

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: 203441
Supporting document/s: 002
Applicant's letter date: November 30, 2011
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Product: Gattex[®] (Teduglutide)
Indication: Short Bowel Syndrome (SBS)
Applicant: NPS Pharmaceuticals
Review Division: Division of Gastroenterology and Inborn Errors
Products (DGIEP)
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1 Executive Summary

1.1 Introduction

Teduglutide is a 33 amino acid peptide that differs from its natural analog, glucagon-like peptide-2 (GLP-2) in the substitution of alanine (in native GLP-2) for glycine at the second position at the N-terminus. This single amino acid substitution provides resistance to *in vivo* degradation of teduglutide by dipeptidyl protease-IV (DPP-IV) resulting in an extended half-life. Teduglutide is manufactured using a recombinant strain of *Escherichia coli*.

Teduglutide has been shown to preserve mucosal integrity by promoting repair and normal growth of the intestine. Teduglutide increased villus height and crypt depth of the intestinal epithelium resulting in enhanced absorptive capacity of the intestine as demonstrated by greater absorption of fluids, electrolytes and nutrients, reduced fecal fluid loss, and diminished diarrhea. In addition, teduglutide accelerated intestinal adaptation, increased nutrient transporter activity, enhanced barrier function in the small intestine and decreased intestinal inflammation. These effects of teduglutide formed the rationale for use in patients with short bowel syndrome (SBS). Short bowel syndrome is characterized by the inadequate absorption of fluid and nutrients in patients who have undergone significant small bowel resection. This NDA was submitted to support the marketing approval of teduglutide for the treatment of SBS in adult patients and for the improvement of intestinal absorption of fluid and nutrients.

1.2 Brief Discussion of Nonclinical Findings

The applicant has conducted adequate nonclinical studies with teduglutide which included pharmacology, safety pharmacology, pharmacokinetics, acute toxicology studies mice, repeated dose toxicology studies in mice (14 days to 26 weeks duration), rats (14 day to 13 weeks duration), Cynomolgus monkeys (14 to 1 year duration), toxicology studies in juvenile minipigs (14 days to 90 days duration), genotoxicity studies (Ames test, chromosome aberration test in Chinese hamster ovary cells, *in vivo* micronucleus test in mice), reproductive toxicology studies (fertility and early embryonic development in rats, embryofetal development in rats and rabbits, and pre and postnatal development in rats), and special toxicology studies (antigenicity and local tolerance studies). Toxicology studies were conducted using the subcutaneous (SC) route, the intended clinical route of administration.

In toxicology studies, teduglutide was administered subcutaneously to mice (26-week treatment) up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg), rats (13-week treatment) up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg), and Cynomolgus monkeys (1-year treatment) up to 25 mg/kg/day (about 500 times the recommended daily human dose of 0.05 mg/kg). The lowest reported AUC (either male or female) values at the highest tested doses in mice (AUC_{0-8h} of 58.5 $\mu\text{g}\cdot\text{hr}/\text{mL}$ at 50 mg/kg/day), rats (AUC_{0-8h} of 36.7

$\mu\text{g}\cdot\text{hr}/\text{mL}$ at 50 mg/kg/day) and monkeys ($\text{AUC}_{0-8\text{h}}$ of 64.6 $\mu\text{g}\cdot\text{hr}/\text{mL}$ at 25 mg/kg/day) were about 250, 156 and 275 times, respectively, the human exposure ($\text{AUC}_{0-\infty}$ of 0.235 $\mu\text{g}\cdot\text{hr}/\text{mL}$) at the proposed clinical dose of 0.05 mg/kg/day.

In pivotal repeated dose toxicology studies, major treatment-related effects were related to the pharmacological activity of teduglutide which were seen in all species. In the 26-week study in mice at 2, 10 and 50 mg/kg/day, major treatment-related histopathological changes were seen at all doses in the small and large intestine (epithelial and villus hypertrophy and hyperplasia), gall bladder (epithelial hypertrophy and hyperplasia accompanied by subacute inflammation), sternal bone marrow (myeloid hyperplasia) and injection site (inflammation and necrosis). In the 13-week study in rats at 10, 25 and 50 mg/kg/day, major treatment-related histopathological changes were seen at all doses in the small and large intestine (mucosal hypertrophy and hyperplasia) and injection site (inflammation and necrosis). In the 1-year study in Cynomolgus monkeys at 1, 5 and 25 mg/kg/day, major treatment-related histopathological changes were seen at all doses in the small and large intestine (mucosal hyperplasia), stomach (mucosal hyperplasia), pancreas (hypertrophy/hyperplasia of the pancreatic duct epithelium), liver and gall bladder (epithelial hypertrophy and hyperplasia of the bile duct in the liver and mucosal hypertrophy/hyperplasia of the gall bladder) and injection site (inflammation and necrosis).

Teduglutide was also tested in juvenile minipigs in 14-day and 90-day toxicology studies up to 25 mg/kg/day (about 500 times the recommended daily human dose of 0.05 mg/kg). In the 14-day study at 5 and 25 mg/kg/day, major treatment-related histopathological changes were seen at all doses in the nonglandular stomach (mucosal hyperplasia associated with ulceration/erosion), small and large intestinal tract (hyperplasia), gall bladder (mucosal hyperplasia), bile duct (mucosal hyperplasia) and injection site (inflammation and necrosis). In the 90-day study at 1, 5 and 25 mg/kg/day, major treatment-related histopathological changes were observed at all doses in the small intestines (minimal/slight villous hypertrophy), gall bladder (cystic mucosal hyperplasia at all doses), extrahepatic bile duct (cystic mucosal hyperplasia), and injection site (inflammation and necrosis). In the 90-day study, teduglutide increased the P wave, PR, QT (mid and high dose) and RR intervals at all doses in males at Week 13 compared to respective controls. The ECG changes were predominantly seen in males; however, the plasma exposure (AUC) to teduglutide was higher in females than that in males at Week 13 at all doses. In addition, these changes were seen at only one time point (Week 13), in one species and the magnitude of these changes were small and these changes were also not dose-related. Moreover, there were no significant treatment-related effects on QTc (Fridericia) in either sex. The QTc values were comparable across all groups. Overall, these ECG changes are not meaningful and not toxicologically significant.

Teduglutide was negative in the Ames test, *in vitro* chromosomal aberration test in Chinese hamster ovary (CHO) cells, and *in vivo* mouse micronucleus test. In a 2-year carcinogenicity study by subcutaneous route in Wistar Han rats at 3, 10 and 35 mg/kg/day (about 60, 200 and 700 times the recommended daily human dose of 0.05

mg/kg, respectively), teduglutide caused statistically significant increases in the incidences of adenomas in the bile duct and jejunum of male rats. There were no drug related tumor findings in females. A 2-year mouse carcinogenicity study is ongoing. By virtue of its mechanism of action (intestintrophic activity or growth promoting pharmacological effect) and the findings of the carcinogenicity study in rats, teduglutide has the potential to cause hyperplastic changes including carcinogenicity in humans.

In the subcutaneous fertility and early embryonic development study in rats at 2, 10 and 50 mg/kg/day, teduglutide did not show any adverse effects on early embryonic development or fertility parameters up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg). In the subcutaneous embryofetal development study in rats at 2, 10 and 50 mg/kg/day, teduglutide was not teratogenic up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg). In the subcutaneous embryofetal development study in rabbits at 2, 10 and 50 mg/kg/day, teduglutide was not teratogenic up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg). In the subcutaneous pre and postnatal development study in rats at 10, 25 and 50 mg/kg/day, teduglutide did not show any significant adverse effect on pre and postnatal development up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg).

Overall, nonclinical safety of teduglutide has been adequately tested in several toxicology studies. Nonclinical studies conducted with teduglutide provide adequate assurance of safety and support its proposed use at the intended therapeutic dosage and in accordance with the proposed product labeling. However, by virtue of its mechanism of action (intestintrophic activity or growth promoting pharmacological effect) and the findings of the carcinogenicity study in rats, teduglutide has the potential to cause hyperplastic changes including carcinogenicity in humans.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical standpoint, this NDA is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The draft labeling of Gattex[®] conforms to the content and format of labeling for human prescription drugs under 21CFR201.57(b)(1). However, the following changes are recommended.

8.1 Pregnancy

Applicant's Version:

8.1 Pregnancy

Pregnancy Category B. (b) (4)
(b) (4) *there are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, (b) (4) should be used during pregnancy only if clearly needed.*

Evaluation: The text is not in accordance with 21CFR 201.57(c)(9)(i)(A)(2). The pregnancy category B is acceptable. However, the text should be modified as recommended below to reflect the dose. In addition, the findings of the pre and postnatal development study in rats should be incorporated in this section.

Recommended Version:

8.1 Pregnancy

Pregnancy Category B

Reproduction studies with teduglutide have been performed in pregnant rats at subcutaneous doses up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg) and in rabbits at subcutaneous doses up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg). These studies did not reveal any evidence of impaired fertility or harm to the fetus due to teduglutide. A pre and postnatal development study in rats showed no evidence of any adverse effect on pre and postnatal development at subcutaneous doses up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg). There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, teduglutide should be used during pregnancy only if clearly needed.

8.3. Nursing Mothers

Applicant's Version:

8.3 Nursing Mothers

It is unknown whether (b) (4) is excreted in human milk. (b) (4) concentration in milk was (b) (4) of the plasma concentration following a single SC injection of 25 mg/kg. (b) (4) The use of GATTEX during nursing should be avoided.

Evaluation: The text is in not accordance with 21CFR 201.57(c)(9)(iii) 8.3. Since teduglutide is excreted through breast milk and teduglutide has tumorigenic potential, the text should be modified as follows as per the CFR.

