CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206829Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on N206829a ceftolozane/tazocatam (CXA-201)

From: A. Jacobs

Nov 3, 2014

1. I concur that there are no Pharm-tox related approval issues.

2. An increased incidence of stillbirths was associated with tazocatam in rats, and thus a pregnancy category C seems more appropriate than B.

3. I have conveyed various comments to the reviewer and supervisor and they will address them as appropriate.

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/s/

ABIGAIL C JACOBS
11/03/2014

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	206829
Supporting document/s:	SD#1
Applicant's letter date:	02/13/2014
CDER stamp date:	02/14/2014
Product:	Ceftolozane/tazobactam (CXA-201)
Indication:	Complicated intra-abdominal infections and complicated urinary tract infections
Applicant:	Cubist Pharmaceuticals Inc.
Review Division:	Division of Anti-infective Products
Reviewer:	James S. Wild, Ph.D.
Supervisor/Team Leader:	Wendelyn J. Schmidt, Ph.D.
Division Director:	Sumathi Nambiar, M.D.
Project Manager:	Maureen P. Dillon-Parker

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

This application is a 505(b)(2) NDA for the antibacterial combination product, ceftolozane/tazobactam (Zerbaxa™) which has not been approved in any countries outside the United States. Ceftolozane is a new cephalosporin with activity against *Pseudomonas aeruginosa* and other common Gram-negative pathogens. Tazobactam is a well-established β-lactamase inhibitor that is a component of the currently marketed drug, Zosyn® (piperacillin/tazobactam).

1.2 Brief Discussion of Nonclinical Findings

Ceftolozane in 28-day studies in both rats and dogs with doses as high as 1000 mg/kg/day, produced dose-dependent nephritic changes in the form of hyaline droplet formation in proximal tubules of the renal cortex. This form of kidney pathology, which is observed with other cephalosporin antibiotics, is thought to represent an adaptation allowing compound disposition via lysosomes. Hyaline droplet formation was a consistent but reversible effect. In the absence of toxicologically meaningful degeneration or necrosis of renal tubular epithelium or substantial changes in relevant clinical pathology parameters including serum BUN, creatinine, inorganic phosphorus and/or urine volume, the hyaline-droplet formation was not considered adverse in adult animals.

However, in a non-GLP, range-finding, study in juvenile rats, ceftolozane-related kidney findings, in addition to hyaline droplet formation, included tubular basophilia and renal cortical fibrosis. These effects are consistent with tubular cell loss and regeneration, and suggest ceftolozane administration in juvenile animals produces kidney toxicity that is not apparent in adults. Currently Zerbaxa™ is not proposed for treatment of patients less than 18 years of age. If pediatric clinical trials preliminary to approval for treatment of pediatric patients are proposed in the future, kidney function should be monitored.

The primary pathology associated with tazobactam administration in the rat and dog, repeated-dose, IV-combination studies as well as published 6-month repeated-dose studies in rat (intraperitoneal administration) and dog (IV administration) was a dose-dependent increase in liver weights and liver histopathology consistent with the accumulation of liver glycogen and increased smooth endoplasmic reticulum. The histopathology occurred diffusely in liver sections, was reversible, and was characterized by accumulation of pale, eosinophilic, foamy to finely vacuolated material within the cytoplasm of hepatocytes. In rat studies, dose-dependent serum chemistry changes including decreased triglycerides, albumin, and glucose and increased globulin and potassium were considered to be related to the liver changes and glycogen accumulation. However, because changes were generally of low magnitude, reversible, and not associated with toxicologically meaningful degeneration or necrosis of hepatocytes or biologically meaningful changes in liver enzymes, the changes were not considered adverse. Higher doses of tazobactam were also associated with dose-dependent decreases in hematocrit, hemoglobin, and red blood cell counts, as well as

occasional increases in platelets and the percent of lymphocytes. However, the hematology changes were generally mild, reversible, and did not extend to bone marrow pathology.

In repeated-dose combination studies with administration of ceftolozane plus tazobactam as well as each compound alone in rats (1-month) and dogs (2-weeks), new or augmented toxicities were not observed. Also, in both rats and dogs, plasma concentrations of ceftolozane and tazobactam were not substantially changed when the compounds were administered in combination, and plasma concentrations for both agents did not substantially increase or decrease with repeated dosing.

The weight of evidence from full batteries of genetic toxicity assays suggests minimal potential for genotoxicity in humans for the combination of ceftolozane and tazobactam as well as each component alone.

In embryo-fetal studies, neither ceftolozane nor tazobactam was teratogenic or caused fetal toxicity. However, both compounds produced limited adverse effects in F_1 generation pups in pre-postnatal studies (decreased auditory startle response for ceftolozane and increased still-births and reduced postnatal body weights for tazobactam) suggesting the benefits of administration of Zerbaxa™ to pregnant women particularly at higher than recommended doses should be weighed against its potential subset of adverse effects in offspring.

Taken as a whole, the nonclinical toxicology data suggests relative safety for clinical administration of Zerbaxa™ at the recommended dose of 4500 mg/kg/day (3000 mg/kg/day ceftolozane and 1500 mg/kg/day tazobactam). The primary effects of each compound in nonclinical studies with adult animals, hyaline droplet formation in the kidneys for ceftolozane and liver glycogen accumulation for tazobactam, were reversible and are not expected to be clinically adverse. Other toxicities that occurred in nonclinical studies, cecal enlargement, injection site reactions, and histamine release are not expected to substantially impact clinical administration. The nonclinical data suggests that ceftolozane is not greatly antigenic, but like other β-lactam antibiotics has the potential to elicit allergic reactions.

1.3 Recommendations

1.3.1 Approvability

This product is approvable from a Pharmacology/Toxicology perspective for the proposed antibacterial treatment indications in adults.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The Sponsor's draft labeling language and the Reviewer's proposed labeling language are shown below. The ceftolozane and tazobactam data supporting the Reviewer's exposure margin comparisons are shown in Table 1 and Table 2.

8.1 Pregnancy

Sponsor's Draft Labeling Language

Pregnancy Category B.

There are no adequate and well-controlled trials in pregnant women with either ceftolozane or tazobactam. Because animal reproduction studies are not always predictive of human response, ZERBAXA™ should be used during pregnancy only if the potential benefit outweighs the possible risk.

(b) (4)

Reviewers Proposed Labeling Language

Pregnancy Category C.

There are no adequate and well-controlled trials in pregnant women with either ceftolozane or tazobactam. Because animal reproduction studies are not always predictive of human response, ZERBAXA should be used during pregnancy only if the potential benefit outweighs the possible risk.

Embryo-fetal development studies performed with ceftolozane in mice and rats up to doses of 2000 and 1000 mg/kg/day, respectively, revealed no evidence of harm to the fetus. The mean plasma exposure (AUC) values associated with these doses are approximately 19 (mice) and 11 (rats) times the mean daily human ceftolozane exposure in healthy adults at the clinical dose of 1 gram thrice-daily. It is not known if ceftolozane crosses the placenta in animals.

In a pre- postnatal study in rats, ceftolozane administered during pregnancy and lactation (Gestation Day 6 through Lactation Day 20) was associated with a decrease in auditory startle response in postnatal day 60 male pups at maternal doses of greater than 300 mg/kg/day. The plasma exposure (AUC) associated with the NOAEL dose of

100 mg/kg/day in rats is approximately equal to the mean human ceftolozane exposure in healthy adults at the clinical dose of 1 gram thrice-daily.

In an embryo-fetal study in rats, tazobactam administered at doses up to 3000 mg/kg/day (approximately 19 times the recommended human dose based on body surface area comparison) did not produce maternal or fetal toxicity. In rats, tazobactam was shown to cross the placenta. Concentrations in the fetus were less than or equal to 10% of those found in maternal plasma.

In a pre-postnatal study in rats, tazobactam administered intraperitoneally twice daily at the end of gestation and during lactation (Gestation Day 17 through Lactation Day 21) produced significantly more stillbirths with a tazobactam dose of 1280 mg/kg/day (approximately 8 times the recommended human dose based on body surface area comparison). No effects on the development, function, learning or fertility of F_1 pups were noted, but postnatal body weights for F_1 pups delivered to dams receiving 320 and 1280 mg/kg/day tazobactam were significantly reduced 21 days after delivery. F_2 generation fetuses were normal for all doses of tazobactam. The NOAEL for reduced F_1 body weights was considered to be 40 mg/kg/day (approximately 0.3 times the recommended human dose based on body surface area comparison).

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's Draft Labeling Language

Long-term carcinogenicity studies in animals have not been conducted with ZERBAXA™, ceftolozane, or tazobactam.

(b) (4)

(b) (4)

Reviewers Proposed Labeling Language

Long-term carcinogenicity studies in animals have not been conducted with ZERBAXA, ceftolozane, or tazobactam.

ZERBAXA was negative for genotoxicity in an *in vitro* mouse lymphoma assay and an *in vivo* rat bone marrow micronucleus assay. In an *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, ZERBAXA was positive for structural aberrations.

Ceftolozane was negative for genotoxicity in an *in vitro* microbial mutagenicity (Ames) assay, an *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, an *in vivo* mouse micronucleus assay, and an *in vivo* unscheduled DNA synthesis (UDS) assay. Ceftolozane was positive for mutagenicity in an *in vitro* mouse lymphoma assay.

Tazobactam was negative for genotoxicity in an *in vitro* microbial mutagenicity (Ames) assay, an *in vitro* chromosomal aberration assay in Chinese hamster ovary cells, and an *in vivo* rat bone marrow micronucleus assay.

Ceftolozane had no adverse effect on fertility in male or female rats at intravenous doses up to 1000 mg/kg/day. The mean plasma exposure (AUC) value at this dose is approximately 8 times the mean daily human ceftolozane exposure value in healthy adults.

In a rat fertility study with intraperitoneal tazobactam, male and female fertility parameters were not significantly affected at doses ≤ 640 mg/kg/day (approximately 4 times the recommended clinical daily dose based on body surface area comparison).

Table 1: Summary of NOAEL Values and Exposure Margins Associated with the CXA-101 Developmental and Reproductive Toxicology Studies.

Study	Doses mg/kg/day)	NOAEL (mg/kg/day)	HED[a] (mg/kg/day)	CXA-101 AUC (µg·h/mL)	Exposure Margin[e,f,g]
CXA-101 Fertility Study in Rats	Male 100, 300, 1000 mg/kg/day	1000	161.3	1584 1604 Mean = 1594[b]	8.8
	Female 100, 300, 1000 mg/kg/day	1000	161.3	1201 1360 Mean = 1281[b]	7.0
CXA-101 Embryo-Fetal Study in Mice	Maternal 300, 1000, 2000 mg/kg/day	2000	162.6	3536[c]	19.4
	Fetus	2000	162.6	3536[c]	19.4
CXA-101 Embryo-Fetal Study in Rats	Maternal 100, 300, 1000	300	48.4	678[d]	3.7
	Fetus	1000	161.3	2013[d]	11.0
CXA-101 Pre-Postnatal Study in	Maternal 100, 300, 1000	1000	161.3	2013[d]	11.0

Rats	F_1	100	16.1	230 [d]	1.3
	F_2	1000	161.3	2013 [d]	11.0

[a] NOAEL values were divided by 12.3 in mice and 6.2 in rats to determine the human equivalent dose values based on relative body surface area.

[b] Based on the mean Day 28 plasma AUC measurements in Study No.: CXA201-T-001: A 28 Day Intravenous Toxicity Study in Sprague-Dawley Rat, and Study No.: GLR050748: 4-Week Intravenous Dose Toxicokinetic Study of FR264205 in Rats.

[c] Based on the plasma $AUC_{0\text{-last}}$ measurement for pregnant mice in Study No.: CX.101.TK.002: CB-500,101: A GLP Intravenous Toxicokinetic Study in Pregnant CD-1 Mice.

[d] Based on the plasma $AUC_{0\text{-}24h}$ measurement for pregnant rats in Study No.: CX.101.TK.001: CB-500,101: A GLP Intravenous Toxicokinetic Study in Pregnant Sprague-Dawley Rats.

[e] The clinical daily dose of CXA-101 is 1000 mg TID (3000 mg/day). For an average 60 kg human, the daily dose is 50 mg/kg/day.

[f] The clinical AUC in healthy adults after 10 days of intravenous administration of 1000 mg CXA-101 TID (3000 mg/day) is 182 (µg•h/mL).

[g] All of the exposure margins are based on nonclinical and clinical AUC comparisons.

Table 2: Summary of NOAEL Values and Exposure Margins Associated with the Tazobactam Developmental and Reproductive Toxicology Studies.

Study	Doses	NOAEL or LOAEL (mg/kg/day)	HED [a] (mg/kg/day)	Tazobactam $AUC_{0\text{-}24h}$ (µg·h/mL)	Exposure Margin [b,c,d]
Tazobactam Fertility Study in Rats	Male	640	103.2	Not measured	4.1
	Female	640	103.2		4.1
Tazobactam Embryo-Fetal Study in Rats	Maternal 125, 500, and 3000 mg/kg/day	500 (due to purported weight loss in the high-dose group)	80.6	Not measured	3.2
	Fetus	3000	483.9	Not measured	19.4
Tazobactam Pre- Postnatal Study in Rats	Maternal 40, 320, 1280 mg/kg/day	< 40 (cecal enlargement) 320 (increased still births in the high-dose group)	51.6	Not measured	2.1
	F_1	40	6.5	Not measured	0.26

[a] NOAEL values were divided by 12.3 for mice and 6.2 for rats to determine the human equivalent dose (HED) values based on relative body surface area.

[b] The clinical daily dose of tazobactam is 500 mg TID (1500 mg/day). For an average 60 kg human, the daily dose is 25 mg/kg/day.

[c] The clinical AUC in healthy adults after 10 days of intravenous administration of 500 mg tazobactam TID (1500 mg/day) is 25 (µg•h/mL).

[d] All of the exposure margins are based on body surface area comparisons.

2 Drug Information

2.1 Drug

CAS Registry Number: 936111-69-2

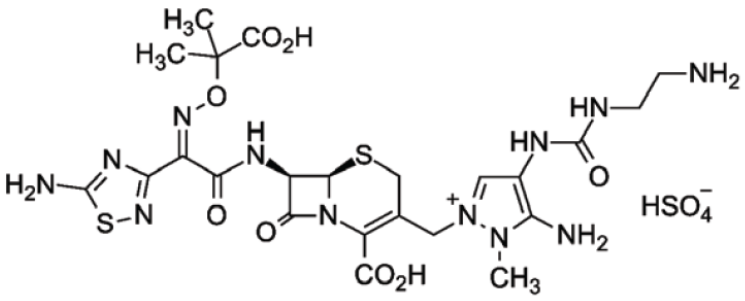
Generic Name: Ceftolozane sulfate

Code Name: CXA-101; FR264205

Chemical Name: 1*H*-Pyrazolium,5-amino-4-[[[(2-aminoethyl)amino]carbonyl]amino]-2[[(6*R*,7*R*)-7-[[(2*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-,sulfate (1:1)

Molecular Formula/Molecular Weight: $C_{23}H_{30}N_{12}O_8S_2 \bullet H_2SO_4$/764.77 g/mole

Structure or Biochemical Description



Pharmacologic Class: antibacterial

CAS Registry Number: 89785-84-2

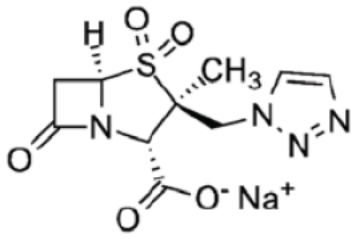
Generic Name: tazobactam

Code Name: KB0112

Chemical Name: Sodium;(2*S*,3*S*,5*R*)-3-methyl-4,4,7-trioxo-3-(triazol-1-ylmethyl)-4λ^6-thia-1-azabicyclo[3.2.0]heptanes-2-carboxylate

Molecular Formula/Molecular Weight: $C_{10}H_{11}N_4NaO_5S$/322.28 g/mole

Structure or Biochemical Description



Pharmacologic Class: antibacterial, β-lactamase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 104490

2.3 Drug Formulation

The drug substance formulation for ceftolozane (CXA-101) is shown in Table 3.

Table 3: The Drug Product Formulation for Ceftolozane/Tazobactam (Sponsor's Table)

Component		Quality Standard[a]	Function	Nominal Composition mg per Vial
Ceftolozane DPI[b]	Ceftolozane Sulfate	In-House Section 3.2.S.4.1	Active	1147
	Citric Acid, (b) (4)	USP	(b) (4)	21
	Sodium Chloride	USP	Stabilizing Agent	487
	L-Arginine	USP	(b) (4)	600[c] (b) (4)
Tazobactam Sodium[d]		In-House Section 3.2.S.4.1	Active	537
(b) (4)				
Total Weight				2792

[a]Quality standard abbreviations: USP = United Stated Pharmacopeia; NF = National Formulary. Refer to Section 3.2.P.4.1 for additional information on excipient quality.

[b]Actual amount of ceftolozane DPI will vary based on the measured potency.

(b) (4) 600 mg per vial is considered a representative total amount.

[d]Actual weight of tazobactam sodium will vary based on the measured potency.

(b) (4)

2.4 Comments on Novel Excipients

All of the excipients, (b) (4) citric acid, sodium chloride, and L-arginine have been used at higher levels in approved intravenous products. The percentage of each

excipient contained in the ceftolozane/tazobactam drug product and approved injection products is shown in Table 4 below.

Table 4: The Percentage of Each Excipient in the CXA-101 Product, and the Percentages Previously Used in Approved Products.

Excipent	Amount (Percent)[b] in the Ceftolozane/Tazobactam Drug Product	Maximum Percentage in an Approved Intravenous Product[a]
(b) (4) Citric Acid	21 mg (0.75%)	42%
Sodium Chloride	487 mg (17.4%)	45%
L-Arginine	600 mg (21.5%)	88%

[a] Values obtained from FDA Database: "Inactive Ingredient Search for Approved Drug Products."
[b] Percentage of the (b) (4) mg total weight/unit product

2.5 Comments on Impurities/Degradants of Concern

The ceftolozane drug substance specification is shown below in Table 5. The drug substance contains multiple impurities and/or degradation products which have been qualified for their acceptance criteria and genotoxic potential. Two residual solvents present in the release substance, (b) (4) and (b) (4) are also controlled according to threshold levels identified in ICH Guideline Q3C(R5): "Impurities: guideline for Residual Solvents.

The ceftolozane drug substance impurities and degradation products, proposed acceptance criteria and the qualification levels calculated by the Sponsor for a 3 g/day clinical dose are shown below in Table 6.

The percent impurity values for all of the CXA-101 impurities/degradants used in the qualifying nonclinical studies (CX.101.TX.031 and GLR050690; both 28-day rat studies) are shown Table 7. The batches of CXA-101 used to qualify the impurities in the nonclinical studies were Batch #139-121211 and #44042000022 in Study # CX.101.TX.031 and Batch # 3F02 in Study #GLR050690.

The qualification levels calculated by the Reviewer using CXA-101 NOAEL values of 1000 mg/kg/day for both 28-day, repeated-IV-dose rat studies (CX.101.TX.031 and GLR050690) which employed multiple different batches of CXA-101 are shown in Table 8.

Table 5: Ceftolozane Sulfate Drug Substance Specification. (Sponsor's Table)

Test	Acceptance Criteria	Analytical Procedure
Appearance	White to Off-White Powder	Visual (TM-190)
Identification, IR	IR spectrum of Ceftolozane sulfate Sample Conforms to IR spectrum of Ceftolozane sulfate Standard	IR (TM-191)
Identification, HPLC by RT	Retention Time of Ceftolozane Sample Peak is Consistent with Retention Time of Ceftolozane Standard Peak	HPLC (TM-197)
Color of Solution	Less Colored than or equal to Reference Y_6	Colorimetry (EP 2.2.2 and TM-192)
(b) (4)	(b) (4)	(b) (4) (TM-193)
Optical Rotation		Polarimeter (TM-195)
Residue on Ignition		USP <281> (TM-221)
(b) (4)		IC (TM-222)
Heavy Metals		USP <231>
Ceftolozane Potency, as is		HPLC (TM-196)
Ceftolozane Assay, Anhydrous and Sulfuric acid free (% w/w)		
Purity (% Area)		HPLC (TM-197)
Related Substances (% Area) Specified, Identified Impurities (b) (4) Each Unspecified Impurity Total Impurities		
Residual Solvent (b) (4)		GC (TM-199)
Residual (b) (4) Content		IC (TM-335)

Table 6: CXA-101 Impurities and Degradants and the Studies Supporting Qualification of Acceptance Criteria. (Sponsor's Table)

Impurity Name	Proposed Acceptance Criteria (% Area)	Range in ceftolozane sulfate (Release)	Qualified level for maximum dose of 3g per day

(b) (4)

Table 7: Percent of Impurities in CXA-101 Batches Used in Nonclinical Studies
(Sponsor's Table)

Peak (CB Number; RRT)	Lot			
	400142G	3F02	4404200022	139-121211

(b) (4)

Table 8: The Percentages of Impurities and Degradants in Nonclinical Studies, Related Impurity NOAEL and HED Values and Calculated Qualification Level for Each Impurity for the Recommended Daily Dose of CXA-101.

Impurity/ Qualifying Study/ Drug Lot.	Percent of impurity in the Study Batch	Impurity NOAEL (mg/kg/day)[a]	HED (mg/kg/day)[b]	Percent Impurity per 3 g daily human dose[c]
[redacted] (b) (4)				

NOAEL = no adverse effect level; HED = human equivalent dose

a For each study and impurity, the impurity NOAELs are calculated by multiplying the CXA-101 NOAEL (1000 mg/kg/day for both qualifying studies) by the percent impurity contained in source batch of CXA-101 used in the study.

b The impurity NOAEL values for the rat studies were converted to HED values using a

conversion factor (divide by 6.2) based on the relative body surface areas of rats and humans.
[c] The percent impurity contained in 3 grams of ceftolozane administered in the recommended clinical dose is calculated by multiplying the HED by the average 60 kg human body weight then dividing the product by 3000 mg, then finally multiplying by 100 to derive a percent value.

Reviewer Comment: *The qualified impurity levels calculated by the Sponsor (shown in* Table 6*) are larger than the values calculated by the Reviewer (shown in* Table 8*) because the Sponsor used 70 kg as the average human body weight instead of the recommended 60 kg used by the Reviewer.*

Residual Solvents

Multiple solvents are used in the manufacture of the CXA-101 drug substance (Table 9). Of these, only (b) (4) is a class I solvent and its monitored levels in multiple batches fell below the 2 ppm concentration limit specified in the ICH Q3C(R5) guideline. Of the class 2 and class 3 solvents most are purged to below the levels specified in the ICH Q3C(R5) guideline at manufacturing stages before the release product and most were shown to occur at very low levels when measured in multiple batches of CXA-101.

Three residual solvents are measured the ceftolozane release product to make sure levels remain below acceptable limits (Table 10). One of these solvents, (b) (4) is listed in the ICH Q3C(R5) Guideline, "Impurities: Guideline for Residual Solvents," as a solvent for which no adequate toxicological data was found. The guideline indicates that "manufacturers should supply justification for residual levels of these solvents in pharmaceutical products."

The Sponsor has provided justification for the acceptance criteria of (b) (4)

Based on these results, the Sponsor calculated the permitted daily exposure (PDE) for (b) (4) in CXA-101 drug substance according to the algorithm included in ICH Q3C(R5) in Appendix 3: Methods for Establishing Exposure Limits. The Sponsor's calculations are shown below. If an average human body weight of 60 kg is substituted for 70 kg the PDE can be recalculated as (b) (4) which equals (b) (4) of a 3000 mg daily dose. As shown in Table 10, the Sponsor has specified the acceptance criteria for (b) (4) in CXA-101 as (b) (4) and in the batches where (b) (4) was measured the amounts ranged from (b) (4) to (b) (4) While a more

comprehensive toxicity assessment for [redacted] (b) (4) is desirable, based on the data available, the acceptance criteria for [redacted] (b) (4) in CXA-101 is adequate.

Table 9: Residual Solvents Used in the Manufacture of CXA-101. (Sponsor's Table)

Solvent	Class	Structure	Acceptable Limit (mg/day)[a]	Actual Levels in Ceftolozane Lots
[redacted] (b) (4)				

[a] Acceptable levels of solvents based on the ICH guidance document" Impurities: Guideline for Residual Solvents Q3C (R5).
[b] Data obtained with ceftolozane sulfate DS lots 44063700022, 44063700032, 44063700042, 44063700052, 44063700062, 44063700013, 44063700023, and 44063700033.
Abbreviations: DS = drug substance; LOQ = limit of quantification; ppm = part per million.

The Sponsor's Calculation of the Permitted Daily Exposure for [redacted] (b) (4) in CXA-101 Drug Substance

PDE = (Lowest Observed Effect Level [LOEL] x Weight) ÷ (F1xF2xF3xF4xF5) where:
LOEL = 75 mg/kg/day;
An average body weight of 70 kg was utilized for calculations as this is believed to accurately reflect the average weight of an adult individual;
F1 = 5 (extrapolation from rats to humans);
F2 = 10 (variability between individuals);

F3 = 1 (reproductive study in which the whole period of organogenesis was covered);
F4 = 1 (fetal toxicity associated with maternal toxicity);
F5 = 5 (moderate severity of effects that are reversible).
Based on these data points, the PDE = (b) (4)

(b) (4)

Table 10: Residual Solvents Measured in the CXA-101 Drug Substance Release.
(Sponsor's Table)

Entry	Substance/Formula/MW/Designation	Process Use	Measured Levels in Drug Substance
1	(b) (4)		
2			
3			

Genetic Toxicity
In addition to testing in repeated-dose studies to qualify each CXA-101 impurity/degradant for general toxicity, each impurity/degradant was also assessed for genetic toxicity in Leadscope and DEREK *in silico* assessments and all the impurities were found to have negative results. In addition CXA-101 Batch #s 400142G (forced degradation product of CXA-101) and 3F02 were used in the extensive panel of *in vitro* genetic toxicity studies with negative results.

Tazobactam Drug Substance Impurities

The tazobactam sodium drug substance specifications are shown in Table 11. The only tazobatam impurity that occurs at levels requiring reporting and greater than the 0.15% qualification threshold is [redacted] is structurally equivalent to the major metabolite for tazobactam, tazobactam M-1. The acceptance criteria of [redacted] for [redacted] in the tazobactam drug substance and ceftolozane/ tazobactam drug product is based on the qualified level established in the USP monograph for [redacted] In addition, the structure of [redacted] was assessed for mutagenic potential using *in silico* assays (DEREK and leadscope), and [redacted] showed no structural alerts suggestive of mutagenic potential.

Table 11: Tazobactam Sodium Drug Substance Specifications. (Sponsor's Table)

Test	Acceptance Criteria	Analytical Procedure
Appearance	White to Off-white Powder	Visual ([redacted]-KB0112A-01)
Identification, HPLC by RT	The retention time of the major peak in the chromatogram of the assay preparation corresponds to those in the chromatogram of the standard preparation in assay	HPLC ([redacted]-KB0112A-02)
Identification, Sodium	Positive to the test for sodium	USP <191> ([redacted]-KB0112A-02)
Identification, IR	IR Spectrum of the sample corresponds to spectrum of tazobactam sodium CRS	IR ([redacted]-KB0112A-02)
Visible Foreign Matter	Should not exist	USP <1> ([redacted]-KB0112A-03)
Particulate Matter Particles [redacted] Particles	[redacted]	USP <788> ([redacted]-KB0112A-04)
pH		USP <791> ([redacted]-KB0112A-07)
Clarity of Solution		Visual ([redacted]-KB0112A-05)
Color of Solution		Visual ([redacted]-KB0112A-06)
[redacted]		USP <921> ([redacted]-KB0112A-08)
Specific Optical Rotation (calculated on [redacted] basis)		USP <781> ([redacted]-KB0112A-09)
Residue on Ignition		USP <281> ([redacted]-KB0112A-10)
Heavy Metals		USP <231> ([redacted]-KB0112A-11)
Assay (calculated on [redacted] basis)		HPLC ([redacted]-KB0112A-12)
Related Substances [redacted]		HPLC ([redacted]-KB0112A-13)
Residual Solvents [redacted]		GC ([redacted]-KB0112A-14)
Bacterial Endotoxin		USP <85> ([redacted]-KB0112A-15)
Sterility		USP <71> ([redacted]-KB0112A-16)

Abbreviations: CFU = colony forming units; EP = European Pharmacopoeia; EU = Endotoxin Unit; GC = Gas Chromatography; HPLC = High-performance liquid chromatography; IC = Ion chromatography; IR = Infrared; RT = Retention Time; USP = United States Pharmacopeia

Ceftolozane/Tazobactam Drug Product Impurities and Degradants

No new ceftolozane-related impurities are known to be generated in the manufacture of the ceftolozane/tazobactam drug product. All of the ceftolozane- and tazobactam-related impurities that are not degradants are controlled in the drug substance process and are not assessed in the ceftolozane/tazobactam drug product. The ceftolozane degradants and the single tazobactam degradant (b) (4) are qualified and controlled in the drug product as indicated below in Table 12. The proposed specifications would support a maximum dosage of up to 6 g/day ceftolozane and 3g/day tazobactam, twice the proposed therapeutic dose of 3 g/day ceftolozane and 1.5 g/day tazobactam.

All of the ceftolozane-related degradants are qualified up to specified levels by their use in nonclinical toxicology studies. The qualified level for (b) (4)

Reviewer Comment: *the qualified levels for* (b) (4) *shown in Table 12 are higher than the values calculated above because* (b) (4)

Table 12: Qualification levels and Proposed Acceptance Criteria for the Degradants in the Drug Product, Ceftolozane/Tazobactam. (Sponsor's Table)

Impurity Name	Proposed Commercial Acceptance Criteria for Release of Ceftolozane/ Tazobactam DP (% Area)	Proposed Commercial Acceptance Criteria on Stability for Ceftolozane/ Tazobactam DP (% Area)	Range in Ceftolozane/ Tazobactam DP Batch Analysis (Release) (% Area)	Proposed Commercial Acceptance Criteria for Ceftolozane DPI /Tazobactam Sodium (% Area)	Qualified Level 3g dose (% Area) (Tox Study)	Qualified Level Up to a 6g dose (% Area)
(b) (4)						

Container Closure System: Extractables and Leachables

The containers used in the preparation of the ceftolozane/tazobactam drug product include a [redacted] for the ceftolozane drug product intermediate (DPI), and the container closure system. The container closure is composed of a glass vial (20 ml, Type I, clear glass, molded), a 20 mm [redacted] stopper [redacted] and a 20 mm aluminum crimp cap with a purple [redacted] seal. All of these products have been used as containers in previously approved drug products and all have Type III drug master files. The Sponsor, Cubist, has obtained the right of reference for all of the drug master files.

Because all of the components have been used previously in approved products, the leachables and extractables from each component in aqueous solutions are considered qualified. The only leachables or extractables that would require further qualification are those that might arise from interaction of ceftolozane DPI with the [redacted] or from interaction of the ceftolozane/tazobactam drug product with the components of the container closure system.

Extraction studies were performed with the [redacted] and each individual component of the container closure system. In addition, a controlled extraction study was performed to evaluate and identify the potential leachable compounds that could arise from interaction of the drug product with the container closure system. In this study, the drug product was stored inverted at 40°C for two months to simulate a 24-month drug product leachable profile when stored refrigerated.

The volatile leachables detected in the controlled extraction study are shown in Table 13. All of the six volatile leachables except two were also detected as volatile extractables from the [redacted] stopper. The two exceptions, [redacted] and [redacted] are respectively present in the tazobactam and ceftolozane drug substances at release and thus are not considered to be new leachable compounds.

Other semi-volatile organic leachables were also detected as shown in Table 14. Of these 13 leachables, 10 were also found as extractables from the [redacted] The three new semi-volatile compounds, [redacted] were not considered related to the container closure as they were reportedly detected at low levels in either the ceftolozane or tazobactam drug substances.

The overall results suggest that no new leachables arose from interaction between the drug product and the container closure system, and the [redacted] and the container closure system are considered acceptable for packaging of the ceftolozane/tazobactam drug product.

Table 13: Summary of the Volatile Leachables Observed in the Controlled Extraction Study with the Drug Product and the Container Closure System.
(Sponsor's Table)

Retention Time (minutes)	Name	CAS #	Identification Level	µg/Vial	µg/Day[1]	Toxicology Assessment
(b) (4)						

Abbreviations: CAS = Chemical Abstract Service
[1]Based upon a dosage up to 6 vials per day
[2]Detected in extractable experiment

Table 14: Summary of the Semi-Volatile Leachables Observed in the Controlled Extraction Study with the Drug Product and the Container Closure System.
(Sponsor's Table)

Retention Time (minutes)	Name	CAS #	Identification Level	µg/vial	µg/Day[1]	Toxicology Assessment
(b) (4)						

Abbreviations: CAS= Chemical Abstract Service
[1]Based upon a dosage up to 6 vials per day
[2]Detected in the (b) (4) extractable experiment

2.6 Proposed Clinical Population and Dosing Regimen

Proposed Clinical Population: ZERBAXA™ (ceftolozane/tazobactam) is indicated for the treatment of patients ≥18 years of age suffering from complicated intra-abdominal infections, and complicated urinary tract infections including pyelonephritis.

Dosing Regimen: The recommended dosage of ZERBAXA is 1.5 grams (1 gram of ceftolozane and 500 mg of tazobactam) every 8 hours by IV infusion over 1 hour. The duration of dosing (up to 14 days) can vary with the treated indication and, in patients with reduced renal function, doses are lowered.

2.7 Regulatory Background

The ceftolozane/tazobactam combination product was first submitted in IND 104490 in 7/01/2009. Many of the nonclinical studies were reviewed in the 2/23/2011 nonclinical safety review for IND 104490.

3 Studies Submitted

3.1 Studies Reviewed

Safety Pharmacology (All of these studies were reviewed in the 2/23/2011 nonclinical safety review for IND 104490)

1. Neuropharmacological Profile (NPP) of CXA-101 in rats. Study Report No.: CXA101-T-002.
2. Convulsion activity of FR264205 by intracerebroventricular injection on mouse and rat. Study Report No.: CRE060244.
3. Effects of CXA-101 on cloned hERG potassium channels expressed in human embryonic kidney cells. Study report No.: 080125-DMK
4. Cardiovascular evaluation of intravenous administered CXA-101 in conscious telemetered dogs. Study Report No.: CXA101-T-001.
5. Safety pharmacology of FR264205 on rat. Study Report No.: CRE060242.
6. Evaluation of respiratory function following intravenous administration of CXA-101 in rats. Study Report No.: CXA101-T-003.
7. Histamine releasing and hemolysis activities of FR264205. Study Report No.: CRE060243.

Pharmacokinetics

Analytical Methods (these studies are only briefly summarized)

1. Determination of FR264205 in Dog Kidney Using High-performance Liquid Chromatography (HPLC) (Study No.: CRD050185).
2. Determination of FR264205 in Rat Kidney Using High-performance Liquid Chromatography (HPLC) (Study No.: CRD050186).
3. Validation of Concentration Level Confirmation Test (HPLC) for Dosing Solutions of FR264205 (Vehicle: Physiological Saline Solution with Sodium Hydroxide Added) (Study No.: GLR030563).
4. Validation of HPLC Concentration Determination Procedures for FR264205 in Physiological Saline Solution with Sodium Hydroxide (Study No.: GLR030797).
5. Validation of Concentration Level Confirmation Test (HPLC) for Dosing Solutions of FR264205 (Vehicle: Water for Injections) (Study No.: GLR040072).

6. Validation of Concentration Level Confirmation Test (HPLC) for Dosing Solutions of FR264205 1g Preparation for Injection (Vehicle: Physiological Saline Solution) (Study No.: GLR040155).
7. Validation of Concentration Level Confirmation Test (HPLC) for Dosing Solutions of FR264205 (Vehicle: Physiological Saline Solution with Sodium Hydrogen Carbonate Added) (Study No.: GLR040253).
8. Validation of Dosing Solution Preparation Procedures and Concentration Determination Procedures of FR264205 and FR264205 Product for 1 g Injection (Study No.: GLR040849).
9. Validation of a Method for the Determination of CXA-101 in Dog Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC08B-0302).
10. Validation of a Method for the Determination of Tazobactam in Dog Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC08B-0339).
11. Validation of a Method for the Determination of CXA-101 in Rat Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC08B-0348).
12. Validation of a Method for the Determination of Tazobactam in Rat Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC08B-0348).
13. Validation of a Method for the Determination of CXA-101 (FR264205) in Modified Saline Formulations Using High-Performance Liquid Chromatography with Ultraviolet Detection (Study No.: MC08F-0009).
14. Cross-validation of a Method for the determination of CXA-101 (FR264205) in Water Formulations Using High-Performance Liquid Chromatography with Ultraviolet Detection (Study No.: MC08F-0049).
15. Validation of a Method for the Determination of Tazobactam in Modified Saline Formulations Using High-performance Liquid Chromatography with Ultraviolet Detection (Study No.: MC08F-0151).
16. Evaluation of the Extended Stability of CXA-101 in Dog Plasma using High-Performance Liquid Chromatography with Mass Spectrometric detection (Study No.: MC09B-0051).
17. Evaluation of the Extended Stability of Tazobactam in Dog Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC09B-0052).
18. Evaluation of the Extended Stability of CXA-101 in Rat Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC09B-0053).
19. Evaluation of the Extended Stability of Tazobactam in Rat Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC09B-0054).
20. Evaluation of the Extended Stability of Tazobactam in Acidified Neonatal Rat Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC12B-0271).

21. Evaluation of the Extended Stability of CXA-101 in Acidified Neonatal Rat Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC13B-0006).
22. Validation of a Method for the Determination of CXA-101 in Mouse Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC10B-0094).
23. Validation of a Method for the Determination of CXA-101 in Acidified Neonatal Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric detection (Study No.: MC12B-0105).
24. Validation of a Method for the Determination of Tazobactam in Acidified Neonatal Rat Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC12B-0106).

Absorption

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. Non-clinical pharmacokinetics: pharmacokinetics of FR264205 after intravenous administration in rats. Study Report No.: CRD060001.
2. Non-clinical pharmacokinetics: pharmacokinetics of FR264205 in dogs. Study Report No.: CRD060002.
3. Non-clinical pharmacokinetics: determination of FR264205 concentration in rat kidney in “four-week intravenous dose toxicity study of FR264205 in rats followed by a 4-week recovery period.” Study Report No.: CRD060003.
4. Intravenous infusion pharmacokinetic study of CXA-101 and tazobactam in Beagle dogs. Study Report # CXA201-P-001.

Reviewed in This Document

5. CXA-101: A Non-GLP Pharmacokinetic Study in Male Sprague-Dawley Rats. Study No.: CX.101.PK.002
6. Tazobactam: A Non-GLP Single Dose Pharmacokinetic Study in the Presence or Absence of CXA-101 Following a Single Subcutaneous or Intraperitoneal Administration to Male Sprague-Dawley Rats. Study No.: CX.101.PK.005.
7. CB-500,101: A GLP Intravenous Toxicokinetic Study in Pregnant Sprague Dawley Rats. Study No.: CX.101.TK.001.
8. CB-500,101: A GLP Intravenous Toxicokinetic Study in Pregnant CD-1 Mice.
9. CXA-101: A Non-GLP Single IV Dose Pharmacokinetic Study in Female Long Evans Rats. Study No.: CXA.101.PK.001.

Distribution

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. Distribution and excretion of ^{14}C-FR264205 after single intravenous dosing in rats. Study Report No.: CRD050182.
2. Non-clinical pharmacokinetics: *in vitro* transfer of FR264205 into blood cells in mice, rats, dogs, and humans. Study report No.: CRD050180.
3. Non-clinical pharmacokinetics: *in vitro* protein binding of FR264205 in mouse, rat, dog, and human serum and in human plasma. Study report No.: CRD050181.
4. Non-clinical pharmacokinetics: determination of FR264205 concentration in dog kidney in “four week intravenous dose toxicity study of FR264205 in dogs followed by a 4-week recovery period.” Study Report No.: CRD060004.

Reviewed in This Document

5. Non-clinical Pharmacokinetics: Determination of FR264205 Concentration in Rat Kidney in "Four-week Intravenous Dose Toxicity Study of FR264205 in Rats Followed by a 4-Week Recovery Period. Study No.: PCM04072.
6. FR264205 Concentration in Rat Plasma, Liver, and Kidneys at Necropsy in a 4-Week Toxicity Trial. Study No.: MB010101.
7. Plasma Protein Binding of FR264205. Study No.: PH021246.
8. Transport of FR264205 Into the Brain (Measurement of Concentrations in Rat Brain and Plasma. Study No.: PH030485.
9. Toxicokinetics of FR264205 in a 4-week Preliminary Toxicity Test in Rats. Study No.: PH031092.
10. Accumulation of FR264205 in the Rat Kidney. Study No.: PH031094.
11. Intrarenal Concentrations of Anti-Psuedomonas aeroginosa Cephem [drug] after 10 Consecutive Doses in Mice. Study No.: PH031095.

Metabolism

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. Composition of metabolites in plasma, urine, feces, and bile of rats after intravenous dosing of ^{14}C-FR264205. Study Report No.: CRD050183
2. Composition of metabolites in kidney of rats after intravenous dosing of ^{14}C-FR264205. Study Report No.: CRD050184.
3. CXA-101: *In vitro* evaluation of CXA-101 as a direct inhibitor of human cytochrome P450 enzymes. Study Report No.: CXA101-P-001.

