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APPLICATION NUMBER:

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NON-CLINICAL 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

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Product: Ozenoxacin Cream 1%
Indication: Topical treatment of Impetigo
Applicant: Ferrer Internacional, S.A., Barcelona, Spain
(US Licensing partner is Medimetriks
Pharmaceuticals, Inc. New Jersey)
Review Division: Division of Anti-infective Products
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1 Executive Summary

1.1 Introduction

Ferrer Internacional, S.A. has developed Ozenoxacin, a non-fluorinated quinolone antibiotic with bactericidal activity against pathogens commonly involved in acute bacterial skin and skin structure infections (ABSSSI). Ozenoxacin cream has been developed for short term topical use in the treatment of impetigo, a bacterial infection of the superficial epidermis that most often affects children. The proposed pharmaceutical form of ozenoxacin for commercial use is a 10 mg/g (1%) cream.

1.2 Brief Discussion of Nonclinical Findings

Ozenoxacin 1% had a low potential for dermal irritation in rats, rabbits and minipigs after repeated application. It was a moderate eye irritant after a single exposure in rabbits, but the animals recovered from the irritation. In addition, ozenoxacin cream and its vehicle were negative for contact sensitization potential in the murine local lymph node assay. Ozenoxacin 1% cream was found to have minimal systemic exposure with plasma concentrations less than the lowest level of quantitation when applied topically to intact and abraded skin in minipigs. In the 4-week repeat-dose study in minipigs, topical administration of ozenoxacin 1% cream on intact and abraded skin, was generally well tolerated, causing only mild erythema noted in all dose groups with the greatest incidence in the vehicle control groups, which however, decreased by the end of the 28-day recovery period. There were no systemic target organs of toxicity for ozenoxacin. In-vivo safety pharmacology studies showed that the drug had no effects on the cardiovascular, respiratory and central nervous system. Ozenoxacin did not have effects on fertility and reproduction in male and female rats but did exhibit minimal developmental toxicity as seen by significant delays in the ossification of rib pairs, significant alterations in the numbers of thoracic and lumbar vertebrae and low fetal weight in rats and rabbits. However, rabbits were more sensitive to developmental toxicity by ozenoxacin occurring at maternally toxic levels, which could be attributed to the antibacterial effect of ozenoxacin on the GI flora of these animals, and have been observed in similar studies of other antibacterial drugs. Safety pharmacology studies and reproductive/developmental toxicity studies were performed using the oral form of the drug and hence, findings in these studies are of limited relevance to the toxicity of ozenoxacin administered topically with minimal systemic exposure.

1.3 Recommendations

1.3.1 Approvability: From a Pharmacology/Toxicology perspective, there is no objection to the approval of Ozenoxacin (1% cream) for dermal application in the treatment of impetigo in adults and children aged 2 months and older.

1.3.2 Additional Non Clinical Recommendations: None

1.3.3 Labeling

Section 8.1 of the label was modified (b) (4)

A brief note on the nonclinical findings was added to the Risk Summary statement. (b) (4)

8.1 Pregnancy

Risk Summary

There are no available data on the use of (b) (4) in pregnant women to inform a drug associated risk. Systemic absorption of (b) (4) in humans is negligible following topical administration of (b) (4) (up to twice the concentration of the marketed formulation) [see *Clinical Pharmacology (12.3)*]. Due to the negligible systemic exposure, it is not expected that maternal use of (b) (4) will result in fetal exposure to the drug. Animal reproduction studies were not conducted with (b) (4). However, toxicity studies conducted in pregnant rats and rabbits administered the oral form of ozenoxacin showed no significant adverse developmental effects (at >10,000 times the maximum human plasma concentration seen with dermal application of ozenoxacin). The estimated background risk of major birth defects and miscarriage for the indicated population are unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

8.2 Lactation

Risk Summary

No data are available regarding the presence of ozenoxacin in human milk, the effects of ozenoxacin on the breastfed infant or on milk production. However, breastfeeding is not expected to result in exposure of the child to ozenoxacin due to the negligible systemic absorption of ozenoxacin in humans following topical administration of (b) (4). The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for (b) (4) and any potential adverse effects on the breast-fed child from (b) (4) or from the underlying maternal condition.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate carcinogenic potential have not been conducted with ozenoxacin.

Ozenoxacin demonstrated no genotoxicity when evaluated *in vitro* for gene mutation and/or chromosomal effects in the Ames test, mouse lymphoma cell assay, or when evaluated *in vivo* in a rat micronucleus test with demonstrated systemic exposure.

Oral doses of ozenoxacin did not affect mating and fertility in male and female rats treated up to 500 mg/kg/day (about 8500 and 16,000 times respectively, the maximum human plasma concentration seen with dermal application of ozenoxacin 1% cream).

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

245765-41-7

2.1.2 Generic Name

Ozenoxacin

2.1.3 Code Name

GF-001001-00, GF-001001-00-TC-12

2.1.4 Chemical Name

1-Cyclopropyl-8-methyl-7-(5-methyl-6-methylamino-pyridin-3-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

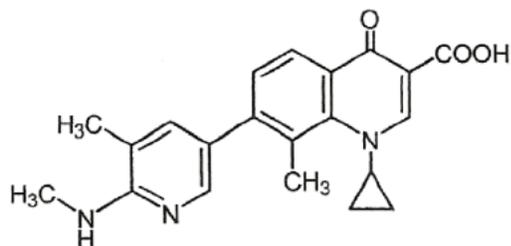
2.1.5 Molecular Formula/Molecular Weight

C₂₁H₂₁N₃O₃ / 363.41

2.1.6 Pharmacologic Class

Quinolone (non-fluorinated) antimicrobial

2.1.7 Structure or Biochemical Description:



2.2 Relevant IND, DMF:

IND 105567 (Ozenoxacin cream); DMF 25023 (Ozenoxacin)

2.3 Drug Formulation

Table 1 Drug Formulation for Ozenoxacin Cream, 1%

Ozenoxacin	1% w/w	(b) (4)
Benzoic acid		(b) (4)
(b) (4) (octyl dodecanol)		(b) (4)
Peglicol 5 oleate		(b) (4)
Pegoxol 7 stearate		(b) (4)
		(b) (4)
Stearyl alcohol		(b) (4)
Propylene glycol		(b) (4)
Purified water,		(b) (4)

2.4 Comments on Novel Excipients

There were no novel excipients in the drug product formulation. However, the excipient Peglicol 5 Oleate (b) (4) constitutes (b) (4) % of the ozenoxacin cream, which (b) (4) % w/w level found in other FDA approved topical cream/emulsion products. However, this level was qualified in the nonclinical studies (RR-080614-01 and RR-090768-01) conducted with the clinical formulation of ozenoxacin to support the clinical development of this drug product. None of the studies suggested that components of the cream are inappropriate for their intended use in this product. (Refer meeting – preliminary comments under IND 105567 dated 1/14/2016).

2.5 Comments on Impurities/Degradants of Concern

The drug substance impurities (b) (4) are proposed at NMT (b) (4)% which is above the ICH qualification threshold. With the current clinical dose, a daily exposure of (b) (4) mg of each impurity is expected (i.e. (b) (4) mg/kg/day or (b) (4) mg/m² of the impurities since ozenoxacin is applied dermally at (b) (4) mg/day or (b) (4) mg/cm² per day per 100 cm² to patients). However, the Applicant conducted a rat micronucleus assay to assess the genotoxic potential of the impurities and to qualify the amount of (b) (4) at the clinical dose. The micronucleus assay (Study RR-090779-01) reviewed below in section 7, tested the impurities (b) (4) orally in rats up to 2000 mg/kg/day (human equivalent dose of 320 mg/kg/day or 12,000 mg/m²) and showed no clastogenic effects or cytotoxicity to the bone marrow.

Therefore, the impurities were tested at about (b) (4) times the human topical dose of Ozenoxacin cream, 1%, based on a body surface area comparison, and found to be non-genotoxic. Hence, the micronucleus assay qualifies the impurities at the Applicant's proposed limits of NMT (b) (4)%.