Recommended Version:

8.3 Nursing Mothers

It is unknown whether teduglutide is excreted in human milk. Teduglutide is excreted in the milk of lactating rats and the highest concentration in the milk was 2.9% of the plasma concentration following a single subcutaneous injection of 25 mg/kg. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions to nursing infants from teduglutide and because of the potential for tumorigenicity shown for teduglutide in rats, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Applicant's Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility



Evaluation: The format is in accordance with 21CFR 201.57(c)(14)(i) 13.1. However, the text should be modified as proposed below to reflect the findings of the rat carcinogenicity study.

Recommended Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a 2-year carcinogenicity study in Wistar Han rats at subcutaneous doses of 3, 10 and 35 mg/kg/day (about 60, 200 and 700 times the recommended daily human dose of 0.05 mg/kg, respectively), teduglutide caused statistically significant increases in the incidences of adenomas in the bile duct and jejunum of male rats.

Teduglutide was negative in the Ames test, chromosomal aberration test in Chinese hamster ovary cells, and *in vivo* mouse micronucleus assay.

Teduglutide at subcutaneous doses up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg) was found to have no adverse effect on fertility and reproductive performance of male and female rats.

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 197922-42-2

Generic Name: Teduglutide

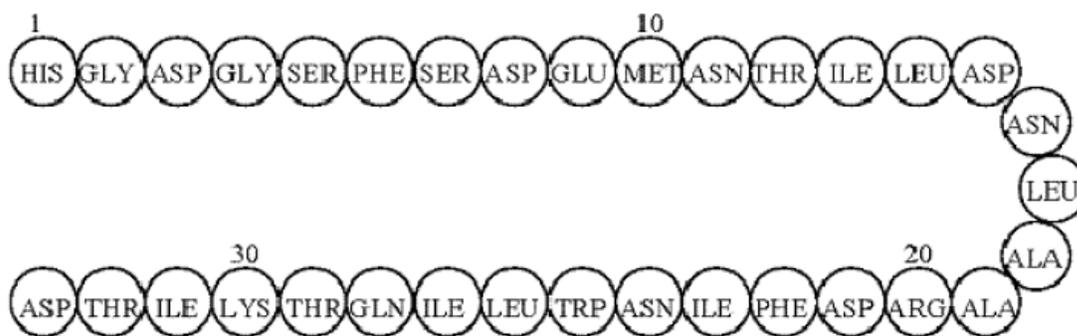
Code Name: ALX-0600

Chemical Name: L-histidyl-L-glycyl-L-aspartyl-L-glycyl-L-seryl-L-phenylalanyl-L-seryl-L-aspartyl-L-glutamyl-L-methionyl-L-asparaginyl-L-threonyl-L-isoleucyl-L-leucyl-L-aspartyl-L-asparaginyl-L-leucyl-L-alanyl-L-alanyl-L-arginyl-L-aspartyl-L-phenylalanyl-L-isoleucyl-L-asparaginyl-L-tryptophanyl-L-leucyl-L-isoleucyl-L-glutamyl-L-threonyl-L-lysyl-L-isoleucyl-L-threonyl-L-aspartic acid

Molecular Formula/Molecular Weight: C₁₆₄H₂₅₂N₄₄O₅₅S/3752 Daltons

Structure or Biochemical Description: Teduglutide is an analog of naturally occurring human GLP-2, a peptide secreted by L cells of the distal intestine. Like GLP-2, teduglutide is 33 amino acids in length with an amino acid substitution of alanine by glycine at the second position of the N-terminus of GLP-2. The structure of teduglutide is shown below (from page 2 of Section 2.3.S.1 of the electronic submission). Teduglutide was manufactured using a recombinant strain of *Escherichia coli*.

Figure 2.3.S.1-1: Primary Sequence of Teduglutide



Pharmacologic Class: Glucagon-like-peptide-2 (GLP-2) receptor agonist

2.2 Relevant IND/s, NDA/s, and DMF/s

1. IND 58,213 (ALX-0600, NPS Pharmaceuticals)

2.3 Drug Formulation

Teduglutide for injection is supplied in a sterile, single-use 3-mL, USP Type I glass vial containing 5 mg of teduglutide as a white lyophilized powder. The lyophilized powder is intended for reconstitution with 0.5 mL of sterile Water for Injection, USP immediately before administration by subcutaneous injection. The reconstituted product is a clear, colorless to light straw-colored solution (10 mg/mL), which also contains the following excipients: (b) (4) sodium phosphate, (b) (4) L-histidine, and (b) (4) mannitol. The reconstituted solution has a pH of approximately 7.4. The composition of the drug product is shown below (from page 1 of 2.3.P.1).

Table 2.3.P.1-1: Composition of Teduglutide for Injection

Name of Ingredients	Function	Quality Standard	Quantity per Vial
Teduglutide Drug Substance	Active Ingredient	NPS in-house standard	5 mg
L-Histidine	(b) (4)	USP	3.88 mg
Mannitol	(b) (4)	USP	15 mg
Monobasic Sodium Phosphate, Monohydrate	(b) (4)	USP	0.644 mg
Dibasic Sodium Phosphate, Heptahydrate	(b) (4)	USP	3.434 mg
Water for Injection	(b) (4)	USP	(---) ^a

NPS = NPS Pharmaceuticals; USP = United States Pharmacopeia; NF = National Formulary

(b) (4)

2.4 Comments on Novel Excipients

The excipients used in the manufacture of teduglutide for injection are all compendial. The quality standard for each excipient is shown in the table (from page 1 of Section 2.3.P.4 of the electronic submission) below. No novel excipients were used in the manufacture of the drug product.

Table 2.3.P.4-1: Specifications for Teduglutide for Injection Excipients

Excipients	Quality Standard
L-Histidine	USP
Mannitol	USP
Monobasic Sodium Phosphate, Monohydrate	USP
Dibasic Sodium Phosphate, Heptahydrate	USP
Water for Injection ^a	USP

(b) (4)

(b) (4)

2.5 Comments on Impurities/Degradants of Concern

The potential process and product-related impurities in the teduglutide drug substance (DS) were controlled as per the International Conference on Harmonization (ICH) guideline Q6B: Product-related impurities and Process-related impurities. The major product-related impurities have a lower biological activity than teduglutide. The biological activities of the minor impurities were not determined. Product-related impurities could arise by chemical or physical degradation of teduglutide, molecular

rearrangement such as truncation, isomerization, incorporation of incorrect amino acids during biosynthesis or incomplete post-translational processing.

The release specifications for the DS are shown in the following table (from page 4 of Section 3.2.R.3.P of the submission). Impurity A and B are teduglutide-related impurity and related in sequence to teduglutide molecule (parent drug) and the specifications are acceptable from a nonclinical standpoint. Impurity A

(b) (4), not more than (NMT) (b) (4); (b) (4), NMT
(b) (4); (b) (4) and (b) (4), NMT
(b) (4); (b) (4), NMT
(b) (4); (b) (4)
(b) (4); NMT (b) (4); (b) (4), NMT
(b) (4); unspecified impurity, NMT (b) (4); Total impurity, NMT (b) (4) and B (b) (4)
(b) (4), NMT (b) (4); (b) (4) Impurity, NMT (b) (4) were
considered qualified based on calculated exposure in toxicology studies.

Table 3.2.R.3.P-2: Teduglutide Drug Substance Release Specification

Test Name	Method Number	Acceptance Criteria
Appearance	Visual (QC-ANP-GLP-1005)	Clear, colorless to light straw-colored liquid
Identity ^a (RP-HPLC)	QC-ANP-GLP-5006	The retention time of the teduglutide peak corresponds to that of the standard preparation as obtained in the Assay
Identity by peptide map ^a (Enzymatic and RP-HPLC peptide map)	QC-ANP-GLP-2110	(b) (4)
pH (Potentiometric)	USP <791> (QC-ANP-GLP-1005)	6.9 to 7.9
Teduglutide concentration/Peptide content (RP-HPLC)	QC-ANP-GLP-5006	(b) (4)
Biological activity (Adenylate cyclase bioassay)	QC-ANP-GLP-5011	(b) (4)
(b) (4) (ELISA)	QC-ANP-GLP-0501	(b) (4)
(b) (4) (RP-HPLC)	QC-ANP-GLP-5010	(b) (4)
Impurity A (RP-HPLC) Specified Impurities	QC-ANP-GLP-5008	(b) (4)

Table 3.2.R.3.P-2: Teduglutide Drug Substance Release Specification

Test Name	Method Number	Acceptance Criteria
Impurity B (RP-HPLC)	QC-ANP-GLP-5007	
Specified Impurities		(b) (4)
Endotoxins ^a	QC-ANP-GLP-1001 (USP <85>)	NMT (b) (4)

(b) (4) ELISA = enzyme-linked immunosorbent assay; EU = endotoxin units; NMT = not more than;

RP = relative potency; RP-HPLC = reverse phase high-pressure liquid chromatography

^a Tested only at release

^b Coeluting impurities

The following table (from page 2 of 3.2.S.3.2) shows the potential product-related impurities.

Table 3.2.S.3.2-1: Potential Product-Related Impurities for Teduglutide

Category	Impurity Name	Sequence ^a	Molecular Weight
Truncated Teduglutide Impurities			(b) (4)
Degradation Products			
Incompletely Processed Teduglutide Peptides ^c			
Genetic Variant of Teduglutide			(b) (4)

The majority of the product-related impurities have been identified to be truncated forms of the peptide. Fifteen principal impurities have been identified in the drug substance. Several of these peaks were co-eluted with other impurities and were quantified as a group of impurities. Good Laboratory Practice (GLP) compliant toxicology studies ranging from 14 days to one year in duration have been conducted in multiple species.

The following table (from page 104 of Section 2.6.6 of the submission) shows impurity profiles for the drug product used in the majority of these toxicology studies.