Reviewed in This Document

4. CXA-101: A non-GLP evaluation of induction potential of cytochrome P450 isoforms by CXA-101 in cultured cryopreserved human hepatocytes. Study Report # CX.101.DM.001.
5. CXA-101: A non-GLP evaluation of induction potential of cytochrome P450 isoforms 1A2 and 3A4 by CXA-101 in cultured cryopreserved human hepatocytes. Study No.: CX.101.DM.002.
6. Tazobactam: A non-GLP evaluation of induction potential of cytochrome P450 isoforms 1A2, 2B6, and 3A4 by tazobactam in cultured cryopreserved primary human hepatocytes. Study Report # CX.101.DM.007.
7. Tazobactam: A non-GLP evaluation of inhibition potential of cytochrome P450 isoforms in human liver microsomes. Study Report No.: CX.101.DM.008.
8. CXA-101: A non-GLP evaluation of inhibition potential of cytochrome P450 isoforms in human liver microsomes. Study Report No.: CX.101.DM.009.
9. Tazobactam M-1: A non-GLP evaluation of induction potential of cytochrome P450 isoforms 1A2, 2B6, 3A4 by tazobactam M-1 in cultured cryopreserved primary human hepatocytes. Study Report No.: CX.101.DM.010.
10. Tazobactam M-1: A non-GLP in vitro evaluation of inhibition potential of seven major cytorchrome P450 isoforms in human liver microsomes. Study Report No.: CX.101.DM.011.
11. CXA-101: A non-GLP in vitro evaluation of induction potential of cytochrome P450 isoform 2B6 by CXA-101 in cultured cryopreserved human hepatocytes. Study Report No.: CX.101.DM.013.

12. CXA-101: A non-GLP *in vitro* evaluation of time-dependent inhibition potential of cytrochrome P450 isoforms in human liver microsomes. Study Report No.: CX.101.DM.017.
13. Tazobactam: A non-GLP *in vitro* evaluation of reversible inhibition potential of six major cytochrome P450 isoforms in human liver microsomes. Study Report No.: CX.101.DM.018.
14. Tazobactam: A non-GLP in vitro evaluation of induction potential of cytochrome P450 isoforms 1A2, 2B6, and 3A4 by tazobactam in cultured cryopreserved primary human hepatocytes. Study Report No.: CX.101.DM.020.
15. CXA-101: A non-GLP *in vitro* evaluation of induction potential of cytochrome P450 isoform 2B6 by CXA-101 in cultured cryopreserved primary human hepatocytes. Study Report No.: CX.101.DM.021.

Excretion

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. Distribution and excretion of ^{14}C-FR264205 after single intravenous dosing in rats. Study Report No.: CRD050182-E.

Reviewed in This Document

2. Excretion of FR264205 in the urine and bile of rats. Study Report No.: PH021244.
3. Urinary excretion of FR264205 in Dogs. Study Report No.: PH021583.

Toxicology

Single-Dose

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. Single intravenous dose toxicity study of FR264205 in rats. Study Report No.: GLR050849.
2. Single intravenous dose toxicity study of FR264205 in dogs. Study Report No.: GLR060015.

Reviewed in This Document

3. General signs and plasma histamine concentrations in dogs receiving a single intravenous dose of antipseudomonal cephalosporin drug (other tests). Study Report No.: GLR030108.
4. Dosage establishment testing for the micronucleus testing of FR264205 Drug Product Forced Degradant in Mice (other testing). Study Report No.: GLR040367.

Repeated-Dose

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. 4-week intravenous dose toxicity study of FR264205 in rats followed by a 4-week recovery period. Study Report No.: GLR050690.
2. Preliminary 2-week intravenous dose toxicity study of FR264205 in dogs. Study Report No.: GLR050697.
3. 4-week intravenous dose toxicity study of FR264205 in dogs followed by a 4-week recovery period. Study Report No.: GLR050729.
4. 4-week intravenous dose toxicokinetic study of FR264205 in rats. Study Report No.: GLR050748.

5. CXA-101: A 28 day intravenous toxicity study in Sprague-Dawley rat. Study Report No.: CXA201-T-001.

Reviewed in This Document

6. Ceftolozane (CXA-101): A 28-Day Intravenous (Slow Push) Injection Toxicity and Toxicokinetic Study in Sprague Dawley Rats with a 28-Day Recovery Period. Study Report No.: CX.101.TX.031.
7. A 14-day twice daily intravenous (15 minute) infusion combination toxicity study of CXA-101 and tazobactam in Beagle dogs. Study Report No.: CXA201-T-005.
8. A Six-Month Intraperitoneal Repeated Dose Toxicity Study of TazoBactam/Piperacillin and Tazobactam in Rats. Hayashi, Y., Yada, H., Auletta, C.S., Daly, I.W., Knezevich, A.L., and Cockrell, B.Y.: The Journal of Toxicological Sciences, 19 (supplement II): 155-176 (1994).
9. A Six-Month Intravenous Repeated Dose Toxicity Study of Tazobactam/Piperacillin and Tazobactam in Dogs. Hayashi, Y., Yada, H., Blair, Laughlin, K.A., Blanchard, G.L., Tucek, P.C. and R.G. Geil, The Journal of Toxicological Sciences, 19 (supplement II): 177-197 (1994).

Genotoxicity

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. Genotoxicity Study of FR264205-Bacterial Reverse Mutation Test. Study Report No.: GLR050752.
2. Gene mutation assay of FR264205 with mouse lymphoma cells (MLA). Study Report No.: GLR050528.
3. Genotoxicity Study of FR264205-chromosomal aberration test using cultured mammalian cells. Study Report No.: GLR050845.
4. *In vivo/in vitro* unscheduled DNA synthesis (UDS) assay with FR264205 in rat hepatocytes. Study Report No.: GLR040362.
5. Genotoxicity study of FR264205-micronucleus test in mice. Study Report No.: GLR050847.

Reviewed in This Document

6. *In vitro* Mammalian Chromosome Aberration Test (CXA-101/Tazobactam at a 2:1 ratio. Study Report No.: CXA-201-T-002.
7. *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay). Study Report No.: CXA201-T-003.
8. Rat Bone Marrow Erythrocyte Micronucleus Test Following Intravenous Administration of CXA-101 and Tazobactam. Study Report No.: CXA201-T-004.
9. Mutagenicity Tests of Tazobactam/Piperacillin, Tazobactam and Piperacillin: Reverse Mutation Assay. Ohuchida, A, Taniguti, A; Yasuhide, K., Yasuhiro M., Kashihara, A., and Omae, S.: The Journal of Toxicological Sciences, Vol. 19, Supplement II, 263-280, 1994.
10. Mutagenicity Tests of Tazobactam/Piperacillin, Tazobactam and Piperacillin: Chromosomal Aberration Assay. Ohuchida, A, Taniguti, A; Yasuhide, K., Yasuhiro M., Kashihara, A., and Omae, S.: The Journal of Toxicological Sciences, Vol. 19, Supplement II, 263-280, 1994.
11. Mutagenicity Tests of Tazobactam/Piperacillin, Tazobactam and Piperacillin: Micronucleus Assay. Ohuchida, A, Taniguti, A; Yasuhide, K., Yasuhiro M.,

Kashihara, A., and Omae, S.: The Journal of Toxicological Sciences, Vol. 19, Supplement II, 263-280, 1994.

Reproductive Toxicity

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. An intravenous injection dose range-finding study of the effects of CXA-101 on embryo/fetal development in mice. Study Report No.: CXA101-T-005.
2. An intravenous injection study of the effects of CXA-101 on embryo/fetal development in rats. Study Report No.: CXA101-T-006.

Reviewed in This Document

3. CB-500,101: A GLP Intravenous Fertility and Early Embryonic Development to Implantation Study in Rats. Study No.: CX.101.TX.002
4. Reproductive and developmental Toxicity Studies of Tazobactam/Piperacillin or Tazobactam. Sato, T., Lochry, E.A., Hoberman, A.M., Christian, M.S.: The Journal of Toxicological Sciences, Vol. 19, Supplement II: 199-214 (1994).
5. CB-500,101: A GLP Intravenous Embryo/Fetal Development Study in Mice. Study Report No.: CX.101.TX.001.
6. Reproductive and Developmental Toxicity Studies of Tazobactam/Piperacillin or Tazobactam (2) – Teratological study in rats with intravenous administration. Sato, T., Lochry, E.A., Hoberman, A.M., and Christian, M.S.**:** The Journal of Toxicological Sciences, Vol. 19, Supplement II, 215-232 (1994).
7. CXA-101: A GLP Intravenous Pre- and Postnatal Development Study, Including Maternal Function, in Rats. Study Report No.: CX.101.TX.012.
8. Reproductive and Developmental Toxicity Studies of Tazobactam/Piperacillin or Tazobactam (3) – Perinatal and Postnatal Study in Rats with Intraperitoneal Administration. Sato, T., Hoberman, A.M., and Christian, M.S.: The Journal of Toxicological Sciences, Vol. 19, Supplement II, 233-247 (1994).

Other Toxicity Tests

Antigenicity and Immunotoxicity

Reviewed in the 2/23/2011 nonclinical safety review for IND 104490

1. Preliminary antigenicity study of FR264205 in Mice. Study No.: GLR050674.
2. Preliminary antigenicity study of FR264205 formulation in guinea pigs. Study No.: GLR050758.

Reviewed in This Document

3. Antigenicity Tests (Screening Tests) of FR264205 in Guinea Pigs. Study Report No.: GLR020057.
4. FR264205 Guinea Pig Antigenicity Test (Study Report No.: GLR050096).
5. Popliteal lymph node assay (PLNA) of FR264205 using mice. Study Report No.: GLR050863.
6. Histamine Releasing and Hemolysis Activities of FR264205 (Study No.: CRE060243).

Skin and Ocular Sensitization

Reviewed in This Document

1. A GLP Repeat Dose Phototoxicity Study to Determine the Effects on Eyes and Skin in Pigmented Rats Following Once Daily Intravenous Administration. Study Report No.: CX.101.TX.013.

2. CXA-101 (Ceftolozane): A Skin Sensitization Study (Buehler Method) in Guinea Pigs (Study Report No.: CXA.101.TX.027).
3. An Acute Dermal Irritation Study in Rabbits (Study Report No.: CX.101.TX.028).

Juvenile Studies

Reviewed in This Document

1. Ceftolozane/Tazobactam: A Non-GLP Subcutaneous 14-Day Dose-Range Finding Juvenile Toxicity and Toxicokinetic Study in Postnatal Day (PND) 4 Sprague-Dawley Rats. Study No.: CXA.101.TX.033.

Impurities

Reviewed in This Document

1. 4-Week Intravenous Dose Toxicity Study of Forced Degradation Product of FR264205 Formulaiton in Rats. Study Report No.: GLR050749.
2. Genotoxicity Study of Forced Degradation Product of FR264205 Formulation – Bacterial Reverse Mutation Test. Study No.: GLR050753.
3. Genotoxicity Study of Forced Degradation Product of FR264205 Formulation – Chromosomal Aberration Test Using Cultured Mammalian Cells. Study Report No.: GLR050846.
4. Genotoxicity Study of Forced Degradation Product of FR264205 Formulation – Micronucleus Test in Mice. Study Report No.: GLR050848.

3.2 Studies Not Reviewed

1. Tazobactam Acid Formulation: Determination of pH, Concentration, and Overnight Stability (Study No.: AR: 2013-1018).
2. Method of Thin Layer Chromatography for FR264205 (Study No.: CRD030146).
3. Preliminary Testing: Assay of Blood Plasma Concentration of FR264205 in Rats using HPLC (Study No.: CRD030156).
4. Procedures for Assay of FR264205 Freebase Concentrations in Dog Plasma with High-Performance Liquid Chromatography (HPLC) (Study No.: CRD030189).
5. Preparative Details of [^{14}C]FR264205, CFQ13548, and CFQ13715 (Study No.: CRD040155).
6. Validation of Procedures for Assay of FR264205 Freebase Concentrations in Rat Plasma with HPLC (Study No.: CRD040167).
7. Assay Method: Determination of FR264205 Free Base in Rat Plasma Using High-performance Liquid Chromatography (HPLC) (Study No.: CRD-5-178).
8. Assay Method: Determination of FR264205 in Dog Plasma Using High-performance Liquid Chromatography (HPLC) (Study No.: CRD050179)
9. Stability of CXA-101 and Tazobactam Under the In Vitro Culture Conditions Utilized for In Vitro Tox Studies (Study No.: Cx.101.AN.001).
10. Characterization of FR264205 Test Substance (Lot No.: GLP-3F02) (Study No.: GLR030596).
11. Validation of Procedures for Characterizing FR264205 Test Article (Study No.: GLR030520).
12. Validation of Property Test for 1 g Test Substance FR264205 Preparation for Injections (Study No.: GLR030852).
13. Stability Study of FR264205 in Dosing Solution (Vehicle: Water for Injection) (Study No.: GLR040156).

14. Stability Testing of FR264205 Test Article (Lot No.: GLP-3F02) (Study No.: GLR030597).
15. Stability Study of FR264205 in Form of Dose Solutions (Medium: Saline Solution Combined with Sodium Hydroxide) (Study No.: GLR030640).
16. Characterization Tests of Test Substance FR264205 Drug Product for 1 g Injection (Lot No.: GLP-103041K) and its Degradants (Lot No.: GLP-400142G) (Study No.: GLR040277).
17. Stability Study of Test Substance FR264205 1g Preparation for Injections (Lot No.: GLP-103041K) (Study No.: GLR040278).
18. Stability in Dosing Solution of FR264205 Product for 1g Injection (Lot No.: GLP-103041K0 and its Degradants (Lot No. GLP-400142G; Vehicle, Physiological Saline Solution) (Study No.: GLR040279).
19. Stability Study of FR264205 in Dosing Solution (Vehicle: Physiological Saline Solution with Sodium Bicarbonate) (Study No.: GLR040310).
20. Methods of Dosing Solution Preparation for Safety Studies of FR264205 Drug Substance (1 mg/ml and 200 mg/ml DMSO solutions) (Study No.: GLR040499).
21. Stability of FR264205 in Dosing Solution (Vehicle: DMSO) (Study No.: GLR040592).
22. Determination of CXA-101 and Tazobactam Concentrations in Rat Plasma from "CXA-101: A 28 Day Intravenous Toxicity Study in the Sprague-Dawley Rat" (Study No.: MC08B-0387).
23. Partial Validation of a Method for the Determination of CXA-101 (FR264205) in Modified Saline Formulations using High-performance Liquid Chromatography with Ultraviolet (UV) Detection (Study No.: MC08F-0145).
24. Partial Validation of a Method to Determine the Interference of Tazobactam in CXA-101 in Modified Saline Formulations Using High-Performance Liquid Chromatography with Ultraviolet Detection (Study No.: MC08F-0150).
25. Partial Validation of a Method for the Determination of CXA-101 and Tazobactam in Dog Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC09B-0065).
26. Determination of CXA-101 Concentrations in Mouse Plasma from "In Vivo Evaluation of CXA-101 ± Tazobactam Versus Piperacillin-Tazobactam Using Human Simulated Exposures Against Phenotypically Diverse Gram-Negative Organisms (Study No.: MC09B-0170).
27. Determination of CXA-101 and Tazobactam Concentrations in Dog Plasma from "A 14-Day Twice Daily Intravenous (15-Minute) Infusion Combination Toxicity Study of CXA-101 and Tazobactam in Beagle Dogs" (Study No.: MC09B-0279).
28. Evaluation of the Stability of CXA-101/Tazobactam in Modified Saline Formulations Using High-Performance Liquid Chromatography with Ultraviolet Detection (Study No.: MC09F-0049).
29. Partial Validation of a Method for the Determination of CXA-101 and Tazobactam in Dog Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC09B-0065).
30. Non-Interference Evaluation of Tazobactam and Tazobactam M-1 on the Analysis of CXA-101 in Mouse Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC10B-0215).

31. Determination of the Solubility of Ceftolozane (CXA-101) in Modified Saline Solution (Study No.: MC10F-0005).
32. Qualification of a Method for the Determination of CXA-101 Concentrations in Supplemented Hank's Balanced Salt Solution (HBSS) and Analysis of Samples (Study No.: MC11B-0289).
33. Qualification of a Method for the Determination of Tazobactam Concentrations in Supplemented Hank's Balanced Salt Solution (HBSS) and Krebs Buffer and Analysis of Samples (Study No.: MC11B-0321).
34. Method Qualification for the Determination of CXA-101 and Tazobactam in Mouse Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MC12B-0091).
35. Qualification of a Method for the Determination of Tazobactam Concentrations in Methanol:Water (3:1, v/v) and Analysis of Samples (Study No.: MC12R-0017).
36. Method for the Determination of CXA-101 (FR264205) in Modified Saline Formulations Using High-Performance Liquid Chromatography with Ultraviolet Detection (Study No.: MN08007).
37. Method for the Determination of CXA-101 and Tazobactam in Dog Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MN08141).
38. Method for the Determination of Tazobactam in Supplemented Hank's Balanced Salt Solution (HBSS) Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MN11100).
39. Method for the Deermination of CXA-101 in Acidified Neonatal Rat Plasma Using Derivatization, Solid Phase Extraction and High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MN12070).
40. Method for the Determination of Tazobactam in Acidified Neonatal rat Plasma Using High-Performance Liquid Chromatography with Mass Spectrometric Detection (Study No.: MN12071).
41. Stability Study of CXA-201 (Ceftolozane and Tazobactam) in Modified Saline Formulations (Study No.: WIL-339127).
42. Partial Validation of a Method to Determine the Interference of Tazobactam in CXA-101 in Modified Saline Formulations Using High-Performance Liquid Chromatography With Ultraviolet Detection (Study No.: PF08F-0150).
43. Pharmacokinetic Study of an Investigational Cephalosporin, CXA-101: Plasma Kinetic, Computer-controlled Simulation, and Tissue Concentrations in Lung, Bone, and Bone Marrow. Study No.: CXA201-P-002.
44. 4-Week Intravenous Dose Toxicokinetic Study of FR264205 in Rats. Study No.: TX043006.
45. Plasma concentration of FR264205 in Rats. Study No.: PH021245.
46. Blood Plasma Concentration of FR264205 in Dogs (PK). Study No.: PH021531.
47. Blood Plasma Concentrations of FR264205 in Mice (20 mg/kg IV). Study No.: PH030332.
48. CXA-101: A Non-GLP In Vitro Assessment of Inhibitor Potential on Human OAT1, OAT3, OCT1, OCT2, OATP1B1 and OATP1B3 Uptake Transporters. Study No.: CX.101.DM.004.

49. Investigation of spleen pathology and serum IgM following 5 consecutive administrations of [Drug] in BALB/c and BDF1 mice. Study Report No.: MB020034
50. Tazobactam: A Non-GLP In Vitro Assessment of Inhibition Potential on Human OAT1, OAT3, OCT1, OCT2, OATP1B1 and OATP1B3 and of Substrate Potential of Human OAT1, OAT3, and OCT2 Uptake Transporters. Study No.: CX.101.DM.006.
51. Tazobactam M-1: A Non-GLP In Vitro Assessment of Inhibitor Potential on Human MDR1, BCRP, BSEP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, and OCT2 Transporters. Study No.: CX.101.DM.012.
52. Determination of Binding of Tazobactam, Tazobactam M-1 and CXA-101 to Human Liver Microsomal Proteins. Study No.: CX.101.DM.014.
53. Study Concerning the Growth-Inhibiting Effects of FR264205 on the Bacteria Used in Reverse Mutation Assays (Other Testing). Study Report No.: GLR030690.
54. Chromosomal Abnormality Testing of FR264205 Using CHL Cells (Preliminary Testing). Study No.: GLR010686.
55. Mouse Lymphoma TK Assay of FR264205 (other testings). Study No: GLR010704.
56. Micronucleus Study of FR264205 in Mice (Preliminary Testing). Study Report No.: GLR020123.
57. *In vivo/in vitro* irregular DNA synthesis (UDS) study of FR264205 utilizing the hepatic cells of rats. Study Report No.: GLR020149.
58.

3.3 Previous Reviews Referenced

The nonclinical safety review for IND 104490 (Submitted to DARRTS on 2/23/2011).

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology information for CXA-101 and tazobactam was reviewed by the microbiology reviewer.

4.2 Secondary Pharmacology

The potential for secondary, off-target pharmacological effects was assessed for celftolozane (Study No.: CX.101.SP.001), tazobactam (Study No.: CX.101.SP.002), and the tazobactam major metabolite (tazobactam M1; Study No.: CX.101.SP.003) *in vitro* in assays assessing binding to 130 enzyme and receptor targets. All of these studies are reviewed below.

Ceftolozane at a concentration of 766 μg/ml (approximately 13-fold greater than the mean clinical tazobactam C_{max} value) produced more than 50% inhibition of binding to 8 receptor targets (histamine H3, opioid delta 2, opioid kappa 1, opioid mu, purinergic P2Y, sigma 1, cholecystokinin CCK1 and neurokinin NPY1). Ceftolozane was also shown to inhibit more than 50% of the activity of 8 enzymes including several

phosphodiesterases (PDE10A2, PDE2A, PDE3B, PDE4A1A, PDE5A1) as well as protein kinases Akt1, protein kinase MEK1, and histone deacetylase SIRTUINI. Tazobactam at a concentration of 448 μg/ml (approximately 20-fold greater than the mean clinical tazobactam C_{max} value) produced more than 50% inhibition to 1 target MEK1. Tazobactam M1 at a concentration of 30 μg/ml (approximately 20-fold greater than the mean clinical tazobactam M1 C_{max} value) produced more than 50% inhibition of 2 targets, binding to cannabinoid CB1 and the activity of phosphodiesterase PDE3A.

Study Title: CXA-101: A Non-Clinical *In Vitro* Receptor Binding Screen (Study No.: CX.101.SP.001).

Methods
Ceftolozane at a concentration of 766 μg/ml was tested in a panel of 130 in vitro receptor and enzyme assays under serum free conditions.

Results
CXA-101 (766 μg/ml) inhibited greater than 50% binding or enzyme activity of a total of 16 out of 130 tested targets. The affected targets and their percent inhibition are shown below in Table 15. The Sponsor indicated that the CXA-101 test concentration was 13.4-fold greater than the mean clinical C_{max} of 57 μg/ml.

Table 15: Receptor and Enzyme Targets Inhibited by > 50% by CXA-101 *In Vitro*.

Target	Percent Inhibition
Histone deacetylase SIRTUIN1	105.52%
Protein kinase MEK1	100.15%
Cholecystokinin CCK1	96.14%
Opioid mu	88.76%
Protein kinase Akt1	84.29%
Phosphodiesterase PDE2A	66.00%
Neurokinin NPY1	61.81%
Phosphodiesterase PDE3B	61.00%
Purinergic P2Y	58.99%
Opioid kappa 1	56.57%
Phosphodiesterase PDE5A1	56.60%
Histamine H3	56.19%
Phosphodiesterase PDE4A1A	55.00%
Sigma 1	51.52%
Opioid delta 2	50.80%
Phosphodiesterase PDE10A2	50.24%

Study Title: Tazobactam: A Non-GLP *In Vitro* Receptor Binding Screen (Study No.: CX.101.SP.002).

Methods
Tazobactam at a free-base concentration of 448 μg/ml was tested in a panel of 130 *in vitro* receptor and enzyme assays under serum-free conditions.

Results
Tazobactam (448 μg/ml) inhibited the activity of only one of the 130 targets by more than 50%, protein kinase MEK1. The Sponsor indicated that the tested concentration of tazobactam (448 μg/ml) was approximately 20-fold greater than the mean clinical C_{max} value of tazobactam (approximately 22 μg/ml).

Study Title: Tazobactam M1: A Non-GLP In Vitro Receptor Binding Screen (Study No.: CX.101.SP.003).

Methods
Tazobactam M1, the primary metabolite of tazobactam, at a concentration of 30 μg/ml, was tested in a panel of 128 *in vitro* receptor and enzyme assays under serum free conditions.

Results
Tazobactam M1 (30 μg/ml) inhibited the activity or binding of two targets. Cannabinoid CB-specific binding was inhibited by 57.10% and phosphodiesterase PDE3A specific activity was inhibited by 51.0%. The Sponsor indicated that the tested concentration of tazobactam M1 (30 μg/ml) was approximately 20-fold greater than the mean C_{max} value of tazobactam M1 (approximately 1.5 μg/ml) associated with the clinical dose of tazobactam.

4.3 Safety Pharmacology

CNS, cardiovascular, and respiratory safety pharmacology studies were submitted in support of NDA 206829 as well a specialized safety pharmacology study examining ceftolozane-related effects on histamine release and hemolysis. CXA-101 did not produce neuropharmacological effects or changes in body temperature in rats at IV doses of 68.9, 207, and 689 mg/kg (Study No.: CXA101-T-002), and its convulsive activity when administered into the cerebral spinal space was 24 and 7.4 times lower than that of cefoselis in mice and rats respectively (Study No.:CRE060244). CXA-101 produced almost no inhibition of the hERG channel at concentrations of up to 1 mM (Study No.: 080125-DMK). In addition, intravenous CXA-101 produced transient increases in blood pressure at 1000 mg/kg and transient decreases in heart rate at 300 and 1000 mg/kg in rats (Study No.: CRE060242). In conscious telemetered dogs, no changes in QT or QTc intervals were noted at any of the test doses (30, 100, 300 mg/kg) of CXA-101 (Study No.: CXA101-T-001). Intravenous CXA-101 did not induce any biologically relevant effects on respiratory rate, tidal volume, or minute volume in conscious rats (Study No.: CXA-101-T-003). Intravenous CXA-101 in doses as high as 1000 mg/kg did not increase plasma histamine levels in rats, and concentrations of ≤ 0.3 mg/ml did not induce histamine release above spontaneous background levels in human peripheral blood cells (Study No.: CRE060243). At concentrations of ≤ 0.1 mg/ml, CXA-101 did not induce hemolysis in human whole blood (Study No.: CRE060243).

All of the safety pharmacology studies were reviewed in the 2/23/2011 nonclinical safety review for IND 104490.

5.1 PK/ADME

Methods of Analysis

HPLC/MS/MS (for both compounds) or HPLC/UV (for tazobactam) methods were developed and validated for the measurement of CXA-101 and tazobactam in saline, water (CXA-101 only), mouse, rat, and dog plasma, and neonatal rat plasma. The limit of detection for CXA-101 was 0.1 μg/ml with good linearity and specificity in the range of 0.1 to 50 μg/ml. The limit of detection for tazobactam was 0.02 μg/ml with a dynamic range of 0.02 to 10 μg/ml. In addition, CXA-101 was measured in rat and dog kidney using non-validated HPLC methods with a dynamic range of 0.1 to 100 μg/ml for both species.

In rat plasma, CXA-101 was shown to be stable for 37 days at both -20°C and -70°C, and tazobactam was stable at -70°C for 93 days. In separate studies using dog plasma, CXA-101 was shown to be stable for 37 days at both -20°C and -70°C, and tazobactam was shown to be stable at -70°C for 37 days.

^{14}C-FR264205 was used in distribution studies, and the plasma and tissue CXA-101 content and its major metabolites was determined by radio-HPLC methodology. The radiochemical purity of ^{14}C-labeled CXA-101 was determined to be 98% with a specific radioactivity of 592 MBq/mmol.

Absorption

CXA-101 absorption following single IV administration was examined in rats (Study No.: CRD060001) and dogs (Study No.: CRD060002). Kidney concentrations of CXA-101 were determined in conjunction with a 4-week repeated-dose toxicology studies in rats (Study No.: CRD060003) and dogs (Study No.: CRD060004). The toxicokinetics of CXA-101 and tazobactam administered singly and in combination was examined following a single SC or IP dose studies in rats (CX.101.PK.005) and dogs (CXA201-P-001), a 4-week repeated-dose toxicology study in rats (CXA201-T-001), and a 2-week repeated-dose toxicology study in dogs (CXA201-T-005). Study Nos.: CRD060001, CRD060002, CRD060003, CRD060004, and CXA201-P-001 were reviewed in the 2/23/2011 nonclinical safety review for IND 104490, and Study Nos. CX.101.PK.005, CXA201-T-001 and CXA201-T-005 are reviewed in this document for NDA 206829 in section 5.1 PK/ADME or section 6.1 Repeated-dose Toxicity.

CXA-101 Absorption

In general, CXA-101 C_{max} and AUC values increased in a dose-proportional manner for mice, rats, and dogs following single- or repeated-IV dosing of 10 to 2000 mg/kg CXA-101 with no major gender differences or indications of substantial accumulation with repeated dosing. Plasma $t_{1/2}$ values were on the order of 1 hour or slightly less for rats and dogs. Estimated volume of distribution values in rats (194 ml/kg) and dogs (~250 ml/kg) suggested distribution beyond the vascular compartment in both species. In studies examining accumulation of CXA-101 in the kidneys of rats (Study No.:

CRD060003) and dogs (Study No.: CRD060004) after 4-weeks of dosing, CXA-101 accumulated in the kidneys of both species in a roughly dose-dependent manner, and kidney levels were not detectable 4 weeks after the end of dosing.

Absorption of CXA-101 and Tazobactam Following Combination Dosing

In a single-dose dog study (Study No.: CXA201-P-001) where CXA-101 and tazobactam were administered intravenously in combination, tazobactam pharmacokinetic values were not altered, but CXA-101 C_{max} and AUC values were reduced approximately 10% with combination administration. However, plasma $t_{1/2}$ values for CXA-101 were not changed with the combination administration. In a rat study employing single intraperitoneal or subcutaneous doses of tazobactam alone or in combination with CXA-101 (Study No. CX.101.PK.005), plasma tazobactam C_{max} and AUC values decreased by 29% and 18% respectively and $t_{1/2}$ values increased by approximately 24% when the compounds were administered in subcutaneously in combination. In a 28-day repeated-dose toxicology study in rats (CXA201-T-001), CXA-101 C_{max} and AUC values did not change substantially, but tazobactam C_{max} and AUC values decreased 57% and 54% respectively after 28 days of dosing compared to the first day of dosing. In a similar repeated-dose study with dogs (CXA201-T-005), plasma C_{max} and AUC values for CXA-101 were increased by 14% and 27% respectively but tazobactam C_{max} and AUC values were decreased approximately 19% and 26% respectively following combination dosing for 14 days compared to the first day of dosing.

Study Title: Tazobactam: A Non-GLP Single Dose Pharmacokinetic Study in the Presence or Absence of CXA-101 Following a Single Subcutaneous or Intraperitoneal Administration to Male Sprague-Dawley Rats (Study No.: CX.101.PK.005).

Methods

In this non-GLP study, the plasma exposure of tazobactam and CXA-101 were determined in male rats following single subcutaneous or intraperitoneal administration of tazobactam (75 mg/kg) alone or in combination with CXA-101 (75 mg/kg tazobactam/150 mg/kg CXA-101). Tazobactam and CXA-101 concentrations in plasma were measured using a validated LC/MS/MS technique with a lower limit of quantitation of 0.004 μg/ml.

Table 16: Study Design for Study No.: CX.101.PK.005. (Sponsor's Table)

Group No.	No. of Animals	Substance	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Dosing Regimen/Route
1	3	Tazobactam	**75**	**15**	5	Subcutaneous
2	3	Tazobactam/ CXA-101	**75/150**	**15/30**	5	Subcutaneous
3	3	Tazobactam	**75**	**15**	5	Intraperitoneal
4	3	Tazobactam/ CXA-101	**75/150**	**15/30**	5	Intraperitoneal

Results

Plasma tazobactam C_{max} and AUC values were moderately reduced and plasma $t_{1/2}$ values were moderately increased when tazobactam was administered in combination with CXA-101 by the subcutaneous or intraperitoneal routes compared to tazobactam administered alone (Table 17). The plasma C_{max} and AUC_{inf} values for tazobactam were respectively reduced 29% and 18% following subcutaneous administration of the combination versus tazobactam alone. Similarly, following intraperitoneal administration, the plasma C_{max} and AUC_{inf} values for tazobactam were respectively reduced 6% and 27% for the combination versus tazobactam alone. Plasma C_{max} and AUC values were increased 47% and 46% respectively when tazobactam in combination with CXA-101 was administered subcutaneously compared to intraperitoneal administration. Plasma $t_{1/2}$ values were increased 24% and 43% for tazobactam administered in combination compared to alone for the subcutaneous and intraperitoneal routes respectively. The plasma $t_{1/2}$ values increased 122% (tazobactam alone) and 155% (combination) for the intraperitoneal route compared to the subcutaneous route of administration. Clearance rates were not calculated; the changes in exposure and $t_{1/2}$ values may have been due to changes in tazobactam clearance.

With results similar to that of tazobactam, plasma C_{max}, and AUC_{inf} values for CXA-101 were reduced by approximately 40% following intraperitoneal (IP) administration of the combination (75/150 mg/kg tazobactam/CXA-101) compared to values associated with subcutaneous (SC) administration. The mean plasma $t_{1/2}$ value for CXA-101 associated with IP administration (0.804 hours) was approximately 25% longer than the $t_{1/2}$ value following SC administration.

Table 17: Summary of Mean Plasma Pharmacokinetic Parameters for Tazobactam in Male Sprague Dawley Rats Following a Single Subcutaneous or Intraperitoneal Administration of Tazobactam (75 mg/kg) Alone or in Combination with CXA-101 (150 mg/kg). (Sponsor's Table)

				Tazobactam				
Group	Route	Nominal Dose (Tazobactam/ CXA-101)		C_{max}	T_{max}	AUC_{0-8hr}	AUC_{inf}	$T_{1/2}$
		mg/kg		ug/mL	hr	ug*hr/mL	ug*hr/mL	hr
1	SC	75/0	Mean	58.2	0.250	35.4	35.4	0.378
			SD	9.1	0.0	5.8	5.8	0.11
2	SC	75/150	Mean	41.3	0.194	29.0	29.0	0.471
			SD	5.6	0.096	1.7	1.7	0.034
3	IP	75/0	Mean	30.0	0.333	27.1	27.1	0.841
			SD	26	0.14	23	23	0.82
4	IP	75/150	Mean	28.1	0.139	19.9	19.9	1.20
			SD	25	0.096	17	17	1.4

Table 18: Summary of Mean Plasma Pharmacokinetic Parameters for CXA-101 in Male Sprague Dawley Rats Following a Single Subcutaneous or Intraperitoneal Administration of Tazobactam (75 mg/kg) in Combination with CXA-101 (150 mg/kg). (Sponsor's Table)

				CXA-101				
Group	Route	Nominal Dose (Tazobactam/ CXA-101)		C_{max}	T_{max}	AUC_{0-8hr}	AUC_{inf}	$T_{1/2}$
		mg/kg		ug/mL	hr	ug*hr/mL	ug*hr/mL	hr
2.00	SC	75/150	Mean	259	0.417	409	409	0.641
			SD	34	0.14	27	27	0.032
4.00	IP	75/150	Mean	147	0.500	233	233	0.804
			SD	120	0.43	190	190	0.038

Distribution

CXA-101 distribution following a single IV administration was examined in rats (Study No.: CRD050182-E). *In vitro* protein binding (Study No.: CRD050181) and blood cell transfer (Study No.: CRD050180) of CXA-101 was examined in serum and plasma (protein binding) or whole blood (blood cell transfer) from mice, rats, dogs, and humans. In addition the transport of CXA-101 into rat brain after a single IV administration (Study No.: PH030485) and the accumulation of CXA-101 in rat kidney following daily IV administrations for 1, 3, and 5 days (Study No.: PH031094). Study Nos.: CRD050182-E, CRD050181, and CRD050180 were reviewed in the 2/23/2011 nonclinical safety review for IND 104490, and Study Nos. PH030485 and PH031094 are reviewed in the present document for NDA 206829.

CXA-101 Distribution
In the rat mass-balance study (Study No.: CRD050182-E), the radioactivity profile in plasma and blood followed similar biphasic elimination patterns with a plasma $t_{1/2}$ for the first two hours after administration of less than 1 hour, and a second phase $t_{1/2}$ of more than 50 hours. At all time-points the ratio of radioactivity in blood compared to plasma was less than 1. The highest tissue concentrations of radioactivity occurred in the kidney, followed by the urinary bladder, plasma, blood, skin, and lung. Measureable quantities of radioactivity were found in the kidney but not plasma 336 hours after administration. Excretion proceeded primarily in urine with 96.5% of dosed radioactivity being detected in urine at 96 hours after dosing compared to 2.2% in feces and 0.4% in bile at 48 hours after dosing. In the rat study examining CXA-101 transport into the brain (Study No.: PH030485), plasma and brain concentrations increased in an approximately dose linear manner, but brain C_{max} concentrations were approximately 800-fold lower than plasma C_{max} concentrations. However, the brain $t_{1/2}$ values was approximately 10 times as long as the plasma $t_{1/2}$. CXA-101 concentrations in the kidney (Study No.: PH031094) were also shown to increase with dose as well as duration of dosing up to 5 days of dosing with the high dose (300 mg/kg/day) when kidney accumulation appeared to saturate. CXA-101 demonstrated low plasma-protein binding (Study No.: PCDM0300304). The *in vitro* protein binding of CXA-101 was 10-20% for mouse, rat, dog, and human plasma. The *in vitro* blood to plasma concentration ratio for CXA-101 was approximately 60% for mice, rats, dogs, and humans (Study No.: PCDM0300304). Approximately 10% of the CXA-101 transferred into red blood cells for each species.

Tazobactam Distribution
The Zosyn® label indicates that tazobactam is widely distributed into tissues and body fluids including intestinal mucosa, gallbladder, lung, female reproductive tissues (uterus, ovary, and fallopian tubes), interstitial fluid, and bile. Tazobactam is also reportedly 30% bound to plasma proteins.

Study Title: Transport of FR264205 Into the Brain (Measurement of Concentrations in Rat Brain and Plasma). (Study No.: PH030485)

Methods
In this non-GLP study conducted in Japan, Sprague Dawley rats (4/group/timepoint) received single intravenous doses of 500, 1000, and 1500 mg/kg FR264205 (CXA-101). Following administration, blood and whole brain samples were obtained from the rats receiving 1000 mg/kg CXA-101 at 0.083, 0.25, 0.5, 1, 3, and 6 hours after administration and at 0.25 hours after administration for the 500 and 1500 mg/kg/doses. Concentrations of CXA-101 in plasma and the supernatant of whole brain homogenate were determined using an HPLC method with limits of determination of 0.4 μg/g in brain and 0.39 μg/ml in plasma.

Results
CXA-101 concentrations in plasma and brain increased in an approximately dose-linear manner following a single IV administration (Table 19). The brain-to-plasma AUC ratio of CXA-101 was 0.013 suggesting modest drug transport into the brain (Table 20).

CXA-101 distributed rapidly to the brain, but the $t_{1/2}$ in the brain was approximately 10-fold greater than in plasma.

Table 19: Plasma and Brain Concentrations of CXA-101 After Intravenous Administration in Rats.

Dose (mg/kg)	Time after Dosing					
	0.083 hrs	0.25	0.5	1	3	6
Plasma						
500		862 ± 161				
1000	2671 ± 484	1435 ± 266	757 ± 484	263 ± 49	2.92 ± 0.91	ND
1500		2313 ± 83				
Brain						
500		1.79 ± 0.43				
1000	3.04 ± 0.63	3.00 ± 0.16	2.97 ± 0.77	2.18 ± 0.38	1.66 ± 0.30	1.15 ± 0.20
1500		5.12 ± 1.29				

Unit: μg/ml in plasma; μg/g tissue in brain
ND: < 0.39 μg/ml in plasma; 0.40 μg/g tissue in brain

Table 20: Pharmacokinetic Parameters After Intravenous Administration of 1000 mg/kg CXA-101 in Rats. (Sponsor's Table)

Sample	Co (μg/mL)	$t_{½}$ (hr)	AUC (mg • hr/mL)	CLt (mL/min/kg)	Vdss (L/kg)
Plasma n = 4	3638.09	0.31	1400.89	11.90	0.30

Sample	Cmax (μg/g)	tmax (hr)	$t_{½}$ (hr)	AUC (mg • hr/g)
Brain n = 4	3.04	0.083	3.38	18.63

Study Title: Accumulation of FR264205 in the Rat Kidney (Study No.: PH031094)

Methods

In this non-GLP study conducted in Japan, male Sprague-Dawley rats (6/group/dose duration) were administered single daily intravenous doses of 100 or 300 mg/kg CXA-101 for 1, 3, or 5 days. Upon removal 24 hours after the final dose, kidneys were homogenized (3 ml PBS/1g tissue) and the concentration of CXA-101 in the kidney was determined using an HPLC method.

Reviewer Comment: *The brief methods included in the study report mistakenly indicates livers were removed, not kidneys.*

Results

Kidney concentrations of CXA-101 increased with dose and duration and in a roughly linear fashion with dose except for 5 days of dosing with the high dose where kidney concentrations increased in a less than dose-proportional manner (Table 21). The

results suggest that CXA-101 accumulated in kidney, but the accumulation was saturated after 5 days of dosing with 300 mg/kg CXA-101.

Table 21: Mean Concentrations of CXA-101 in Rat Liver After 1, 3, or 5 Days of Dosing with 100 or 300 mg/kg/day.

Duration of Dosing	Mean Kidney Concentration (μg/g tissue)	
	100 mg/kg CXA-101	300 mg/kg CXA-101
Single Dose	40.4 ± 6.3	134.1 ± 17.4
3 Daily Doses	87.4 ± 6.6	289.7 ± 40.3
5 Daily Doses	156.6 ± 29.9	247.8 ± 32.4
Kidney concentrations were measured 24 hours after the final dose administration. Values are expressed as Mean ± SD.		

Metabolism

CXA-101 metabolites in kidney, plasma, urine, feces, and bile were examined following a single IV dose of 20 mg/kg ^{14}C-CXA-101 in rats (Studies No.: CRD050183 and CRD050184). CXA-101 was examined *in vitro* for its ability to directly inhibit (Study No.: CX.101.DM.017) or induce (Study No.: CX.101.DM.001) multiple isoforms of human CYP450 enzymes. Similar studies were conducted for tazobactam (inhibition: Study No.: CX.101.DM.008; induction: Study No.: CX.001.DM.007) and tazobactam M-1 (inhibition: Study No.: CX.101.DM.011; induction: Study No.: CX.101.DM.010). Study Nos. CRD050183 and CRD050183 were reviewed in the 2/23/2011 nonclinical safety review for IND 104490, and the rest of the studies are reviewed below.

In rats, CXA-101 was shown to produce 11 unidentified metabolites, but in a pattern also seen in humans, the extent of metabolism was minimal and unchanged CXA-101 accounted for most of the total compound in plasma, urine, feces, and bile. In rats, 1 unidentified metabolite appeared in small amounts in plasma and feces and 4 of the metabolites appeared at minimal levels in urine, bile, and kidney. Also in rats, CXA-101 glucuronide or sulphate conjugates were not detected in urine or bile. In CYP450 assays, CXA-101 did not significantly inhibit or induce specific CYP450 isozymes.