2.6 Proposed Clinical Population and Dosing Regimen

Ozenoxacin 1% cream is intended for short term topical treatment of impetigo in adults and children aged 2 months and older. Each gram of the cream contains 10 mg of ozenoxacin, and is to be applied as a thin layer to the affected area (up to 100 cm² in adult and pediatric patients 12 years of age and older or 2% total body surface area in pediatric patients aged 2 months to 11 years) twice daily for 5 days.

2.7 Regulatory Background

The initial IND was submitted in February 2010. Three meetings were held with the Applicant to discuss the developmental program for ozenoxacin cream, 1%. FDA meeting minutes were provided for each of these meetings. The first meeting was a pre-IND held on September 28, 2009. In the meeting, the agency agreed that no carcinogenicity studies would be required for this drug product. (Refer meeting minutes in IND 105567 dated 10/9/2009); the second meeting was an End of Phase 2 held on June 10, 2011, and the third meeting was a pre-NDA on January 13, 2016. In the pre-NDA correspondence, the agency qualified the amount of (b) (4)% w/w) in the formulation. (Refer section 2.4 above).

3 Studies Submitted

3.1 Studies Reviewed

The following studies were reviewed by Dr. Amy Ellis under IND 105567. Some of the relevant data from these studies have been summarized in the sections below and in the Integrated Summary section of this review.

1. GF-001001-00 Cream Formulation (GF-001001-00-TC-28 and GF-001001-00-TP-02): Dermal and Ocular Tolerance Study in Male Rabbits (RR-080568-01)
2. GF-001001-00 Creams (GF-001001-00-TC-28, GF-001001-00-TC-12, and GF-001001-00-TC-32): Murine Local Lymph Node Assay (MLLNA) for the Assessment of Contact Sensitization Potential (RR-080614-01)
3. GF-001001-00: A 28-Day Toxicity Study in Rats by the Dermal Route with a 14-Day Recovery Period (RR-040149-01)
4. GF-001001-00: A 28-Day Toxicity Study in Minipigs by the Dermal Route with a 14-Day Recovery Period (P-040150-01)
5. GF-001001-00: 4-Week Toxicity Study in Beagle Dogs with Repeated Oral Administration and a 2-Week Recovery Period (RR-030078-01)
6. GF-001001-00: 28-Day Repeated Dose Oral Toxicity Study in Rats with 14-Day Recovery Period (RR-030050-01)
7. GF-001001-00: Bacterial Reverse Mutation Test (RR030070-01)
8. GF-001001-00: *In Vivo* Rat Bone Marrow Micronucleus Assay (RR-040128-01)
9. GF-001001-00: Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells (RR-040098-01)
10. GF-001001-00: Determination of Phototoxicity in Albino Guinea Pigs (RR-030086-01)
11. GF-001001-00: Determination of Photoallergenicity in Albino Guinea Pigs (Including Information about Allergenicity, Photoirritation, and Irritation) (RR-040119-01)
12. GF-001001-00: Contact Hypersensitivity in Albino Guinea Pigs, Maximization Test (RR- 040157-01)

The following studies were reviewed for this NDA:

1. GF-001001-00: Effect on the Cardiovascular System and ECG in Conscious Dogs Monitored by Telemetry (RR-050413-01)
2. Effects of GF-001001-00 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (RR-050414-01)
3. GF-001001-00: Study on the Respiratory Parameters in the Freely Moving Conscious Rat by Whole Body Plethysmography (RR-050415-01)
4. GF-001001-00: Comprehensive Observational Assessment. Irwin Test in the Rat (RR-050411-01)
5. GF-001001-00 Cream (GF-001001-00-TC-12 and GF-001001-00-TC-29) and Ointment Formulations (GF-001001-00-PD-13 and GF- 001001-00-PD-21): Comparative Study of Exposure After 14-Day Repeated Dose Administration by the Dermal Route to Minipigs (RR-080574-01)
6. GF -001001-00: The Disposition and Tissue Distribution of Total Radioactivity in the Rat Following Intravenous and Dermal Administration of [¹⁴C] (RR-080604-01)
7. GF-001001-00 Cream Formulation (GF-001001-00TC-12): A 28-Day Dermal Toxicity Study in Minipigs with a 14-Day Recovery Period. (RR-090768-01).
8. GF-001001-00: Oral (Gavage) Fertility and General Reproduction Toxicity Study in Rats (RR-090725-01).
9. GF-001001-00: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Behavioral/Functional Evaluation. (RR-090726-01).
10. GF-001001-00: Developmental Toxicity Study in Rats by Oral (Gavage) Route. (RR-090722-01).
11. GF-001001-00: Oral (Stomach Tube) Developmental Toxicity Study in Rabbits, Including a Satellite Toxicokinetic Evaluation (RR-090724-01)
12. GF-001001-00 impurities (^{(b) (4)}): In Vivo Rat Bone Marrow Micronucleus Assay (RR-090779-01).

3.2 Previous Reviews Referenced

Filing review for current application dated 8/16/2016; IND 105567 non-clinical reviews by Dr. Amy Ellis dated 9/22/2009, 10/1/2009, 6/14/2011 and 8/26/2011.

3.3 Studies not reviewed

1. GF-001001-00: Effect Of Intravenous Infusion On QT-Interval In Anaesthetized Guinea Pigs. (RR-040107-01)
2. GF-001001-00: Dermal Formulation 1% Study Of The In Vitro Percutaneous Absorption Through Juvenile Minipig Skin. (RR-100866-01)
3. GF-001001-00. Pharmacokinetic Plasma Levels After Repeated Topical Administration In Rats And Its Correlation With In Vivo Antimicrobial Activity. (RR-030090-01).
4. GF-001001-00: Pharmacokinetics After Single Topical, Oral And Intravenous Administration To Dogs (RR-040095-01)
5. GF-001001-00: The Disposition of Total Radioactivity in the Minipig Following Intravenous and Dermal Administration of [¹⁴C]. (RR-100829-01)
6. GF-001001-00: Determination of the In Vitro Binding of [¹⁴C] to the Plasma Proteins and Blood Cell Partitioning in Mouse, Rat, Dog, Rabbit and Human. (RR-090688-01)
7. GF-001 001-00: Species comparison metabolism with freshly isolated rat, mouse, rabbit, dog, Cynomolgus monkey, mini-pig and human hepatocytes. RR-070499-01).
8. GF-001001-00: Pharmacokinetics After Single Topical, Oral And Intravenous Administration To Rats. (RR-040096-01).
9. GF-001001-00: Acute I.V. Toxicity study in rats. (I-000028-02).
10. GF-001001-00: 2-Week Dose-Range-Finding Study In Beagle Dogs By Oral Administration (RR-030069-01).

4 Pharmacology

4.1 Primary Pharmacology

Ozenoxacin acts by inhibiting DNA replication by blocking bacterial enzymes, DNA gyrase and topoisomerase IV. Primary Pharmacology studies to assess efficacy of ozenoxacin 1% have been reviewed by the clinical microbiology reviewer, Dr. Avery Goodwin.

4.2 Secondary Pharmacology

No secondary pharmacology studies were conducted with ozenoxacin.

4.3 Safety Pharmacology

Reviewer's notes: Safety pharmacology studies with ozenoxacin (GF-001001-00) have been briefly summarized below. The findings of this study are of limited relevance to this drug product because of its lack of systemic exposure when administered via the dermal route.

Cardiovascular system:

In the GLP hERG assay (Study: RR-050414-01), two concentrations of GF-001001-00 (1.7 and 17 μ M) were tested. Slight hERG channel inhibition at 17 μ M was observed as indicated by reduced amplitude of outward tail currents (18.0%). IC₅₀ of the drug was not determined. The positive control terfenadine at 60 nM produced 74.6% inhibition of hERG potassium current. In the GLP in-vivo cardiovascular study (Study RR-050413-01), four male and four female dogs were administered vehicle and 3 doses of GF-001001-00 (50, 250, and 500 mg/kg) with a 7 day washout period between each treatment until each animal received all treatments in ascending order. All treatments were administered once orally. Only descriptive statistics were done to analyze QT intervals and other cardiovascular parameters. A board certified veterinary cardiologist conducted a qualitative review of ECGs obtained prior to dosing, 1.5-2.5 and 19-20 hours post-dose and found no qualitative ECG abnormalities that were caused by the drug.