Table 27 Levels of Principal Impurities in Drug Product Batches Used in Repeat Dose Toxicology Studies

Species	Duration	NOAEL (mg/kg/day)	Impurity (%)
Mouse	26 weeks ^a	25/50	(b) (4)
Rat	14 days ^b	50	
Rat	14 days	50	
Rat	13 weeks	50	
Monkey	52 weeks	5	
Human ^c	P3 studies	0.05 ^d	

^a Animals dosed with 25 mg/kg/day for 2 weeks and 50 mg/kg/day for the duration of the study.

^b Study comparing multiple strains of rats.

^c The highest value for each individual impurity that was measured in drug product batches used in all of the Phase 3 clinical studies.

^d Clinical dose.

P3 = Phase 3.

As shown in the above table, impurity profiles for drug substance used in the majority of these toxicology studies have been determined and margins of exposure (MOE) based upon individual impurity exposure (AUC) at the no-observable-adverse-effect level (NOAEL) in animals relative to human exposure (AUC) at the therapeutic dose (0.05 mg/kg/day) of the commercial drug product have been calculated. Based upon the calculated MOE, the impurities were considered to be qualified from a nonclinical standpoint. Similarly, MOE based upon release specifications for each of the principal impurities identified in the drug substance relative to human exposure were determined and the calculated ratios support the release specifications from the nonclinical perspective. The following tables (from page 105 of Section 2.6.6 of the submission) show the release specifications and margins of exposure based on AUC comparisons for principal impurities.

Table 29 Release Specifications for Principal Impurities in Drug Substance and Drug Product

Release Specification (NMT %)	
(b) (4)	

NMT = Not more than.

Table 28 Margins of Exposure (Animal to Human Ratio) for Principal Impurities in Drug Product Batches

Species	Duration	MOE by Impurity
(b) (4)		
Mouse	26 weeks	
Rat	14 days ^a	
Rat	14 days	
Rat	13 weeks	
Monkey	52 weeks	

^a Study comparing multiple strains of rats.

Similarly, MOE based upon release specifications for each of the principal impurities identified in the drug product (DP) relative to human exposure (AUC) were determined. The following table (from page 106 of Section 2.6.6 of the submission) shows the MOE (animal to human AUC ratio) for principal impurities based on release specifications for drug product.

Table 30 Margins of Exposure (Animal to Human Ratio) for Principal Impurities Based on Release Specifications for Drug Product

Species	Duration	MOE by Impurity
		(b) (4)
Mouse	26 weeks	
Rat	14 days ^a	
Rat	14 days	
Rat	13 weeks	
Monkey	52 weeks	

^a Study comparing multiple strains of rats.

From a nonclinical standpoint, these margins of exposure calculated from the toxicology studies adequately support the proposed release specifications.

2.6 Proposed Clinical Population and Dosing Regimen

Gattex[®] is indicated for the following:

- Treatment of adult patients with Short Bowel Syndrome (SBS)
- Improvement of intestinal absorption of fluid and nutrients

The recommended once daily dose is 0.05 mg/kg by subcutaneous injection, alternating sites between 1 of the 4 quadrants of the abdomen, or into alternating thighs or alternating arms.

2.7 Regulatory Background

The following are the major regulatory milestones.

1. The pre-IND 58,213 meeting dated October 20, 1998 (FDA meeting minutes dated October 20, 1998)
2. End of Phase 2 (EOP2) meeting dated October 6, 2003 (FDA meeting minutes dated November 5, 2003)
3. Telecon to discuss pre-clinical program on December 19, 2003 (FDA meeting minutes dated January 16, 2004)
4. Type C meeting to discuss clinical pharmacokinetics package on June 6, 2006 (FDA meeting minutes dated July 7, 2006)
5. Type C meeting to discuss statistical and regulatory issues on January 23, 2007 (FDA meeting minutes dated February 12, 2007)

6. Type C meeting to discuss development plan on January 18, 2008 (FDA meeting minutes dated January 25, 2008)
7. Type B Pre-NDA meeting on July 14, 2008 (FDA meeting minutes dated August 19, 2008)
8. Pre-NDA Chemistry Manufacturing and Control (CMC) meeting on October 19, 2010 (FDA meeting minutes dated November 30, 2010)
9. Type B Pre-NDA meeting to discuss content and format of the NDA on April 25, 2011 (FDA meeting minutes dated May 23, 2011)

3 Studies Submitted

3.1 Studies Reviewed

The following studies were reviewed as shown in the table below.

STUDY	STUDY/REPORT NO.	REVIEW PAGE #
PHARMACOLOGY*****		20
ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION		49
ABSORPTION		49
Bioavailability of teduglutide following IV and SC administration to rabbits	7203-105*	50
Bioavailability of teduglutide following IV and SC administration to Cynomolgus monkeys	7203-106*	52
Bioavailability of teduglutide following IV and SC administration to mice	7203-107*	51
Pharmacokinetic study in juvenile minipigs	51170*	53
Comparative study in Wistar and SD rats following single SC injection	800759	58
Pharmacokinetic study after single IV or SC bolus dose of teduglutide to male SD rats	10101-R*****	59
DISTRIBUTION		61
Lacteal excretion and placental transfer of teduglutide following administration of SC doses to lactating rats and pregnant rabbits	7203-104*	61
Determination of teduglutide concentration in cerebrospinal fluid and plasma following SC bolus doses to male SD rats	10102-R*****	64
TOXICOLOGY		65
Acute		65
Mice		65
Single, SC	88614*****	66
Subacute/Subchronic/Chronic		66
Mouse		66
14-Day, SC	88617*****	67
1-Month, SC	88730	70
90-Day, SC	0470MN12.001*	76
26-Week, SC	7203-112*	81
Rat		86
14-Day, SC	02-2776***	87
14-Day, SC, Wistar Han, Fischer-344 and SD rats	800869****	91

13-Week, SC, with Dietary optimization	800069***	99
Minipig		108
14-Day, SC, Juvenile	51153*	109
90-Day, SC, Juvenile	66585	115
Cynomolgus Monkey		129
3-Day, SC	88616	130
14-Day, SC	88619*****	130
1-Month, SC	88729*****	133
13-Week, SC	7203-100**	138
12-Month, SC	1368-100*	153
GENOTOXICITY		169
Ames test	88665*****	170
Chromosome aberration test in Chinese hamster ovary (CHO) cells	88666*****	172
<i>In vivo</i> micronucleus test in mice, SC	AA65WK.112.BTL*	175
CARCINOGENICITY		179
Rat, 2-Year, SC	80070*****	180
REPRODUCTIVE TOXICITY		212
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Segment I, SC	98357*	213
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Acute IV/perivenous/intraarterial tolerance study in rabbits	7203-101**	233
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Hemolytic potential and blood compatibility study	7203-102**	238
Induction of delayed hypersensitivity response	0711XA27.001*****	239
Immunogenicity study in New Zealand white rabbit	47063*****	241
Exploratory mechanistic toxicology study in Cynomolgus monkey on potential increase of CRP	519157	242

*: Reports reviewed under IND 58,213 (pharmacology review dated December 19, 2006)

** : Reports reviewed under IND 58,213 (pharmacology review dated April 1, 2004)

***: Reports reviewed under IND 58,213 (pharmacology review dated December 5, 2003)

****: Reports reviewed under IND 58,213 (pharmacology review dated June 16, 2005)

*****: Report reviewed under IND 58,213 (pharmacology review dated October 14, 2008)

*****: Reports reviewed under IND 58,213 (pharmacology review dated May 24, 2002)

3.2 Studies Not Reviewed

Analytical methods and validation reports submitted in Section 4.2.2.1 of the submission were not reviewed.

3.3 Previous Reviews Referenced

1. Pharmacology review of IND 58,213 dated May 24, 2002
2. Pharmacology review of IND 58,213 dated December 5, 2003
3. Pharmacology review of IND 58,213 dated April 01, 2004
4. Pharmacology review of IND 58,213 dated June 16, 2005
5. Pharmacology review of IND 58,213 dated December 19, 2006
6. Pharmacology review of IND 58,213 dated October 14, 2008

Above reviews of IND 58,213 were incorporated in the appropriate sections of this review.

4 Pharmacology

4.1 Primary Pharmacology

Review of primary pharmacology studies are incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

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ALX-0600, a 33 amino acid peptide differs from natural GLP-2 in the substitution of a glycine molecule for alanine molecule at position 2, is a recombinant analog of human regulatory gut glucagon-like peptide-2 (GLP-2). ALX-0600 synthesized from E. coli (b)(4)

(b)(4) was proposed to be used in the present trial. Because of its resemblance to GLP-2, sponsor speculated that ALX-0600 could exert an intestinotrophic effect (increase in diameter, weight and hyperplasia of intestine, DNA and protein concentrations) and proposed the present clinical study. The preclinical pharmacology studies submitted with the present application are reviewed here.

Primary Pharmacology

1. **Mechanism of Action:** ALX-0600 and GLP-2 caused the stimulation and proliferation of epithelial cells in a regulated manner and, found to increase the crypt-to-villus height, mucosal mass and consequently absorptive surface area of intestines. GLP-2 hormone was demonstrated to act through a cell-surface G-protein coupled receptor and was responsible for the stimulation of cAMP production in cells and expressing GLP-2 receptors (GLP-2R; (b)(4)). ALX-0600 was shown to act by GLP-2R expression in stomach, small intestines and large intestines. It exhibited intestinotrophic effects in vitro and in vivo studies. Sponsor claimed that cDNA cloning and functional characterization of human and rat GLP-2 receptors showed that these receptors were (b)(4) similar in their amino acids sequence. One of the genomic DNA clones from human GLP-2R gene was similar to (b)(4) and was thought to be a genetic factor in inherited bowel disease.