Like CXA-101, tazobactam and tazobactam M-1 did not significantly inhibit or induce a range of CYP450 isozymes. The Zosyn® label indicates that tazobactam is metabolized into a single metabolite (M1) that lacks pharmacological and antibacterial activities. According to the label, protein binding to the tazobactam M1 metabolite is negligible.

Study Title: CXA-101: A Non-GLP In Vitro Evaluation of Time-Dependent Inhibition Potential of Cytochrome P450 Isoforms in Human Liver Microsomes. (Study No.: CX.101.DM.017)

Methods

This non-GLP study was conducted by (b) (4) in 2013. CXA-101 at concentrations of 0, 10, 50, 100, 500, 1000, 3000, and 6000 μg/ml was incubated with and without NADPH in the presence of pooled human liver microsomes (HLM). As part of an IC_{50} shift assay, after 30 minutes, a

portion of each incubation (with and without NADPH) was transferred to a secondary incubation containing a CYP-isoform specific substrate in order to measure isoform-specific catalytic activity. The isoform-specific substrates and incubation times are shown below in Table 22. Substrate metabolites were quantified using an LC/MS/MS method.

Table 22: Incubation Conditions for the CYP450 Inhibition Assay in Study No.: CX.101.DM.017. (Sponsor's Table)

P450 Isoform	Substrate	Substrate Conc. (IC_{50})	HLM Conc. Pre-incubation	HLM Conc. Final	Incubation Time
CYP1A2	Phenacetin	40 µM	2.0 mg/mL	0.2 mg/mL	10 min
CYP2B6	Bupropion	80 µM	1.0 mg/mL	0.1 mg/mL	5 min
CYP2C8	Amodiaquine	1.5 µM	0.2 mg/mL	0.02 mg/mL	5 min
CYP2C9	Diclofenac	5 µM	0.5 mg/mL	0.05 mg/mL	5 min
CYP2C19	(S)-Mephenytoin	40 µM	1.5 mg/mL	0.3 mg/mL	10 min
CYP2D6	Dextromethorphan	5 µM	1.0 mg/mL	0.1 mg/mL	5 min
CYP3A4	Midazolam	3 µM	0.2 mg/mL	0.02 mg/mL	5 min
CYP3A4	Testosterone	50 µM	0.5 mg/mL	0.05 mg/mL	10 min

Positive control inhibitors were also incubated with hepatocytes with the isoform-specific substrates to confirm the inhibition of isoform-specific activity within an acceptable range (Table 23).

Table 23: Positive Control Inhibitors, Acceptance Criteria and Results from this Study. (Sponsor's Table)

P450 Isoform	Time-dependent inhibition		
	Positive Control	Acceptable range IC_{50} value* (µM)	IC_{50} value results in this study (µM)
CYP1A2	Furafylline	0.0037-0.082	0.032
CYP2B6	Ticlopidine	0.033-0.18	0.15
CYP2C8	Gemfibrozil glucuronide	0.083-9.3	0.68
CYP2C9	Tienilic acid	0.027-0.14	0.069
CYP2C19	S-Fluoxetine	0.66-13	2.3
CYP2D6	Paroxetine	0.017-0.17	0.063
CYP3A4/ Midazolam	Azamulin	0.0016-0.013	0.0064
CYP3A4/ Testosterone	Azamulin	0.0037-0.038	0.020

* IC_{50} value after a 30 min preincubation (with NADPH) calculated based on inhibitor concentrations in the secondary incubation. Acceptance ranges were determined based on historical Gentest^{SM} CYP inhibition data as the mean ± 3 SD of all IC_{50} values obtained for each isoform from April, 2008 through Jan, 2012.

Results

Compared to solvent control incubations, the percent of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 enzyme activity remaining after pre-incubation of 6000 µg/ml CXA-101 ranged from 77% to 100% at the highest concentration. No enhancement in enzyme inhibition was observed when CXA-101 was pre-incubated with NADPH compared to incubations without NADPH suggesting that the inhibition that did occur relative to control incubations was not due to specific inhibition of any of the

CYP isoforms. IC_{50} values could not be calculated due to the lack of inhibition. CYP2C8 was inhibited by more than 50% with 6000 μg/ml CXA-101, but this effect occurred only in the absence of NADPH (Table 24). Inhibition in the presence of NADPH was at most 76%. Since NADPH is required for CYP activity, it was concluded that CXA-101 did not inhibit specific CYP2C8 activity.

Table 24: Inhibition of CYP2C8 Enzyme (Amodiaquine N-deethylase) Activity With and Without NADPH Pre-incubation. (Sponsor's Table)

Preincub. conc. [μg/mL]	Final conc. [μg/mL]	Preincubation without NADPH			Preincubation with NADPH		
		A % Remaining	B % Remaining	**Mean % Remaining**	A % Remaining	B % Remaining	**Mean % Remaining**
6000	600	48%	45%	46%	81%	76%	78%
3000	300	77%	77%	77%	93%	93%	93%
1000	100	86%	100%	93%	106%	97%	101%
500	50	95%	91%	93%	115%	98%	106%
100	10	97%	100%	98%	109%	101%	105%
50	5	91%	88%	90%	108%	99%	103%
10	1	103%	98%	100%	104%	99%	101%
0	0	100%	100%	100%	100%	100%	100%

Study Title: Tazobactam: A Non-GLP Evaluation of Inhibition Potential of Cytochrome P450 Isoforms in Human Liver Microsomes (Study No.: CX.101.DM.008).

Methods

This non-GLP study was conducted by (b) (4) in 2012. Human liver microsomes were incubated at 37°C with vehicle or tazobactam in concentrations of 10, 25, 50, 100, 250, 500, and 1000 μg/ml in the presence of an NADPH-regenerating system and specific substrates (Table 25) for 5 or 10 minutes. Incubations were performed in duplicates. The specific CYP450 isoform products were analyzed by an LC/MS method. Positive control inhibitors (Table 25) were also tested to confirm the patency of each assay.

Table 25: Assay Conditions for Specific CYP450 Isoforms. (Sponsor's Table)

P450 Isoform	Substrate	Substrate Conc. (IC_{50})	HLM Conc.	Incubation Time
CYP1A2	Phenacetin	40 μM	0.2 mg/mL	10 min
CYP2B6	Bupropion	80	0.1 mg/mL	5 min
CYP2C8	Amodiaquine	1.5 μM	0.02 mg/mL	5 min
CYP2C9	Diclofenac	5 μM	0.05 mg/mL	5 min
CYP2C19	(S)-Mephenytoin	40 μM	0.3 mg/mL	10 min
CYP2D6	Dextromethorphan	5 μM	0.1 mg/mL	5 min
CYP3A4	Midazolam	3 μM	0.02 mg/mL	5 min
CYP3A4	Testosterone	50 μM	0.05 mg/mL	10 min

Table 26: IC_{50} Acceptance Ranges for Each Positive Control Used to Confirm Inhibiton Patency for Specific CYP450 Isoforms. (Sponsor's Table)

P450 Isoform	Direct Inhibition	
	Positive Control	Acceptable range * IC_{50} value (µM)
CYP1A2	7,8-Benzoflavone	0.0014 - 0.068
CYP2B6	Ketoconazole	0.46 -13.0
CYP2C8	Montelukast	0.0071 - 0.19
CYP2C9	Sulfaphenazole	0.17 - 1.3
CYP2C19	S-Benzylnirvanol	0.13 - 1.3
CYP2D6	Quinidine	0.023 - 0.18
CYP3A4/ Midazolam	Ketoconazole	0.0039 - 0.15
CYP3A4/ Testosterone	Ketoconazole	0.0056 - 0.087

Acceptance ranges were determined based on historical BD Gentest^{SM} CYP inhibition data as the mean ± 3SD of all IC_{50} values obtained for each isoform through December 31, 2011.

Results

All of the positive control inhibitors produced inhibition of specific CYP450 isoform activity within acceptable ranges. Tazobactam did not substantially (>80% compared to the vehicle control) inhibit the activity of CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 at any of the test concentrations (10, 25, 50, 100, 250, 500, and 1000 µg/ml). Tazobactam did inhibit CYP3A4 activity by 14%, 33%, 45%, and 59% using midazolam as a substrate and 5%, 11%, 20%, and 25% using testosterone as a substrate at concentrations of 100, 250, 500, and 1000 µg/ml respectively (Table 27 and Table 28). The tazobactam IC_{50} for inhibition of CYP3A4 was estimated to be > 500 µg/ml.

Table 27: Inhibition of CYP3A4 by Tazobactam Using Midazolam as a Substrate. (Sponsor's Table)

Tazobactam Conc. (µg/mL)	Percent remaining activity		
	Replicate 1	Replicate 2	Mean
0	100%	100%	100%
10	96%	98%	97%
25	92%	91%	92%
50	92%	88%	90%
100	84%	87%	86%
250	68%	66%	67%
500	55%	55%	55%
1000	42%	40%	41%

Table 28: Inhibition of CYP3A4 by Tazobactam Using Testosterone as a Substrate. (Sponsor's Table)

Tazobactam Conc. (µg/mL)	Percent remaining activity		
	Replicate 1	Replicate 2	Mean
0	100%	100%	100%
10	111%	102%	106%
25	102%	95%	98%
50	95%	95%	95%
100	96%	95%	95%
250	88%	91%	89%
500	82%	77%	80%
1000	77%	72%	75%

Study Title: CXA-101: A Non-GLP Evaluation of Induction Potential of Cytochrome P450 Isoforms by CXA-101 in Cultured Cryopreserved Human Hepatocytes. (Study No.: CX.101.DM.001)

Methods

This non-GLP study was conducted by (b) (4) beginning on Oct 18, 2010. Primary cryopreserved hepatocytes from three donors were plated and exposed to CXA-101 (100, 300, and 1000 µg/ml) for 3 days. CYP induction relative to vehicle control incubations was assessed by measuring the activity of CYP1A2, CYP2B6, and CYP3A4. Each condition was performed in triplicate. In addition, the positive control inducers shown in Table 29 were tested under the same conditions to confirm the induction potential of each tested CYP isoform in the hepatocytes. A positive control toxicant, tamoxifen, was also applied to inhibit cell viability and confirm the sensitivity of an MTT assay used to confirm hepatocyte viability before and after initiation of the induction assays.

Table 29: Positive Control Inducers for the Tested CYP Isoforms. (Sponsor's Table)

Endpoints	Positive Control Inducer	Final Concentration
CYP1A2	β-Naphthoflavone	20 µM
CYP3A4	Rifampicin	20 µM
CYP2B6	Phenobarbital	2 mM
Toxicity (MTT)	Tamoxifen	50 µM

Results

CXA-101 was not cytotoxic and did not inhibit the viability of any of the hepatocyte cultures after 3 days of incubation. In contrast, tamoxifen completely suppressed cell viability. According to the FDA draft guidance: "Drug Interaction Studies, Study Design, Data Analysis, and Implications for Dosing and Labeling," a drug candidate is considered an enzyme inducer *in vitro* if it produces an increase in enzyme activity of more than 40% of the positive-control inducer. Based on this criteria, CXA-101 did not

induce the activity of CYP1A2, CYP2B6 or CYP3A4 in any of the three human hepatocyte cultures. Relative to positive control inductions, CXA-101 produced inductions <1%, <1% to 3%, and <1% of the positive control inductions for CYP1A2, CYP2B6, and CYP3A4 respectively.

Study Title: Tazobactam: A Non-GLP Evaluation of Induction Potential of Cytochrome P450 Isoforms 1A2, 2B6, 3A4, by Tazobactam in Cultured Cryopreserved Primary Human Hepatocytes. (Study No.: CX.001.DM.007)

Methods

Primary hepatocytes from three human donors were incubated with 0 (vehicle control = saline), 15, 50, 150, and 500 μg/ml concentrations of tazobactam or a positive control agent, tamoxifen (50 μM; CYP1A2), phenobarbital (1 mM; CYP2B6), or rifampicin (10 μM; CYP3A4) for approximately 72 hours. After 72 hours of exposure, some cultures of hepatocytes were tested for viability (MTT assay), and other identical cultures were then incubated with specific P450 substrates for 30-60 minutes to assess P450 induction which was evaluated by measurement of specific metabolites (Table 30) in incubation supernatants with LC-MS/MS and by mRNA expression in the conditioned hepatocytes.

Table 30: Specific Substrates and Metabolite Products for CYP450 Isoforms in Study No.: CX.001.DM.007.

CYP-450 Isozymes	Specific Substrate	Metabolite
CYP1A2	Phenacetin	Acetamidophenol
CYP2B6	Bupropion	Hydroxybupropion
CYP3A4	Testosterone	6β-hydroxy-testosterone

Results

Tazobactam was shown to be stable in the incubation solutions and did not produce substantial cytotoxicity at any concentration with 92% cell viability observed at the highest concentration of 500 μg/ml. Also, tazobactam did not cause induction of CYP1A2, CYP2B6, CYP3A4 activity above vehicle control levels or cause induction above 1-2% of the respective positive control inducer responses. Similarly, none of the tazobactam concentrations increased mRNA expression levels above vehicle control levels. The Sponsor noted that the highest tested concentration of tazobactam, 500 μg/ml was approximately 23-fold greater than the expected clinical C_{max} value.

Study Title: Tazobactam M1: A Non-GLP *In Vitro* Evaluation of Inhibition Potential of Seven Major Cytochrome P450 Isoforms in Human Liver Microsomes. (Study No.: CX.001.DM.011)

Methods

Pooled human liver microsomes were incubated with tazobactam M-1 metabolite at concentrations of 0 (sodium phosphate), 2, 5, 10, 20, 35, 75, and 150 μg/ml in the presence of a NADPH-regenerating system and substrates specific for CYP450 isoforms (Table 31). Incubations were ended at specified times with stop solution and concentrations of isoform-specific metabolites were measured using LC/MS/MS. In addition to incubations with tazobactam, positive control incubations were also performed (Table 32).

Table 31: Assay Conditions for Study No.: CX.001.DM.011. (Sponsor's Table)

P450 Isoform	Substrate	Substrate Conc. (IC_{50})	HLM Conc.	Incubation Time
CYP1A2	Phenacetin	40 µM	0.2 mg/mL	10 min
CYP2B6	Bupropion	80 µM	0.1 mg/mL	5 min
CYP2C8	Amodiaquine	1.5 µM	0.02 mg/mL	5 min
CYP2C9	Diclofenac	5 µM	0.05 mg/mL	5 min
CYP2C19	(S)-Mephenytoin	40 µM	0.3 mg/mL	10 min
CYP2D6	Dextromethorphan	5 µM	0.1 mg/mL	5 min
CYP3A4	Midazolam	3 µM	0.02 mg/mL	5 min
CYP3A4	Testosterone	50 µM	0.05 mg/mL	10 min

Results

Tazobactam M1 did not inhibit the activity of any of the CYP450 isozymes at concentrations up to 150 µg/ml. The greatest dose-dependent inhibition was 22% inhibition of CYP2B6 and 24% inhibition of CYP2D6 produced by 150 µg/ml tazobactam. In contrast, the highest concentrations of all of the positive controls produced 70 to 96% inhibition. The IC_{50} values for tazobactam were considered to be > 150 µg/ml for all of the tested CYP450 isozymes. According to the Sponsor, the 150 µg/ml concentration is on the order of 100-fold greater than the mean plasma C_{max} values for tazobactam M1 (1.5 µg/ml) expected with clinical administration of tazobactam.

Table 32: Isoform-Specific Positive Controls, Acceptance Criteria and Results. (Sponsor's Table)

P450 Isoform	Direct Inhibition		
	Positive Control	Acceptable range [a] IC_{50} value (µM)	Results for this study IC_{50} value (µM)
CYP1A2	7,8-Benzoflavone	0.0014 - 0.068	0.016 µM
CYP2B6	Ketoconazole	0.46 -13.0	8.9
CYP2C8	Montelukast	0.0071 - 0.19	0.027
CYP2C9	Sulfaphenazole	0.17 - 1.3	0.28
CYP2C19	S-Benzylnirvanol	0.13 - 1.3	0.16
CYP2D6	Quinidine	0.023 - 0.18	0.080
CYP3A4/ Midazolam	Ketoconazole	0.0039 - 0.15	0.028
CYP3A4/ Testosterone	Ketoconazole	0.0056 - 0.087	0.018

[a] Acceptance ranges were determined based on historical CYP inhibition data from the Testing Facility as the mean ± 3 SD of all IC_{50} values obtained for each isoform from June 28, 2007 through December 31, 2011.

Study Title: Tazobactam M-1: A Non-GLP Evaluation of Induction Potential of Cytochrome P450 Isoforms 1A2, 2B6, and 3A4 by Tazobactam M-1 in Cultured Cryopreserved Primary Human Hepatocytes. (Study No.: CX.101.DM.010)

Methods

Cryopreserved hepatocytes from 3 donors were incubated with 0 (sodium phosphate vehicle), 10, 30, or 75 μg/ml tazobactam M-1 or a positive control agent, omeprezole (50 μM; CYP1A2), phenobarbital (1 mM; CYP2B6), or rifampicin (10 μM; CYP3A4) for approximately 72 hours. After 72 hours of exposure, some cultures of hepatocytes were tested for viability (MTT assay), and other identical cultures were then incubated with specific P450 substrates for 30-60 minutes to assess P450 induction which was evaluated by measurement of specific metabolites (Table 33) in incubation supernatants with LC-MS/MS and by mRNA expression (measured with RT-PCR) in the conditioned hepatocytes.

Table 33: Specific Substrates and Metabolite Products for CYP450 Isoforms in Study No.: CX.001.DM.010.

CYP-450 Isozymes	Specific Substrate	Metabolite
CYP1A2	Phenacetin	Acetamidophenol
CYP2B6	Bupropion	Hydroxybupropion
CYP3A4	Testosterone	6β-hydroxy-testosterone

Results

Tazobactam M-1 was shown to be stable in the incubation solutions and did not produce substantial cytotoxicity at any concentration with 92% cell viability observed at the highest concentration of 75 μg/ml. Also, tazobactam did not cause induction of CYP1A2, CYP2B6, CYP3A4 activity above vehicle control levels or cause induction above 1-2% of the respective positive control inducer responses. Similarly, none of the tazobactam concentrations increased mRNA expression levels above vehicle control levels. The Sponsor noted that the highest tested concentration of tazobactam M-1, 75 μg/ml was approximately 50-fold greater than the expected plasma C_{max} value for tazobactam M-1 (1.5 μg/ml) expected with clinical tazobactam administration.

Excretion

Excretion of radioactive CXA-101 was examined in rats (Study Nos.: CRD050182 and PH021244) and dogs (Study No.: PH021583). Study No.: CRD050182 was reviewed in the in the 2/23/2011 nonclinical safety review for IND 104490, and the other two studies are reviewed below.

CXA-101 plasma and blood elimination followed a biphasic elimination pattern with a plasma $t_{1/2}$ for the first two hours after elimination of 0.35 hours, and a $t_{1/2}$ for 48 to 96 hours after administration of 54.45 hours. This pattern may indicate that CXA-101 redistributes from the kidney to plasma following the initial phase of elimination. In rats and dogs, radioactive CXA-101 elimination occurred primarily in urine with over 75% of the radioactivity detected in urine in both species 24 hours after intravenous dosing. In rats, 96 hours after dosing, 96.5% of radioactivity was detected in urine compared to 2.2% in feces and 0.4% in bile after 48 hours.

Study Title: Excretion of FR264205 in the urine and bile of rats. (Study No.: PH021244)

Methods

In this non-GLP study, three male rats, 6-weeks of age, received intravenous administrations of 20 mg/kg CXA-101 followed by urine collection at intervals of 0-3, 3-6, and 6-24 hours after administration. Bile was also sampled during the same time intervals. CXA-101 was measured using HPLC analysis.

Results
Over 75% of the administered CXA-101 was detected in urine within the first 3 hours of administration (Table 34). CXA-101 was not detected in bile at levels above the detection limit of 1.2 μg/ml)

Table 34: Percent of CXA-101 Excreted in Rat Urine. (Sponsor's Table)

Drug Name	Administered Dose (mg/kg)	No. of cases	Body weight (g)	Percent of urinary excretion (%)			Percent of urinary excretion Total (%)
				Measurement time (hr)			
				0-3	3-6	6-24	
FR264205	20	3	196.7 ± 1.7	75.84 ± 1.30	0.25 ± 0.23	0.11 ± 0.06	76.20 ± 1.49

Mean ± standard deviation

Study Title: Urinary Excretion of FR264205 in Dogs. (Study No.: PH021583).

Methods
In this non-GLP study conducted in Japan, 3 Beagle dogs received intravenous injections of CXA-101 (FR264205; 20 mg/kg) and their urine was collected at intervals of 0-3, 3-6, and 6-24 hours after administration. CXA-101 concentrations in urine were measured using an HPLC method.

Results
Like CXA-101 excretion in rats, over 75% of the total dose of CXA-101 was excreted in urine in dogs with the majority of excretion occurring in the first 3 hours (Table 35).

Table 35: Percent of Total CXA-101 Excreted in Urine in Dogs. (Sponsor's Table)

Sample	Dosage (mg/kg)	Sample size	Weight (kg)	Excretion rate (%)			Excretion rate total (%)
				Measuring time (hr)			
				0-3	3-6	6-24	
FR264205	20	3	12.8 ± 0.6	66.97 ± 6.86	9.03 ± 1.52	0.84 ± 0.32	76.84 ± 8.58

Mean ± standard deviation

Other Pharmacokinetic Studies

Study Title: Determination of Binding of Tazobactam, Tazobactam M-1 and CXA-101 to Human Liver Microsomal Proteins. (Study No.: CX.101.DM.014).

Methods

Human liver microsomes were incubated with test compounds in triplicate at 37°C then the degree of test article (CXA-101, tazobactam, and tazobactam M-1) binding to microsomes was assessed. Control compounds (chlorpromazine, imipramine, and warfarin) were also assessed for binding to microsomes (Table 36).

Table 36: Study Design and Incubation Conditions for Binding to Human Liver Microsomal Proteins. (Sponsor's Table)

Compound	Conc. (µg/mL)	Microsomes Conc. (mg/mL)	Quantitation (Standard Curve)
Tazobactam	500, 1000	0.02	Yes
Tazobactam M-1	3	0.2	No*
CXA-101	1000	0.02	Yes
Chlorpromazine	3	0.02	Yes
Imipramine	3	0.02	Yes
Warfarin	3	0.02	Yes

*Data were calculated using peak area ratios.

Results

As shown in Table 37, CXA-101 exhibited 21% binding to human liver microsomes (HLM) at 1000 µg/ml. Tazobactam at concentrations of 500 and 1000 µg/ml demonstrated 7.8% and no binding respectively to HLM. Tazobactam M-1 at a concentration of 3 µg/ml did not bind to microsomal proteins. According to the Sponsor, the expected order of binding to microsomal proteins for the control compounds is chlorpromazine (higher) > imipramine (intermediate) > warfarin which is consistent with the observed results.The results indicate that at all three test compounds have limited or no binding to human liver microsomes, but do not conclusively indicate whether any of the compounds have a limited capacity to interact with CYP450 enzymes.

Table 37: The Percent Binding and Percent Recovery of the Test Compounds Following Incubation with Human Liver Microsomes and Dialysis. (Sponsor's Table)

Compound	Conc. (µg/mL)	% Bound	% Recovery (in Assay)	% Recovery (in Matrix)
Tazobactam	500	7.8	157.6	118.8
Tazobactam	1000	-0.4	219.7	220.2
Tazobactam M-1	3	-9.8	99.2	96.3
CXA-101	1000	21.0	46.9	48.9
Chlorpromazine	3	27.8	27.5	29.5
Imipramine	3	3.0	75.4	70.8
Warfarin	3	-1.4	98.1	114.3

6 General Toxicology

6.1 Single-Dose Toxicity

CXA-101 was administered to rats and dogs in single-dose toxicity studies. In rats, CXA-101 at doses of 1000 and 2000 mg/kg did not produce death or pronounced toxicity (Study No.: TX031008). Dogs did not experience death at doses of 500 and 2000 mg/kg, but acute toxicity was observed in the form of vomiting, flushed auricle, prone position, decreased spontaneous motility, swelling of the head, purplish coloration of the head, as well as increased serum GPT activity, and decreased serum calcium (Study No.: TX031007). The flushing, swelling, and skin discoloration were assumed to be due to acute histamine release, although this parameter was not measured. In another study, clinical signs and also plasma histamine concentrations were examined in dogs following a single intravenous dose of CXA-101 (Study No.: TX039019). Study Nos. TX031008 and TX031007 were reviewed in the 2/23/2011 nonclinical safety review for IND 104490, and Study No.: TX039019 is reviewed below.

Study Title: General Signs and Plasma Histamine Concentrations in Dogs Receiving a Single Intravenous Dose of an Antipseudomonal Cephalosporin Drug (other tests). (Study No.: TX039019, Study Report No.: GLR030108)

Methods
Single-intravenous doses of 100, 300, and 1000 mg/kg FR264205 (CXA-101) were administered to Beagle dogs (1/sex/dose), and general clinical signs and changes in plasma histamine concentrations were assessed. Animals were observed before and continuing to 1 hour after dosing for flushing, swelling, and related clinical signs. Clinical signs that occurred were observed until resolution. Blood was collected before, immediately after, and 15 minutes after dosing for each animal, and plasma histamine concentrations were assessed by ELISA.

Results
While the plasma histamine results were not consistent, both of the higher doses of CXA-101 (300, 1000 mg/kg) were associated with higher histamine concentrations in at least one of the two test animals compared to baseline measurements (Table 38). Increased plasma histamine concentrations grossly correlated with clinical signs consistent with elevated histamine.

Table 38: Clinical Signs and Plasma Histamine Concentrations in Dogs Receiving a Single Intravenous Dose of CXA-101.

Dose (mg/kg)	Animals	Clinical Signs	Plasma Histamine Concentrations (nM)		
			Baseline	Immediately After Dosing	15 minutes After Dosing
100	Male	None	< 1.0	< 1.0	< 1.0
	Female	None	< 1.0	< 1.0	< 1.0
300	Male	Vomiting	< 1.0	94.4	12.7
	Female	Vomiting	1.33	1.25	5.62
1000	Male	None	2.36	< 1.0	< 1.0

	Female	Flushing	2.34	208	150

6.2 Repeat-Dose Toxicity

CXA-101 was administered intravenously in GLP-compliant, 4-week, repeated-dose studies in rats (Study Nos.: GLR050690 and CX.101.TX.031) and dogs (Study No.: GLR050729). In addition, repeated-dose studies with CXA-101 and tazobactam were conducted in adult (4-week intravenous study; CXA201-T-005) and juvenile (14-day subcutaneous study: Study No.: CXA.101.TX.033) rats and dogs (14-day intravenous study; Study No.: CXA201-T-005). Study Nos.: GLR050690, GLR050729, and CXA201-T-005 were reviewed in the 2/23/2011 nonclinical safety review for IND 104490, and Study Nos.: CX.101.TX.031, CXA.101.TX.033, and CXA201-T-005 are reviewed below.

Ceftolozane in 28-day studies in both rats and dogs with doses as high as 1000 mg/kg/day, produced dose-dependent nephritic changes in the form of hyaline-droplet formation in proximal tubules of the renal cortex. This form of kidney pathology, which is observed with other cephalosporin antibiotics, is thought to represent an adaptation allowing compound disposition via lysosomes. Hyaline-droplet formation was a consistent but reversible effect.

The primary pathology associated with tazobactam administration in the rat and dog 28 day, repeated-dose, IV-combination studies was a dose-dependent increase in liver weights and liver histopathology consistent with the accumulation of liver glycogen and increased smooth endoplasmic reticulum. The histopathology occurred diffusely in liver sections and was reversible. In rat studies, dose-dependent serum chemistry changes including decreased triglycerides, albumin, and glucose and increased globulin and potassium were considered to be related to the liver changes and glycogen accumulation. Higher doses of tazobactam were also associated with dose-dependent decreases in hematocrit, hemoglobin, and red blood cell counts, as well as occasional increases in platelets and the percent of lymphocytes.

An important finding in both of the CXA-101/tazobactam combination studies is that co-administration of the two compounds did not modify CXA-101-related or tazobactam-related toxicity in an additive or synergistic manner.

Two repeated-dose studies of 6-months duration, one in rats and one in dogs were conducted with tazobactam and the results were published in the literature and reviewed below. In the first study (Hayashi *et al.*, J Toxicol Sci, 19: 155-176, 1994), male and female Sprague Dawley rats were administered tazobactam (40, 80, and 160 mg/kg/day) BID by intraperitoneal injection for 6 months. Key findings for this study were decreased reticulocytes and increased erythrocytes in males and females receiving 80 and 160 mg/kg tazobactam, decreased triglycerides in males receiving 160 mg/kg tazobactam, cecal enlargement in males and females receiving 160 mg/kg tazobactam, increased liver weights in males and females receiving 160 mg/kg and males receiving 80 mg/kg, and hepatocellular glycogen accumulation in the males of the 80 and 160 mg/kg groups. The cecum and hepatic changes were largely reversible after

a one-month recovery period. Based on the hepatic changes, the NOAEL for tazobactam in this study was considered to be 40 mg/kg/day in both males and females.

In the second study (Hayashi *et al.*, J Toxicol Sci, 19: 177-197, 1994), male and female Beagle dogs were administered 40, 80, and 160 mg/kg/day tazobactam in BID intravenous infusions for a period of 6 months. Key findings for this study were pronounced hepatocellular aggregate accumulation composed of increased SER and cytoplasmic glycogen for male and female dogs administered 80 mg/kg/day and above. After one-month recovery, the hepatic changes were resolving. Based on the hepatic changes, the NOAEL for tazobactam for this study was considered to be 40 mg/kg/day for both males and females.

Study title: Ceftolozane (CXA-101): A 28-Day Intravenous (Slow Push) Injection Toxicity and Toxicokinetic Study in Sprague Dawley Rats with a 28-Day Recovery Period.

Study no.:	CX.101.TX.031
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 4, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101 (Clinical Development Process): Lot No.: 440420 0004 1, purity of 98.2%; CXA-101 (Forced-degraded Commercial Process): Lot No.: 139-121211, purity of 90.1%; CXA-101 (Commercial Process): Lot No.: 440420 0002 2, purity of 96.3%.

Key Study Findings

In this study, the clinical formulation of CXA-101, commercial batches of CXA-101 and forced degradation batches of CXA-101 were examined for toxicity. The primary findings were dose-related histopathology (renal hyaline droplets in males and renal tubular cell vacuolation in females) of marginal toxicological relevance. In general all three batches of CXA-101 produced comparable results.

Methods

Doses:	See Table 40.
Frequency of dosing:	Once daily
Route of administration:	Intravenous
Dose volume:	5 ml/kg
Formulation/Vehicle:	0.9% sodium chloride
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	Main Study: 10/sex/group

Age:	Approximately 7-weeks old upon initiation of dosing.
Weight:	At initiation of dose administration: Males: 180 to 232 g (Main Study) and 177 to 231 g (Toxicokinetic Study); Females: 138 to 189 g (Main Study), and 145 to 179 g (Toxicokinetic Study).
Satellite groups:	Recovery Study: 5/sex/group for Groups 1, 5, and 8. Toxicokinetic animals: See Table 41; 3/sex/group for Group 1, 9/sex/group for Groups 2A, 6A, 7A, and 8A.
Unique study design:	Animals received vehicle or test articles (Table 39) once daily by intravenous slow push (no less than 60 seconds, no more than 2 minutes) via the tail vein for 28 days through the day prior to the primary necropsy (Main Study animals). Recovery animals were maintained an additional 28 days. Toxicokinetic animals received a single dose on Study Day 0 at multiple time points.
Deviation from study protocol:	Multiple study protocol deviations were noted. However, none of the deviations was considered to have altered the results or compromised the integrity of the study.

Table 39: Designated Treatments and Drug Batches Used for Each Study Group.
(Sponsor's Table)

Group	Treatment	Designation on Tables
1/1A	Vehicle (0.9% sodium chloride)	0 mg/kg/day
2/2A	1000 mg/kg/day ceftolozane Lot 440420 0004 1 (clinical development process)	1000 mg/kg Lot 1
3	100 mg/kg/day ceftolozane Lot 139-121211 (forced degraded commercial process)	100 mg/kg Lot 2
4	300 mg/kg/day ceftolozane Lot 139-121211 (forced degraded commercial process)	300 mg/kg Lot 2
5	1000 mg/kg/day ceftolozane Lot 139-121211 (forced degraded commercial process)	1000 mg/kg Lot 2
6/6A	100 mg/kg/day ceftolozane lot 440420 0002 2 (commercial process)	100 mg/kg Lot 3
7/7A	300 mg/kg/day ceftolozane lot 440420 0002 2 (commercial process)	300 mg/kg Lot 3
8/8A	1000 mg/kg/day ceftolozane lot 440420 0002 2 (commercial process)	1000 mg/kg Lot 3

Table 40: Study Design for the Main Study Groups in Study No.: CX.101.TX.031. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals[a]	
				Males	Females
1	Vehicle	0	5	15	15
2	Ceftolozane (clinical development process)	1000	5	10	10
3	Ceftolozane (forced-degraded commercial process)	100	5	10	10
4	Ceftolozane (forced-degraded commercial process)	300	5	10	10
5	Ceftolozane (forced-degraded commercial process)	1000	5	15	15
6	Ceftolozane (commercial process)	100	5	10	10
7	Ceftolozane (commercial process)	300	5	10	10
8	Ceftolozane (commercial process)	1000	5	15	15

[a] = Ten animals/sex/group were assigned to the primary necropsy. The remaining animals in Groups 1, 5, and 8 were assigned to a minimum of 28 days of recovery.

Table 41: Study Design for the Toxicokinetic Groups in Study No.: CX.101.TX.031. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals	
				Males	Females
1A	Vehicle	0	5	3	3
2A	Ceftolozane (clinical development process)	1000	5	9	9
6A	Ceftolozane (commercial process)	100	5	9	9
7A	Ceftolozane (commercial process)	300	5	9	9
8A	Ceftolozane (commercial process)	1000	5	9	9

Observations and Results

Table 42: Observation Schedule for Study No.: CX.101.TX.031

Parameter Evaluated	Schedule
Mortality and Clinical Signs	All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed prior to dose administration,

	and at approximately 15 minutes and 1-2 hours following dose administration. During the recovery period, animals were observed once daily. In addition, detailed physical examinations were performed on all animals 1 week prior to randomization, on the day of randomization, weekly during the study period, and on the days of the scheduled necropsies. Injection sites were observed weekly during the study period.
Body Weights	Individual body weights were recorded 1 week prior to randomization, on the day of randomization, Study Day 0, twice weekly during dosing, once weekly during the recovery period, and on the day prior to the scheduled necropsies. Final body weights (fasted overnight) were recorded in the Necropsy day for the Main Study animals.
Food Consumption	Individual food consumption was recorded prior to randomization, on the day of randomization, on Study Day 0 prior to dosing, twice weekly throughout the dosing period, and once weekly during the recovery period.
Clinical Pathology	Blood and urine samples for hematology, coagulation, serum chemistry and/or urinalysis were collected from 10 animals/sex/group at the primary necropsy (Day 28) and from all remaining animals (exclusive of Toxicokinetic animals) in Groups 1, 5, and 8 at the Recovery necropsy on Day 56.
Ophthalmic Examinations	Ocular examinations were conducted on all animals during acclimation, near the end of the dosing period (Day 25 or 26), and near the end of the recovery period (Day 49 or 50).
Toxicokinetics	Blood was collected from 3 animals/sex/group at 5 minutes after dosing for Group 1A and at approximately 5 and 30 minutes, and 1, 2, 4, and 8 hours after dosing for Groups 2A, 6A, 7A, and 8A.
Necropsy	Day 28 for Main Study animals and Day 56 for Recovery animals.

Mortality

No CXA-101-related mortality occurred for any of the CXA-101 batches. Three animals (1 high-dose Main Study female and 1 male and 1 female high-dose animals in the Toxicokinetic Study) were found dead, but the deaths were thought to be related to mechanical trauma with internal hemorrhage (Main Study female: finding of red fluid on the abdominal cavity) or mechanical insult related to the jugular vein bleeding procedure (Toxicokinetic animals).

Clinical Signs

No CXA-101-related clinical signs occurred for any of the CXA-101 batches.

Body Weights

Body weights and body weight gains were not significantly affected by CXA-101 administration.

Feed Consumption

Mean food consumption was not affected by CXA-101 administration.

Ophthalmoscopy

All ocular examinations were conducted using an indirect ophthalmoscope and slit-lamp biomicroscope preceded by pupillary dilation with a mydriatic agent.

No ophthalmic lesions indicative of toxicity were observed in any ceftolozane-treated group.

ECG: not performed

Hematology

The measured hematology and coagulation parameters are shown in Table 108.

Mean red blood cell counts were slightly lower (-0.9% to -6.5%) than values for the control group for all of the CXA-101 groups regardless of dose or drug batch, but the only significant reduction occurred for the high-dose group females receiving the forced degradation CXA-101 product (Table 43). Reticulocyte counts were higher for all CXA-101 groups with significant increases for the mid- and high-dose groups (males and females) receiving the forced degradation CXA-101 product. However, the changes in red cell parameters were not consistently dose-dependent, and all values reportedly fell within the (b) (4) historical control range. Therefore the results were not considered toxicologically relevant.

The absolute and percent neutrophil counts were also lower at times to a significant degree than control values particularly for females (in females: -18 to -46% for absolute neutrophils; -28 to -49% for relative neutrophils). However the reductions were not distributed in a dose-related manner, and all values were within the (b) (4) historical control range suggesting a lack of toxicological relevance. Correlating with the reduction in neutrophils, percent lymphocytes numbers were increased, at times significantly. However, as for the neutrophil numbers, lymphocyte counts were not increased in a dose-dependent manner and all values fell within the (b) (4) historical control range.

Among coagulation parameters, APTT was slightly, but significantly reduced for the mid- and high-dose females receiving the commercial process CXA-101 (-6.4 to -7.8%) and the forced degradation product CXA-101 (-7.8% for both groups), but the magnitude of decreased clotting time was low enough to be of questionable toxicological significance. Prothrombin time was not significantly decreased relative to control values for any treatment group.

In recovery animals all of the changed hematology and coagulation parameters either partially or fully reversed.

Table 43: Hematology Parameters With Changes From Control Values. (Sponsor's Table)

Group (N=10)	1	2	3	4	5	6	7	8
Dosage (mg/kg/day)	0	1000 Lot 1	100 Lot 2	300 Lot 2	1000 Lot 2	100 Lot 3	300 Lot 3	1000 Lot 3
Males								
Red Blood Cell Count	8.67	8.43	8.36	8.48	8.40	8.40	8.59	8.36
(percent difference)		(-2.8)	(-3.6)	(-2.2)	(-3.1)	(-3.1)	(-0.9)	(-3.6)
(b) (4) Historical Control Range for RBC: 7.24-9.23								
Abs. Reticulocyte Count	171.8	199.1	170.3	195.2	198.1*	180.1	182.3	196.8
(percent difference)		(15.9)	(-0.9)	(13.6)	(15.3)	(4.8)	(6.1)	(14.6)
(b) (4) Historical Control Range for Reticulocytes: 84.8-355.2								
Percent Reticulocytes	2.0	2.4	2.1	2.3*	2.4*	2.2	2.1	2.4
(percent difference)		(20.0)	(5.0)	(15.0)	(20.0)	(10.0)	(5.0)	(20.0)
(b) (4) Historical Control Range for Percent Reticulocytes: 1.0-4.7								
Females								
Red Blood Cell Count	8.89	8.52	8.73	8.49	8.31*	8.77	8.35	8.57
(percent difference)		(-4.2)	(-1.8)	(-4.5)	(-6.5)	(-1.3)	(-6.1)	(-3.6)
(b) (4) Historical Control Range for RBC: 7.14-8.95								
Abs. Reticulocyte Count	154.6	190.7	196.0	204.3*	215.8**	172.4	213.0	196.6
(percent difference)		(23.4)	(26.8)	(32.1)	(39.6)	(11.5)	(37.8)	(27.2)
(b) (4) Historical Control Range for Reticulocytes: 66.1-287.4								
Percent Reticulocytes	1.8	2.2	2.3	2.4*	2.6**	2.0	2.6	2.3
(percent difference)		(22.2)	(27.8)	(33.3)	(44.4)	(11.1)	(44.4)	(27.8)
(b) (4) Historical Control Range for Percent Reticulocytes: 0.8-3.9								

Lot 1: Ceftolozane manufactured using the clinical development process
Lot 2: Ceftolozane manufactured using the commercial process followed by forced degradation
Lot 3: Ceftolozane manufactured using the commercial process
Abs. = Absolute
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Clinical Chemistry

The measured serum chemistry parameters are shown in Table 109.

Changes in serum chemistry parameters were not considered to be related to test article administration and/or toxicologically relevant. Slightly lower (-3.8% to -12.0%) globulin levels and corresponding slight elevations (3.6% to 10.2%) in albumin/globulin ratios were present in both genders administered all dose levels and all preparations of CXA-101. These changes remained after the recovery period for high-dose animals receiving the commercial product CXA-101 (males and females) and forced degradation product CXA-101 (females only). However, because of the low magnitude of the changes, and because the changes exhibited no dose-dependency, the changes were not considered to be toxicologically relevant. Serum phosphorus levels were slightly but

significantly elevated in Main Study high-dose females (+ 5%; commercial process CXA-101) and in Recovery Study high-dose males (+ 7%; forced degradation CXA-101). Elevated serum phosphorus was not considered related to CXA-101 administration because of the inconsistent manner of the occurrence in only one gender, and because the levels were within the (b) (4) historical control range.

Urinalysis

The measured urinalysis parameters are shown in Table 110.

No changes in any urinalysis parameters were considered to be related to CXA-101 administration.

Gross Pathology

Pale kidneys were observed in some mid- and high-dose males administered all of the different batches of CXA-101 (Table 44). Females were not similarly affected. Also injection site scabbing was observed in all CXA-101 groups for males and most groups for females. For males, the pale kidneys correlated with increased hyaline droplets and higher kidney weights.