Considering minimal systemic exposure with this drug product via the dermal route and no drug-related adverse cardiovascular events in the repeat-dose dermal toxicology study on intact and abraded skin in minipigs, from a Pharmacology/Toxicology perspective, qualitative analysis of the data (Study RR-050413-01) was considered sufficient and the Applicant was not asked for any further evaluation of the data.

Respiratory system:

In a GLP study (Study RR-050415-01) using 6 male SD rats/treatment group, 3 doses of the drug GF-001001-00 (125, 850 and 2000 mg/kg) was administered by oral gavage as a single dose. There were no toxicologically relevant effects of the drug on tidal volume, respiratory rate and minute volume in any of the treatment groups compared to controls.

Central Nervous System:

Oral administration of GF-001001-00 at dose levels of 125, 850 or 2000 mg/kg produced no behavioral or physiological changes in rats when compared to vehicle treated animals (Study RR-050411-01).

5. Pharmacokinetics/ADME

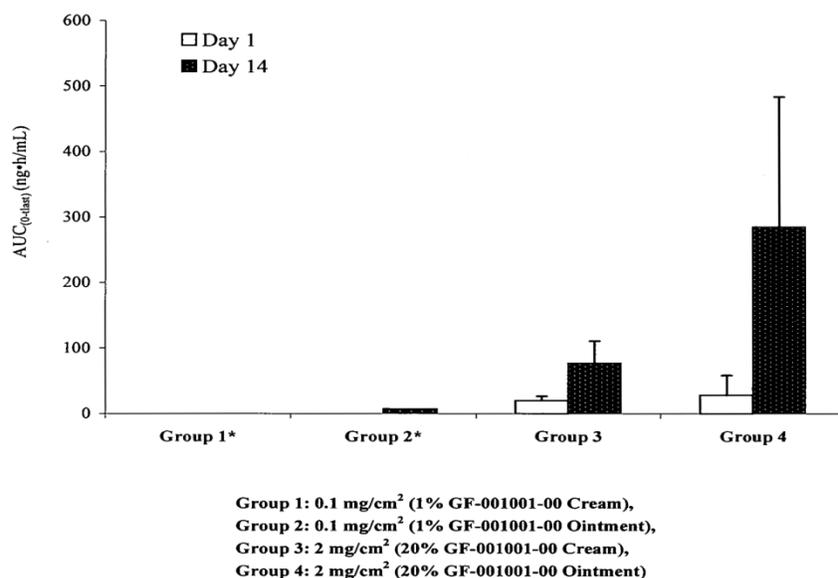
5.1 PK/ADME

PK/ADME was analyzed in a study conducted in male SD rats administered a single dermal application of radiolabeled GF-001001-00 at a dose volume of 100 mg/animal to achieve a dose level of 0.2 mg/cm² (Study RR-080604-01). Following dermal administration, total radioactivity concentrations were below the lower limit of quantitation in the plasma, liver, and other general tissues. Main route of elimination after dermal application was through feces accounting for a mean of 2.5% of the applied dose indicating low absorption of the drug via the skin. GF-001001-00 was excreted mainly unchanged indicating low metabolism when administered dermally.

PK study (Study RR-080574-01) via a dermal route of administration was performed using 1% (intended clinical dose) and 20% (maximum feasible dose) of GF-001001-00 in minipigs administered once daily for 14 days. Quantifiable levels of GF-001001-00 were not observed in the plasma on the first day of the study at the low dose level (1% or 0.1 mg/cm²) following dermal application of GF-001001-00 cream or ointment. However, repeated once daily administration of GF-001001-00 cream and ointment for 14 days was found to result in considerable increase (see table 2, below) in the systemic exposure parameters (C_{max} and AUC_{0-t}) at the high dose level (2 mg/cm²). At the high dose level, overall systemic exposure parameters were approximately 4-10 times higher on Day 14 than on Day 1, suggesting accumulation of the test article on repeated dosing at this dose. $T_{1/2}$ of the test article ranged between 4.01 and 6.69 hours.

Table 2 Comparison of AUC_{0-t} after repeated dermal administration

Figure 3 Comparison of $AUC_{(0-tlast)}$ GF-001001-00 in the Male Gottingen Minipig Plasma Following Repeated Dermal Dose Administration of GF-001001-00



5.2 Toxicokinetics

Toxicokinetic studies are summarized in Sections 6, 7 and 9 under each study reviewed.

6 General Toxicology

Review of studies submitted for IND 105567

Repeat-dose toxicity studies were conducted with GF-001001-00 prior to the initiation of clinical trials to determine systemic exposure and target organs of toxicity. These studies were reviewed by Dr. Amy Ellis for IND 105567. The dermal irritation potential of 2% ozenoxacin cream and its vehicle was mild in male rabbits when the test articles were applied on the skin for 6 hours daily for 2 weeks. Abrading the skin did not significantly enhance irritation potential. Ozenoxacin was moderately irritating to the rabbit eye after a single exposure without wash-out, but the eyes recovered within 72 hours of instillation.

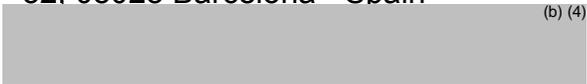
Daily dermal application of ozenoxacin ointment to rats at doses of 1%, 6.3% and 40% for 28 days were locally well-tolerated and did not cause clinical signs of systemic toxicity. TK data showed that the drug was mildly absorbed over time, resulting in accumulation and modest dose-dependent systemic exposure after repeated dosing for 28 days. Very slight erythema was observed at the site of application of the high dose rats more frequently than controls or groups exposed to lower concentrations of ozenoxacin. The NOAEL in this study was considered to be the mid dose of 6.3%.

Daily dermal application of ozenoxacin ointment to minipigs at doses 1%, 6.3% and 40% for 28 days were locally well-tolerated and did not cause clinical signs of systemic toxicity. There were no skin changes at the site of application that appeared related to ozenoxacin. TK data suggested variable absorption of ozenoxacin among animals in the same group and indicated that systemic exposure after repeated dosing was modest. The NOAEL in this study was considered to be the high dose of 40%.

Once daily oral ozenoxacin doses of up to 450 mg/kg/day in dogs (reduced to 350 mg/kg/day on Day 15) administered for 28 days were associated with mortality and CNS effects (tremor, vomiting and convulsions). A dose of 150 mg/kg was associated with sporadic vomiting. There were no microscopic changes that revealed target organs other than the CNS in this study. The NOAEL in this study was considered by the reviewer to be 50 mg/kg, based on the possibility that some episodes of emesis at 150 mg/kg were related to CNS effects of ozenoxacin. Considering minimal systemic exposure from topically administered ozenoxacin cream, 1%, there is likely no risk for drug related CNS effects to patients.

6.2 Repeat-Dose Toxicity

Study title: GF-001001-00 Cream Formulation (GF-001001-00TC-12): A 28-Day Dermal Toxicity Study in Minipigs with a 14-Day Recovery Period

Study no.:	RR-090768-01
Study report location:	Ferrer Internacional S.A., Juan de Sada, 32. 08028 Barcelona - Spain
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	6/7/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GF-001001-00 1% Cream Formulation, 001490, 100.3%

Key Study Findings

No toxicologically relevant effects of GF-001001-00 were observed at any of the doses tested.

Methods

Doses:	0, 2, 4 mg/kg/day
Frequency of dosing:	Once daily to no less than 10 % of total body surface area
Route of administration:	Dermal on intact and abraded skin
Dose volume:	10, 5 and 10 mg/cm ² for 0, 2 and 4 mg/kg/day
Species/Strain:	Gottingen minipig
Number/Sex/Group:	5/sex/group
Age:	12 weeks
Weight:	Males: 5.6-8.11 kg; females: 6.06-8.11 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Dermal administration of GF-001001-00 cream formulation at 1% was evaluated for 28 days in Gottingen minipigs followed by a 15-day recovery period. The test article cream was applied for approximately 24 hours to normal and abraded skin sections corresponding to no less than 10% of the total body surface area to achieve maximal exposure conditions. The dose levels were selected based on the use of the intended clinical formulation (GF-001001-00 cream formulation 1%). The low and high doses corresponded with 10 and 22 times the intended human dose proposed for the clinical

trials during the drug development (1 gm/day of 1% GF-001001-00 cream formulation considering a 60 kg patient).