(b)(4)

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(b) (4)



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(b) (4)

6. Regulation of Biological Activity of GLP-2 in Vivo by Dipeptidyl Peptidase IV (DPP IV): Rat GLP-2 at the dose of 5 or 50 ug/day (in 2 divided daily doses) in Fishers 344 rats for 10 days produced intestinotrophic effects. The enzyme deficient rat serum showed expected effects of GLP-2 but when native rat GLP-2 incubated with human placental DPP-IV, only little effect was seen. This lack of effect was attributed to rapid inactivation of GLP-2 by DPP-IV. When it was administered in the mouse, an increase in height of mucosal epithelium as well as small bowel weight was observed. GLP-2 when incubated with serum from DPP-IV deficient rats, a significant increase in the intestinotrophic effect was reported showing that the effect was DPP-IV mediated and had limited intestinotrophic effect.

7. Direct Effects of GLP-2 and ALX-0600 on Epithelial Functions in CD-1 Mice: In CD-1 mice subcutaneous injection of either 0, 5 ug GLP-2 or ALX-0600 was administered for 10 days. On day 11, the animals were weighed, sacrificed, and entire small intestine (pylorus to cecum) was removed. The intestines flushed of its materials, washed, dried and weighed. Jejunal segments were excised for morphology by light microscopy and electron microscopy. GLP-2 and ALX-0600 produced a significant ($p < 0.001$) increase in total weight of mucosal weight by 33 and 50% respectively and, protein contents in mucosal scrapings by 59.2 and 65.7% respectively. Total mucosal and villus height were larger by 25 and 35% in mice treated with GLP-2 and ALX-0600, respectively. The crypt depth was not affected in animals given any of these agents. The crypt ratio was significantly increased ($p < 0.05$). The electron microscopy examination revealed that GLP-2 or ALX-0600 treatment doubled microvillus length of the enterocytes. ALX-0600 treatment produced similar but only more intense effects on intestinal morphology and enterocytic morphology (See following table taken from sponsor's submission vol 1:5, pp 274):

Table 1. Effect of GLP-2 and ALX-0600 treatment on mucosal and enterocytic morphology

Treatment	Mucosal Height ¹	Villus Length ¹	Crypt Depth ¹	Microvillus Length ²	Enterocyte Width ²	Enterocyte Length ²
PBS	653 ± 33	541 ± 31	114 ± 5	1.20 ± 0.08	5.17 ± 0.20	17.57 ± 0.47
GLP-2	816 ± 26*	688 ± 28*	121 ± 6	2.27 ± 0.07*	4.83 ± 0.22	29.00 ± 0.43*
ALX	880 ± 22*	762 ± 22*	118 ± 6	2.31 ± 0.11*†	4.19 ± 0.16*†	32.75 ± 0.40*†

All measurements are expressed as μm .

Values represent means \pm SEM

¹ light microscopy, n = 7-8 mice per group

² electron microscopy, n = 20 enterocytes per group

* p < 0.05 compared to PBS-treated control mice

† p < 0.05 compared to GLP-2 treated mice.

8. Intestinal Response to Growth Factors Administered alone or in Combination with Human (Gly 2) Glucagon-Like Peptide-2: CD-1 mice were treated with a peptide alone, i.e., GLP-2 (2.5 ug), ALX-0600 (2.5 ug), IGF-1 (40 ug), hGH (25 ug), LRIGF-1 (25 ug or 40 ug), IGF-II (40 ug), EGF (1 mg) administered twice daily for 10 to 14 days. In other groups of animals, ALX-0600 was administered with one of these peptides. The greatest increase in the small bowel mass was seen in rats treated with ALX-0600. Large bowel mass was increased more in animals included in combination of ALX-0600 with IGF-1 or LR-IGF-1 or hGH treatment groups. Villus height in proximal and distal jejunum and large bowel was seen in mice treated with ALX-0600 alone. ALX-0600 in combination with growth hormones exerted non-specific intestinotropic effects.

9. Pharmacological Effects of Subcutaneous Injection of ALX-0600 for 14 Days in Mice, Rats, Dogs and Monkeys:

a. Mice: The intestinotropic effects of ALX-0600 were pronounced and dose dependent from 1.25 to 2.5 ug/kg, sc doses. The effect was greater in mouse small intestine (40 to 50%) than in the colon (ED_{50} = 1.28 ug/day). A plateau was reached within 2 weeks of administration. In order to evaluate the doses and regimen which could maintain the increased small intestine weight, a group of mice was given dose escalating treatment of 0, 0.625, 1.25, 2.5 or 5.0 ug/day ALX0600. The lowest dose was escalated to next higher dose every second day of the study. The second group was given 5 ug ALX0600 every two days for 14 days. An increase of 71% was seen in small intestines weight

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after 14 days of treatment. The maintenance dose from 0.625, 1.25 and 2.5 ug ALX-0600 bid for next 14 days did not further increase the weight this indicating that the intestinotrophic effect was maintained for only 2 weeks.

Rats: ALX-0600 at daily doses of 0, 0.025, 0.1 and 0.25 mg/kg for 14 days exerted intestinotrophic effect. A pronounced and dose dependent increase in weight of duodenum, jejunum and ileum and, increased mucosal DNA and protein contents were seen in the treated rats. The maximum increase of 50-55, 10, 70 and 70% weight of colon, small intestine, duodenum and jejunum, respectively was noted. Villus height was increased by 50 % in animals treated with high dose of 0.25 mg/kg ALX-0600 and its effect was greater in small intestines than the colon ($ED_{50}=1.28$ ug/day). There was an increase in the protein content and DNA concentrations at all doses. Intestinal absorption of D-xylose was slightly affected at the start and end of the 18 hr study duration. Only a slight but insignificant effect on the xylose absorption was seen in the animals.

Beagle Dogs: ALX-0600 at the dose of 0.4 mg/kg/day, S.C. for 14 days (0.2 mg/kg/bid) in a dog did not produce significant pharmacological effects. The observed multiple dark areas in the proximal portion of jejunum were associated with slight multifocal hemorrhage. Sponsor claimed that these effects were not treatment related. The quantities of d-xylose excreted in the urine on day 7 and day 13/14 were similar. ALX-0600 did not produce an effect on the absorption of xylose. The study indicated that the dog was not a suitable animal model for studying the effect of the compound.

The experiment was repeated in dogs by selecting isoflurane as an anesthetic. The peak inspiratory flow and tidal volume were respectively, increased by 28.7% and 21.8% after 30 min of the administration of the compound. The effect on the respiratory rate and tidal volume was not seen.

c. Cynomolgus Monkeys: ALX-0600 at the dose of 0.4 mg/kg/day, p.o. for 14 days (0.2 mg/kg/BID, 8 hr apart) did not produce any significant pharmacological effects in the monkey. An increase in the absorption of xylose was reported. Thus the monkey was a suitable animal model for determining the effect of the compound on the xylose absorption.

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10. **Augmentation of Adaptive Response to Massive Intestinal Resection in Rats:** In rats with 25% of the mid jejunoileum intact, a treatment of 2 mg/kg ALX-0600 twice daily for 21 days produced an increase in diameter, total intestinal and mucosal weights/cm, crypt-villus height and sucrase activity. The protein contents and DNA/cm in the remaining ileum and jejunum in response to resection and, a significant additive response in duodenum were reported. ALX-0600 increased the absorption of xylose in control and resected rats. All these responses were due to stimulation of cell synthesis and enhanced in the rate and magnitude of proximal intestinal adaptation due to massive resection. The sucrase activity expressed/mg protein or DNA contents were not changed.

11. **Effect of Human (Gly²) GLP-2 on Colonic Injury in Experimental Colitis Model in Mouse:** In dextran-induced colitis in CD1/Balb/c mice (induced with 5% dextran sulfate for 9 to 10 days), a weight loss, reduced colon length, crypt depletion and loss of colonic mucosal integrity along with damaged mucosal area were reported. The administration of 350 or 750 ng ALX-0600 along with dextran solution (in drinking water) prevented or reduced these changes. The histological changes were 2 to 3 times greater in DS treated animals than DS+ALX-0600 treated animals. Thus ALX-0600 exerted a protective effect in dextran-induced colitis mice by preventing or reducing the changes in the weight and length of large intestines, stomach and small intestines.

12. **Effect of GLP-2 on NSAID-Induced Intestinal Damage in Rats:** Indomethacin-induced (5 or 10 mg/kg, p.o.) intestinal damage in rats (SD or/and Wistar strain) was associated with an increase in urinary laculose-mannitol ratio (intestinal permeability damage). Simultaneous treatment with 2.5 ug bid ALX-0600 prevented or reduced the damage and decreased the permeability. Thus ALX-0600 produced useful effect in inflammatory condition.

13. **Indomethacin induced murine enteritis in CD1 mice:** In indomethacin induced murine enteritis, ALX-0600 2.5 ug bid decreased mortality from 50% to 20-30% which was accompanied by intestinotrophic effect in small intestine. Indomethacin induced reduction in villus height and, increase in fluidity, myeloperoxidase activities and the production of cytokines (TNF α , IL-2, IFN γ , and IL-10) were also prevented by a pretreatment with the compound.

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14. **Prevention of TPN-Induced Hypoplasia in Rats:** Total parenteral nutrition (TPN-2.5 ml/hr) in Fishers 344 rats via external jugular vein caused intestinal hypoplasia and atrophy. ALX-0600 (1.0 ug/kg) or GLP-2 (0.6 ug/ml) administration at the time of start of TPN infusion was seen to prevent TPN induced changes in small intestine villus height and villus crypt ratio. The co-infusion of ALX-0600 and GLP-2 with TPN did not affect immunosuppression and bacterial translocation. The organ weight of liver, heart were only slightly changed and not important. The compounds could prevent TPN induced histopathological changes, reduction of protein contents and DNA contents (excepting of duodenal).