Table 44: Incidence of Pale Kidneys and/or Injection Site Scabbing. (Sponsor's Table)

Group (N=10)	1	2	3	4	5	6	7	8
Dosage (mg/kg/day)	0	1000 Lot 1	100 Lot 2	300 Lot 2	1000 Lot 2	100 Lot 3	300 Lot 3	1000 Lot 3
Males								
Pale kidneys	0	3	0	3	5	0	2	7
Injection site, scabbing	0	3	2	1	3	2	1	1
Females								
Injection site, scabbing	0	2	2	0	1	0	4	2

Lot 1: Ceftolozane manufactured using the clinical development process
Lot 2: Ceftolozane manufactured using the commercial process followed by forced degradation
Lot 3: Ceftolozane manufactured using the commercial process

Organ Weights

The organs that were weighed are shown in Table 111.

CXA-101-related increases in kidney weights occurred in all high-dose males and females receiving the commercial process CXA-101 or forced degradation product CXA-101 (Table 45). Higher kidney weights correlated with cytoplasmic hyaline droplets in proximal convoluted tubule cells in males and with slight cytoplasmic vacuolation in proximal tubule cells in females.

Table 45: Kidney Weight Changes in Main Study Animals. (Sponsor's Table)

Group (N=10)	1	2	3	4	5	6	7	8
Dosage (mg/kg/day)	0	1000 Lot 1	100 Lot 2	300 Lot 2	1000 Lot 2	100 Lot 3	300 Lot 3	1000 Lot 3
Males								
Absolute weight	3.15	3.38	3.30	3.42	3.79**	3.36	3.37	3.54
(percent difference)		(7.3)	(4.8)	(8.6)	(20.3)	(6.7)	(7.0	(12.4)
[redacted] Historical Control Range 2.09-3.82 +/- 2 S.D.								
Relative to body	0.844	0.941	0.913	0.942**	1.016**	0.904	0.959**	0.960**
(percent difference)		(11.5)	(8.2)	(11.6)	(20.4)	(7.1)	(13.6)	(13.7)
[redacted] Historical Control Range 0.676-1.061 +/- 2 S.D.								
Relative to brain	157.0	172.2	167.0	174.3	191.4**	166.6	169.8	176.5
(percent difference)		(9.7)	(6.4)	(11.0)	(21.9)	(6.1)	(8.1)	(12.4)
[redacted] Historical Control Range 111.927-187.497 +/- 2 S.D.								
Females								
Absolute weight	2.00	2.10	2.08	1.97	2.21	2.09	1.99	2.12
(percent difference)		(5.0)	(4.0)	(-1.5)	(10.5)	(4.5)	(-0.5)	(6.0)
[redacted] Historical Control Range 1.43-2.34 +/- 2 S.D.								
Relative to body	0.885	0.932	0.893	0.877	1.002**	0.932	0.892	0.949*
(percent difference)		(5.3)	(0.9)	(-0.9)	(13.2)	(5.3)	(0.8)	(7.2)
[redacted] Historical Control Range 0.682-1.052 +/- 2 S.D.								
Relative to brain	107.5	116.0	110.8	107.0	121.4*	115.0	109.4	115.0
(percent difference)		(7.9)	(3.1)	(-0.5)	(12.9)	(7.0)	(1.8)	(7.0)
[redacted] Historical Control Range 78.365-125.165 +/- 2 S.D.								

Lot 1: Ceftolozane manufactured using the clinical development process
Lot 2: Ceftolozane manufactured using the commercial process followed by forced degradation
Lot 3: Ceftolozane manufactured using the commercial process
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Histopathology

Adequate Battery: Yes, see Table 111.

Peer Review
Yes

Histological Findings

In males, hyaline droplets were noted in proximal tubule cells in the kidney. In females, kidney findings were most compatible with clear vacuoles rather than hyaline droplets (Table 46). No bone marrow changes were noted that might correlate with the minimal changes in RBC and reticulocyte counts.

Table 46 Incidence of Kidney Histopathology in Main and Recovery Study Males and Females. (Sponsor's Table)

	Males							
Group (N=10)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dosage (mg/kg/day):	**0**	**1000 Lot 1**	**100 Lot 2**	**300 Lot 2**	**1000 Lot 2**	**100 Lot 3**	**300 Lot 3**	**1000 Lot 3**
Kidney (Day 28) [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Hyaline Droplets	2	10	3	10	10	4	5	10
Minimal	2	2	3	2	0	4	5	4
Mild	0	5	0	8	5	0	0	6
Moderate	0	3	0	0	5	0	0	0
Kidney (Day 56) [a]	**5**	**0**	**0**	**0**	**5**	**0**	**0**	**5**
Hyaline Droplets	0	-	-	-	0	-	-	1
Minimal	-	-	-	-	-	-	-	1

	Females							
Group (N=10)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dosage (mg/kg/day):	**0**	**1000 Lot 1**	**100 Lot 2**	**300 Lot 2**	**1000 Lot 2**	**100 Lot 3**	**300 Lot 3**	**1000 Lot 3**
Kidney (Day 28) [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**11**
Vacuolation, Tubular	0	0	0	0	4	0	0	5
Minimal	-	-	-	-	3	-	-	4
Mild	-	-	-	-	1	-	-	1
Kidney (Day 56) [a]	**5**	**0**	**0**	**0**	**5**	**0**	**0**	**4**
Vacuolation , Tubular	0	-	-	-	0	-	-	0

[a] = Number of tissues examined from each group.
Lot 1: Ceftolozane manufactured using the clinical development process
Lot 2: Ceftolozane manufactured using the commercial process followed by forced degradation
Lot 3: Ceftolozane manufactured using the commercial process

Special Evaluation

None

Toxicokinetics

Toxicokinetics were determined for the 1000 mg/kg Clinical Development batch of CXA-101 and all three doses of the Commercial Process batch of CXA-101. The results are shown below in Table 47.

Table 47: CXA-101 Toxicokinetic Parameters Following IV Administration of 3 Different Batches of CXA-101. (Sponsor's Table)

Ceftolozane Dosage	1000 mg/kg Clinical Development		100 mg/kg Commercial		300 mg/kg Commercial		1000 mg/kg Commercial	
	Males	Females	Males	Females	Males	Females	Males	Females
Study Day 0								
AUC_{last} (µg·h/mL)	1820	2750	187	154	522	448	3560	1640
SE AUC_{last} (µg·h/mL)	87.9	514	6.35	4.62	14.9	20.6	181	66.8
D.N. AUC_{last}	1.82	2.75	1.87	1.54	1.74	1.49	3.56	1.64
AUC_{inf} (µg·h/mL)	NR	NR	187	155	523	448	3560	NR
C_0 (µg/mL)	4650	10000	442	433	1380	993	11200	3970
C_{max} (µg/mL)	3530	6600	351	329	1050	784	7990	3080
D.N. C_{max}	3.53	6.60	3.51	3.29	3.50	2.61	7.99	3.08
T_{max} (h)	0.083	0.083	0.083	0.083	0.083	0.083	0.083	0.083
K_{el} (h^{-1})	NR	NR	1.86	2.31	1.88	1.86	0.935	NR
$T_{1/2}$ (h)	NR	NR	0.37	0.30	0.37	0.37	0.74	NR
Cl (mL/h/kg)	NR	NR	534	644	574	670	281	NR
V_Z (mL/kg)	NR	NR	287	279	306	360	301	NR

D.N. = Dose-normalized; units for D.N. AUC_{last} and D.N. C_{max} are (µg·h/mL)/(mg/kg) and (µg/mL)/(mg/kg), respectively.
Clinical = Ceftolozane Lot 440420 0004 1 clinical development process.
Commercial =Ceftolozane Lot 440420 0002 2 commercial process.
NR = Not reportable.

Dosing Solution Analysis

Multiple samples from each dosing formulation were collected including samples from the first and last days of dosing and retained for analysis.

All of the analyzed dosing formulations were found to contain 95.9% to 108% of the nominal concentrations of the test articles.

Study title: A 14-Day Twice Daily Intravenous (15 Minute) Infusion Combination Toxicity Study of CXA-101 and Tazobactam in Beagle Dogs

Study no.:	CXA201-T-005
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 14, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101 (FR264205), Lot # 020010, purity of 98.1%; tazobactam, Lot # 090813102, purity of 99.9%.

Key Study Findings

No CXA-101 or tazobactam-related toxicities were observed in Beagle dogs at combination doses as high as 300 mg/kg/day CXA-101 combined with 150 mg/kg/day tazobactam administered BID for 14 days in 15-minute infusions. The high dose was considered to be the NOAEL.

Methods

Doses:	0 (Group 1), 300 mg/kg/day CXA-101 alone (Group 2), 150 mg/kg/day tazobactam alone (Group 3), 100 m/kg/day CXA-101 with 50 mg/kg/day tazobactam (Group 4), and 300 mg/kg/day CXA-101 with 150 mg/kg/day tazobactam (Group 5).
Frequency of dosing:	Twice daily
Route of administration:	Intravenous infusion over approximately 15 minutes into the cephalic vein of a forelimb.
Dose volume:	Infusion rate of 0.333 ml/kg/min for approximately 15 minutes
Formulation/Vehicle:	0.9% sodium chloride for injection
Species/Strain:	Beagle dogs
Number/Sex/Group:	3/sex/group
Age:	Approximately 7 months old at receipt.
Weight:	At initiation of dosing: 8.8 to 12.1 kg for males; 7.4 to 10.0 kg for females.
Satellite groups:	none
Unique study design:	Test articles (CXA-101 and/or tazobactam) were administered BID for 14 consecutive days (Days 0-13). The BID daily doses were separated by approximately 8 hours. Surviving animals were euthanized for necropsy on Day 14.
Deviation from study protocol:	Multiple deviations from the study protocol were noted including infusion times outside the protocol-specified range. However, the deviations were not considered to have altered the results or integrity of the study.

Observations and Results

Table 48:Schedule of Observations for the 14-Day Toxicology Study in Dogs.

Measured Parameters	Schedule
Mortality	All animals were observed twice daily for mortality and morbundity
Clinical Signs	Clinical observations were performed prior to each infusion (in the morning and afternoon), at the end of each infusion period, and approximately 1 to 2 hours following each infusion. In addition, detailed physical examinations were conducted on all animals at least once during acclimation, prior to

	randomization, weekly during the treatment period, and prior to the scheduled necropsy.
Body Weights	Individual body weights were recorded at least weekly during the pretest period and throughout the study.
Food Consumption	Individual food consumption was recorded at least weekly during the pretest period and throughout the study.
Clinical Pathology	Blood and urine samples were collected from all dogs prior to initiation of dose administration and the scheduled necropsy on Day 14.
Ophthalmic Examinations	Ocular examinations were conducted on all animals prior to initiation of dosing and near the end of the treatment period.
Electrocardiograms (ECG)	ECGs were recorded prior to randomization and on Days 11 (males) and 10 (females).
Toxicokinetics	Blood samples for toxicokinetics were collected from all animals prior to the first daily infusion, just prior to the end of the first daily infusion, and at approximately 5, 15, and 30 minutes, and 1, 2, 4, and 8 hours after completion of the infusion on Days 0 and 13.
Necropsy	Day 14

Mortality
All animals survived until the scheduled necropsy

Clinical Signs
No test article-related clinical signs were observed.

Body Weights
Body weights were not significantly altered by any of the test-article administrations.

Feed Consumption
Food consumption was not significantly altered by any of the test-article administrations.

Ophthalmoscopy
All ocular examinations were conducted using an indirect ophthalmoscope and slit-lamp biomicroscope preceded by pupillary dilation.

No ophthalmic lesions indicative of toxicity were observed in any of the test article groups.

ECG
Multilead (I, II, III, aVR, aVL, aVF, and V2) ECGs were recorded for all animals for measurements of heart rate, and PR, RR, QRS, QT, and QTcV (Van de Waters correction).

No test-article related changes in any of the measured or calculated ECG parameters were observed.

Hematology
The hematology and coagulation parameters that were assessed are listed in Table 108.

No significant changes in any of the hematology or coagulation parameters were attributed to any of the test article administrations.

Clinical Chemistry
The assessed serum chemistry parameters are listed in Table 109.

None of the significant changes in the clinical chemistry parameters were attributed to test-article administration. A few parameters were significantly altered in test-article groups compared to control values. Serum phosphorus was significantly lower than control values in males in Groups 3 (150 mg/kg/day tazobactam, -14% compared to control) and 4 (100/50 mg/kg/day CXA-101/tazobactam; -16% compared to control). In contrast, serum phosphorus was significantly higher than control values for females in Groups 4 (+27% compared to control) and 5 (300/150 mg/kg/day CXA-101/tazobactam; +18% compared to control). For all of the changes, the values associated with test article administration were reportedly within the historical control range, and the changes were not considered to be clearly related to test-article administration.

Urinalysis
The urinalysis parameters that were assessed for this study are listed in Table 110.

No test-article related changes in any of the urinalysis parameters were observed.

Gross Pathology
None of the gross pathology observations were considered to be related to test-article administration.

Organ Weights
The organs that were weighed in this study are listed in Table 111.

No significant changes in organ weights relative to control values were observed.

Histopathology

Adequate Battery: Yes, the tissues and organs that were assessed microscopically for histopathology are listed in Table 111.

Peer Review: No

Histological Findings
One Group 5 female exhibited a moderate perivascular cellular infiltrate composed primarily of macrophages with lesser amounts of lymphocytes in the meninges and Virchow-Robbin space. Infiltrates were observed in the cerebrum, cerebellum, and in the perivascular space of grey matter in the spinal cord. According to the Sponsor, a mixed inflammatory infiltrate in the brain has been reported in the (b) (4) historical control

database in 2/139 male dogs. Also two kinds of idiopathic inflammatory diseases in dogs (granulomatous meningoencephalitis and nonsuppurative/granulomatous meningoencephalitis) have been reported in the literature to occur sporadically in Beagle dogs. Given this background data, and the incidence in a single animal in the current study, the brain inflammation was not considered to be clearly related to the Group 5 treatment.

Other findings occurring in all groups including control animals included injection site hemorrhage and inflammation and epididymis changes. The epididymis changes including hypospermia and luminal cell debris, and changes in testes (hypoplasia, hypospermia and luminal debris in seminiferous tubules) occurred in all groups including the vehicle control group and were considered related to the variable sexual maturity of the male dogs in the study.

Special Evaluation: none reported

Toxicokinetics
CXA-101 administered in combination with tazobactam produced AUC exposures that increased in a roughly dose-proportional manner with a single (Day 1) dose or when administered for 13 days (Table 49). CXA-101 did not accumulate in plasma after 13 days of dosing when administered alone or in combination with tazobactam at the lowest doses (Group 4). Approximately 24% accumulation of CXA-101 occurred after 13 days of dosing of the high-dose combination compared to Day 1 plasma levels. In correlation with this finding, plasma clearance (Cl) and volume of distribution (Vss) of CXA-101 were slightly lower for the high-dose combination after 13 days of dosing compared to Day 1 values, suggesting saturation of clearance pathways. Also plasma $t_{1/2}$ values were similar for all groups, but slightly higher after 13 days of dosing compared to Day 1 values. No gender-specific differences in the toxicokinetic values for CXA-101 were apparent (data not shown).

Table 49: Summary of Plasma Toxicokinetic Parameters for CXA-101. (Sponsor's Table)

	Day 0			Day 13		
Parameter	**Group 2**	**Group 4**	**Group 5**	**Group 2**	**Group 4**	**Group 5**
AUC_{0-8}[a] (µg•h/mL)	855 (13.8)	293 (17.9)	755 (17.5)	735 (19.3)	271 (16.4)	934 (17.4)
AUC_{last} (µg•h/mL)	855 (13.8)	293 (17.9)	755 (17.5)	735 (19.3)	271 (16.4)	934 (17.4)
$AUC_{0-\infty}$ (µg•h/mL)	856 (13.8)	294 (17.9)	756 (17.6)	736 (19.4)	272 (16.5)	936 (17.5)
C_{max} (µg/mL)	903 (15.6)	342 (43.3)	783 (8.5)	817 (14.9)	300 (13.0)	931 (21.3)
T_{max}[b] (h)	0.23 (0.22 - 0.23)	0.26 (0.22 - 0.33)	0.23 (0.22 - 0.23)	0.23 (0.23 - 0.23)	0.23 (0.22 - 0.23)	0.23 (0.22 - 0.25)
$t_{1/2}$ (h)	0.882 (8.3)	0.859 (7.3)	0.851 (12.6)	0.906 (4.8)	0.962 (12.3)	0.901 (14.0)
MRT_{∞} (h)	1.07 (4.9)	1.02 (9.4)	1.03 (18.1)	1.04 (11.5)	1.01 (13.4)	1.15 (17.4)
CL (L/h/kg)	0.178 (13.6)	0.175 (17.9)	0.203 (16.8)	0.210 (16.8)	0.189 (15.7)	0.165 (15.9)
V_{ss} (L/kg)	0.190 (12.7)	0.177 (15.2)	0.204 (4.7)	0.215 (10.1)	0.188 (14.8)	0.186 (13.2)

[a] - also referred to as AUC_{ss} on study day 13
[b] - Median (min - max)
N = 6 for all groups
Group 2: 300 mg/kg/day CXA-101
Group 4: 100/50 mg/kg/day CXA-101/Tazobactam
Group 5: 300/150 mg/kg/day CXA-101/Tazobactam

When administered alone (Group 3) or in combination with CXA-101 (Groups 4 and 5), plasma tazobactam appeared to increase in a dose-proportional manner when administered for one day, but in a more than dose-proportional manner when administered for 13 days (Table 50). Plasma tazobactam appeared to accumulate approximately 20% with 13 days of dosing with the high combination dose (Group 5) compared to a single combination dose. Plasma clearance also decreased slightly with 13 days of combination dosing with the high combination dose (Group 5) compared to a single combination dose suggesting saturation of clearance pathways may have contributed to tazobactam plasma accumulation. No gender-specific differences in the toxicokinetic values for tazobactam were apparent (data not shown).

Table 50: Summary of Plasma Toxicokinetic Parameters for Tazobactam.
(Sponsor's Table)

	Day 0			Day 13		
	Group 3	Group 4	Group 5	Group 3	Group 4	Group 5
AUC_{0-8}[a] (µg•h/mL)	251 (25.7)	75.7 (22.2)	229 (15.2)	219 (17.3)	70.9 (21.3)	275 (10.0)
AUC_{last} (µg•h/mL)	251 (25.7)	75.3 (22.2)	229 (15.2)	219 (17.3)	70.9 (21.1)	275 (10.0)
$AUC_{0-\infty}$ (µg•h/mL)	251 (25.7)	75.5 (22.2)	229 (15.2)	219 (17.3)	73.4 (21.3)[c]	275 (10.0)
C_{max} (µg/mL)	289 (15.2)	117 (49.8)	283 (9.5)	284 (20.7)	99.9 (15.4)	339 (21.3)
t_{max}[b] (h)	0.22 (0.22 - 0.23)	0.23 (0.22 - 0.33)	0.23 (0.22 - 0.23)	0.23 (0.22 - 0.25)	0.23 (0.22 - 0.23)	0.23 (0.22 - 0.25)
$t_{1/2}$ (h)	0.749 (6.3)	0.619 (20.1)	0.738 (9.2)	0.808 (6.6)	0.785 (20.2)[c]	0.790 (11.2)
MRT_{∞} (h)	0.750 (13.1)	0.685 (14.8)	0.790 (16.7)	0.826 (10.5)	0.728 (19.4)[c]	0.875 (15.6)
CL (L/h/kg)	0.315 (24.6)	0.347 (25.7)	0.334 (15.9)	0.353 (20.2)	0.366 (20.4)	0.275 (10.0)
V_{ss} (L/kg)	0.233 (23.5)	0.235 (22.1)	0.258 (4.0)	0.287 (11.9)	0.251 (14.9)[c]	0.239 (13.9)

[a] - Also referred to as AUC_{ss} on study day 13
[b] - Median (min - max)
[c] - Animal 7188 not included in calculation of summary statistics, N = 5
N = 6 for all group with the exceptions as noted
Group 3: 150 mg/kg/day Tazobactam
Group 4: 100/50 mg/kg/day CXA-101/Tazobactam
Group 5: 300/150 mg/kg/day CXA-101/Tazobactam

Dosing Solution Analysis
The actual concentrations of the dose formulations were found to contain 97.4% to 101% of the nominal CXA-101 concentrations and 95.8% to 98.7% of the nominal tazobactam concentrations. Neither test article was detected in the vehicle formulation.

Study title: Ceftolozane/Tazobactam: A Non-GLP Subcutaneous 14-Day Dose-Range Finding Juvenile Toxicity and Toxicokinetic Study in Postnatal Day (PND) 4 Sprague-Dawley Rats.

Study no.:	CXA.101.TX.033
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 2013
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CXA-101, Batch No.: 440420 0002 2, purity of 96.3%; tazobactam, batch #

090813102, purity of 99.6%.

Key Study Findings

- Kidney and liver findings were consistent with previous effects of CXA-101 and tazobactam in adult animals. However the kidney findings were not limited to hyaline droplet formation as has been noted in adults but also included tubular basophilia, and renal cortical fibrosis. These effects are consistent with cell loss during dosing and subsequent regeneration, effects that did not occur in adult rats.
- The results suggest CXA-101 may be more toxic to the kidneys of juvenile rats as opposed to adult rats.
- The NOAEL for this study was considered to be the low combination dose of 50 mg/kg/day CXA-101 and 25 mg/kg/day tazobactam associated with plasma AUC values of 657 µg·hr/mL CXA-101 and 145 µg·hr/mL tazobactam (approximately 3.6 and 5.8 fold the clinical exposures for CXA-101 and tazobactam respectively in healthy adults).

Methods

Doses:	CXA-101/tazobactam (mg/kg/day): 0/0, 50/25. 300/150, 1000/500
Frequency of dosing:	Once daily (into two sites) from PND 4 to 17.
Route of administration:	Subcutaneous injection
Dose volume:	10 ml/kg
Formulation/Vehicle:	Sterile saline, pH adjusted to 5.4 to 5.9
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	6/sex/group
Age:	Post natal day (PND) 4 at the start of dosing
Weight:	PND 4 body weights were: 6.1 to 12.3 g for male pups and 5.1 to 11.9 g for female pups.
Satellite groups:	Toxicokinetic animals: 3/sex for Group 1, and 18/sex/group for Groups 2-4
Unique study design:	CXA-101/tazobactam combinations and vehicle control formulations were administered to male and female pups via subcutaneous injection into the scapular and mid-dorsal area once daily from PND 4 to 17. Study animals were euthanized on PND 18.
Deviation from study protocol:	Multiple protocol deviations occurred, but none was considered to have altered the results or compromised the integrity of the study.

Table 51: Study Design for Study No.: CXA.101.TX.033. (Sponsor's Table)

Group No.	Test Material	Ceftolozane / Tazobactam Dose Level (mg/kg/day)	Ceftolozane/ Tazobactam Dose Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Animals			
					Main Study		Toxicokinetic Study	
					Males	Females	Males	Females
1	Control	0/0	0/0	10	6	6	3	3
2	Ceftolozane / Tazobactam	50/25	5/2.5	10	6	6	18	18
3	Ceftolozane / Tazobactam	300/150	30/15	10	6	6	18	18
4	Ceftolozane / Tazobactam	1000/500	100/50	10	6	6	18	18

Observations and Results

Table 52: Observation Schedule for Study No.: CXA.101.TX.033

Measured Parameters	Schedule
Mortality	All pups were observed twice daily for mortality and morbundity
Clinical Signs	Clinical observations were performed at least once daily during the acclimation period, daily before each dose, and on the day of the scheduled necropsy. In addition, after each dose, observations were recorded within 20 minutes for the first 6 days and 1-2 hours after dosing and at the end of the working day for all dosing days.
Body Weights	Individual body weights were recorded on PND 1, daily from PND 4 to 17, and at the scheduled necropsy.
Clinical Pathology	Blood samples for hematology and serum chemistry analysis were collected from all Main Study animals prior to the scheduled necropsy on PND 18.
Toxicokinetics	Blood samples for toxicokinetics were collected from all surviving toxicokinetic animals. Blood samples were collected from 3 rats/sex in Group 1 at approximately 0.5 hours postdose, and from 3 rats/sex/timepoint for rats in Groups 2 to 4 at approximately 0, 0.25, 0.5, 1, 3, and 8 hours after dosing.
Necropsy	PND 18

Mortality

No treatment-related deaths occurred. Two control toxicokinetic animals (one male and one female) were found on PND 6 (male) and PND 8 (female).

Clinical Signs
Treatment-related clinical signs were noted only in the high-dose group (Group 4: 1000/500 mg/kg/day CXA-101/tazobactam. Clinical signs included decreased motor activity, lost or impaired righting reflex, and ataxia and occurred only on PND 12-16 generally only on one day for each rat. Red and/or purple discoloration also occurred at injection sites for all but one of the high-dose animals on the first three days of dosing, but not subsequently. This injection-site discoloration was considered by the Sponsor to be an artifact of the dosing procedure.

Body Weights
Body weights were not affected for any of the CXA-101/tazobactam dose groups compared to the control group.

Femur Lengths
Right and left femurs were excised and measured for length (point of the lateral condyle to the point of the greater trochanter) from Main Study animals following sacrifice on PND 18.

Right and left femur lengths were not affected by CXA-101/tazobactam administration at any dose. Mean ± SD values for control and treatment male and female rats ranged from 14.0 ± 0.7 to 14.9 ± 0.3 millimeters with no trends as to treatment.

Hematology
The measured hematology and coagulation parameters are shown in Table 108. No substantial effects on any hematology or coagulation parameters related to the administration of ceftolozane/tazobactam were observed.

Clinical Chemistry
The measured serum chemistry parameters are shown in Table 109.

Values for serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, cholesterol, and triglycerides were decreased for the male and female mid- and high-dose groups compared to controls. However the differences were slight (approximately 10% to 35% reductions), and not considered toxicologically relevant.

Reviewer Comment: *In this non-GLP study, statistical analysis of hematology and clinical chemistry differences was not performed.*

Gross Pathology
No treatment-related gross pathology findings were reported.

Organ Weights
The organs that were weighed are shown in Table 111

Absolute liver weights were higher in the mid-dose (+6% for males, +8% for females) and high-dose (+11% for males and +17% for females) compared to the control group.

Similar increases also occurred for relative liver weights. In male and female rats respective absolute weights of paired kidneys were 12% and 7% higher for mid-dose animals and 24% and 21% higher for high-dose animals compared to the control group. Similar values occurred for relative kidney weights.

Histopathology

Adequate Battery: Yes, see Table 111.

Peer Review
No

Histological Findings
High-dose male and female rats demonstrated kidney cortical tubular vacuolation (4/6 males and 6/6 females), basophilic staining (3/6 males and 2/6 females), and minimal to mild cortical fibrosis (1/6 males and 2/6 females). Cortical tubular vacuolation was also noted to occur in 4/6 mid-dose females. Low level hyaline droplet formation was also noted in the proximal convoluted tubular epithelium of 2/6 high-dose male rats. Epithelial cell necrosis was not observed, but tubular basophilia and renal cortical fibrosis is consistent with previous cell loss and regeneration that may have occurred during dosing.

Reviewer Comment: *The CXA-101-related kidney effects appear to have more severe consequences than that seen in adult rats. Further study is needed, but it may be that CXA-101 is more toxic to the kidneys of young animals compared to adults.*

Mild centrilobular hepatocellular hypertrophy which correlated with the increased liver weights was observed in 5/6 and 1/6 high-dose males and females respectively and 1/6 male mid-dose animals. No associated necrosis was observed.

Special Evaluation: none reported

Toxicokinetics
Plasma AUC exposures for both CXA-101 and tazobactam were both approximately dose proportional (Table 53 and Table 54), and no gender differences were apparent (data not shown).

Table 53: Toxicokinetic Parameters for CXA-101 in Male and Female Rats on PND 17. (Sponsor's Table)

Nominal Dose Ceftolozane/ Tazobactam (mg/kg)	Cmax (µg/mL)	AUC (µg·hr/mL)	AUC∞ (µg·hr/mL)	Cmax/Dose (µg/mL)/(mg/kg)	AUC∞/Dose (µg·hr/mL)/(g/kg)
50/25	81.5	119	124	1.74	2.65
300/150	416	657	657	1.57	2.48
1000/500	1280	2250	2250	1.40	2.47

Table 54: Toxicokinetic Parameters for Tazobactam in Male and Female Rats on PND 17. (Sponsor's Table)

Nominal Dose Tazobactam/ Ceftolozane (mg/kg)	Cmax (µg/mL)	AUC (µg·hr/mL)	AUC∞ (µg·hr/mL)	Cmax/Dose (µg/mL)/(mg/kg)	AUC∞/Dose (µg·hr/mL)/(mg/kg)
25/50	32.9	29.7	29.7	1.25	1.13
150/300	184	145	145	1.22	0.965
500/1000	644	640	641	1.25	1.24

Dosing Solution Analysis
In dose formulations of the CXA-101/tazobactam combinations, CXA-101 actual concentrations were found to be 88.4 to 102% of the target concentrations, and tazobactam actual concentrations were found to be 100 to 111% of the target concentrations.

Tazobactam

Study Title: A Six-Month Intraperitoneal Repeated Dose Toxicity Study of Tazobactam/Piperacillin and Tazobactam in Rats. Hayashi, Y., Yada, H., Auletta, C.S., Daly, I.W., Knezevich, A.L., and Cockrell, B.Y.: The Journal of Toxicological Sciences, 19 (supplement II): 155-176 (1994).

Reviewer Comment: *This manuscript was published in Japanese, but completely translated into English. Combination doses of tazobactam and piperacillin was also administered to rats in this study, but these results were not considered in this review.*

Key Study Findings

- Numerous hematomas were observed in the colon and mesenteries of animals treated with tazobactam for 6 months, but not after recovery. This effect correlated with cecal enlargement in high-dose animals. Body weights and food intake were not affected.
- PAS-positive staining material identified histochemically and ultrastructurally as glycogen was observed in the livers of mid- (80 mg/kg/day) and high- (160 mg/kg/day) males. Liver glycogen deposits were reduced after the Recovery Period suggesting reversibility.
- Exclusive of the cecal effects, the NOAEL was considered to be the high-dose of 160 mg/kg/day.

Methods

Doses:	0 (normal saline), 40, 80, 160 mg/kg/day tazobactam.
Frequency of dosing:	Twice per day approximately 5 hours apart
Route of administration:	intraperitoneal
Dose volume:	5 ml/kg/injection; 10 ml/kg/day
Formulation/Vehicle:	Sodium bicarbonate in distilled water.
Species/Strain:	SD (CD) rats

Number/Sex/Group:	Main Study animals: 20/sex/group were necropsied at 6 months.
Age:	Approximately 2 months old
Weight:	At the start of the study: males 175 to 218 g; females: 140 to 172 g.
Satellite groups:	Recovery animals: 10/sex/group (no Recovery animals in the 40 mg/kg tazobactam group)
Unique study design:	Male and female rats were intraperioneally administered saline or tazobactam twice per day for 6 months. Main Study animals were euthanized and necropsied after 6 months. Recovery animals were necropsied one month later.
Deviation from study protocol:	Not reported.

Observations and Results

Table 55: Schedule of Observations for the 6-Month Tazobactam Study in Rats.

Measured Parameters	Observation Schedule
Mortality and clinical signs	All the animals were observed twice daily for deaths and general condition.
Body weight	Body weight was checked at the start of dosing, and once per week thereafter.
Food consumption	Food consumption was checked at the start of dosing, and once per week thereafter.
Opthalmic Examinations	Animals received ophthalmic examinations before dosing at 1, 3, and 6 months after the start of dosing.
Clinical Pathology	Before dosing, and 1, 3, and 6 months after the start of treatment and after recovery, blood was collected via the orbital vein from 20 rats/sex/group that had been fasted overnight. Blood samples were processed for hematology and serum chemistry analysis. Urine samples were also collected over period of 16 hours overnight while the animals were deprived of water.
Necropsy	After 6 months of dosing, Main Study animals were necropsied and Recovery animals were necropsied one month later.

Mortality

During the dosing period 3 control animals died, as well as 1 low-dose (40 mg/kg/day) female and 1 mid-dose (80 mg/kg/day) male. The deaths were not considered related to tazobactam administration. All animals died 3-6 weeks after the start of dosing.

Clinical Signs

No clinical signs associated with tazobactam administration were reported.

Body Weights
No changes in body weight that were significantly different than control values were noted in animals treated with tazobactam.

Feed Consumption

Reportedly no tazobactam dose-related changes in food consumption occurred.

Ophthalmoscopy
The eyelids and conjunctiva of all animals were macroscopically examined and animals received an indirect ophthalmoscope examination of the cornea, anterior chamber, lens, iris, vitreous body, retina, and optic papilla.

Reportedly, no tazobactam-related ocular changes occurred.

ECG: Not performed

Hematology
The hematology parameters that were assessed were as shown in Table 108.

Reticulocytes were increased after 6 months of dosing with 80 (+50%) and 160 (+72%) mg/kg/day doses of tazobactam. However the changes were not statistically significant, and the changes were reversed after Recovery.

Clinical Chemistry

The serum chemistry parameters that were assessed were as shown in Table 109.

Triglycerides were significantly decreased in high-dose males (-49%) 6 months after the start of dosing, and in females from all three tazobactam groups 3 months after the start of dosing, but not in a dose-dependent manner in females (-33% to -38%). Triglycerides were reduced but not to a significant degree in tazobactam-treated females at 6-months, and a trend toward decreased triglycerides persisted in tazobactam-treated females but not males after the Recovery period.

Urinalysis
Urinalysis included assessment of the parameters shown in Table 110.

Reportedly, no tazobactam-related changes occurred.

Gross Pathology

Cecums were enlarged (mild to severe) in females receiving 160 mg/kg/day tazobactam. Cecum enlargement reversed during Recovery.

Organ Weights

Assessed organs were as listed in Table 111.

Absolute and/or relative liver weights were significantly increased in males receiving 80 (11% relative increase) and 160 (24% absolute weight increase; 26% relative increase)

mg/kg/day of tazobactam and in females given 160 mg/kg/day of tazobactam (11% relative increase). After the recovery period, hepatic weight changes were not clearly resolved except in high-dose females.

Histopathology

Adequate Battery: Yes. The tissues examined for histopathology are listed in Table 111

Peer Review
No

Histological Findings
Numerous hematomas accompanied by moderate inflammation were noted in the colon and mesenteries of animals treated with tazobactam at the end of dosing. This effect was not reported after the recovery period. An accumulation of PAS-positive material identified histochemically and ultrastructurally as glycogen was observed in the livers of males receiving 80 and 160 mg/kg/day of tazobactam alone. After recovery, the liver glycogen deposits moderately reduced suggesting the effect was reversible.

Special Evaluation: none reported

Toxicokinetics: not reported

Dosing Solution Analysis: not reported

Study Title: A Six-Month Intravenous Repeated Dose Toxicity Study of Tazobactam/Piperacillin and Tazobactam in Dogs. Hayashi, Y., Yada, H., Blair, Laughlin, K.A., Blanchard, G.L., Tucek, P.C. and R.G. Geil, The Journal of Toxicological Sciences, 19 (supplement II): 177-197 (1994).

Reviewer Comment: *This manuscript was published in Japanese but entirely translated in English. Combination treatments with tazobactam/piperacillin were also examined in this manuscript, but only the tazobactam effects are discussed by the Reviewer.*

Key Study Findings

- Increased red spots in cecal mucosa were noted in high-dose (160 mg/kg/day) animals.
- A primary finding was the presence of cytosolic aggregates positive for eosinophilic and PAS staining in liver hepatocytes and around the bile canaliculi of high-dose males and females. This finding was consistent with glycogen accumulation, was not associated with liver degeneration or necrosis and decreased after the Recovery period.
- Exclusive of the cecal effects, the NOAEL was considered to be 160 mg/kg/day.

Study no.:	Not identified in the publication
Study report location:	Published manuscript
Conducting laboratory and location:	Drug Safety Laboratory, Taiho Pharmaceutical Co., Ltd., 224-2,

	Ebisuno, Hirashi, Kawauchi-cho, Tokushima 771-01, Japan; and International Research and Development Corp., Mattawan, MI 49071, USA.
Date of study initiation:	Not identified. The manuscript was accepted on June 4, 1994.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Tazobactam, the lot and purity were not identified.

Methods

Doses:	0 (normal saline), 40, 80, 160 mg/kg/day tazobactam
Frequency of dosing:	Twice per day
Route of administration:	Intravenous, infusion rate of 5 ml/min
Dose volume:	10 ml/kg (5 ml/kg twice per day)
Formulation/Vehicle:	Distilled water
Species/Strain:	Beagle dogs
Number/Sex/Group:	Main Study animals: 4/sex/group were necropsied after 6 months of dosing.
Age:	6 to 9 months old
Weight:	At the start of dosing: males: 7.6 to 13.4 kg; females: 6.2 to 10.4 kg.
Satellite groups:	Recovery animals: 2/sex/group except for the low-dose Tazobactam group.
Unique study design:	Male and female Beagle dogs were administered saline or tazobactam twice a day by intravenous infusion for 6 months. Main Study animals were necropsied after 6 months of dosing and recovery animals were necropsied 1 month later.
Deviation from study protocol:	Study protocol deviations were not identified.

Observations and Results

Table 56: Observation Schedule for the 6-Month Tazobactam Study in Dogs.

Measured Parameters	Observation Schedule
Mortality and clinical signs	All the animals were observed twice daily for deaths and signs of toxicity, diarrhea, and vomiting. In addition animals were examined once per week for mobility/activity, changes in urinary organs, respiratory organs, and oral cavity and eyes, and tumors. Before dosing and 2, 6, 10, 14, 18, 22, and 26 weeks after the start of dosing animals and during the recovery period, the cranial, cervical, thoracic and abdominal regions, external reproductive organs, skin, extremities, heart

	sounds, respiratory sounds and general condition were assessed.
Body weight	Body weight was checked at the start of dosing, and once per week thereafter.
Food consumption	Food consumption was measured once per week.
Body temperature	Body temperature was measured 5 times before dosing, once a week during the dosing period (about 1 hour after the first daily dose), and at the end of the recovery period.
Blood pressure	Blood pressure was measured before dosing and at 1, 3, and 6 months after the start of dosing (about 1 hour after the first daily dose) and at the end of the Recovery period.
ECG	ECG was recorded by the standard extremity lead (I, II, III) and augmented unipolar extremity lead (aV_R, aV_L, aV_F) before dosing, 1, 3, and 6 months after the start of dosing, and at the end of the Recovery period.
Opthalmic Examinations	Animals received ophthalmic examinations before dosing, at 1, 3, and 6 months after the start of dosing, and at the conclusion of the Recovery period.
Clinical Pathology	Blood samples were collected for hematology and serum chemistry analysis, and urine samples were collected from fasted animals overnight before dosing, 1, 3, and 6 months after the start of dosing, and after the recovery period.
Necropsy	After 6 months of dosing, Main Study animals were necropsied, and Recovery animals were necropsied one month later.

Mortality
No animals died during the study

Clinical Signs
No tazobactam-related clinical signs were noted.

Body Weights
No changes in weight gain were noted in the tazobactam-treated animals compared to control animals.

Feed Consumption
Food consumption in high-dose females was mildly lower in the last 3 months of the dosing period and remained depressed in the Recovery period.

Ophthalmoscopy
No ophthalmic changes were noted.

Body Temperature and Blood Pressure
No tazobactam-related changes in body temperature or blood pressure were noted.

ECG
Tazobactam-related changes in ECG readings were not observed.

Hematology
The assessed hematology and coagulation parameters are shown in Table 108.

Red blood cell, hemoglobin levels, and hematocrit levels were decreased at 1 and/or 3 months of dosing in the low- and/or mid-dose tazobactam groups in males and females, but no effects were noted at 6-months or after the Recovery period or in high-dose animals. Platelets were not affected by dosing with tazobactam compared to pre-dosing values or control values.

Clinical Chemistry
The assessed serum chemistry parameters are shown in Table 109.

Decreased total protein (-10% in males, 20% in females) and globulin (-19% in males; 33% in females) was observed in high-dose males and females after 6 months of dosing. Values returned to control values after the Recovery period. AST values were significantly decreased in high-dose females at the 6-month time-point (-30%) and after Recovery (-33%), but no changes were noted in high-dose males.

Urinalysis
The assessed urinalysis parameters are shown in Table 110

No tazobactam-related changes in any urinalysis parameters were noted.

Gross Pathology
A greater incidence of red spots was noted in the cecal mucosa of males and females in all the tazobactam groups compared to controls.

Organ Weights
The list of organs that were weighed are shown in Table 111.

The absolute weight of ovaries and uterus and relative weight of uterus were increased in high-dose females.

Histopathology

Adequate Battery: Yes; see Table 111

Peer Review
No

Histological Findings
Hepatic cytosolic aggregates which were positive for eosinophilic and PAS staining were observed in the livers of high-dose males and females. Similar staining also occurred around the bile canaliculi. The finding decreased after the Recovery period. Electron microscopy analysis revealed more SER and glycogen granules in the bile canaliculi and hepatocyte nuclei in the high-dose males and females.

Special Evaluation: None

Toxicokinetics: Not performed

Dosing Solution Analysis: Not reported

7 Genetic Toxicology

CXA-101 was negative in all *in vitro* and *in vivo* genetic toxicity studies except the mouse lymphoma assay. CXA-101 with and without metabolic activation was not mutagenic in the Ames test (Study No.: GLR050752), and did not induce unscheduled DNA synthesis in rat hepatocytes (Study No.: GRL040362). *In vitro* in a Chinese hamster lung fibroblasts cell line (Study No.: GLR050845), and *in vivo* in a mouse micronucleus assay (Study No.: GLR050847), CXA-101 was not clastogenic. In the *in vitro* mouse lymphoma gene mutation assay (Study No.: GLR050528), CXA-101 produced a statistically significant dose-dependent increase in gene mutation frequency in the continuous 24-hour assay without S9 activation and in the 4-hour assay with S9 activation. However, given the negative results in the other genotoxicity assays including the *in vivo* micronucleus assay, CXA-101 is considered to have a low potential for genotoxicity in humans. Study Nos.: TX037148, GRL040362, TX037143, TX037139, and GLR050528 were reviewed in the 2/23/2011 nonclinical safety review for IND 104490.

