite AL-91591 (report # 0013949)			
Time (hours)	Finafloxacin (ng eq/g)	AL-91591 (ng eq/g)	Percentage finafloxacin:AL-91591
Wistar rat plasma			
0.5	1920	102	5.3 %
1	1560	47	3.0 %
2	384	ND	NC
4	126	BLQ	NC
Long-Evans rat plasma			
0.5	1630	26	1.6 %
1	1320	29	2.2 %
2	754	20	2.7 %
4	147	BLQ	NC
Beagle dog plasma			
0.5	970	ND	NC
1	2060	BLQ	
2	3360	ND	
4	4260	BLQ	
ND: not detected BLQ: detectable but below the limit of quantitation NC: not calculated Values rounded by this reviewer for readability			
Urinalysis (0 to 12 hours post-dose): <ul style="list-style-type: none"> • The N-sulfate conjugate of finafloxacin was: <ul style="list-style-type: none"> ○ 34% of total radioactivity in the Wistar rats ○ 43% of total radioactivity in the Long-Evans rats ○ 1.7% of total radioactivity in the Beagle dogs • AL-91591 was ≤ 0.5% of the total radioactivity in both rats strains in the dogs 			
Figure 3: Proposed metabolic pathways of finafloxacin (report # 0013949)			

(b) (4)



Title	Identification of Metabolites of AL-60371 in Plasma of Human, Dog and Rat Following Oral Administration of AL-60371																				
Report #	TDOC-0015412																				
Report date	July 23, 2013																				
Key findings	<ul style="list-style-type: none"> Analysis of human plasma following oral dosing found AL-91591 and two minor metabolites AL-91591 and the M2 metabolite were present in plasma at levels more than 10% of the parent compound 																				
Analysis of volunteer plasma	<ul style="list-style-type: none"> Plasma samples were collected from healthy volunteers at 0, 1, 2, and 4 hours after a single oral dose of finafloxacin hydrochloride (200 mg tablet), trial # C-10-007 Analysis of the plasma detected finafloxacin and three metabolites. The predominant metabolite was AL-91591 (the β-glucuronide ester) The structures of the other two metabolites, M1 and M2, were not provided <p>Table 18: Percentage of analyte (by MS/MS) relative to the amount of finafloxacin detected at 1 hour (report # 0015412)</p> <table border="1"> <thead> <tr> <th>Analyte</th> <th>1 hour</th> <th>2 hours</th> <th>4 hours</th> </tr> </thead> <tbody> <tr> <td>Finafloxacin</td> <td>100 %</td> <td>71 %</td> <td>38 %</td> </tr> <tr> <td>M1</td> <td>5.6 %</td> <td>3.0 %</td> <td>1.7 %</td> </tr> <tr> <td>M2</td> <td>29 %</td> <td>15 %</td> <td>6.9 %</td> </tr> <tr> <td>AL-91591</td> <td>66 %</td> <td>16 %</td> <td>25 %</td> </tr> </tbody> </table>	Analyte	1 hour	2 hours	4 hours	Finafloxacin	100 %	71 %	38 %	M1	5.6 %	3.0 %	1.7 %	M2	29 %	15 %	6.9 %	AL-91591	66 %	16 %	25 %
Analyte	1 hour	2 hours	4 hours																		
Finafloxacin	100 %	71 %	38 %																		
M1	5.6 %	3.0 %	1.7 %																		
M2	29 %	15 %	6.9 %																		
AL-91591	66 %	16 %	25 %																		
Dog and rat experiment	<ul style="list-style-type: none"> Male Wistar rats and male Beagle dogs received a single oral dose of 10 mg/kg finafloxacin (Free base) [number of animals not reported] Plasma was collected 1 and 2 hours post-dose Finafloxacin was detected in the pooled rat plasma and the pooled dog plasma, but no metabolites were detected 																				

Title	Chromatographic Profiles of [¹⁴C]AL-60371 Following Incubation With Rat, Rabbit, Monkey, and Human Liver Microsomes and Hepatocytes
Report #	TDOC-0016052
Key findings	<ul style="list-style-type: none"> Incubation of finafloxacin with hepatocytes from humans, rat, rabbit, and monkeys observed minor metabolism /degradation, (b)(4) of parent drug by 3 hours. No clear species differences were apparent No metabolism/degradation was detected when finafloxacin was incubated with hepatic microsomes from humans, rat, rabbit or monkeys for up to 60 minutes
Method notes:	<ul style="list-style-type: none"> Test concentrations of 2.2 μg/ml (5 μM) or 10.9 μg/ml (25 μM)

	<ul style="list-style-type: none"> The activity of the microsomes and hepatocytes was verified with positive controls.
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Title:	Quantitation of AL-60371 and AL-91591 in Rat, Dog and Human Plasma Samples Following Oral Administration
Report #:	TDOC-0016434
Key findings	<ul style="list-style-type: none"> Pooled plasma samples from healthy volunteers, collected under trial C-10-007, were compared to samples from male Wistar rats and male beagle dogs.
Method notes	<ul style="list-style-type: none"> Nineteen healthy volunteers received a single 200 mg oral dose of finafloxacin hydrochloride (AL-60371), and plasma samples were collected (from 4 volunteers/time point) at 0.5, 1, 2 and 4 hours post-dose. Rats (2/time point; 8 total) and 2 dogs received a single 10 mg/kg solution by oral gavage, with the same schedule of plasma sampling. Plasma samples were analyzed for the parent drug (AL-60371) and the metabolite AL-91591
Results	<ul style="list-style-type: none"> For each species, the proportion of metabolite to parent drug (% M/P) was relatively constant for 0.5 to 4 hours Humans plasma samples had substantially more metabolite than either the rat (5.5-fold) or the dog (116-fold)
<p>Figure 6: Mean concentrations of finafloxacin and the AL-51591 metabolite in human, rat, and dog plasma following single oral doses (report # 0016434)</p>	

Time (h)	Rat Plasma Concentration (ng/mL)		
	AL-60371	AL-91591	%M/P ^a
0.5	946	127	13.4
1	1215	150	12.3
2	659	72.1	10.9
4	212	24.5	11.6
Time (h)	Dog Plasma Concentration (ng/mL)		
	AL-60371	AL-91591	%M/P ^a
0.5	645	2.66	0.412
1	1510	7.31	0.484
2	3445	20.8	0.604
4	5265	41.6	0.790
Time (h)	Human Plasma Concentration (ng/mL)		
	AL-60371	AL-91591	%M/P ^a
0.5	1660	1232	74.2
1	1591	1161	73.0
2	1094	687	62.8
4	466	264	56.7

^a%M/P stands for percentage of AL-91591 plasma concentration relative to AL-60371 plasma concentration.

5.4 Excretion

The Sponsor submitted to excretion studies (NDA module 4.2.2.5 Excretion)

Title	Excretion and Mass Balance of Total Radioactivity in Pigmented Male Long-Evans Rats Following a Single Intravenous Dose of [¹⁴C]AL-60371A	
Report #	TDOC-0015404	
Key findings	<p>Following a single iv dose in male rats:</p> <ul style="list-style-type: none"> Excretion was rapid: 92% within the first 24 hours post-dose The primary route of excretion was urine (45.4% in the first 12 hours post-dose, and an additional 12.1% in the subsequent 96 hours) Excretion in feces was substantial: 38.7% from 0-120 hours 	
Report details	Report date	October 23, 2012
	Study laboratory	(b) (4)
	GLP status	Not GLP
	Test article	<ul style="list-style-type: none"> Radiolabeled [¹⁴C]finafloxacin hydrochloride lot # CFQ41156 specific activity of 59 mCi/mmol (126 µCi/mg) purity 97.5%
Methods	Formulation	<ul style="list-style-type: none"> 6.0% finafloxacin intravenous solution (equivalent to 5.55 mg/ml as free base) Magnesium chloride (b) (4) (b) (4) Water q.s. pH 8.0 ± 0.1
	Sex and species	Male Long-Evans rats
	Dosing	<ul style="list-style-type: none"> A group of 5 rats received a single i.v. dose of [¹⁴C]finafloxacin hydrochloride, equivalent to 2 mg/kg of free base (0.36 ml/kg volume) Dose administered via a tail vein Animals were not fasted
	Sample collection	<ul style="list-style-type: none"> Urine, feces, and expired air were collected at intervals of 0-12 hours, 12-24 hours, and then for the subsequent four 24 hour periods Cage rinse was collected every 24 hours for the first 5 days
Results	<ul style="list-style-type: none"> Excretion was rapid: 92% within the first 24 hours post-dose The primary route of excretion was urine (45.4% in the first 12 hours post-dose, and an additional 12.1% in the subsequent 96 hours) Excretion in feces was substantial: 38.7% from 0-120 hours Trivial amounts of radiation were collected in the expired air 	

	<ul style="list-style-type: none"> ○ 0.03% of total radiation at 0-12 hours ○ 0.04% of total radiation from 0-120 hours ○ These values might be due to exhaled radiolabel, or might be aerosol formed from urine 																																			
	<p>Table 19: Mean cumulative percent of radioactive dose recovered from male Long-Evans rats following a single i.v. dose of [¹⁴C]-finafloxacin hydrochloride (report # 0015404)</p> <table border="1"> <thead> <tr> <th>Collection interval (hours)</th> <th>Cumulative total</th> <th>Urine</th> <th>Feces</th> <th>Cage rinse^a</th> </tr> </thead> <tbody> <tr> <td>0-12</td> <td>52.4</td> <td>45.4</td> <td>6.93</td> <td>-</td> </tr> <tr> <td>0-24</td> <td>92.0</td> <td>55.4</td> <td>31.8</td> <td>4.77</td> </tr> <tr> <td>0-48</td> <td>100</td> <td>57.0</td> <td>38.0</td> <td>5.15</td> </tr> <tr> <td>0-72</td> <td>101</td> <td>57.3</td> <td>38.4</td> <td>5.26</td> </tr> <tr> <td>0-96</td> <td>102</td> <td>57.4</td> <td>38.6</td> <td>5.50</td> </tr> <tr> <td>0-120</td> <td>102</td> <td>57.5</td> <td>38.7</td> <td>NA</td> </tr> </tbody> </table> <p>^a Cage rinse was collected from 0-24 hours for 5 days (i.e. no 0-12 hour time point, no 96-120 hour time point)</p>	Collection interval (hours)	Cumulative total	Urine	Feces	Cage rinse ^a	0-12	52.4	45.4	6.93	-	0-24	92.0	55.4	31.8	4.77	0-48	100	57.0	38.0	5.15	0-72	101	57.3	38.4	5.26	0-96	102	57.4	38.6	5.50	0-120	102	57.5	38.7	NA
Collection interval (hours)	Cumulative total	Urine	Feces	Cage rinse ^a																																
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0-96	102	57.4	38.6	5.50																																
0-120	102	57.5	38.7	NA																																

Title	Secretion of Total Radioactivity in Pigmented Lactating Long-Evans Rats Following a Single Oral Dose of [¹⁴C]AL-60371A	
Report #	TDOC-0015405	
Key findings	<p>Lactating female Long-Evans rats received a single oral dose of 2 mg/kg finafloxacin at 12 days postpartum.</p> <ul style="list-style-type: none"> • From 0.5 to 12 hours post-dose, the concentration of finafloxacin was higher in milk than in the mothers' blood or plasma. • [Concentrations in the plasma of nursing offspring were not measured] 	
Report details	Report date	September 28, 2012
	Study laboratory	(b) (4)
	GLP status	Not GLP
	Test article	<ul style="list-style-type: none"> • Radiolabeled [¹⁴C] finafloxacin hydrochloride (AL-60371A) • Lot # CFQ41156 • specific activity of 59 mCi/mmol (126 μCi/mg) • purity 97.5%
Method notes	Formulation	<p>(b) (4) magnesium chloride</p> <p>(b) (4)</p> <p>Water q.s. pH 8.0 ± 0.1</p>

	Species and model	<ul style="list-style-type: none"> • 28 female Long Evans rats (HsdBlu:LE) were “time-pregnant” by the supplier, single-housed and allowed to acclimate for 20 days prior to dosing • [No mention is made of delivery; presumably dams were allowed to deliver naturally) • On postpartum day (PPD) 8, litters were culled to 8 pups • The day before milk collection, litters were culled to 4 pups • On the day of milk collection (PPD12), the remaining 4 pups were removed from the dams 4 hours before milk collection • To stimulate lactation, dams received a single sc injection of oxytocin approximately 15 minutes before milking • Dams were anesthetized during milking • Immediately following milking, dams were euthanized, and blood was collected 																																			
	i.v. dose	2 mg/kg (as free base), target volume 3.64 ml/kg																																			
	Time points	After dosing, 4 dams/time point were used to collect data for the 0.5, 1, 4, 8, 12, 24 and 48 hour time points (total of 28 rats)																																			
Results	<p>Table 20: Finafloxacin concentration in the blood, plasma, and milk of lactating Long-Evans rats following a single oral dose (report # 0015405)</p> <table border="1" data-bbox="418 1171 1427 1518"> <thead> <tr> <th rowspan="2">Time (hours)</th> <th colspan="3">Finafloxacin concentration (ng/g)</th> </tr> <tr> <th>Blood</th> <th>Plasma</th> <th>Milk</th> </tr> </thead> <tbody> <tr> <td>0.5</td> <td>430 ± 86</td> <td>439 ± 86</td> <td>1020 ± 220</td> </tr> <tr> <td>1</td> <td>360 ± 87</td> <td>357 ± 81</td> <td>1220 ± 394</td> </tr> <tr> <td>4</td> <td>64 ± 30</td> <td>66 ± 31</td> <td>378 ± 175</td> </tr> <tr> <td>8</td> <td>6.1 ± 3.3</td> <td>6.8 ± 3.3</td> <td>44 ± 27</td> </tr> <tr> <td>12</td> <td>2.3 ± 1.9</td> <td>3.2 ± 1.7</td> <td>17.3 ± 11.3</td> </tr> <tr> <td>24</td> <td>BLQ</td> <td>BLQ</td> <td>BLQ</td> </tr> <tr> <td>48</td> <td>BLQ</td> <td>BLQ</td> <td>BLQ</td> </tr> </tbody> </table> <p>BLQ: below the limit of quantitation Values expressed as means ± standard deviation, and rounded by this reviewer for readability.</p>		Time (hours)	Finafloxacin concentration (ng/g)			Blood	Plasma	Milk	0.5	430 ± 86	439 ± 86	1020 ± 220	1	360 ± 87	357 ± 81	1220 ± 394	4	64 ± 30	66 ± 31	378 ± 175	8	6.1 ± 3.3	6.8 ± 3.3	44 ± 27	12	2.3 ± 1.9	3.2 ± 1.7	17.3 ± 11.3	24	BLQ	BLQ	BLQ	48	BLQ	BLQ	BLQ
Time (hours)	Finafloxacin concentration (ng/g)																																				
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12	2.3 ± 1.9	3.2 ± 1.7	17.3 ± 11.3																																		
24	BLQ	BLQ	BLQ																																		
48	BLQ	BLQ	BLQ																																		
	LLOQ	<ul style="list-style-type: none"> • Blood: 1.22 ng/g • Plasma: 0.939 ng/g • Milk: 1.20 ng/g 																																			

6 General Toxicology

6.1 Topical otic dosing (repeat dose)

The Applicant submitted two rabbit studies investigating topical otic dosing.

Study title: 14 Day Intra-Auricular Repeated Dose Study in New Zealand White Rabbits with Finafloxacin HCl (AL-60371A) and an Ofloxacin Marketed Comparator

Study no.: TDOC-0011256
 Study report location: NDA module 4.2.3.2 Repeat-dose toxicity
 Conducting laboratory and location: Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134
 Date of study initiation: February 2010
 GLP compliance & QA statement: Yes, signed
 Drug, lot #, and % purity: Finafloxacin hydrochloride (AL-60371A), 99.9% pure

% finafloxacin	Buffer	Lot #
0.3%	Acetate, pH 5.75	10-58275-1
0.3%	Phosphate, pH 7.5	10-58276-1
0.6%		10-58277-1
1.0%		10-58278-1

Key Study Findings

- Rabbits were dosed left ear only, topically, 4 drops/dose, 2 doses/day, for 2 weeks.
- All finafloxacin doses were well tolerated. This reviewer concludes that the procedure (i.e. holding the left ear after each dose to allow the test article to drain) caused the background incidence of ear canal effects observed by otoscopy and histopathology.
- Two formulations were tested:
 - 0.3% finafloxacin in acetate buffer, pH 5.75, was not associated with toxicity (no systemic histopathology was performed)
 - Finafloxacin in the phosphate buffer (pH 7.5) was associated with increased incidence of minimal-to-mild epithelial necrosis of the ear canal. Therefore, no NOAEL was identified for the phosphate buffer.
 - Neither formulation is comparable to the clinical drug product. As the Applicant noted (NDA module 1.11.2 Safety Information Amendment submitted 8/13/2014), this early otic studies (b) (4)

- Endpoints included otic evaluation (of the external auditory canal for skin reactions), gross pathology, collection of the ears together with the skull section including the temporal bone for histopathology of the left and right external ear canal, tympanic membrane, bulla, Eustachian tube, and middle ear ossicles.
- Systemic endpoints included body weight, clinical pathology (hematology, clinical chemistry, coagulation), full systemic gross pathology, and select organ weights and histology (adrenal, brain, heart, kidney, liver, ovary, spleen, testes) for the saline control, vehicle control, and 1% finafloxacin group.
- Systemic TK was evaluated.

Methods

Doses:

Group #	Formulation	% finafloxacin	Drop size (4 drops BID)	Total daily dose (mg/animal)
1	Saline (0.9% sodium chloride)	0	(b) (4)	0
2	Acetate vehicle control (pH 5.75)	0	(b) (4)	0
3	Phosphate vehicle control (pH 7.5)	0	(b) (4)	0
4	Acetate vehicle control (pH 5.75)	(b) (4)	(b) (4)	(b) (4)
5	Phosphate vehicle control (pH 7.5)	(b) (4)	(b) (4)	(b) (4)
6		(b) (4)	(b) (4)	(b) (4)
7		(b) (4)	(b) (4)	(b) (4)
8	Commercial ofloxacin otic solution	0	(b) (4)	(b) (4)

Formulation/Vehicle:

- The saline control (group 1) had no ingredients other than water and sodium chloride.
- The ofloxacin otic solution (group 8) was obtained commercially (0.9% sodium chloride, 0.0025% benzalkonium chloride)
- The formulations for groups 2-7 had (b) (4) % magnesium chloride and (b) (4) % benzalkonium chloride.
- Groups 2 and 4 had (b) (4)
- Groups 3 and 5-7 had (b) (4)
- For reference, the clinical formulation has (b) (4) % hydroxyethyl cellulose (b) (4) % sodium chloride (not present in the vehicles),

(b) (4) % magnesium chloride ((b) (4))
0.005% BAC (one half of the vehicle concentration), pH 6.0.

Frequency of dosing: Twice daily for 14 days

Route of administration: Into the external left ear canal. The pinna was held for ~ 10 seconds after each dose to allow the dose to drain down the canal before the rabbit could shake.

Species/Strain: New Zealand White rabbit (*Oryctolagus cuniculus*)

- Age range 3 to 5 months at study initiation
- Body weight range 2.7 to 3.3 kg at study initiation

Number/Sex/Group: 3/sex/dose

Age: 3 to 5 months at study initiation

Weight: 2.7 to 3.3 kg at study initiation

Dosing Solution Analysis

- A certificate of analysis for finafloxacin hydrochloride was provided (report page 67), which listed specification for 8 different impurities.
- The amount of finafloxacin was measured for each lot pre-study and post-study. The results were acceptable (105% to 107% of nominal), and no degradation was detected.
- Homogeneity was not evaluated. Particles were not observed pre-study, but were observed post-study in the finafloxacin-containing lots (report page 69).
 - This issue was part of the rationale for the subsequent rabbit study (report # TDOC-0013365).
 - Systemic TK for this study confirms exposure, despite precipitation in the finafloxacin lots.

Observations and Results

Mortality

- No premature mortalities occurred.
- Checks were performed twice daily.

Clinical Signs

- No treatment-related clinical signs were apparent.
- Clinical signs were assessed pre-dose, and once (after the last dose) on D3, D7, D14 and D15
- The list of clinical observations noted for any animal was: abnormal stool, abnormal urination, discoloration, bruise, pustule, scab, scratch, squinting

Body Weights

- No treatment-related changes apparent.
- Body weight was measured pre-dose, D3, D7, D10, D14, and (with fasting) on D15

Hematology and Clinical Chemistry

- No treatment-related changes apparent.
- Blood was collected for hematology, clinical chemistry and coagulation on D13 only.

Otic Evaluation

- No treatment-related effects were apparent.
- Both ears were cleaned with alcohol wipes and photographed [no data from the photographs were mentioned in the report]. An otoscope was placed into the external auditory canal, and the skin was viewed on a video monitor. Dermal reactions were scored for erythema and eschar formation (arbitrary 0-4 scale), edema formation (arbitrary 0-4 scale), and external exudate (0-3 scale).
- Photography and skin reaction scoring pre-dose, and (between doses) on D1, D4, D8 and D13
- The otic evaluation results are not easily interpreted: all groups (including the saline control) had individual irritation scores ranging from none to severe (0-4) and edema scores ranging from none to moderate (0-3). The mean score for each group was moderate (2.29 to 3.38).

Gross Pathology

- No treatment-related effects were apparent.

Organ Weights

- No treatment-related effects were apparent.

Histopathology

Adequate Battery: Yes.

- The selection of systemic tissues was based on the results of the oral rat toxicity studies: adrenal, brain, heart, kidney, liver, lungs, ovary, spleen, and testes.
- Gross pathology and histology included the “bullas” and “ear (pinna)”. The report (page 55, pages 373-374) specifies that the ears were harvest “such that both pinna and the skull section containing both temporal bones are removed and preserved as one unit with the intact nasal bridge in 10% neutral buffered formalin.”
- The histopathology report lists findings explicitly for the left and right ‘external ear canal’, ‘tympanic membrane’, ‘bulla’, ‘Eustachian tube’, ‘middle ear ossicles’.

Peer Review: No. Pathology was conducted by a board-certified veterinary pathologist, (b) (6), DVM, PhD, (b) (6).

Histological Findings

- The only effect associated with finafloxacin was minimal-to-mild epithelial necrosis of the ear canal, detected in 0 or 1/group/sex for the 0.3%, 0.6% and 1% finafloxacin groups (phosphate formulations)
- Comparing the left (treated) ear to the right (untreated) ear, exposure to saline and vehicle (with or without finafloxacin) was associated with increased incidences of :
 - minimal-to-mild edema of the dermis of the bony ear canal
 - minimal-to-mild epithelial hyperplasia of the bony and cartilaginous ear canal
 - minimal sebaceous gland hyperplasia of the cartilaginous ear canal
 - minimal serocellular exudate of the bony ear canal
 - minimal hyperplasia of the epithelium of the tympanic membrane
 - minimal mixed or heterophilic inflammation of the tympanic membrane
- Comparing the vehicles and treated groups to saline, the vehicles were associated with increased incidence of minimal-to-mild acantholysis of the epidermis of the cartilaginous external ear
- Note: no finafloxacin- or vehicle-related effects were apparent for the bulla or middle ear ossicles.

Toxicokinetics

- Blood was collected on D1 and D14 just prior to the second dose (0 hours) and after the second dose at 0.5, 1 and 3 hours. LLOQ = 0.05 ng/ml.
- Individual animal T_{max} was usually the 0.5 hour time point, and was occasionally the 1 hour time point
- This reviewer notes that the C_{max} values for rabbits receiving 0.3% finafloxacin are comparable to the clinically reported C_{max} values (see section 11 of this review)

Table 21: Serum TK for the 14-day topical otic rabbit study with finafloxacin hydrochloride comparing formulations (report # TDOC-0011256)

Group	Study Day		C _{max} (ng/mL)	AUC _{0-3h} (ng/mL*h)
Group 4 0.3% Finafloxacin (as FB) in Acetate, pH 5.75	1	Mean	2.76	6.59
		Stdev	1.21	2.93
	14	Mean	3.92	9.05
		Stdev	1.52	3.43
Group 5 0.3% Finafloxacin (as FB) in Phosphate, pH 7.5	1	Mean	2.53	6.25
		Stdev	0.654	2.01
	14	Mean	3.96	8.87
		Stdev	1.56	3.34
Group 6 0.6% Finafloxacin (as FB) in Phosphate, pH 7.5	1	Mean	6.23	14.9
		Stdev	2.00	4.68
	14	Mean	8.73	19.0
		Stdev	7.85	15.7
Group 7 1.0% Finafloxacin (as FB) in Phosphate, pH 7.5	1	Mean	6.86	16.9
		Stdev	2.76	7.04
	14	Mean	8.45	20.7
		Stdev	2.25	5.39

FB = Free Base

Study title: AL-60371 (Finafloxacin): A Two-Week b.i.d. Topical Otic Toxicology Study with Two-Week Recovery Groups in New Zealand White Rabbits

Study no.: TDOC-0013365
 Study report location: NDA module 4.2.3.2 Repeat-Dose Toxicity
 Conducting laboratory and location: Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134
 Date of study initiation: January 2011
 GLP compliance & QA statement: Yes, signed
 Drug, lot #, and % purity: Finafloxacin (free base), purity 100.3% (calculated), lot # 15664-48

Key Study Findings

- The dosing schedule was the same as the previous rabbit study: rabbits were dosed left ear only, topically, 4 drops/dose, 2 doses/day for 2 weeks.

- The rationale for this study was to dose with finafloxacin (free base) rather than finafloxacin hydrochloride, in the phosphate formulation, and to assess recovery
- The NOAEL was the high-dose, 1.2% finafloxacin (2.79 mg/animal/day). No treatment-related histopathology in the tympanic membrane or ear canal was apparent.
 - Notably, this study did not detect epithelial necrosis of the ear canal in any animal (as was observed in the previous rabbit study, report # TDOC-001256)

Methods

- Doses:
- 0, 0.15%, 0.3%, 0.6%, 1.2% finafloxacin (free base)
 - Based on the measured average drop size, these doses are the equivalent of 0, 0.36, 0.67, 1.41, 2.78 mg/animal/day

Frequency of dosing: Twice daily, approximately 6 hours apart, daily for 14 days

- Route of administration:
- Topical otic route (via drop-tainer) to the left external ear canal
 - As with the previous rabbit study, the pinna was held for approximately 10 seconds after each dose to allow for drainage down the canal before the rabbit could shake

Dose volume: Each dose was 4 drops (average drop size ranged from 28.0 to 29.6 µl)

Formulation/Vehicle:

Component	Formulation tested in this study	Clinical formulation
Finafloxacin	0 to 1.2%	0.3%
Tyloxapol	(b) (4)	(b) (4)
Hydroxyethyl cellulose		
Sodium chloride		
Magnesium Chloride		
(b) (4)		
Benzalkonium Chloride (BAC)		
Sodium hydroxide and/or hydrochloric acid	(b) (4)	(b) (4)
pH		

- **Note:** This formulation is slightly different from the clinical formulation (more tyloxapol and BAC, no magnesium chloride)
- **Note:** Although the formulation included sodium

hydroxide and hydrochloride acid and q.s. for pH, no mention of the pH was identified in the study report.

This is a study limitation.

Species/Strain: New Zealand White rabbits (*Oryctolagus cuniculus*)
 Number/Sex/Group:

- Main-group: 3/sex/dose (necropsy D15)
- Recovery-group: 2/sex/dose (necropsy D29)

 Age: 5.5 to 6 months at study initiation
 Weight: 3.2 to 3.6 kg at study initiation

Dosing Solution Analysis

- Test article characterization was performed before and after dosing, and degradation ((b)(4)%) was apparent (from report page 68).
- This degradation did not affect the overall outcome of the study or the interpretability of the results.

Table 22: Test article characterization for the 14-day topical otic rabbit study with finafloxacin detected degradation over time (report # 0013365)

Lot Number	10-61622-1		10-61615-1		10-61617-1		10-61619-1		10-61621-1	
FID	118328		118329		118325		118326		118327	
Labeled Concentration	Vehicle		0.15%		0.3%		0.6%		1.2%	
AL-60371	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Identity	-	-	+	+	+	+	+	+	+	+
Strength ¹	N/A	N/A	113.5	104.5	111	106	111.5	104	111	104.5

¹Average of replicates

Observations and Results

Mortality

- No premature mortalities occurred.
- Animals were checked twice daily.

Clinical Signs

- No treatment-related effects on clinical signs were apparent.
- Post-dose observations were made approximately 15 minutes after the second daily dose
- Cage side observations were made on D1, D2, D4, D11, D18 and D25
- Rabbits were removed from cage for clinical signs assessment prior to start of dosing, and on D3, D7, D15, D22 and D29

Body Weights

- No treatment-related effects on body weight were apparent.