There were no drug-related deaths in the study and no target organs were identified. No test article-related dermal irritation was noted at the test site. Low-grade erythema (grade 1-2) was noted in all dose groups in both sexes with the greatest incidence in both control groups. By the end of recovery period, the erythema had resolved completely. Desquamation was noted in all treatment groups including intact and abraded controls. In addition, red, raised areas were noted within the test site, primarily in the intact and abraded control groups. In general, dermal findings in the intact and abraded dose groups were comparable.

No toxicologically relevant adverse effects were noted on body weight changes, qualitative food consumption, ophthalmology, electrocardiography and clinical pathology parameters in the treatment groups compared to the controls. No toxicologically relevant histopathological findings were noted in the limited panel of tissues evaluated (brain, kidneys, liver, lung, ovaries, abraded and intact skin sites, testes, and thymus). Considering minimal systemic exposure with the dermal application of GF-001001-00 cream formulation 1%, histopathological evaluation of the limited panel of tissues was considered sufficient. TK analysis on day 1 revealed all animals were below the lower limit of quantitation (0.5 ng/ml), but on day 28, one female animal that received 4 mg/kg/day on abraded skin had plasma concentration of 0.52 ng/ml of the drug which is slightly above detection limits of the drug in the plasma at 1 hour post-dose but not at 3, 6, 12 and 24 hours post-dose. This minimal systemic exposure is not toxicologically relevant because of the low amount present at only one time-point. Based on these results, the no-observed-adverse-effect level (NOAEL) for both local and systemic toxicity was considered to be 4 mg/kg/day for both intact and abraded animals.

7 Genetic Toxicology

Genotoxicity studies including in-vitro Ames and mouse lymphoma assays and an in-vivo rat micronucleus assay were reviewed by Dr. Amy Ellis for IND 105567. In the Ames assay, ozenoxacin did not induce reverse mutation regardless of metabolic activation. However, the drug did induce cytotoxicity at the 2 higher concentrations of drug tested and therefore the data from this assay are of limited value. Concentrations <0.0125 µg/plate did not cause cytotoxicity in the Ames assay. In the mouse lymphoma assay, 2-3-fold increase in mutation frequency was observed with 24-hour treatment time at concentrations >185.0 µg/ml. However, the Applicant was not asked to repeat the assay before commencement of clinical trials because several marketed quinolone antimicrobial drug products have tested positive in in-vitro genotoxicity assays. In the in-vivo rat micronucleus assay, ozenoxacin did not induce micronuclei in PCEs from the bone marrow of rats when administered once daily by oral gavage on consecutive days at doses up to 2000 mg/kg. Systemic exposure to oral ozenoxacin was confirmed by TK analysis. Based on the negative in-vivo micronucleus assay, ozenoxacin cream, 1% is considered non-mutagenic for dermal application in the treatment of impetigo.

7.4 Other Genetic Toxicity Studies

Study Title: GF-001001-00 impurities (b) (4) *In Vivo* Rat Bone Marrow Micronucleus Assay

Study no.: RR-090779-01
 Study report location: Ferrer Internacional S.A., Juan de Sada, 32, 08028 Barcelona - Spain
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 6/7/2011
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4), STD-08-05 (b) (4) %
 (b) (4), STD-08-09 (b) (4) %
 (b) (4), 09-01, (b) (4) %
 Positive Control: Cyclophosphamide; 068K1131
 Vehicle Control: 1% CMC and 0.1% Tween 80 in water

Key Study Findings

The impurities (b) (4) did not show clastogenicity or cytotoxicity in the bone marrow at the doses tested.

Methods

Doses: 0, 200, 500, 2000 mg/kg/day; Positive Control (Cyclophosphamide): 60 mg/kg/day;
 Frequency of dosing: 2 doses, once daily, 24 hours apart
 Route of administration: Oral gavage
 Dose volume: 20 ml/kg/day
 Species/Strain: Hsd:SD rats
 Number/Sex/Group: 5 males/group
 Satellite groups: TK study; 3 males/treatment group
 Unique study design: None
 Deviation from study protocol: None

Observation & Results:

The impurities of GF-001001-00, (b) (4) did not induce signs of clinical toxicity in the animals treated at dose levels up to 2000 mg/kg/day. The impurities did not induce significant increases in micronucleated polychromatic erythrocytes (PCE) in the bone marrow of the rats at any of the doses of the impurities tested as shown in the

table above indicating that the impurities were not clastogenic at the doses tested. The impurities did not induce a significant decrease in the PCE:NCE ratio at any of the doses tested indicating that they were not cytotoxic to the bone marrow. The positive control, cyclophosphamide, induced a significant increase in micronucleated PCEs compared to controls, indicating clastogenicity and hence, validity of the assay. TK analysis (shown in table below) showed plasma concentrations at 2 and 4 hours post-dosing on day 2 indicating that adequate systemic exposure was achieved prior to bone marrow analysis.

Table 3 Toxicokinetic summary for impurities (b) (4)

Compound	Dose (mg/kg/day)	Exposure Levels (ng/mL) (mean ± SD)	
		Sampling Time After Second Dosage	
		2 hours	4 hours
(b) (4)	200	172,00 ± 80	98,60 ± 79
	500	351,33 ± 118	555,00 ± 394
	2000	3134,33 ± 2076	2860,00 ± 802
	200	487,00 ± 29	423,00 ± 113
	500	424,00 ± 112	369,00 ± 115
	2000	331,67 ± 50	348,33 ± 88
	200	868,67 ± 334	330,00 ± 76
	500	1646,67 ± 361	865,67 ± 86
	2000	48233,33 ± 24955	16693,33 ± 22200

9 Reproductive and Developmental Toxicology

Reviewer's notes: The reproductive and developmental toxicology study findings were of limited relevance as the proposed clinical formulation is a 1% topical cream with minimal systemic exposure, whereas the studies summarized below were conducted by oral gavage.

9.1 Fertility and early Embryonic Development

Study title: Oral (Gavage) Fertility and General Reproduction Toxicity

Study in Rats

Study no.: RR-090725-01
 Study report location: Ferrer Internacional S.A., Juan de Sada,
 32. 08028 Barcelona - Spain
 Conducting laboratory and location: (b) (4)
 Date of study initiation: November 10, 2009
 GLP compliance: Yes
 QA statement: Yes

Drug, lot #, and % purity: GF-001001-00; A06052E; 99.9%

Key Study Findings

- There were no effects of GF-001001-00 on mating, fertility and litter parameters.
- Systemic exposure was seen at all doses of GF-001001-00 and C_{max} was achieved within half an hour of dose administration. Terminal elimination half-life ($T_{1/2}$) of ozenoxacin ranged from 4.54 to 6.43 hours, increasing slightly in a dose-dependent manner.

Methods

Doses: 0, 125, 250, 500 mg/kg/day
Frequency of dosing: Once daily
Dose volume: 10 ml/kg
Route of administration: Oral
Formulation/Vehicle: 0.1% Polysorbate 80 and 1%
Carboxymethylcellulose sodium
Species/Strain: Crl:CD(SD)
Number/Sex/Group: 25/sex/group
Satellite groups: 3/sex/group – TK study
Deviation from study protocol: None that affects the conclusion of the study

Observations and Results

Male rats were administered the test article and/or the vehicle once daily beginning 28 days before cohabitation (maximum 21 days) and continuing through the day before sacrifice. Female rats were administered the test article and/or the vehicle once daily beginning 15 days before cohabitation (maximum 21 days) and continuing through gestation day 7. Doses were selected on the basis of a dose-range developmental toxicity study and a 28-day repeat-dose oral toxicity in rats.