In other study, ALX-0600 at i.v. doses of 0.025, 0.125 and 0.25 mg/kg/day was demonstrated to prevent TPN-induced hypoplastic responses in a dose related manner. The restorative effects of GLP-2 and ALX-0600 in TPN-induced hypoplasia were studied in rats given infusion of TPN for initial 5 days and then either GLP-6 or ALX-0600 infusion along with TPN infusion for additional 5 days. The animals treated with ALX-0600 showed an increase in gut weight and protein contents in the duodenum, jejunum and ileum by 90% and colon by 24%. The protein contents were not restored to the control levels and colon contents were not increased. TPN induced depressed cellular immunity and increased bacterial translocations to mesenteric nodes were not affected by ALX-0600 treatment.

15. **Prevention of TPN-Induced Hypoplasia in Tumor Bearing Rats:** In Fishers 344 rats, a subcutaneous injection of methylcholanthrene was given to induce tumors. After 19 days of induction of tumor, these animals were catheterized and given saline for 3 days and TPN (2.5 ml/hr) for next 9 days. The hypoplasia and intestinal atrophy induced by TPN were prevented by ALX-0600 (1 ug/ml) or GLP-2 (0.1 ug/ml). A decrease in protein and DNA contents by TPN was prevented. The reversed changes were produced in small intestine villus height and villus crypt ratio. TPN induced immunosuppression and increased bacterial colony formation in spleen and lymph nodes were not affected by either ALX-0600 or GLP-2.

16. **Effect of ALX-0600 on Radiation Induced Intestinal Damage in the Rats:** The rats exposed to 10, 12 or 15 Gy radiation in the abdominal region by anterior/posterior parallel pair γ -irradiation (^{60}Co) had increased jejunal myeloperoxidase activity (indicator for inflammation). The enzymatic activity was

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reduced in animals treated with 100 ug/kg, i.v. ALX-0600. The MOP activities of ileum and colon were not affected by radiation. The increase in intestinal tissue conductance by radiation was decreased by 100 ug/kg ALX-0600.

17. **Effect of ALX-0600 on Intestinal Permeability and Enterocolitis in IL-10 Deficient Mice:** ALX-0600 in interleukin-10 (IL-10) deficient mice administered every 12 hr from 72 hr after birth to 10 weeks of age decreased intestinal permeability (measured by sucrose, lactulose:mannitol and sucralose (1,6-dichloro-1,6, dideoxy-beta D-fructofuranosyl-4-chloro-4-deoxy-alpha-galacopyranoside) excretion after ingestion of these sugars). IL-10 deficiency associated with increased MOP activity was reduced by ALX-0600 but increased cytokines levels associated with IL-10 deficiency were not affected.

18. **Reduction of Chemotherapy associated Mortality and Enhancing Cell Survival in Cell Expressing Transfected GLP -2 Receptors:** A 3-days pretreatment with 10 ug of GLP-2 in CD1 mice significantly decreased 400 mg/kg 5-FU induced crypt cell apoptosis in the jejunum and colon. This improved the animal survival during the test.

19. **Proliferative Effect on Intestinal Epithelial Cells:** IEC6 and IEC18 (isolated from neonatal rat small intestines, human HT29 and SW620 colon adenocarcinoma cell lines (like T84 tumor cell line) were cultured with 10 and 500 ng/ml ALX-0600 for 7 days. No significant effects were seen in any of the cell lines indicating that it did not stimulate cell division in these cell lines.

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17. Effect of ALX-0600 on Intestinal Permeability and Enterocolitis in IL-10 Deficient Mice: ALX-0600 in interleukin-10 (IL-10) deficient mice administered every 12 hr from 72 hr after birth to 10 weeks of age decreased intestinal permeability (measured by sucrose, lactulose:mannitol and sucralose (1,6-dichloro-1,6, dideoxy-beta D-fructofuranosyl-4-chloro-4-deoxy-alpha-galacopyranoside) excretion after ingestion of these sugars). IL-10 deficiency associated with increased MOP activity was reduced by ALX-0600 but increased cytokines levels associated with IL-10 deficiency were not affected.

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19. Proliferative Effect on Intestinal Epithelial Cells: IEC6 and IEC18 (isolated from neonatal rat small intestines, human HT29 and SW620 colon adenocarcinoma cell lines (like T84 tumor cell line) were cultured with 10 and 500 ng/ml ALX-0600 for 7 days. No significant effects were seen in any of the cell lines indicating that it did not stimulate cell division in these cell lines.

Intestinotrophic Effects of Teduglutide in Dogs (PH02-003)

The objective of this study was to evaluate the intestinotrophic effects of daily teduglutide treatment for 10 days in the gastrointestinal tract (GIT) of adult Beagle dogs. In this study, adult male and female dogs were administered by once daily SC injection of saline (vehicle) or teduglutide at 0.3 mg/kg or 1.0 mg/kg for 10 days. Intestinotrophic responses were measured as increased tissue weight and morphological parameters.

Small intestinal weight was significantly increased at both doses. Stomach weight tended to increase at 1 mg/kg when compared to vehicle controls, but the increase was not statistically significant. Colon weight was not significantly different between groups. Small intestinal length tended to increase at both doses of teduglutide. Mucosal thickness significantly increased in the duodenum and jejunum at 0.3 mg/kg or 1 mg/kg and significantly increased in the stomach pylorus and colon at 1 mg/kg. Mucosal thickness in the stomach (fundus) was similar in all groups. Villus height significantly

increased in the jejunum at both doses and in the stomach (fundus) at 1 mg/kg. There were less pronounced increases in villus height in the stomach pylorus and duodenum at both doses. Crypt depth significantly increased in the duodenum at both doses and significantly increased in the stomach only at 1 mg/kg. There were no significant differences in crypt depth in the jejunum or stomach fundus. No intestinotrophic effects (mucosal thickness, villus height, crypt depth) were observed in the ileum of teduglutide treated animals. Overall, teduglutide caused increase in small intestinal weight, mucosal thickness of the jejunum and duodenum, villus height and crypt depth in the duodenum and stomach at both tested doses.

Effects of Teduglutide on Small Intestinal Epithelium Following Irradiation (PH03-001)

The objective of the study was to examine the potential protective effects of teduglutide following cytotoxic exposure to irradiation in the normal small intestinal epithelium of BDF1 male mice. The level of protection was determined by the number of stem cells in the crypt that survived as measured by the crypt microcolony assay. Protection was demonstrated by a shift to the right in the survival curve and an increased protection factor at a given dose. Relative to exposure to irradiation, several teduglutide pre-treatment and post-treatment regimens were tested (0.2 to 1.6 mg/kg BID) as shown in the table (from page 4 of the report) below.

Summary of Experimental Conditions of Crypt Survival Studies

Experiment Number	Treatment groups	Dose regimen (mg/kg/day)	Duration of administration relative to irradiation	Radiation doses (Gy)
E1680	None		Irradiation only	11, 12, 14, 15, 16
E1640	Saline Saline Saline		4 days post-irradiation 6 days pre-irradiation 6 days pre-treatment and 4 days post-treatment	14
E1646	Saline		6 days pre-irradiation	14
E1668	Saline		4 days post-irradiation	14
E1673	ALX-0600 Saline	0.2, bid	6 days pre-treatment	11, 12, 14, 15, 16
E1679	ALX-0600 Saline	0.2, bid	4 days post-treatment	11, 12, 14, 15, 16
E1698	ALX-0600 Saline	0.2, bid	6 days pre-treatment and 4 days post-treatment	11, 12, 14, 15, 16
E1703	ALX-0600 Saline	0.2, bid	14 days pre-treatment	11, 12, 14, 15, 16
E1715	ALX-0600 Saline	0.2, bid	14 days pre-treatment and 4 days post-treatment	11, 12, 14, 15, 16
E1755 (Normal light cycle)	ALX-0600 Saline Saline None	0.2, 0.4, 0.8, 1.6	2 days pre-treatment 2 days pre-treatment 2 days pre-treatment no pre-treatment	14 14 No exposure to irradiation 14
E1756 (Reverse light cycle)	ALX-0600 Saline Saline None	0.2, 0.4, 0.8, 1.6	2 days pre-treatment 2 days pre-treatment 2 days pre-treatment no pre-treatment	14 14 No exposure to irradiation 14

Administration of teduglutide (0.2 mg/kg BID) for 6 or 14 days prior to exposure ranging from 12 to 15 Gy γ -radiation significantly increased crypt stem cell survival in BDF1 mice compared to vehicle controls. The levels of protection (protection factor) ranged from 1.0 (no effect at the lowest radiation dose of 11 Gy) to 2.2 (at 15 Gy). A protection factor of 2.0 meant two-fold more crypts survived a given dose of radiation if the treatment with teduglutide precedes the radiation. Administration of teduglutide (0.2 mg/kg BID) for 6 days prior and 4 days following irradiation doses ranging from 11 to 16 Gy significantly increased stem cell survival, with a protection factor ranging between 1.2 and 1.6 compared to the saline treated group. Administration of teduglutide (0.2 mg/kg BID) for 4 days following the doses of radiation ranging from 11 to 16 Gy did not increase crypt stem cell survival when compared to the saline treated group.

Overall, exposure to teduglutide significantly increased the levels of crypt survival and provided modest protection to the small intestine following high doses of radiation. A maximum of two-fold more crypts survived a given dose of radiation if treatment with teduglutide preceded the radiation. No significant differences were observed between 6 day and 14 day pre-treatment paradigms. There was no significant improvement in the levels of protection if teduglutide was continued following the dose of radiation (during the crypt regeneration phase). Teduglutide given only following irradiation provided no protection against intestinal irradiation damage.