- Body weight was measured pre-dose, D3, D7, D10, D15, D17, D21, D24 and D29

Otic Evaluation

- No treatment-related effects were apparent. The severity of erythema in the left (treated ear) increased as the study progressed. This reviewer speculates the erythema was procedure-related.
- For this study, otic evaluations were made under anesthesia. An otoendoscope was inserted into the external auditory canal of each ear (left and right). The ear canal skin was scored for erythema and eschar formation, edema formation, and exudate (same scale as used for the previous rabbit study, report # TDOC-0011256)
- Otoendoscopic examination of the skin was scored pre-dose, D1, D3, D7, D14, D21 and D28

Hematology and Clinical Chemistry

- Blood was collected on D11 and D28 for hematology, clinical chemistry, and coagulation.

Gross Pathology

- Necropsy was limited to the ear (pinna) and bullae middle ear tissues.
- No treatment-related effects were apparent.

Histopathology

Peer Review: Yes. The pathologist was (b) (6), DVM, PhD, (b) (6). The peer review pathologist was (b) (6), DVM, PhD, (b) (6).

Histological Findings

- Tissues examined were the left and right: bulla, Eustachian tube, external ear canal, middle ear ossicles, and tympanic membrane.
- No treatment-related effects apparent in the left/right bulla, left/right Eustachian tube, or left/right middle ear ossicles.
- The author considered incidences $\geq 0.3\%$ to be treatment-related: minimal heterophilic inflammation of the tympanic membrane epidermis and stroma; minimal vacuolation of the epidermis of the external ear canal. This reviewer disagrees: these incidences appear to reflect background variability, and no finafloxacin-effect is apparent.

Toxicokinetics

- Blood was collected from main-group animals (3/sex/dose) on D1 and D14 after the last daily dose, at 0 (i.e. within 15 minutes after dosing), 0.5, 1, and 3 hours
- A sex difference was not apparent

- Comparing D1 for the 0.3% and 0.6% groups, no difference is apparent, suggesting that the nominal doses might not have been received.

Table 23: TK results for the 14-day topical otic rabbit study with finafloxacin (free base) (report # 0013365)

Finafloxacin group	Day	C _{max} (ng/ml)	AUC _{0-3 hours} (ng*hr/ml)
0.15%	1	4.22	10.3
	14	5.39	12.8
0.3%	1	8.58	22.0
	14	12.7	29.1
0.6%	1	8.97	23.4
	14	18.1	42.8
1.2%	1	16.9	44.8
	14	28.6	68.2

6.2 Middle-ear dosing (Single-dose and Repeat-Dose)

The applicant submitted two non-GLP single-dose middle-ear dosing studies in chinchillas, and two GLP repeat-dose middle-ear dosing studies in guinea pigs. Toxicity endpoints focused on hearing loss and middle ear necropsy.

Study title: Ototoxicity Screening Study with Finafloxacin (in either Zinc Chloride or Magnesium Chloride) Vehicles in Chinchillas

Study no.: TDOC-0010418
 Study report location: NDA module 4.2.3.1 Single-Dose Toxicity
 Conducting laboratory and location: Alcon Research Ltd. 6201 S. Freeway, Fort Worth, Texas 76134
 Date of study initiation: September 2009
 GLP compliance & QA statement: No
 Drug, lot #, and % purity: Finafloxacin (as free base), purity not reported.

Test article	Lot #
0.3% finafloxacin in acetate with (b) (4), pH 5.5	09-56649-1
0.3% finafloxacin in acetate with (b) (4)% magnesium chloride, pH 5.5	08-56651-1

Key Study Findings

- No finafloxacin-related toxicity was observed following a single dose (0.6 mg/kg) administered directly into the middle ear (transbullar)
- The magnesium chloride-based vehicle was associated with local toxicity (with or without finafloxacin). This formulation is not comparable to the topical clinical formulation.
- A positive control (Zinotic®) exhibited local toxicity on the middle ear (circling, head tilt and nystagmus)

Methods

- | | |
|--|---|
| Species, sex,
group size | <ul style="list-style-type: none"> • Male Laniger chinchillas (<i>Chinchilla laigera</i>) • 3/group (18 total) <ul style="list-style-type: none"> ○ 5 to 8 months of age at time of study ○ For the two finafloxacin-treated groups, the average body weight was 553 g |
| Surgical
procedure and
route of
administration: | <ul style="list-style-type: none"> • Group-housed (3 to 5 per cage) • Under anesthesia, the skin was prepared (hair plucked, surgical scrub) over both ears • An ~ 1 cm incision was made into the skin over the upper chamber of the middle ear cavity (bulla), to reach the thin bony covering of the superior chamber of the bulla • A 16 gauge needle was inserted through the bone, into the superior chamber of each bulla, resulting in an ~ 2 mm diameter hole • After instillation of test article, the tissue and skin were closed with sutures. |
| Right ear doses
(single dose, at
time of surgery): | Sodium chloride (0.9%), all animals (i.e. procedure control) |

Left ear doses (single dose, at time of surgery): All formulations had (b) (4) % sodium acetate, pH 5.5. Additionally:

Group #	Finafloxacin	Formulation (+0.68% sodium acetate)
1	0	3.3% mannitol
2	0	(b) (4)
3	0.33% [1.6 mg total dose]	(b) (4)
4	0	(b) (4)
5	0.3% [1.6 mg total dose]	(b) (4) % magnesium chloride, (b) (4)
6	0	ZinOtic®

Note: these formulations are not directly comparable to the topical otic clinical formulation ((b) (4) % tyloxapol, (b) (4) % hydroxyethyl cellulose, (b) (4) % sodium chloride, (b) (4) % magnesium chloride, 0.005% BAC, pH 6.0).

- Dose volume: 500 µl/ear
- Positive control:
- The report authors state that ZinOtic® is a commercially marketed product.
 - This reviewer did not find mention of ZinOtic® at the FDA webpage or Orange Book, but a literature search found consistent results: Zinotic® is chloroxylenol/pramoxine/zinc acetate, a combination medication indicated for treatment of outer ear infection.
 - Chloroxylenol is an antibacterial and antifungal agent.
 - Praxmoxine is an anesthetic.
 - Common side-effects reported with topical otic dosing are mild burning, irritation, redness, stinging, or dryness.
- Endpoints:
- Clinical observations (pre-dose, during recovery from anesthesia, 20 minutes and 1 hour post-dose, periodically until necropsy on D4)
 - Body weight (pre-dose and necropsy only)
 - Gross necropsy of the brain and bulla
 - [Note: no TK measured]

Observations and Results

- For the average body weight of 553 g, the 0.33% dose = 0.55 mg/kg of finafloxacin

- No finafloxacin-related weight loss was observed
 - All groups exhibited weight-loss from D-1 to D4, attributed to the withholding of food the morning of surgery
 - The ZinOtic® group lost more weight (-7.75%) than the other groups (-1.7 to -3.9%), indicating a treatment-effect
- No finafloxacin-related clinical signs were observed
 - The ZinOtic® group also exhibited more circling, head tilt, and nystagmus (from D1 to D4) compared to the other groups, indicating middle ear toxicity or irritation
 - Eyelid twitches were observed in all groups post-dose, and persisted longer in the groups treated with magnesium chloride (to D3) and ZinOtic® (to D4) compared to the other groups (D1 or D2 only)

Study title: Exploratory Ototoxicity Dose Response Evaluation of Finafloxacin Administered via Transbullar Injection in Chinchillas

Study no.: TDOC-0011059
 Study report location: NDA module 4.2.3.1 Single-Dose Toxicity
 Conducting laboratory and location: Alcon Research Ltd.
 6201 S. Freeway, Fort Worth, Texas 76134
 Date of study initiation: January 2010
 GLP compliance & QA statement: No
 Drug, lot #, and % purity: Finafloxacin (as free base), purity not reported

% finafloxacin	Buffer	Lot #
0.33%	Acetate	10-57936-1
0.33%	Phosphate	10-57937-1
0.66%	Phosphate	10-57938-1
1.1%	Phosphate	10-57939-1

Key Study Findings

- As expected, single-dose administration of 500 µl of fluid into the middle ear of chinchillas resulting in hearing loss (consistent with other published chinchilla middle ear studies). The vehicle tested in this study is not comparable to the topical otic clinical formulation.
- Beyond the vehicle-related hearing loss, finafloxacin (1.1%, equivalent to 5.5 mg/animal, and 9.2 mg/kg) was not associated with additional hearing loss.
- Although the finafloxacin-treated group caused mild-to-moderate hearing loss compared to sham, the relevance of this finding to patients is unclear, because this hearing loss is attributable to the vehicle formulation, and this study did not test the clinical formulation.
- This experiment assessed only a limited panel of endpoints (hearing loss, body weight, clinical signs, brain and bulla gross pathology)

Methods

- Species, sex, group size
 - Male Laniger chinchillas (*Chinchilla laigera*)
 - 3/group (21 total)
 - 6 to 8 months of age at time of study
 - Average body weight for finafloxacin-treated animals was 596 g
 - Individually housed
- Surgical procedure and route of administration:
 - The same procedure was used in study as in the previous chinchilla study (report # TDOC-0010418, reviewed above).
 - Under anesthesia, the skin was prepared, and then cut to expose the thin bony covering of the superior chamber of the bulla
 - A 16 gauge needle was inserted through the bone, into the superior chamber of each bulla, resulting in an ~ 2 mm diameter hole
 - After instillation of test article, the tissue and skin were closed with sutures.
- Right ear doses (single dose, at time of surgery):
 - Sodium chloride (0.9%), groups 2-7s (i.e. procedure control)
- Left ear doses (single dose, at time of surgery):
 - Group 1 was sham surgery.
 - For groups 2-7, all formulations had (b) (4) % magnesium chloride and (b) (4) % benzalkonium chloride.
 - Additionally:

Group #	Finafloxacin	Formulation
2	0	(b) (4)
3	0	
4	0.33% (1.7 mg/ear)	
5	0.33% (1.7 mg/ear)	
6	0.66% (3.3 mg/ear)	
7	1.1% (5.5 mg/ear)	

- Note: These formulations are not directly comparable to the topical otic clinical formulation: (b) (4) % tyloxapol (b) (4) % hydroxyethyl cellulose (b) (4) % sodium chloride (b) (4) % magnesium chloride (b) (4) % 0.005% BAC (preservative), pH 6.0.
 - These formulations have (b) (4) fold more magnesium

chloride. Additionally, the purpose of the mannitol is also to (b)(4).

- These formulations have (b)(4) the amount of BAC, and may therefore exhibit more local irritation.
- These formulations lack the tyloxapol and hydroxyethyl cellulose. To the extent that solubility/precipitation affect the local irritation of these formulations, the data may not be relevant to the topical otic clinical formulation.

Dose volume: 500 µl/ear for groups 2-7

- Endpoints:
- Clinical observations (pre-dose, during recovery after surgery including 20 and 60 minutes post-dose; in the afternoon after surgery, and twice daily thereafter)
 - Body weight (pre-dose and on D4)
 - Auditory brainstem response (ABR)
 - On day 4, animals were sacrificed and gross evaluation of the brain and bullae were performed
 - [Note: no TK measured]

- ABR details
- Left ears only of groups 1, 3, and 7
 - Pre-screen and D4 (prior to necropsy)
 - Frequencies measured: 2, 4, 8 and 12 kHz
 - Sound pressure levels (dB): 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25 and 20
 - Procedure (report pages 59-63):
 - In a sound-proof room, animals were anesthetized and kept warm with a heating pad
 - Three sterile 25 5/8 gauge needles inserted subcutaneously: under each pinna and at the vertex of the head
 - A microphone was positioned at the entrance of the left ear canal (i.e. embedded in an ear plug)
 - The subject animal hears one frequency at a time, at successive intensities
 - The raw data are waveforms, from which the authors calculate the intra-aural threshold, and then the threshold shift (terminal threshold minus prescreen threshold)
 - The author reports that humans have a normal variability of 0-25 dB threshold shifts (page 20), and identified shifts in the threshold of more than 20 dB as being indicative of “mild to moderate hearing loss”
 - For the ABR methodology used in these experiments, this reviewer concurs that threshold increases ≥ 20 dB are potentially biologically significant

Observations and Results

- No treatment-related clinical observations were apparent. A few animals exhibited head shake and eye squinting post-surgery, which this reviewer attributes to the surgical procedure.
- No treatment-related effects on body weight were apparent. All groups exhibited weight-loss from D-1 to D4, attributed to the withholding of food the morning of surgery.
- No brain lesions were noted for any animal.
- No finafloxacin-related changes to the bullae were apparent. Compared to the sham-treated animals, the other animals had fluid-filled bullae (as expected) and exhibited higher incidences of red blood cells and white blood cells in the bullae (attributable to the surgical procedure).
- ABR:
 - The sham-controls exhibited shifts in the auditory threshold from -6.7% (i.e. background improved hearing) to + 10% (i.e. background hearing loss)
 - The vehicle control group exhibited loss of hearing at 8 and 12 kHz (15 to 25 dB), but no significant loss at 2 kHz and 4 kHz.
 - Comparing the pre-dose to D4 results for the 1.0% finafloxacin group:
 - No significant hearing loss was observed at 2 kHz (mean threshold shift of 6.7 dB), 4 kHz (mean threshold shift of 11.7 dB)
 - At 8 kHz, one (of 3) animals exhibited a threshold shift of 25 dB, which is considered biologically significant.
 - At 12 kHz, all three animals exhibited biologically significant shifts in the threshold (mean of 36.7 dB, range 30 to 45 dB)
 - Comparing the mean vehicle threshold at D4 for each frequency versus the finafloxacin thresholds at D4 (mean and individual) for each frequency, no additional hearing loss is attributable to finafloxacin:
 - At 2 kHz: mean change of +3 dB (range 2 to 8)
 - At 4 kHz: mean change of -2 dB (range 0 to -5)
 - At 9 kHz: mean change of 0 dB (range -8 to + 7)
 - At 12 kHz: mean change of + 7 dB (range 0 to 10)
 - The 1.1% dose of finafloxacin is the equivalent of 5.5 mg/animal, and 9.2 mg/kg of body weight.

Table 24: Auditory brainstem response (ABR) thresholds following a single instillation into the left middle ear of male chinchillas (report # 0011059)

Sham Surgery

Animal	2 kHz			4 kHz			8 kHz			12 kHz		
	Threshold (dB)		Shift									
	Pre	Day 4		Pre	Day 4		Pre	Day 4		Pre	Day 4	
1001	50	40	-10	35	40	5	20	30	10	25	35	10
1002	50	45	-5	40	35	-5	30	25	-5	35	45	10
1003	45	40	-5	35	30	-5	25	15	-10	15	25	10
Mean	48.3	41.7	-6.7	36.7	35.0	-1.7	25.0	23.3	-1.7	25.0	35.0	10.0
SD	2.89	2.89	2.89	2.89	5.00	5.77	5.00	7.64	10.41	10.00	10.00	0.00

0.50 mL Finafloxacin Phosphate Vehicle pH 7.5

Animal	2 kHz			4 kHz			8 kHz			12 kHz		
	Threshold (dB)		Shift									
	Pre	Day 4		Pre	Day 4		Pre	Day 4		Pre	Day 4	
3001	55	50	-5	45	55	10	25	30	5	25	55	30
3002	55	50	-5	35	40	5	20	40	20	25	45	20
3003	50	55	5	40	55	15	25	45	20	25	50	25
Mean	53.3	51.7	-1.7	40.0	50.0	10.0	23.3	38.3	15.0	25.0	50.0	25.0
SD	2.89	2.89	5.77	5.00	8.66	5.00	2.89	7.64	8.66	0.00	5.00	5.00

0.50 mL 1.0% Finafloxacin in Phosphate pH 7.5

Animal	2 kHz			4 kHz			8 kHz			12 kHz		
	Threshold (dB)		Shift									
	Pre	Day 4		Pre	Day 4		Pre	Day 4		Pre	Day 4	
7001	45	55	10	30	45	15	15	30	15	20	50	30
7002	45	50	5	40	50	10	20	45	25	15	60	45
7003	55	60	5	40	50	10	25	40	15	25	60	35
Mean	48.3	55.0	6.7	36.7	48.3	11.7	20.0	38.3	18.3	20.0	56.7	36.7
SD	5.77	5.00	2.89	5.77	2.89	2.89	5.00	7.64	5.77	5.00	5.77	7.64

Study title: AL-60371 Otic (Finafloxacin): A One-Month Twice Daily Transbullar Middle Ear Dose Ototoxicity Study in Guinea Pigs

Study no.: TDOC-0013396

Study report location: NDA module 4.2.3.2 Repeat-Dose Toxicity

Conducting laboratory and location: Alcon Research, Ltd., 6201 South Freeway, Fort Worth, Texas 76134

Date of study initiation: January 2011

GLP compliance & QA statement: Yes, signed

Drug, lot #, and % purity: Finafloxacin (hydrochloride salt), purity not reported

- 0.6% suspension: lot # 10-61630-1
- 1.2% suspension: lot # 10-61652-1

Key Study Findings

- Twice-daily transbullar administration of finafloxacin to male guinea pigs resulted in no apparent hearing loss

- No NOAEL for local toxicity was observed: the low-dose (0.6 mg/day) was associated with minimal hyperplasia of the tympanic membrane, minimal hemorrhage, and increased incidence of pericanular bone fibrosis. The high-dose (1.2 mg/day) was also associated with mild thickening of the tympanic membrane, more severe (mild) tympanic membrane hyperplasia, and more severe (moderate) pericanular bone fibrosis, and increased incidence of pericanular bone proliferation.
 - Recovery was not assessed.
- As expected, the positive control (gentamycin) caused hearing loss and local toxicity (to the left bulla and left cochlea)
- Like the chinchilla hearing-loss study (report # TDOC-0011059), this guinea pig study also had only a limited panel of endpoints (hearing loss, body weight, clinical signs, brain and bulla gross pathology)
- Design and reporting limitations (detailed below) regarding test article homogeneity and delivery reduce the usefulness of this study to support safety

Methods

- Doses: Nominally 0, 0.6% (0.6 mg/animal/day ~0.7 mg/kg/day), 1.2% (1.2 mg/animal/day, ~ 1.5 mg/kg/day), or positive control (4 mg/day of gentamycin)
- Species and sex: 4/group male Hartley-outbred guinea pigs (*Cavia porcellus*)
- 4 months old at study initiation
 - Weight range 527 to 969 grams at study initiation
 - Single-housed
- Surgical procedure to allow for transbullar (middle ear) dosing: A subcutaneous access port was placed in the left ear of each animal, to allow test article administration into the middle ear. The description of the procedure is not completely clear. From the report (page 58):
- A “vertex screw” was implanted “approximately 1 cm caudal to the bregma on the midline of the cranium to serve as an anchor
 - The dosing port of the cannula was implanted subcutaneously in the thoracic vertebral region of the back, and the cannula extended to loop around the vertex screw
 - An incision behind the left ear exposed the tympanic bulla; a small hole was “delicately chipped in the bulla slightly lower than midpoint”, and the cannula was “carefully fed into the middle ear and fixed in place”
 - The cannula void volume was approximately 75 μ l
 - A pressure equalization shunt was placed to vent the middle ear into the “subcutaneous compartment proximal to the implanted ear”, and then the cannula and shunt were “cemented or glued in place and all incisions” closed
 - The right ear did not receive surgery, and was used as an untreated control for the histopathology evaluation.

Frequency of dosing and dose volume:

- The left ear was dosed twice daily with 0, 0.6%, or 1.2% suspension (50 µl/dose) for 29 days, followed with approximately 50 µl/dose volume of air “to assure that the dose was not retained within the catheter”
- Animals were sacrificed on D30
- The D14 morning dose was skipped (so as to not interfere with ABR)

Formulation/
Vehicle:

Component	Vehicle	0.6% suspension	1.2% suspension	Clinical formulation (listed for context)
Fluorfenacin hydrochloride salt	0	0.66% (equivalent to 0.6% free base)	1.32% (equivalent to 1.2% free base)	0.3%
Tyloxapol	(b) (4)			
Hydroxyethyl cellulose				
Sodium chloride				
Magnesium chloride				
BAC				
pH				

- Note: the formulations tested in this study are not identical to the topical otic clinical formulation. The increased amount of tyloxapol (b) (4) and BAC may change the local effects. (b) (4)

Endpoints:

- Commercial gentamycin (40 mg/ml) was used as the positive control
- Checks for mortality and morbidity
- Clinical observations
- Body weight (pre-dose, and then 10 measurements between D1 and D30)
- Left ear auditory brainstem response (ABR) pre-dose, D14 and D30
- The brain, bullae (left and right ears), and skin around the access port were collected for gross pathology and histology
- [Note: no TK measured]

Details for the ABR endpoint

- ABR was measured for four frequencies: 4, 8, 12 and 16 kHz.
- For each frequency, animals experienced a series of decreasing intensities of sound stimuli at 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, and 20 db.
- The lack of details regarding the methodology limits the

usefulness of the ABR data (e.g. electrode placement, use/absence of anesthesia).

- The report indicates that:
 - calibrated “Tucker-Davis Technologies equipment was used to perform the ABRs” (page 15, 52, 57),
 - hair shaving was performed as needed (page 53),
 - the worksheet template (report page 60) lacks details
 - Two standard operating procedures, PROC_3632 and PROC-3634 were referenced (page 53) but not provided
- Based on the lack of methodological details, it is not feasible to understand the sensitivity of the ABR methods used in this study. In the absence of data, this reviewer considered changes in the auditory threshold of ≥ 20 dB to be potentially biologically significant.

Observations and Results

- Note: because the catheter volume was reported as 75 μ l (report page 58), the approximately 50 μ l of air to push the test article through the catheter (report pages 13, 14, 50, 51) would have been inadequate.
 - With twice daily dosing (50 μ l fluid followed by 50 μ l air for each dose), some of the administered dose might have remained in the catheter, until the subsequent dose. If some of the drug volume drained from the catheter into the middle ear slowly, rather than as a bolus, then the PK might have been inconsistent.
 - Theoretically, some of a dose may have exited the middle ear through the pressure equalization shunt.
 - If some doses reached the middle ear as a bolus while other were partial bolus, with some possibly trickling in over time, then the T_{max} , C_{max} and AUC parameters may not be consistent.

Dosing Solution Analysis

- The dosing solutions were analyzed pre-study and post-study for pH, BAC content, and finafloxacin content (data on report page 68).
 - The amount of finafloxacin detected was within 111% of nominal.
- No certificate of analysis, purity information, or homogeneity testing results was provided in the study report.
- “The vehicle and finafloxacin suspension were briefly vortexed prior to dose administration” (report page 13), suggesting that the suspensions were evidently not homogenous. It is not clear if, or to what extent, the introduction of air bubbles into these test articles contributed to the slight differences in local irritation noted between saline and vehicle (below)

Results

- No treatment-related mortalities, brain or skin lesions were observed.
- Finafloxacin was not associated with changes to clinical signs, body weight, or bulla gross pathology.
- Comparing to the right bullae, the left bullae had evidence of surgery and dosing: thickened bulla, thickened mucosa, mild-to-moderate bone proliferation, and neutrophil inflammation.
- The positive control, gentamycin, was toxic:
 - Clinical signs (decreased food consumption, decreased feces production)
 - Weight loss
 - Clear hearing loss at all four frequencies (2/4 guinea pigs lost hearing completely)
 - Gross pathology (gray discoloration of the bulla)
 - Histology in the external ear canal (hemorrhage, epidermis hyperplasia) and cochlea (hair cell loss, spiral ganglion necrosis, acellular limbus spiralis)
- Histology of the left (treated) bulla associated finafloxacin with hyperplasia and thickening of the tympanic membrane, minimal hemorrhage, increased incidence of pericanular fibrosis
 - Note: the author did not consider any of these changes to be treatment-related. Bone proliferation was noted for all groups (evidence of healing), and was listed separately from pericanular bone proliferation.
 - Note: this reviewer considers the increased incidence of foreign bodies to be potentially related to deposition of the test article from suspension (i.e. not clear toxicity)

Table 25: Selected left (treated) bulla histology for the 30-day middle-ear dosing guinea pig study (report # 0013396)

Lesion	Severity	Vehicle	0.6%	1.2%	Gentamycin (positive control)
Left bulla					
Hemorrhage	Minimal	0/4	3/4	2/4	1/4
Fibrosis, pericanular	Mild	1/4	4/4	0/4	1/4
	Moderate	1/4	0/4	3/4	3/4
Hyperplasia, tympanic membrane, external epithelium	Minimal	0/4	1/4	0/4	0/4
	Mild	0/4	0/4	2/4	3/4
Thickened tympanic membrane	Minimal	0/4	0/4	0/4	1/4
	Mild	0/4	0/4	2/4	2/4
Foreign body	Present	0/4	3/4	3/4	3/4
Fibrosis, bone, pericanular	Mild	1/4	4/4	0/4	1/4
	Moderate	1/4	0/4	3/4	3/4
Proliferation, bone, pericanular	Mild	1/4	2/4	0/4	0/4
	Moderate	1/4	0/4	3/4	3/4

Bolding added to emphasize finafloxacin-related effects.

- ABR results:
 - The vehicle groups had biologically significant shifts in the ABR threshold from pre-dose to D14 and D30 (14-38dB)
 - Comparing the finafloxacin groups to vehicle on D14 and D30, none of the finafloxacin-treated animals exhibited ABR threshold shifts more than 20 dB:
 - Two-low dose animals exhibited a shift beyond 20 dB at 4 kHz
 - One high-dose animal exhibited a shift beyond 20 dB at 16 kHz
 - The range of threshold shift in finafloxacin-treated animals (8-31dB) did not exceed that of the vehicle controls, indicating that the effects on hearing loss are likely vehicle-mediated.
 - Given that the formulation used in this nonclinical study is not the clinical formulation, relevance to patient safety is unclear.

Table 26: Left (treated) ear ABR results for D14 and D30 of the 30-day middle-ear dosing guinea pig study (report # 0013396)

Day	Frequency	Mean ABR threshold shift from pre-dose			
		Vehicle	0.6% finafloxacin	1.2% finafloxacin	Gentamycin (positive control)
D14	4 kHz	21 ± 3	10 ± 9	20 ± 15	36 ± 14
	8 kHz	23 ± 15	13 ± 25	28 ± 18	48 ± 23
	12 kHz	14 ± 31	8 ± 18	21 ± 13	44 ± 28
	16 kHz	33 ± 32	20 ± 18	23 ± 13	73 ± 28
D30	4 kHz	25 ± 29	29 ± 25	10 ± 17	39 ± 13
	8 kHz	30 ± 19	31 ± 12	16 ± 8	54 ± 26
	12 kHz	16 ± 23	13 ± 10	11 ± 3	50 ± 27
	16 kHz	38 ± 25	15 ± 4	20 ± 16	73 ± 28

Values are presented as the mean (± standard deviation) shift from prescreen (i.e. prior to the surgical procedure for middle ear dosing)

Table 27: Left (treated) ABR results for treated groups compared to vehicle for the 30-day middle-ear dosing guinea pig study (report # 0013396)

Day	Frequency	Mean ABR threshold shift (treatment minus vehicle ABR threshold)		
		0.6% finafloxacin	1.2% finafloxacin	Gentamycin (positive control)
D14	4 kHz	-7	1	18
	8 kHz	-9	5	29
	12 kHz	-10	3	25
	16 kHz	1	1	34
D30	4 kHz	8	-13	16
	8 kHz	3	-14	28

	12 KHz	-8	-10	29
	16 KHz	11	9	29

Positive values ≥ 20 dB are potentially concerning for hearing loss. Negative values are not concerning (i.e. indicate increased sensitivity to sound)

Study title: 28-Day Definitive Ototoxicity Evaluation in Guinea Pigs with AL-60371

Study no.:	TDOC-0016033
Study report location:	NDA module 4.2.3.2 Repeat-Dose Toxicity
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 24, 2011
GLP compliance & QA statement:	Yes, signed <ul style="list-style-type: none"> The ABR evaluation was not done under GLP
Drug, lot #, and % purity:	Finaxofacin (free based; AL-60371), purity not reported. <ul style="list-style-type: none"> 0.3% suspension: lot # 10-61629-1 0.6% suspension: lot # 10-61630-1 1.2% suspension: lot # 10-61652-1

Key Study Findings

- Male and female guinea pigs were dosed twice daily via instillation into the middle ear with saline control, vehicle control, three dose levels of finaxofacin, or a positive control (neomycin) for 28 days.
- As expected, neomycin caused hearing loss (at all three frequencies measured, at both time points measured) and microscopic damage to the bulla and cochlea
- Hearing loss:
 - The saline control did not cause hearing loss.
 - The author concluded, and this reviewer concurs, that the exposure to vehicle was associated with slight hearing loss (at all three frequencies measured, at both time points) as compared to the saline control group.
 - Using a change in the ABR threshold of ≥ 20 dB as a benchmark for biologically-meaningful hearing loss, finaxofacin was not shown to exacerbate the vehicle-induced hearing loss, under the conditions tested in this study.
 - For finaxofacin-treated males, biologically meaningful increases were observed on D15 for the mid-dose group (+25 dB) and the high-dose group (+21 dB) versus pre-dose. No biologically-meaningful increases were observed at D29, indicating acclimation

to repeat-dosing occurred. This reviewer attributes this hearing loss to vehicle, rather than finafloxacin.