No drug-related mortality occurred during the course of this study. 1 male rat in the control group was found dead, cause of death unknown. 2 male rats in each of the vehicle control group and 250 mg/kg/day dosage group were euthanized before the study terminated, but the deaths were unrelated to drug administration and were determined to be caused by gavage accident or accidental limb injury (250 mg/kg/day). All female rats survived to scheduled sacrifice.

Incidences of soft or liquid feces at ≥ 125 mg/kg/day in male rats and at 500 mg/kg/day in female rats were observed with ozenoxacin treatment only during the pre-mating dosage period, not detected in the vehicle control. 7 of 25 male rats in the 500 mg/kg/day dosage group also had slight excess salivation.

Body weight gains in male rats were transiently reduced in each treatment group during the first week of the dosing period (74%, 76% and 72% of the vehicle control group in

the 125, 250 and 500 mg/kg/day dose groups respectively), which resolved thereafter until the end of the dosing period. However, in females, body weight gains in the 125, 250 and 500 mg/kg/day dose groups were reduced for the entire pre-mating dosing period (79%, 75% and 73% of the vehicle controls in the 125, 250 and 500 mg/kg/day dose groups respectively), returning to control levels during the gestation period. Absolute and relative feed consumption values in male and female rats were transiently, but significantly reduced (10-15%) in all treatment groups during the first week of dosing, compared to vehicle treatment, returning to control levels for the remainder of the dosing period. GF-001001-00 did not affect terminal body weights, the weights of the reproductive organs or sperm parameters (number and percent motile sperm, number of non-motile sperm and total sperm count from the vas deferens and cauda epididymal sperm count and density) in male rats or estrous cyclicity in female rats at any of the doses tested.

There were no effects of GF-001001-00 on mating or fertility and litter parameters (i.e. numbers of days in cohabitation, rats that mated, number of pregnancies per number of rats that mated, rats with confirmed mating dates during the first or second week of cohabitation and number of pregnancies per number of rats in cohabitation). Caesarean-sections were performed on gestational day 13 and the litter averages for corpora lutea, implantations, percentage of preimplantation loss, viable and nonviable embryos and the percentage of post-implantation loss of treatment groups were not different from controls. All placentae appeared normal.

$T_{1/2}$ for GF-001001-00 ranged from 4.54 to 6.43 hours increasing slightly in a dose-dependent manner. For both sexes, exposure to GF-001001-00 generally increased between the 125 to 500 mg/kg/day dosage levels less than proportionally to the dose. A very slight plasma accumulation of GF-001001-00 was observed at the 250 and 500 mg/kg/day doses in both sexes. Based on these data, the NOAEL was determined to be 500 mg/kg/day for both sexes. In sum, the reviewer agrees that GF-001001-00 does not affect fertility or reproduction in male and female rats at doses up to 500 mg/kg/day.

Table 4 Toxicokinetic summary of fertility and general reproductive toxicity study in rats:

Text Table 3. Mean Toxicokinetic Parameters for GF-001001-00 in Male Rats								
Dosage Level (mg/kg/day)	DS 1				DS 28			
	C_{max} (ng/mL)	T_{max} (h)	AUC _(0-t) (ng•hr/mL)	$T_{1/2}$ (h)	C_{max} (ng/mL)	T_{max} (h)	AUC _(0-t) (ng•hr/mL)	$T_{1/2}$ (h)
125	4230	0.5	14,298	4.60	2257	0.50	21,677	5.76
250	4970	0.50	22,928	4.54	5230	2.00	75,268	4.76
500	4190	0.50	30,582	6.06	5693	2.00	75,025	6.08

DS = Day of Study

Text Table 4. Mean Toxicokinetic Parameters for GF-001001-00 in Female Rats								
Dosage Level (mg/kg/day)	DS 1				DS 14			
	C _{max} (ng/mL)	T _{max} (h)	AUC _(0-t) (ng•hr/mL)	T _{1/2} (h)	C _{max} (ng/mL)	T _{max} (h)	AUC _(0-t) (ng•hr/mL)	T _{1/2} (h)
125	6140	0.50	15,112	RNR	2841	0.50	9185	RNR
250	5173	0.50	22,341	5.44	6223	0.50	37,093	5.05
500	7827	0.50	31,203	6.43	11,060	0.556	49,088	4.86

DS = Day of Study

RNR = results not reported because the AUC(0-inf) was extrapolated by more than 20% or Rsq was <0.800.

9.2 Embryonic Fetal Development

Study title: Developmental Toxicity Study in Rats by Oral (Gavage) Route

Study no.: RR-090722-01
 Study report location: Ferrer Internacional S.A., Juan de Sada, 32, 08028 Barcelona - Spain
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 7/26/2009
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: GF-001001-00; A07428E; 100.1%

Key Study Findings

- GF-001001-00 caused significant delays in the ossification of rib pairs with associated significant alterations in the numbers of thoracic and lumbar vertebrae, respectively.
- An overall reduction in fetal body weight was observed in all treatment groups of GF-001001-00

Methods

Doses: 0, 125, 250, 500 mg/kg/day
 Frequency of dosing: Once daily from gestation day 7 through 17
 Dose volume: 10 ml/kg
 Route of administration: Oral
 Formulation/Vehicle: 0.1% Polysorbate 80 and 1% Carboxymethylcellulose sodium
 Species/Strain: Crl:CD(SD)

Number/Sex/Group: 25/sex/group
 Satellite groups: 4/sex (control) and 8/sex (treatment) – TK study
 Deviation from study protocol: None that impacted the results of the study

Observations and Results:

Doses were selected on the basis of a dose-range study in rats. No mortality occurred in this study. No drug-related adverse clinical signs were observed. There were no effects of the drug on maternal body weight gains or feed consumption throughout the dosing period. 25/25 rats in the control and 125 mg/kg/day groups and 23/25 rats in 250 and 500 mg/kg/day groups were pregnant and Caesarean-sectioning was done on gestation day 21. No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by GF-001001-00 in any of the dose groups compared to controls. All doses of GF-001001-00 caused significant delays in the ossification of rib pairs with associated significant alterations in the numbers of thoracic and lumbar vertebrae, respectively. There was an overall slight reduction in fetal body weight observed in all treatment groups of GF-001001-00. The average number of ossified caudal vertebrae was significantly decreased in all treatment groups, while the average number of ossified sternal centers, hindlimb metatarsals and hindlimb phalanges were significantly decreased in the 500 mg/kg/day dose group, compared to the controls. Systemic exposure was observed and C_{max} achieved within half an hour to 2 hours in pregnant female rats at all doses of GF-001001-00 on gestational days 7 and 17. C_{max} increased, less than dose proportionally on gestation days 7 and 17 and slight dose-dependent drug accumulation seen by day 17. $T_{1/2}$ ranged from 4.4 - 6.9 hours. Based on this, the Applicant reported the maternal and fetal NOAEL as 500 mg/kg/day. Considering this is a topical administration of ozenoxacin, the reviewer agrees that the design of the study, doses selected and the NOAEL are adequate.

Table 5 Summary of fetal ossification sites in rats:

Text Table 2. Summary of Treatment-Related Changes in Ossification Site Averages					
Parameter	Dosage Group				Historical Control Data ^a
	0 (Vehicle) mg/kg/day	125 mg/kg/day	250 mg/kg/day	500 mg/kg/day	
Thoracic Vertebrae	13.16±0.16	13.02±0.05**	13.02±0.06**	13.01±0.03**	13.08 (13.03-13.18)
Lumbar Vertebrae	5.83±0.16	5.97±0.07**	5.98±0.06**	5.96±0.08**	5.91 (5.81-5.96)
Caudal Vertebrae	7.80±0.90	7.01±0.58**	7.04±0.51**	6.68±0.55**	7.54 (6.75-8.05)
Ribs (Pairs)	13.13±0.13	13.02±0.05**	13.01±0.03**	13.01±0.03**	13.07 (13.02-13.90)
Sternal Centers	3.99±0.04	3.99±0.04	3.99±0.04	3.94±0.10*	3.99 (3.93-4.00)
Hindlimb: Metatarsals	4.94±0.10	4.84±0.22	4.93±0.08	4.81±0.18**	4.85 (4.50-5.00)
Hindlimb: Phalanges	6.69±1.18	6.08±0.94*	6.52±0.93	5.73±0.79**	6.29 (5.43-6.97)

a. Testing Facility Historical Control Data for January 2007 to January 2009 (46 studies, 973 litters examined and 7147 fetuses examined)

* = significantly different from the vehicle control group value ($p \leq 0.05$).