Effect of Teduglutide on Intestinal Permeability in a TNBS-induced Ileitis Guinea Pig Model (PH04-002)

The objective of this study was to examine the potential therapeutic effects of teduglutide in a Guinea pig 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced ileitis model. Ethanol was administered to increase permeability of the small intestinal epithelium, which allowed TNBS (as a hapten) to gain access to the submucosa. A chronic inflammatory response was subsequently observed within the wall of the intestine that mimicked the characteristics of human Crohn's disease. Baseline permeability was determined at Day -7 and Day -3, prior to TNBS exposure. Inflammation was induced on Day 0 in the ileum of the Guinea pigs by a single intra-ileal injection of TNBS (40 mg/mL in 10% ethanol). Animals were allowed to recover, and randomized to receive either teduglutide or vehicle (phosphate buffered saline) by SC injection (0.1 mg/kg, BID for 18 days) following TNBS exposure. Gastric and small intestinal permeability were assessed on Days 3, 7, 10, 14 and 18 post-treatment.

Administration of TNBS increased the small intestinal permeability, as determined by an increase in the lactulose:mannitol ratio. Teduglutide significantly accelerated the reversal of increased intestinal permeability to baseline levels by Day 7. A further reduction below baseline in intestinal permeability was observed in animals treated at Day 10. Vehicle-treated animals returned to baseline permeability by Day 14. Gastric permeability, as measured by sucrose excretion, was unaffected by TNBS. No significant changes in gastric permeability were observed by treatment with teduglutide. These results indicated that teduglutide did not reduce maximal increased TNBS-induced small intestinal permeability. However, teduglutide appeared to accelerate the reversal of TNBS-induced increased small intestinal permeability.

A 14-Day Comparative Pharmacology Study of Teduglutide Administered by Continuous Intravenous Infusion or Subcutaneous Injection in Female Rats (PH04-024)

Methods: The objective of this study was to compare the effects of teduglutide on the gastrointestinal tract in female rats following administration by continuous IV infusion (at 0.5 or 2.0 mg/kg/day) or twice daily SC injection (0.5 mg/kg/day) for 14 consecutive days. The following parameters were evaluated: clinical signs, body weight, food consumption, pharmacokinetics, macroscopy, small and large intestine weight and length and histopathology of the small and large intestine. The following table (from page 1 of the study report) shows the study design.

Group No. Identification	Dose Level (mg/kg/day)	Route	No. of Animals	
			Main	Pharmacokinetic
1 Control*	0	iv	6	
2 ALX-0600	0.5	iv	6	6
3 ALX-0600	2.0	iv	6	6
4 ALX-0600	0.5**	sc	6	6

* Phosphate Buffer with mannitol and L-histidine

** 2 equal doses of 0.25 mg/kg

Results: There were no mortality and treatment-related clinical signs. There were occasional reductions in daily food intake with a consequent reduction in overall body weight gain following the SC route of administration. Both routes of administration were associated with statistically significant increases in intestinal weights (small intestine: 125%, 115% and 188% of control in Groups 2, 3 and 4, respectively; large intestine: 135%, 133% and 120% of control in Groups 2, 3 and 4, respectively), length (small intestine: 117%, 114% and 107% of control in Groups 2, 3 and 4, respectively; large intestine: 107%, 114% and 104% of control in Groups 2, 3 and 4, respectively) and thickness. Histopathology findings included treatment-related mucosal hyperplasia in the small intestine at 0.5 mg/kg/day IV and in the small and large intestines at 2 mg/g/day IV or 0.5 mg/kg/day SC. This change was characterized by an elongation of the villi and/or crypts lined by high columnar and basophilic epithelial cells, with an increased number of mitotic figures in the crypts. Mucosal hyperplasia was seen in all segments of the small intestine (duodenum, jejunum, and ileum) of all treated animals. This change was more prominent in the proximal small intestine (duodenum and jejunum) with both SC and IV routes of administration. There was a dose-related increase of the duodenal and jejunal mucosal hyperplasia following IV administration. There were no apparent differences of mucosal hyperplasia in the small or large intestines between SC and IV groups at the same dosage. Mucosal hyperplasia correlated with the thickening of the intestinal wall seen at necropsy. Minimal cecal and colonic mucosal hyperplasia without increased mitotic activity was seen in some animals at 2 mg/kg/day IV and in one animal at 0.5 mg/kg/day SC. This change correlated with the thickening of the wall seen grossly in the cecum. The following table (from page 15 of the report) shows the incidences of mucosal hyperplasia.

Text Table 3: Incidence and extent of mucosal hyperplasia in ALX-0600-treated animals

Group Identification	2	3	4
Duodenum	6/6	6/6	6/6
Grade 2	3	2	4
Grade 3	3	4	2
Jejunum	6/6	6/6	6/6
Grade 2	3	2	4
Grade 3	3	4	2
Ileum	6/6	6/6	6/6
Grade 1	1	-	-
Grade 2	5	6	6
Cecum	0/6	3/6	1/6
Grade 1	-	3	1
Colon	0/6	3/6	1/6
Grade 1	-	3	1

Incidence results are in bold.

Grade 1 - minimal; Grade 2 - slight; Grade 3 - moderate

The IV route of administration appeared to cause a greater response on intestinal weight at 0.5 mg/kg/day than the same dosage given SC. At 0.5 mg/kg/day IV, there was significant increase in small intestinal length compared to the control group. In addition, a tendency towards increased small intestinal length at 2.0 mg/kg/day IV and 0.5 mg/kg/day SC injection was observed. Similarly, a tendency towards increased large intestinal length in animals from all groups was observed compared to the control group.

The steady-state plasma concentration of teduglutide at 0.5 mg/kg/day IV was 40.2 ng/mL on Day 2 and 50 ng/mL on Day 14. The steady-state plasma concentration of teduglutide at 2.0 mg/kg/day IV was 154 ng/mL on Day 2, while no teduglutide exposure was observed on Day 14. The total daily exposure (AUC) to teduglutide was higher in the IV group ($AUC_{0-\infty}$ 965-1200 ng.hr/mL) than in the group receiving the same dose of 0.5 mg/kg/day teduglutide by SC injection ($AUC_{0-\infty}$ 297-384 ng.hr/mL). The following table (from page 10 of the PK report) shows the PK data.

Table 3 Summary of pharmacokinetic parameters for ALX-0600

Dose Route	Group	Dose Level (mg/kg/d)	Day	C_{max} (ng/mL)	t_{max} (h)	C_{ss} (ng/mL)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{1/2}$ (h)
iv infusion	2	0.5	2	NA	NA	40.2	NA	965 ^a	NA
			14	NA	NA	50	NA	1200 ^a	NA
iv infusion	3	2.0	2	NA	NA	154	NA	3693 ^a	NA
			14	NA	NA	ND	ND	ND	NA
sc	4	0.5	2	315	0.5	NA	296 ^b	297 ^b	0.45
			14	416	0.5	NA	384 ^b	384 ^b	0.41

NA = Not applicable

ND = Not determined

^a Over a 24-hour period

^b following the first daily dose

In conclusion, both routes of administration were associated with greater intestinal weights and intestinal wall thickening, and caused intestinal mucosal hyperplasia. The IV route appeared to cause a greater response on intestinal weight at 0.5 mg/kg/day than the same dosage given SC.

A 14-Day Comparative Study on the Effects of Teduglutide Administered by Continuous Subcutaneous Infusion or Subcutaneous Injection on the Gastrointestinal Tract (GIT) of the Female Rat (SP06-008-0600)

Methods: The objective of this non-GLP exploratory study was to compare the effects of teduglutide on the GIT in female rats following administration by continuous SC infusion at 0.5, 2.0 or 10 mg/kg/day or once or twice daily SC injection at 0.5 mg/kg/day or 0.25 mg/kg twice daily for 14 consecutive days. The following parameters were examined: clinical signs, body weight, food consumption, pharmacokinetics, macroscopy, small and large intestine weight and length and histopathology of the small and large intestine. The study design is shown below (from page 14 of the report).

<u>Group No.</u> <u>Identification</u>	<u>Dose Level</u> <u>(mg/kg/day)</u>	<u>Dose</u> <u>Concentration</u>	<u>Dose</u> <u>Volume</u>	<u>No. of Animals</u> <u>Females</u>
1 Control*	0	0 mg/mL	1 mL/kg/h	6
2 ALX-0600*	0.5	0.0208 mg/mL	1 mL/kg/h	6
3 ALX-0600*	2.0	0.0833 mg/mL	1 mL/kg/h	6
4 ALX-0600*	10	0.4167 mg/mL	1 mL/kg/h	6
5 ALX-0600 ⁺	0.5	0.8 mg/mL	0.625 mL/kg/day	9
6 ALX-0600 [#]	0.5	0.4 mg/mL	1.25 mL/kg/day	9

* Test article was administered by continuous subcutaneous infusion

+ Once-daily dose of 0.5 mg/kg or 0.625 mL/kg/dose administered by subcutaneous injection. Dilution of 20 mg/mL stock solution made using Phosphate Buffer with mannitol and L-histidine vehicle to prepare 0.8 mg/mL concentration.

2 equal doses of 0.25 mg/kg or 0.625 mL/kg/dose approximately 8 hours apart. Dilution of 20 mg/mL stock solution made using Phosphate Buffer with mannitol and L-histidine vehicle to prepare 0.4 mg/mL concentration.