- Because the formulation tested in this study is slightly different from the clinical formulation, and because the route of administration is different (topical otic for patients, middle-ear instillation for these guinea pigs), the relevance of the vehicle-mediated hearing loss to patient safety is unclear.
- No treatment related cochlear histology changes were reported in the finafloxacin- or vehicle-treated groups.
- Necropsy of the middle ear:
 - Compared to saline, vehicle caused middle ear ossicle fibrosis, epithelial hyperplasia, and thickened mucosa.
 - A no observed effect level (NOEL) was not identified. Finafloxacin was associated grossly with bulla thickening, which correlated microscopically with increased severity of periosteal bone proliferation.
 - In males, finafloxacin was also associated with increased incidence of thickened tympanic membrane stroma.
 - Based on the model (i.e. insertion of a catheter into the middle ear), it is not clear to this reviewer whether these effects are toxic, or represent enhancement of healing. Therefore, this reviewer did not identify a NOAEL or LOAEL for finafloxacin histopathology in this study.

Methods

- | | |
|--------------------------|--|
| Doses: | <ul style="list-style-type: none"> ● Saline negative control (0.9% sodium chloride) ● Vehicle negative control ● 0.3, 0.6 and 1.2% finafloxacin (free base) ● 10% neomycin (positive control for ototoxicity) |
| Frequency of dosing: | Twice daily, 8 hours \pm 30 minutes apart, for 28 days |
| Dose volume: | 50 μ l |
| Route of administration: | <ul style="list-style-type: none"> ● Intratympanic route via transbullar catheter ● Left ear only ● Bolus infusion ● Note: this route of administration is essentially similar to the route used for the chinchilla studies and the previous guinea pig study. This report describes the route as "intratympanic", but administration was not through the tympanic membrane. ● Note: the report does not mention flushing of the catheter, or placing a pressure equalization shunt (as was done for the previous guinea pig study, report # TDOC-001339) |
| Description of surgery: | <ul style="list-style-type: none"> ● Animals were fasted the morning prior to surgery, and received prophylactic antibiotics |

- Under anesthesia, the skin was shaved, then a midline skin incision was made from the medial canthus caudally, then continuing to the base of the left ear to expose the lateral cranium
- Four screws were implanted to anchor the catheter and for the ABR analysis
- The surface of the tympanic bulla was exposed, and “a small hole... slightly lower than the midpoint of the bulla” by “carefully” chipping the bone to expose the middle ear
- A 22 gauge angiocatheter was inserted in the middle ear, glued to the bone, and then carboxylate cement was used to further secure the catheter and cover the opening.
- After securing the catheter to the skull screws, the incision was sutured closed.

Formulation/Vehicle: Suspension with (b) (4) % hydroxyethyl cellulose, (b) (4) % benzalkonium chloride (b) (4) %, pH 5.5, and:

Component	Finafloxacin			
	0%	0.3%	0.6%	1.2%
Tyloxapol	(b) (4)			
Sodium chloride	(b) (4)			

Compared to the topic otic clinical formulation, these formulations had more tyloxapol and BAC, less magnesium chloride, and a lower pH. (b) (4)

(b) (4)

Species/Strain: Male and female Albino Hartley guinea pigs, 5 to 7 weeks old at start of dosing

Number/Sex/Group: 5/sex/dose

- Endpoints:
- Observations for morbidity, mortality and injury
 - Clinical signs prior to each dose (twice daily) and prior to termination (D30)
 - Daily body weights
 - Ooscopic evaluation and physical examination (pre-dose and D29)
 - ABR evaluation: pre-dose, D15, D29 (4, 10 and 20 kHz)
 - Left ear gross pathology and histology of the cochlea, middle ear ossicles, and tympanic membrane were conducted for all animals
 - Right ear gross pathology and histology (of the

cochlea, middle ear ossicles, and tympanic membrane) was performed only for the saline control, the vehicle control group, and the high-dose group

- ABR details
- [No TK evaluation]
 - Animals were fasted the morning of ABR evaluation, and were anesthetized with xylazine and ketamine, and placed in enclosures for ABR
 - For the ABR test method used for this study, the author considered hearing > 15 dB as “suggestive” of a detectable difference, and > 20 dB as potentially biologically significant (page 269); this reviewer concurs.

Dosing Solution Analysis

- The finafloxacin, vehicle, and positive control were used as received from the Sponsor.
- Analysis of dosing formulations was conducted (report page 167), pre- and post-study concentrations were 106% to 110% of nominal, and no degradation was observed
- Lot reports were provided (report pages 158-160), describing the formulation, but no purity data were provided. Homogeneity of the test articles was not assessed.

Results

- No premature deaths occurred.
- No treatment-related clinical signs were apparent, for finafloxacin or neomycin.
- Decreased weight gain was observed for the neomycin-treated animals

Auditory Brainstem Response (ABR) results

- For two animals, a procedural error (faulty connection to ground) affected the D15 data; these two animals were retested on D17. This did not affect the interpretation of the overall study results.
- The positive control, neomycin, caused mild-to-moderate (35-53 dB threshold elevation) hearing loss in both males and females at both time points, and at all three frequencies measured (4, 10 and 20 kHz), compared to pre-test. Moreover, biologically-significant hearing loss was clearly apparent for neomycin at D15 and D29 comparing neomycin to saline (33 to 47 dB hearing loss) and vehicle (24 to 38 dB hearing loss)
- No hearing loss was observed for the 0.9% saline group (comparing pre-test to D15 and D29)
- Vehicle effect:

- For females, the vehicle formulation was associated with mild hearing loss in females at both D15 (19 to 30 Db) and D29 (22 to 38 dB) compared to pretest, at all three frequencies.
- For males, vehicle was not associated with meaningful hearing loss (9 to 15 dB hearing loss at D15 and D29 compared to pre-dose)
- There are three approaches to evaluating the finafloxacin groups: (1) comparing ABR threshold after treatment (D15 or D29) to ABR threshold at pre-dose, (2) comparing treated group ABR threshold to vehicle threshold at each time point, and (3) comparing the shift from baseline for vehicle versus treated at D15 and D29
 - Comparing post-dose to pre-dose for the finafloxacin-treated groups:
 - For males, biologically meaningful increases were observed on D15 for the mid-dose group (+25 dB) and the high-dose group (+21 dB). No biologically-meaningful increases were observed at D29, indicating acclimation.
 - For females, no biologically meaningful effect was observed (range -32 to 19 dB on D15; range -33 to 15 dB on D29)
 - Comparing the finafloxacin groups to vehicle, no biologically-meaningful effect on the ABR threshold was apparent:
 - For finafloxacin-treated females, the change in ABR threshold (7 to 18 dB on D15, 14 to 23 dB on D29) was less than the ABR threshold change for vehicle-treated females.
 - For males at D15, the mean differences between treated and vehicle ranged from 0 to 10 dB
 - For males at D29, the mean differences between treated and vehicle ranged from -6 dB (improved hearing) to 6 dB hearing loss
 - Comparing the shift from baseline (pre-dose value) for the vehicle group versus the treated groups:
 - For males, no biologically meaningful hearing loss was apparent: range was -8 dB (improved hearing) to 11 dB hearing loss on D15, and -12 to 9 dB hearing loss on D29
 - For females, no biologically meaningful hearing loss was apparent: range was negative (improved hearing) for all frequencies: -11 to -19 dB for D15 and -15 to -26 dB for D29
- The clinical formulation (shown in Table 2 of this review, above) is different (this study's formulation had the same (b) (4) % hydroxyethyl cellulose, more tyloxapol and benzalkonium chloride, no magnesium chloride, and approximately the same amount of sodium chloride). (b) (4) to reduce precipitation of the suspension.
 - Based on the difference in formulation, the relevance of the vehicle-related hearing loss to patient safety is unclear.

Table 28: Male auditory brainstem response thresholds following intratympanic dosing in the guinea pig (report # 0016033)

Summary of Auditory Brainstem Response Values, Left Ear, dB - MALE																			
Frequency	Study Interval	0.9% Saline			100 mg/mL Neomycin			0 mg/mL AL-6031			3 mg/mL AL-6031			6 mg/mL AL-6031			12 mg/mL AL-6031		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	36.0	10.84	5	35.6	5.55	5	35.2	4.76	5	33.2	6.06	5	32.8	3.27	5	39.4	11.48	5
	Day 15	33.6	8.44	5	72.0	5.70	5	45.8	7.68	4	45.4	9.81	5	45.8	17.78	5	48.2	9.68	5
	Day 29	31.4	5.27	5	69.4	3.44	5	44.6	8.59	5	40.4	7.92	5	42.0	9.41	5	42.6	11.63	5
10 kHz	Pretest	24.0	4.64	5	23.8	5.26	5	24.4	3.21	5	23.2	1.92	5	22.6	1.14	5	22.4	2.70	5
	Day 15	26.8	6.42	5	60.2	10.57	5	33.5	6.45	4	33.2	10.13	5	39.4	11.10	5	36.2	15.14	5
	Day 29	21.8	3.90	5	61.2	3.70	5	37.2	8.98	5	32.6	5.98	5	32.8	4.15	5	31.0	14.95	5
20 kHz	Pretest	13.0	6.60	5	15.4	6.19	5	13.2	2.59	5	9.0	2.55	5	12.0	3.67	5	12.6	3.78	5
	Day 15	16.4	7.30	5	59.8	8.58	5	27.5	6.19	4	27.6	11.59	5	37.2	17.37	5	33.8	20.54	5
	Day 29	14.2	2.17	5	60.8	8.81	5	22.4	6.11	5	27.6	10.01	5	28.6	7.54	5	28.0	18.15	5

Table 29: Female auditory brainstem response thresholds following intratympanic dosing in the guinea pig (report # 0016033)

Summary of Auditory Brainstem Response Values, Left Ear, dB - FEMALE																			
Frequency	Study Interval	0.9% Saline			100 mg/mL Neomycin			0 mg/mL AL-6031			3 mg/mL AL-6031			6 mg/mL AL-6031			12 mg/mL AL-6031		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	32.4	2.30	5	33.8	2.17	5	33.2	2.77	5	34.2	4.87	5	37.0	5.39	5	33.6	2.19	5
	Day 15	37.2	5.89	5	70.8	6.98	5	52.2	14.43	5	33.4	4.51	5	40.2	8.56	5	39.6	11.15	5
	Day 29	29.2	2.17	5	71.2	6.30	5	56.0	20.58	5	32.6	8.29	5	39.2	5.22	5	37.4	6.91	5
10 kHz	Pretest	20.8	3.11	5	22.0	3.32	5	20.8	1.79	5	21.2	2.77	5	21.6	1.67	5	20.6	3.21	5
	Day 15	30.6	8.20	5	67.4	4.77	5	42.6	13.72	5	25.8	4.32	5	31.8	9.55	5	28.2	7.26	5
	Day 29	22.6	5.90	5	69.4	4.39	5	47.2	13.07	5	28.0	9.59	5	33.2	3.56	5	27.2	2.17	5
20 kHz	Pretest	11.0	3.81	5	9.2	2.77	5	9.0	2.00	5	9.8	2.17	5	12.8	4.09	5	12.2	4.32	5
	Day 15	21.2	8.90	5	60.2	3.90	5	39.4	13.11	5	22.2	8.35	5	32.0	17.10	5	28.2	11.43	5
	Day 29	14.2	11.14	5	62.0	8.31	5	47.4	13.78	5	24.8	12.03	5	26.2	6.57	5	24.2	7.19	5

Gross pathology of the middle ear

- Gross evaluation of the middle ear noted a finafloxacin dose-response for thickened bulla, described as mild-to-moderate proliferation of the periosteal bone and minimal-to-moderate thickened mucosa. All right bullae were grossly normal (as expected, since the right bullae were untreated).

Table 30: Selected middle-ear gross pathology for the 28-day guinea pig study (report # TDOC-0016033)

Left bulla thickened	Saline	0	3 mg/ml	6 mg/ml	12 mg/ml	Neomycin (positive control)
Males	0/5	2/5	2/5	5/5	5/5	4/5
Females	1/5	1/5	3/5	2/5	4/5	3/5

Histopathology of the middle ear

- Compared to saline, vehicle caused middle ear ossicle fibrosis (minimal-to-moderate), epithelial hyperplasia (minimal-to-mild), and thickened mucosa (minimal-to-moderate)
- Finafloxacin and neomycin were associated with increased severity of periosteal bone proliferation. The author (page 289) notes that this effect was prominent, and correlated with the gross observation of thickened bulla.
- In males (but not females), vehicle/finafloxacin (but not neomycin) was associated with thickened tympanic membrane stroma.
- In males and females, finafloxacin was associated with tympanic membrane foreign bodies.
 - For one female (control # 1006), the individual animal sheet explains that the foreign material is glue fragments (i.e. from the surgical procedure). No explanation was identified for the other animals. This reviewer speculates that the foreign bodies may be test article precipitate.
- No cochlear damage was apparent for finafloxacin or vehicle.
- Neomycin caused clear lesions in the middle ear (luminal neutrophilic inflammation, squamous metaplasia of the mucosal epithelium, hyperplasia of the squamous epithelium of the external ear canal, mild-to-marked necrosis of the tympanic membrane, ulcer of the tympanic membrane) and cochlea (marked hair cell loss, hypocellularity of the spiral limbus and spiral ganglion, neuronal necrosis of the spiral ganglion, axonal degeneration of the cochlear nerve, cell debris)

Table 31: Selected male left bulla histopathology for the 28-day guinea pig study (report # 0016033)

Dose →	Severity	Saline	0	3 mg/ml	6 mg/ml	12 mg/ml	Neomycin (positive control)
Thickened tympanic membrane stroma	1	0/5	2/5	1/5	2/5	1/5	1/5
	2		0/5	0/5	2/5	2/5	0/5
Foreign body, tympanic membrane	1	0/5	0/5	3/5	4/5	3/5	0/5
	2			1/5	0/5	0/5	
Tympanic membrane, internal	2	0/5	0/5	0/5	1/5	0/5	0/5

surface ulcer							
Fibrosis, middle ear ossicles	1	0/5	0/5	2/5	0/5	0/5	0/5
	2		1/5	1/5		1/5	
	3		0/5	0/5		0/5	3/5
Epithelial hyperplasia	1	0/5	0/5	1/5	1/5	0/5	0/5
	2		5/5	4/5	4/5	4/5	4/5
Proliferation periosteal bone	1	3/5	0/5	0/5	0/5	0/5	1/5
	2	1/5	4/5	3/5	3/5	0/5	0/5
	3	0/5	1/5	2/5	2/5	5/5	4/5
Thickened mucosa	1	1/5	1/5	0/5	0/5	4/5	1/5
	2	0/5	4/5	4/5	4/5	0/5	4/5
	3		0/5	1/5	1/5	1/5	3/5

Grade 1 = minimal, grade 2 = mild, grade 3 = moderate

Table 32: Selected female left bulla histopathology for the 28-day guinea pig study (report # 0016033)

Dose →	Severity	Saline	0	3 mg/ml	6 mg/ml	12 mg/ml	Neomycin (positive control)
Thickened tympanic membrane stroma	1	0/5	2/5	0/5	0/5	0/5	0/5
	2		0/5		2/5	0/5	1/5
	3		1/5		0/5	1/5	0/5
Foreign body, tympanic membrane	1	1/5	2/5	3/5	2/5	3/5	0/5
	2	0/5	0/5	0/5	3/5	0/5	
Tympanic membrane internal surface ulcer	1	0/5	1/5	0/5	0/5	0/5	0/5
Tympanic membrane, epithelial hyperplasia	2	0/5	0/5	1/5	0/5	1/5	0/5
Epithelial hyperplasia	1	1/5	3/5	1/5	4/5	1/5	0/5
	2	2/5	0/5	1/5	0/5	0/5	2/5
Proliferation periosteal bone	1	1/5	1/5	0/5	0/5	0/5	0/5
	2	0/5	3/5	3/5	0/5	1/5	1/5
	3	1/5	1/5	2/5	5/5	3/5	4/5
Thickened mucosa	1	3/5	0/5	3/5	0/5	1/5	0/5
	2	1/5	4/5	2/5	5/5	3/5	4/5
	3	0/5	1/5	0/5	0/5	0/5	1/5

Grade 1 = minimal, grade 2 = mild, grade 3 = moderate

- For two animals, a procedural error (faulty connection to ground) affected the D15 data; these two animals were retested on D17. This did not affect the interpretation of the overall study results.

6.3 Systemic-Route Toxicity

The systemic route general toxicity studies were not fully reviewed for this indication. One study (report #8245014) is relevant to labeling for impairment of fertility.

Title: Finafloxacin: 4 Week Intravenous (Infusion) Administration Toxicity Study in the Rat Followed by an 11 Week Treatment-free Period

Report #: 8245014

Summary:

- Wistar rats were dosed with 0, 30, 40, 50 or 60 mg/kg/day by intravenous infusion daily for 4 weeks. Group sizes: 10/sex/dose main-group; 5/sex/dose recovery (5 week recovery); 12/sex/dose for TK.
- Reportedly for main-group animals:
 - In males at ≥ 40 mg/kg/day, the authors reported change in sperm parameters (count, percent motile, velocity and straightness) when compared to concurrent controls
 - In males at 50 and 60 mg/kg: higher level of tubular atrophy in the testes; higher incidence of cellular debris in the epididymis
 - In males at 60 mg/kg, a higher incidence of prostate contraction was reported.
 - 50 and 60 mg/kg/day, compared with controls.
 - Lower spleen hematopoiesis in males at 60 mg/kg
- The authors report complete recovery after the 5 week washout period.

From report page 461:

Table 33: TK for the 30 mg/kg intravenous dose in rats (report # 8245014)

	Day 1		Day 7		Week 4	
	Male	Female	Male	Female	Male	Female
C _{max} (ng/mL)	34900	34600	38100	39900	41400	48600
t _{max} (h)	0.25	0.25	0.25	0.25	0.25	0.25
t _{1/2} (h)	NR	3.47	NR	NR	NR	4.71
Cl (mL/hr/kg)	527	624	597	606	591	641
V _z (mL/kg)	3400	3120	3100	8760	4190	4350
V _{ss} (mL/kg)	1100	915	951	1790	1040	925
AUC _{0-τ} (hr.ng/mL)	56600	48000	50100	47900	50500	46700
AUC _{0-tlast} (hr.ng/mL)	56600	48000	50100	47900	50500	46700
AUC _{0-∞} (hr.ng/mL)	NR	48100	NR	NR	NR	46800
C _{max} /D	1160	1150	1270	1330	1380	1620
AUC/D	1890	1600	1670	1600	1680	1560
RA _{AUC}	NA	NA	0.885	1.00	0.892	0.97
RA _{Cmax}	NA	NA	1.09	1.15	1.19	1.40

NR – No result calculable

NA – Not applicable

7 Genetic Toxicology

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

Study title: Reverse mutation assay with BYK60621 using bacteria (*Salmonella typhimurium* and *Escherichia coli*)

Study no.: 198/2000

Study report location: NDA module 4.2.3.3.1 Genotoxicity *in vitro*

Conducting laboratory and location:

(b) (4)

Date of study initiation: June 20, 2000

GLP compliance and QA statement: Yes, signed

Drug, lot #, and % purity: BYK60621, batch # 503606, purity 99.8%

Key Study Findings

- Finafloxacin was clearly positive in strain TA 102 ± S9 (a *Salmonella* strain sensitive to base pair substitution). Fluoroquinolones as a class tend to be positive in only strain TA 102.

- The activity of the S9 was not verified. The usefulness of the + S9 results are limited, because it is not clear if the S9 used in these experiments would have metabolically activated finafloxacin.

Methods

- Strains:
- 5 strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, TA100 and TA102)
 - 1 strain of *Escherichia coli* (WP2uvrA)

Concentrations in definitive studies:

Experiment	Strains	Concentrations (µg/plate)
Plate-incorporation	TA98	0.01, 0.032, 0.1, 0.32, 1.0 and 3.16
	All others	0.001, 0.0032, 0.01, 0.032, 0.1, 0.32 and 1.0
Preincubation	TA 1535	0.0003, 0.001, 0.0032, 0.01, 0.032, 0.1
	TA100	0.0002, 0.0005, 0.001, 0.0025, 0.005, 0.01
	All others	0.001, 0.0032, 0.01, 0.032, 0.1, 0.32 and 1.0

Basis of concentration selection: A preliminary cytotoxicity dose-ranging study in TA98 and TA100 observed loss of lawn at 1 µg/plate for TA98, and at 0.032 µg/plate for TA100

Negative control: vehicle

Positive controls:

compound	S9	strains
Sodium azide (NaN3)	-	TA 1535, TA100
4-nitro-o-phenylene-diamine (4-NOPD)	-	TA1537, TA98
Methyl methane sulfonate (MMS)	-	TA102, WP2 uvrA
2-aminoanthracene (2-AA)	+	All strains

Formulation/Vehicle: Dimethyl sulfoxide (DMSO)
 Incubation & sampling time: • For the pre-incubation test, test article was incubated with sterile buffer or S9 for 60

minutes at 37°C prior to adding it to the overlay agar

- For both test methods, plates were incubated for at least 48 hours prior to reading

Study Validity

- Both the plate incorporation (direct plating) and pre-incubation methods were tested.
- Dose selection was adequate.
 - It is not clear to this reviewer why the plate incorporation assay tested 3.16 µg/plate in TA98 (presumably to verify the results of the preliminary cytotoxicity test). Because 5 lower concentrations were also tested, inclusion of the 3.16 µg/plate dose for TA98 is not a study limitation.
 - Based on the preliminary cytotoxicity experiment, limiting the top dose for TA100 to 0.1 µg/plate is acceptable.
- The use of 2-AA as the only positive control + S9 is a study limitation.
 - The rat S9 liver microsomal fraction was prepared in-house (from male Wistar rats induced with phenobarbital and β-naphthoflavone) and stored frozen prior to use. The report makes no claim of testing to verify the potency of the S9.
 - Per OECD 471 Bacterial Mutation Test¹, "2-Aminoanthracene should not be used as the sole indicator of the efficacy of the S9-mix. If 2-aminoanthracene is used, each batch of S9 should also be characterized with a mutagen that requires metabolic activation by microsomal enzymes".

Results

- Finafloxacin was clearly positive ± S9 in strain TA102.
 - The plate incorporation assay detected a dose response with increasing activity from 0.032 to 0.1 µg/plate (and cytotoxicity apparent at 0.32 and 1.0 µg/plate) in the plate incorporation assay
 - The pre-incubation assay similarly detected a dose response, with increased activity at 0.1 and 0.032 µg/plate (and cytotoxicity apparent ≥ 0.1 µg/plate)
 - Maximal increase was 4.5-fold over control, and the increases were outside the laboratory's historical control range for TA102
- Finafloxacin was negative in each other strain tested at each concentration ± S9
- The positive and negative controls gave the expected responses

¹ OECD Guidelines for the Testing of Chemicals. Section 4 Health Effects. Test 471: bacterial reverse mutation test. July 1997. Accessed online via: http://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en

Table 34: Bacterial reverse mutation assay: strain TA102 results (report # 198/2000)

Dose group (µg/plate)	Plate-incorporation results		Preincubation results	
	-S9	+S9	-S9	+S9
Water	255 ± 22	301 ± 26	246 ± 10	294 ± 34
DMSO	241 ± 30	295 ± 35	220 ± 20	245 ± 31
0.001	264 ± 10	305 ± 32	252 ± 16	345 ± 11
0.0032	304 ± 5	362 ± 1	272 ± 15	360 ± 20
0.01	438 ± 36	565 ± 38	486 ± 18	818 ± 49
0.032	608 ± 144	768 ± 106	800 ± 18	1084 ± 36
0.1	1089 ± 97	659 ± 169	542 ± 31	355 ± 41
0.32	20 ± 9	28 ± 25	0 ± 0	0 ± 0
1.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Positive control	1515 ± 137	879 ± 129	1699 ± 98	704 ± 82

Values expressed as mean ± standard deviation

7.2 In Vitro Assays in Mammalian Cells

7.2.1

Study title: Cell mutation assay at the thymidine kinase locus in mouse lymphoma L5178Y cells with BYK60621

Study no.:	146/2001
Study report location:	NDA module 4.2.3.3.1 Genotoxicity <i>in vitro</i>
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	April 18, 2001
GLP compliance & QA statement:	Yes, signed
Drug, lot #, and % purity:	BYK60621, batch # 505822, purity 98.3%

Key Study Findings

- Clearly positive ± S9, with dose-responses apparent

Methods

- Cell line: Mouse lymphoma cell line L5178Y
- Concentrations in definitive study:
- S9: 125, 250, 750, 1000 µg/ml
 - +S9: 125, 250, 500, 1000, 2000, 2500 µg/ml
- Basis of concentration selection: Based on a preliminary cytotoxicity test, the authors identified the following as having "strong toxicity" (not explicitly defined; these dose levels had total suspension growth < 5%, and relative suspension growth < 50%):

- 4 hours –S9: 500 µg/ml
- 4 hours +S9: 1000 µg/ml
- 24 hours –S9: 250 µg/ml

Negative control: 0.5% DMSO in culture medium

- Positive control:
- -S9: MMS
 - +S9: 3-methylcholanthrene

Formulation/Vehicle: DMSO

Incubation & sampling time: Standard methodology (4 hours ±S9)

Study Validity

- The mouse lymphoma assay (MLA) is a well characterized assay to identify mutagens.
- The study design appears valid and adequate. The microwell method was used.
- This reviewer consulted the guidance OECD 476 in vitro Mammalian Cell Gene Mutation Test² and the FDA/CFSAN guidance³.
 - In accordance with the FDA/CFSAN guidance, the authors excluded concentrations which reduced relative total growth (RTG) below 10% of the control. This approach is acceptable, and does not change the overall interpretation of the results.

Results

- The authors conclude, “A substantial and dose dependent increase of the mutant frequency” ± S9 was observed, and this reviewer concurs.
- The authors consider an increase in mutant colonies ≥ 2x to be a positive response.

Table 35: Mouse lymphoma assay data (report # 146/2001)

Concentration (µg/ml)	First experiment				Second experiment			
	RCE	RTG	Mutant colonies	Induction factor	RCE	RTG	Mutant colonies	Induction factor
-S9								
0	100	100	86	1x	100	100	145	1x
13	77.5	43.9	534	6.2x	84.9	54.5	812	5.6x

² OECD Guidelines for the Testing of Chemicals, Section 4 Health Effects. Test No. 476: in vitro Mammalian Cell Gene Mutation Test. July 1997. Accessed online via: http://www.oecd-ilibrary.org/environment/test-no-476-in-vitro-mammalian-cell-gene-mutation-test_9789264071322-en

³ FDA/CFSAN. Redbook 2000: IV.C.1.c Mouse Lymphoma Thymidine Kinase Gene Mutation Assay. Updated April 2006. Accessed online via: <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm078336.htm>

125	100	66.3	138	1.6x	72.6	75.0	278	1.9x
250	85.3	56.2	106	1.2x	64.8	73.8	292	2.0x
500	75.1	39.1	225	2.6x	61.2	62.0	550	3.8x
750	63.7	30.1	307	3.6x	46.4	52.9	568	3.9x
1000	70.7	25.2	383	4.5x	32.4	351	562	3.9x
Positive control	77.5	43.9	534	6.2x	84.9	54.5	812	5.6x
+S9								
0 (medium)	100	100	59	1x	100	100	112	1x
0 (with DMSO)	100	100	72	1x	100	100	138	1x
125	91.7	57.6	158	2.7x	125.4	59.0	213	1.9x
250	74.7	45.9	157	2.6x	103.3	35.9	291	2.6x
500	58.3	34.5	206	3.5x	98.4	41.9	393	3.5x
1000	<i>40.8</i>	<i>1.7</i>	<i>3739</i>	<i>63x</i>	<i>50.4</i>	<i>10.5</i>	<i>1051</i>	<i>9.3x</i>
2000	<i>1.1</i>	<i>0</i>	<i>9721</i>	<i>164x</i>	<i>4.7</i>	<i>1.2</i>	<i>617</i>	<i>5.5x</i>
Positive control	77.0	21.6	630	8.8x	68.9	59.0	349	2.5x

- RCE: relative cloning efficiency
- RTG: relative total growth
- Bolding: positive results
- Italics: the two high doses (1000 and 2000 µg/ml) +S9 are considered not biologically relevant, despite the dramatic increase in mutant colonies, because the RTG was less than 10% of control RTG.
- No clear shift from large to small colonies was apparent with treatment.
 - The authors concluded that finafloxacin +S9 increased the ratio of small to large colonies; however, this reviewer disagrees.
 - An increase in the ratio of small to large colonies would suggest chromosomal aberration damage.