The combination of CXA-101 and tazobactam in a 2:1 ratio was negative for genotoxicity in an *in vitro* mouse lymphoma assay (CXA-201-T-003) and an *in vivo* rat bone marrow micronucleus assay (CXA-201-T-004). In an *in vitro* chromosomal aberration assay using Chinese hamster ovary cells, the combination was positive for structural aberrations (CXA-201-T-002). The weight of evidence suggests CXA-101 combined with tazobactam in a 2:1 ratio has a low potential for genetic toxicity. Study Nos.: CXA-201-T-002, CXA-201-T-003, CXA-201-T-004 are reviewed below.

In literature reports reviewed below, tazobactam was shown to be negative for mutagenicity or clastogenesis in an Ames assay, a chromosomal aberration assay, and an *in vivo* micronucleus assay (Ohuchida *et al.*, J Toxicol Sci, 19, suppl II: 263-280, 1994).

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Test

Study no.:	CXA-201-T-002
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 30, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101, Lot No.: 020010, purity of 98.51%; tazobactam, Lot # 05/08, purity of 98.0%

Key Study Findings

- In the 4-hour incubation with S9 activation, the percentage of cells with structural aberrations was significantly increased in a dose-dependent manner by the combination of CXA-101 and tazobactam combined in a 2:1 ratio.
- Also in the 20-hour incubation without S9 activation, the percentage of cells with structural aberrations was significantly increased in a dose-dependent manner.
- The percentage of cells with numerical aberrations was not increased by any CXA-101/tazobactam concentrations in any of the incubation conditions.

Methods

Cell line:	Chinese hamster ovary (CHO-K_1) cells
Concentrations in definitive study:	As noted in Table 57.
Basis of concentration selection:	The test article concentrations used in the definitive assay were based on the results of a preliminary toxicity assay.
Negative control:	Saline
Positive control:	Mitomycin C (MMC) for incubations in the absence of S9 activation, Cyclophosphamide (CP) for incubations in the presence of S9 activation.
Formulation/Vehicle:	Saline
Incubation & sampling time:	In assays in the absence of S9 activation, cells were exposed to test articles for 4 hours or 20 hours. In assays with S9 activation, cells were incubated with test articles for 4 hours. For all assays, after the test article incubation, cells were washed then incubated with colchicine (0.1 μg/ml) for 2 hours before cell harvest.

Table 57: Test Article Concentrations for the Definitive Chromosome Aberration Assay. (Sponsor's Table)

Treatment Condition	Treatment Time	Recovery Time	Dose levels (CXA-101/Tazobactam) (μg/mL)
Non-activated	4 hr	16 hr	625/312.5, 1250/625, 2500/1250, 5000/2500
	20 hr	0 hr	100/50, 250/125, 500/250, 1000/500, 1500/750, 2500/1250, 2750/1375
S9-activated	4 hr	16 hr	50/25, 100/50, 200/100, 400/200, 450/225, 500/250, 550/275

Study Validity

In this study, the following criteria for a valid test were fulfilled.

1. The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control.

2. The percentage of cells with chromosome aberrations in the positive control must be statistically increased relative to the solvent control.

Results

In the definitive assays, 29% cytotoxicity (cell growth relative to the solvent control) occurred at the highest test concentrations, 5000 μg/ml CXA-101/2500 μg/ml tazobactam, after 4 hours of incubation without S9 activation, and the mitotic index at this dose level was reduced 11% compared to the solvent control. After 4-hours incubation in the presence of S9 activation, 66% cytotoxicity and a 42% reduction in the mitotic index occurred at the high dose of 1000 μg/ml CXA-101/500 μg/ml tazobactam concentrations. After 24 hours of incubation without S9 activation, 58% cytotoxicity and a 43% reduction in the mitotic index occurred with the high dose of 3000 μg/ml CXA-101/1500 μg/ml tazobactam.

The percentage of cells with structural aberrations was not increased relative to the solvent control in the 4-hour incubation without S9 activation (Table 58). However, in the 4-hour incubation with S9 activation, the percentage of cells with structural aberrations were significantly increased by 4.0% and 10% relative to the solvent control at dose levels of 500/250 and 1000/500 μg/ml for CXA-101 and tazobactam respectively. Also in the 20-hour incubation without S9 activation, the percentage of cells with structural aberrations were significantly increased 5 and 23% relative to the solvent control at dose levels of 2000/1000 and 3000/1500 μg/ml for CXA-101/tazobactam respectively. The percentage of cells with numerical aberrations was not increased by any CXA-101/tazobactam concentrations in any of the incubation conditions (Table 58).

In all incubation conditions, the positive control agents produced significantly greater structural aberrations but not numerical aberrations compared to solvent controls.

Table 58: The Effect of CXA-101/Tazobactam in a 2:1 Ratio on Structural and Numerical Aberrations in CHO-K_1 Cells. (Sponsor's Table)

Treatment μg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
Saline for injection	-S9	4	12.9	200	200	0.000	±0.000	2.0	0.0
CXA-101/Tazobactum at 2:1 ratio									
1250/625	-S9	4	11.3	200	200	0.000	±0.000	2.0	0.0
2500/1250	-S9	4	12.3	200	200	0.000	±0.000	2.5	0.0
5000/2500	-S9	4	11.5	200	200	0.015	±0.122	4.0	1.5
MMC, 0.2	-S9	4	7.2	200	50	0.340	±0.688	2.0	24.0**

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Saline for injection	+S9	4	12.7	200	200	0.010	±0.100	5.5	1.0
CXA-101/Tazobactam at 2:1 ratio									
250/125	+S9	4	10.3	200	200	0.025	±0.157	6.5	2.5
500/250	+S9	4	8.8	200	200	0.045	±0.231	7.0	4.0*
1000/500	+S9	4	7.4	200	200	0.125	±0.413	8.0	10.0**
CP, 10	+S9	4	3.7	200	50	0.480	±0.839	3.5	32.0**
Saline for injection	-S9	20	12.8	200	200	0.005	±0.071	3.5	0.5
CXA-101/Tazobactam at 2:1 ratio									
1000/500	-S9	20	10.7	200	200	0.015	±0.122	3.5	1.5
2000/1000	-S9	20	8.3	200	200	0.050	±0.218	4.0	5.0**
3000/1500	-S9	20	7.3	200	100	0.340	±0.685	3.0	23.0**
MMC, 0.1	-S9	20	6.3	200	100	0.290	±0.640	2.5	21.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's Exact test.

Study title: In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Study no.:	CXA-201-T-003
Study report location:	Electronic transmission
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	May 4, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101, Lot No.: 020010, purity of 98.51%; tazobactam, Lot # 05/08, purity of 98.0%

Key Study Findings
Mutant frequencies tended to increase with increasing CXA-101/tazobactam (2:1 ratio) concentrations in all the assay conditions with and without S9 activation. However, none of the increases were ≥ 90 mutants per 10^6 cells above the mutant frequency for the respective solvent controls which was considered to be the criteria for a positive result.

Methods

Cell line:	L5178Y cells
Concentrations in definitive study:	As noted in Table 59.

Basis of concentration selection:	The test article concentrations used in the definitive assay were based on the results of a preliminary toxicity assay.
Negative control:	Saline
Positive control:	Methyl methanesulfonate (MMS) for incubations in the absence of S9 activation; 7,12-dimethylbenz(a)anthracene (DMBA) for incubations in the presence of S9 activation.
Formulation/Vehicle:	Saline
Incubation & sampling time:	Cells were incubated with test article concentrations for 4 hours with and without S9 activation and for 24 hours without S9 activation.

Table 59: CXA-101/Tazobactam Concentrations Tested in the Definitive Assay

Assay Conditions	S9 Activation	CXA-101/Tazobactam Concentration
4-hour incubation	without	1000/500, 2000/1000, 3000/1500, 4000/2000, 5000/2500
	with	
24-hour incubation	without	5/2.5, 10/5.0, 25/12.5, 50/25, 75/37.5, 100/50

Study Validity

In this study, the following criteria for a valid test which were fulfilled.

1. The average spontaneous mutant frequency of the solvent (or vehicle) control cultures must be within 35 to 140 TFT-resistant mutants per 10^6 surviving cells. A lower range is considered acceptable if small colony recovery is demonstrated. The average cloning efficiency of the solvent or vehicle controls must range between 65 to 120% and the total suspension growth must be between 8-32% for the 4-hour exposures and 20-180% for the 24 hour exposures.
2. The mutant frequency for at least one dose of the positive controls must meet the criteria for a positive response and induce an increase in small colony mutants according to the following criteria: induced mutant frequency (IMF) positive control ≥ 300 x 10^{-6} mutants with 40% small colonies or small colony IMF for positive control ≥ 150 x 10^{-6}.
3. Cultures treated with a minimum of four concentrations of test article must be attained and their mutant frequencies measured. The highest test article concentration must produce 80 to 90% toxicity unless limited by solubility or if the maximum required concentration (5000 μg/ml) is attained.

Results

In the preliminary toxicity assays, for 4-hour incubations with or without S9 activation, the maximum concentration tested (5000 μg/ml CXA-101 and 2500 μg/ml tazobactam) did not produce a precipitate. Suspension growth in the absence of S9 activation was 55% relative to the solvent control and 14% with S9 activation. Without activation in the

24-hour exposure, relative suspension growth was 7% at 150/75 mg/ml CXA-101/tazobactam and 0% at concentrations ≥ 500/250 μg/ml with no visible precipitate at any concentration.

Mutant frequencies tended to increase with increasing CXA-101/tazobactam concentrations in all the assay conditions with and without S9 activation. However, none of the increases were ≥ 90 mutants per 10^6 cells above the mutant frequency for the respective solvent controls which was considered to be the criteria for a positive result (Table 60, Table 61, and Table 62). In contrast, mutant frequencies were increased by ≥ 90 mutants per 10^6 cells above the mutant frequency for the respective solvent controls for all of the positive control agents in all assay conditions. Under the conditions of this study none of the fixed 2:1 ratio CXA-101/tazobactam concentrations were considered to be mutagenic.

Table 60: Summary Data for L5178Y/TK+/- Mouse Lymphoma Cell Treated with CXA-101/Tazobactam for 4 Hours in the Absence of S9 Activation. (Sponsor's Table)

DOSE LEVEL**** (μg/mL)	PRECIP.	TOTAL SUSP. GROWTH	% SUSP. GROWTH	TFT COLONIES				VC COLONIES				TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% TOTAL GROWTH
				PLATE COUNTS				PLATE COUNTS						
				1	2	3	MEAN	1	2	3	MEAN			
SOLVENT 1		18.4	100	23	33	18	**25**	176	171	159	**169**	29	N/A	100
SOLVENT 2		20.4		23	36	42	**34**	150	170	157	**159**	42		
1000/500 A		16.6	85	18	14	24	**19**	214	196	194	**201**	19	**-17**	**105**
1000/500 B		18.8	96	27	26	26	**26**	134	127	150	**137**	38	**3**	**81**
2000/1000 A		18.9	97	33	22	42	**32**	157	119	124	**133**	49	**13**	**79**
2000/1000 B		17.0	87	24	23	36	**28**	153	116	148	**139**	40	**4**	**74**
3000/1500 A		14.7	76	33	31	32	**32**	130	119	124	**124**	51	**16**	**57**
3000/1500 B		15.8	81	31	27	28	**29**	150	191	194	**178**	32	**-4**	**89**
4000/2000 A		11.5	59	68	58	64	**63**	111	136	138	**128**	99	**63**	**46**
4000/2000 B		11.0	57	79	47	75	**67**	134	144	138	**139**	97	**61**	**48**
5000/2500 A		10.4	53	87	69	79	**78**	182	183	132	**166**	95	**59**	**54**
5000/2500 B		9.7	50	73	73	92	**79**	138	119	133	**130**	122	**86**	**39**
POSITIVE CONTROL: Methyl methanesulfonate (MMS) (μg/mL)														
20		9.4	48	107	109	118	**111**	27	38	56	**40**	552	**516**	**12**
15		10.4	53	152	173	178	**168**	125	88	104	**106**	317	**282**	**34**
MEAN SOLVENT TOTAL SUSPENSION GROWTH: 19.4														
MEAN SOLVENT CLONING EFFICIENCY: 82%														
MEAN SOLVENT MUTANT FREQUENCY: 36 (PER 10^6 CELLS)														

Solvent = 0.9% saline A and B or 1 and 2 are duplicate cultures

**** - Concentrations presented as: μg/mL CXA-101 / μg/mL Tazobactam

Table 61: Summary Data for L5178Y/TK+/- Mouse Lymphoma Cell Treated with CXA-101/Tazobactam for 4 Hours With of S9 Activation. (Sponsor's Table)

DOSE LEVEL**** (µg/mL)	PRECIP.	TOTAL SUSP. GROWTH	% SUSP. GROWTH	TFT COLONIES				VC COLONIES				TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% TOTAL GROWTH
				PLATE COUNTS				PLATE COUNTS						
				1	2	3	MEAN	1	2	3	MEAN			
SOLVENT 1		13.1	100	43	42	42	**42**	137	193	185	**172**	49	N/A	100
SOLVENT 2		13.6		31	36	42	**36**	199	193	188	**193**	38		
1000/500 A		10.7	80	52	41	35	**43**	159	178	199	**179**	48	**4**	**78**
1000/500 B		10.9	81	52	23	36	**37**	191	179	*	**185**	40	**-3**	**83**
1500/750 A		8.7	65	55	42	52	**50**	173	206	217	**199**	50	**7**	**71**
1500/750 B		8.6	64	55	23	35	**38**	201	196	156	**184**	41	**-3**	**65**
2000/1000 A		6.4	47	38	49	55	**47**	165	180	211	**185**	51	**8**	**48**
2000/1000 B		6.1	46	40	35	24	**33**	162	176	*	**169**	39	**-4**	**42**
3000/1500 A		4.5	34	63	43	46	**51**	156	153	196	**168**	60	**17**	**32**
3000/1500 B		3.8	31	56	54	61	**57**	156	192	199	**182**	63	**19**	**31**
4000/2000 A		1.7	20	55	51	41	**49**	147	146	176	**156**	63	**19**	**17**
4000/2000 B		2.1	22	45	51	55	**50**	184	159	194	**179**	56	**13**	**22**
5000/2500 A		1.6	19	47	50	36	**44**	136	142	164	**147**	60	**17**	**16**
5000/2500 B		1.5	19	70	75	87	**77**	170	160	176	**169**	92	**48**	**17**
POSITIVE CONTROL: 7,12-dimethylbenz(a)anthracene (DMBA) (µg/mL)														
1.25		0.8	12	246	243	251	**247**	87	113	107	**102**	482	**439**	**7**
1		1.8	21	240	180	220	**213**	146	116	119	**127**	336	**293**	**15**
MEAN SOLVENT TOTAL SUSPENSION GROWTH: 13.4														
MEAN SOLVENT CLONING EFFICIENCY: 91%														
MEAN SOLVENT MUTANT FREQUENCY: 43 (PER 10^6 CELLS)														

Solvent = 0.9% saline A and B or 1 and 2 are duplicate cultures

**** - Concentrations presented as: µg/mL CXA-101 / µg/mL Tazobactam

Table 62: Summary Data for L5178Y/TK+/- Mouse Lymphoma Cell Treated with CXA-101/Tazobactam for 24 Hours in the Absence of S9 Activation. (Sponsor's Table)

DOSE LEVEL**** (µg/mL)	PRECIP.	TOTAL SUSP. GROWTH	% SUSP. GROWTH	TFT COLONIES PLATE COUNTS 1	2	3	MEAN	VC COLONIES PLATE COUNTS 1	2	3	MEAN	TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% TOTAL GROWTH
SOLVENT 1		20.1	100	52	47	55	51	133	124	114	124	83	N/A	100
SOLVENT 2		20.3		32	43	68	48	155	125	157	146	65		
5/2.5 A		21.1	104	56	59	43	53	162	152	151	155	68	-6	120
5/2.5 B		21.0	104	41	41	47	43	155	130	123	136	63	-11	105
10/5.0 A		20.6	102	46	58	42	49	153	152	169	158	62	-13	120
10/5.0 B		22.5	111	49	38	51	46	183	144	128	152	61	-14	125
25/12.5 A		16.6	82	51	49	61	54	114	125	114	118	91	17	71
25/12.5 B		17.1	85	59	40	50	50	129	97	87	104	95	21	65
50/25 A		15.2	75	110	51	107	89	139	136	128	134	133	59	75
50/25 B		15.9	79	56	50	86	64	118	97	110	108	118	44	63
75/37.5 A		8.0	39	43	73	35	50	104	105	123	111	91	17	32
75/37.5 B		6.7	33	33	31	65	43	127	107	110	115	75	1	28
100/50 A		1.2	0	+				+						
100/50 B		1.3	0	+				+						
POSITIVE CONTROL: Methyl methanesulfonate (MMS) (µg/mL)														
7.5		12.5	62	192	197	192	194	64	84	86	78	497	422	36
5		16.5	81	170	134	184	163	127	97	87	104	314	240	63
MEAN SOLVENT TOTAL SUSPENSION GROWTH: 20.2														
MEAN SOLVENT CLONING EFFICIENCY: 67%														
MEAN SOLVENT MUTANT FREQUENCY: 74 (PER 10^6 CELLS)														

Solvent = 0.9% saline A and B or 1 and 2 are duplicate cultures

+ - Too toxic to clone **** - Concentrations presented as: µg/mL CXA-101 / µg/mL Tazobactam

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

Study title: Rat Bone Marrow Erythrocyte Micronucleus Test Following Intravenous Administration of CXA-101 and Tazobactam

Study no: CXA-201-T-004
Study report location: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: May 1, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CXA-101, Lot # 020010, purity of 98.51%;
Tazobactam, Lot # 05/08, purity of 98.0%

Key Study Findings

A single IV dose of a combination of CXA/tazobactam in a 2:1 ratio at doses up to 2000/1000 mg/kg did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in the bone marrow of male rats.

Methods

Doses in definitive study:	For definitive assay: 500/250, 1000/500 and 2000/1000 mg/kg CXA-101/tazobactam. Confirmatory assay: 2000/1000 mg/kg CXA-101/tazobactam. See Table 63.
Frequency of dosing:	Single Dose
Route of administration:	Intravenous
Dose volume:	10 ml/kg
Formulation/Vehicle:	0.9% sodium chloride
Species/Strain:	Sprague Dawley Rats
Number/Sex/Group:	In the definitive study, 7 groups of 5 male rats each. In the confirmatory study one group of 5/sex.
Satellite groups:	None
Basis of dose selection:	The doses for the definitive assay were based on the survival of the animals in the confirmatory assay receiving 2000/1000 mg/kg CXA-101/tazobactam.
Negative control:	0.9% sodium chloride
Positive control:	Cyclophosphamide (CP; 40 mg/kg)

Table 63: Study Design for the Definitive Study for Study No.: CXA-201-T-004.
(Sponsor's Table)

Treatment (10 mL/kg)	Number of Male Rats Dosed	Number of Male Rats Used for Bone Marrow Collection at	
		24 hrs post-dose	48 hrs post-dose
Negative Control: 0.9% Sodium chloride for injection	10	5	5
Test Article: CXA-101/ Tazobactam (2:1 ratio)			
Low dose: 500 mg/kg CXA-101/ 250 mg/kg Tazobactam	5	5	-
Mid dose: 1000 mg/kg CXA-101/ 500 mg/kg Tazobactam	5	5	-
High dose: 2000 mg/kg CXA-101/ 1000 mg/kg Tazobactam	10	5	5
Positive Control: CP (40 mg/kg)	5	5	-

Study Validity

The following criteria for a valid test were fulfilled:

1. The incidence of micronucleated polychromatic erythrocytes in the vehicle control group was within the historical vehicle control range.

2. The incidence of micronucleated polychromatic erythrocytes in the positive control group was significantly increased relative to the vehicle control group.

Results
In the definitive assay, none of the CXA-101/tazobactam dose groups demonstrated reductions in the ratio of polychromatic erythrocytes (PCEs) to total erythrocytes compared to the concurrent control groups at 24 or 48 hours after dosing. Also none of the CXA-101/tazobactam dose groups produced significant increases in the incidence of micronucleated PCEs compared to the concurrent control groups at 24 or 48 hours after dose administration. In contrast, the positive control agent, cyclophosphamide (40 mg/kg), reduced the ratio of PCEs to total erythrocytes and induced a statistically significant increase in the incidence of micronucleated PCEs relative to the vehicle control group (Table 64).

Table 64: Summary of Bone Marrow Micronucleus Results Following a Single Intravenous Administration of CXA-101 and Tazobactam (2:1 ratio) in Sprague Dawley Rats. (Sponsor's Table)

Treatment (10 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/ PCE Scored
0.9% Sodium Chloride	M	24	5	0.540 ± 0.06	---	0.4 ± 0.22	4 / 10000
500 mg/kg CXA-101/250 mg/kg Tazobactam	M	24	5	0.502 ± 0.02	-7	0.0 ± 0.00	0 / 10000
1000 mg/kg CXA-101/500 mg/kg Tazobactam	M	24	5	0.514 ± 0.06	-5	0.1 ± 0.22	1 / 10000
2000 mg/kg CXA-101/1000 mg/kg Tazobactam	M	24	5	0.536 ± 0.04	-1	0.2 ± 0.27	2 / 10000
Cyclophosphamide monohydrate 40 mg/kg	M	24	5	0.400 ± 0.02	-26	16.7 ± 2.64	*167 / 10000
0.9% Sodium Chloride	M	48	5	0.554 ± 0.05	---	0.0 ± 0.00	0 / 10000
2000 mg/kg CXA-101 / 1000 mg/kg Tazobactam	M	48	5	0.557 ± 0.07	1	0.1 ± 0.22	1 / 10000

*Statistically significant increase, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

Tazobactam

Study Title: Mutagenicity Tests of Tazobactam/Piperacillin, Tazobactam and Piperacillin. Ohuchida, A, Taniguti, A; Yasuhide, K., Yasuhiro M., Kashihara, A., and Omae, S.: The Journal of Toxicological Sciences, Vol. 19, Supplement II, 263-280, 1994.

Reviewer Comment: *This manuscript was published in Japanese but entirely translated into English. Combination treatments with tazobactam/piperacillin were also examined in this manuscript, but only the tazobactam effects are discussed by the Reviewer.*

Study title: Reverse Mutation Assay
Study no.: Not identified

Study report location:	Reported in Ohuchida *et al.*, 1994
Conducting laboratory and location:	Drug Safety Research Laboratory, Taiho Pharmaceutical Co., Ltd., 224-2Ehisuno, Hiraidhi, Kawauchi-cho, Tokushima 771-01, Japan
Date of study initiation:	Accepted for publication on June 1, 1994
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Tazobactam, Lot Nos.: 6J23 and 7D24, purity not reported.

Key Study Findings

Tazobactam at concentrations of ≤ 1000 μg/plate was negative for mutagenesis in *Salmonella typhimurium* strains TA100, TA98, TA1535 and TA1537 and *Escherichia coli* strain WP2uvrA in an Ames assay.

Methods

Strains:	*Salmonella typhimurium* strains TA100, TA98, TA1535 and TA1537 and *Escherichia coli* strain WP2uvrA
Concentrations in definitive study:	10, 25, 50, 100, 250, 500, and 1000 μg/plate with and without S9 activation.
Basis of concentration selection:	Pretesting revealed cytotoxicity occurred at tazobactam doses ≥ 1000 μg/plate.
Negative control:	Sodium bicarbonate
Positive control:	N-ethyl-N-nitro-N-nitrosoguanidine (ENNG), 2-nitrofluorene (2NF), 9-aminoacridine (9AA), and 2 –amonoanthracene (2AA).
Formulation/Vehicle:	Sodium bicarbonate
Incubation & sampling time:	Plates were incubated for 48 hours at 37°C.

Study Validity

Validation criteria for the reverse mutation assay were not described in the publication.

Results

The results were considered positive for mutagenicity when the mean number of colonies per plate was two or more times greater than those of the vehicle control group with dose-dependent increases.

For all of the test strains, tazobactam at any concentration did not stimulate increased numbers of revertant colonies compared to the vehicle control group with or without S9 activation.

Study title: Chromosomal Aberration Assay

Study no.:	Not identified
Study report location:	Reported in Ohuchida *et al.*, 1994
Conducting laboratory and	Drug Safety Research Laboratory, Taiho

location:	Pharmaceutical Co., Ltd., 224-2Ehisuno, Hiraidhi, Kawauchi-cho, Tokushima 771-01, Japan
Date of study initiation:	Accepted for publication on June 1, 1994
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Tazobactam, Lot Nos.: 6J23 and 7D24, purity not reported.

Key Study Findings
Tazobactam at concentrations as high as 10 mM (3.0 mg/ml) did not increase the frequency of structural or numerical aberrations in a chromosome aberration assay.

Methods

Cell line:	Chinese hamster lung-derived cultured cells (CHL).
Concentrations in definitive study:	Treatment with 0.313, 0.625, 1.25, 2.5, 5.0 and 10.0 mM tazobactam; examination of slides from cells treated with 2.5, 5.0, and 10 mM tazobactam.
Basis of concentration selection:	Pretesting revealed 20% cytotoxicity occurred at tazobactam doses ≥ 3000 μg/ml (10 mM).
Negative control:	Normal saline
Positive control:	Mitomycin C (MMC) without metabolic activation and N-nitrosodimethylamine (DMN) for metabolic activation.
Formulation/Vehicle:	Normal saline
Incubation & sampling time:	Cells and tazobactam were incubated together for 24 or 48 hours without S9 activation and for 6 hours with S9 activation. Before harvest, cells were incubated with colcemid for 2 hours.

Study Validity
Validation criteria for the chromosome aberration assay were not described in the publication.

Results
The criteria for determining positive results for structural abnormalities exclusive of gaps were: negative results for 4% or less, equivocal results for 4% to 8% and positive results for 8% and above. The percentage of polyploidy cells considered to be negative for numerical aberrations was 5% or less, 5% to 10% for equivocal results, and 10% polyploidy cells or above for a positive numerical aberration result.

The structural aberration frequency for the tazobactam concentrations in incubation conditions with and without S9 activation did not exceed 1.5%, which was not different

than the structural aberrations frequencies for the control group (0-1.5%). None of the tazobactam concentrations produced numerical aberrations at a frequency above the negative control value (0.5%).The positive control agents, MMC and DMN, produced structural aberrations in 68.5% of cells without S9 activation (MMC) and structural aberrations in 29% of cells with S9 activation (DMN). However, neither positive control agent produced numerical aberrations (polyploidy) in more than 1.5% of cells which was not different than negative control values.

Study title: Micronucleus Assay

Study no:	Not identified
Study report location:	Reported in Ohuchida *et al.*, 1994
Conducting laboratory and location:	Drug Safety Research Laboratory, Taiho Pharmaceutical Co., Ltd., 224-2Ehisuno, Hiraidhi, Kawauchi-cho, Tokushima 771-01, Japan
Date of study initiation:	Accepted for publication on June 1, 1994
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Tazobactam, Lot No.: 7D22N, purity not reported.

Key Study Findings

Tazobactam in single IV doses of ≤ 5000 mg/kg did not induce clastogenesis in rat bone-marrow cells in a micronucleus assay.

Methods

Doses in definitive study:	0, 625, 1250, 2500, and 5000 mg/kg
Frequency of dosing:	Single dose
Route of administration:	intravenous
Dose volume:	10 to 12.5 ml/kg
Formulation/Vehicle:	Distilled water
Species/Strain:	CRj:CD-1 Mice
Number/Sex/Group:	6 male mice/group
Satellite groups:	none
Basis of dose selection:	Based on findings from a preliminary study.
Negative control:	Distilled water
Positive control:	Mitomycin C (MMC), 2 mg/kg.

Study Validity

The criteria for study validity for the micronucleus assay were not described in the publication.

Results

A frequency of 10 or fewer micronucleated polychromatic erythrocytes (MNPCE) per 6,000 (0.166%) observed cells was considered to be negative, and a frequency of 18 or more MNPCE per 6,000 cells (0.30%) was considered to be positive for clastogenesis.

Cytotoxicity was also assessed by counting the number of polychromatic erythrocytes (PCEs) per 1000 red blood cells (RBCs).

The frequency of MNPCE ranged from 0.02% to 0.17% for the tazobactam treatment groups with the highest percentage occurring for the low tazobactam dose with no dose-dependent increases. The highest dose of tazobactam (5000 mg/kg) reduced the PCE/RBC ratio to 33.4% compared to 52.9% for the vehicle control group indicating weak erythroblast cytotoxicity at this dose but not at the lower tazobactam doses. The positive control agent, MMC (2 mg/kg), produced a frequency of MNPCE of 3.80% for a clear positive clastogenesis result.

8 Carcinogenicity

Ceftolozane and tazobactam are only recommended for short-term administration (≤ 14 days). Consequently nonclinical carcinogenicity assessments were not recommended for either compound or the combination.

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicology studies with CXA-101 included: a male and female fertility study in rats (Study No.: CX.101.TX.002), embryo-fetal studies in mice (Study No.: CXA101-TX.001) and rats (Study No.: CXA101-T-006), and a pre- postnatal study in rats (Study No.: CX.101.TX.012). The Sponsor indicated that due to an expectation of prohibitive maternal toxicity, an embryo-fetal study in rabbits was not conducted. Literature reports of reproductive and developmental toxicology studies with tazobactam included a fertility study, an embryo-fetal study, and a pre- postnatal study in rats. All of the studies are reviewed below.

In a rat fertility study, ceftolozane had no adverse effects on fertility in males or females at intravenous doses up to 1000 mg/kg/day. The mean plasma exposure (AUC) value at this dose is approximately 8 times the mean daily clinical ceftolozane exposure values. In a rat fertility study with intraperitoneal tazobactam (Sato *et al.*, J Toxicol Sci, 19, Suppl II: 199-214, 1994), male and female fertility parameters were not significantly affected at doses ≤ 640 mg/kg/day (approximately 4 times the recommended clinical daily dose based on body surface area comparison).

Embryo-fetal development studies performed in mice and rats with cepftolozane doses up to 2000 and 1000 mg/kg/day, respectively, revealed no teratogenicity and no evidence of harm to the fetus. The mean plasma exposure (AUC) values associated with these doses are approximately 19 (mice) and 11 (rats) times the mean daily human ceftolozane exposure at the clinical dose of 1 gram administered three times per day. It is not known if ceftolozane crosses the placenta in animals. In an embryo-fetal study in rats, tazobactam administered at doses up 3000 mg/kg/day (approximately 19 times the recommended human dose based on body surface area comparison) did not produce maternal toxicity, or fetal toxicity or teratogenicity (Sato *et al.*, J Toxicol Sci, 19, Suppl II: 215-232, 1994). In rats, tazobactam was shown to cross the placenta. Concentrations in the fetus were less than or equal to 10% of those found in maternal plasma.

In a pre- postnatal study in rats, ceftolozane administered during pregnancy and lactation (Gestation Day 6 through Lactation Day 20) was associated with a decrease in auditory startle response in postnatal day 60 male and female pups at maternal doses of ≥ 300 mg/kg/day. The plasma exposure (AUC) associated with the NOAEL dose of 100 mg/kg/day in rats is approximately equal to the mean human ceftolozane exposure at the clinical dose of 3 grams/day. In a pre-postnatal study in rats, tazobactam administered intraperitoneally twice daily at the end of gestation and during lactation (Gestation Day 17 through Lactation Day 21) produced decreased maternal food consumption at the end of gestation and significantly more stillbirths with a tazobactam dose of 1280 mg/kg/day (Sato *et al.*, J Toxicol Sci, 19, Suppl II: 233-247, 1994). No effects on the development, function, learning or fertility of F_1 pups were noted, but the postnatal body weights for F_1 pups from dams receiving 320 and 1280 mg/kg/day tazobactam were significantly reduced 14 and 21 and 7, 14, and 21 days after delivery respectively. F_2 generation fetuses were normal for all doses of tazobactam. The NOAEL for reduced F_1 body weights was considered to be 40 mg/kg/day (approximately 0.3 times the recommended human dose based on body surface area comparison). Exclusive of reduced body F_1 generation body weights, the NOAEL was considered to be 320 mg/kg/day or approximately equal to the recommended human dose based on body surface area comparisons.

9.1 Fertility and Early Embryonic Development

Study title: CB-500,101: A GLP Intravenous Fertility and Early Embryonic Development to Implantation Study in Rats

Study no.:	CX.101.TX.002
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	May 25, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101 (CB-500,101), Lot # 440420 0001 0, purity of 98.32%

Key Study Findings

In a male and female fertility study, CXA-101 did not produce any adverse effects on male and female reproductive performance, fertility, or intrauterine embryonic survival. The NOAEL values for males and females were considered to be the high dose of 1000 mg/kg/day.

Methods

Doses:	0, 100, 300, and 1000 mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	5 ml/kg
Route of administration:	Intravenous
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	Crl:CD(SD) rats

Number/Sex/Group:	See Table 65
Satellite groups:	None
Study design:	Male rats were dosed during Study Days 0-59, a minimum of 28 days prior to cohabitation through the day prior to euthanasia, for a total of 59-60 doses. The females were dosed during Study Days 14-48, a minimum of 14 days prior to cohabitation through Gestation Day (GD) 7, for a total of 22-35 doses.
Deviation from study protocol:	Deviations from the study protocol were noted. However, none was considered to have altered the results or compromised the integrity of the study.

Table 65: Study Design for the Rat Fertility Study (Sponsor's Table)

Group	Test Article	Dose (mg/kg)	Number of Animals		Frequency/Dose Route
			Males	Females	
1	Vehicle Control	0[a]	25	25	Once daily/slow bolus intravenous injection
2	CB-500,101	100	25	25	
3	CB-500,101	300	25	25	
4	CB-500,101	1000	27[b]	25	

[a] = The vehicle (0.9% sterile saline for injection) was administered to Group 1.
[b] = A total of 27 males were assigned to Group 4, which included 2 potential replacement males. Due to difficulty dosing 2 males on the first day of dosing, which resulted in injury to the tail, it was not certain if these 2 males would be able to be dosed for the remainder of the study; therefore, potential replacement males were added to this group on study day 1. Any extra males exceeding 25 males/group following the pre-mating treatment period were not paired for breeding.

Observations and Results

Table 66: Observation Schedule for Study No.: CX.101.TX.002

Parameters	Schedule
Mortality and Clinical Signs	All animals were observed twice daily once in the morning and once in the afternoon. Individual clinical observations were recorded daily. Each male and female was also observed for clinical signs at the time of dose administration and 15 and 60 minutes after dosing.
Injection Site Observations	Injection sites were examined daily for erythema, swelling, and other dermal findings.
Body Weights	Individual male body weights were recorded twice weekly throughout the study and prior to the scheduled euthanasia. Individual female body weights were recorded twice weekly until evidence of copulation or until euthanasia for females that did not mate. Once evidence of mating was observed, female body weights

	were recorded on GDs 0, 3, 7, 10, 13, and 15.
Food Consumption	Individual food consumption was recorded twice weekly until cohabitation. Food intake was not recorded during the mating period. Following mating, male food consumption was measured on a twice-weekly basis until the scheduled euthanasia and female food consumption was measured on GDs 0, 3, 7, 10, 13, and 15.
Estrous Cycles	Vaginal lavages were performed daily and the slides were evaluated microscopically to determine the stage of estrus of each female for 10 days prior to CXA-101 administration and continuing until evidence of copulation was observed.
Necropsy	All females were euthanized on GD15.

Mortality

No CXA-101-related mortalities occurred at any dose.

Clinical Signs

Other than injection-site reactions, no CXA-101-related clinical signs were observed at any dose. Injection-site reactions were noted for males in the mid- and high-dose groups. Very slight erythema was noted in 16 mid-dose and 22 high-dose animals as well as desquamation in 2 and 5 animals in the same respective groups. Greater degrees of erythema (slight to severe) were noted in 11 high-dose males and very slight to slight edema was noted in 4 high-dose males. Similar findings were observed in females. These reactions were considered to be localized effects and not reflective of systemic toxicity.

Body Weight

No CXA-101-related changes in mean body weights, body weight gains, and cumulative body weight changes were noted in the 100, 300, and 1000 mg/kg/day groups during the premating period or throughout the entire study.

Feed Consumption

No consistent CXA-101-related changes in food consumption were noted during the premating period, during cohabitation, and throughout the entire study. Transient significant decreases in food consumption were observed sporadically in CXA-101-treated groups, but no corresponding weight changes occurred.

Toxicokinetics: Not performed

Dosing Solution Analysis

The analyzed dosing formulations were 102% to 105% of the target concentrations.

Necropsy

For males, no CXA-101-related gross pathology was evident at necropsy and no organ weight changes were observed. In females no CXA-101-related gross pathology was observed. Higher absolute and relative (to brain weight) ovarian weights for females in the 100 mg/kg/day group were noted, but higher ovarian weights did not occur in the higher- (mid and high) dose groups.

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

Male Fertility Parameters: One male in the mid-dose group did not sire a litter. However, overall, CXA-101 administration at any dose did have any significant effects on male reproductive performance (Table 67). Also CXA-101 administration did not significantly affect any spermatogenesis endpoints including mean testicular and epididymal sperm numbers and sperm production rate, motility, and morphology.

Table 67: Male Reproductive Performance Parameters. (Sponsor's Table)

	Dosage Level (mg/kg/day)				(b) (4) HC[a]
Parameter	0	100	300	1000	Mean (Range)
Male Mating Index (%)	96.0	100.0	96.0	100.0	96.8 (84.0-100.0)
Male Fertility Index (%)	96.0	100.0	96.0	96.0	91.1 (60.0-100.0)
Male Copulation Index (%)	100.0	100.0	100.0	96.0	94.4 (71.4-100.0)

[a] = (b) (4) historical control data
None significantly different from the concurrent control group using the Chi-Square test.

Female Fertility Parmameters: No significant CXA-101-related effects on any female reproductive performance parameters were noted (Table 68).

Table 68: Female Reproductive Performance Parameters. (Sponsor's Table)

	Dosage Level (mg/kg/day)				(b) (4) HC[a]
Parameter	0	100	300	1000	Mean (Range)
Female Mating Index (%)	100.0	100.0	100.0	100.0	98.2 (86.7-100.0)
Female Fertility Index (%)	100.0	100.0	100.0	96.0	93.2 (60.0-100.0)
Female Conception Index (%)	100.0	100.0	100.0	96.0	95.0 (65.2-100.0)
Estrous Cycle Length (days)	4.2	4.2	4.2	4.1	4.4 (3.6-5.8)
Pre-Coital Interval (days)	2.9	2.7	3.0	2.4	2.9 (1.8-4.8)

[a] = (b) (4) historical control data
None significantly different from the concurrent control group using the Chi-Square test or Dunnett's test.

Gestation Day 15 Laparohystoerectomy

Intrauterine survival of embyros was not affected by CXA-101 administration. Parameters including pre-implantation loss, postimplantation loss, mean number of viable embryos, mean number of corpora lutea, and mean number of implantation sites were not significantly changed.

Study Title: Reproductive and developmental Toxicity Studies of Tazobactam/Piperacillin or Tazobactam. Sato, T., Lochry, E.A., Hoberman, A.M., Christian, M.S.: The Journal of Toxicological Sciences, Vol. 19, Supplement II: 199-214 (1994).

Reviewer Comment: *This manuscript was published in Japanese but entirely translated into English. Combination treatments with tazobactam/piperacillin were also examined in this manuscript, but only the tazobactam effects are discussed by the Reviewer.*

Key Study Findings

- In males, tazobactam had no effect on epididymal and testes weight or mating and fertility indexes.
- In females, tazobactam did not produce changes in the female estrus cycle length, days in cohabitation, mating index, fertility index, fetal body weight, or fetal gender ratio.
- In female rats that underwent caesarean section, the number of surviving offspring was reduced to a non-significant degree with the high-dose (640 mg/kg/day) of tazobactam. In high-dose females that were allowed to proceed to delivery without caesarean section, the number of implantations was significantly decreased, and this finding was accompanied by a significant increase in the number of still births and a non-significant decrease in the number of surviving offspring compared to control values.

Methods

Doses:	40/160, 160/640, and 320/1280 mg/kg/day of tazobactam/piperacillin and 40, 160, 640 mg/kg/day tazobactam
Frequency of dosing:	Twice per day
Dose volume:	5 ml/kg
Route of administration:	intraperitoneal
Formulation/Vehicle:	0.9% saline
Species/Strain:	Crl:CD Sprague Dawley rats
Number/Sex/Group:	Main Study animals: 26/sex/group
Satellite groups:	None
Study design:	Tazobactam/piperacillin or tazobactam alone was administered by the intraperitoneal route to male and female Sprague-Dawley rats. Males were dosed for 70 days prior to and during cohabitation for a total of 92 days. Females were dosed for 15 days prior to and during cohabitation until 21 days of gestation in dams undergoing cesarean section or giving birth naturally, and until 25 days of gestation in dams who did not give birth.
Deviation from study protocol:	Protocol deviations were not reported in the publication.

Observations and Results

Table 69: Observation Schedule for the Fertility Study with Tazobactam in Rats

Parameter	Schedule

Mortality and Clinical Signs	Rats were observed daily for moribundity and mortality.
Body Weights and Gravid Uterine Weights	Individual male and female body weights were recorded daily during the treatment period.
Food Consumption	Food consumption was recorded weekly in males and weekly during cohabitation and daily during gestation and nursing for females.
Laparhysterectomy	10-13 dams from each group were necropsied on Day 21 of gestation.

Mortality
No animals died as a result of treatment with tazobactam.

Clinical Signs
A significant increase in inguinal small masses was noted in the tazobactam mid- and high-dose males.

Body Weight
No changes were noted in body weights for males. In females receiving the high dose of tazobactam (640 mg/kg/day), weight gain was significantly inhibited before mating and during gestation, but weight gain increased significantly during the nursing period.

Feed Consumption
No changes in food consumption were noted for males. For females, food consumption was decreased before mating in the low- and high-dose tazobactam groups, and during gestation for the high-dose females.

Toxicokinetics: Not performed

Dosing Solution Analysis: Not reported

Necropsy
Males: Cecal prominence was noted in the tazobactam groups but not to a degree significantly greater than controls.

Females: A significant dose-dependent increase in the prominence, discoloration, or hemorrhage of sites in the cecum or colon was noted for the high-dose of tazobactam.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)
Males: All males were necropsied after the cohabitation period and the testes and epididymis were weighed. No changes in epididymal and testes weight were noted. The male mating and fertility indexes were near 100% and not significantly different than control values for all the tazobactam treatment groups.