** = significantly different from the vehicle control group value ($p \leq 0.01$).

Table 6 Toxicokinetic summary of pregnant female rats:

Text Table 3. Summary of Toxicokinetic Parameters in Pregnant Female Rat Plasma Following Oral Gavage of GF-001001-00						
Dosage Group/Level	C _{max} (ng/mL)	AUC(0-t) (ng•h/mL)	AUC(0-inf) (ng•h/mL)	T _{max} (h)	T _{last} (h)	T _{1/2} (h)
Day 7 of Gestation						
II 125 mg/kg/day	2580	16883	17225	0.50	24.0	4.47
III 250 mg/kg/day	13350	49698	52449	0.50	24.0	6.96
IV 500 mg/kg/day	9365	61334	a	0.50	24.0	a
Day 17 of Gestation						
II 125 mg/kg/day	5730	34584	35880	0.50	24.0	5.58
III 250 mg/kg/day	10545	76317	80038	2.00	24.0	6.18
IV 500 mg/kg/day	17675	147105	154085	2.00	24.0	5.40

a. Values were not reported because the AUC(0-inf) was extrapolated by more than 20% or RSQ was <0.8.

Study title: Oral (Stomach Tube) Developmental Toxicity Study in Rabbits, Including a Satellite Toxicokinetic Evaluation

Study no.: RR-090724-01

Study report location: Ferrer Internacional S.A., Juan de Sada, 32, 08028 Barcelona - Spain

Conducting laboratory and location:

(b) (4)

Date of study initiation: 7/26/2009

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: GF -001001-00; A07428E; 100.1%

Key Study Findings

- Significant increase in Post-implantation loss.
- Delays in skeletal development occurred the 2 higher doses
- Fetal body weights were significantly reduced at the 2 higher doses

Methods

Doses:	0, 5, 15, 40 mg/kg/day
Frequency of dosing:	Once daily from gestation day 7 through 19
Dose volume:	5 ml/kg
Route of administration:	Oral via stomach tube
Formulation/Vehicle:	0.1% Polysorbate 80 and 1% Carboxymethylcellulose sodium
Species/Strain:	New Zealand White [Hra:(NZW)SPF] rabbit
Age/Weight:	5-7 months; 2.5 – 5.5 kg
Number/Sex/Group:	20 females/group
Satellite groups:	4 females/treatment – TK study
Deviation from study protocol:	None that impacted the conclusion of the study

Observations and Results:

Doses were selected on the basis of a dose-range study in rabbits. There were no drug-related deaths. 20/20, 19/20 and 18/20 females in the 0, 5 and 15 and 40 mg/kg/day groups were pregnant. 2 females aborted on gestational day 21 and were sacrificed and 1 female delivered on gestational day 29. Caesarean-sections were carried out on gestational day 29 in 20, 19, 16 and 17 pregnant does in the control, 5, 15 and 40 mg/kg/day groups. Clinical observations attributed to GF-001001-00 included scant feces and orange urine (5, 15 and 40 mg/kg/day), no feces in the cage pan (15 and 40 mg/kg/day) and soft or liquid feces (40 mg/kg/day). Significant loss in maternal body weight occurred in the 15 and 40 mg/kg/day dosage groups compared to the vehicle control group. Maternal body weight gains were 71%, 37% and 17% of the vehicle control group in the 5, 15 and 40 mg/kg/day dosage groups, respectively from gestational days 7-29. Corresponding to reductions in body weight, absolute and relative feed consumption values were significantly reduced. Absolute feed consumption values in the 5, 15 and 40 mg/kg/day dosage groups were 82%, 60% and 48% of the vehicle control from gestational days 7-29.

Post-implantation loss which includes the percentage of post-implantation loss, the percentage of does with any resorptions and the percentage of dead or resorbed conceptuses per litter was significantly increased in the 40 mg/kg/day dose group, in comparison to the vehicle control group value (2.5%, 5.5% and 12.7% in 5, 15 and 40 mg/kg/day group compared to 1.5% in vehicle controls). Does with resorptions were significantly increased in 40 mg/kg/day (11%) compared to controls (3%). Delays in skeletal development occurred at 15 and 40 mg/kg/day, and included significant reductions in the average number of ossified hyoid bodies at 40 mg/kg/day, the average number of ossified forelimb metacarpals and hindlimb phalanges at 40 mg/kg/day and the average number of ossified forelimb phalanges at 15 and 40 mg/kg/day compared to controls. Fetal body weights were significantly reduced in the 15 and 40 mg/kg/day dose groups compared to controls (males and females combined at 15 and 40 mg/kg/day were 37.9% and 34.9% compared to 43.5% in controls). GF-001001-00 did not produce any overt gross external, soft tissue or skeletal malformations or variations at any dosage level. The Applicant concluded that the maternal and developmental

toxicities observed were due to the antibacterial activity of the drug (a quinolone antibiotic). Rabbits in general are more sensitive to the developmental effects of a wide range of antibacterials as a consequence of alterations in the maternal gut flora, resulting in adverse developmental effects in their offspring. Based on these data, the maternal and fetal NOAEL is 5 mg/kg/day. This corresponds to AUC values of 787 and 974 ng.hr/mL and C_{max} values of 277 and 352 ng/mL on gestational days 7 and 19, respectively. The fetal toxicities observed occurred only at maternally toxic levels. The reviewer agrees with this conclusion.

Table 7 Toxicokinetic summary of pregnant female New Zealand white rabbits:

Text Table 3. Toxicokinetic Parameters of GF-001001-00 in Pregnant New Zealand White Rabbit Plasma Following Oral (Stomach Tube) Administration of GF-001001-00							
Dosage Level (mg/kg/day)	Study Day	T_{max}^a (h)	C_{max} (ng/mL)	AUC_(0-t) (ng·h/mL)	T_{1/2} (h)	C_{max}/D	AUC_(0-t)/D
5	DG 7	0.50	277 ± 149	787 ± 458	6.80 ± 3.97	55.4 ± 29.8	157 ± 91.5
15	DG 7	0.50	1208 ± 969	2807 ± 1811	8.12 ± 2.16	80.5 ± 64.6	187 ± 121
40	DG 7	0.50	3943 ± 3530	9253 ± 6663	6.70 ± 1.27	98.6 ± 88.3	231 ± 167
5	DG 19	0.50	352 ± 189	974 ± 368	6.20 ± 2.16	70.3 ± 37.8	195 ± 73.5
15	DG 19	1.25	443 ± 218	3099 ± 1206	-	29.5 ± 14.5	207 ± 80.4
40	DG 19	0.50	1881 ± 1438	9939 ± 4965	-	47.0 ± 35.9	248 ± 124

a. Median value reported for T_{max}.

DG = day of gestation

- Not calculated because no results were reported in these dosage groups at this timepoint because the AUC(0-inf) was extrapolated by more than 20% or R_{sq} was <0.800.

9.3 Prenatal and Postnatal Development

Study title: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Behavioral /Functional Evaluation

Study no.:	RR-090726-01
Study report location:	Ferrer Internacional S.A., Juan de Sada, 32, 08028 Barcelona - Spain
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	11/3/2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GF -001001-00; A07428E; 100.1%

Key Study Findings

There were no toxicologically relevant effects of GF-001001-00 in the F₀, F₁ or F₂ generations.