7.2.2

Study title: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre^R fluctuation technique

Study no.:	2808/17 (280817)
Study report location:	NDA module 4.2.3.3.1 Genotoxicity <i>in vitro</i>
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 9, 2007
GLP compliance & QA statement:	Yes, signed
Drug, lot #, and % purity:	Fluorouracil hydrochloride (BAY 35-3377), batch # CBC00319 (505821), purity 93.2% (b) (4) of the impurities is (b) (4) concentrations were adjusted appropriately)

Key Study Findings

- Clearly positive \pm S9, concentration-response apparent

Methods

Cell line:	L5178 tk ^{+/−} (3.7.2C) mouse lymphoma cells
Concentrations in definitive study:	<ul style="list-style-type: none"> Experiment 1 tested 0, 75, 100, 125, 150 and 200 μg/ml \pm S9 Experiment 2(-S9 only) tested: 0, 25, 80, 90, 100, 110, 120 μg/ml Experiment 3 (-S9 only) tested: 0, 20, 40, 60, 90, 100, 110, 120 μg/ml
Basis of concentration selection:	24 hours –S9 cytotoxicity range finder
Negative control:	Media
Positive control:	<ul style="list-style-type: none"> -S9: 4-nitroquinoline 1-oxide (NQO) +S9: benzo[a]pyrene (BaP)
Formulation/Vehicle:	Cell culture media
Incubation & sampling time:	4 hours \pm S9, 24 hours –S9

Study Validity

- The design and reporting of the study appear adequate to support the conclusion that fluorouracil was positive under the conditions tested.
- The cytotoxicity range finder did not evaluate a 4 hour time point, and Experiment 1 (4 hour exposure \pm S9) did not test sufficiently high concentrations. Per OECD 476, concentrations should be tested that result in 10 to 20% RTG. However,

because the highest dose tested at 4 hours, 200 µg/ml, was positive ± S9, higher doses are not needed to confirm the positive result.

Results

- Finafloxacin was positive after 4 hours of exposure, ± S9, and after 24 hours exposure –S9.
- From the study report (page 25):

Table 36: Mutagenicity results from the second MLA assay (report # 2808/17)

Experiment 1 (4 hour treatment in the absence and presence of S-9)

Treatment (µg/mL)	-S-9		Treatment (µg/mL)	+S-9	
	% RTG	MF§		% RTG	MF§
0	100!	69.28!	0	100	73.35
75	111	95.07	75	120	88.83
100	99	69.51	100	104	92.08
125	63	175.08	125	87	103.62
150	65	165.66	150	84	165.37
200	41	300.08 #	200	78	225.89 #
Linear trend		***	Linear trend		***
NQO			BP		
0.15	46	739.17	2	84	494.03
0.20	30	989.69	3	42	858.80

Experiments 2 and 3 (24 hour treatments in the absence of S-9)

Treatment (µg/mL)	Experiment 2 -S-9			Treatment (µg/mL)	Experiment 3 -S-9		
	% RTG	MF§			% RTG	MF§	
0	100	63.81		0	100	71.70	
25	91	62.06		20	101	70.95	
80	30	203.26 #		40	106	93.92	
90	22	364.34 #		60	71	165.65	
100	16	391.33 #		80	57	207.55 #	
110	11	473.54 #		90	19	354.06 #	
120	10	442.95 #		100	16	447.43 #	
				110	14	467.74 #	
				120	12	487.19 #	
Linear trend		***		Linear trend		***	
NQO				NQO			
0.05	32	655.71		0.05	14	969.80	
0.10	8	1518.83 ♦		0.10	5	1798.86 ♦	

§ 5-TFT resistant mutants/10⁶ viable cells 2 days after treatment
 ! Based on one replicate only
 # The MF of the test concentration exceeds the sum of the mean control MF plus GEF
 *, **, *** Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively
 ♦ RTG was <10% (but acceptable response observed for NQO at 0.05 µg/mL)

- No clear shift in colony size with finafloxacin treatment was apparent.
- The author concluded that finafloxacin hydrochloride induced mutations ± S9, at ≥ 125 µg/ml at 4 hours ± S9 and ≥ 60 µg/ml at 24 hours –S9.

7.2.3

Study title: Bay 35-3377. V79/HPRT-test *in vitro* for the detection of induced forward mutations

Study no.:	PH 30802
Study report location:	NDA module 4.2.3.3.1 Genotoxicity <i>in vitro</i>
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 4, 2000
GLP compliance & QA statement:	Yes
Drug, lot #, and % purity:	Fluorouracil hydrochloride (BAY 35-3377; BYK 60621), batch # 503605, content 91.6%

Key Study Findings

- This study detected evidence of mutagenic potential for fluorouracil
 - The authors and Applicant consider this study to be negative
- Dose selection was inadequate. Because the results are positive, this study is acceptable (i.e. a positive result does not warrant verification in an adequately designed study).
 - For an experiment to be valid, several replicates should have relative total growth (RTG, a measure of cytotoxicity relative to controls) in the 10-20% range.
 - Lack of mutagenicity at RTGs above 20% may be a false negative.
 - Evidence of mutagenicity at RTGs below 10% may be a false positive.
 - Because the results are positive, this study is acceptable (i.e. a positive result does not warrant verification in an adequately designed study).
- Five experiments were conducted, but the first was halted due to excessive toxicity.
- The four completed experiments (two –S9, two +S9) each exhibited individual replicates that were positive (i.e. total counts above the concurrent and historical negative control ranges).

Methods

Cell line:	V79 Chinese hamster lung cells
Basis of concentration selection:	Preliminary cytotoxicity range-finder (5 hour exposure ±S9, then incubated 6 to 8 days to allow colony development)
Negative control:	No treatment and DMSO

- Positive control: • EMS –S9
 • DMBA +S9
- Formulation/Vehicle: DMSO
- Incubation & sampling time: • Cells were exposed for 5 hours, then washed and replated
 • Petri dishes of replated cells were incubated for 6 days to determine cytotoxicity
 • Flasks of replated cells were reseeded on D3 (into flasks) and then D6 (in Petri dishes)
 • Petri dishes were incubated with or without 6-TG (with to select mutants; without to determine cloning efficiency) or without 6-TG for 6 to 8 days

Study Validity

- The HPRT test is a valid assay for identifying mutagens. OECD 476 addresses the HPRT test, and was consulted in the review of this study report.
- The study design and reporting generally appears adequate, except for concentration selection.
 - As the table below shows, the preliminary cytotoxicity range finder did not identify any concentration within the 10-20% cytotoxicity range –S9, and only one concentration in the 10-20% cytotoxicity range +S9 (1000 µg/ml)

Table 37: Preliminary cytotoxicity data for the V79/HPRT-test (report # PH 30802)

± S9	Concentration (µg/ml)	Colony number (% control)
+	≤ 250	93 to 100
	500	95.5
	1000	59.0
	≥ 2000	≤ 1.4
-	≤ 250	88 to 100
	500	92.4
	1000	17.0
	≥ 2000	≤ 1.1

- This reviewer considers the author's criteria for mutagenicity to be inadequate, because they are overly weighted to avoid positives and allow negatives (including false negatives):
 - The author's criteria for a positive result included:
 - Both parallel cultures positive
 - Increase "at least two to three times that of the highest negative or vehicle control value"

- The author's criteria for concluding a test article to be mutagenic included reproducibility of a positive result in both experiments.
- The content of the test article was reported as 91.6%, and the purity was not provided. This omission is a minor study limitation.
- A stability test of 0.05 mg/ml and 200 mg/ml in culture media for 5 hours detected no degradation.
- These experiments were conducted in 2000. The authors provided historical control data from 1996 to 1999. The 1999 data (from report page 25) are copied:

Table 38: 1999 historical control data for the V79/HPRT test (report # PH 30802)

1999

Compound and S9 Mix		Number of Trials	Mean MF	SD	Range
None	-	30	1.9	± 1.5	0.4 - 6.5
Water	-	4	3.5	± 2.8	0.8 - 7.2
Ethanol	-	8	2.4	± 1.8	0.6 - 5.1
DMSO	-	18	1.8	± 2.6	0.2 - 4.5
EMS	-	30	612.0	± 208.3	229.7 - 1001.5
None	+	36	2.3	± 3.8	0.5 - 15.8
Water	+	4	4.8	± 4.0	1.7 - 10.6
Ethanol	+	8	3.5	± 3.9	0 - 10.6
DMSO	+	24	1.2	± 1.2	0.3 - 5.0
DMBA	+	36	64.6	± 38.8	16.7 - 184.2

Results

- The authors conducted three separate experiments –S9, the initial was cancelled due to high cytotoxicity.
- The first experiment (-S9) detected mutagenicity at only one concentration, 750 µg/ml).
 - This concentration was relatively non-cytotoxic (relative total growth [RTG] more than 20% of control)
 - The mutant colony counts for both replicates were above the historical control ranges (1996-1999)
- The second experiment (-S9) detected mutagenicity at only one concentration, 1250 µg/ml
 - Only one of the two replicates yielded cytotoxicity in the 10-20% range, and this replicate had a clearly elevated mutant colony count compared to concurrent and historical controls
- For the third experiment (+S9), the only two replicates with RTG between 10 and 20% had increased mutant colony counts compared to controls
 - One of two at 1000 µg/ml, and one of two at 2000 µg/ml
- For the fourth experiment (+S9), three individual replicates were positive (increased mutant colony counts above concurrent and historical controls).
 - No dose-response apparent for mutagenicity

Table 39: Results from the V79/HPRT test (report # PH 30802)

Experiment	S9	Concentration (µg/ml)	Relative total growth (% vehicle control)			Mutant colonies (per 1600 cell counted total for 8 dishes/replicate)		
			1 st replicate	2 nd replicate	Mean	1 st replicate	2 nd replicate	Mean
1	-	Negative control	75.1	78.4	77	4	12	8
	-	Vehicle control	100	100	100	20	6	13
	-	Positive control	79.3	49.3	64	587	300	444
	-	250	98.9	108.0	103	16	10	13
	-	500	80.0	59.3	70	9	8	9
	-	750	29.4	36.6	33	81	21	51
	-	1000	15.4	9.5	12	4	10	7
	-	1250	6.7	11.5	9	15	8	12
	-	1500	5.8	5.6	6	5	12	9
2	-	Negative control	77.6	99.1	88	8	2	5
	-	Vehicle control	100	100	100	6	7	7
	-	Positive control	24.9	30.3	28	779	542	661
	-	125	99.2	128.1	114	0	2	1
	-	250	63.2	76.8	70	2	2	2
	-	500	50.6	61.5	56	0	3	2
	-	750	54.9	61.3	58	3	6	5
	-	1000	33.2	31.8	33	12	13	13
	-	1250	29.5	18.4	24	7	39	23
3	+	Negative control	82.2	101.3	92	2	4	3
	+	Vehicle control	100	100	100	3	1	2
	+	Positive control	107.3	63.7	86	68	50	59
	+	200	160.8	67.8	114	3	1	2
	+	400	129.4	92.0	111	2	6	4
	+	600	119.5	64.5	92	4	5	5
	+	800	75.3	70.7	73	8	4	6
	+	1000	91.7	18.9	55	11	23	17
	+	1200	19.5	\$	\$	36	10	23
4	+	Negative control	98.2	84.2	91	8	15	12
	+	Vehicle control	100	100	100	13	13	13

	+	Positive control	73.7	43.0	58	117	142	130
	+	200	129.4	121.6	126	27	7	17
	+	400	139.6	100.1	120	11	13	12
	+	600	61.0	109.1	85	11	6	9
	+	800	42.1	45.2	44	16	21	19
	+	1000	28.1	28.3	28	23	5	14
	+	1200	47.5	15.9	32	5	6	6

- \$: not calculable to due low cell numbers at seeding (less than 1.5×10^6)
- Cells marked with grey background show cytotoxicity in the 10-20% of vehicle control range
- Mutant frequency values marked in **bold** are considered by this reviewer to be higher than concurrent negative and vehicle controls.
- Note: Means calculated (and rounded) by this reviewer. For the mutant colony count, the authors prepared 8 dishes per replicate, and counted 200 cells/dish. Experiment # assigned by this reviewer for convenience of review (they were done separately in the order: #1, #3, #2, #4)

7.2.4

Study title: BAY 365-3377. Photo-V79/HPRT-test in vitro for the detection of induced forward mutations	
Study no.:	PH-30910
Study report location:	NDA module 4.2.3.3.1 Genotoxicity <i>in vitro</i>
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 14, 2000
GLP compliance & QA statement:	Yes, signed
Drug, lot #, and % purity:	Fluorouracil hydrochloride (BAY 35-3377; BYK 60621), batch # 503605, content 91.6%

Key Study Findings

- The study design was inadequate and is not useful from a regulatory perspective.
- The purpose of this assay was to evaluate the mutagenicity of fluorouracil in the presence of UV-irradiation.
 - The experiments were shown to be insensitive for detection of mutagens. In the absence of radiation, the negative and positive control mutant frequencies yielded expected responses.

- With radiation, the vehicle control mutant frequencies were high – comparable or above the positive control mutant frequencies.

Methods

- This HPRT test is similar to the previous one reviewed above (report # PH- PH 30802): same laboratory, test article, methodology
- -S9 only

Cell line:	V79 Chinese hamster lung cells
Basis of concentration selection:	Preliminary cytotoxicity assay
Negative control:	Vehicle (DMSO)
Positive control:	<ul style="list-style-type: none">• EMS (a directly-acting alkylating agent)• 8-methoxypsoralen (8-MOP), a compound requiring light activation to be mutagenic
Irradiation:	<ul style="list-style-type: none">• UVA approximately 130 mJ/cm²• UVB approximately 5 mJ/cm²
Incubation & sampling time:	<ul style="list-style-type: none">• Experiment 1 (direct irradiation method): cells were treated with test article, then immediately irradiated with both UVA and UVB (lid off), then cultured for 5 hours prior to washing and replating into dishes with 5-TG for selection of mutants.• Experiment 2 (preincubation method): cells were treated with test article, incubated in the dark for 20 minutes, irradiated with UVA and UVB (lid off), then cultured for 4:40 prior to washing and replating into dishes with 5-TG for selection of mutants.

Study Validity

- The study is not valid, because the irradiated positive control responses were not $\geq 3x$ higher than the irradiated negative controls
 - Note: the report's acceptance criteria specified that the non-irradiated positive control should exceed the non-irradiated vehicle control by at least 3-fold. However, the report does not explicitly state acceptance criteria for the irradiated positive versus negative control.
- The standard aspects of the HPRT assay appear valid, but the attempt to evaluate photoactivation is inadequate.

- The UV doses were reportedly chosen (page 13) because they induce “low but significant increases of the mutant frequency in irradiated vehicle control cells above the background frequency of unirradiated vehicle control cells”
 - No historical control data for irradiated cells were provided (e.g. negative or positive controls)
 - It is not clear that the doses of UV radiation chosen were appropriate for the intended purpose
- Experiments were only done –S9, ostensibly because “so far there is no compound known which is exclusively photomutagenic after addition of S9 mix.”
- The failure to include EMS as an additional positive control + irradiation, or 8-MOP without irradiation, significantly limits the interpretability of the study results.

Results

- For experiment 1, the irradiated negative control response overlapped the positive control response. Therefore, the experiment is not valid.
- For experiment 2, one of the positive control replicates was lost (due to cytotoxicity), and the other positive control was only 2.1x the irradiated control average. Therefore, experiment 2 is also invalid.
- The Applicant (module 2.6.6. Toxicology Written Summary) notes that these data suggest a protective effect for finafloxacin against UV-induced mutagenicity. This activity signal may be relevant to future dermal/ocular exposure.

Table 40: Results from the photo-HPRT assay (report # PH-30910)

Experiment	Irradiation	Test article	Total mutant colonies (8 dishes, 200 cells/dish counted)	
			First replicate	Second replicate
1	None	Vehicle	2	1
		EMS	780	510
	Direct irradiation (UVA + UVB)	Vehicle	42	33
		8-MOP	76	39
		Finafloxacin (125 to 1250 µg/ml)	1 to 18	
2	None	Vehicle	14	10
		EMS	440	450
	Preincubation (UVA + UVB)	Vehicle control	26	50
		8-MOP	not cloned due to cytotoxicity	81
		Finafloxacin (62.5 to 1000 µg/ml)	5 to 46	

7.2.5

Study title: Micronucleus test with V79 cells *in vitro* with BYK60621

Study no.:	276/2000
Study report location:	NDA module 4.2.3.3.1 Genotoxicity <i>in vitro</i>
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	September 29, 2000
GLP compliance & QA statement:	Yes, signed
Drug, lot #, and % purity:	Finafloxacin hydrochloride, batch # 503606, purity 99.8%

Key Study Findings

- Finafloxacin was clearly clastogenic in this *in vitro* micronucleus assay.
- The author concluded, “A dose-dependent increase in micronuclei was bound in both experiments with S9 mix in the concentration range of 500 to 1000 µg/ml”, and this reviewer concurs.
- The tests were inconclusive for clastogenicity –S9 ≥ 250 µg/ml

Methods

Cell line:	V7 Chinese hamster cell line
Concentrations in definitive study:	<ul style="list-style-type: none"> • -S9: 125, 250, 500 µg/ml • +S9: 250, 500, 650, 750, 1000 µg/ml
Basis of concentration selection:	Higher doses than these were cytotoxic, and cell counts were too low to analyze. The report does not specify the range of concentrations attempted.
Negative control:	Untreated and 1% DMSO
Positive control:	<ul style="list-style-type: none"> • -S9: mitomycin C • +S9: cyclophosphamide
Incubation period	Cells were exposed to test article for 3 hours + S9 (followed by 21 hour recovery) or 24 hours –S9 (no recovery time after treatment)
Mitotic shake off method (MSO)	<ul style="list-style-type: none"> • 16 hours after seeding into flasks, cells were treated with test article, ± S9. • After the incubation period, the flasks were then beaten vigorously against a drawer, so “mitotic cells are shaken off” into the supernatant. • The supernatant was plated into dishes.

- Mixed population method (MIP)
- Three hours later, the cells were fixed, stained, and scored.
 - Cells were seeded into dishes. Thirty minutes later, cells were treated with test article, \pm S9.
 - After the incubation period, cells were fixed, stained and scored (i.e. without replating).

Study Validity

- The test method and reporting appear adequate. OECD 487 guideline⁴ was consulted for the review of this report.
 - Instead of using a cytokinesis blocker, the assay scored the formation of clones to calculate the proliferation index (PI); this approach is acceptable under OECD 487.
 - Because the proliferation index data were not provided for the higher concentrations (e.g. 650 and 750 μ g/ml $-$ S9, and whatever doses were tested $+$ S9), it is not possible to verify that dose selection was adequate. However, because clear positive results were detected, testing higher doses is not warranted.
- The responses of the negative and positive controls appear reasonable.
 - No historical control data were provided.
- 1000 cells/well counted for identification of micronuclei. The number of cells in mitosis was also recorded.
- 2000 cells/well counted to determine proliferation index.
- The MIP method clearly enriched the number of cells in mitosis (as intended) compared to the MSO method.
- No statistical analyses were reported.

Results

- The author concluded, and this reviewer concurs, that finafloxacin was clearly positive in both $+$ S9 experiments, \geq 500 μ g/ml
- The $-$ S9 finafloxacin treated groups had \sim 2 to 3x more micronuclei than the negative controls at 250 and 500 μ g/ml. The responses are clearly higher numerically than concurrent negative controls, but the magnitudes of the increases are of unclear biological relevance. Therefore, the results are considered inconclusive for finafloxacin $+$ S9.

⁴ OECD Guidelines for the Testing of Chemicals, Section 4 Health Effects. Test No. 487: *in vitro* mammalian cell micronucleus test. July 2010. Accessed online via: http://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264091016-en

Table 41: Results for the in vitro micronucleus assay (report # 276/2000)

Experiment	Concentration (µg/ml)	Micronuclei (per 1000 cells counted)		% in mitosis	
		Replicate 1	Replicate 2	Replicate 1	Replicate 2
MIP -S9	Untreated control	12	-	3.7%	-
	Vehicle control	15	12	3.7%	7.1%
	Positive control	107	206	1.7%	1.6%
	125	13	13	2.7%	1.1%
	250	22	23	3.9%	4.2%
	500	23	24	3.5%	3.7%
MSO -S9	Untreated control	10	-	0.7%	-
	Vehicle control	13	11	0.1%	0.2%
	Positive control	83	230	1.1%	0.1%
	125	22	23	1.4%	0.4%
	250	28	18	0.2%	0.8%
	500	19	21	NR	0.3%
MIP +S9	Untreated control	7	-	3.6%	-
	Vehicle control	11	10	3.4%	2.1%
	Positive control	111	114	0.3%	0.3%
	250	15	13	2.7%	1.6%
	500	24	39	0.6%	0.4%
	650	39	26	1.3%	1.2%
	750	54	55	1.0%	1.9%
	1000	78	60	0.9%	1.6%
MSO +S9	Untreated control	11	-	0.5%	-
	Vehicle control	8	11	1.0%	0.8%
	Positive control	237	229	0.6%	2.3%
	250	15	17	0.9%	0.9%
	500	21	29	2.0%	0.5%
	650	39	35	2.3%	1.1%
	750	48	62	1.3%	1.1%
	1000	146	116	0.1%	0.2%

“-“ only one replicate for each of the untreated control groups

NR: number of mitoses was not reported (omission is not addressed by the authors)

Bolding: added to emphasis positive replicates

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

7.3.1

Study title:	(b) (4) 14-1881. Micronucleus test pilot study
Study no:	PH 25561
Study report location:	NDA module 4.2.3.3.2 <i>Genotoxicity in vivo</i>
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 5, 1996
GLP compliance:	No
Drug, lot #, and % purity:	<ul style="list-style-type: none"> • Finafloxacin (no batch # or purity information) • Note: The Sponsor (module 2.6.6) reports that (b) (4) 14-1881 is a synonym for finafloxacin.

Key Study Findings

- This pilot *in vivo* mouse micronucleus study is inadequate for regulatory purposes, because it was not done under GLP, and lacked negative and positive controls (needed to verify the sensitivity of the assay)
- Finafloxacin was negative for clastogenicity in this pilot study.
- No evidence of bone marrow toxicity at the nominal oral dose, 1000 mg/kg.

Methods

Species/Strain:	Hsd/Win:NMRI mice
Formulation/Vehicle:	A "emulsion ... suspension" of finafloxacin in 0.5% aqueous Cremophor
Dosing:	5 male and 5 female mice received a single oral gavage dose of 2000 mg/kg finafloxacin
Dose volume:	20 ml/kg
No controls	No positive or negative controls groups were included in this pilot study
Harvest and analysis	<ul style="list-style-type: none"> • 24 hours after dosing, femoral marrow was collected • Slides were prepared, stained, and evaluated. • 1000 polychromatic erythrocytes (PCEs) were counted per animal. • The number of normal erythrocytes (NCEs) per 1000 PCEs was noted, as a measure of bone marrow toxicity

- The numbers of micronucleated PCEs (MNPCEs) and micronucleated NCEs were tabulated

Study Validity

- This pilot non-GLP study was not intended to be fully valid.
- To achieve the desired dose (2000 mg/kg in 20 ml/kg), the concentration of the dosing solution would have been 100 mg/ml. From the reporting, it is not clear to this reviewer how well finafloxacin suspends into 0.5% cremophor, or how much of this nominal dose would have been absorbed systemically following gavage.
- The authors provided historical negative control data from 1990 to 1993.

Results

- After the single oral 1000 mg/kg dose, animals “showed apathy until sacrifice” but “feeding behavior was normal.”
 - No other details provided
 - No premature mortality
- The ratio of NCE/PCE does not suggest bone marrow toxicity
- No evidence of clastogenicity was detected. For the 10 treated animals, the mean number of MNPCEs = 1.4; the range was 0 to 3.

7.3.3

Study title: Testing of BYK60621 for mutagenic activity in the mouse by means of the micronucleus test with oral administration

Study no:	70/2001
Study report location:	NDA module 4.2.3.3.2 <i>Genotoxicity in vivo</i>
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	November 22, 2000
GLP compliance & QA statement:	Yes, signed
Drug, lot #, and % purity:	Finafloxacin hydrochloride (BYK60621), batch # 503606, purity 99.8%

Key Study Findings

- Finafloxacin was positive for induction of micronuclei in this study.
- The authors concluded, and this reviewer concurs that finafloxacin induced MN PCE in males at 1000 and 2000 mg/kg at the 24 hour sampling time in the second experiment.

Methods

Doses:	0, 500, 1000, 2000 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	20 ml/kg
Formulation/Vehicle:	Aqueous suspension of (b) (4) Xanthan /100 ml
Species/Strain:	NMRI mice
Number/Sex/Group:	<ul style="list-style-type: none"> • First experiment: 5/sex/dose/time point • Second experiment: 10 males/dose time point
Harvest time points:	24 and 48 hours post-dose
Evaluation:	<ul style="list-style-type: none"> • Counting of 2000 PCEs per animal, to determine the # of MN PCE per 2000 PCE • Counting at least 1000 NCEs, to determine the ratio of PCE to NCE
Basis of dose selection:	Limit test
Negative control:	vehicle
Positive control:	Cyclophosphamide

Study Validity

- OECD 474⁵ was consulted in the review of this report

Results

- The author concludes that the second experiment clearly showed treatment-related clastogenicity at 24 hours (1000 and 2000 mg/kg) and 48 hours (2000 mg/kg only). This reviewer concurs.
- The author considered the results of the first experiment to be inconclusive for the 24 hour males. This reviewer disagrees – the results for males at 24 hours are negative (i.e. concurrent control values appear incidentally low, compared to the other control values reported)
- Based on the magnitude of responses, the author concluded that the overall response was “weakly positive”.

Table 42: Results for the mouse micronucleus study (report # 70/2001)

Experiment and sample time	Sex	Individual MN PCE and total counts (per 2000 PCEs)					
			Vehicle control	Finafloxacin hydrochloride (mg/kg)			Positive control (CPA)
				500	1000	2000	
1 st	F	Individual	0	2	2	1	20

⁵ OECD Guidelines for the Testing of Chemicals, Section 4 Health Effects. Test No. 474: Mammalian Erythrocyte Micronucleus Test. July 1997. Accessed online via: http://www.oecd-ilibrary.org/environment/test-no-474-mammalian-erythrocyte-micronucleus-test_9789264071285-en

experiment 24 hr		counts	0	3	2	4	26
			3	3	3	4	33
			4	4	4	4	39
			10	5	4	5	41
		sum	17	17	15	18	159
		P value	-	-	-	0.221	0.004
1 st experiment 24 hr	M	Individual counts	1	0	0	3	34
			1	3	1	4	40
			2	5	4	5	46
			2	6	7	5	59
			3	8	11	5	61
		sum	9	22	23	22	240
		P value	-	-	-	0.071	0.004
1 st experiment 48 hr	F	Individual counts	1	0	0	1	-
			1	1	1	1	
			2	1	2	2	
			3	1	2	3	
			3	3	3	4	
		sum	10	6	8	11	
		P value	-	-	-	0.371	
1 st experiment 48 hr	M	Individual counts	1	1	1	2	-
			2	2	2	2	
			3	3	4	4	
			5	3	5	5	
			13	6	8	6	
		sum	24	15	20	19	
		P value	-	-	-	0.362	
2 nd experiment 24 hr	M	Individual counts	0	0	2	3	16
			0	0	2	4	31
			1	1	2	4	36
			1	3	4	4	53
			1	3	4	4	75
			2	3	4	4	
			3	3	5	5	
			3	5	6	5	
			4	5	7	6	
			6	6	7	11	
		sum	21	29	43	50	211
		P value	-	0.208	0.011	< 0.001	< 0.001
2 nd experiment 48 hr	M	Individual counts	0	0	0	2	-
			1	0	0	2	
			1	1	1	2	
			1	1	2	3	
			2	1	2	3	
			2	1	2	4	

			2	2	3	4
			2	2	4	4
			3	2	5	6
			5	5	6	8
		sum	19	15	25	38
		P value	-	-	0.311	0.006

The p-values presented in this table are the author's: using the Jonckreere-Teprstra test to compare control and the finafloxacin doses; using the Wilcoxon Test (one-sided) to compare vehicle and positive control.

"-" denotes where the author did not report statistics or did not run a concurrent positive control group.