Females: The number of luteal bodies, number of implantations, number of early and late resorptions, dead fetuses, and surviving fetuses were measured. The female estrus cycle length, days in cohabitation, mating index, and fertility index did not differ significantly from the control group for any tazobactam group (Table 70). Also no

differences were noted compared to the control group for fetal body weight or gender ratio for any of the tazobactam groups. Although the number of surviving fetuses was reduced in the high-dose tazobactam group, the difference from the control value was not significant.

For high-dose female rats that were allowed to proceed to delivery without caesarean section, the number of implantations was significantly decreased, and this finding was accompanied by a non-significant decrease in the number of surviving offspring (Table 71). Also the number of still births for high-dose females (15) was significantly higher than the 5 stillbirths that occurred for control females. All other delivery parameters were not changed by treatment with tazobactam.

Table 70: Caesarean Data in Dams Treated with Tazobactam in the Rat Fertility Study.

Parameter	Control Group	Tazobactam (mg/kg/day)		
		40	160	640
Number of dams	10	11	12	12
Number of corpora lutea - mean ± SD	15.4 ± 1.6	15.4 ± 2.2	15.8 ± 3.4	14.8 ± 2.2
Number of implantations - mean ± SD	15.1 ± 1.3	14.7 ± 2.0	14.4 ± 2.9	12.8 ± 2.7
Number of total dead fetuses (%)	17 (11.8)	23 (14.3)	20 (12.1)	25 (15.7)
Number of early resorptions	7	9	13	15
Number of late resorptions	10	14	7	10
Number of live fetuses (mean ± SD)	134 (13.4 ± 2.4)	139 (12.6 ± 2.5)	153 (12.8 ± 3.3)	129 (10.8 ± 2.4)
Sex ratio (Male/litter)	0.53	0.47	0.49	0.52
Body weight (g) of live fetuses				
Male – mean ± SD	5.41 ± 0.31	5.26 ± 0.35	5.34 ± 0.40	5.10 ± 0.34
Female - mean ± SD	5.21 ± 0.29	4.89 ± 0.46	4.98 ± 0.42	4.79 ± 0.29

Table 71: Delivery Data in Dams Treated with Tazobactam in the Rat Fertility Study.

Parameter	Control Group	Tazobactam (mg/kg/day)		
		40	160	640
Number of dams	13	12	13	11
Duration of gestation	23.2 ± 0.4	23.1 ± 0.5	22.8 ± 0.4	23.3 ± 0.5
Number of implantation sites - mean ± SD	15.1 ± 2.6	14.4 ± 1.6	13.7 ± 3.6	11.4 ± 2.6*
Delivery rate[a]	100	100	100	91.7

Live-born pups (mean ± SD)	125 (10.4 ± 3.8)	139 (11.6 ± 3.4)	153 (11.8 ± 3.4)	72 (7.2 ± 3.4)
Still-born pups (%)	5 (3.8)	7 (4.8)	6 (3.8)	15 (17.2)**
Sex ratio (Male/litter)	0.57	0.45	0.44	0.51
Body weight (g) of live born pups – mean ± SD	6.4 ± 0.7	6.4 ± 1.0	6.0 ± 0.8	6.4 ± 0.5
Mortality (%) per litter				
Day 1	0.0 ± 0.0	1.0 ± 3.4	0.6 ± 2.3	10.0 ± 31.6
Day 2-4	1.5 ± 3.5	1.4 ± 3.2	1.7 ± 4.4	3.2 ± 4.9
Day 5-7	0.7 ± 2.4	0.0 ± 0.0	0.4 ± 1.6	0.0 ± 0.0
Day 8-14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 15-21	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Viability index - %	97.6	97.1	96.7	94.4
Lactation index - %	100.0	100.0	100.0	100.0

[a] Percentage relative to the number of pregnant rats.

*, **: Significantly different from control (P < 0.05, 0.01)

9.2 Embryonic Fetal Development

Study title: CB-500,101: A GLP Intravenous Embryo/Fetal Development Study in Mice.

Study no.: CX.101.TX.001, (b) (4)-339063
Study report location: Electronic transmission
Conducting laboratory and location: (b) (4)
Date of study initiation: May 26, 2010
GLP compliance: Yes
, QA statement: Yes
Drug, lot #, and % purity: CB-500,101 (CXA-101), lot # 440420 0001 0, purity of 98.32%.

Key Study Findings

Once daily administration of CXA-101 to pregnant mice during GDs 6-15 was not associated with any maternal or fetal toxicity. The NOAEL value was considered to be the high dose of 2000 mg/kg/day.

Methods

Doses: 0, 300, 1000, 2000 mg/kg/day
Frequency of dosing: Once per day
Dose volume: 10 ml/kg
Route of administration: intravenous
Formulation/Vehicle: Sterile saline for injection
Species/Strain: Crl:CD1(ICR) mice

Number/Sex/Group:	25 females per group
Satellite groups:	none
Study design:	CXA-101 or vehicle was administered intravenously to pregnant CD-1 mice during gestation days (GD) 6-15. On GD 18, each surviving female received a laparohysterectomy and the uteri, placentae and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded.
Deviation from study protocol:	Multiple deviations were noted; however, none was considered to have altered the results or compromised the integrity of the study.

Table 72: Study Design for Study No.: CX.101.TX.001. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle Control	0	10	28[a]
2	CB-500, 101	300	10	25
3	CB-500, 101	1000	10	25
4	CB-500, 101	2000	10	25

[a] = A total of 28 females were assigned to Group 1 as 3 females were found dead following 1 or 2 doses and were subsequently replaced.

Observations and Results

Table 73: Observation Schedule for Study No.: CX.101.TX.001

Parameter	Schedule
Mortality and Clinical Signs	All mice were observed twice daily for moribundity and mortality. Individual clinical signs were recorded from GD0 to GD18 (prior to dose administration during the treatment period). Mice were also observed for clinical signs at the time of dose administration and approximately 15 minutes and 1 hour following dose administration.
Injection Site Observations	Injection sites were examined for erythema and swelling beginning with the initiation of dose administration until euthanasia for all study animals.
Body Weights and Gravid Uterine Weights	Individual maternal body weights were recorded on GD 0 and GD 6-18. Gravid uterine weights were measured on GD 18, the laparhysterectomy date.
Food Consumption	Individual food consumption was recorded on GD 0 and GDs 6-18.
Laparhysterectomy	A gross necropsy was performed on females that died or aborted during the course of the study. The number and location of implantation sites, corpora

	lutea, and viable fetuses were recorded. All surviving females including one female that delivered were euthanized on GD 18.

Mortality
No CXA-101-related mortality was observed during the study. One mid-dose and four control females were found dead in their dosing restrainers. As no clinical signs and no dose-dependent effects were apparent, the mid-dose death was not considered CXA-101 related.

Clinical Signs
One mid-dose female was found with a partially cannibalized fetus on GD17, but the same female had 14 live fetuses *in utero*. As no abortions were observed in the 2000 mg/kg/day group, this abortion was not considered CXA-101 related. Injection site reactions occurred in all three CXA-101 dose groups. Dose-dependent occurrences of very slight to slight erythema and edema, desquamation, subcutaneous hemorrhage, and/or encrustation were noted for 1, 6, 17, and 22 females in the control, 300, 1000, and 2000 mg/kg/day groups. The findings were localized to the immediate injection sites and not considered to be an indication of systemic toxicity. No other maternal clinical signs were noted.

Body Weight
No significant effects of CXA-101 on mean maternal body weights, body weight gains, net body weights, net body weight gains, and gravid uterine weights in the 300, 1000, and 2000 mg/kg/day groups were noted.

Feed Consumption
Mean maternal food consumption (g/animal/day or g/kg/day) was not affected by CXA-101 at any dose.

Toxicokinetics
Toxicokinetics were not performed with this study, but toxicokinetics were performed in a companion GLP study in pregnant CD-1 mice (Study No.: CX.101.TX.002). In the companion study, pregnant female mice received daily intravenous doses of CXA-101 (300, 1000, and 2000 mg/kg/day) from Gestation Day (GD) 6 until GD 15.

For both the GD 6 and GD 15 toxicokinetic measurements, plasma AUC values increased in a roughly dose-linear manner (Table 74). Plasma half-life values were similar (range of 0.35 to 0.46 hours) for all doses on GD 6 and 17. Plasma clearance (Cl) and estimated volume of distribution (V_z) values were similar for all doses on GD 6. However, while the Cl and Vz values were similar at all doses on GD 15, the values were approximately 40% lower than the respective values on GD 6 suggesting saturation of CXA-101 excretion pathways and also tissue accumulation. In agreement with the reduced plasma clearance, plasma AUC values were increased approximately 1.5 to 2 fold on GD 15 compared to GD 6.

Table 74: Toxicokinetic Parameters for CXA-101 After IV Administration in Pregnant Mice. (Sponsor's Table)

CB-500,101 Dosage (mg/kg/day)	300	1000	2000[a]
		Gestation Day 6	
C_0 (µg/mL)	1240	4704	8494 (4845)
$AUC_{0\text{-last}}$ (µg•h/mL)	307	1019	1899 (1531)
$AUC_{0\text{-}\infty}$ (µg•h/mL)	307	1019	1899 (1531)
$t_{1/2}$ (h)	0.41	0.41	0.35
Cl (mL/h/kg)	976	981	1053 (1306)
V_z (mL/kg)	581	580	532 (660)
		Gestation Day 15	
C_0 (µg/mL)	1672	5191	9506
$AUC_{0\text{-last}}$ (µg•h/mL)	519	1647	3536
$AUC_{0\text{-}\infty}$ (µg•h/mL)	520	1648	3538
Accumulation Ratio	1.7	1.6	1.9 (2.3)
$t_{1/2}$ (h)	0.46	0.41	0.36
Cl (mL/h/kg)	577	607	565
V_z (mL/kg)	387	362	296

[a] = Toxicokinetic parameters in parentheses are without exclusion of the out-of-trend value for female no. 76342 in the 2000 mg/kg/day group at 0.083 hours following dose administration on gestation day 6.

Dosing Solution Analysis

The actual concentrations of the dosing formulations were within 90% to 110% of the nominal concentrations. CXA-101 was not detected in the vehicle formulation.

Necropsy

The thoracic, abdominal, and pelvic cavities were opened by a ventral mid-line incision, and the contents were examined in the scheduled necropsy on GD 18.

No CXA-101-related internal findings were observed at dosage levels of 300, 1000, and 2000 mg/kg/day.

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

GD 18 Laparohysterectomy: The uterus and ovaries were exposed and the number of corpora lutea in each ovary was recorded. The uterus was weighed and opened and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. The placentae were also examined.

As shown in Table 75, values for mean litter pre- and post-implantation loss, viable fetuses, fetal sex ratios for the CXA-101 low-, mid- and high-dose groups did not differ significantly from the control group and were within the historical control ranges. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss were similar across all groups. Mean fetal weights in the mid-dose group (1.29 grams for males and females combined) were lower than control values (1.35 g combined) and the differences for males were statistically significant (1.31 for mid-dose males versus 1.38 for control males). However, high-dose values were similar to control values, and all the values were within the historical control range (1.21 to 1.41 g).

Table 75: Summary of Mouse Fetal Data at the Scheduled Necropsy. (Sponsor's Table)

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	135	134	269	0	10	5	15	284	301	17	NA	20
MEAN	6.8	6.7	13.5	0.0	0.5	0.3	0.8	14.2	15.1	0.8	1.35	
S.D.	1.97	1.59	2.09	0.00	0.69	0.64	1.07	1.88	1.99	0.93	0.076	
S.E.	0.44	0.36	0.47	0.00	0.15	0.14	0.24	0.42	0.44	0.21	0.017	
2 TOTAL	157	171	328	1	8	3	12	340	375	35	NA	25
MEAN	6.3	6.8	13.1	0.0	0.3	0.1	0.5	13.6	15.0	1.4	1.34	
S.D.	1.54	1.86	1.94	0.20	0.56	0.33	0.59	1.89	2.16	1.44	0.086	
S.E.	0.31	0.37	0.39	0.04	0.11	0.07	0.12	0.38	0.43	0.29	0.017	
3 TOTAL	134	135	269	0	26	3	29	298	325	27	NA	22
MEAN	6.1	6.1	12.2	0.0	1.2	0.1	1.3	13.5	14.8	1.2	1.29	
S.D.	2.52	2.32	3.75	0.00	3.42	0.47	3.40	2.50	2.67	1.15	0.084	
S.E.	0.54	0.49	0.80	0.00	0.73	0.10	0.72	0.53	0.57	0.25	0.018	
4 TOTAL	146	151	297	2	16	2	20	317	346	29	NA	24
MEAN	6.1	6.3	12.4	0.1	0.7	0.1	0.8	13.2	14.4	1.2	1.34	
S.D.	2.43	2.03	2.46	0.28	1.49	0.28	1.79	1.89	2.34	1.18	0.090	
S.E.	0.50	0.41	0.50	0.06	0.30	0.06	0.36	0.39	0.48	0.24	0.018	

None significantly different from control group
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 300 MG/KG/DAY 3- 1000 MG/KG/DAY 4- 2000 MG/KG/DAY

Offspring (Malformations, Variations, etc.)

Each viable fetus was examined externally, individually sexed, and weighed before being euthanized. The eyes, palate, and external orifices for each viable fetus were examined, and the fetuses received a visceral and skeletal examination. Nonviable fetuses where decomposition was limited were examined, the crown-rump length measured, weighed and sexed. Crown-rump measurements and the degree of autolysis were recorded for late resorptions which also received a gross external examination if possible.

The number of fetuses(litters) available for morphological evaluation were 269(20), 328(25), 269(21), and 297(24) in the control, 300, 1000, and 2000 mg/kg/day groups respectively (Table 76). Malformations were observed in 6(6), 4(3), 2(2), and 4(4) fetuses (litters) in the same respective groups. As the litter incidence of external, visceral or skeletal malformations did not increase in the CXA-101 groups, CXA-101 was not considered to have induced malformations.

External malformations were noted in 5(5), 4(3), 2(2), and 4(4) fetuses(litters) in the control, low-, mid-, and high-dose groups respectively. Cleft palate was observed in two control fetuses, two low-dose fetuses, one mid-dose fetus, but no high-dose fetuses. Similarly tarsal flexure was noted in two control, one mid-dose, and three high-dose fetuses. None of the external malformations occurred at a significantly higher incidence in the CXA-101 treatment groups compared to the control group. Also no external developmental variations were observed to occur in the CXA-101 treatment groups.

The only visceral malformations were an absent right kidney and ureter in a control fetus, and a nodule in the jejunum in one low-dose fetus. The only visceral development variation also occurred in a single control fetus and consisted of major blood vessel variation.

Skeletal malformations included fused sternebrae in a control fetus and the cleft palates described above. Skeletal variations (Table 77) occurred at similar mean litter proportions for all groups and at proportions within the historical control ranges.

Table 76: Summary of the Absolute Numbers of Fetuses and Litters with External, Visceral, and Skeletal Malformations. (Sponsor's Table)

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	269	328	269	297	20	25	21	24
CLEFT PALATE	2	2	1	0	2	1	1	0
CARPAL AND/OR TARSAL FLEXURE	2	0	1	4	2	0	1	4
OPEN EYELID	1	2	0	0	1	2	0	0
NUMBER EXAMINED VISCERALLY	269	328	269	297	20	25	21	24
KIDNEY AND URETER ABSENT	1	0	0	0	1	0	0	0
NUMBER EXAMINED SKELETALLY	269	328	269	297	20	25	21	24
STERNEBRAE FUSED	1	0	0	0	1	0	0	0
TOTAL NUMBER WITH MALFORMATIONS								
EXTERNAL :	5	4	2	4	5	3	2	4
SOFT TISSUE :	1	0	0	0	1	0	0	0
SKELETAL :	1	0	0	0	1	0	0	0
COMBINED :	6	4	2	4	6	3	2	4

1- 0 MG/KG/DAY 2- 300 MG/KG/DAY 3- 1000 MG/KG/DAY 4- 2000 MG/KG/DAY

Table 77: Summary of the Absolute Numbers of Fetuses and Litters with External, Visceral, and Skeletal Variations. (Sponsor's Table)

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	269	328	269	297	20	25	21	24
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	269	328	269	297	20	25	21	24
MAJOR BLOOD VESSEL VARIATION	1	0	0	0	1	0	0	0
NUMBER EXAMINED SKELETALLY	269	328	269	297	20	25	21	24
ACCESSORY SKULL BONE(S)	29	29	25	40	13	14	12	14
7TH CERVICAL RIB(S)	49	53	68	54	13	18	15	18
14TH RUDIMENTARY RIB(S)	69	58	57	66	16	19	13	21
25 PRESACRAL VERTEBRAE	1	10	10	3	1	5	4	2
7TH STERNEBRA	9	11	7	11	5	8	5	6
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	25	14	8	13	13	9	7	6
14TH FULL RIB(S)	34	16	25	39	11	10	12	12
27 PRESACRAL VERTEBRAE	1	0	0	0	1	0	0	0
STERNEBRAE WITH THREAD-LIKE ATTACHMENT	0	0	1	0	0	0	1	0
BENT RIB(S)	0	0	0	1	0	0	0	1
CIRCULAR AREA OF UNOSSIFICATION IN STERNEBRA(E)	2	0	0	0	1	0	0	0
EXTRA SITE OF OSSIFICATION ANTERIOR TO CERVICAL ARCH #2	1	0	0	0	1	0	0	0
VERTEBRAL CENTRA UNOSSIFIED	0	0	1	0	0	0	1	0

1- 0 MG/KG/DAY 2- 300 MG/KG/DAY 3- 1000 MG/KG/DAY 4- 2000 MG/KG/DAY

Study title: An Intravenous Injection Study of the Effects of CXA-101 on Embryo/Fetal Development in Rats.

Study no.:	CXA101-T-006
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 24, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101, Lot # 3F02, purity of 98.4%

Key Study Findings

- Mean maternal body weights were significantly reduced in the high-dose (1000 mg/kg/day) group during the treatment period.
- No other maternal toxicity was noted.
- No CXA-101-related changes occurred in postimplantation loss, live litter size, mean fetal weight, fetal sex ratios, or fetal malformations at any dose.
- The NOAEL for maternal toxicity was considered to be 300 mg/kg/day and the NOAEL for embryo/fetal development was considered to be 1000 mg/kg/day.

Methods

Doses:	0, 100, 300, and 1000 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Intravenous infusion into the lateral tail vein
Formulation/Vehicle:	0.9% sodium chloride for injection, pH 5.5 to 6.0
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25 females/group
Satellite groups:	none
Study design:	See Table 78. Female rats approximately 13 weeks old were administered intravenous doses of vehicle or CXA-101 once daily from gestation days (GD) 6 through 17. On GD 20 a laparohysterextomy was performed on each female. Uteri, placentae, and ovaries were examined and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded.
Deviation from study protocol:	Multiple protocol deviations were noted; however, none was considered to have altered the results or compromised the integrity of the study.

Table 78: Study Design for the Rat Embryo-Fetal Study. (Sponsor's Table)

Group Number	Test Article	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Females
1	Vehicle	0	5	25
2	CXA-101	100	5	25
3	CXA-101	300	5	25
4	CXA-101	1000	5	25

Observations and Results

Table 79: Observation Schedule for Study No.: CXA101-T-006

Parameter	Schedule
Mortality and Clinical Signs	All rats were observed twice daily for moribundity and mortality. Individual clinical signs were recorded from GD0 to GD20 (prior to dose administration during the treatment period). Rats were also observed for clinical

	signs at the time of dose administration and approximately 1 hour following dose administration.
Injection Site Observations	Injection sites were examined for erythema and swelling beginning with the initiation of dose administration until euthanasia for all study animals.
Body Weights and Gravid Uterine Weights	Individual maternal body weights were recorded on GD 0 and daily from GD 6- GD 18, and on GD20 Gravid uterine weights were measured on GD 20, the laparhysterectomy date.
Food Consumption	Individual food consumption was recorded on GD 0, GDs 6-18, and GD 20.
Laparhysterectomy	A gross necropsy was performed on females that died or aborted during the course of the study. The number and location of implantation sites, corpora lutea, and viable fetuses were recorded. All surviving females were euthanized on GD 20.

Mortality
All of the study animals survived until the scheduled necropsy on GD 20.

Clinical Signs
No clinical signs were noted during the daily observations. For the observations 1 hour after dose administration, yellow material on the urogenital or ventral abdominal areas and red material around the nose was noted to occur sporadically in a few high-dose females during GDs 9-12. These findings did not persist to the daily observations.

Body Weight
Group mean body weights were calculated based on recorded individual body weights. Mean body weights were calculated for each corresponding measurement interval and also for GD 6-9, 9-12, 12-18, and 6-18.

Maternal body weights gains (mean values) for the high-dose females (64 grams) were significantly lower than the control value (73 grams) for the overall treatment period and also during GDs 12-18. Mean body weight values were similar for all the groups during the treatment period.

Gravid uterine weights for all of the CXA-101 groups were not significantly different than control values.

Feed Consumption
Food intake was reported as g/animal/day or g/kg/day for the corresponding body weight change interval.

Sporadic but significant reductions in food consumption relative to control values were observed for the high-dose females on GDs 6-7, 7-8, and 6-9.

Toxicokinetics

Toxicokinetics were not performed with this study, but toxicokinetics were performed in a companion GLP study in pregnant Sprague-Dawley rats (Study No.: CX.101.TX.001). In the companion study, pregnant female rats received daily intravenous doses of CXA-101 (100, 300, and 1000 mg/kg/day) from Gestation Day (GD) 6 until GD 17.

As in the mouse embryo-fetal study with CXA-101, plasma AUC values increased in a roughly dose-linear manner on both the first (GD 6) and last days (GD 17) of dosing, and plasma $t_{1/2}$ values were similar for all doses and at all time-points (Table 80). However, in a similar pattern to that observed in pregnant mice, plasma clearance (Cl) and V_z values decreased on GD 17 compared to GD 6 suggesting saturation of CXA-101 excretion pathways and tissue accumulation. A corresponding effect was increased plasma AUC values on GF 17 compared to GD 6.

Table 80: Toxicokinetic Parameters for CXA-101 After IV Administration in Pregnant Rats. (Sponsor's Table)

CB-500,101 (CXA-101) Dosage (mg/kg/day)	100	300	1000
		Gestation Day 6	
C_0 (µg/mL)	332	1062	3977
$AUC_{0\text{-last}}$ (µg·h/mL)	169	521	1767
$AUC_{0\text{-}\infty}$ (µg·h/mL)	169	522	1770
$t_{1/2}$ (h)	0.41	0.45	0.44
Cl (mL/h/kg)	590	575	565
V_Z (mL/kg)	348	372	362
		Gestation Day 17	
C_0 (µg/mL)	612	1759	5015
$AUC_{0\text{-last}}$ (µg·h/mL)	229	677	2010
$AUC_{0\text{-}24}$ (µg·h/mL)	230	678	2013
Accumulation Ratio	1.4	1.3	1.1
$t_{1/2}$ (h)	0.40	0.40	0.46
Cl (mL/h/kg)	436	443	497
V_Z (mL/kg)	250	257	330

Dosing Solution Analysis
The actual dose concentrations were within 99-101% of the nominal concentrations.

Necropsy
All surviving females were euthanized on GD 20, and the thoracic, abdominal, and pelvic cavities were opened by ventral midline incision and the contents examined.

No CXA-101-related gross pathology was reported for the GD 20 necropsy.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
The uterus and ovaries were then exposed and excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed and opened, and the

number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded.

Intrauterine growth and survival parameters including postimplantation loss, live litter size, mean fetal body weights, fetal sex ratios, and mean numbers of corpora lutea were not significantly altered by CXA-101 administration (Table 81).

Table 81: Summary of Rat Fetal Data at the GD 20 Necropsy. (Sponsor's Table)

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	181	175	356	0	22	0	22	378	398	20	NA	23
MEAN	7.9	7.6	15.5	0.0	1.0	0.0	1.0	16.4	17.3	0.9	3.6	
S.D.	2.05	2.25	1.68	0.00	0.77	0.00	0.77	1.24	1.64	0.92	0.21	
S.E.	0.43	0.47	0.35	0.00	0.16	0.00	0.16	0.26	0.34	0.19	0.04	
2 TOTAL	208	194	402	0	14	2	16	418	433	15	NA	25
MEAN	8.3	7.8	16.1	0.0	0.6	0.1	0.6	16.7	17.3	0.6	3.5	
S.D.	2.10	2.13	1.41	0.00	0.77	0.28	0.81	1.40	1.95	0.96	0.24	
S.E.	0.42	0.43	0.28	0.00	0.15	0.06	0.16	0.28	0.39	0.19	0.05	
3 TOTAL	187	173	360	0	18	0	18	378	400	22	NA	24
MEAN	7.8	7.2	15.0	0.0	0.8	0.0	0.8	15.8	16.7	0.9	3.5	
S.D.	1.47	1.84	1.74	0.00	0.79	0.00	0.79	1.82	2.41	1.18	0.22	
S.E.	0.30	0.38	0.36	0.00	0.16	0.00	0.16	0.37	0.49	0.24	0.04	
4 TOTAL	195	176	371	0	14	0	14	385	433	48	NA	25
MEAN	7.8	7.0	14.8	0.0	0.6	0.0	0.6	15.4	17.3	1.9	3.6	
S.D.	2.08	2.56	2.30	0.00	0.71	0.00	0.71	2.42	2.43	2.06	0.26	
S.E.	0.42	0.51	0.46	0.00	0.14	0.00	0.14	0.48	0.49	0.41	0.05	

None significantly different from control group
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

Offspring (Malformations, Variations, etc.)

Each fetus was examined externally, individually sexed, weighed, and euthanized. External examination included: examination of eyes, palate, and external orifices. Crown-rump measurements and the degree of autolysis were recorded for late resorptions, and a gross external examination was performed. Each viable fetus was subjected to a visceral examination. All carcasses were eviscerated, fixed, then macerated and examined for skeletal malformations or variations. External, visceral, and skeletal findings were recorded as developmental variations or malformations. The numbers of fetuses(litters) available for morphological evaluation were 356(23), 402(25), 360(24), and 371(25) in the control, 100, 300, and 1000 mg/kg/day groups, and malformations were observed in 3(2), 1(1), 3(2) and 1(1) fetuses(litters) for the same respective groups. The number of external, visceral and skeletal malformations and variations were similar for all the groups and CXA-101 was not considered to have caused malformations or variations.

External malformations were noted in 1, 0, 2, and 0 fetuses in the control, 100, 300, and 1000 mg/kg/day groups respectively as shown in Table 82. No external developmental variations were observed.

No visceral malformations were observed in any of the CXA-101 treatment groups. One fetus in the control group was observed to have multiple malformations including a malpositioned vena cava, lobular dysgenesis of the lungs, situs inversus, and transposition of the great vessels. As shown in Table 83, visceral developmental variations included renal papilla(e) not developed and/or distended ureter(s) in 1(1) control, 3(3) low-dose and 8(4) mid-dose fetuses(litters), and a liver accessory lobule in

2(2) low-dose fetuses(litters). However, none of these variations occurred in any of the high-dose fetuses suggesting no relationship to CXA-101 administration.

Skeletal malformations including vertebral anomalies, rib anomalies, and sternoschisis occurred in single animals in each of the control and treatment groups (Table 82). The skeletal malformations in the CXA-101 treatment groups were not considered to be related to CXA-101 administration because they did not occur in a dose-dependent manner, occurred in litter proportions that were within the historical control ranges, and were not significantly different than the concurrent control group. Multiple skeletal variations were noted in the control group and CXA-101 treatment groups (Table 83). However, like the skeletal malformations, the variations did not occur in a dose-dependent manner, occurred in litter proportions that were within the historical control ranges and were not significantly different than the concurrent control group. Therefore the skeletal variations were also not attributed to CXA-101 administration.

Table 82: Summary of the Absolute Number of Fetuses and Litters with Malformations. (Sponsor's Table)

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	356	402	360	371	23	25	24	25
UMBILICAL HERNIATION OF INTESTINE	0	0	1	0	0	0	1	0
OMPHALOCELE	1	0	0	0	1	0	0	0
VERTEBRAL AGENESIS	0	0	1	0	0	0	1	0
NUMBER EXAMINED VISCERALLY	356	402	360	371	23	25	24	25
MALPOSITIONED VESSEL	1	0	0	0	1	0	0	0
LUNGS- LOBULAR DYSGENESIS	1	0	0	0	1	0	0	0
SITUS INVERSUS	1	0	0	0	1	0	0	0
TRANSPOSITION OF THE GREAT VESSELS	1	0	0	0	1	0	0	0
NUMBER EXAMINED SKELETALLY	356	402	360	371	23	25	24	25
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	0	0	1	0	0	0	1	0
RIB ANOMALY	1	0	0	1	1	0	0	1
STERNOSCHISIS	0	1	0	0	0	1	0	0
TOTAL NUMBER WITH MALFORMATIONS								
EXTERNAL :	1	0	2	0	1	0	1	0
SOFT TISSUE :	1	0	0	0	1	0	0	0
SKELETAL :	1	1	1	1	1	1	1	1
COMBINED :	3	1	3	1	2	1	2	1

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

Table 83: Summary of the Absolute Number of Fetuses and Litters with Variations. (Sponsor's Table)

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	356	402	360	371	23	25	24	25
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	356	402	360	371	23	25	24	25
RENAL PAPILLA(E) NOT DEVELOPED AND/OR DISTENDED URETER(S)	1	3	8	0	1	3	4	0
LIVER- ACCESSORY LOBULE(S)	0	2	0	0	0	2	0	0
NUMBER EXAMINED SKELETALLY	356	402	360	371	23	25	24	25
14TH RUDIMENTARY RIB(S)	34	24	46	39	14	10	9	13
CERVICAL CENTRUM #1 OSSIFIED	64	71	42	58	17	17	19	17
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	2	0	0	0	2	0	0	0
REDUCED OSSIFICATION OF THE SKULL	2	5	0	1	2	3	0	1
REDUCED OSSIFICATION OF THE VERTEBRAL ARCHES	1	4	0	0	1	3	0	0
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	5	6	2	7	4	4	2	4
7TH CERVICAL RIB(S)	3	4	2	2	3	3	1	2
REDUCED OSSIFICATION OF THE 13TH RIB(S)	7	0	0	1	1	0	0	1
25 PRESACRAL VERTEBRAE	3	0	0	0	1	0	0	0
14TH FULL RIB(S)	2	0	1	1	1	0	1	1
27 PRESACRAL VERTEBRAE	2	1	1	4	1	1	1	3
HYOID UNOSSIFIED	0	2	0	3	0	2	0	2
UNCO-OSSIFIED VERTEBRAL CENTRA	0	0	0	1	0	0	0	1
PUBIS UNOSSIFIED	1	0	0	0	1	0	0	0
ACCESSORY SKULL BONE(S)	0	0	0	1	0	0	0	1
BENT RIB(S)	0	1	0	0	0	1	0	0
REDUCED OSSIFICATION OF THE RIB(S)	0	1	0	0	0	1	0	0

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 300 MG/KG/DAY 4- 1000 MG/KG/DAY

Study title: Reproductive and Developmental Toxicity Studies of Tazobactam/Piperacillin or Tazobactam (2) – Teratological study in rats with intravenous administration. Sato, T., Lochry, E.A., Hoberman, A.M., and Christian, M.S.**:** The Journal of Toxicological Sciences, Vol. 19, Supplement II, 215-232 (1994).

Study no.:	Not reported
Study report location:	Published article
Conducting laboratory and location:	Drug Safety Laboratory, Taiho Pharmaceutical Co., Ltd., 224-2 Ebisuno, hiraishi, kawauchi-cho, Tokushima 771-01, Japan; and Argus Research laboratories, Inc., 2025 Ridge Road, Perkasie, Pennsylvania 18944, USA.
Date of study initiation:	Not reported. Accepted for publication on June 1, 1994
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Tazobactam, Batch No.: 6818B46, purity was not reported.

Reviewer Comment: *This manuscript was published in Japanese but entirely translated into English. Combination treatments with tazobactam/piperacillin were also examined in this manuscript, but only the tazobactam effects are discussed by the Reviewer.*

Key Study Findings

- Maternal body weights were decreased in high-dose (3000 mg/kg/day) females and food consumption was reduced in all tazobactam-treatment groups.

- No tazobactam-related fetal toxicity, including significantly reduced fetal weights, or increased fetal malformations or variations were noted.

Methods

Doses:	0, 125, 500, and 3000 mg/kg/day
Frequency of dosing:	Twice per day
Dose volume:	5 ml/kg
Route of administration:	Intravenous into tail veins at a rate of 2 ml/min
Formulation/Vehicle:	Distilled water for injection
Species/Strain:	Crl:CD(SD) mice
Number/Sex/Group:	35-37 pregnant rats/group
Satellite groups:	none
Study design:	Pregnant female rats (6/group) were administered tazobactam from Gestation Day (GD) 7 to 17. Approximately two thirds of the rats underwent caesarean section on GD 21 and the remaining third were allowed to deliver naturally.
Deviation from study protocol:	Not reported in the publication

Reviewer Comment: *One design inconsistency was that the vehicle control group was administered 0.9% saline but tazobactam was dissolved and administered in distilled water for injection.*

Observations and Results

Table 84: Observation Schedule for the Rat Embryo-Fetal Study with Tazobactam

Parameter	Schedule
Mortality and Clinical Signs	Dams were observed daily throughout the study period.
Body Weights and Gravid Uterine Weights	Maternal body weights were recorded on GD 0 and daily from GD 7- GD 21 for caesarean section dams and for 21 days after delivery for the dams allowed to deliver naturally.
Food Consumption	Individual food consumption was recorded on GD 0, GDs 6-18, and GD 20.
Caesarean Section	Approximately two thirds of dams (21 to 24 per group) underwent caesarean section on GD 21 with the remaining third of dams (9-13) allowed to deliver naturally.
Necropsy	The caesarean section dams were necropsied GD 21. The dams that delivered naturally were euthanized and necropsied 21 days after delivery. Dams for which no delivery was confirmed were euthanized and necropsied on GD 25. Any dams that died during the treatment period were also necropsied.

Mortality

No deaths occurred as a result of tazobactam administration.

Clinical Signs
Clinical signs for the high-dose tazobactam group included: urine stains on abdominal fur, and administration-site irritation.

Body Weight
Body weight gain was inhibited during gestation in the high-dose tazobactam group.

Reviewer Comment: *The narrative in the publication indicates that maternal body weight gain was reduced during gestation in the high-dose tazobactam group, but no numerical data is provided. Data is depicted in a figure which does not include statistical analysis.*

Feed Consumption
Food consumption was decreased in the tazobactam groups during Days 7-18 of gestation, but appeared to return to control levels by Day 21 of gestation.

Toxicokinetics: Not performed

Dosing Solution Analysis: Not reported

Necropsy
No tazobactam-related gross pathology was noted.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)
No significant changes in the number of corpora lutea, implantations, dead fetuses, early or late resorptions, or live fetuses were attributed to tazobactam administration at any dose level. In addition fetal weights and the fetal sex ratios were not altered by tazobactam administration.

Offspring (Malformations, Variations, etc.)
All of the offspring data was calculated for all of the fetuses in a group, but not on a per-litter basis.

External Malformations: One fetus had a club foot and tail constriction, and one fetus had a short body, both in in the mid-dose (500 mg/kg/day) tazobactam group.

Visceral Malformations and Variations: No visceral malformations were identified. Visceral variations included one fetus with mild pelvic expansion in the mid-dose tazobactam group.

Skeletal Malformations and Variations: One mid-dose tazobactam fetus exhibited thoracic vertebral hypoplasia below the lumbar vertebrae. Multiple skeletal variations were noted in all the tazobactam groups; however, the variations occurred at a low level and generally did not occur in a dose-dependent manner and/or at a high incidence.

Table 85: External, Visceral, and Skeletal Malformations and Variations in the Rat Embryo-Fetal Study with Tazobactam.

Malformation or Variation Parameter	Tazobactam Dose (mg/kg/day)

	0 (control)	125	500	3000
External Examination				
Number of fetuses examined	304	314	364	311
Number of fetuses with major anomalies	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)
Short body	0	0	1 (0.3)	0
Thread-like tail	0	0	0	0
Bent tail	0	0	0	0
Number of fetuses with minor anomalies	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)
Rotated inward of rear limbs	0	0	1 (0.3)	0
Constriction band present at base of tail	0	0	1 (0.3)	0
Visceral Examination				
Fetuses examined	148	152	177	152
Fetuses with major anomalies	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fetuses with minor anomalies	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
Slight dilation of renal pelvis	0	0	1 (0.6)	0
Moderate dilation of lateral ventricles	0	0	0	0
Skeletal Examination				
Number of Fetuses examined	156	162	187	158
Number of Fetuses with skeletal anomalies	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Incomplete ossification of lumbar, sacral, and caudal vertebrae	0	0	1 (0.5)	0
Fusion of cervical arches and centra	0	0	0	0
Fusion of thoracic centra	0	0	0	0
Hemivertebrae of thoracic vertebra	0	0	0	0
Number of fetuses with skeletal variations	0 (0.0)	3 (1.9)	3 (1.6)	1 (0.6)
Cervical rib	0	1 (0.6)	1 (0.5)	0
Enlarged suture of nasals-frontal	0	0	1 (0.5)	0
Bifid thoracic centrum	0	1 (0.6)	1 (0.5)	0
Wavy ribs and/or hypoplasia ribs	0	1 (0.6)	0	0
Incomplete ossification of sternabrae	0	0	0	2 (1.2)

Incomplete ossification of pubic bone	0	0	0	1 (0.6)
Data shown as Mean (± SEM)				

9.3 Prenatal and Postnatal Development

Study title: CXA-101: A GLP Intravenous Pre- and Postnatal Development Study, Including Maternal Function, in Rats.

Study no.:	CX.101.TX.012
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 23, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101, Lot No.: 440420 0004 1, purity of 98.2%

Key Study Findings

- Exclusive of injection-site reactions, the NOAEL for maternal toxicity was considered to be the highest dose of 1000 mg/kg/day.
- Maternal administration of 300 and 1000 mg/kg/day was associated with significantly reduced and delayed auditory startle responses in F_1 males with similar non-significant differences in females on postnatal day 60, but not on postnatal day 20. Based on this effect, the NOAEL for F_1 toxicity was considered to be 100 mg/kg/day.
- Maternal CXA-101 administration produced no other toxic effects on F_1- or F_2-generation offspring.

Methods

Doses:	0, 100, 300, and 1000 mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	5 ml/kg
Route of administration:	Intravenous Injection (slow bolus over 1 minute) via a lateral tail vein.
Formulation/Vehicle:	Sterile saline for injection
Species/Strain:	Sprague-Dawley [Crl:CD(SD)] rats
Number/Sex/Group:	25 females/group
Satellite groups:	none
Study design:	See Table 86 below. Pregnant females were administered vehicle or CXA-101 from Gestation Day 6 through Lactation Day 20 for a total of 36 to 38 doses. Females that failed to deliver were dosed through post-mating Day 24 for a total of 19 doses. All females were allowed to deliver naturally and rear their young to weaning postnatal day (PND) 21.

Deviation from study protocol: Deviations from the study protocol were noted. However, none was considered to have altered the study results or compromised the study integrity.

Table 86: Study Design for the Rat Pre- Postnatal Study with CXA-101. (Sponsor's Table)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle	0	5	25
2	CXA-101	100	5	25
3	CXA-101	300	5	25
4	CXA-101	1000	5	25

Observations and Results

F_0 Dams

Survival: Rats were observed twice daily, once in the morning and once in the afternoon for moribundity and mortality.

No CXA-101-related mortality was observed at any dosage level. One low-dose female death on GD 12 was attributed to restraint procedures. One high-dose female death occurred on Lactation Day (LD) 0 after delivery of 15 pups.

Clinical signs: Individual clinical observations were recorded daily (prior to dosing) from GD 0 until necropsy.

In high-dose females CXA-101 injection sites were associated with very slight to severe erythema (up to 21 females), very slight to moderate edema (up to 9 females), desquamation (6 females), blanching (4 females), subcutaneous hemorrhage (8 females) and/or necrosis (4 females). These adverse findings were considered to be localized effects of high-dose CXA-101. No injection–site effects were noted in the low- or mid-dose females, and no other clinical signs were observed.

Body weight: Individual body weights were measured on GDs 0, 6, 9, 12, 15, 18, and 20 and on lactation days (LDs) 0 (when possible), 1, 4, 7, 10, 14, 17, and 21.

No CXA-101-related effects were noted on mean body weights or body weight gains at any dose during gestation or during lactation.

Feed consumption: Individual maternal food consumption was recorded on GDs 0, 6, 9, 12, 15, 18, and 20 and on LDs 1, 4, 7, 10, 14, 17, and 21.

Mid- and high-dose females had significantly less mean food (g/animal/day) consumption compared to control animals for the entire gestation treatment period (GD 6 to GD 20) and at other periods within the gestation treatment period. For the entire gestation treatment period food consumption was significantly

reduced by 6% and 7% for the mid- and high-dose groups respectively. However, the reduced food consumption did not result in corresponding weight loss and thus was not considered adverse. Mean food consumption (g/animal/day) was not significantly reduced for the low-dose (100 mg/kg/day) group during gestation and food consumption was not significantly reduced for any CXA-101 group during lactation.

Uterine content: A detailed gross necropsy was performed on each female that did not survive to the scheduled euthanasia, and the number and location of corpora lutea and implantation sites were recorded. Also a gross pathology was performed on all surviving females with viable pups on LD 21 and those females that did not deliver by post-mating Day 25. The thoracic, abdominal, and pelvic cavities were opened and the contents examined. For females that delivered or had macroscopic evidence of implantation, the numbers of former implantation sites were recorded.

On LD 21, the mean numbers of former implantation sites (15.9) and pups born (14.9) in the 1000 mg/kg/day group were significantly higher than in the control group (14.1 and 13.1 respectively), but in the historical control range. No CXA-related effects were noted at any dose for live litter size, percentage of males per litter at birth, and postnatal survival.