Methods

Doses:	0, 125, 250, 500 mg/kg/day
Frequency of dosing:	Once daily from gestational day 7 through lactation day 20
Dose volume:	10 ml/kg
Route of administration:	Oral
Formulation/Vehicle:	0.1% Polysorbate 80 and 1% Carboxymethylcellulose sodium
Species/Strain:	Crl:CD(SD)
Number/Sex/Group:	25/sex/group
Satellite groups:	3/sex/group – TK study
Deviation from study protocol:	None that impacted the conclusion of the study

F₀ Generation Rats:

Female rats assigned to the main study were administered the test article and/or the vehicle once daily from gestational day 7 through lactation day 20. Female rats assigned to TK analysis were given the test article and/or the vehicle once daily from gestational day 7 through lactation day 14. After acclimation, 137 virgin female rats were cohabitated with 137 breeder male rats, one male rat per female rat. The cohabitation period consisted of a maximum of five days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be at gestational day 0 and assigned to individual housing. Rats were observed for viability at least twice each day of the study and for clinical observations and general appearance weekly during acclimation and on gestational day 0. The rats were also examined for clinical observations, abortions, premature deliveries and deaths before and 90 ± 30 minutes after dosage administration and on the day sacrifice occurred. Body weights were recorded weekly during the acclimation period, on gestational day 0, daily during the dosage period and at sacrifice. Feed consumption values were recorded on gestational days 0, 7, 10, 12, 15, 18, 20 and 25 and lactation days 1, 4, 7, 10 and 14. Because pups begin to consume maternal feed on or about lactation day 14, feed consumption values were not tabulated after lactation day 14. Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation (gestational day 0 to the day the first pup was observed), litter sizes (all pups delivered) and pup viability at birth. Maternal behavior was evaluated on lactation days 1, 4, 7, 14, 18 and 21.

F₁ Generation Rats:

Day 1 of lactation (postpartum) was defined as the day of birth and was also the first day on which all pups in a litter were individually weighed (pup body weights were

recorded after all pups in a litter were delivered and groomed by the dam). Each litter was evaluated for viability at least twice daily. The pups in each litter were counted once daily. Clinical observations were recorded once daily during the pre-weaning period. Pup body weights were recorded on lactation days 1 (day of birth), 4, 7, 14, 18 and 21. At approximately 90 days of age, the F₁ generation rats within each dosage group were assigned to cohabitation, one male rat per female rat, based on random unit tables, with the exclusion of sibling matings. The cohabitation period consisted of a maximum of 21 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be at gestational day 0 and assigned to individual housing. One female rat not mated within the first 14 days of cohabitation was assigned an alternate male rat from the same dosage group that had mated; this female rat had not mated after completion of the 21-day cohabitation period and was considered to be at gestational day 0 on the last day of cohabitation and assigned to individual housing.

Observations and Results

F₀ Dams:

Doses were selected on the basis of a dose-range developmental toxicity study in rats. No drug-related mortalities occurred. 1 female in the 500 mg/kg/day dose was found dead due to gavage error. Significant increase in animals with soft or liquid feces in the 500 mg/kg/day group was observed. Body weight gains were significantly reduced in all treatment groups for the entire dosing period (gestation day 7 to 20). The reductions were 20%, 10% and 15% less than vehicle controls in the 125, 250 and 500 mg/kg/day dose groups, respectively. Absolute and relative feed consumption values were significantly reduced in the treatment groups for the entire gestation dosage period (gestation days 7 to 20) and overall for the entire gestation period (gestation days 0 to 20), compared to the vehicle control group values. Body weight gains increased significantly on gestation days 7 to 10 at 250 and 500 mg/kg/day and overall for the entire lactation period (days 1 to 21). Absolute feed consumption values of the treatment groups were between 103-108% of the vehicle control group on lactation days 1 to 14. Pregnancy occurred in 24/25 (control, 125 and 250 mg/kg/day groups) and 23/25 (500 mg/kg/day) mated female rats. All pregnant dams delivered litters. The average pup body weight per litter was significantly reduced (10-15 % reduction) in each of the treatment groups on days 1, 4 and 7 postpartum, in comparison to the vehicle control group values. By day 14 postpartum, the pup body weights in the 125 and 250 mg/kg/day dosage groups were comparable to the vehicle control group values; however, pup body weight reductions at 500 mg/kg/day persisted until day 21 postpartum. TK analysis (shown in the table below) in the pups on day 14 postpartum showed that systemic exposure in the pups reached a maximum after 8 hours and T_{1/2} was shown to be 17.2 hours indicating that the drug is transferred via lactation.

F₁ Generation:

All F₁ generation rats survived to scheduled sacrifice. There were no toxicologically relevant effects of GF-001001-00 on feed consumption, sexual maturation, learning and memory, mating and fertility, male reproductive organ weights or caesarean-sectioning parameters in the F₁ generation rats. There were no gross external soft tissue or skeletal developmental malformations at any dose level tested. Slightly reduced fetal body weight and delays in skeletal development were observed, presumably due to maternal oral exposure and the maternal gastrointestinal antibiotic effects of the drug. There were no apparent effects on gestation, parturition, lactation or maternal behavior at any dose level tested. There was quantifiable systemic exposure in pup plasma on day 14 postpartum in all dose groups (Table 9). Overall, plasma exposure in pups increased with the increase in the dose administered to the dams. Therefore, the NOAEL for viability and growth in the offspring was also established at 500 mg/kg/day.

Table 8 Toxicokinetic summary of F₁ generation pup plasma on Day 14

Text Table 3. Toxicokinetic Parameters of GF-001001-00 in F₁ Generation Pup Plasma on Day 14 Postpartum Following Oral Gavage of GF-001001-00 to F₀ Generation Female ^(b)⁽⁴⁾CD(SD) Rats							
Group No.	Dosage Level (mg/kg/day)	T _{max} (h)	C _{max} (ng/mL)	AUC _(0-t) (ng·h/mL)	T _{1/2} (h)	C _{max} /D	AUC _{(0-t)/D}
II	125	8.00	15.1	220	NE	0.121	1.76
III	250	8.00	25.3	714	17.2	0.101	2.86
IV	500	0.50	48.1	1372	RNR	0.0963	2.74

NE: Parameter not estimatable from data set.

RNR: Result not reported because the AUC_(0-inf) was extrapolated by more than 20% or R_{sq} was <0.800.

F₂ Generation:

There were no toxicologically relevant effects of GF-001001-00 on live fetuses, fetal body weights and resorbed conceptuses in the F₂ generation litters. Overall, the study was adequate and the reviewer agrees with the NOAEL of 500 mg/kg/day for the F₁ and F₂ generations in this study.

11 Integrated Summary and Safety Evaluation

Ozenoxacin is a non-fluorinated quinolone that inhibits bacterial DNA replication by blocking the bacterial DNA gyrase and topoisomerase IV enzymes. Ozenoxacin 1% cream is intended for topical treatment of impetigo in adults and children aged 2 months

and older. The drug has shown activity against gram-positive and gram-negative strains of bacteria. In-vitro and in-vivo safety pharmacology studies were conducted to evaluate cardiovascular and respiratory effects after administration of ozenoxacin. Slight hERG channel inhibition was observed at 17 μM indicated by reduced amplitude of outward tail currents (18.0%); no IC_{50} of the drug was determined. In the in-vivo cardiovascular study in dogs, there were no qualitative ECG abnormalities detected with oral administration of the drug. Oral administration of ozenoxacin to rats up to 2000 mg/kg did not induce significant changes in respiratory parameters or cause any adverse effects on the central nervous system, general behavior, and on motor activity in the treated animals. Considering the negligible systemic absorption and lack of findings, ozenoxacin is not expected to cause any CNS, cardiovascular, or respiratory effects in patients.

Ozenoxacin cream 1% appears to have a low potential for dermal irritation. When 1% ozenoxacin cream or its vehicle was applied daily to the intact and abraded skin of minipigs for 4 weeks, the application sites exhibited low-grade erythema (grade 1-2). Abrading the skin did not significantly enhance irritation potential. Minimal systemic exposure was seen in minipigs with the 1% topical cream formulation. The drug was moderately irritating to the rabbit eye after a single exposure but the eyes recovered within 72 hours of instillation. In addition, neither ozenoxacin cream (0.5%, 1%, or 2%) nor its vehicle was a sensitizer in the murine local lymph node assay. Considering the general tolerance of ozenoxacin when applied topically to animals at far higher concentrations than planned in the clinic, the risk for dermal irritation with topical administration of Ozenoxacin cream, 1% cream to patients appears to be low.