Pharmacokinetic Analysis

The study laboratory performed a stand-alone PK study (report # 89/2001) to support this micronucleus study

- **Title: Single dose toxicokinetics of BYK60621 in mice following oral administration of 500 and 2000 mg/kg BYK60621 within the scope of a micronucleus test for mutagenic activity**
- Single oral gavage dose (20 ml/kg)
- 5/sex mice received vehicle
- 20/sex mice received 500 or 2000 mg/kg of finafloxacin hydrochloride.
- Same formulation (b)(4)% Xanthan suspension)
- However, a different batch (# 505172, purity 96.1%) was used from the batch used in the micronucleus study.
- The author concluded that a less-than-dose proportional increase in AUC and C_{max} was apparent:

Table 43: PK parameters for the single-dose oral mouse PK study (report# 89/2001) done to support the mouse micronucleus study (report # 70/2001)

PK parameter	500 mg/kg		2000 mg/kg	
	Male	Female	Male	female
AUC (mg*h/L)	337	247	860	712
C_{max} (mg/L)	47	46	115	82
T_{max} (hr)	1	1	1	0.5
$t_{1/2}$ (hr)	2.6	2.1	Not calculable	

7.3.3

Study title: Induction of micronuclei in the bone marrow of treated mice

Study no:	2808/10
Study report location:	<ul style="list-style-type: none"> NDA module 4.2.3.3.1 Genotoxicity <i>in vitro</i> Note: the module location is an error; this is an <i>in vivo</i> study
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 21, 2007
GLP compliance & QA statement:	Yes, signed
Drug, lot #, and % purity:	Finafloxacin hydrochloride, batch # CBC00319 (505821), purity 100%

Key Study Findings

- Finafloxacin was clearly positive in this assay: statistically significant increases in MN PCE were observed at all three dose levels at the 24-hour time point, and a linear trend was apparent.
- Finafloxacin was negative at the one dose level measured at 48 hours after dosing.
- Note: the author considered this study to be negative at both time points, based on the results of a fourth statistical analyses (using the Wilcoxon Rank Sum Test)
- This third study was conducted to resolve the findings of the previous two experiments (i.e. report # 70/2001). This study was intended to test a larger dose group, with larger numbers of cells scored.

Methods

Species/Strain:	Male CD-1 mice [out-bred CrI:CD-1 (ICR)] <ul style="list-style-type: none"> • Weight range 29 to 40 g on the first day of dosing
Dose levels	<ul style="list-style-type: none"> • 0, 1000, 2000, or 4000 mg/kg of finafloxacin hydrochloride, with harvest 24 hours later • 0 or 4000 mg/kg of finafloxacin hydrochloride, with harvest 48 hours later • Positive control (40 mg/kg cyclophosphamide, CPA), with harvest 24 hours later
Route of administration:	Oral gavage
Dose volume:	20 ml/kg
Formulation/Vehicle:	<ul style="list-style-type: none"> • (b) (4) Xanthan / 100 ml • Stirred continuously before and during dosing

- Analysis
- 4,000 PCE/mouse were evaluated for each animal
 - PCE, NCE, and MN PCE were tabulated

Study Validity

- No certificate of analysis (COA) for the test article was provided. As the author notes (on page 15), the COA provided from (b) (4) to the study laboratory is for a different batch.
- A concentration analysis was performed: animals were dosed on June 12, 2007, and samples were collect to verify concentration. The actual concentrations were 88 to 98% of nominal.
- This reviewer verified the accuracy of the author's statistical analyses.

Results

- No treatment-related clinical signs or changes in body weight after treatment were apparent.
- This reviewer concludes that finafloxacin exhibited clastogenicity at all three dose levels tested (1000, 2000, 4000 mg/kg) at 24 hours compared to vehicle control
- At 24 hours, the tests for statistical significance found:
 - Statistically significant difference ($p \leq 0.005$) differences for each finafloxacin-group from each other and the control group by the Chi Squared (X^2) heterogeneity test.
 - Statistically significant difference ($p \leq 0.005$) by X^2 2x2 contingency testing for the high-dose group versus vehicle control.
 - Statistically significant ($p \leq 0.01$) linear trend test. $Z = 2.952$.
 - All of the author's evaluation criteria to consider the test article clastogenic (page 24) were met.
- Because the two X^2 tests and the trend test were all positive at 24 hours, the author also analyzed the data using the Wilcoxon Rank Sum Test (more commonly referred to as the Mann-Whitney U test), which did not detect statistical differences for the finafloxacin groups.
 - Based on the lack of statistical significance in the Wilcoxon Rank Sum Test, the author concluded that finafloxacin was negative.
 - The author did not provide a scientific justification for relying upon the Wilcoxon Rank Sum Test, rather than the previous statistical tests, as the criterion for identifying a positive response.
- At 48 hours, the MN PCE values for control versus the single treatment-group were not statistically significant.

Table 44: Results of the male-only mouse micronucleus study (report # 2808/10)

Sample time	Treatment	Total # of PCE scored	Total # of MN PCE	% MN PCE	X^2 heterogeneity statistical significance	X^2 2x2 contingency (versus vehicle control) statistical	Wilcoxon Rank Sum Test statistical significance
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						significance	
24 h	Vehicle control	40,000	13	0.0325	NS	-	-
	1000 mg/kg	40,000	17	0.0425	$P \leq 0.05$	NS	NS
	2000 mg/kg	40,000	23	0.0575	$P \leq 0.05$	NS ($p = 0.096$)	NS
	4000 mg/kg	40,000	31	0.0775	$P \leq 0.001$	$P \leq 0.05$ ($p = 0.0066$)	NS
	Positive control	36,000 ^a	206	0.515	Not calculated	$P \leq 0.001$	$P \leq 0.001$
48 h	Vehicle control	40,000	36	0.090	NS	NS	-
	2000 mg/kg	40,000	52	0.13	NS	NS ($p = 0.088$)	NS

^a For one of the 10 positive controls, PCEs could not be differentiated from NCEs.

NS: Not statistically significant

NC: not calculated

Note: p-values in parentheses were calculated by this reviewer (X^2 2x2 contingency test, two-tailed)

Discussion of the statistical analysis

- The X^2 test is a robust test for detecting statistical significance of rare events, such as MN PCEs per PCE scored.
- In contrast, because the Wilcoxon Rank Sum Test's nonparametric design intentionally does not account for the magnitude of the difference between responders and nonresponders, it is not a robust method for detecting the biological significance of large increases in MN PCE. This method measures the consistency of the response, rather than the intensity of the response.
 - The Wilcoxon Rank Sum Test essentially interprets the individual animal MN PCE data at 24 hours as:
 - For controls, the highest value was 3, and three of the ten individuals had scores of 3.
 - The low-dose group and high-dose group each had three of ten individuals with scores above 3 (not statistically significant by Wilcoxon)
 - The mid-dose had only two of ten scores above 3 (not statistically significant by Wilcoxon)
 - The positive control (CPA) had 8/9 mice above 3 (statistically significant by Wilcoxon)
 - Another way to interpret the data is to note that the low-, medium, and high-dose groups at 24 hours increased the MN PCE response by 31%, 77% and 2.4x that of controls. This way highlights the biological relevance of the magnitude of the increases.

Table 45: Individual data (24 hour time point) for the confirmatory micronucleus study (report # 2808/10)

Data for Finafloxacin hydrochloride, 24 hour sample time

Treatment (mg/kg)	Animal Number	PCE Count	NCE Count	% PCE	Total PCE Count	MN PCE	% MN PCE
Vehicle control	912	329	671	32.90	4000	0	0.00
	918	385	615	38.50	4000	2	0.05
	950	328	672	32.80	4000	3	0.08
	930	477	523	47.70	4000	0	0.00
	943	342	658	34.20	4000	1	0.03
	940	405	595	40.50	4000	1	0.03
	901	382	612	38.43	4000	0	0.00
	926	420	580	42.00	4000	3	0.08
	913	399	601	39.90	4000	0	0.00
	916	391	609	39.10	4000	3	0.08
Finafloxacin hydrochloride (1000)	928	363	637	36.30	4000	0	0.00
	945	364	636	36.40	4000	1	0.03
	919	433	567	43.30	4000	0	0.00
	921	452	548	45.20	4000	1	0.03
	906	327	673	32.70	4000	2	0.05
	949	322	678	32.20	4000	4	0.10
	909	415	585	41.50	4000	0	0.00
	923	405	595	40.50	4000	5	0.13
	902	336	664	33.60	4000	4	0.10
	946	253	747	25.30	4000	0	0.00
Finafloxacin hydrochloride (2000)	934	326	674	32.60	4000	6	0.15
	925	439	561	43.90	4000	1	0.03
	927	286	714	28.60	4000	1	0.03
	914	405	595	40.50	4000	1	0.03
	922	292	708	29.20	4000	2	0.05
	933	366	634	36.60	4000	7	0.18
	911	416	584	41.60	4000	2	0.05
	905	438	562	43.80	4000	2	0.05
	904	449	551	44.90	4000	1	0.03
	903	393	607	39.30	4000	0	0.00
Finafloxacin hydrochloride (4000)	944	397	603	39.70	4000	2	0.05
	942	400	600	40.00	4000	0	0.00
	907	339	661	33.90	4000	0	0.00
	937	455	545	45.50	4000	9	0.23
	936	361	639	36.10	4000	2	0.05
	910	423	577	42.30	4000	2	0.05
	935	320	680	32.00	4000	2	0.05
	941	383	617	38.30	4000	6	0.15
	929	371	629	37.10	4000	8	0.20
	920	284	716	28.40	4000	0	0.00

8 Carcinogenicity

No carcinogenicity testing has been performed for finafloxacin. This reviewer concludes that carcinogenicity studies are not warranted to support the proposed indication (otic dosing for 7 days).

Per the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) S1A Guideline on the Need for Carcinogenicity Studies for Pharmaceuticals (ICH S1A),

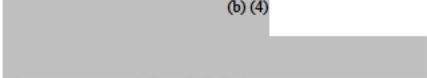
- “Carcinogenicity studies should be performed for any pharmaceutical whose expected clinical use is continuous for at least 6 months”
- “Pharmaceuticals showing poor systemic exposure from topical routes in humans may not need studies by the oral route to assess the carcinogenic potential to internal organs.”

9 Reproductive and Developmental Toxicology

The Applicant submitted three studies related to fertility and embryofetal toxicity.

9.1 Fertility and Early Embryonic Development

Study title: BYK60621, Study for effects on male and female fertility and early embryonic development in the rat, p.o.

Study no.:	49/2006
Study report location:	NDA module 4.2.3.5.2 Embryo-fetal development
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	January 12, 2001
GLP compliance & QA statement:	No. No mention of GLP or QA was found in the report
Drug, lot #, and % purity:	Finafloxacin hydrochloride (BYK60621), batch # 505832, purity 99.6%

Key Study Findings

- For male fertility:
 - NOAEL = 100 mg/kg/day
 - LOAEL = 500 mg/kg/day, based on reduced sperm count and drastic-to-total loss of sperm motility, delayed coitus and failure to achieve coitus within 3 weeks of mating
- For female fertility:
 - NOAEL = 100 mg/kg/day

- The effect of 500 mg/kg/day on female fertility could not be assessed (because the mated males were infertile)
- The authors report that the number of corpora lutea in high-dose females was zero.
- TK was not evaluated
 - The Applicant notes (NDA module 2.6.6.1) that the oral dose of 100 mg/kg/day in the repeat-dose rat study (report # 197/2000) had a C_{max} of 13.9 mg/L, approximately 171,000-fold higher than the highest C_{max} detected following otic clinical dosing.

Methods

- Doses: 0, 20, 100, 500 mg/kg/day
- Frequency of dosing:
 - Males were dosed once daily from 70 days prior to mating to day 15 post coitum (pc)
 - Females were dosed from 14 days prior to mating to day 15 pc
- Dose volume: 10 ml/kg
- Route of administration: Oral gavage
- Formulation/Vehicle:
 - Suspension of finafloxacin hydrochloride in purified water, with (b) (4) of xanthan gum, pH 7 to 7.5
 - Vehicle is water + (b) (4) of xanthan gum, pH 7 to 7.5
- Species/Strain: Wistar rats
 - Males 6 weeks of age at start of treatment, and 16 weeks of age at start of mating
 - Females 12 weeks of age at start of treatment, and 14 weeks of age at start of mating
- Number/Sex/Group: 28/sex/dose
- Note: Dosing was staggered by two weeks.
 - Half the males were started 1/22/2001, and the other half started on 2/05/2001
 - Half the females started dosing on 3/19/2001, and the other half on 4/02/2001
- Study design:
 - D1 pc was defined as the day spermatozoa were detected in the vaginal smear
 - Mating was overnight each day until mating was confirmed, to a maximum of 3 weeks
 - For each dose levels, females were only mated with males from the same dose level

Inadequacy comments

NOTE: This study does not meet ICH M3(R2) recommendations for an adequate assessment of fertility.

- No mention of GLP or QA was found in the report. Per ICH M3(R2):
 - “A male fertility study ... should be completed before the initiation of large scale or long duration clinical trials (e.g., phase 3 trials).”
 - “Nonclinical studies that specifically address female fertility...should be completed to support inclusion of WOCBP [women of child bearing potential] in large-scale or long-duration clinical trials (e.g., phase 3 trials).”
- The cover page says “Closure Report ... cause for closure of study: project was terminated in 2005 for non toxicological reasons.” This implies that the study director may have intended the study to be GLP, and the report is not final.
 - Note: the study laboratory, (b) (4) was reportedly acquired by (b) (4) in December 2006. (b) (4), was acquired by (b) (4) in 2011.
 - These experiments were done January-May 2001, and the report was closed in May 2006.
- A certificate of analysis is referenced (page 6) but was not provided.
- During mating, daily vaginal smears for detection of sperm were not reported.
 - It is not clear from the individual animal data that animals were mated daily for 21 days, as claimed in the Materials and Methods section.
- The identity of the study director and report author are not clear, and this lack of clarity reduces confidence in the study report.
 - The name of the author is not typed on the report, and may be the person in charge of animal husbandry, rather than the study director:
 - Dr. (b) (4) is listed as the Study Director (page 1 and 3), Dr. (b) (4) was responsible for animal husbandry (page 3), and (b) (4) was the responsible technician (page 3).
 - The name of the author is not printed, but the signature reads “(b) (4)”.
 - The signature of the study director is dated May 11, 2006, and is clearly not (b) (4).
 - The page has a signature of the (b) (4)
 - Many of the results tables have the note, “Requested by: (b) (4)”

Observations and Results

Mortality

- No premature mortalities occurred.

Clinical Signs

- No treatment-related clinical signs were apparent.
- Clinical signs were evaluated daily.
- Motility and respiration were monitored immediately before dosing and at 3-4 hours post-dose

Body Weight

- No treatment-related differences in body weight apparent.
 - High-dose females exhibited reduced body weight, due to the absence of pregnancies in this group.
- Body weight was measured for males twice per week
- Body weight was measured for females twice per week until mating, and then daily after a positive vaginal smear

Dosing Solution Analysis

- The report explicitly states (page 7) that sample analysis was not performed because the project was terminated.
- No analysis for concentration, homogeneity, or stability was provided. The report (page 6) notes that the test article has (b) (4), and (b) (4)% total impurities, with “unknown impurities, largest single value: (b) (4)%”.
- Reportedly “stable when stored refrigerated” (page 6)

Female Fertility and Female Necropsy Endpoints

- Dams were euthanized on D15 pc.
 - Necropsy endpoints were: number of implantations/dam, location of implantations, number of living fetuses/dam, number of corpora lutea, preimplantation loss (difference between the number of corpora lutea and the number of implantation sites per animal), postimplantation loss (difference between the number of implantation sites and the number of living fetuses)
 - Fertility endpoints were: copulation rate (number of females mated/number of females paired), fertility rate (number of pregnant females/number of females mated), vaginal smear (taken daily in the morning for estrus cycle and evidence of spermatozoa)
- For female fertility, NOAEL = 100 mg/kg, LOAEL = 500 mg/kg
 - No clearly treatment-related effect on estrus cycling:
 - The number of estrus events during premating (i.e. 14 days) was reported (page 52): most females had 3 or 4 estrus events.
 - For the high-dose group, the proportion with 3 instead of 4 events appeared slightly higher, but not of sufficient magnitude to be clearly treatment-related or clearly adverse.
 - “The mean number of corpora lutea was reduced to zero in the 500 mg/kg/day group.” (pdf page 19). No corpora lutea data are provided in

the summary table (pdf page 47), but the number of corpora lutea is reported as zero for the high-dose group in the individual data table (pdf page 131).

- For rats^{6,7}, the estrus cycle is 4 to 6 days, during which corpora lutea are formed spontaneously at each ovulation period (i.e. regardless of mating), but which do not usually secrete progesterone unless mating (sterile or fertile) has occurred.
- The authors conclude “Female fertility at a dose of 500 mg/kg/day could not be evaluated as the males of this group were not able to mate.” (page 2). Therefore, it is not clear to this reviewer whether:
 - the number of corpora lutea was truly zero,
 - or the authors did not check, based on the sperm effects at 500 mg/kg
 - or the authors mean only that no corpora lutea supporting pregnancy were detected.
- Fertility rate was 0% in the 500 mg/kg group. As a corollary, the precoital interval increased; the fertility rate, implantations, and living fetuses fell to zero.
- The low-dose group had 4 dams not pregnant: # 107, 148, 301 and 334 (report pdf page 146). This reviewer did not find in the report a listing of which individual females were mated to which individual males. Because two low-dose males had sperm abnormalities (# 7 – 0 sperm count, 0 sperm motility; # 52 – low sperm count, 0 sperm motility), this reviewer attributes two of the non-pregnancies to the males. No treatment-related effect on female fertility is apparent at 20 mg/kg.

Table 46: Female rat fertility parameters (report # 49/2006)

Parameter	Descriptor	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Number of estrus events during the pre mating period	Mean	3.39	3.54	3.46	3.32
	N= 1	1/28	0/28	0/28	0/28
	N= 2	0/28	1/28	1/28	1/28
	N= 3	14/28	11/28	15/28	17/28
	N = 4	13/28	16/28	13/28	10/28
# of females without sperm detected in vaginal smear		0/28	0/28	0/28	3/28
# pregnant		27/28	24/28	27/28	0/28
# not pregnant		1/28	4/28	1/28	28/28
Total litter lost		0/28	0/28	1/28	0/28

⁶ Greenwald GS, Rothchild I. 1968. Formation and maintenance of corpora lutea in laboratory animals. J. Animal Sci. S1:139-162. Accessed via:

http://www.journalofanimalscience.org/content/27/Supplement_1/139.long

⁷ Hilliard J. 1973. Corpus luteum function in guinea pigs, hamsters, rats, mice and rabbits. Biology of Reproduction. 8:203-221. Accessed via:

<http://www.bioreprod.org/content/8/2/203.full.pdf+html>

Number of corpora lutea	Total	347	315	350	0
	Litter mean	12.9	13.1	13.5	0
Number of implantation sites	Total	318	291	324	0
	Litter mean	11.8	12.1	12.5	0
% pre-implantation loss	Litter mean	8.1 %	7.7 %	7.3 %	-
Number of intrauterine deaths (dead fetuses)	Total	0	2	0	0
	Litter mean	0	0.1	0	0
Early resorptions	Total	3	7	6	-
	Litter mean	0.1	0.3	0.2	-
Late Resorptions	Total	4	10	9	-
	Litter mean	0.1	0.4	0.3	-
Total post-implantation loss	Total	7	19	15	0
	Litter mean	0.3	0.8	0.6	0
% post-implantation loss	Litter mean	2.2 %	6.5 %	4.6 %	-
# of live fetuses	Total	311	272	309	0
	Litter mean	11.5	11.3	11.9	0
% live fetuses/ implantations	Litter mean	97.8%	93.5 %	95.4 %	-

Note: "-" for the high-dose reflects the lack of pregnancy in this group.

Male Fertility and Male Necropsy Endpoints

- Males were sacrificed after the corresponding female exhibited pregnancy.
- Fertility endpoints were percent motility (2 x 100 sperms were counted per male), and sperm count (number of sperm per gram of caudal epididymides), organ weight (testes, caudae epididymis)
- No treatment-related changes were apparent for testes or epididymis weight
- At 500 mg/kg:
 - Sperm count was reduced by 50%
 - Essentially no spermatozoa motility was apparent

- This reviewer compiled the dates when sperm was detected in the vaginal smear (from the individual animal data, report pdf pages 146-149)
 - These data suggest that the high-dose was associated with decreased male sexual performance.
 - Increased number of days of co-habitation needed to achieve coitus.
 - Lower copulation index and fertility index compared to controls
 - NOAEL for male sexual performance = 100 mg/kg

Table 47: Selected male fertility endpoints for the rat fertility study (report # 49/2006)

Group	0 mg/kg	20 mg/kg	100 mg/kg (NOAEL)	500 mg/kg
No. of Females:				
<i>Paired</i>	28	28	28	28
<i>Mated</i>	28	28	28	25
<i>Pregnant</i>	27	24	27	0
Nominal pre-Coital Interval (days)	3.6	3.9	3.2	5.1
Copulation Index (%)	100	100	100	89%
Fertility Index	96	86	96	0

- Comments on the adequacy of the vaginal smear data:
 - Cohabitation was begun on D15, after 14 days of treatment.
 - The report (page 1; pages 8 and 9 [pdf pages 10 and 11]) indicates that pairs were mated “up to 3 weeks, 7 days/week”
 - The report states that “vaginal smears were taken daily in the morning” (report page 11 [pdf page 13]) until a positive smear was detected
 - The individual animal data (as tabulated below) suggest that the protocol was not followed:
 - Smear data are only reported for 14/28 dams/group after the first day of mating (i.e. mating on D15, smear on D16)
 - Of the remaining 14/group not smeared on D16, most but not all have smear data for D17
 - Subsequent smear data are missing for some animals
 - No smear data were reported for D20-21, D27-30, or D34-36.
 - Theoretically, some of the missing data may be due to uninterpretable data (i.e. smears were not readable), rather than data not being collected. However, this possibility seems unlikely (i.e. more likely to read the smear as negative, no sperm detected, rather than unreadable). This reviewer concludes that the animals were only mated 13 out of 19 consecutive days.
 - The apparent failure to mate pairs daily for 21 days is a study limitation.

- The apparent failure to take daily vaginal smears is a minor study limitation.

Table 48: Detection of sperm in vaginal smears for the rat fertility study (report # 49/2006)

Day relative to start of dosing	Sperm in vaginal smear detected			
	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
16	4/14	3/14	6/14	4/14
17	7/18	6/17	7/18	4/16
18	10/14	8/16	6/14	3/13
19	2/5	6/10	6/9	6/14
Total 1 st week	23	23	25	17
20, 21	Not reported			
22	0/1	0/0	0/0	0/0
23	2/5	1/4	0/3	0/8
24	1/3	2/3	3/3	6/8
25	1/2	2/2	0/0	1/3
26	1/1	0/0	0/0	0/2
Total 2 nd week	5	5	3	7
27, 28, 29, 30	Not reported			
31	0/0	0/0	0/0	0/2
32	0/0	0/0	0/0	0/4
33	0/0	0/0	0/0	1/4
Total 3 rd week	0	0	0	1
34, 35, 36	Not reported			
Total (D16 to D33)	28/28	28/28	28/28	25/28

Table tabulated by this reviewer from the reported individual female sperm in vaginal smear data (pages 146-149; summary data not provided in the report). The denominator reflects the number with data (i.e. not all females were checked daily until sperm was detected).

Table 49: Sperm count and sperm motility decreased in high-dose males in the fertility study (report # 49/2006)

Parameter	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Testes weight (g)	3.51 ± 0.34	3.43 ± 0.54	3.48 ± 0.24	3.53 ± 0.57
Cauda epididymis weight (g)	11.72 ± 3.09	11.90 ± 3.08	11.09 ± 3.46	9.95 ± 3.22
Sperm count range (N per gram of cauda epididymis)	5.5 to 36.5	0 to 29.5	3.5 to 28.5	0 to 15.5
Sperm count (mean ± SD)	14.9 ± 6.7	15.4 ± 6.7	15.6 ± 5.5	6.4 ± 3.7

# with sperm count = 0	0/28	1/28	0/28	1/28
Sperm motility (mean \pm SD)	71.4 \pm 13.1	62.8 \pm 21.0	62.5 \pm 12.2	0.21 \pm 0.63
Sperm motility range (%)	37 to 87	0 to 84	39 to 85	0 to 3
# with sperm motility = 0%	0/28	2/28	0/28	24/28

Table tabulated by this reviewer from the reported individual animal data (pages 151-154; summary data not provided in the report)

9.2 Embryonic Fetal Development

9.2.1 Rabbit range-finder

Report # 37/2001 (page 4) mentions a range-finding embryofetal study, which was not submitted to the NDA. Briefly:

- Pregnant female rabbits (1/group, strain not reported) received 1, 5, 10 or 15 mg/kg finafloxacin hydrochloride.
- Route not reported (presumably iv)
- Duration of dosing not reported (presumably pc D to pc D18)
- At 1 mg/kg, one fetus was “severely malformed... spina bifida, phocomelia”
 - The author (of report 37/2001) considered this “spontaneous” because similar effects were not observed in the higher-dose litters. Based on the weight-of-evidence (i.e. rare observation consistent with the exencephaly observed in later studies), this reviewer concludes that finafloxacin was teratogenic in the range-finder.
- No teratogenic effects were reported for other fetuses
- The NOAEL for maternal weight loss and decreased food consumption was 1 mg/kg
 - No additional information on weight loss/reduced weight gain were provided
 - Regarding reduced food consumption:

Table 50: Reduced food consumption in the rabbit range-finding embryofetal study was the basis of dose selection for the GLP- rabbit embryofetal study

Dose	Reported reduction in food consumption
1 mg/kg	“not affected”
5 mg/kg	“about 50% during the whole treatment period”
10 mg/kg	“about only 20%”
15 mg/kg	“about 20-30%”

- No effects on fetal weight were reported for any dose.

- Based on this range-finder, the doses of 1, 3 and 9 mg/kg/day were selected for the GLP rabbit embryofetal study (report # 37/2001)

9.2.2 Rabbit GLP embryofetal study

Study title: BYK60621, Study for effects on embryo-fetal development in the rabbit, i.v.

Study no.:	37/2001
Study report location:	NDA module 4.2.3.5 Reproductive and Developmental Toxicity
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 29, 2000
GLP compliance & QA statement:	Yes, signed
Drug, lot #, and % purity:	Fluoroquinolone hydrochloride (BYK60621), batch # 505172, purity > 07.13%

Key Study Findings

- No NOAEL for developmental toxicity, based on observations in the low-dose group of exencephaly and enlarged fontanel, paw hyperflexure, missing lumbar vertebra-7, missing lumbar arch-7, and sternbra-1 fusion. These effects were also observed at the higher doses, although without a dose-response.
 - The author considered the increased incidence of retinal folds and swollen mucous membrane in the mid- and high-dose pups to be treatment-related. This reviewer disagrees – these effects are not clearly treatment-related.
- For litter data endpoints, the NOAEL was the mid-dose (3 mg/kg) and the LOAEL was the high-dose, 9 mg/kg, based on increased preimplantation loss.
- For maternal toxicity, the NOAEL was the high-dose, 9 mg/kg.
 - Reduced food consumption was observed at all dose levels, and is considered evidence of pharmacologic activity, rather than toxicity, because no apparent effect on body weight was observed.
 - This reviewer disagrees with the author's findings (the author concluded that the maternal NOAEL = 3 mg/kg/day, and the maternal LOAEL = 9 mg/kg/day based on decreased food consumption and body weight)
 - This reviewer concludes that the results of this study are relevant to patient safety. If fluoroquinolone had caused biologically-significant maternal weight loss (perhaps due to pharmacological activity against gut bacteria), then the relevance of the results would have been a review question. Because no biologically-significant maternal weight loss was observed, the dose-selection does not appear too high.
- TK was not assessed in this study.

Methods

- Doses: 0, 1, 3, or 9 mg/kg/day (equivalent to 0, 2.5, 7.5 and 22.6 $\mu\text{mol/kg}$)
- Frequency of dosing: Daily from pc D6 through pc D18 (13 days total)
[Note: this duration is incorrectly reported by the Applicant in the Toxicology summaries, NDA modules 2.6.6 and 2.6.7, as pc D6 to pc D17]
- Dose volume: 0.5 ml/kg
- Route of administration: i.v. slow bolus over 15 seconds
- Formulation/Vehicle: Water for injection, pH adjusted to 8.0 to 8.5
- Species/Strain: Female Himalaya rabbits, Chbb:HM
- Number/Sex/Group:
- Vehicle-control: 16
 - Low-dose (1 mg/kg): 19
 - Mid-dose (3 mg/kg): 15
 - High-dose (9 mg/kg): 17
- Study design:
- Females were received and mated with untreated males. The day after mating = pc D1. The initiation of dosing was spread over 25 days (depending on when mating occurred)
 - Dams were dosed from pc D6 to D18, and euthanized on pc D29

- Note: no mention of randomization was found in the study report, and the report is not clear as to why the group sizes were uneven. This reviewer infers (based on report page M10 [pdf page 18], survey of animal material) that 20 females/group were randomized prior to mating, and that only pregnant animals were dosed.