Necropsy observation: No CXA-101-related internal macroscopic findings were observed at the scheduled necropsies at any dose. The only gross pathology was related to injection site trauma (1 female with scabbing, 3 females with missing tails) in the high-dose group.

Toxicokinetics: Not performed.

Dosing Solution Analysis: All the dosing formulations (measured three times during dosing) were within 98.9 to 103% of the target concentration. CXA-101 was not detected in the vehicle formulation.

Other: During the period of parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. Beginning on PND 0, the day parturition was initiated, pups were sexed and examined for gross malformations and the number of stillborn and live pups was recorded.

No CXA-101-related effects were noted on mean gestation lengths or the process of parturition at any dosage level. No signs of dystocia were noted at any dose.

No CXA-101-related effects were noted at any dosage level on the mean number of pups born, live litter size, percentage of males per litter at birth, and postnatal survival. The mean number of pups born and live litter size in the 1000 mg/kg/day group was significantly higher compared to the control group, but the values were within the historical control range and the result was not considered to be toxicologically relevant.

F_1 Generation

Survival: Pups (litters) were found dead in the following proportions: 3(3), 10(9), 5(5) and 6(5) for the vehicle control and low-, mid- and high-dose CXA-101 groups respectively. In the same respective groups, 6(5), 7(6) 2(2) and 12(9) pups (litters) were found missing and presumed to have been cannibalized. These results do not depict a dose-dependent increase in pup deaths associated with CXA-101 administration. Also gross pathology or microscopic necropsies of the dead pups did not evidence a CXA-101-related effect.

Clinical signs: The general physical condition of all F_1 pups was unaffected by F_0 maternal CXA-101 administration. One mid-dose male was euthanized in extremis on PND 47 due to clinical signs of hypoactivity, hunched posture, red material around nose and decreased defecation. In the absence of similar results in high-dose animals, the clinical signs in the single animal were not attributed to CXA-101 administration.

Body weight: Mean body weights on postnatal day 1 (PND1) for male (6.4 grams) and female pups (6.1 grams) in the high-dose group were significantly lower compared to vehicle control pups (7.0 grams for males and 6.6 grams for females). However, the high-dose litters were also larger than controls suggesting the lower birth weights may have been related to larger litter sizes. Also, by PND 4 no weight differences were apparent.

During the premating period for F_1 pups (PND 21-84), mean body weight gains and mean body weights for males and females in the mid- and high-dose groups were slightly lower than for the control group. Differences were significant for mid- and high-dose females for body weight gains and for high dose females for mean body weights. However the weight differences were transient, and the mean body weights for high-dose males and females were similar to the control group by the time of necropsy on PND 126 for males or LD4 for females.

Gestation: Mean F_1 maternal body weights and body weight gains were unaffected by F_0 maternal CXA-101 administration during gestation.

Lactation: Mean F_1 maternal body weights and body weight gains were not affected by F_0 maternal CXA-101 administration during lactation.

Feed consumption: Food consumption in F_1 offspring was not described.

Physical development: Balanopreputial Separation: The mean ages of attainment of balanopreputial separation were similar for all groups with values of 44.4, 46.1, 44.0, and 45.5 days in the control and 100, 300, and 1000 mg/kg/day CXA-101 groups respectively. Mean body weights at the age of attainment were also similar for all groups; 232.4 g for the control group and 245.9, 234.8 and 234.4 for the 100, 300, and 1000

mg/kg/day CXA-101 groups respectively.

Vaginal Patency: The mean ages of attainment of vaginal patency (33.1, 33.1, 32.4, and 32.6 days) and the mean body weights at the age of attainment (114.4 112.9, 112.1, and 108.5 g) were not significantly different for the control, and CXA-101 low-, mid-, and high-dose groups respectively.

Neurological assessment:

Auditory Startle Response

Methods: An auditory startle response test was performed on one rat/sex/litter (from 25 litters/group, if possible) on PND 20 (end of the preweaning period) and PND 60 (sexual maturity). The same animals were tested at each interval. The measured startle response measurements were mean overall peak (MAX) in Newtons (N), average (AVE), and time to peak values (T_{MAX}) in milliseconds.

Results: Significantly lower MAX and AVE values for the F_1 auditory startle response and significantly higher T_{max} were noted for the F_1 males in the CXA-101 300 and 1000 mg/kg/day groups (F_0 maternal dosing) on PND 60 compared to the control group. However, the significantly different auditory startle values with the exception of the male MAX values all fell within historical control ranges. F_1 females in the 300 and 1000 mg/kg/day groups showed similar trends for the mean overall MAX and AVE values but the differences did not reach statistical significance. At PND 20 no CXA-101-related differences in MAX, AVE, or T_{max} values were noted for males or females. Also no effects were noted in the pattern of habituation response over the entire 50-trial session in adult animals. The auditory startle responses are summarized in Table 87 below.

Table 87: Auditory Startle Response Measurements for F_1 Pups in the Pre-Postnatal Study with CXA-101. (Sponsor's Table)

Interval	Parameter	HC (M)	CXA.101.TX.012 (M)	HC (F)	CXA.101.TX.012 (F)
PND 60	MAX (N)	0.872 to 1.344	Group 1: 1.084 Group 2: 1.025 Group 3: 0.813* Group 4: 0.780*	0.674 to 1.063	Group 1: 0.867 Group 2: 0.733 Group 3: 0.707 Group 4: 0.680
	T_{MAX} (ms)	44.072 to 54.965	Group 1: 47.0 Group 2: 46.6 Group 3: 53.4* Group 4: 50.4*	47.585 to 54.225	Group 1: 52.1 Group 2: 51.5 Group 3: 54.1 Group 4: 51.5
PND 20	MAX (N)	1.022 to 1.206	Group 1: 1.275 Group 2: 1.360 Group 3: 1.490 Group 4: 1.118	1.028 to 1.318	Group 1: 1.289 Group 2: 1.144 Group 3: 1.522 Group 4: 1.322
	T_{MAX} (ms)	61.570 to 62.191	Group 1: 63.0 Group 2: 62.6 Group 3: 62.4 Group 4: 62.4	60.667 to 62.733	Group 1: 62.4 Group 2: 61.8 Group 3: 61.2 Group 4: 62.6

M = Males; F = Females; HC = Historical Control
* = Significantly different from concurrent control group at $p \leq 0.036$ using repeated measures analysis

Locomotor Activity

Methods: Locomotor activity was assessed for one rat/sex/litter (from

25 litters/group, if possible) on PND 21 and 61. The same animals were tested separately at each interval. Total locomotor activity was defined as a combination of fine motor skills and ambulatory motor activity.
Results: Locomotor activity patterns (total activity as well as ambulatory activity counts) in F_1 animals were not affected by F_0 maternal CXA-101 administration at all dosage levels when evaluated on PND 21, and in animals approaching sexual maturity on PND 61. No remarkable shifts in the pattern of habituation occurred in any of the CXA-101-treated groups when the F_1 animals were compared to the control group. Overall, differences from the control group were not significantly different, within the historical control ranges, and/or did not occur in a dose-related manner.

Biel Maze Swimming Trials
Methods: Beginning on PND 22 and PND 62, swimming ability and learning and memory were assessed for one rat/sex/litter (from 25 litters/group, if possible) using a water-filled 8-unit T-maze.
Results: Swimming ability, times to criterion during the learning and memory trials, and the mean total number of errors committed during the various phases of evaluation were similar in all the CXA-101-treated groups and the control group. CXA-101 high-dose males for Trial 1 (Path A) during the PND 62 testing demonstrated a lower mean escape time compared to controls, but this enhanced ability did not reflect the overall pattern of learning for this group for Path A.

Reproduction: No CXA-101-related effects on F_1 reproductive performance were observed at any dose level. Male parameters (mating index, fertility index, and copulation index) and female parameters (mating index, fertility index, conception index, cycle length, and pre-coital interval) were not significantly changed relative to the control group or the historical control range.

Other: No internal gross pathology that could be attributed to maternal CXA-101 administration was noted in the pups found dead. One low-dose pup had underdeveloped renal papillae and 1 high-dose pup had a pale liver, but these were considered isolated occurrences unrelated to CXA-101 administration. Similarly, no CXA-101-related internal gross pathology was noted in the necropsy of the pups selected for histopathology or the nonselected pups on PND 21 or in pups euthanized due to the death of the dam. One high-dose pup was observed to have an accessory spleen.

Kidneys from 25 F_1 pups not selected for breeding (1 pup/sex/litter from each group where possible) were collected and prepared for histopathology. No CXA-101 related histopathology of the kidneys was noted.

F_2 Generation

Survival: The mean number of pups born, live litter size, percentage of males

	per litter at birth, and postnatal survival between birth and PND 0 (relative to number born), PND 0 to 1 and 1 to 4, and from birth to PND 4 were unaffected by the F_0 maternal CXA-101 administration at all dosage levels. Pups (litters) that were found dead were 7(5), 10(7), 5(4), and 29(7) in the control, 100, 300, and 1000 mg/kg/day groups, respectively. No internal findings that could be attributed to F_0 maternal CXA-101 administration were noted at the necropsies of pups that were found dead. Also zero (0), 7(5), 0(0), and 13(3) pups (litters) in the same respective groups were missing and presumed to have been cannibalized. The mean litter proportions for postnatal survival in the 1000 mg/kg/day group from PND 1 to 4 and during birth to PND 4 were lower but not significantly lower than the control group. This was attributed to the total litter loss of one litter on PND 1. No other total litter losses and no other evidence of pup survival effects were noted.
Body weight:	Mean pup body weights and body weight changes in the 100, 300, and 1000 mg/kg/day group males and females were unaffected by F_0 maternal CXA-101 administration throughout the postnatal period. No statistically significant differences from the control group were noted.
External evaluation:	External gross pathology (examination of the body, ears, eyes, nose and oral/dental) related to F_0 maternal CXA-101 administration were not observed.
Male/Female ratio:	The percentage of males per litter at birth was similar in all groups.
Other:	The animals found dead were examined internally. Milk was present in the stomachs of similar numbers of pups in all groups. In addition, the only other findings were pale livers in two high-dose pups and pale spleen in one high-dose pup.

Study title: Reproductive and Developmental Toxicity Studies of Tazobactam/Piperacillin or Tazobactam (3) – Perinatal and Postnatal Study in Rats with Intraperitoneal Administration. Sato, T., Hoberman, A.M., and Christian, M.S.: The Journal of Toxicological Sciences, Vol. 19, Supplement II, 233-247 (1994).

Study no.:	Not reported in the publication
Study report location:	Contained in published manuscript
Conducting laboratory and location:	Drug Safety Laboratory, Taiho Pharmaceutical Co., Ltd., 224-2 Ebisuno, hiraishi, kawauchi-cho,Tokushima 771-01, Japan; and Argus Research Laboratories, Inc., 2025 Ridge Road, Perkasie, Pennsylvania 18944 USA.
Date of study initiation:	Not reported; Accepted for publication on June 6, 1994.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Tazobactam, Batch No.: 6818B46, purity not reported.

Reviewer Comment: *This manuscript was published in Japanese but entirely translated into English. Combination treatments with tazobactam/piperacillin were also examined in this manuscript, but only the tazobactam effects are discussed by the Reviewer.*

Key Study Findings

Findings included:

- Dams in the tazobactam groups exhibited greater large intestine, cecal, or uterine redness, hemorrhage, prominence, and visceral fusion with pleural fluid accumulation etc. compared to the control group.
- At maternal doses of 320 and/or 1280 mg/kg/day, stillbirths were increased (1280 mg/kg/day group only), decreased F_1 pup weights were observed during lactation (both groups), and pup weights were significantly but transiently decreased in the early postweaning period (1280 mg/kg/day group only).
- No other developmental effects for F_1 pups were observed.
- The NOAEL for maternal toxicity was considered to be < 40 mg/kg/day based on the cecal enlargement, but the NOAEL for maternal reproduction was considered to be 320 mg/kg/day and the NOAEL for F_1 toxicity was considered to be 40 mg/kg/day based on reduced F_1 pup weights.

Methods

Doses:	0, 40, 320, and 1280 mg/kg
Frequency of dosing:	Twice per day
Dose volume:	5 ml/kg
Route of administration:	intraperitoneal
Formulation/Vehicle:	Distilled water
Species/Strain:	Crl:CD(SD) BR rats
Number/Sex/Group:	22 dams/sex/group
Satellite groups:	None

Study design:	Female dams were administered tazobactam or vehicle from gestation day (GD) 17 until 21 days after weaning.
Deviation from study protocol:	Study protocol deviations were not reported.

Observations and Results

F_0 Dams

Survival:	Animals were observed daily throughout the study period for mortality.
	One dam in the 320 mg/kg/day tazobactam group and one in the 1280 mg/kg/day group died 5 and 20 days after delivery respectively.
Clinical signs:	Animals were observed daily throughout the study period for clinical signs.
	The mid-dose dam that died 5 days after delivery experienced difficulties with delivery and all offspring died. Necropsy revealed fetuses left in the womb upon death, fusion of the vagina and uterus, cecum, small and large intestines, pleural fluid accumulation and adrenomegaly. Necropsy of the high-dose dam that died 20 days after delivery revealed fusion of the abdominal wall with the bladder and cecum and areas of relatively large hemorrhaging in the cecum. Surviving animals did not demonstrate clinical signs.
Body weight:	Body weights were determined on Days 0, 7, and 14 of gestation and then daily from GD 17 until 21 days after delivery.
	Weight gain from Day 17 to Day 21 of gestation decreased to a nonsignificant degree in the high-dose group. During the postnatal period weight gain in the mid- and high-dose groups was significantly higher compared to the control group.
Feed consumption:	Food consumption was determined on Days 0, 7, and 14 of gestation and then daily from GD 17 until 21 days after delivery.
	Food consumption from GD 17 to GD 21 was significantly decreased in all the tazobactam groups compared to the control group. No significant tazobactam-related changes in food consumption were noted during the postnatal period.
Uterine content:	The number of implantation sites did not vary between groups.
Necropsy observation:	Dams were sacrificed and necropsied 21 days after delivery.
	Necropsy of all of the tazobactam groups revealed greater large intestine, cecal, or uterine redness, hemorrhage, prominence and visceral fusion with pleural fluid accumulation compared to the control group.
Toxicokinetics:	Not performed
Dosing Solution Analysis:	Not reported

Other:	The number of stillbirths (17.2%) were significantly greater in the high-dose tazobactam group and the total liveborn fetuses (80.8%) were significantly decreased compared to the control group (11.4% stillborn, 87.5% liveborn).

F_1 Generation

Survival:	The fetal survival rate 7 days after birth was significantly lower than the control group in the low- and mid-dose tazobactam groups, but not in the high-dose group.
Clinical signs:	Notable clinical signs beyond the control group were not observed.
Body weight:	Postnatal body weight was significantly lower than control weights 14 and 21 days after birth in the mid-dose tazobactam group, and 7, 14, and 21 days after birth in the high-dose tazobactam group.
	In the post-weaning period, offspring body weight was significantly lower 3 to 5 weeks after birth in males and 3 weeks after birth in females in the high-dose tazobactam group. Later weights were not different than the control.
Feed consumption:	There was no significant difference in the weaning rate between the control group and any of the tazobactam groups.
	In the post-weaning period, food consumption was significantly greater 1 week after weaning in high-dose females but not in other tazobactam-treated animals or at other time periods.
Physical development:	None of the physical or functional developmental parameters including surface righting, pinna unfolding, eye opening, acoustic startle, air righting, pupil constriction, day of testes descent, and day of vaginal opening were altered in any of the tazobactam groups compared to the control group.
Neurological assessment:	The results of the passive avoidance test and the water maze test did not indicate significant differences between the tazobactam groups and control values.
Reproduction:	No tazobactam-related changes in copulation rate, impregnation rate, testes weight or epididymal weight were observed.
	Caesarean section data for F_1 generation dams indicated no tazobactam-related differences for the number of luteal bodies, number of implantations, number of dead fetuses, number of surviving offspring, fetal body weight, and gender ratio compared to control F_1 values.
Other:	Reportedly, no effects on the next generation (F_2) fetuses were found.

10 Special Toxicology Studies

Antigenicity

Study Title: Antigenicity Tests (Screening Tests) of FR264205 in Guinea Pigs (Study No.: GLR020057).

Methods
CXA-101 (FR264205) at a concentration of 10 mg/kg alone or a 1:1 emulsion with Freund's Complete Adjuvant (FCA) was administered subcutaneously in the axillary region to each of 5 male Hartley guinea pigs once per week for four weeks. The negative control group received normal saline plus FCA, and the positive control group received bovine serum albumin (BSA) plus FCA.
Skin Reaction Test: Twenty days following the initial sensitization with CXA-101, the animals were given CXA-101 (50 μg/0.1 ml/site) subcutaneously at dorsal skin sites. Skin signs were observed 24 and 48 hours after administration.
Systemic Anaphylactic Reaction: Thirty-five days following the initial sensitization with CXA-101, the animals were given 20 mg/kg of CXA-101 intravenously to induce systemic anaphylactic reaction.
Passive Skin Anaphylactic Reaction (PCA Reaction): Thirty days after the initial sensitization, serum was drawn from the sensitized animals to use to conduct a 24-hour allogenic PCA reaction using 1 mg/kg of CXA-101-egg albumin as an inducing antigen.

Results
Skin Reaction Test: No skin reactions were observed in any animals sensitized with CXA-101 or CXA-101 plus FCA then challenged with subcutaneous CXA-101. Positive control animals sensitized with BSA plus FCA then challenged with subcutaneous BSA demonstrated erythema.
Systemic Anaphylactic Reaction: No anaphylaxis was noted in any of the animals sensitized with CXA-101 or CXA-101 plus FCA then challenged with intravenous CXA-101. In contrast all of the animals that were sensitized with BSA plus FCA then challenged with BSA exhibited anaphylactic signs including cyanosis and 4 animals subsequently died.
PCA Reaction: All of the serums were negative for reactions (antibody value < 4) for animals sensitized with CXA-101 or CXA-101 plus FCA then challenged with CXA-101 plus egg antigen as were the serums of negative control animals. In contrast, animals sensitized with BSA plus FCA then challenged with BSA demonstrated a high antibody value (>512) in their pooled serum.

Study Title: FR264205 Guinea Pig Antigenicity Test (Study No.: GLR050096).

Methods
Male guinea pigs were subcutaneously injected with 10 mg/kg of FR264205 (CXA-101) three times at 2-week intervals. Freund's complete adjuvant (FCA) was included with the first injection and the second and third injections included Freund's incomplete adjuvant (FIA). The negative control group received saline and the positive control groups received penicillin G potassium (PCG) or bovine serum albumin (BSA) according to the same schedule of injections as CXA-101.

Elicitation: Administration for elicitation for skin reaction was performed 7 days after the final active sensitization, 14 days after the final active sensitization for ASA reaction, and about 24 hours after passive sensitization for 24-hour PCA reaction. Positive control animals were elicited with the same substances used for sensitization or in the case of PCG, animals were elicited with PCG or PCG plus albumin from guinea pig serum (GPA).

Skin Reactions: Actively sensitized animals were challenged intradermally 7 days after the final sensitization with the same agent as was used for sensitization. The diameters of erythema and necrosis reactions on the administration sites were measured 24 and 48 hours after challenge. If the average length of the long diameter and short diameter was 5 mm or more, it was determined to be positive.

Active Systemic Anaphylactic (ASA) Reactions: Fourteen days after the final active sensitization, the corresponding challenge antigen was administered intravenously. Animals were observed for one hour after challenge for manifestation of anaphylactic symptoms according to the scale shown in Table 88.

Table 88: Criteria for Determination of the Degree of Anaphylactic Symptoms. (Sponsor's Table)

Grade	Symptoms Observed
0:	Normal
1:	Anxiety, tremors, sneezing, scratching the nose, piloerection, urination, defecation
2:	Mild respiratory difficulty, motor dysfunction
3:	Severe respiratory distress, convulsion
4:	Death

24-Hour Passive Skin Anaphylactic Reaction (24-hour PCA reaction): Blood was collected (and processed to serum) from the actively sensitized guinea pigs in each group on Day 12 after the active sensitization. Serum was diluted with saline then administered dermally into the dorsal region of two naïve guinea pigs (4 guinea pigs for the PCG group) from each group for passive sensitization. Guinea pigs that were passively sensitized were given eliciting antigen solution intravenously in the front paws 24 hours after passive sensitization followed by 2% Evan's blue solution intravenously. Thirty minutes after administration for elicitation, the animals were euthanized and dorsal skin was peeled off. Pigmented spots on the underside of the skin were measured. If the diameter of a pigmented spot was 5 mm or more, it was determined to be positive, and the maximum dilution ratio showing a positive reaction was determined to be the antibody value.

Results

Skin Reactions: None of the guinea pigs that were sensitized dermally with normal saline demonstrated skin reactions. Guinea pigs sensitized subcutaneously with CXA-101 did not show positive reactions when administered intradermal CXA-101 or saline. Guinea pigs sensitized with PCG did not show skin reactions when elicited with PCG, but BSA sensitized animals demonstrated positive skin reactions upon elicitation with BSA.

ASA Reaction Tests: Four out of 10 guinea pigs subcutaneously sensitized with CXA-101 showed systemic anaphylactic symptoms (tremors, piloerection, nose scratching,

and dyskinesia) with grades of 1 and 2 after CXA-101 elicitation. Guinea pigs sensitized with normal saline did not show systemic anaphylactic symptoms (grade = 0) upon elicitation with CXA-101, PCG, PCG-GPA, and BSA. The animals sensitized with PCG did not show anaphylactic symptoms after PCG elicitation, but all 5 of the animals elicited with PCG-GPA demonstrated anaphylaxis (grade 4) resulting in death 7 minutes after elicitation. The 2 guinea pigs sensitized with BSA had anaphylactic reactions (grades 3 and 4) upon elicitation with BSA, and one animal died.

PCA Assays: In the 24-hour PCA reaction tests 2/10 animals exhibited positive reactions, and in these animals, antibody titers were elevated (4X and 8X). Guinea pigs sensitized with serum from guinea pigs sensitized with normal saline did not demonstrate positive reactions after elicitation with CXA-101, PCG, PCG-GPA, and BSA. Guinea pigs passively sensitized with serum obtained guinea pigs subcutaneously sensitized with PCG (n =8) showed positive reactions and elevated antibody titers (4X to 64X) upon elicitation with PCG, and positive reactions and elevated antibody titers (64X and 128X) upon elicitation with PCG-GPA (n = 10). The 2 guinea pigs that received passive sensitization with serum obtained from guinea pigs subcutaneously sensitized with BSA showed positive PCA reactions and elevated antibody titers (1024X and 4096X) upon elicitation with BSA.

Other Toxicities

Study Title: CXA-101: A GLP Repeat Dose Phototoxicity Study to Determine the Effects on Eyes and Skin in Pigmented Rats Following Once Daily Intravenous Administration. (Study No.: CX.101.TX.013)

Methods

CXA-101 was administered in IV (tail vein) doses of 0, 100, 300, and 1000 mg/kg CXA-101 once daily for 4 days (n = 5) followed by exposure to UV radiation from a xenon lamp (after the last daily dose) on the eyes and skin of Crl:LE (Long-Evans) pigmented rats (Table 89). The vehicle, saline (daily for four days) and a positive control agent, 8-methoxypsoralen (8-MOP; administered once on Day 4), were also administered, and one group of rats receiving high-dose CXA-101 was treated with sham-UVR exposure.

Table 89: Experimental Design for the Phototoxicity Study in Rats. (Sponsor's Table)

Group	Number of Rats	Descriptor	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	Instrumental UVR Dose (Minimal Erythema Dose)	Interval Between Dose and UVR Exposure[a] (minutes)
1	5	Vehicle	0	0	5	0.5	5±3
2	5	CXA-101	100	20	5	0.5	5±3
3	5	CXA-101	300	60	5	0.5	5±3
4	5	CXA-101	1000	200	5	0.5	5±3
5	5	CXA-101	1000	200	5	0	N/A[b]
6	5	8-MOP	10	2	5	0.5	60 ± 10

a. Interval based on the T_{max} of CXA-101 in the eyes and skin. The actual exposure time of the group was based on the median dosing time of the group.
b. Sham exposure; rats were sedated and restrained but not exposed to UVR.

UVR was administered for approximately one-half hour to deliver a 0.5 minimal erythema dose (MED). Animals were anesthetized and their eyes were taped open. Animals were assessed for clinical signs and skin reactions, body weights, body weight changes, ophthalmology, and histopathology. Animals were euthanized 7-days after the first dose.

Results and Observations

Mortality and Clinical Signs

All animals were monitored constantly during anesthesia and UVR exposure until recovery from anesthesia and at 1 hour, 4 hours and 1, 2, and 3 days after UVR exposure.

No CXA-101-related deaths occurred during the study. Two vehicle control and one positive control animal were found dead after UVR exposure on Day 4. No obvious cause of death was apparent and the deaths were attributed to anesthesia. No clinical signs were attributed to CXA-101 administration.

Skin Reactions

Rats were assessed for skin reactions at 1 hour, 4 hours and daily for three days after UVR exposure according to the grades shown below in Table 90.

Table 90: Grading Values for Skin Reactions. (Sponsor's Table)

Observations	Grade	Description
Erythema	• Grade 1 • Grade 2 • Grade 3	• Barely perceptible light redness • Distinct redness • "Beet red" color
Edema	• Grade 1 • Grade 2 • Grade 3	• Mild, raised <1 mm • Moderate, raised 1 mm to 2 mm • Marked, raised >2 mm
Flaking	• Grade 1 • Grade 2 • Grade 3	• Barely perceptible scales[a] • Distinct scales[a] • Pronounced flaking with denuded[b] sites
Ulceration	NA	1 = Yes[c]
Scab	NA	1 = Yes[c]

NA = not applicable
a. Scale - small, thin dry exfoliation shed from the upper layer of the skin.
b. Denude - to remove the protective layer.
c. Were documented as per Standard Operating Procedures.

No skin reactions indicative of phototoxicity were observed after four consecutive daily administrations of the vehicle or any of the doses of CXA-101 or in the sham-UVR exposure group. In the positive control group, skin reactions were observed. In lightly pigmented skin sites, erythema grade 1 lesions occurred in 4/4 surviving animals in the 8-MOP group. In darkly pigmented skin sites, erythema grade 1 lesions occurred in 2/4 surviving animals.

Body Weights

Body weights were recorded once during acclimation, once each day of formulation administration, and once on each day of observation and before scheduled euthanasia.

Ophthalmic Examinations

Ophthalmology examinations were performed for all rats before assignment to study and for all phototoxicity phase rats three days after UVR exposure. Visible ocular structures (including lens and fundus oculi) were assessed with indirect ophthalmology using a hand-held lens. Slit lamp biomicroscopy was performed to evaluate the lids, adnexal structures, cornea, anterior chamber, lens, and anterior vitreous.

No ophthalmological observations indicative of phototoxicity were observed in the vehicle or CXA-101 treatment groups. In contrast, the positive control group receiving 8-MOP and UVR exhibited diffuse corneal edema with intraocular structures not visible (3 of 4 animals) and mild diffuse corneal edema (1/4 animals) and cataracts in (2/4 animals). Corneal dystrophy observed in all rats including those in the vehicle control group was considered to be a characteristic observation in Long-Evans rats, and inferior focal retinopathy, observed in all groups of rats exposed to UVR was not considered related to CXA-101 administration.

Histopathology

Tissues assessed for histopathology included eyes, kidney, dorsal skin, gross lesions/masses, injection sites on the tail, heart, liver, spleen, and lungs. Tissues from all animals were assessed. Tissue section examinations were performed by a board-certified veterinary pathologist, and a histopathology peer review was performed.

Reviewer Comment: *The study report indicates that the pathology report was not signed and dated by the study pathologist, and indicates that interpretation of the histopathology results was based on incomplete raw data. However, reportedly, the conclusions of the study pathologist were confirmed by the peer review pathologist.*

No CXA-101-related microscopic changes indicative of phototoxicity were observed. Ocular histopathology that was observed (as noted above), occurred in the vehicle control group and was considered to be common pathology in the test species or a direct result of the UVR exposure. Lesions in the positive control group receiving 8-MOP and UVR, showed corneal lesions with bilateral moderate to marked neutrophilic infiltration of the cornea in 50% of the animals combined with instances of damage to the corneal epithelium and endothelium, and mild edema of the corneal stroma. In some positive control animals, early signs of cataracts, minimal unilateral hyperplasia of the central lenticular epithelium (2 of 4 animals) and minimal hyperplasia of the central lenticular epithelium (1 of 4 animals) were observed.

Study Title: CXA-101 (Ceftolozane): A Skin Sensitization Study (Buehler method) in Guinea Pigs. (Study No.: CXA.101.TX.027)

Methods

For all animals in the Induction Phase, the left scapular surface was clipped the day prior to each dose of CXA-101. Each dose of CXA-101 (0.4g) was mixed 1:1 with deionized water to form a paste and applied to clipped skin regions with a Hill Top chamber. On each day of dosing, each animal received one application to the clipped region, and the dose was repeated at the same site once per week for 3 weeks for a total of 3 six-hour exposures (last dose on Day 15; Table 91). In the Challenge Phase, all animals were administered the challenge dose on Day 29, 2 weeks following the last induction exposure on Day 15. At least 24-hours prior to dosing, the hair was clipped from the flank of each animal and on the day of Challenge dosing, each animal received on application of CXA-101 paste applied with a Hill Top chamber for 6-6.5 hours. Dermal irritation scores were conducted 24 hours and 48 hours following the Challenge dose.

Table 91: Study Design for the Skin Sensitization Study in Guinea Pigs. (Sponsor's Table)

Table B. Group Assignments (Induction and Challenge Phases)

Group Number	Treatment	Dose Level	Number of Animals[a] Induction Phase Male	Induction Phase Female	Challenge Phase Male	Challenge Phase Female
2	Control/Ceftolozane[b]	0.4 g at 100%	5	5	5	5
3	Ceftolozane	0.4 g at 100%	10	10	10	10

[a]The same animals used during the Induction Phase (Days 1, 8, and 15) were used during the Challenge Phase (Day 29)
[b]Treated during the Challenge Phase only

The scoring system for dermal irritation is shown in Table 92. Scores of ≤ 0.5 were considered equivocal.

Table 92: Dermal Irritation Scoring System. (Sponsor's Table)

Table C. Sensitization Scoring Method	
Score	Observation
0	No visible change
0.5	Very faint erythema, usually patchy
1	Faint erythema, usually confluent
2	Moderate erythema
3	Severe erythema with or without edema

Other measures, Severity Index (SI) and Sensitization Index (SII) were also calculated. The SI scores for dermal irritation were calculated as: the sum of the sensitization scores at an interval/total number of animals. The SII was determined for the control and test groups according to the equation: SII = (the number of animals showing a positive response at 24 and 48 hours x 100)/ (total number of animals).

Table 93: Sensitization Index Scores (Sponsor's Table)

Table E. Sensitization Classification	
Sensitization Index (%)	Classification
0	Nonsensitizer
>0-8	Weak sensitizer
9-28	Mild sensitizer
29-64	Moderate sensitizer
65-80	Strong sensitizer
81-100	Extreme sensitizer

Results

Following induction and challenge, no dermal irritation was observed. All of the dermal sensitization scores for control and CXA-101 treated animals were 0.

Study Title: CXA-101 (Ceftolozane): An Acute Dermal Irritation Study in Rabbits. (Study No.: CX.101.TX.028)

Methods

CXA-101 (0.5g) was moistened with deionized water (1:1) to form a paste. The paste was placed on a gauze patch and applied to a 1 inch square shaved area of skin on the right sides of three rabbits. The application was wrapped with gauze for 4 hours, then dermal irritation scores for all three animals were assessed at approximately 30 to 60 minutes, 24, 48, and 72 hours following the exposure. Skin irritation was scored according to the Draize scale shown below in Table 94.

Table 94: Draize Scale for Skin Irritation. (Sponsor's Table)

Table B. Edema Formation	
Score	Observation
0	No edema
1	Very slight edema (barely perceptible)
2	Slight edema (edges of area well-defined by definite raising)
3	Moderate edema (raised approximately 1 mm)
4	Severe edema (raised more than 1 mm and extending beyond area of exposure)

Table C. Erythema and Eschar Formation	
Score	Observation
0	No erythema
1	Very slight erythema (barely perceptible)
2	Well-defined erythema
3	Moderate to severe erythema
4	Severe erythema (beet redness) to slight eschar formation (injuries in depth)

Results
All of the rabbits survived until euthanasia at the end of the experiment. Reportedly, no dermal irritation was present in any animal at any time point.

Study Title: Histamine Releasing and Hemolysis Activities of FR264205 (Study No.: CRE060243)

Methods
Histamine Release Assessment: Peripheral white blood cells were isolated from two healthy adult males. Cells were incubated with CXA-101 (FR264205) at concentrations of 0.3, 1.0, and 3.0 mg/ml at 37°C for 45 minutes. The negative control treatment was saline and the positive control treatment was 0.3 μg/ml anti-IgE solution. Total histamine content in each cell suspension was estimated by freezing and thawing the cells. Experiments were performed in duplicate, and histamine was quantified by ELISA.
Hemolysis Assessment: Whole blood obtained from two healthy adult males was diluted 10 fold with saline then incubated with 50 ml of CXA-101 (0.3, 1, and 3 mg/kg), saline as the negative control, or the positive control, amphotericin-B. Maximum hemolysis was induced by the addition of water. Experiments were performed in triplicate.

Results
CXA-101 did not stimulate the release of histamine above the level of the negative control for any of the concentrations tested (Table 95). The positive control stimulated histamine release of approximately 4- to 10-fold above negative control levels.

Table 95: Effect of CXA-101 on Histamine Released From Human Peripheral White Blood Cells. (Sponsor's Table)

	Concentration (mg/mL)	Histamine concentration (nM)	
		Volunteer #1	Volunteer #2
FR264205	0.3	4.74	3.61
	1	4.78	2.87
	3	5.70	4.31
Anti-IgE	3×10^{-4}	49.8	14.8
Spontaneous release	-	4.94	3.25
Total Histamine Content	-	478	365

CXA-101 did cause appreciable hemolysis (Table 96).

Table 96: The Effect of CXA-101 on Hemolysis on Human Whole Blood. (Sponsor's Table)

	Concentration (mg/mL)	Relative hemolysis activity (%)	
		Volunteer #1	Volunteer #2
FR264205	0.3	<1	<1
	1	<1	<1
	3	<1	<1
Amphotericin-B	0.3	88.2	89.4
Total Hemolysis	-	100	100

Impurities

Impurity qualification studies were performed with a forced degradation product of CXA-101 (Lot # GLP-400142G). The amount of each CXA-101 degradant and impurity in Lot # GLP-400142G is shown in Table 97 below.

Table 97: Levels of Impurities in the Lots of CXA-101 Used for the Toxicity Studies Including the Study Using the Forced Degradation Product of CXA-101.
(Sponsor's Table)

Peak (CB Number; RRT)	Lot			
	400142G	3F02	4404200022	139-121211
[redacted] (b) (4)				

[a] Identified as [redacted] (b) (4) on Certificate of Analysis.

Study title: 4-Week Intravenous Dose Toxicity Study of Forced Degradation Product of FR264205 Formulation in Rats.

Study no.:	TX043007, GLR050749
Study report location:	Electronic transmission
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	April 19, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101 forced degradation product, Lot # GLP-400142G, see Table 98 below for purity.

Table 98: Description and Purity of the CXA-101 Forced Degradation Product, # GLP-400142G. (Sponsor's Table)

Item	Pre-treatment	Post-treatment
Description	White mass	White mass
Identification	Conforms	Conforms
Related substances	Individual (max): (b) (4) (b) (4) Total: (b) (4) (b) (4)	Individual (max): (b) (4) (b) (4) Total: (b) (4)
Purity	87.3%	86.8%

Key Study Findings

- Other than the accumulation of hyaline droplets in the tubular epithelial cells of the renal cortex of rats, no CXA-related adverse effects were observed.
- The hyaline droplet accumulation was not considered adverse because no correlating degenerative or necrotic changes in the kidney or changes in renal function were noted.
- The NOAEL value was considered to be the highest administered dose of 300 mg/kg/day.

Methods

Doses:	0, 100, 300 mg/kg/day CXA-101 (forced degradation product)
Frequency of dosing:	Once per day
Route of administration:	intravenous
Dose volume:	5 ml/kg
Formulation/Vehicle:	saline
Species/Strain:	Crj:CD(SD) IGS rats
Number/Sex/Group:	10/sex/group
Age:	6 weeks old
Weight:	220 - 240 g for males; 150 - 164 g for females
Satellite groups:	none
Unique study design:	See Table 99.
Deviation from study protocol:	Several deviations from the study protocol were noted. However, none of the deviations were considered to have altered the results or compromised the integrity of the study.

Table 99: Study Design for Study No.: TX043007

Group/Dose (mg/kg/day)	**Dose Volume**	**Animal Number**	
		Male	**Female**
Control Group - saline	5 ml/kg	10	10

CXA-101 (100)	5 ml/kg	10	10
CXA-101 (300)	5 ml/kg	10	10

Observations and Results

Table 100: Observation Schedule for Study No.: TX043007.

Parameter	Schedule
Mortality and Clinical Signs	Animals were observed for clinical signs 3 times/day during the treatment period (once before dosing and twice after dosing), and once on the day of necropsy.
Body Weight	Body weight was measured on Day 1 of dosing and once weekly during the treatment period (before dosing on the designated days) and on the day of necropsy.
Food Consumption	Food consumption was measured once weekly during the treatment period before dosing on the designated days.
Clinical Pathology	
Hematology	Blood was collected for measurement of hematology and coagulation parameters from fasted animals on the day after the final dosing in conjunction with the necropsy.
Serum Chemistry	Blood was collected at the same time as the blood for hematology analysis.
Urinalysis	Urine was collected from 5 animals/sex/group for 24 hours during Week 4 of dosing from animals with free access to water.
Ophthalmology	Before dosing, during Week 4, 5 animals/sex/group received ocular examinations.
Necropsy	Animals were euthanized for necropsy the day after the last day of dosing (Day 29).

Mortality

No mortality occurred

Clinical Signs

No test article-related clinical signs were noted.

Body Weights

Changes in body weight were similar in all groups.

Feed Consumption

Food consumption was similar in all groups.

Ophthalmoscopy

No ocular abnormalities were observed in control or treatment groups.

ECG: Not performed

Hematology

The hematology and coagulation parameters listed in Table 108 were assessed.

No changes in any hematology or coagulation parameters attributed to treatment with CXA-101 Forced Degradation Product were observed. Significant differences in reticulocyte ratio, percent lymphocytes, percent segmented cells, percent monocytes, and fibrinogen were observed in CXA-101 treatment groups compared to control values. However, the changes did not occur in a dose-dependent manner or individual values were within the historical control range.

Clinical Chemistry

The clinical chemistry parameters listed in Table 109 were assessed.

Total serum cholesterol was significantly increased by 23% in the 300 mg/kg/day (high-dose) group. Also significant differences in serum total protein, ALB/GLB ratio, glucose, and creatinine were observed in CXA-101 treatment groups compared to control values. However, the changes did not occur in a dose-dependent manner or individual values were within the historical control range.

Urinalysis

The urinalysis parameters listed in Table 110 were assessed.

No treatment-related changes in any urinalysis parameters were observed.

Gross Pathology

No treatment-related gross pathology was observed.

Organ Weights

Increases in absolute and relative cecum weights were observed in males and females in the mid- and high-dose groups. Significant differences in the absolute and/or relative weights of prostate, spleen, thymus, lungs, and salivary gland were observed in CXA-101 treatment groups compared to control values. However, other than the cecum weight changes, the organ weight changes did not occur in a dose-dependent manner or fell within the historical control range and consequently were not considered to be toxicologically relevant.

Histopathology

Adequate Battery: Yes the tissues examined for histopathology are listed in Table 111.

Peer Review

Yes

Histological Findings

Proximal tubule hyaline droplets in the renal cortex were found in 2/10 males in the 300 mg/kg group. In addition, injection site reactions were noted in all groups with increased incidence and severity compared to the control group occurring in high-dose males and females. Injection site effects included: perivascular hemorrhage, infiltration of

inflammatory cells, fibrosis, and intimal thickening. All other histopathology which was also observed in control animals was considered to be consistent with spontaneous lesions known to occur in rats.

Special Evaluation: None

Toxicokinetics: Not performed

Dosing Solution Analysis
The pre and post-treatment purity of the drug substance is shown in Table 98. Dosing solutions prepared on Day 1 of dosing and Week 4 of dosing were analyzed for test article concentration. The dosing solution actual concentrations were determined to be 93.1 to 104% of the nominal concentrations.

Study Title: Genotoxicity Study of GLR050753 Forced Degradation Product of FR264205 Formulation – Bacterial Reverse Mutation Test.

Study no.:	TX047091 (Study Report No.: GLR050753)
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 17, 2004
GLP compliance:	Yes (Japanese GLP)
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101 (FR264205), Lot # GLP-400142G, see Table 104 below for purity.

Table 101: Characteristics, Stability, and Purity of CXA-101 Lot # GLP-400142G.
(Sponsor's Table)

Item	Before the start of the study	After use in the study
Description	White masses	White masses
Purity	87.3%	86.0%
Related substances	Total: (b) (4) Individual (max): (b) (4) (b) (4)	Total: (b) (4) Individual (max): (b) (4) (b) (4)
Expiration date	March, 2005	
Storage conditions	(b) (4)	

*: Actual measurement values

Key Study Findings

The forced degradation product for CXA-101 (Lot # GLP-400142G) was not mutagenic in an Ames test using *Salmonella typhimurium* and *Escherichia coli* bacterial strains.

Methods

Strains:	*Salmonella typhimurium* TA100, TA98, TA1535, TA1537, and *Escherichia coli* WP2 uvrA
Concentrations in definitive study:	See Table 102.
Basis of concentration selection:	The concentrations used for the definitive assay with the forced degradation product of CXA-101 were the same as those used in a previous study using an undegraded batch of CXA-101.
Negative control:	Physiological saline
Positive control:	2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2), sodium azide (SAZ), 9 – aminoacridine (9-AAC), and 2-aminoanthracene (2-AAnt): See Table 103.
Formulation/Vehicle:	Physiological saline
Incubation & sampling time:	Each tester strain was incubated for approximately 48 hours with the CXA-101, positive and negative control solutions.