Topically administered ozenoxacin is not readily absorbed through the skin. Negligible amounts of the drug was seen in the plasma after ozenoxacin 1% cream was administered as a topical formulation in minipigs and rats indicating minimal absorption from the skin. Repeated daily administration of GF-001001-00 (ozenoxacin) cream and ointment at the high dose level (20% cream or 2 mg/cm²) for 14 days increased systemic exposure parameters (C_{max} and AUC_{0-t}) considerably in minipigs. At the high dose level (20% cream or 2 mg/cm²), which is 570 fold higher than the human equivalent dose, overall systemic exposure parameters were approximately 4-10 times higher on Day 14 than on Day 1, suggesting accumulation of the test article on repeated dosing at this dose. $T_{1/2}$ of the test article ranged between 4.01 and 6.69 hours. Only very minimal absorption just above the limit of detection was observed in two patients when repeatedly administered at twice the clinically relevant dose. PK/ADME studies conducted with dermal application of radiolabeled GF-001001-00 in rats at 0.2 mg/cm² confirmed radioactivity concentrations below the lower limit of quantification in the plasma, liver, and other tissues. Main route of elimination in rats after dermal application was through feces accounting for a mean of 2.5% of the applied dose indicating low absorption of the drug via the skin. GF-001001-00 was excreted mainly unchanged indicating low metabolism when administered dermally.

Repeat-dose toxicity studies were conducted in minipigs, rats, and dogs to assess systemic exposure following dermal application of GF-001001-00 (ozenoxacin) cream

formulation at 1%. GF-001001-00 cream 1% was topically administered to abraded and unabraded skin (approximately 10% of the total body surface area) of Gottingen minipigs for 28 days followed by a 15-day recovery period. The cream was well tolerated with only minimal dermal erythema (grade 1-2), desquamation, and red, raised areas noted at the test site, in all dose groups in both sexes in both treatment and vehicle control groups. All incidences of erythema, desquamation, and raised red areas had resolved in all groups by the end of the recovery period. All other test parameters in this study were similar between the treatment groups and controls. No toxicologically relevant histopathological findings were noted in the tissues evaluated (brain, kidneys, liver, lung, ovaries, abraded and intact skin sites, testes, and thymus). TK analysis revealed all animals were at or below the lower limit of quantification (0.5 ng/ml) of drug in the plasma at all timepoints. Based on these results, the no-observed-adverse-effect level (NOAEL) for both local and systemic toxicity was considered to be 4 mg/kg/day for both intact and abraded animals.

In repeat-dose toxicity studies conducted with the oral administration of ozenoxacin in dogs and rats, the plasma levels attained in these studies in animals were approximately 40 times the systemic exposure in humans following topical application of ozenoxacin 1% cream. Rats tolerated 4 weeks of oral ozenoxacin doses of up to 500 mg/kg/day without displaying clinical signs of toxicity. Dogs were more sensitive to ozenoxacin than rats. Daily oral ozenoxacin doses of 450 mg/kg/day for 28 days (reduced to 350 mg/kg/day on Day 15) were associated with mortality and CNS effects (tremor, vomiting, convulsions). There were no microscopic changes that revealed target organs other than the CNS in this study. The NOAEL was considered by the reviewer to be 50 mg/kg, based on the possibility that some episodes of emesis at 150 mg/kg were related to CNS effects of ozenoxacin.

Ozenoxacin is not considered to be genotoxic. Genotoxicity studies conducted with ozenoxacin included an in-vitro Ames test, an in vitro mouse lymphoma assay, and an in-vivo rat micronucleus assay. In the Ames assay, ozenoxacin did not induce mutation regardless of metabolic activation but was limited by the cytotoxicity at the highest 2 concentrations of drug tested (the concentrations that generated reliable data were 0.00625 and 0.0125 µg/plate). Therefore, the data from the Ames assay are of limited relevance. In the mouse lymphoma assay, 2-3-fold increase in mutation frequency was observed with 24-hour treatment time at concentrations >185.0 µg/ml. Several approved quinolone antimicrobial drug products have tested positive in in-vitro genotoxicity assays, and hence the Applicant was not asked to repeat the assay. In the in-vivo rat micronucleus assay, ozenoxacin did not induce micronuclei in PCEs from the bone marrow of rats when administered once daily by oral gavage on consecutive days at doses up to 2000 mg/kg. Based on the negative in-vivo micronucleus assay and demonstrated negligible systemic exposure, there is no significant risk for mutagenicity with dermal application in the treatment of impetigo.

Impurities (b) (4) at doses upto 2000 mg/kg/day were found to be non-genotoxic in the micronucleus assay in rats at about (b) (4) times the expected human exposure, thus qualifying them at NMT (b) (4)%, which is above the ICH qualification

threshold for drug substance. Specifically, hind-limb bone marrow cells, extracted from rats that were administered upto 2000 mg/kg/day dose (human equivalent dose of 320 mg/kg/day or 12,000 mg/ m²), did not exhibit increases in PCE, indicating non-clastogenicity at the doses tested. TK analysis confirmed systemic exposure.

Reproductive Toxicology studies using the oral form of the drug were conducted to test the effect of GF-001001-00 on fertility and general reproduction in rats, embryonic and fetal development in rats and rabbits and peri- and postnatal development in rats. However, considering the negligible absorption of topically administered ozenoxacin observed both in repeat-dose animal studies and in patients enrolled in the clinical trials, these findings are considered to be of minimal relevance to patients. In the fertility and general reproduction study in rats, orally administered GF-001001-00 (125-500 mg/kg/day) did not affect terminal body weights of both males and females, the weights of the reproductive organs or sperm parameters in males or estrous cyclicity in females at any of the doses tested. There were no effects of GF-001001-00 on mating or fertility and litter parameters in rats. Embryonic and fetal developmental studies in rats showed an overall reduction in fetal body weight observed in all treatment groups of GF-001001-00 (125, 250 and 500 mg/kg/day). Similarly, in rabbits, fetal body weights were significantly reduced in the 2 higher doses of 15 and 40 mg/kg/day. Fetal toxicities in rats occurred at 500 mg/kg/day dosage group, compared to the controls whereas in rabbit fetuses, delays in skeletal development occurred at 15 and 40 mg/kg/day. TK analysis confirmed systemic exposure in the maternal plasma of rats and rabbits. Maternal toxicities were manifested at 15 and 40 mg/kg/day in the rabbit developmental toxicity study and were likely due to the antibacterial effect of GF-001001-00 on the GI flora of these animals observed in similar studies of other antibacterial drugs. Signs of maternal toxicity included dose-related reductions in body weight gain and food consumption and these were associated with increased post-implantation loss, percentage of does with resorptions and the percentage of dead or resorbed conceptuses per litter in the 40 mg/kg/day dose group. The NOAEL in rats was determined to be 500 mg/kg/day and in rabbits at 5 mg/kg/day. Perinatal/postnatal reproduction toxicity study in rats showed that there were no toxicologically relevant effects of GF-001001-00 on F₀, F₁ or F₂ generations. TK analysis confirmed systemic exposure in the F₁ generation rat pups on day 14 postpartum.

Since systemic exposure in minipigs following dermal administration of ozenoxacin 1% cream on intact and abraded skin was below levels of quantitation, calculations cannot be done to show human margins of exposure based on plasma levels in human and animals. The clinical dose of ozenoxacin (GF-001001-00 1% cream formulation) is 20 mg/day or 0.33 mg/kg/day, and the NOAEL in minipigs based on the repeat-dose toxicity study on intact and abraded skin was found to be 4 mg/kg/day which is about 12 fold the clinical dose based on a body surface area comparison. It should be noted that negligible systemic absorption of ozenoxacin was observed in both healthy volunteers and impetigo patients administered topical ozenoxacin in the submitted clinical trials. The nonclinical studies support the approval ozenoxacin 1% cream via dermal administration for the treatment of impetigo in adult and pediatric patients (aged 2 months to 11 years) twice daily for five days.

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/s/

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03/21/2017

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