Observations and Results

Mortality

- No treatment-related mortalities occurred.
- Two dams were lost due to accidental fractures (sacral bones for a low-dose female on pc D10, vertebral bone for a high-dose female on pc D6).

Clinical Signs

- No treatment-related clinical signs were apparent.
- For does, clinical signs were inspected once daily, with animals in their cages
- Additionally: motility, respiration and irregularities were assessed immediately before and after dosing, and 3-4 hours after dosing

Body Weight

- No change in body weight was apparent.

- The author reports that the high-dose dams exhibited reduced food consumption, reduced body weight, and reduced body weight gain during the first few days of treatment, but values returned to normal by the end of dosing.
- Does were weighed daily from pc D0 to pc D29. Food consumption was measured daily.

Table 51: Finafloxacin did not affect maternal body weight in the GLP rabbit embryofetal study (report # 37/2001)

Pc Day	Mean body weight (percentage change from pc D0 weight)			
	0 mg/kg	1 mg/kg	3 mg/kg	9 mg/kg
6 (first day of dosing)	+2.6%	+1.8%	+1.0%	+1.3%
7	+2.6%	+1.8%	+1.9%	+0.7%
9	+2.9%	+1.9%	+1.4%	+0.7%
12	+3.5 %	+2.4%	+2.3%	+1.8%
15	+5.8%	+4.1%	+4.7%	+3.3%
18 (last day of dosing)	+7.6%	+5.4%	+5.4%	+5.8%
21	+7.7%	+5.6%	+5.6%	+5.6%
29 (day of necropsy)	+15.5%	+12.5%	+12.5%	+12.6%

Note: selected days tabulated (for readability, weights were measured daily)

Feed Consumption

- All groups (including controls) exhibited a decrease in daily food consumption over time
- Finafloxacin-treated groups exhibited decreased food consumption compared to controls. Relatedly, finafloxacin-treated groups exhibited a greater decrease in food consumption compared to baseline, compared to controls.
 - The author reported decreased food consumption for the high-dose, “food consumption was reduced during the first four days after start of treatment compared to the control group” (i.e. D6 to D10)
 - The author’s reasoning is unclear, since the low- and mid-dose also clearly decreased food consumption, and the decrease persisted past pc D10.
 - The decrease in food consumption is consistent with primary pharmacology (i.e. finafloxacin’s antibacterial activity affecting gut bacteria)

Table 52: Finafloxacin decreased food consumption in the rabbit embryofetal study (report # 37/2001)

pc day range for food consumption	Food consumption (relative to control)		
	1 mg/kg	3 mg/kg	9 mg/kg

4 to 5 (pre-dose)	-12%	-0	-8%
5 to 6	-9%	+5%	-14%
6 to 7	-3%	+3%	-32%
7 to 8	-7%	+1%	-31%
8 to 9	-10%	0	-17%
9 to 10	-20%	-18%	-7%
10 to 11	-7%	-3%	-13%
11 to 12	-2%	-6%	-14%
12 to 13	-22%	-17%	-17%
13 to 14	-16%	-5%	-24%
14 to 15	-13%	-7%	-14%
15 to 16	-11%	-1%	-14%
16 to 17	-18%	-2%	-11%
17 to 18 (last day of dosing)	-21%	-4%	-11%
18 to 19	-16%	-12%	-9%
19 to 20	-11%	+ 10%	0
20 to 21	-11%	+ 5%	-5%
21 to 22	-2%	+16%	0
22 to 23	-7%	+ 3%	-5%
23 to 24	-5%	+ 3%	-12%
24 to 25	+ 5%	+ 5%	+1%
25 to 26	-9%	-7%	-9%
26 to 27	-3%	+9%	-5%
27 to 28	+ 3%	+ 6%	+2%
28 to 29	+4%	+ 4%	+2%

Dosing Solution Analysis

The author reports that the test articles were provided by the study sponsor as 1 mg/kg (0.2%), 3 mg/kg (0.6%) and 9 mg/kg (1.8%) solutions, reportedly “slightly turbid” (pdf page 197). The author provided a table (report page N15, pdf page 198) that shows that the measured concentrations were outside the $\pm 10\%$ range:

- the low-dose was 115% and 65% of nominal
- the mid-dose was 113 to 106% of nominal
- the high-dose was 117% to 124% of nominal

Figure 7: Concentration analysis for the rabbit embryofetal study (report # 37/2001)

Concentration of BYK60621 in aqueous solutions

dose	sample		concentration of BYK60621 analysed [%]		
	(nominal) concentration [%]	sampling (date)	replicate		mean
			1	2	
blank	0	01.09.00	n.d. ^{a)}	n.d. ^{a)}	n.d. ^{a)}
low	0.2	01.09.00	0.24	0.23	0.23
middle	0.6	01.09.00	0.69	0.67	0.68
high	1.8	01.09.00	2.08	2.11	2.10
blank	0	20.10.00	n.d. ^{a)}	n.d. ^{a)}	n.d. ^{a)}
low	0.2	20.10.00	0.14	0.11	0.13
middle	0.6	20.10.00	0.64	0.65	0.64
high	1.8	20.10.00	2.35	2.13	2.24

^{a)} n.d. = not detected; detection limit 0.08 mg/ml (0.008%)

Notes:

- The first sampling date was September 9, 2000 and the second sampling date was October 20, 2000.
- The first pc D0 date was August 29 and the end of in-life was December 8, 2000.
- Animals were not dosed concurrently, but rather dosing initiated over a 25-day window.
- Presuming that the top data are the first sample and the bottom data are the second sample, these data suggest that the low- and mid-doses degraded (or precipitated) over time, and suggest that the high-dose was not mixed/stirred adequately for the first-time point.
- Based on this analysis, the low-dose, nominally 1 mg/kg, was actually in the range of 0.65 to 1.15 mg/kg.
- A certificate of analysis is mentioned (pdf page 11), "No. 2000-020 dated 30 May 2000", but the certificate was not provided in the study report.
- The Applicant provided a Response to Information request on August 13, 2014, that confirmed that lack of additional test article characterization data for this study.

Litter Data

- The high-dose group exhibited an increased incidence of pre-implantation loss.
 - The Applicant (NDA module 2.6.6) noted this increase in high-dose preimplantation loss.

- Endpoints were: gravid uterus weight, placental weight, # of corpora lutea, # of implantation sites, pre-implantation loss (# of corpora lutea minus # of implantations), pre-implantation loss rate (difference between the number of corpora lutea and number of implantation sites, divided by the number of corpora lutea), living fetuses/dam, post implantation loss rate (difference between the number of implantation sites and the number of living fetuses, divided by the number of implantation sites)

Table 53: Litter data for the GLP rabbit embryofetal study (report # 37/2001)

Endpoint	Measure	0 mg/kg	1 mg/kg	3 mg/kg	9 mg/kg
# of pregnant fetuses with live fetuses at necropsy		16	18	14	16
# of total resorptions		0	0	0	0
Corpora lutea	Total	141	153	134	154
	Mean per litter	8.8	8.5	9.6	9.6
Implantations	Total	120	137	117	121
	Mean per litter	7.5	7.6	8.4	7.6
Pre-implantation loss	Total	21	16	17	33
	% loss [number of losses/number of corpora lutea]	14.9	10.5	12.7	21.4
Dead fetus	Total	3	7	2	3
	Mean per litter	0.2	0.4	0.1	0.2
Number of early resorptions	Total	2	4	2	2
	Mean per litter	0.1	0.2	0.1	0.1
Number of late resorptions	Total	2	3	4	1
	Mean per litter	0.1	0.2	0.3	0.1
Post-implantation loss	Total	7	14	8	6
	Mean per litter	0.05	0.09	0.07	0.04
Live fetuses	Total	113	123	109	115
	Mean per litter	7.1	6.8	7.8	7.2
	% of implantations	94.17	89.78	93.16	95.04

- No treatment-related effects apparent for fetal sex, litter weight, fetal weight. For placental weight, the mean control weight is higher than the dosed-group means, but this appears to be due to normal variation (i.e. not clearly treatment-related).

Table 54: No treatment-related changes apparent for sex, litter weight, fetus weight, or placental weight in the GLP rabbit embryofetal study (report # 37/2001)

Endpoint	0 mg/kg	1 mg/kg	3 mg/kg	9 mg/kg
# of live fetuses	113	123	109	115
% of male fetuses	45.1%	42.3%	47.7%	52.2%
Mean litter weight (g)	257 ± 74	245 ± 56	282 ± 57	255 ± 68
Mean fetus weight (g)	37 ± 5	36 ± 3	37 ± 4	36 ± 4
Mean placental weight (g)	4.71 ± 0.51	4.37 ± 0.63	4.46 ± 0.46	4.49 ± 0.73

Data presented as means ± standard deviations

Offspring Data

- Endpoints were “behavior and appearance” after delivery by Caesarian section (motility, respiration, circulation, irregularities), sex (determined by internal evaluation of organs), body weight, and fetal morphology (external changes, visceral changes, head, and skeletal changes).
- All fetuses were examined for gross external defects. All heads were examined for external changes, then 50% of heads were fixed in Bouin’s solution for Wilson’s soft sectioning, and the other half of heads were processed for skeletal evaluation (page 16).
- It is not clear from the reporting whether the assessment of visceral defects was adequate. No complete list of visceral organs/tissues assayed was identified in the report
 - ICH S5(R2) specifies that “When using fresh microdissection techniques for soft tissue alterations - which is the strongly preferred method for rabbits - 100% of rabbit fetuses should be examined for soft tissue and skeletal abnormalities.”
 - The report states (pdf page 16) that “Visceral changes ... autopsy of thoracic, abdominal, and cranial cavity of all fetuses”, and the summary table specifies that the “fresh visceral” method was used.
 - The report also states (pdf page 16) that “Skeletal changes ... The fetuses are fixed in alcohol; cleared... and the skeleton stained with alizarin red” by Dawson’s method.
 - Fetuses were separated into two halves, and the data (fresh visceral anomalies and skeletal anomalies) are presented separately for each half. 60/114, 65/123, 58/109 and 62/115 fetuses (for the control, 1, 3, and 9 mg/kg groups respectively), and then the remaining 53/114, 58/123, 52/109 and 53/115 fetuses (for the control, 1, 3, and 9 mg/kg groups respectively)
 - Each litter is represented in both halves (i.e. the authors did not evaluate half of the litters, and then evaluate the other half of the litters).
 - Therefore, data for eye, liver, ovary, thymus, ribs, sternebra, vertebra, shoulder and foreleg, pelvis and hindlimb appear twice (i.e. for both groups of

fetuses separately). The Applicant's Toxicology Tabulated Summary (NDA module 2.6.7) does not appear to correctly tabulate these fetal anomalies (i.e. only captures one of the two groups).

- Data for the skull skeletal defects are only presented for half of animals (the 50/54/57/62 half, on report pages 39-42)
 - Data for the testis only appear once (page 35); this reviewer infers that no testis effects were noted in the other half of fetuses (i.e. the omission is due to a lack of observations, not a lack of checking)
- Note: the authors did not provide summary tabulation of fetal anomalies by litter, which complicates review of this study report. The authors did provide individual animal data.
- The author concluded, and this reviewer concurs, that the following pathologies appear associated with treatment:
 - Exencephaly (external defect)
 - Enlarged fontanel (skeletal defect)
 - Paw hyperflexure (external defect)
 - Missing lumbar vertebra-7 and missing lumbar arch-7 (skeletal defect)
 - Sternebrae-4 not ossified (skeletal defect)
- Additionally, this reviewer considers the increased incidence of sternebra-1 fusion to be treatment-related. This pathology was not discussed by the author.
- The author considered the following to be treatment-related, but this reviewer disagrees (i.e. these are not clearly treatment related):
 - Cataracts (observed during the external examination; i.e. all fetuses checked)
 - Retinal folds (observed after Bouin's staining; i.e. only half of fetuses checked)
 - Nose swollen mucous membrane
 - Suture bone present

Table 55: Selected fetal pathology parameters for the GLP rabbit embryofetal study (report # 37/2001)

Endpoint	Measure	0 mg/kg	1 mg/kg	3 mg/kg	9 mg/kg
Total # of fetuses examined		113 ^a	123	109	115
# of litters examined		16	18	14	16
Paw hyperflexure, left forepaw	# of affected fetuses	0/113	0/123	0/109	1/115 (0.9%)
	# of affected litters	0/16	0/18	0/14	1/16 (6.2%)
Paw hyperflexure, right forepaw	# of affected fetuses	0/113	1/123 (0.8%)	0/109	1/115 (0.9%)
	# of affected litters	0/16	1/18 (5.5%)	0/14	1/16 (6.2%)
Paw hyperflexure, both forepaws	# of affected fetuses	0/113	1/123 (0.8%)	0/109	0/115
	# of affected litters	0/16	1/18 (5.5%)	0/14	0/16

Brain, exencephaly	# of affected fetuses	0/113	2/123 (1.6%)	0/109	0/115
	# of affected litters	0/16	1/18 (5.5%)	0/14	0/16
Cranium, exencephaly	# of affected fetuses	0/113	0/123	1/109 (0.9%)	0/115
	# of affected litters	0/16	0/18	1/16 (7%)	0/16
Lumbar vertebra-7, missing ^b	# of affected fetuses	0/60	3/65 (4.6%)	0/57	2/62 (3.2%)
	# of affected litters	0/16	3/18 (16.6%)	0/14	2/16 (12.5%)
Lumbar arch-7, both, missing ^b	# of affected fetuses	0/60	3/65 (4.6%)	0/57	2/62 (3.2%)
	# of affected litters	0/16	3/18 (16.6%)	0/14	2/16 (12.5%)
Lumbar vertebra-7, missing ^c	# of affected fetuses	0/53	0/58	1/52 (1.9%)	2/53 (3.8%)
	# of affected litters	0/16	0/18	1/14 (7.1%)	2/16 (12.5%)
Lumbar arch-7, both, missing ^c	# of affected fetuses	0/53	0/58	1/52 (1.9%)	2/53 (3.8%)
	# of affected litters	0/16	0/18	1/14 (7.1%)	2/16 (12.5%)
Total # of Lumbar vertebra-7, missing ^d	# of affected fetuses	0/113	3/123 (2.4%)	1/109 (0.9%)	4/115 (3.5%)
Total # of Lumbar arch-7, both, missing ^d	# of affected fetuses	0/113	3/123 (2.4%)	1/109 (0.9%)	4/115 (3.5%)
Sternebra-1, fused ^e	# of affected fetuses	0/60	1/65 (1.5%)	0/57	2/62 (3.2%)
	# of affected litters	0/16	1/18 (5.5%)	0/14	2/16 (12.5%)
Sternebra-1, fused ^f	# of affected fetuses	0/53	1/58 (1.7%)	0/52	1/53 (1.9%)
	# of affected litters	0/16	1/18 (5.5%)	0/14	1/16 (6.2%)
Total, sternebra-1, fused ^d	# of affected fetuses	0/113	2/123 (1.6%)	0/109	3/115 (2.6%)
Sternebra-4, not ossified ^g	# of affected fetuses	9/60 (15%)	24/65 (36.9%)	11/57 (19.3%)	17/62 (27.4%)
	# of affected litters	4/16 (25%)	13/18 (72.2%)	9/14 (64.2%)	9/16 (56.2%)
Sternebra-4, not ossified ^g	# of affected fetuses	7/53 (13.2%)	18/58 (31.0%)	12/52 (23.1%)	11/53 (20.8%)

	# of affected litters	5/16 (31.2%)	11/18 (61.1%)	8/14 (57.1%)	7/16 (43.7%)
Total, stenebra-4, not ossified ^d	# of affected fetuses	12/113 (10.5%)	29/123 (23.6%)	20/109 (18.3%)	18/115 (15.6%)
Fontanel, enlarged	# of affected fetuses	0/60	1/65 (1.5%)	3/57 (5.3%)	0/62
	# of affected litters	0/16	1/18 (5.5%)	3/14 (21.4%)	0/16
Eye, right, cataract	# of affected fetuses	0/60	0/65	1/58 (1.7%)	0/62
	# of affected litters	0/16	0/18	1/14 (7%)	0/16
Eye, both, cataract	# of affected fetuses	0/60	0/65	1/58 (1.7%)	0/62
	# of affected litters	0/16	0/18	1/14 (7%)	0/16
Eye, left, retinal fold	# of affected fetuses	1/52 (1.9%)	2/56 (3.6%)	2/52 (3.8%)	2/53 (3.8%)
	# of affected litters	1/16 (6.2%)	2/18 (11.1%)	2/14 (14.2%)	2/16 (12.5%)
Eye, right, retinal fold	# of affected fetuses	1/52 (1.9%)	2/56 (3.6%)	0/52	3/53 (5.7%)
	# of affected litters	1/16 (6.2%)	2/18 (11.1%)	0/14	3/16 (18.7%)
Eye, both, retinal fold	# of affected fetuses	1/52 (1.9%)	0/56	0/52	2/53 (3.8%)
	# of affected litters	1/16 (6.2%)	0/18	0/14	2/16 (12.5%)
Nose, swollen mucous membrane	# of affected fetuses	0/52	0/56	1/52 (1.9%)	1/53 (1.9%)
	# of affected litters	0/16	0/18	1/14 (7.1%)	1/16 (6.2%)
Skull, sutural bone present	# of affected fetuses	5/60 (8.3%)	4/123 (6.2%)	0/109	8/62 (12.9%)
	# of affected litters	4/16 (25%)	4/18 (22.2%)	0/14	8/16 (50%)

^a The control group had 113 live fetuses. The report occasionally lists data 114 fetuses (i.e. including one of the dead fetuses). This difference does not affect the interpretation of the study results.

^b Lumbar vertebra-7 and lumbar arch-7 data from report page ST22 (pdf page 43)

^c Lumbar vertebra-7 and lumbar arch-7 data from report page ST27 (pdf page 48)

^d This reviewer did not check the individual animal data to determine how many litters were affected.

^e Stenebra-1 data from report page ST24 (pdf page 45)

^f Stenebra-1 data from report page ST29 (pdf page 50)

^g Stenebra-4 data from report page ST25 (pdf page 46)

^h Stenebra-4 data from report page ST30 (pdf page 51)

- Note: Neither the report authors nor the Applicant identified relevant historical control data. Published historical control data for the Himalayan rabbit^{8, 9} support the conclusion that the increased incidences for exencephaly, missing lumbar vertebrae, and fused sternbrae are treatment-related.
- Regarding the exencephaly and enlarged fontanel data:
 - One dam at 1 mg/kg (dam #47) had two fetuses with “external, head, brain, exencephaly”.
 - The head of fetus #9 was examined viscerally, and “only small amounts of brain tissue were present.” (page 7, but no mention of the brain tissue was included in the individual fetal data on pages 120-121).
 - The head of fetus #8 was examined “skeletally” and exhibited “skeletal, skull, fontanel, enlarged” with incomplete ossification of both sphenoid, frontal and parietal bones and the manubrium.
 - One dam at 3 mg/kg (dam #2) had one fetus (position R05) where skeletal examination of the head detected “external, head, cranium, exencephaly” with “skeletal, skull, fontanel, enlarged” and incompletely ossified frontal skull bones.
 - Another dam at 3 mg/kg (dam #12) had a fetus (position L02) with enlarged fontanel and incompletely ossified skull bones (both frontal, parietal, supraoccipital) bones.
 - A third dam at 3 mg/kg (#43) had a fetus (position R02) with enlarged fontanel and incompletely ossified skull bones (both frontal, parietal, interparietal bones)
 - No explanation of the difference between brain exencephaly and cranium exencephaly was identified in the study report.
 - Notably, all four of these fetuses also exhibited incomplete ossification of sternbra 4 (i.e. a developmental delay)
- Regarding the observations of paw hyperflexure:
 - The authors considered the incidences detected on general external examination to be treatment-related, and this reviewer concurs.
 - It is unclear whether this defect had a skeletal component:
 - For evaluation of shoulder and forelimb skeletal defects, the authors noted background incompletely ossified scapula among all groups
 - The pelvis and hindlimb evaluation noted background incompletely ossified pubis, femur, calcaneus (heel bone) and talus (ankle bone).
 - No other forelimb or hindlimb skeletal defects were noted for any animal, and no detailed list of structures to be evaluated was provided.

⁸ Viertel B, Trieb G. 2003. The Himalayan rabbit (*Oryctolagus cuniculus* L.): spontaneous incidences of endpoints from prenatal developmental toxicity studies. *Laboratory Animals* 37:19-36. Accessed online via <http://www.ncbi.nlm.nih.gov/pubmed/12626069>

⁹ Matsuo A, Kast A. 1995 Two decades of control Himalayan rabbit reproductive parameters and spontaneous abnormalities in Japan. *Laboratory Animals* 29(1):78-82. Accessed online via <http://www.ncbi.nlm.nih.gov/pubmed/7707682>

- Therefore, it is unclear whether the skeletal evaluation in this study was adequate for the forelimb bones.

9.3 Embryonic Fetal Development

Study title: BYK60621, Study for effects on embryo-fetal development in the rat, p.o.

Study no.:	63/2001
Study report location:	NDA module 4.2.3.5 Reproductive and Developmental Toxicity
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 20, 2000
GLP compliance & QA statement:	Yes, signed
Drug, lot #, and % purity:	Finaxofacin hydrochloride (BYK60621), batch # 505172, purity 98.5%

Key Study Findings

- This study is inadequate: the concentration, homogeneity, and stability of the test article were not assessed. TK was not assessed (and therefore, dose cannot be estimated from exposure data).

Nominally:

- Maternal NOAEL for general toxicity = 100 mg/kg/day, based on reduced body weight gain and transiently reduced food consumption at 500 mg/kg
- Maternal LOAEL for general toxicity = 500 mg/kg/day, based on reduced body weight gain, and transiently reduced food consumption
- Litter Data NOAEL = 100 mg/kg/day, based on preimplantation losses at 500 mg/kg
- Developmental NOAEL = 30 mg/kg/day
 - Exencephaly observed in one fetus at 100 mg/kg
 - The author dismissed the observed exencephaly as incidental, and therefore concluded that no teratogenicity was detected. This reviewer disagrees. No historical control incidence was provided to support dismissing the relevance of the exencephaly.
- Other developmental toxicities observed at 500 mg/kg:
 - Increased number of incomplete ossifications (sternebrae-1 to sternebrae-3, the xiphisternum, the sacral arches-4, and the metacarpal-5)
 - Increased number of non-ossified sternebrae-4

- Decreased mean litter weight, decreased fetal weight, decreased placental weight
 - Author considers the effects to be growth delays/retardation secondary to reduced fetus and placental weight, and this reviewer agrees.
- No TK [note- the presentation of this study in the Toxicology Tabulated Summary is potentially misleading, since TK estimates were provided based on data from other studies]

Methods

- Doses: 0, 20, 100 or 500 mg/kg/day
- Frequency of dosing:
 - Daily from pc D6 to pc D17
 - day of positive vaginal smear = pc D0
- Dose volume: 10 ml/kg
- Route of administration: Oral gavage
- Formulation/Vehicle: (b) (4) % Xanthan (aqueous suspension), pH 7.0 to 7.5
- Species/Strain: Female Wistar rats
 - 14 weeks old at start of mating
 - Mated 4 days/week until pc D0
- Number/Sex/Group:

Daily dose		# of pregnant females
mg/kg/day	µmol/kg/day	
0	0	21
20	50.2	21
100	251	22
500	1255	25

Dosing Solution Analysis

- “Due to the termination of the project BY377, analysis of the test solutions was not performed” (report page 13 [pdf page 13])
 - This page notes that a certificate of analysis is available, “No. 2000-020 dated 30 May 2000”, but the certificate was not included in the study report.
- The cover page of the report states “Formulation: Analytical characterization of the test item BYK60621 was conducted by (b) (4).” However, no information to support this claim was identified in the report.
- The Quality Assurance (page 5) makes no mention of any QA related to the test article.

Observations and Results

Mortality

- No dams died prematurely
- The dams were dosed pc D6 to pc D17, and euthanized on pc D20

Clinical Signs

- No treatment-related clinical signs were apparent
- Clinical signs were evaluated once daily. Additionally, dams were observed for motility, respiration, and irregularities just prior to dosing and at 3 to 4 hours post-dose

Maternal Body Weight

- Body weight was measured on pc D0, and daily from pc D6 until pc D20
- The mid- and high dose groups exhibited decreased weight gain, decreased final body weight, decreased final body weight without uterus, and decreased gravid uterus weight compared to controls. Dose-responses were apparent.
 - The author only considered the high-dose effects to be treatment-related, but this reviewer disagrees.

Figure 8: Pregnant rat body weight data (report # 63/2001)

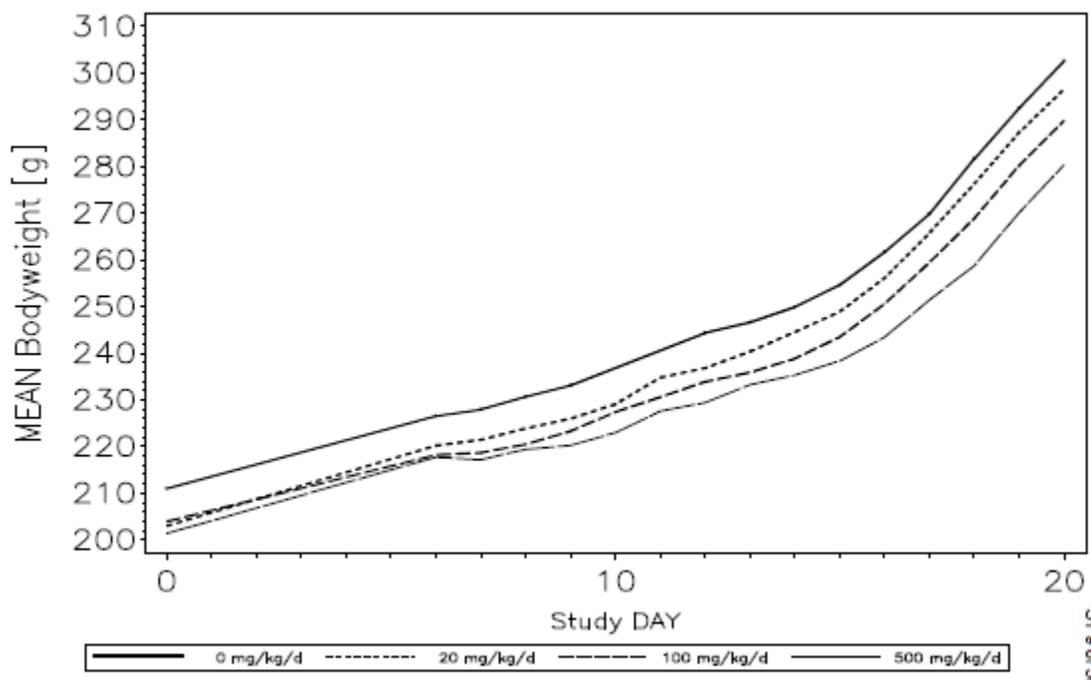


Table 56: Body weight parameters for the rat embryofetal study (report # 63/2001)

Weight measure relative to controls		20 mg/kg	100 mg/kg	500 mg/kg
Body weight gain from D6	to D10	88%	93%	53%
	to D14	107%	92%	78%
	to D18	105%	96%	78%
	to D20	104%	98%	86%
Final body weight (with uterus) (g)		98%	96%	93%
Gravid uterus weight (g)		102%	94%	88%
Final body weight without uterus (g)		97%	96%	94%

Maternal Feed Consumption

- Food consumption was measured daily from pc D0 to pc D20
- Food consumption decreased slightly for the mid- and high-dose groups at start of dosing (pc D6) and returned to normal by pc D12

Dam Necropsy

- Endpoints included gravid uterus weight, placenta weight, number and location of implantations, number of living fetuses, number of corpora lutea
- The rate of preimplantation loss was defined as the difference between the number of corpora lutea and the number of implantation sites, as a percentage of the corpora lutea.
- The rate of postimplantation loss was defined as the difference between the number of implantation sites and the number of living fetuses, as a percentage of the implantation sites.