Results: There were no mortalities or treatment-related clinical signs during the study. Food consumption was reduced occasionally at 2.0 or 10 mg/kg/day by SC infusion and at 0.5 mg/kg/day by SC injection compared to controls. However the body weight was unaffected by the treatment. The administration of teduglutide by SC continuous infusion caused an increase in the weight and length of the small intestine and weight of the large intestine at 0.5, 2.0 or 10 mg/kg/day. The mean percent increases of the weights were higher in the small intestine when compared with the large intestine. Histopathology findings included minimal to moderate mucosal hyperplasia of the small intestine at all doses by SC infusion associated with thickening of the duodenum, jejunum and ileum. Minimal to slight mucosal hyperplasia was also noted in the large intestine of most animals at 2.0 or 10 mg/kg/day. The administration of teduglutide by SC injection (once or twice daily) at a dose of 0.5 mg/kg/day caused an increase in the weight of the small intestine that was more pronounced following twice daily treatment. A trend toward an increase of the mean length of the small intestine was noted in animals treated once or twice daily. These changes were not noted in the large intestine. Minimal to slight mucosal hyperplasia of the small intestine was noted following twice daily treatment, but this finding was observed in the large intestine after both once or twice daily treatment. Macroscopic thickening of the gastrointestinal wall was not observed. There were more pronounced increases in the small intestine weight and length, thickening of the small intestine and a higher incidence of mucosal hyperplasia in the duodenum, jejunum and ileum in animals treated by SC infusion. The following table (from page 29 of the report) shows the incidences of mucosal hyperplasia.

Text Table 7 Incidence and extent of mucosal hyperplasia

Group No.	1	2	3	4	5	6
Number of animals	6	6	6	6	9	9
Mucosal hyperplasia						
Stomach	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>
Grade 1	—	—	—	1	—	—
Duodenum	<i>0</i>	<i>6</i>	<i>6</i>	<i>6</i>	<i>0</i>	<i>5</i>
Grade 1	—	1	—	3	—	5
Grade 2	—	5	5	3	—	—
Grade 3	—	—	1	—	—	—
Jejunum	<i>0</i>	<i>6</i>	<i>3</i>	<i>4</i>	<i>0</i>	<i>1</i>
Grade 1	—	1	1	—	—	—
Grade 2	—	5	2	3	—	1
Grade 3	—	—	—	1	—	—
Ileum	<i>0</i>	<i>5</i>	<i>4</i>	<i>6</i>	<i>1</i>	<i>3</i>
Grade 1	—	4	1	3	1	3
Grade 2	—	1	2	3	—	—
Grade 3	—	—	1	—	—	—
Cecum	<i>0</i>	<i>0</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>5</i>
Grade 1	—	—	3	3	5	5
Grade 2	—	—	—	1	—	—
Colon	<i>0</i>	<i>0</i>	<i>4</i>	<i>6</i>	<i>2</i>	<i>5</i>
Grade 1	—	—	2	1	2	5
Grade 2	—	—	2	5	—	—

Incidence results are in italics.

The average daily exposure (AUC) was similar for both QD and BID dosing regimens. Peak exposure (C_{max}) was dose proportional between the 0.25 and 0.5 mg/kg. The pharmacokinetics of the QD and BID dosing regimens were comparable. The PK parameters are shown in the following table (from page 110 of the report).

Table 2 Summary of teduglutide pharmacokinetics parameters in female rats following subcutaneous administration by infusion or bolus injection

Group	Dose Level (mg/kg/d)	Dose Route	C _{max} (ng/mL)	t _{max} (h)	AUC _{0-t} (ng·h/mL)
2	0.5	sc infusion	34.4	24	5242
3	2.0	sc infusion	142	24	12811
4	10	sc infusion	339	1	24266
5	0.5 (0.5 mg/kg qd)	sc injection	799	0.5	835 ^a
6	0.5 (0.25 mg/kg bid)	sc injection	309	0.5	391 ^{a, b}

^a Represents AUC for a single injection, not the entire treatment period.

^b AUC from first daily dose only.

In conclusion, In female rats, the effect of teduglutide on GIT was more prominent when the drug was administered by continuous SC infusion than by SC injection. There were higher incidences of mucosal hyperplasia following continuous SC infusion compared to SC injection.

Teduglutide Inhibits Contractility in the Colon (SP08-002-0600)

The present *ex vivo* study examined the effects of teduglutide and a variety of GLP-2 analogues on spontaneous contractility of rodent colon. In this study, segments of colon were first equilibrated for 60 min. After stabilization, GLP-2 analogues were added to the organ bath at concentrations range of 10^{-9} M to 10^{-6} M for 5 to 10 minutes. Tissues were washed for 1 hour between each experiment, until spontaneous basal contractility was restored. The data were calculated and expressed as percentage response relative to basal levels. This study also examined the cAMP accumulation using 293-EBNA cells stably expressing the rat GLP-2R (rGLP-2R).

Teduglutide and GLP-2 inhibited contractility of the colon with an IC_{50} of 4.9 nM and 13.5 nM, respectively. GLP-2(3-33), the primary degradation product of GLP-2, blocked the inhibitory effects of teduglutide and GLP-2. In the presence of 10 μ M GLP-2(3-33), teduglutide and GLP-2 inhibited colon contractility with an IC_{50} of 59.9 nM and 142.7 nM, respectively. The rank order of potency of GLP-2 peptide analogues [hGLP(1-33), [Thr insertion]hGLP-2(1-34), Ac-rGLP2(1-33) and [Arg-I]rGLP-2(1-33)] was similar for peptide-induced increases in cAMP levels in 293-EBNA cells expressing the rGLP-2R. Overall, the results of this study demonstrated the ability of teduglutide and GLP-2 to inhibit contractility of the rat colon.

Effects of Teduglutide on Motor Activity in the Canine Gastrointestinal Tract (SP09-001-0600)

The objective of this study was to examine the effects of teduglutide on motor activity in the anesthetized canine (n = 6) GIT segments (antrum, jejunum and ileum or ileal segments). In this study, teduglutide was administered to the perfused segments of the GIT with or without electrical field stimulation (EFS).

Teduglutide (0.1 to 100 μ g) caused concentration-dependent excitation during EFS followed by inhibition, with the most prominent activity occurring in the jejunum and ileum compared to the antrum. In the absence of EFS (i.e., lack of spontaneous basal tone), teduglutide administration only elicited an excitatory effect, but not an inhibitory effect. In isolated ileal segments, teduglutide (300 nM) produced a contraction prior to EFS and enhanced the response to EFS. Cessation of teduglutide administration decreased the responses to EFS. In addition, teduglutide further enhanced tetrodotoxin (TTX)-induced myogenic activity. These results suggested that teduglutide-mediated excitatory and inhibitory responses on the motor activity of the canine GIT may be caused due to its actions on the neurons.

Intestinotrophic Effects of Teduglutide in Ferrets (SP10-001-0600)

The objective of this study was to evaluate the efficacy of teduglutide in stimulating intestinotrophic activity in female ferrets. In this study, two parameters were evaluated, which included intestinal weight and mucosal thickness. Female ferrets (n = 4/group) were administered SC teduglutide (0.1 to 0.5 mg/kg BID) or vehicle 0.1% TFA (trifluoroacetic acid/water) for 10 days. An additional group of 4 ferrets was treated with teduglutide at 0.5 mg/kg BID for 20 days. Plasma samples were collected for the detection of intact residual GLP-2 or teduglutide following the last dose and with 18 hours of fasting. After administration of the last dose of teduglutide or vehicle control, the ferrets were fasted for approximately 18 hours, euthanized and then sacrificed for necropsy. The small and large intestine were removed and weighed. Crypt and villus height were measured for sections of proximal jejunum, distal jejunum, distal ileum and colon.

Teduglutide increased the absolute and relative intestinal weight in a dose-related manner. The mean increases in relative intestine weight compared to the vehicle control were 22.9% and 39.4% at 0.10 and 0.5 mg/kg BID, respectively. When a 0.5 mg/kg BID group was dosed for 20 days, the increase in relative intestinal weight compared to the vehicle controls ranged from 39% to 44%. Statistically significant increases in small intestinal and colonic mucosal thickness were also seen at 0.5 mg/kg BID at Day 10 and Day 20. The following tables (from page 10 and 11 of the report) show the intestinal weights and thickness following teduglutide administrations.

Table 3-1 Mean Absolute and Relative Intestinal Weights Following 10 Consecutive Days of Administration

Treatment Group (mg/kg/bid)	Absolute Intestine Weight (g) ^a	Increase in Absolute Intestine Weight ^b (%)	Relative Intestine Weight (%) ^a	Increase in Relative Intestine Weight ^b (%)
0	22.11 ± 0.91	-	2.74 ± 0.09	-
0.1	27.34 ± 0.94*	23.7	3.37 ± 0.12*	22.9
0.5	27.62 ± 0.92*	24.9	3.82 ± 0.18*	39.4

* Significantly increased from vehicle control (p≤ 0.01)

^a mean ± SE

^b relative to control

Table 3-2 Mean Absolute and Relative Intestinal Weights Following 20 Consecutive Days of Administration

Treatment Group (mg/kg)	Absolute Intestine Weight (g) ^a	Increase in Absolute Intestine Weight ^b (%)	Relative Intestine Weight (%) ^a	Increase in Relative Intestine Weight ^b (%)
0	22.05 ± 0.25	-	2.76 ± 0.06	-
0.5	32.74 ± 1.96*	48.5	4.0 ± 0.26*	44.9

* Significantly increased from vehicle control (p≤ 0.01)

^a mean ± SE

^b relative to control

Table 3-3 Mean Crypt Plus Villus Length in the Small Intestine or Mucosal Thickness in the Colon

Treatment Group (mg/kg bid)	Proximal Jejunum (μM)	Distal Jejunum (μM)	Distal Ileum (μM)	Colon (μM)
0 ^a	839.6 \pm 73.7	856.5 \pm 38.3	795.8 \pm 35.1	271.2 \pm 20.9
0.1 ^a	939.6 \pm 157.4	898.8 \pm 118.5	748.1 \pm 89.9	281.7 \pm 19.2
0.5 ^a	986.4 \pm 79.5*	1040.5 \pm 142.6*	853.1 \pm 54.8	322.4 \pm 27.0*
0 ^b	903.5 \pm 42.2	746.8 \pm 74.8	554.8 \pm 10.7	257.6 \pm 4.7
0.5 ^b	1129.9 \pm 80.8*	1092.9 \pm 52.7*	890.0 \pm 28.2*	338.7 \pm 20.2*

* Significantly increased from vehicle control ($p \leq 0.05$)

^a – animals treated for 10 days

^b – animals treated for 20 days

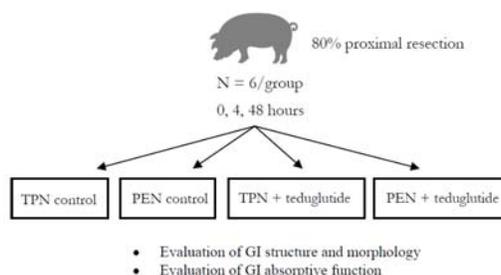
Plasma GLP-2 levels were minimal and there were no statistically significant differences in GLP-2 levels between the dose groups. There was no apparent accumulation of teduglutide in the plasma after a twice daily treatment at 0.5 mg/kg for 20 days.