Table 102: CXA-101 (Forced Degradation Product) Concentrations Used in the Definitive Ames Assays. (Sponsor's Table)

Experiment I

Tester strain	Dose level (µg/plate)	
	Without metabolic activation	With metabolic activation
TA100	0.313 - 20	0.313 - 20
TA98	0.313 - 20	0.313 - 20
TA1535	0.0781 - 5	0.0781 - 2.5
TA1537	0.0781 - 2.5	0.0781 - 2.5
WP2 uvrA	0.0781 - 2.5	0.0781 - 2.5

Experiment II

Tester strain	Dose level (µg/plate)	
	Without metabolic activation	With metabolic activation
TA100	0.313 - 10	0.313 - 10
TA98	0.313 - 10	0.313 - 10
TA1535	0.0781 - 2.5	0.0781 - 2.5
TA1537	0.0781 - 2.5	0.0781 - 2.5
WP2 uvrA	0.0781 - 2.5	0.0781 - 2.5

Table 103 Positive Control Concentrations for Study No.: GLR050753

Strain	Positive Control	Concentration
Without S9 Activation		
TA100	AF-2	0.01 µg/plate
TA98	AF-2	0.1 µg/plate
TA1535	SAZ	0.5 µg/plate

TA1537	9-AAC	80 μg/plate
WP2uvrA	AF-2	0.01 μg/plate
With S9 Activation		
TA100	2-AAnt	1 μg/plate
TA98	2-AAnt	0.5 μg/plate
TA1535	2-AAnt	2 μg/plate
TA1537	2-AAnt	2 μg/plate
WP2uvrA	2-AAnt	10 μg/plate

Study Validity
The following study validation criteria were fulfilled:

1. The number of viable cells in the bacterial suspension for each test was 0.5 x 10^9 cells/ml or greater.
2. No contaminant bacteria were detected or contamination was negligible.
3. The number of revertant colonies for the solvent control group was within the historical control ranges.
4. The mean number of revertant colonies in the positive control group was at least twice that in the solvent control group.

Results
The test article was judged to be mutagenic when the results fulfilled both of the following criteria.

1. The mean number of revertant colonies increased in a dose-dependent manner to at least twice the number of the solvent control.
2. The result was reproducible in a repeat experiment.

In Experiment I, CXA-101 (forced degradation product) inhibited growth of TA100 and TA98 at ≥ 10 μg/plate, and TA1535, TA1537, and WP2 at ≥ 2.5 μg/plate. No precipitation was observed. The mean number of revertant colonies did not reach at least double that of the solvent control group for any strain with or without S9 activation. Similar results were obtained in Experiment II. In contrast, in both experiments, the positive control agents produced at least twice the number of revertant colonies compared to the concurrent negative control.

Study Title: Genotoxicity Study of Forced Degradation Product of FR264205 Formulation - Chromosomal Aberration Test Using Cultured Mammalian Cells

Study no.:	TX047088 (Study Report No.: GLR050846)
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	June 15, 2004
GLP compliance:	Yes (Japanese GLP)

QA statement:	Yes
Drug, lot #, and % purity:	CXA-101 (FR264205), Lot # GLP-400142G, see Table 104 below for purity.

Table 104: Characteristics, Stability, and Purity of CXA-101 Lot # GLP-400142G.
(Sponsor's Table)

Test Item	Before the start of the study	After use in the study
Description	White masses	White masses
Purity	87.3%	87.5%
Related substances	Total: (b) (4)	Total: (b) (4)
	Individual (max) (b) (4) (b) (4)	Individual (max): (b) (4) (b) (4)
Expiration date	March, 2005	
Storage conditions	(b) (4)	

*: Actual measurement values

Key Study Findings

CXA-101 in 4 and 24 hour assays with and without S9 activation did not produce significantly increased numbers of cells with structural or numerical aberrations compared to the vehicle control group for any of the incubation conditions.

Methods

Cell line:	Chinese Hamster Lung Fibroblast Cell Line.
Concentrations in definitive study:	For the 6 hour incubations with and without S9 activation: 0, 625, 1250, 2500, and 5000 μg/ml; For the 24 hour incubation in the absence of S9 activation: 250, 500, 1000, 1500, 2000, 3000, and 5000 μg/ml; For the 48 hour incubation in the absence of S9 activation: 125, 250, 500, 750, and 1000 μg/ml.
Basis of concentration selection:	Cytotoxicity results from a previous study using undegraded CXA-101 indicated CXA-101 did not inhibit cell growth at the highest tested concentration of 5000 μg/ml after 6 hours incubation, but did produce cytotoxicity with an estimated IC_{50} of 4800 and 470-560 μg/ml after 24 and 48 hours of incubation respectively.
Negative control:	Physiological saline.
Positive control:	Without S9 activation: mitomycin C (MMC); with S9 activation: dimethylnitrosamine (DMN).

Formulation/Vehicle:	Physiological saline.
Incubation & sampling time:	Cells were incubated for 6, 24, and 48 hours in the absence of S9 activation and for 6 hours in the presence of metabolic activation with test solutions. After the initial incubation, cells were washed and incubated in fresh culture medium for an additional 18 hours followed by 2 hours in the presence of colcemid solution.

Study Validity

The following validation criteria were fulfilled for all of the assays in this study.

1. The incidence of cells with structural aberrations or polyploid cells in the vehicle control group was less than 5%.
2. The incidence of cells with structural aberrations excluding gaps in the positive control groups was greater than 10%.

Results

The forced degradation product for CXA-101 inhibited growth in a concentration dependent manner in the 24 and 48 hour incubations with approximately 70% growth inhibition occurring at concentrations of 5000 and 1000 μg/ml respectively. In the 6 hour incubations, cell growth was not inhibited even by the highest concentration of degraded CXA-101 (5000 μg/ml).

Positive results for clastogenicity were considered to have occurred when:

1. The incidence of cells with structural aberrations excluding gap or polyploidy cells increased in a concentration dependent manner.
2. The incidence of cells with structural aberrations excluding gap or polyploidy cells was significantly increased in at least one treatment group compared to the vehicle control group.

None of the test concentrations of degraded CXA-101 produced significantly increased numbers of cells with structural aberrations (excluding gaps) compared to the vehicle control group for any of the incubation conditions. Similarly, the number of polyploid cells was not significantly increased by any of the concentrations of degraded CXA-101 under any of the incubation conditions. In contrast all of the positive control conditions produced significant increases in the number of cells with structural aberrations without changing the number of polyploid cells.

Study title: Genotoxicity Study of Forced Degradation Product of FR264205 Formulation – Micronucleus Test in Mice.

Study no:	TX047079
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4) [redacted]

Date of study initiation:	May 31, 2004
GLP compliance:	Yes (Japanese GLP)
QA statement:	Yes
Drug, lot #, and % purity:	CXA-101 (FR264205) Forced Degradation Product, Lot No.: GLP-400142G, purity shown below in Table 105.

Table 105: Description and Purity of the CXA-101 Forced Degradation Product (Lot No.: GLP-400142G). (Sponsor's Table)

Item	Before the start of the study	After use in the study
Description	White masses	White masses
Related substances	Individual (max) (b) (4)	Individual (max) (b) (4)
	(b) (4)	(b) (4)
	Total: (b) (4)	Total: (b) (4)
Purity	87.3%	87.4%
Expiration date (re-evaluation date)	March, 2005	
Storage conditions	(b) (4)	

Key Study Findings

The CXA-101 Forced Degradation Product (Lot No.: GLP-400142G) at single intravenous doses ≤ 1500 mg/kg was shown to be negative for clastogenic potential in mouse peripheral blood reticulocytes in a micronucleus assay.

Methods

Doses in definitive study:	375, 750, and 1500 mg/kg
Frequency of dosing:	Single dose
Route of administration:	intravenous
Dose volume:	15 ml/kg for the high dose and 10 ml/kg for the other doses.
Formulation/Vehicle:	Physiological saline
Species/Strain:	Crj:CD-1 (ICR) mice
Number/Sex/Group:	5/sex/group
Satellite groups:	none
Basis of dose selection:	Based on the toxicity data from a previous dose-finding study.
Negative control:	Physiological saline
Positive control:	Mitomycin C (MMC; 0.5 mg/kg)

Study Validity

The following study validity criteria were satisfied:

1. The mean incidences of micronucleated reticulocytes in the vehicle (negative control and positive control groups were within the respective historical control ranges.

Results
Positive results were considered to have occurred when both of the following two results were obtained.

1. There was a significant increase in the number of micronucleated reticulocytes for at least one dose of CXA-101 when compared to the historical negative control data.
2. There was a dose-dependent increase in the incidence of micronucleated reticulocytes.

Mean values for the incidence of micronucleated reticulocytes were 1.2 – 2.6% for males and 1.0 – 2.1% for females in the CXA-101 groups. These values were similar for the vehicle control group (1.8% for males and 1.4% for females). Significant differences from the negative control historical data were not observed and the CXA-101 responses did not follow a dose-dependent pattern. In contrast, the mean values for the incidence of micronucleated reticulocytes was 14.6% for males and 11.0% for females in the positive control, MMC, group.

11 Integrated Summary and Safety Evaluation

Secondary Pharmacology

Ceftolozane, tested in a number of assays for its ability to bind receptors and inhibit enzymes, was shown at high concentrations to inhibit binding to several receptors, including specific opioid receptors and also inhibit the activity of 8 enzymes including specific phosphodiesterase isozymes. Ceftolozane at a concentration of 766 μg/ml (approximately 13-fold greater than the mean clinical C_{max} value) produced more than 50% inhibition of binding to 8 receptor targets (histamine H3, opioid delta 2, opioid kappa 1, opioid mu, purinergic P2Y, sigma 1, cholecystokinin CCK1, and neurokinin NPY1). Ceftolozane was also shown to inhibit more than 50% of the activity of 8 enzymes including several phosphodiesterases (PDE10A2, PDE2A, PDE3B, PDE4A1A, PDE5A1) as well as protein kinases Akt1, protein kinase MEK1, and histone deacetylase SIRTUINI.

Tazobactam and its major metabolite, tazobactam M1, inhibited binding to or the activity of a total of 3 targets. Tazobactam at a concentration of 448 μg/ml (approximately 20-fold greater than the mean clinical tazobactam C_{max} value) produced more than 50% inhibition of binding to to 1 target, MEK1. Tazobactam M1 at a concentration of 30 μg/ml (approximately 20-fold greater than the mean clinical tazobactam M1 C_{max} value) produced more than 50% inhibition of 2 targets, binding to cannabinoid CB1 and the activity of phosphodiesterase PDE3A.

For all of the targets, the ceftolozane and tazobactam inhibitory concentrations that were tested greatly exceeded the expected clinical plasma C_{max} values. Also, toxicities related to the affected receptors and enzymes including indications of drug dependency and/or CNS functional changes that might be expected to occur with opioid or cannabinoid receptor interactions were not observed in the safety pharmacology and

general toxicology studies. These results suggest that the off-target effects of the two compounds will not substantially contribute to toxicologically relevant effects associated with clinical administration of Zerbaxa™.

Safety Pharmacology

Ceftolozane was assessed in CNS (neuropharmacological profile, convulsive activity), cardiovascular (hERG activity, cardiovascular effects in rats, cardiovascular evaluation in conscious dogs), and respiratory safety pharmacology studies. In addition, the histamine releasing ability of ceftolozane was assessed in human peripheral blood cells.

Ceftolozane was shown to stimulate convulsions in mice and rats, but only at very high intracerebrospinal-injection doses (428 μg/head in mice and 789 μg/head in rats) which were respectively 24 and 7.4 times higher than the doses of cefoscilis (a marketed cephalosporin) that stimulated convulsions in the same models. Ceftolozane did not produce neuropharmocological effects (siezures, startle-response motor activity, decreased grip strength, immobility), or pain or changes in body temperature at single IV doses as high as 689 mg/kg in rats.

In a hERG assay, ceftolozane did not inhibit hERG activity at concentrations as high as 1 mM, or alter any ECG parameters in conscious dogs at single IV doses ≤ 300 mg/kg. However, one of four dogs receiving 300 mg/kg IV ceftolozane demonstrated a transient increase in heart rate, and rats receiving single IV doses of 100, 320, and 1000 mg/kg ceftolozane transiently demonstrated dose-dependent decreases in both blood pressure (8-27%) and heart rate (8-22%).

Pulmonary function parameters (respiratory rate, tidal volume, and minute volume) were not affected by single IV doses ≤ 689 mg/kg of ceftolozane in rats and ceftolozane concentrations of ≤ 300 μg/ml did not induce human peripheral white blood cells to release histamine above spontaneous release background levels.

The results of the safety pharmacology studies suggest ceftolozane at clinical plasma and brain concentrations should not produce CNS, cardiac, or respiratory toxicity other than possible transient changes in heart rate and blood pressure.

Pharmacokinetics

Absorption

In general ceftolozane C_{max} and AUC values increased in a dose-proportional manner for mice, rats, and dogs following single- or repeated-IV dosing of 10 to 2000 mg/kg CXA-101 with no major gender differences or indications of substantial accumulation with repeated dosing. Plasma $t_{1/2}$ values were on the order of 1 hour or slightly less for rats and dogs. Estimated volume of distribution values in rats (194 ml/kg) and dogs (~250 ml/kg) suggested distribution beyond the vascular compartment in both species. In a single-dose dog study where CXA-101 and tazobactam were administered intravenously in combination, tazobactam pharmacokinetic values were not altered, but CXA-101 C_{max} and AUC values were reduced approximately 10% with combination administration. However, plasma $t_{1/2}$ values were not changed with the combination administration.

Two studies, a single dose pharmacokinetic study in Beagle dogs, and a repeat-dose toxicokinetic analysis in rats, were conducted to examine the pharmacokinetic parameters of CXA-101 and tazobactam administered together or alone. The plasma C_{max} and AUC values changed in a complicated manner when the two compounds were administered together depending primarily on how long the compounds were administered together (Table 106). Generally single combination doses produced lower plasma C_{max} and AUC values compared to individual administration, while repeated combination doses produced higher C_{max} and AUC values compared to the repeated individual administrations. An exception to this pattern is the reduction in tazobactam C_{max} and AUC values after 28-days of combination dosing in rats (Study No.: CXA201-T-001). However, for all patterns, the changes were generally on the order of 20% or less at the highest doses with lesser or no changes with lower doses. The overall results indicate that neither compound greatly altered the pharmacokinetics of the other, and that neither compound substantially accumulated with repeated dosing.

Table 106: Pharmacokinetic and Toxicokinetic Patterns for Ceftolozane (CXA-101) and Tazobactam Administered in Combination and Alone.

Study Description/Doses	Effects
Study No.: CXA201-P-001/ Single IV dose PK study in dogs with CXA-101 and tazobactam administered in combination and alone. **Doses** CXA:101: 10, 50, 100 mg/kg Tazobactam: 5, 25, 50 mg/kg	When CXA-101 and tazobactam were co-administered, the CXA-101 C_{max} was **reduced** 3% to 20% and the mean AUC was **reduced** 9% to 14% across all dose levels compared to the individual agents administered alone. Tazobactam values were not similarly affected and did not greatly change.
Study No.: CX.101.PK.005/ Single SC or IP dose PK study in rats with CXA-101 and tazobactam administered in combination or with tazobactam alone. **Doses** CXA-101: 150 mg/kg Tazobactam: 75 mg/kg	The plasma C_{max} and AUC values for tazobactam were **reduced** 29% and 18% respectively when administered subcutaneously and 6% and 27% respectively when administered intraperitoneally in combination compared to alone.
Study No.: CXA201-T-001/ BID IV administration of CXA-101 and tazobactam in combination and alone for 28 days in rats. **Pertinent Dose Groups (mg/kg/day)** Group 2: 1000 CXA-101 Group 3: 500 tazobactam Group 4: 100 CXA-101/50 tazobactam Group 5: 250 CXA-101/125 tazobactam Group 6: 1000 CXA-101/500 tazobactam	**AUC and C_{max} on Individual Days: Combination Versus Alone (High Doses)** Day 1: Plasma C_{max} and AUC values **were similar** for both CXA-101 and tazobactam in combination compared to alone. Day 28: Plasma C_{max} and AUC values **were similar** for both CXA-101 and tazobactam in combination compared to alone.
	Day 28 vs Day 1 Values (High Doses) Combination: Plasma C_{max} and AUC values were similar for CXA-101, but **decreased** 57% and 54% respectively for tazobactam on Day 28 compared to Day 1. Individual Agents: Similar patterns were evident for the agents administered alone.
Study No.: CXA201-T-005/ BID IV administration of CXA-101 and tazobactam in combination and alone for 14 days in dogs. **Pertinent Dose Groups (mg/kg/day)**	**AUC and C_{max} on Individual Days: Combination Versus Alone (High Doses)** Day 0: Plasma C_{max} and AUC values were **decreased** approximately 12% and 13% respectively for CXA-101 and 9% and 2% respectively for tazobactam in combination compared to alone.

Group 2: 300 CXA-101 Group 3: 150 tazobactam Group 4: 100 CXA-101/50 tazobactam Group 5: 300 CXA-101/150 tazobactam	Day 13: Plasma C_{max} and AUC values were **increased** approximately 14% and 27% respectively for CXA-101 and **decreased** approximately 19% and 26% for tazobactam in combination compared to alone.
	Day 13 vs Day 0 Values (High Doses) Combination: Plasma C_{max} and AUC values were approximately 14% and 24% **increased** respectively for CXA-101 and 19% and 20% **higher** respectively for tazobactam on Day 13 compared to Day 0. Individual Agents: Plasma C_{max} and AUC values were **decreased** approximately 10% and 14% respectively for CXA-101 and 2% and 13% for tazobactam on Day 13 compared to Day 0

Distribution

In a rat mass-balance study with ^{14}C-ceftolozane, the radioactivity profile in plasma and blood followed similar biphasic elimination patterns with a plasma $t_{1/2}$ for the first two hours after administration of less than 1 hour, and a terminal $t_{1/2}$ of more than 50 hours. At all time-points the ratio of radioactivity in blood compared to plasma was less than 1. The highest tissue concentrations of radioactivity occurred in the kidney, followed by the urinary bladder, plasma, blood, skin, and lung. Measureable quantities of radioactivity were found in the kidney but not plasma 336 hours after administration.

In the rat study examining CXA-101 transport into the brain, plasma and brain concentrations increased in an approximately dose-linear manner, but brain C_{max} concentrations were approximately 800-fold lower than plasma C_{max} concentrations. However, the brain $t_{1/2}$ values was approximately 10 times as long as the plasma $t_{1/2}$.

CXA-101 concentrations in the kidney were also shown to increase with dose as well as duration of dosing up to 5 days of dosing with the high dose (300 mg/kg/day) when kidney accumulation appeared to saturate. In studies examining accumulation of CXA-101 in the kidneys of rats and dogs after 4-weeks of dosing, CXA-101 accumulated in the kidneys of both species in a roughly dose-dependent manner and kidney levels were not detectable 4 weeks after the end of dosing.

CXA-101 demonstrated low plasma protein binding. The *in vitro* protein binding of CXA-101 was 10-20% for mouse, rat, dog, and human plasma. The *in vitro* blood to plasma concentration ratio for CXA-101 was approximately 60% for mice, rats, dogs, and humans. Approximately 10% of the CXA-101 transferred into red blood cells for each species.

The Zosyn® label indicates that tazobactam is widely distributed into tissues and body fluids including intestinal mucosa, gallbladder, lung, female reproductive tissues (uterus, ovary, and fallopian tubes), interstitial fluid, and bile. Tazobactam is also reportedly 30% bound to plasma proteins.

Metabolism

In rats, ceftolozane was shown to produce 11 unidentified metabolites, but as in humans, the extent of metabolism was minimal and unchanged ceftolozane accounted for most of the total compound in plasma, urine, feces, and bile. In rats, 1 unidentified metabolite appeared in small amounts in plasma and feces and 4 of the metabolites

appeared at minimal levels in urine, bile, and kidney. Also in rats, ceftolozane glucuronide or sulphate conjugates were not detected in urine or bile. In CYP450 assays, ceftolozane did not significantly inhibit or induce specific CYP450 isozymes.

Like ceftolozane, tazobactam and tazobactam M-1 did not significantly inhibit or induce a range of CYP450 isozymes. The Zosyn® label indicates that tazobactam is metabolized into a single metabolite (M1) that lacks pharmacological and antibacterial activities. According to the label, protein binding to the tazobactam M1 metabolite is negligible.

Excretion

The biphasic elimination pattern may indicate that ceftolozane redistributes from the kidney to plasma following the initial phase of elimination. In rats and dogs, radioactive ceftolozane elimination occurred primarily in urine with over 75% of the radioactivity detected in urine in both species 24 hours after intravenous dosing. In rats, 96 hours after dosing, 96.5% of radioactivity was detected in urine compared to 2.2% in feces and 0.4% in bile after 48 hours.

Both ceftolozane and tazobactam are excreted primarily via urine through glomerular filtration. However, coadministration of the two drugs in rat and dog studies did not substantially alter clearance of either drug suggesting the two drugs do not compete for active tubular secretion pathways.

General and Special Toxicities

Ceftolozane in 28-day studies in both rats and dogs with doses as high as 1000 mg/kg/day, produced dose-dependent nephritic changes in the form of hyaline droplet formation in proximal tubules of the renal cortex. This form of kidney pathology, which is observed with other cephalosporin antibiotics, is thought to represent an adaptation allowing compound disposition via lysosomes. Hyaline droplet formation was a consistent but reversible effect. In the absence of toxicologically meaningful degeneration or necrosis of renal tubular epithelium or substantial changes in relevant clinical pathology parameters including serum BUN, creatinine, inorganic phosphorus and/or urine volume, the hyaline-droplet formation was not considered adverse.

However, in a non-GLP, range-finding, study in juvenile rats, ceftolozane-related kidney findings, in addition to hyaline droplet formation, included tubular basophilia and renal cortical fibrosis. These effects are consistent with tubular cell loss and regeneration. While it is unclear if this is a rat-specific effect, the results suggest ceftolozane administration in juvenile animals may have a greater potential to produce kidney toxicity than administration in adults. Currently Zerbaxa™ is not proposed for treatment of patients less than 18 years of age. If pediatric clinical trials preliminary to approval for treatment of pediatric patients are proposed in the future, kidney function should be monitored.

The primary pathology associated with tazobactam administration in the rat and dog 28-day, repeated-dose, IV-combination studies as well as published 6-month repeated-dose studies in rat (intraperitoneal administration) and dog (IV administration) was a dose-dependent increase in liver weights and liver histopathology consistent with the accumulation of liver glycogen and increased smooth endoplasmic reticulum. The

histopathology occurred diffusely in liver sections, was reversible, and was characterized by accumulation of pale, eosinophilic, foamy to finely vacuolated material within the cytoplasm of hepatocytes. In rat studies, dose-dependent serum chemistry changes including decreased tryglycerides, albumin, and glucose and increased globulin and potassium were considered to be related to the liver changes and glycogen accumulation. However, because changes were generally of low magnitude, reversible, and not associated with toxicologically meaningful degeneration or necrosis of hepatocytes or biologically meaningful changes in liver enzymes, the changes were not considered adverse. Higher doses of tazobactam were also associated with dose-dependent decreases in hematocrit, hemoglobin, and red blood cell counts, as well as occasional increases in platelets and the percent of lymphocytes. However, the hematology changes were generally mild, reversible, and did not extend to bone marrow pathology.

The warnings and precautions section of the Zosyn® (piperacillin plus tazobactam) label warns of bleeding manifestations associated with β-lactam drugs including abnormalities of coagulation, platelet aggregation, and prothrombin time. Both thrombocytopenia and thrombocytosis have been linked to the administration of Zosyn® in literature case-study reports. An analysis of hematology and coagulation data from several nonclinical studies supporting the present NDA for ceftolozane and tazobactam indicates that significant platelet and or coagulation changes associated with the individual or combined administration of the test articles did not consistently occur (Table 107). Also when changes in platelet and/or coagulation parameters did occur, synergism or augmentation associated with combined treatment was not observed.

Table 107: Individual and/or Combined effects of Ceftolozane (CXA-101) and Tazobactam on Platelet and Coagulation Parameters in General Toxicology Studies in Rats and Dogs.

Study Type/ Study No./ Dose Information	Platelet Counts	APTT	PT	Fibrinogen
28-Day Combination Study in Rats/ Study No.: CXA201-T-001 **Pertinent Groups** **Group 2:** 1000 mg/kg/day CXA-101 **Group 3:** 500 mg/kg/day tazobactam **Group 6:** 1000/500 mg/kg/day CXA-101/tazobactam	**Group 3** Males: +34% Females: +28% **Group 6** Males: +29% Females: +31%	**Group 2** Males: -5.5% Females: NSC **Group 3** Males: -9% Females: NSC **Group 6** Males: -9% Females: NSC	**Group 2:** NSC **Group 3:** NSC **Group 6** Males: -5.0% Females: NSC	Not measured
4-week with CXA-101 in rats/Study No.: TX033014 **Doses:** CXA-101 IV doses of 100, 300, 1000 mg/kg/day	NSC	NSC	NSC	NSC
14-Day Combination Study in Dogs/ Study No.: CXA201-T-005 **Doses (mg/kg/day):** 300 CXA-101, 150 tazobactam, 100/50 CXA-101/tazobactam, 300/150 CXA-101/tazobactam	NSC	NSC	NSC	NSC

4-week with CXA-101 in dogs/Study No.: TX033013 **Doses mg/kg/day:** 100, 300, 1000 mg/kg/day CXA-101	NSC	NSC	NSC	NSC
28-Day study with 3 different batches of CXA-101 in rats/ Study No.: CX.101.TX.031 **Doses mg/kg/day:** (three different batches: 100, 300, 1000 CXA-101	NSC	6.4% to 7.8% reductions in females only	NSC	NSC
14-Day Study Combination Study in Juvenile rats/ Study No.: CXA.101.TX.033 **Doses (mg/kg/day):** 50/25 CXA-101/tazobactam, 300/150 CXA-101/tazobactam, 1000/500 CXA-101/tazobactam	NSC	Not measured	Not measured	Not measured
6-month IP dose study with tazobactam in rats. **Doses (mg/kg/day):** 40, 80, 160 mg/kg//day tazobactam	NSC	Not measured	NSC	NSC
6-month IV dose study with tazobactam in dogs. **Doses (mg/kg/day):** 40, 80, 160 mg/kg//day tazobactam	NSC	Not measured	NSC	NSC
NSC = No significant changes				

In repeated-dose combination studies with administration of ceftolozane plus tazobactam as well as each compound alone in rats (1-month) and dogs (2-weeks), new or augmented toxicities were not observed. As in previous studies using the single agents, dose-dependent reversible hyaline-droplet formation in kidneys was observed with the administration of ceftolozane and dose-dependent, reversible glycogen accumulation in liver occurred with tazobactam administration in rats. In the dog combination study using high-doses of 300/150 mg/kg/day ceftolozane/tazobactam tazobactam, toxicities were absent. In both rats and dogs, plasma concentrations of ceftolozane and tazobactam were not substantially changed when the compounds were administered in combination and plasma concentrations for both agents did not substantially increase with repeated dosing.

Other toxicities included cecal enlargement, injection-site reactions, histamine release and antigenicity under sensitizing conditions. Cecal enlargement occurred with both ceftolozane and tazobactam administered alone in rats. This effect, commonly associated with antibiotic treatment in rodents and rabbits, was not reported to become more severe with combination treatment. Severe GI effects are not expected in humans.

Injection-site reactions including erythema, edema, desquamation, subcutaneous hemorrhage, perivascular hemorrhage, perivascular fibrosis, inflammation and scabbing occurred for both ceftolozane and tazobactam but only with repeated-dosing in mice and rats. The effects were dose and concentration dependent. In addition to an absence of injection-site reactions in dogs, no injection-site reactions were reported for the combination rat study where maximal concentrations of ceftolozane and tazobactam were 200/100 mg/ml which is much greater than the concentrations recommended for clinical administration (10 mg/ml ceftolozane and 5 mg/ml tazobactam).

Ceftolozane did not stimulate histamine release *in vitro* in isolated human peripheral white blood cells, and histamine release and/or histamine-related clinical signs were not noted for rats administered ceftolozane. However, in single- and repeated-dose studies, dogs administered ceftolozane demonstrated increased plasma histamine and multiple clinical signs consistent with histamine release including vomiting, weakness, and redness of the ears and oral mucosa. Dogs appear to be more sensitive than other species to ceftolozane-related histamine release, and humans are not expected to be greatly affected.

Ceftolozane also did not stimulate antigenic responses in mice, but guinea pigs actively sensitized to ceftolozane in the presence of Freund's adjuvant experienced systemic anaphylaxis reactions upon re-exposure to ceftolozane alone. The same animals experienced positive antibody titers associated with passive cutaneous antibody reactions. These results suggest that ceftolozane, while not greatly antigenic, has the potential to elicit allergic reactions like other β-lactam antibiotics.

Carcinogenicity and Genetic Toxicity

Ceftolozane and tazobactam are only recommended for short-term administration (≤ 14 days). Consequently nonclinical carcinogenicity assessments were not recommended for either compound. Also the weight of evidence suggests both ceftolozane and tazobactam and their combination do not pose a strong potential for genotoxicity in humans. The combination of ceftolozane and tazobactam (ZERBAXA) was assessed in several *in vitro* and *in vivo* genetic toxicity assays. ZERBAXA was negative for genotoxicity in an *in vitro* mouse lymphoma assay and an *in vivo* rat bone marrow micronucleus assay. In an *in vitro* chromosomal aberration assay using Chinese hamster ovary cells, ZERBAXA was positive for structural aberrations. Similarly, ceftolozane alone was negative for genotoxicity in an *in vitro* microbial mutagenicity (Ames) assay, an *in vitro* chromosomal aberration assay using Chinese hamster lung fibroblast cells, an *in vivo* mouse micronucleus assay, and an *in vivo* unscheduled DNA synthesis (UDS) assay, but positive results for mutagenicity were obtained for ceftolozane in an *in vitro* mouse lymphoma assay. Tazobactam alone was negative for genotoxicity in all assays including in an *in vitro* microbial mutagenicity (Ames) assay, an *in vitro* Chinese hamster lung fibroblast cell chromosomal aberration assay, and an *in vivo* rat bone marrow micronucleus assay.

Reproductive and Developmental Toxicity

In a rat fertility study, ceftolozane had no adverse effects on fertility in males or females at intravenous doses up to 1000 mg/kg/day. The mean plasma exposure (AUC) value at this dose is approximately 8 times the mean daily clinical ceftolozane exposure value. In a rat fertility study with intraperitoneal tazobactam, male and female fertility parameters were not significantly affected at doses ≤ 640 mg/kg/day (approximately 4 times the recommended clinical daily dose based on body surface area comparison).

Embryo-fetal development studies performed in mice and rats with IV ceftolozane doses up to 2000 and 1000 mg/kg/day, respectively, revealed no teratogenicity and no evidence of harm to the fetus. The mean plasma exposure (AUC) values associated with these doses are approximately 19 (mice) and 11 (rats) times the mean daily human ceftolozane exposure at the clinical dose of 1 gram administered three times per day. It

is not known if ceftolozane crosses the placenta in animals. In an embryo-fetal study in rats, tazobactam administered at IV doses up to 3000 mg/kg/day (approximately 19 times the recommended human dose based on body surface area comparison) did not produce maternal toxicity, or fetal toxicity or teratogenicity. In rats, tazobactam was shown to cross the placenta. Concentrations in the fetus were less than or equal to 10% of those found in maternal plasma.

In a pre- postnatal study in rats, IV ceftolozane administered during pregnancy and lactation (Gestation Day 6 through Lactation Day 20) was associated with a decrease in auditory startle response in postnatal day 60 male and female pups at maternal doses of ≥ 300 mg/kg/day. The plasma exposure (AUC) associated with the NOAEL dose of 100 mg/kg/day in rats is approximately equal to the mean human ceftolozane exposure at the clinical dose of 3 grams/day. In a pre-postnatal study in rats, tazobactam administered intraperitoneally twice daily at the end of gestation and during lactation (Gestation Day 17 through Lactation Day 21) produced decreased maternal food consumption at the end of gestation and significantly more stillbirths with a tazobactam dose of 1280 mg/kg/day. No effects on the development, function, learning or fertility of F_1 pups were noted, but the postnatal body weights for F_1 pups from dams receiving 320 and 1280 mg/kg/day tazobactam were significantly reduced 14 and 21 and 7, 14 and 21 days after delivery respectively. F_2 generation fetuses were normal for all doses of tazobactam. The NOAEL for reduced F_1 body weights was considered to be 40 mg/kg/day (approximately 0.3 times the recommended human dose based on body surface area comparison). Exclusive of reduced body F_1 generation body weights, the NOAEL was considered to be 320 mg/kg/day or approximately equal to the recommended human dose based on body surface area comparisons.

Taken as a whole, the nonclinical toxicology data suggests relative safety for clinical administration of Zerbaxa™ at the recommended dose of 4500 mg/kg/day (3000 mg/kg/day ceftolozane and 1500 mg/kg/day tazobactam). The primary effects of each compound in nonclinical studies, hyaline droplet formation in the kidneys for ceftolozane and liver glycogen accumulation for tazobactam are not considered adverse. Other toxicities that occurred in nonclinical studies, cecal enlargement, injection site reactions, and histamine release are not expected to occur with clinical administration. The nonclinical data suggests that ceftolozane is not greatly antigenic, but like other β-lactam antibiotics has the potential to elicit allergic reactions. In embryo-fetal studies, neither ceftolozane nor tazobactam was teratogenic or caused fetal toxicity. However, both compounds produced limited adverse effects in F_1 generation pups in pre-postnatal studies (decreased auditory startle response for ceftolozane and increased still-births and reduced postnatal body weights for tazobactam) suggesting the benefits of administration of Zerbaxa™ to pregnant women particularly at higher than recommended doses should be weighed against its potential subset of adverse effects in offspring.

12 Appendix/Attachments

Table 108: Hematology and Coagulation Parameters

Study No.	CXA201-T-005	CX.101.TX.031	CXA.101.TX.033	Hayashi *et al.*,	Hayashi *et al.*,	TX043007

				1994a	1994b	
Species	**Dog**	**Rat**	**Rat**	**Rat**	**Dog**	**Rat**
Hemoglobin concentration	X	X	X	X	X	X
Hemoglobin distribution width	X	X				
Hematocrit	X	X	X	X	X	X
Erythrocyte count	X	X	X	X	X	X
Platelet count or platelet estimate	X	X	X		X	X
Plateletcrit/ thrombocrit						
Mean platelet volume		X	X			
Mean corpuscular volume	X	X	X	X	X	X
Mean corpuscular hemoglobin	X	X		X	X	X
Mean corpuscular hemoglobin concentration	X		X	X	X	
Red cell distribution width	X	X				
Total leukocyte count	X	X	X	X	X	X
Reticulocyte count (absolute and relative)	X	X	X	X		X (reticulocyte ratio)
Reticulocyte hemoglobin content						
Differential leukocyte count (Absolute and percent neutrophil, lymphocyte, monocyte, eosinophil, basophil counts)	X	X	X	X	X	X
Blood smear for cell morphology (if necessary for interpretation)						
Red cell morphology	X	X				
Activated partial thromboplastin time (APTT)	X	X		X	X	X
Prothrombin time (PT)	X	X		X	X	X

Fibrinogen		X				X

Table 109: Clinical Chemistry Parameters

Study No.	**CXA201 -T-005**	**CX.101 .TX.031**	**CXA.101. TX.033**	**Hayashi *et al.*, 1994a**	**Hayashi *et al.*, 1994b**	**TX0430 07**
Species	**Dog**	**Rat**	**Rat**	**Rat**	**Dog**	**Rat**
Aspartate aminotransferase	X	X	X	X	X	X
Alanine aminotransferase	X	X	X	X	X	X
Alkaline phosphatase	X	X	X	X	X	X
Blood urea nitrogen				X		X
Urea						
Urea Nitrogen	X	X	X		X	
Creatinine	X	X	X	X	X	X
Creatinine kinase						
Glucose	X	X	X	X	X	X
Cholesterol	X	X	X	X	X	X
Triglycerides	X	X	X	X	X	X
Total protein	X	X	X	X	X	X
Albumin	X	X	X	X	X	X
Total bilirubin	X	X	X	X	X	X
Sodium	X	X	X	X	X	X
Sorbitol dehydrogenase	X	X				
Potassium	X	X	X	X	X	X
Chloride	X	X	X	X	X	X
Calcium	X	X	X	X	X	X
Inorganic phosphorus or phosphate	X	X	X	X	X	X
Gamma-glutamyl transferase	X	X	X	X	X	
Glutamate dehydrogenase						
Globulin	X	X	X	X	X	
Albumin/globulin ratio	X	X	X	X		X
Hemolysis, Lipemia, Icterus						
Phospholipid					X	
Uric Acid					X	
Lactic dehydrogenase				X		

Table 110: Urinalysis Parameters

Study No.	**CXA201-T-005**	**CX.101. TX.031**	**CXA.101. TX.033**	**Hayashi *et al.*,**	**Hayashi *et al.*,**	**TX043007**

				1994a	1994b	
Species	**Dog**	**Rat**	**Rat**	**Rat**	**Dog**	**Rat**
Specific gravity	X	X		X	X	X
pH	X	X		X	X	X
Urobilinogen	X	X		X	X	X
Total volume	X	X		X	X	X
Color	X	X			X	X
Clarity	X	X				
Protein	X	X		X	X	X
Glucose	X	X		X	X	X
Ketones	X	X		X	X	X
Bilirubin	X	X		X	X	
Occult blood	X	X		X	X	X
Leukocytes	X	X				
Nitrites	X	X			X	
Microscopy of sediment	X	X		X	X	
Creatinine		X				
Sodium		X				
Potassium		X				
Chloride		X				

Table 111: Histopathology and Organ Weight Inventory

Study #	**CXA201-T-005**	**CX.101.TX.031**	**CXA.101.TX.033**	**Hayashi *et al.*, 1994a**	**Hayashi *et al.*, 1994b**	**TX043007**
Species	**Dog**	**Rat**	**Rat**	**Rat**	**Dog**	**Rat**
Adrenals	X*	X*	X*	X*	X*	X*
Administration site			X			
Aorta	X	X		X	X	X
Bone Marrow smear		X		X	X	X
Bone (sternum, and/or femur and/or rib)	X	X		X	X	X
Brain	X*	X*	X*	X*	X*	X*
Bronchi, main stem						X
Cecum	X	X		X	X	X*
Cervix	X	X				
Colon	X	X		X	X	X
Conjunctiva						
Duodenum	X	X		X	X	
Epididymis	X*	X*		X*	X*	X
Esophagus	X	X		X	X	X
Eye	X	X		X	X	
External ear						

Fallopian tube						
Gall bladder	X				X	
Gross lesions	X	X	X			
Harderian gland						X
Heart	X*	X*	X*	X*	X*	X*
Herder's gland				X		
Hypophysis						
Ileum	X	X		X	X	X
Injection site	X	X				X
Jejunum	X	X		X	X	X
Joint, tibiofemoral						
Kidneys	X*	X*	X*	X*	X*	X*
Lachrimal gland						
Larynx		X		X	X	
Liver	X*	X*	X*	X*	X*	X*
Lungs	X	X	X*	X*	X*	X*
Lymph nodes, inguinal					X	
Lymph nodes, axillary	X	X			X	
Lymph nodes, mediastinal				X	X	
Lymph nodes mandibular	X	X			X	X
Lymph nodes, mesenteric,	X	X		X	X	X
Mammary Gland	X			X	X	X
Muscle (biceps, femoris)						
Nasal cavity						
Nasal turbinates						
Optic nerves	X	X		X		
Ovaries	X*	X*		X*	X*	X*
Oviduct	X	X*				
Pancreas	X	X	X	X	X	X
Parathyroids	X*	*		X*		X
Peripheral nerve	X	X				
Peyer's patches	X	X				
Pharynx		X				
Pituitary	X*	X*		X*	X*	X*
Prostate	X	X		X*	X*	X*

Rectum	X	X		X	X	X
Salivary gland	X	X				*
Sciatic nerve				X	X	X
Seminal vesicles		X		X*		X*
Skeletal muscle	X	X		X	X	X
Skin	X	X		X	X	X
Spinal cord	X	X		X	X	X
Spleen	X*	X*	X*	X*	X*	X*
Sternum					X	X
Stomach	X	X	X	X	X	X
Submandibular Gland (right)				X	X*	X
Sublingual gland						X
Testes	X*	X*		X*	X*	*
Thymus	X*	X*	X*	X*	X*	X*
Thyroid	X*	X*		X*	X*	X*
Tongue	X	X		X	X	
Tonsils					X	
Trachea	X	X		X	X	X
Ureter	X			X	X	
Urethra				X	X	
Urinary bladder	X	X		X	X	X
Uterus	X*	X*		X*	X*	X*
Vagina	X	X		X	X	X
Vertebra, Lumbar						
Zymbal gland						
X = tissue collected for histopathology; * weighed organ						

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/s/

JAMES S WILD
10/23/2014

WENDELYN J SCHMIDT
10/23/2014

I concur with Dr. Wild's assessment of the comprehensiveness of the nonclinical studies, as well as his interpretation of the data.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 206829 **Applicant:** Cubist Pharmaceuticals **Stamp Date:** 2/14/2014 and 4/21/2014

Drug Name: Zerbaxa (ceftolozane/tazobactam) NDA/BLA Type: 505b2

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
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		
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? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	While the labeling sections relative to pharmacology/toxicology are generally appropriate, some changes and additions in labeling language will be needed.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurities for tazobactam will be assessed based on information included in Type II Drug Master File No. (b) (4) for which the Sponsor has rights of reference.
11	Has the applicant addressed any abuse potential issues in the submission?	X		Because of its short duration of clinical administration (≤ 14 days), and because no evidence of dependence or withdrawal was noted in any of the repeated-dose nonclinical toxicology studies, the product is not considered to have a notable abuse potential.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____Yes____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

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

Reviewing Pharmacologist Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

Team Leader/Supervisor Date

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

/s/

JAMES S WILD
06/18/2014

WENDELYN J SCHMIDT
06/19/2014