Table 57: Reproductive parameters for the GLP rat embryofetal study (report # 36/2001)

Endpoint	Measure	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
# of pregnant fetuses with live fetuses at necropsy		21/21	21/21	22/22	25/25
# of total loss, # of dead fetuses, # of uterine scars		0	0	0	0
Corpora lutea	Total	283	279	280	326
	Mean per litter	13.5	13.3	12.7	13.0
Implantations	Total	249	255	257	288
	Mean per litter	11.9	12.1	11.7	11.5
Pre-implantation loss	Rate	11.3%	8.1%	8.0%	11.5%
Live fetuses	Total	237	246	231	270
	Mean per litter	11.3	11.7	10.5	10.8

Post-implantation loss	Rate	4.6%	3.5%	9.5%	6.0%
Early deaths ^a [early resorption]	Total	7	4	10	4
	# of affected litters	6/21	4/21	8/22	4/25
Late deaths ^a [late resorption]	Total	5	5	16	14
	# of affected litters	5/21	5/21	5/22	5/25

^a The authors reported the incidences of “early death” and “late death”. No definition was identified in the study report for either term, but the authors report recording the incidence of early resorption and late resorption (page 10), which is not presented in the report. Considering the summary table data (report pages 59-60) and the individual data (report pages 133-136), this reviewer infers that the authors used “early death” to mean early resorption, and “late death” to mean late resorption.

Offspring Data

- Pc D20 fetuses were weighed and examined for life (motility, respiration, circulation), external changes and irregularities. Sex was assessed by anogenital distance.
- Half of each litter was examined for visceral changes (i.e. fixed in Bouin’s solution)
- The other half of each litter was examined for skeletal changes (i.e. fixed in 100% alcohol, cleared in 1-2% potassium hydroxide solution, and stained with alizarin red)
- For the high-dose group compared to controls, decreases were observed for fetal litter weight (-7%), mean fetus weight (-5%), and placental weight (-28%)

The author noted the clearly treatment-related **incomplete ossifications** for the high-dose group in:

- Sternebrae-1, sternebrae-2 and sternebrae-3
- Xiphisternum
- Sacral arch-4
- Metacarpal-5

Additionally, the following effects observed in the high-dose are also treatment-related:

- One high-dose fetus (dam #9, L05) exhibited **bipartite** manubrium, sternebra-1 and sternebra-3, as well as incomplete ossification of sternebrae 1 and 3, the xiphisternum, and metacarpal 5
- The external defect examination found one mid-dose fetus **with brain exencephaly** (dam #40, fetus R04). This fetus also exhibited [report pages 267-268]:
 - **skull occipital cleaved**
 - **thoracic vertebra-5 cleaved**
 - **shortened skull tympanic annulus**
 - incomplete ossification of multiple bones of the skull, thoracic vertebra, sternebra and xiphisternum, and metacarpals
 - These defects are each concerning, but they are not independent observations

- In the high-dose, two fetuses (dam #75, R06, pdf page 336; dam # 106, L01, pdf page 356) had **missing lumbar vertebra-6** and **missing both lumbar arches**
 - The appearance of the same defect pattern in two different litters indicates these are treatment-related, rather than incidental.

Table 58: Fetal abnormalities reported GLP rat embryofetal study (report # 36/2001)

Endpoint		0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Mean fetal weight (g)		3.57	3.63	3.68	3.40
Mean placental weight (g)		0.53	0.50	0.48	0.38
Total # of fetuses		237	246	231	270
Total # of litter		21	21	22	25
# examined for external defects		237	246	231	270
# examined for visceral defects		116	120	111	129
# examined for skeletal defects		121	126	120	141
Brain, exencephaly (external defect)	# of affected fetuses	-	-	1 (0.4%)	-
	# of affected litters	-	-	1/22	-
Notable skeletal defects					
Lumbar vertebra-6, missing	# of affected fetuses	-	-	-	2 (1.4%)
	# of affected litters	-	-	-	2 (8%)
Lumbar arch-6, both, missing	# of affected fetuses	-	-	-	2 (1.4%)
	# of affected litters	-	-	-	2 (8%)
Sacral arch-4, left, not ossified	# of affected fetuses	-	-	-	3 (2.1%)
	# of affected litters	-	-	-	2 (8%)
Sacral arch-4, left, incomplete ossified	# of affected fetuses	3 (2.5%)	1 (0.8%)	4 (3.3%)	11 (7.8%)
	# of affected litters	3 (14.2%)	1 (.7%)	4 (18.1%)	9 (36%)
Sacral arch-4, right, not ossified	# of affected fetuses	1 (0.8%)	-	1 (0.8%)	5 (3.5%)
	# of affected litters	1 (4.7%)	-	1 (4.5%)	4 (16%)
Sacral arch-4, right, incomplete	# of affected fetuses	4 (3.3%)	4 (3.2%)	3 (2.5%)	9 (6.4%)

ossified	# of affected litters	4 (19%)	2 (9.5%)	3 (13.6%)	7 (28%)
Sacral arch-4, both, not ossified	# of affected fetuses	-	-	-	2 (1.4%)
	# of affected litters	-	-	-	1 (4.0%)
Sacral arch-4, both, incomplete ossified	# of affected fetuses	1 (0.8%)	9 (7.1%)	9 (7.5%)	49 (34.8%)
	# of affected litters	1 (4.7%)	6 (28.5%)	7 (31.8%)	18 (72%)
Manubrium, bipartite; sternebra-1, bipartite; sternebra-3 bipartite	# of affected fetuses	-	-	-	1 (0.7%)
	# of affected litters	-	-	-	1 (4%)
Sternebra-1, incomplete ossified	# of affected fetuses	5 (4.1%)	4 (3.2%)	6 (5%)	9 (6.4%)
	# of affected litters	5 (23.8%)	2 (9.5%)	5 (22.7%)	7 (28%)
Sternebra-2, incomplete ossified	# of affected fetuses	-	3 (2.4%)	3 (2.5%)	10 (7.1%)
	# of affected litters	-	3 (14.2%)	3 (13.6%)	6 (24%)
Sternebra-3, incomplete ossified	# of affected fetuses	6 (5%)	11 (8.7%)	5 (4.2%)	19 (13.5%)
	# of affected litters	5 (23.8%)	7 (33.3%)	4 (18.1%)	13 (52%)
Xiphisternum, incomplete ossified	# of affected fetuses	4 (3.3%)	-	6 (5%)	17 (12.1%)
	# of affected litters	3 (14.2%)	-	5 (22.7%)	11 (44%)
Metacarpal-5, both, not ossified	# of affected fetuses	1 (0.8%)	2 (1.6%)	3 (2.5%)	6 (4.3%)
	# of affected litters	1 (4.7%)	2 (9.5%)	3 (13.6%)	5 (20%)
Metacarpal-5, both, incomplete ossified	# of affected fetuses	30 (24.8%)	51 (40.5%)	42 (35.0%)	69 (48.9%)
	# of affected litters	11 (52.3%)	12 (57.1%)	12 (54.5%)	17 (68.0%)

- Neither the author nor the Applicant provided relevant historical control data. The Wistar rats used in this study were obtained from (b) (4),

(b) (4). Limited published data for the Wistar rat were identified^{10, 11, 12} and the usefulness of these data are limited. For context, the reported incidence of exencephaly in the CDE rat is 0.03% (26/88,270 affected fetuses).¹³

9.4 Prenatal and Postnatal Development

The Applicant reports (NDA module 2.6.6) that no prenatal and postnatal development studies have been conducted with finafloxacin. Per ICH M3(R2) section 11, "In all ICH regions, the pre-postnatal development study should be submitted for marketing approval."

Because otic dosing for 14 days is expected to result in negligible systemic exposure to the mother and no exposure to the embryo/fetus, this reviewer concludes that a pre-postnatal development study is not warranted for the otic indication.

10 Special Toxicology Studies

The Applicant submitted two local tolerance study reports, and four other study reports.

Title	Test for sensitizing properties of BYK60621 in the guinea pig	
Report #	289/2000	
Key findings	Dermal finafloxacin exposure was not sensitizing under the conditions tested	
Report details	Study laboratory	(b) (4)
	File location	NDA module 4.2.3.6 Local Tolerance
	GLP	Yes, signed

(b) (4)

¹¹ Grosz M, Hajdo E, Nyitray M, Druga A. 1995. Control data of Wistar rats CrI: (WL) BR. Abstract in Toxicology Letters, 78(1):37-38. Accessed via:

<http://www.sciencedirect.com/science/article/pii/S0378427495947523#>

¹² Karolina Pal Kutas, Andras Miklos, Janice Nimer, Cecelia Paculba, and Karen L Steinmetz. 2007. Historical control data of CrI:(Wi) BR-Wistar rats in Embryo-fetal Development Study. Abstract in Toxicology Letters 172:S188. Accessed via:

<http://www.sciencedirect.com/science/article/pii/S0378427407006133>

(b) (4)

	compliance & QA statement	
	Study initiation	November 2, 2000
	Test article	Finafloxacin hydrochloride (BYK60621), batch # 505172, 99.7% pure
Methods	Species	Female guinea pig, Dunkin Hartley, CrI: (HA)BR
	Group size:	5 control and 10 treated guinea pigs
	1 st Induction	Shoulders were shaved, and animals received a total of 6 intradermal injections: <ul style="list-style-type: none"> • 2 x 0.1 ml emulsion of Freund's adjuvant in saline • 2 x 0.1 ml of a 0 or 4% solution of finafloxacin in water • 2 x 0.1 ml of a 0 or 2% solution of finafloxacin in water + 50% Freud's adjuvant in saline
	Pretreatment	6 days later, the skin was re-shaved, and a 10% mixture of sodium lauryl sulfate was rubbed into the shoulder area skin
	2 nd induction	<ul style="list-style-type: none"> • The following day, animals were treated dermally with 0.3 ml of Vaseline (controls) or 0.3 ml of 25% finafloxacin in Vaseline • The treatments were applied on aluminum foil strips, which were covered with bandage and tape for 24 hours. • Skin was examined on D11 and 12 for any reaction.
	Challenge	<ul style="list-style-type: none"> • After shaving, 21 days after the first induction, animals were re-treated (same dermal exposure as for the 2nd induction). • Skin was examined on D25, then hair removed. • Skin was examined on D26
Results	<ul style="list-style-type: none"> • No treatment-related clinical signs or body weight changes apparent • No treatment-related skin reactions were observed. 	

Title:	Local tolerance testing in the rabbit after a single intravenous, intraarterial and paravenous administration of finafloxacin versus vehicle	
Report #	419.143.2235	
Key findings	<ul style="list-style-type: none"> • No treatment-related local effects (no erythema, edema, apparent pain, gross pathology or histopathology changes) 	
Report details	Study laboratory	(b) (4)
	File location	NDA module 4.2.3.6 Local Tolerance

	GLP compliance & QA statement	Yes, signed												
	Study initiation	March 18, 2010												
	Test article	Finafloxacin, batch # CBC00371 (60510PIL07), purity 92.7%												
	Vehicle	0.01 M trometamol (Tris buffer), 0.75% sodium chloride, pH 8.5												
Methods	Species	Female New Zealand White rabbits												
	Dosing	Three female rabbits received a total of 6 doses (left ear received vehicle, right ears received finafloxacin): <table border="1" data-bbox="609 672 1429 861"> <thead> <tr> <th>Route</th> <th>Dose (mg/animal)</th> <th>Volume (ml)</th> </tr> </thead> <tbody> <tr> <td>Intravenous</td> <td>0 or 5</td> <td>1</td> </tr> <tr> <td>Intraarterial</td> <td>0 or 5</td> <td>1</td> </tr> <tr> <td>Paravenous</td> <td>0 or 1.25</td> <td>0.25</td> </tr> </tbody> </table>	Route	Dose (mg/animal)	Volume (ml)	Intravenous	0 or 5	1	Intraarterial	0 or 5	1	Paravenous	0 or 1.25	0.25
	Route	Dose (mg/animal)	Volume (ml)											
Intravenous	0 or 5	1												
Intraarterial	0 or 5	1												
Paravenous	0 or 1.25	0.25												
Endpoints:	<ul style="list-style-type: none"> Clinical findings (erythema, edema, pain reaction) Gross and histopathological examination of the injection sites on day 5 (approximately 96 hours after injections) 													
Results	No treatment-related findings													

Title	Cytotoxic, phototoxic, and convulsive potentials of gastrofluoroquinolones analogous compounds <i>in vitro</i>	
Report #	PH 28757	
Key findings	<ul style="list-style-type: none"> Finafloxacin was not phototoxic in mouse fibroblast Balb/c 3T3 cells after UVA irradiation 2 uM of finafloxacin did not affect the excitatory potential of rat hippocampus <i>ex vivo</i> 	
Report details	Study laboratory	(b) (4) [location not provided]
	File location	NDA module 4.2.3.7 Other Toxicity Studies
	GLP compliance	No
	Report date	May 18, 1999
	Test article	<ul style="list-style-type: none"> Finafloxacin (BAY 35-3377), no batch # or purity information reported Ciprofloxacin was tested as a control Five other investigational fluoroquinolones were also

		tested
Cytotoxicity study	Methods	<ul style="list-style-type: none"> • Mouse macrophage (J774.A1) cells were seeded, and then incubated with test article for 72 hours • Viability assessed by Neutral red assay • Report did not specify the concentrations tested
	Results	<p>For finafloxacin:</p> <ul style="list-style-type: none"> • No observed effect concentration = 30 µg/ml • EC₅₀ for cytotoxicity ≥ 100 µg/ml
Phototoxicity study	Methods	<ul style="list-style-type: none"> • Mouse fibroblast Balb/c 3T3.31 cells were seeded, exposed to test articles for 1 hour • then irradiated with either UVA 1.67 mW/cm² for 20 minutes or UVA 2 and J/cm² for 1 hour • then washed and incubated with fresh media for 24 hours • As above, viability assessed by Neutral red assay, and the report did not specify the concentrations tested
	Results	<ul style="list-style-type: none"> • For finafloxacin, no cytotoxicity was observed at either UVA exposure. EC₅₀ > 100 µg/ml • For ciprofloxacin: <ul style="list-style-type: none"> ○ EC₅₀ > 100 µg/ml at UVA 1.67 mW/cm² for 20 minutes ○ EC₅₀ = 30 µg/ml at UVA 2 and J/cm² for 1 hour ○ The author regarded this as “slightly phototoxic”
Hippocampal slice study	Methods	<ul style="list-style-type: none"> • Young female rats (strain not reported) were anesthetized, and hippocampal slices prepared. • Within 1-2 hours of preparation, slices were placed in a test chamber, and the extracellular field potential was measured. • Slices were superfused for 30 minutes with 2 µM of test article
	Results	<ul style="list-style-type: none"> • 2 µM Finafloxacin was inactive (field potential was measured as 104% ±13% of control vehicle) • 2 µM Ciprofloxacin was active (field potential was 131 ± 7% of control vehicle)

Title	BAY-14-1881. Study of photoreactive potential in guinea pigs
Report #	PH 29356
Key findings	<ul style="list-style-type: none"> • Single oral doses of 30 or 100 mg/kg finafloxacin followed by UV-A irradiation (20 J/cm²) caused transient redness of the back and ears of treated female guinea pigs • The author concluded, and this reviewer concurs, that these data

		show that finafloxacin produced a photoreaction
Report details	Study laboratory	(b) (4) [location not provided]
	File location	NDA module 4.2.3.7 Other Toxicity Studies
	GLP compliance	No
	Study initiation	June 25, 1996
	Test article	Finafloxacin ((b) (4) 14-1881, no batch # or purity reported) formulated in phosphate buffered salt solution
Methods	Species	Female guinea pigs (Hsd POC:DH)
	Procedure:	<ul style="list-style-type: none"> • The application area of the skin was shaved • One day later, groups of 5 female guinea pigs received a single dose by oral gavage (1 ml/kg) of 0, 30 or 100 mg/kg finafloxacin • The animals were then irradiated “about 30 minutes with UV-A 20 J/cm²” • For the back, ear and eyelid, skin reactions were rated on an arbitrary 0-3 scale (no reaction to extreme reaction/swelling of the skin) for 3 days • Body weight was measured daily for 3 days
Skin reaction results		<ul style="list-style-type: none"> • For the irradiated control group, just-visible redness was observed on the back at 1 hour post-dose (score of 0.5). No other reactions were observed (back, eyelid, ear) • The treated groups exhibited back redness for 24 hours post-dose, with recovery by D2 • The treated groups showed barely visible ear redness at 1 and 3 hours post-dose, with recovery by 24 hours • No eyelid redness was observed for any animal

Title	Cytotoxicity of BA Y 35-3377 (BYK 60621) on cartilage cells from dogs and man <i>in vitro</i>	
Report #	PH 30691	
Key findings	<ul style="list-style-type: none"> • Finafloxacin was not chondrotoxic under the conditions tested • Fluoroquinolones, as a class, potentially target juvenile cartilage. • This <i>in vitro</i> screening study was performed to assess the potential chondrotoxicity of finafloxacin 	
Report details	Study laboratory	(b) (4) [location not reported]
	File location	NDA module 4.2.3.7 Other Toxicity Studies
	GLP	No

	compliance	
	Report date	2001
	Test article	Finafloxacin (BAY 33-3377; BKY 60621)
Cell types	<ul style="list-style-type: none"> • Dog chondrocytes were isolated from knee joints of 9 month old beagle dogs, used as controls in a separate experiment • Human chondrocytes were obtained from cartilage tissue obtained after surgery 	
Concentrations tested	1, 3, 10, 30, 100 µg/ml of finafloxacin	
Methods	Six assays were performed: <ul style="list-style-type: none"> • Cytotoxicity (neutral red uptake) – incubation time not reported • Mitochondrial dehydrogenase activity (MTT) – incubation time not reported • Mitochondrial inner membrane potential (rhodamine assay) – incubation time not reported • Intracellular ATP determination (72 hour incubation) • Cell proliferation – treatment periods of 2, 24, 48 and 72 hours • Collagen type II content (72 hour incubation) 	
Results	<ul style="list-style-type: none"> • No treatment-effects were observed in dog or human chondrocytes • The no observed effect concentration (NOEC) = 100 µg/ml, the highest dose tested. 	

Title	Comparison of the cytotoxicity of BAY 35-3377, (b) (4) 61-9997 and trovafloxacin in primary hepatocytes cultures of rats	
Report #	PH 31154	
Key findings	<ul style="list-style-type: none"> • Finafloxacin did not induce AST, ALT or LDH release into the media of rat primary hepatocytes exposed <i>in vitro</i> • This screening assay was conducted because some fluoroquinolones target the liver 	
Report details	Study laboratory	(b) (4) [location not reported]
	File location	NDA module 4.2.3.7 Other Toxicity Studies
	GLP compliance	No
	Report date	2001
	Test article	Finafloxacin ((b) (4) 35-3377)
Methods notes	<ul style="list-style-type: none"> • Primary hepatocyte cultures were prepared from male Wistar rats • The hepatocytes were cultured in “collagen sandwich gels” made of rat collagen, to maintain normal enzyme activity • Cells were exposed to 1, 3, 10, 30 or 100 µg/ml of finafloxacin for 7 days (media changed daily), or to other test articles, and then 	

	<p>grown without test article exposure for an additional 7 days</p> <ul style="list-style-type: none"> Media was collected from the cells daily, and measured for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) Note: No traditional method of cytotoxicity / viability was measured.
Result	Finafloxacin did not induce any treatment-related changes at any dose. NOEC = 100 µg/ml, the highest dose tested.

11 Integrated Summary and Safety Evaluation

11.1 Introduction

Finafloxacin is a member of the fluoroquinolone class of antibiotics

- Review of the Applicant's microbiology data is outside the scope of this review, and is deferred to the Clinical Microbiology discipline.
- Like other fluoroquinolones, the mechanism of action of finafloxacin is inhibition of bacterial DNA gyrase and topoisomerase IV, resulting in inhibition of bacterial cell division and bacterial cell death.

Finafloxacin was developed to retain biological activity at pH < 7, to treat sites of local infection

- AOE is swimmer's ear, inflammation and infection of the external ear canal. The Applicant reports that physiological pH is ~ 7.4, and the infected external ear canal has a local pH of ~ 6.0.
- Reportedly, ciprofloxacin and levofloxacin have reduced activity at pH 5.8 versus *P. aeruginosa* and *S. aureus* compared to finafloxacin (NDA module 2.4 Nonclinical Overview).
- Finafloxacin is amphoteric; its activity is dependent on the pH of the surrounding medium. The Applicant reports that their data demonstrate that finafloxacin's optimal pH range of 5.8 to 6.2, with reduced activity at pH (b) (4).

11.2 Topical otic safety

The safety of the AOE indication is supported by two 14-day toxicology studies in New Zealand rabbits, both conducted in compliance with good laboratory practices (GLP). Following twice-daily topical otic dosing (4 drops/dose, ~ 30 µl drop size):

- The first study detected minimal-to-mild local toxicity with finafloxacin hydroxide in phosphate buffer at pH 7.5. The high-dose (1.0% finafloxacin, ~ 2.18mg/animal/day) was the no observed adverse effect level (NOAEL) for systemic toxicity. This first rabbit study included clinical pathology (hematology, clinical chemistry, coagulation), and necropsy (organ weights, gross pathology and histopathology) for the ears (pinna and bulla contents), adrenals, brain, heart, kidney, liver, lungs, ovary, spleen, and testes.

- The second study tested finafloxacin (free base). The NOAEL was the highest dose tested, 1.2% (~ 2.78 mg/animal/day). Clinical pathology was assessed, but necropsy was limited to the ears (pinna and bulla).

Patient dose	Rabbit doses	Exposure margin
0.3%, 4 drops twice daily for seven days (0.72 mg/ear/day)	1.0%, 4 drops twice daily for 14 days (tolerable local effects, systemic NOAEL)	3.3-fold
	1.2% (NOAEL for local toxicity)	4-fold

11.3 Safety of middle ear dosing

The nonclinical toxicity data associate direct instillation of finafloxacin and its vehicle into the middle ear with mild hearing loss and local toxicity.

- Recovery has not been assessed.
- For these four studies, animals underwent surgery to expose the bulla (i.e. dosing was through the bony covering, not through the tympanic membrane)
- Using ≥ 20 dB hearing loss as the benchmark for biologically significant hearing loss, no significant hearing loss was attributable to finafloxacin exposure.

The chinchilla has been used as a research model for ototoxicity.

- Two single-dose studies were conducted in chinchillas, and finafloxacin was not associated with typical signs of middle ear toxicity (i.e. eyelid twitches, unsteady gait, head shake, head tilt, circling, or nystagmus) at doses up to 1.1% (5.5 mg/ear).
- Only the second study measured hearing loss, at one finafloxacin dose level (1.1%, 5.5 mg/ear). The auditory brainstem response (ABR) assay detected hearing loss attributable to the test vehicle compared to sham control.

Two GLP guinea pig studies were conducted, with direct middle-ear dosing.

- The first (28-day) study tested finafloxacin hydrochloride. The NOAEL for hearing loss was the high dose, 1.2% (~1.2 mg/animal/day). However, this study did not identify a NOAEL for local toxicity. The lowest dose (0.6%, ~0.6 mg/animal/day) was associated with minimal hyperplasia of the tympanic membrane, minimal hemorrhage, and increased incidence of pericanular bone fibrosis.
- The second (30-day) study tested finafloxacin free base in a different formulation (similar to the clinical formulation). Compared to saline control, the vehicle control was associated with hearing loss (at all frequencies tested) and local toxicity (bulla thickening, periosteal bone proliferation, and thickened tympanic membrane stroma). Finafloxacin did not exacerbate these toxicities.

Additional notes:

- This NDA is for AOE. The supporting clinical trials were conducted in patients with an intact tympanic membrane, but the Applicant's draft label does not propose to limit the patient population. These nonclinical middle-ear dosing data are potentially concerning for patients that lack an intact tympanic membrane (because more drug and vehicle might reach the middle ear).
- For humans, the lower range of hearing is as low as 12 Hz (for children and some adults); the upper range is ~ 20 kHz in most people. Below the audible range, the "infrasonic range" of ~ 4 to 16 Hz can be felt as physical body vibrations. Reportedly¹⁴, chinchillas hear ~ 50 Hz to 33 kHz. Reportedly¹⁵, guinea pigs hear ~ 54 Hz to 50 kHz.
- The decibel (dB) is a logarithmic unit; the energy necessary to increase sound pressure increases as a square of the sound. Therefore, the absolute ABR threshold as well as the ABR shift is important for review consideration. Zero to 10 dB may be considered the auditory threshold; sounds below 35 dB may be considered quiet; 40 dB is distracting; 45 to 60 dB is normal conversation.

Consideration of the excipients:

- As shown in table 2 above, the clinical otic formulation contains (b) (4) % tyloxapol, (b) (4) % hydroxyethyl cellulose, (b) (4) % sodium chloride, (b) (4) % magnesium chloride, 0.005% benzalkonium chloride (BAC) pH 6.0.
- The CDER Inactive Ingredient Guide¹⁶ lists:
 - Tyloxapol: multiple approved uses for ophthalmic solution and suspensions, up to 0.3%, and for topical suspension up to 0.05%
 - Hydroxyethyl cellulose: otic solutions up to 0.25%, buccal film up to 29.88 mg, ophthalmic exposure (solution and suspension) up to 0.686%, topical exposure (solution, gel, lotions) up to 1.75%, oral exposures (tablet, capsule, syrup) up to 3%
 - Magnesium chloride: intraocular exposures at 0.03%, intravenous injection at 10.2%, subcutaneous injection at 0.125%
 - BAC: auricular (otic) exposures up to 0.275%, ophthalmic exposures up to 0.9%, topical exposures up to 0.2%, intra-articular injection up to 0.02%, intramuscular injection up to 0.02%, nasal sprays up to 12.5%, inhalation solution up to 20%
- These previously allowed exposures to these excipients provides additional support for considering this NDA's proposed uses to be safe.

¹⁴ Hefner RS, Hefner HE. 1991. Behavioral hearing range of the chinchilla. Hear Res. 52(1):13-16.

¹⁵ Louisiana State University webpage: How well do dogs and other animals hear? Accessed via: <http://www.lsu.edu/deafness/HearingRange.html>

¹⁶ The internal confidential version was used to compile this section of the review, accessed via <http://intranetapps.test.fda.gov/scripts/iig/queryiig.cfm> . Note that a publically-accessible version is accessible via <http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm>

11.4 Systemic-route general toxicity studies

The nonclinical systemic-route general toxicity studies were not fully reviewed for this indication.

General systemic route-toxicology studies were conducted in rats and dogs. Wistar rats received oral doses as high as 500 mg/kg for 4 weeks, and intravenous (iv) doses as high as 320 mg/kg for 14 days. Beagle dogs received oral doses as high as 90 mg/kg for 4 weeks, and iv doses as high as 45 mg/kg for 14 days. In dogs, the oral no observed adverse effect level (NOAEL) is 10 mg/kg, based on arthrotoxicity. In rats, the oral NOAEL is 30 mg/kg, based on male reproductive system toxicity (decreased sperm counts and motility; toxicity to the testis, epididymis, prostate). The liver was identified as a potential target organ for systemic administration.

11.5 Safety pharmacology

Safety pharmacology studies were not required to support the AOE indication, but studies were completed to support other routes of exposure, and these studies were submitted to this NDA for completeness. The results are not concerning for the AOE indication.

11.6 Human equivalent dose (HED) and exposure margin notes

- The proposed dose is “4 drops into the affected ear twice daily for seven days. ... For patients requiring use of an otowick, the initial dose can be doubled (to 8 drops), followed by 4 drops instilled into the affected ear twice daily”
- The annotated draft label (NDA module 1.14.1) submitted in the original NDA (4/25/2014) reports (in section 12.2 Pharmacokinetics) that “Finafloxacin plasma concentrations ^{(b) (4)} repeated ototopical doses of TRADENAME (finafloxacin otic suspension), 0.3%. In healthy subjects administered 4 drops in each ear [twice] daily for seven days quantifiable finafloxacin concentrations were observed in ^{(b) (4)} 2 of 14 subjects ^{(b) (4)}; and these concentrations were just above the quantitation limit (0.05 ng/mL).”
 - This reviewer defers to the Clinical Pharmacology reviewer regarding the review of this claim.
- The annotated label references clinical trial # C-10-007 (report # TDOC-0015247).
 - From the clinical report (page 4): “Following a single ototopical dose of AL-60371 Otic Suspension, 0.3% in both ears, the 1 and 4 hours post-dose samples on Day 1 from Subject 1018 showed plasma levels of 0.0534 ng/mL and 0.0603 ng/mL, respectively. Following twice daily ototopical dosing in both ears for 7.5 days, the 12 hour post-dose sample on Day 8 from Subject 1011 showed a plasma level of 0.0812 ng/mL. Plasma samples from these 2 subjects at all other sampling time points were below the quantitation limit.”
 - The LLOQ is reported as 0.05 ng/ml
 - Samples were measured on D1 and D8 pre-dose and post-dose at 10, 20, 30 45 minutes, 1, 2, 4, 6, 8 and 12 hours

- Patient 1018 had measurable concentrations at 1 hour (but not 2 hours) and at 4 hours
- Many of the 12 hour time points were listed as “not evaluable” rather than “present >LLOQ”. This reviewer did not find an explanation for why the 12 hour samples were not evaluable (e.g. were the other samples detectable but below LLOQ, and the 12 hour samples were non-detect?). One sample is listed as “No Sample... Missing”, suggesting that non-evaluable was not used to mean missing.
- PK data from a second trial is also available, trial # C-10-022 (TDOC-0015502)
 - This trial reports quantifiable concentrations: 0.12, 0.100, 0.0735, 0.141, 0.234 ng/ml, as well as the data from the previous trial (0.0535 to 0.0812 ng/ml)
 - From the report (page 3):

“Pharmacokinetic Results:
Quantifiable AL-60371 concentrations (> 0.05 ng/mL) were observed in plasma samples from 2 of the 36 patients. Plasma concentrations of AL-60371 were not quantifiable (< 0.05 ng/mL) in all other samples collected. As a result, there were insufficient data to determine pharmacokinetic parameters. Individual patient listings of AL-60371 concentrations are presented in Table 14.2.-1 (page 55).

Patient 3006 (male, 4 drops without otowick) had quantifiable levels in his 0.5 (0.12 ng/mL), 1 (0.100 ng/mL) and 2 hour (0.0735 ng/mL) plasma samples. Patient 2109 (female, 8 drops with otowick) had quantifiable levels in her 1 (0.141 ng/mL) and 2 hour (0.234 ng/mL) plasma samples. Similarly low plasma levels were also observed in healthy patients (without otowicks) who were administered 4 drops per ear of AL-60371 Otic Suspension, 0.3% (Alcon study C-10-007). In the healthy patients, 2 of 14 patients had quantifiable concentrations at 3 time points ranging from 0.0534 ng/mL to 0.0812 ng/mL.

Systemic exposure following ototopical doses of AL-60371 Otic Suspension, 0.3% is extremely low with levels in nearly all samples collected being below the quantitation limit of a sensitive mass spectrometry assay with a lower quantitation limit of 0.05 ng/mL (50 picograms/mL). Due to insufficient plasma concentration data, it is not possible to characterize the systemic pharmacokinetic parameters of AL-60371.”

As a benchmark for AOE labeling, this reviewer chooses the clinical plasma value of **0.234 ng/ml** for repeat-dose 0.3% ototopical dosing, the highest plasma level detected after 8 drops (i.e. with otowick).

- The higher plasma concentration following use of the otowick is expected, because the patients receive 8 drops instead of 4.
- The otowick will only be used for the first dose, and therefore using it as a benchmark is protective.
- Patient 3006 received half the otowick dose (i.e. 4 drops without otowick), and the highest plasma concentration detected was 0.12 mg/ml (slightly more than half). This finding supports the conclusion that 0.234 ng/ml is a valid measurement.

11.7 Assessment of fertility

One of the topical otic rabbit studies assessed testes histology; no finafloxacin-toxicity was apparent.

The results of systemic-route administration are not relevant to otic dosing. For context:

- The rat toxicity studies consistently observed finafloxacin-toxicity in the male reproductive tract. A 4-week iv study (report # 8245014) identified the NOAEL as 30 mg/kg, based on decreased sperm count and motility ≥ 40 mg/kg. The C_{max} for males at 30 mg/kg was 34,900 ng/ml.
- A stand-alone fertility study in rats was conducted. The NOAEL for male and female fertility was 100 mg/kg/day. The male 500 mg/kg group was completely infertile, and exhibited reduced sperm count, essentially no sperm motility. TK was not assessed.
- Clinical systemic TK has a lower limit of quantitation (LLOQ) of 0.05 ng/ml. Plasma levels following exposure to 0.3% finafloxacin are usually below the LLOQ; the range of quantified concentrations is 0.0535 to 0.234 ng/ml. Comparing the systemic C_{max} for the rat NOAEL (34,900 ng/ml) to the highest detected patient plasma concentration (0.234 ng/ml), the exposure margin for male infertility is at least 149,000-fold.

11.8 Assessment of developmental toxicity

In oral-dosing embryofetal studies, finafloxacin was clearly teratogenic.

- A rabbit oral range-finding study (1 female/dose level) was conducted. At 1 mg/kg, one fetus was “severely malformed... spina bifida, phocomelia”. Maternal weight loss and reduced food consumption were observed ≥ 5 mg/kg.
- For the rabbit GLP embryofetal study:
 - No developmental NOAEL. The low-dose of 1 mg/kg was associated with exencephaly and enlarged fontanel, paw hyperflexure, missing lumbar vertebra-7, missing lumbar arch-7, and sternebra-1 fusion.
 - The NOAEL for reproductive toxicity was 3 mg/kg (based on increased preimplantation loss at 9 mg/kg).
 - NOAEL for maternal general toxicity was 9 mg/kg, the highest dose tested. Reduced food consumption was apparent at all dose levels, and is considered evidence of pharmacologic activity rather than toxicity because body weight was not affected.
 - This reviewer notes that the doses tested were lower than have been tested for other fluoroquinolone drugs in the rabbit embryofetal assay.
 - The rabbit study (report # 37/2001) reports that a range-finding embryofetal study was conducted, 1 to 15 mg/kg, with reduced food consumption observed.
 - The GLP rabbit embryofetal study tested doses of 1, 3 and 9 mg/kg.
 - Based on the observation of terata in both the range-finder and confirmatory GLP study, this reviewer concludes that dose selection was adequate.

- It is notable, per ICH S5(R2) section 3.1 Dosages, that neither study reported that the doses tested reduced body weight, reduced body weight gain, or caused specific target organ toxicity.
- For the rat GLP embryofetal study:
 - Developmental NOAEL = 30 mg/kg. LOAEL = 100 mg/kg, based on exencephaly. Additional toxicities at 500 mg/kg.
 - Reproductive NOAEL = 100 mg/kg, LOAEL = 500 mg/kg based on increased preimplantation loss
- Estimating exposure margins for these developmental toxicity dose levels is difficult, because these studies did not measure systemic TK.
 - The Applicant notes an oral dose of 100 mg/kg in a 4-week rat oral repeat dose toxicity study (report # 197/2000) resulted in a C_{max} of 13.9 mg/L (13,900 ng/ml). Using this parameter as a benchmark, the exposure margin (from the clinical plasma concentration of 0.234 ng/ml) would be 59,400-fold.
 - The same 4-week rat oral repeat-dose toxicity study tested an oral dose of 20 mg/kg, which resulted in a C_{max} of 3.83 mg/L (3830 ng/ml), a 16,368-fold exposure margin. These data were used to interpolate a C_{max} for the rat embryofetal study's 20 mg/kg dose.
 - No rabbit TK data are available for finafloxacin. The rat toxicity studies tested doses much higher than 1 mg/kg. However, the pregnant rat radiolabel distribution study (report # 0026507) gave a single oral gavage dose of 2 mg/kg on gestation day (GD) 12 or 18. Using the GD 12 C_{max} as a benchmark (622 ng/ml), the exposure margin would be 2658-fold.
 - The above empirical data points were used to extrapolate a plasma C_{max} at 1 mg/kg for the rabbit embryofetal studies

Table 59: Exposure margin calculations for the fertility and developmental toxicity studies

Exposure	Calculations		Exposure margin (compared to the patient benchmark of 0.234 ng/ml)
Clinical exposure	LLOQ	0.05 ng/ml	
	Range (when quantifiable)	0.0535 to 0.234 ng/ml	
	Benchmark for exposure margin calculation	0.234 ng/ml	
Male rat fertility	NOAEL = 30 mg/kg	C_{max} = 34,900 ng/ml	149,145 x
Female rat teratogenicity	Rat dose of 20 mg/kg from another study (used to interpolate)	Female C_{max} = 3830 ng/ml	16,368 x

	NOAEL = 30 mg/kg	C_{max} estimated at 5089 ng/ml	21,748 x
	Rat dose of 100 mg/kg from another study	Female C_{max} = 13,900 ng/ml	59,400 x
	LOAEL = 100 mg/kg	C_{max} = 13,900 ng/ml	59,400 x
Rabbit teratogenicity	Rat 2 mg/kg dose (used to extrapolate)	C_{max} = 622 to 809 ng/ml	2658 x (at 622ng/ml)
	LOAEL = 1 mg/kg	C_{max} estimated at 311 ng/ml	1329 x
	High-dose = 9 mg/kg	C_{max} estimated at 1870 ng/ml	7991 x

Shaded cells represented interpolated/extrapolated C_{max} levels (based on oral dose rat TK data).

11.9 Assessment of potential phototoxicity

- Finafloxacin was not phototoxic under the conditions tested *in vitro* (report # PH 28757) or *in vivo* (report # PH 29356).
- A photo-HPRT assay (report # PH-30910) was attempted, but the test conditions were not adequate to evaluate photomutagenicity.

11.10 Genotoxicity

Overall, finafloxacin was positive for mutagenicity *in vitro*, and for clastogenicity *in vitro* and *in vivo*.

Table 60: Genotoxicity summary

Report #	GLP status	Assay	P/T reviewer's conclusions			Author's conclusions	
			±	Adequacy (Y/N)	Notes	±	Notes
198/2000	Y	Ames	+	Y	+ in TA102 only	+	Same as P/T reviewer
146/2001	Y	Mouse lymphoma assay (MLA) <i>in vitro</i>	+	Y	<ul style="list-style-type: none"> Clearly positive ± S9 dose-responses apparent No colony size shift 	+	<ul style="list-style-type: none"> as P/T reviewer concluded that finafloxacin caused a large→small colony shift
2808/17	Y	Mutagenicity in mammalian cells <i>in vitro</i> (TK locus)	+	Y	<ul style="list-style-type: none"> positive ± S9 dose-responses apparent 	+	Same as P/T reviewer
PH 30802	Y	Mutagenicity in mammalian cells <i>in vitro</i> (HPRT locus)	+	<ul style="list-style-type: none"> adequate to support a positive finding would be inadequate if the study were negative 	<ul style="list-style-type: none"> positive ± S9 sensitivity of the study limited by concentration selection 	-	Author did not consider the increases "biologically relevant" because the assessment criteria were not met (i.e. positive responses, when observed, were in only one but not both of the parallel cultures)
PH-30910	Y	Photo-activation test for mutagenicity in mammalian cells <i>in vitro</i> (HPRT locus)	No call	Inadequate	<ul style="list-style-type: none"> irradiated negative and positive controls overlapped No evidence of a positive response for finafloxacin 	-	Author did not comment on the overlap
276/2000	Y	<i>In vitro</i> micronucleus assay	+	Y	<ul style="list-style-type: none"> Clear positive + S9 only Dose-response apparent 	+	Same as P/T reviewer
PH 25561	N	Pilot mouse micronucleus	-	<ul style="list-style-type: none"> Inadequate Not GLP 		-	

		assay		<ul style="list-style-type: none"> No positive or negative controls to verify assay sensitivity 			
70/2001	Y	Mouse micronucleus assay	+	Y	Positive in males only, at 24 hr and 48 hr	+	Same as P/T reviewer
2808/10	Y	Mouse micronucleus assay	+	Inadequate, because only male mice were tested (i.e. to interrogate the results of 70/2001)	<ul style="list-style-type: none"> Positive at 24 hours (dose response apparent) Negative at 48 hours 	-	Because the first two statistical tests detected statistically significant differences at 24 hours, the author conducted a third statistical test

The Applicant notes (module 2.6.6 Toxicology Written Summary) that finafloxacin is a member of the fluoroquinolone class, which “as gyrase inhibitors suggest the risk of class-related genotoxic effect.”

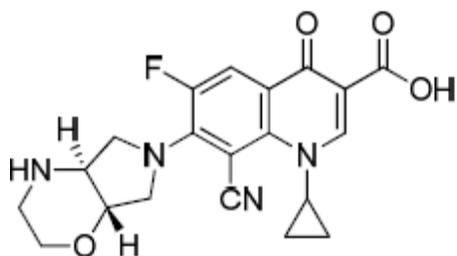
The Applicant considered third micronucleus study (report # 2808/10) to be negative, and therefore dismissed the positive results of the second micronucleus study (report 70/2001).

11.11 Structure-activity relationship (SAR) notes

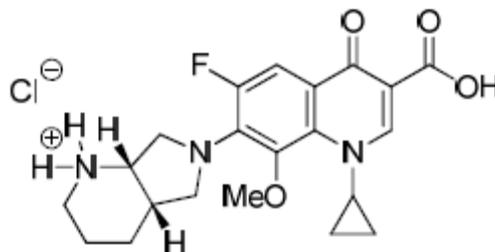
- The Applicant claims (NDA module 2.3 Quality Overall Summary) that finafloxacin is a fourth generation topical fluoroquinolone, with:
 - “enhanced activity against bacteria in an acidic environment (pH 5.5 - 6.2)”
 - Fluoroquinolone mechanism of action: “inhibition of both DNA gyrase and topoisomerase IV”
 - Enhanced Gram-positive activity relative to second generation fluoroquinolones

During the NDA review, FDA requested clarification regarding which fluoroquinolones the Applicant considered relevant for class effect claims regarding genotoxicity and carcinogenicity. The Applicant (Safety Information Amendment, submitted 8/13/2014) identified moxifloxacin:

Figure 9: Comparison of the structures of finafloxacin and moxifloxacin



Finafloxacin (MOPY-11)

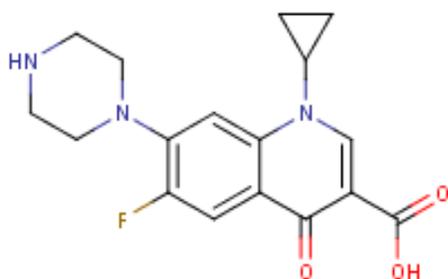


Moxifloxacin Hydrochloride

The Sponsor

previously noted (report # FIN0806) the structural similarity between finafloxacin, pradofloxacin¹⁷ (a veterinary quinolone), and ciprofloxacin¹⁸.

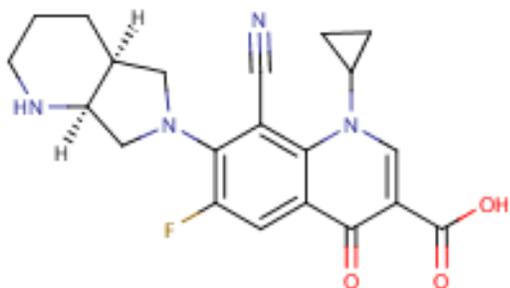
Figure 10: Structure of ciprofloxacin



¹⁷ For the new animal drug application (NADA) 141-344, a published version of the freedom of information act summary was accessed via:
<http://www.fda.gov/downloads/animalveterinary/products/approvedanimaldrugproducts/oiadruugsummaries/ucm338132.pdf>

¹⁸ Bayer Healthcare's NDA 019537 label dated 8/14/2013 accessed via:
http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/019537s082,020780s040lbl.pdf

Figure 11: Structure of pradofloxacin



12 Appendix/Attachments

Appendix 1. Labeling notes and deliberative comments

The Applicant submitted the original NDA on 4/25/2014, with draft labeling. As of 8/08/2014, no revised labeling has been submitted by the Applicant.

As benchmarks, the following labels were considered:

- The 2009 label for Cetraxal® (ciprofloxacin otic solution) for treatment of acute otitis externa¹⁹
- The 2003 label for Floxacin Otic® (ofloxacin otic solution for otic use in treatment of infections caused by susceptible isolates)²⁰
- For moxifloxacin, Bayer's 2013 label of oral use (Avelox®)²¹ and Alcon's 2011 label for ophthalmic use (Vigamox®)²²

Key: red/strike-through for notable deletions from the Applicant's text. Blue/bold for small additions in the P/T recommendations.

¹⁹ The 2009 label for NDA 021918 was accessed via:

www.accessdata.fda.gov/drugsatfda_docs/label/2009/021918lbl.pdf

²⁰ The 2003 label for NDA 20-799/S-012 was accessed via:

www.accessdata.fda.gov/drugsatfda_docs/label/2003/20799slr012_floxin_lbl.pdf

²¹ The 2013 label for NDA 021085/S-057 accessed via:

www.accessdata.fda.gov/drugsatfda_docs/label/2011/021598s017lbl.pdf

²² The 2011 label for NDA 21-598/S-017 was accessed via:

www.accessdata.fda.gov/drugsatfda_docs/label/2011/021598s017lbl.pdf

Applicant's 4/25/2014 label	P/T recommended wording	P/T notes
8.1 Pregnancy		
<p>(b) (4)</p> <p>Pregnancy Category C:</p>	<p>8.1 Pregnancy <i>Pregnancy Category C.</i> <i>Risk Summary</i> There are no adequate or well-controlled studies with [NAME] in pregnant women. Finafloxacin was shown to be teratogenic in rabbits and rats following oral administration. Neural tube defects and skeletal anomalies in both species and limb anomalies in rabbits were observed at exposures estimated to be 1300 (b) (4) times the maximum human systemic exposure following topical otic administration of 0.3% finafloxacin. Because animal studies are not always predictive of human responses, [NAME] should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.</p>	<p>The header (b) (4) should be struck.</p> <p>Added Risk Summary and standard PLR language.</p>
<p>(b) (4)</p>	<p><i>Animal Data</i> In rabbit embryofetal studies, no adverse maternal toxicity was observed at oral doses up to 9 mg/kg/day (estimated 8000 times the maximum human systemic exposure following topical otic administration with 0.3% finafloxacin). Fetal toxicity was observed at the lowest dose tested, 1 mg/kg/day (estimated to be 1300 times the maximum human systemic exposure following topical otic administration with 0.3% finafloxacin), and included exencephaly, enlarged fontanel, spina bifida, phocomelia, paw hyperflexure, missing lumbar vertebra, missing lumbar arch, and sternebra fusion.</p>	<ul style="list-style-type: none"> The Applicant omitted mention of the (b) (4). This appears to be an oversight. (b) (4)
<p>(b) (4)</p>	<p>In a rat embryofetal study, no adverse maternal toxicity was observed at oral doses up to 100 mg/kg/day (estimated 60,000 times the maximum human systemic exposure following topical otic administration with</p>	<p>The Applicant did not consider the single incidence of (b) (4) to be treatment-related, but did not provide supporting historical control data. The public historical control data identified indicate that</p>

<p>(b) (4)</p>	<p>0.3% fluroxacin). The developmental no observed adverse effect level (NOAEL) was 30 mg/kg (estimated 22,000 times the maximum human systemic exposure following topical otic administration with 0.3% fluroxacin). Exencephaly was observed in one fetus at 100 mg/kg. At 500 mg/kg, additional developmental toxicities were observed including increased preimplantation loss, decreased fetal weight, decreased placental weight, increased incidence of non-ossified sternebrae, and delayed ossifications in the sternebrae, xiphisternum, sacral arches, and metacarpals.</p>	<p>(b) (4) is rare in rats, and therefore supports classifying this observation as treatment-related.</p> <p>(b) (4)</p>
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8.3 Nursing Mothers

<p>(b) (4)</p>	<p>Fluroxacin has been identified in the milk of nursing rats following oral administration. Following oral administration of 2 mg/kg fluroxacin to nursing rats, concentrations of fluroxacin in rat milk were higher (up to 6.5-fold) than that detected in maternal plasma. Fluroxacin was not detected in milk or plasma at 24 hours post dose.</p> <p>The human systemic concentration of [NAME] following topical otic treatment is low [see Clinical Pharmacology (12.3)]. It is not known whether topical otic administration could result in sufficient systemic absorption to produce detectable quantities in the human milk.</p>	<ul style="list-style-type: none">• Report # TDOC-0015405• (b) (4)
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	<p>Caution should be exercised when finafloxacin is administered to a nursing mother.</p>	
<p>12.1 Mechanism of Action</p>		
<p>(b) (4)</p>	<p>[This reviewer defers to Clinical Microbiology]</p>	<ul style="list-style-type: none"> • P/T had requested additional information to support the mechanism of action (MOA) claim, which the Applicant provided (8/13/2014). • Note that other labels have a shorter MOA, e.g. "Moxifloxacin is a member of the fluoroquinolone class of anti-infective drugs (See 12.4 Microbiology)."
<p>13. NONCLINICAL TOXICOLOGY 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility</p>		
<p>(b) (4)</p>	<p>Animal studies have not been conducted to determine the carcinogenic potential of finafloxacin.</p>	<ul style="list-style-type: none"> • Applicant's proposed language is accurate. • Mentioning (b) (4) would be administratively problematic if another class member is subsequently shown to be carcinogenic. • (b) (4)
<p>(b) (4)</p>	<p>Finafloxacin was shown to be genotoxic and clastogenic <i>in vitro</i>, with and without metabolic activation, and <i>in vivo</i>.</p> <p>In a bacterial reverse mutation assay, finafloxacin was positive in only one strain (TA102).</p>	<ul style="list-style-type: none"> • (b) (4)

<p>(b) (4)</p> <p>[Redacted]</p>	<p>Finafloxacin was positive in mammalian cell culture assays: mouse lymphoma cell forward mutation assays, a mutagenicity assay in V79 Chinese hamster lung cells, and a micronucleus test in V79 cells.</p> <p>Finafloxacin was clastogenic in mouse micronucleus studies.</p>	<p>(b) (4)</p> <ul style="list-style-type: none">• The Applicant's proposed labeling is incorrect: <p>(b) (4)</p>
<p>(b) (4)</p> <p>[Redacted]</p>	<p>An oral rat fertility study detected a NOAEL for male and female fertility of 100 mg/kg/day (estimated 60,000 times the maximum human systemic exposure following topical otic administration with 0.3% finafloxacin). At 500 mg/kg/day, males were completely infertile due to low sperm count and sperm immotility.</p> <p>General toxicity studies in rats have confirmed sperm toxicity following oral and intravenous dosing. Following intravenous dosing, the NOAEL for sperm toxicity was 30 mg/kg/day (150,000 times the maximum human exposure following topical otic administration with 0.3% finafloxacin).</p>	<p>(b) (4)</p> <ul style="list-style-type: none">• [Redacted]

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ANDREW J MCDOUGAL
09/25/2014

LORI E KOTCH
09/25/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA Number: 206307

Applicant: Alcon Research, Ltd.

Stamp Date: April 25, 2014

Drug Name:

NDA Type: 505(b)1

- **Finafloxacin otic suspension**
- **Xtoro (proposed)**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		Only a partial audit was performed for filing. Two reports were identified that were not completely legible.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations?	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?		X	<ul style="list-style-type: none"> • The rat fertility study (report # 492006) was not conducted in accordance with GLP. At the pre-IND meeting (for IND 110576, held 11/3/2011), P/T concurred that the existing data (from IND (b) (4)) support filing and approval. • Yes for the other pivotal toxicology studies (Toxicology Written Summary, module 2.6.6.1)

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	Not applicable from a P/T perspective		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?		X	P/T data are presented either without reference to the clinical dose, using C _{max} , or based on administered dose (mg/kg). The feasibility of calculating P/T doses based on comparative plasma levels will be a review issue.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		This is a review issue.
11	Has the applicant addressed any abuse potential issues in the submission?	Not applicable, from a P/T perspective		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	Not applicable		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE?

YES

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

1. Provide human equivalent dose (HED) estimates for both pediatric and adult patients calculated by AUC and C_{max}, for each dose in each GLP toxicology study to the NDA, or indicate where in the NDA this information is located. Please provide the calculations and assumptions used.
2. Regarding the draft label Section 13.1 Carcinogenesis, mutagenesis, impairment of fertility, the support for the statement (b) (4) was not identified in the NDA. Either indicate where in the NDA the list was provided, or provide to the NDA a list of each fluoroquinolone in the class which you consider relevant to the evaluation of the carcinogenic potential of finafloxacin.
3. Regarding the draft label Section 13.1 Carcinogenesis, mutagenesis, impairment of fertility, the support for the statement (b) (4) was not identified in the NDA. Either indicate where in the NDA the list was provided, or provide to the

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA a list of each class to which you refer, including each class member which you consider relevant to the evaluation of the genotoxic potential of finafloxacin.

4. Regarding the draft label section 13.1 Animal Toxicology and/or Pharmacology, the language does not use the term [REDACTED] ^{(b) (4)}, although the term is used elsewhere in the NDA (e.g. module 2.6.6), and is used in the labels of other fluoroquinolones. Upon review of the NDA, Pharmacology/Toxicology may recommend explicit use of the term [REDACTED] ^{(b) (4)} in the finafloxacin label for class consistency. If there is a reason not to use [REDACTED] ^{(b) (4)}, please explain.
5. From a nonclinical perspective, the differences between AL-60371 and AL-60371A are unclear. In the Pharmacology Written Summary (module 2.6.2.1), you reported that “Finafloxacin hydrochloride (AL-60371A), the hydrochloride salt of finafloxacin, free base (AL-60371), was used in early *in vitro* studies. Due to stability issues, the free base was used in later *in vivo* studies.” The ongoing review notes that AL-60371A was used for oral dosing in the healthy volunteer trial # C-10-007, as well as one safety pharmacology study, several pharmacokinetic studies, and the 14-day GLP rabbit study (TDOC-0012256).
 - a. Either indicate where an explanation of the two test articles is provided in the NDA, or provide to the NDA an explanation and comparison of the physical and ADME properties of AL-60371 and 60371A.
 - b. Please verify that BYK60621 (CAS # 209342-41-6) is finafloxacin free base.
6. Initial review found that several of the nonclinical toxicology study reports (e.g. # 197/2000, 202/2009, 45/2001, 63/2001) have GLP comments regarding the computer systems that raise questions about the validity of the reports. It is not entirely clear why these comments were added to the reports. For each report with non-GLP computer systems: define and explain each abbreviation used to describe the computer problems; explain what was outside GLP; and explain if/how the issues affected the collection and analysis of data.
 - a. E.g. The 14-day rat study (report # 202/209) states (page 6), “Comment: Computer system usage and maintenance of the department FIT/OI (standard commercial components) did not fulfill GLP requirements. However, general system features and application-specific software design, documentation and validation assure data integrity.”
 - b. E.g. the rat 4-week oral toxicity study (report # 197/2000) states (page 5), “Comment: Computer system usage and maintenance of the department AOI (standard commercial components) do not fulfill GLP requirements.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

However, general systems features and application-specific software design, documentation and validation assure data integrity.”

- c. E.g. The rat embryofetal study (report # 63/2001) and the 2-week dog study (report # 45/2001) each state on page 5 that “Computer system usage and maintenance of the department ADI, FIT/OI or HIS, respectively (standard commercial components) did not fulfill GLP requirements before 1st January 2006. However, general systems features and application-specific software design, documentation and validation assure data integrity.”
7. Two of the (b) (4) study reports (#202/2009 and #45/2001) have lines of illegible text (presumably due to pdf rendering); the meaning is not always clear from the context. Submit fully legible study reports to the NDA.
8. Initial review of the GLP toxicology studies did not identify complete information regarding the test article characterization for all reports [e.g. certificates of analysis (COA), strength, purity, individual impurities, homogeneity, and stability]. For report # 49/2006 (non-GLP) and each GLP toxicology report lacking this information, either indicate where in the NDA the information is located, or provide the information to the NDA.
 - a. For the non-GLP rat fertility and early embryonic development study (report # 49/2006), review noted the summary information (page 8) but has not found the supporting information.
 - b. E.g. the rat embryofetal study (report # 63/2001). Review noted the summary information (page 13), with the statement “analysis of the test solutions was not performed.”
 - c. E.g. the rabbit embryofetal study (372001). Review noted the summary statements (page 11) but has not find supporting information.
 - d. E.g. the Ames study (report # 198/2000).
 - e. E.g. the two guinea pig studies (report # 0013396 and # 1108012).
 - f. E.g. a rat 2-week study (report # 202/2009).
 - g. E.g. the 4-week rat study (report #197/2000) mentioned in the draft label (section 8.1).
 - h. E.g. the iv dog study (report # 45/2001).
9. For the 4-week rat study (report #197/2000) and the iv dog study (report # 45/2001), the composition of the test article vehicle and the control test article was not clear upon initial review. Please indicate where in the study reports these data are located, or provide them to the NDA.
10. For the toxicology study reports, ensure that all sections written in German already have English translations in the study report, or provide English

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

translations to the NDA. For example, for report # 372001, it is not clear whether page 186 is completely translated.

11. Regarding the proposed draft label section 12.1 Mechanism of action, the initial review noted the submission of the three publications (Emrich et al. 2010; Higgins et al. 2010; Dalhoff et al. 2011). If you have other information supporting the proposed language “finafloxacin ... targets bacterial DNA gyrase and topoisomerase IV enzymes. ... Evidence that finafloxacin targets DNA gyrase and/or topoisomerase IV was shown”, please either indicate where in the NDA the data are located, or provide these data to the NDA.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ANDREW J MCDOUGAL
06/04/2014

LORI E KOTCH
06/04/2014