The Effects of Teduglutide on Small and Large Intestinal Adaptation in a Neonatal Piglet Model of Short Bowel Syndrome (SP10-002-0600)

The aim of this study was to assess the adaptive role of teduglutide in a neonatal piglet model of SBS and to determine if teduglutide and partial enteral nutrition (PEN) have any synergistic effect in treating intestinal injury in infants. The neonatal piglet model was chosen for the following reasons: the 2-day old piglet undergoes a period of rapid growth and development; a piglet can double its body weight within a week of birth which represents approximately 4 to 5 months of growth in a human infant; and pig's GIT has several digestive, absorptive and metabolic similarities to humans (Morgan W, Yardley J, et al., 1987, Total Parenteral Nutrition and Intestinal Development: A Neonatal Model. J Ped Surg, 22:541-545; Stoddart RW and Widdowson EM, 1976, Changes in the Organs of Pigs in Response to Feeding for the First 24 h after Birth. III. Fluorescence Histochemistry of the Carbohydrates of the Intestine, Biol Neonate, 9:18-27). The acute 4 h and 48 h time points were selected to assess immediate adaptive changes occurring in the intestine. The chronic 7-day time point was selected to assess long term developmental changes. These time points have been established in the literature as a means to identify potential dysregulation between structural and functional adaptations (Bartholome AL, Albin DM et al., 2004, Supplementation of Total Parenteral Nutrition with Butyrate acutely increase structural Aspects of Intestinal Adaptation after an 80% Jejunal Resection in Neonatal Piglets, JPEN, 28:210-222). The chronic time point was considered relevant as more than one complete turnover of an entire crypt-villus structure can occur (Thompson J and Sudan D, 2008, Intestinal Lengthening for Short Bowel Syndrome. Adv Surg, 42:49-61) during this time.

In this study, structural and functional adaptations of the residual intestine were quantified in neonatal piglets (body weight 1.57 to 1.59 kg) following an 80% jejuno-ileal resection, randomized to the following four groups: 1) TPN (total parenteral nutrition), 2) 80% PN + 20% enteral nutrition (PEN), 3) TPN with teduglutide, or 4) PEN with teduglutide. Teduglutide was administered at 1 mg/kg/day in 35 mM sodium phosphate (dibasic), 50 mM D-histidine in 3% w/v D-mannitol buffer and was delivered *via* the central line. At 4 hours, 48 hours or 7 days following surgery, tissues were harvested for the analysis of intestinal structural (segment weight and length for small and large intestine including villus height and crypt depth) and functional indices (nutrient transport). The following diagram shows the study design (from page 11 of the study report).

Figure 3-1 Experimental Design



teduglutide treatment, PEN feeding, and the combination of teduglutide administration and PEN feeding resulted in significant improvements in crypt-villus architecture in the small intestine. Teduglutide increased villus height by 20% in the duodenum, 20% in the jejunum, and 12% in the ileum, irrespective of the time point and route of administration. Teduglutide and PEN showed synergistic effect as villus height was numerically greatest in this group at all time points. Teduglutide increased crypt depth in the ileum and colon, when compared to vehicle-treated groups. Similar to effects observed on villus height, crypt depth was numerically greatest at 7-day for both teduglutide and PEN group. Functionally, duodenal, jejunal and ileal glucose transport and jejunal glutamine transport was increased at 4 h post resection, regardless of route of administration. Based on these findings, teduglutide appeared to cause improvements in structural and transient functional aspects of the neonatal pig intestine following surgery. When teduglutide and PEN were co-administered, the combination appears to have synergistic effect on villus height.

6-Week Study of the Effects of Mid-Small Bowel Resection on the Gastrointestinal Tract in Cynomolgus Monkeys (XGW00006)

Methods: The objective of this study was to determine the relationship between clinical findings, influence on body weight, hematology, and clinical chemistry parameters to the morphological changes in the GIT of Cynomolgus monkeys following surgical mid-small bowel resection. The relationship between the histological observations and citrulline levels (an amino acid produced by enterocytes as a biomarker of a reduced enterocyte mass; Crenn P, Coudray-Lucas C, Thuillier F, et al., 2000, Postabsorptive Plasma Citrulline Concentration is a Marker of Absorptive Enterocyte Mass and Intestinal

Failure in Humans, *Gastroenterology*, 119:1496-505; Jeppesen PB, Gilroy R, et al., 2011, Randomised Placebo-Controlled Trial of teduglutide in Reducing Parenteral Nutrition and/or Intravenous Fluid Requirements in Patients with Short Bowel Syndrome, *Gut*, 60:902-914) was also examined. In this study, Cynomolgus monkeys (n = 8/sex, 2.4 to 4.0 years of age for the males and 2.6 to 3.8 years of age for the females, and weighing 2.2 to 3.8 kg for the males and 2.4 to 2.9 kg for the females at Week -1 of the study) were assigned to groups as shown in the table below (from page 13 of the report).

Group No.	Number of Males/Females	Treatment	Number Necropsied:	
			Day 30	11 Jan 2007 ¹
1	2/2	Control (no surgery)	0	0
2	6/6	Mid-small bowel resection	3/3	3/3

¹ All final necropsies were performed on the same day, January 11, 2007, which corresponds to Set A Day 37, Set B Day 36, Set C Day 35, and Set D Day 32.

Animals underwent either no surgical treatment (Group 1) or a surgical mid-small bowel resection (Group 2) on Day 1. The animals were observed for mortality and morbidity (twice daily), clinical signs (once daily) and body weight (Week -1 and weekly thereafter). Blood samples were collected for clinical pathology, including serum chemistry and hematology (prior to surgery and on Days 2, 4, 8, 15, 28, and prior to necropsy). Blood samples were also collected for GLP-2 and citrulline analyses (prestudy and on Days 2, 4, 8, 15, and 28). Group 1 animals were released to the Testing Facility animal colony after the final data were collected on Day 37 or 35. Six animals from Group 2 (3 per sex) were euthanized on Day 30, and the remaining 6 animals from Group 2 (3 per sex) were euthanized on Day 37, 36, 35, or 32. At termination, a full necropsy was conducted on all Group 2 animals sacrificed on Day 30, and select tissues were collected for histopathology.

Results: Treatment-related clinical signs in Group 2 animals included emesis (3 of 12 animals), watery feces (9 of 12 animals), and low food consumption (7 of 12 animals). These were most prevalent in the first 11 days following surgery, and recovered in most animals within two weeks. One male (# 2003) had a distended abdomen on Day 25. In Group 2, loss of body weight during the first 2 weeks post-surgery was considered as the result of the mid-small bowel resection. In addition, there were significant surgery-related reductions in phosphorus and cholesterol as well as mild reductions in albumin and gamma glutamyltransferase (GGT) that persisted through Day 28. Surgery associated changes in hematology changes included reductions in red blood cell counts on Day 8, hemoglobin on Days 8 and 15, and hematocrit on Day 8 compared to control. Prior to resection, mean plasma citrulline levels were approximately 75 μ M. By the second day after resection, citrulline levels decreased to about 20 μ M and then slowly recovered to about 40 μ M by Day 28. In general, citrulline levels in non-resected animals remained relatively unchanged throughout the 28-day period. Treatment-related histopathological findings included increased villus density and height within all segments of the small bowel, cellular hypertrophy of biliary epithelium in the liver,

mucosal hyperplasia and mononuclear cell infiltrates of the gallbladder and common bile duct. The increase in citrulline level was consistent with the reported increase in intestinal hyperplasia/hypertrophy due to a compensatory rise in endogenous GLP-2 following intestinal resection in the rat (Ljungmann K, Hartmann B, et al. 2001, Time-Dependent Intestinal Adaptation and GLP-2 Alterations after Small Bowel Resection in Rats, *Am J Physiol Gastrointest Liver Physiol*, 281:G779-G785). Overall, as per the sponsor, the hyperplastic changes observed in the small bowel, liver, gall bladder, and common bile duct were part of a natural response following intestinal resection likely due in part to the pharmacology of endogenous GLP-2.

4.2 Secondary Pharmacology

Cross Reactivity Screening of Teduglutide Against G-Protein Coupled Receptors (SP08-001-0600)

The objective of this study was to determine the selectivity of teduglutide against various G-protein coupled receptor (GPCR) binding sites. In addition, functional cross-reactivity against the most closely related GPCR to the GLP-2R and GLP-1R was tested. Activity was measured either by displacement of [³H]-radioligands or activation of cAMP (cyclic adenosine monophosphate) production in transfected cells expressing the target receptor.

Teduglutide did not exhibit significant interactions with representative GPCRs at 10 μ M and 30 μ M concentrations. At the highest tested concentration, teduglutide inhibited [³H]-radioligand binding of standard compounds against serotonin receptors 1A, 1B, 1D, 2A, 2C, 6 or 7 by less than 17%. Similarly, teduglutide displaced binding at the dopamine receptors subtypes D1, D2, D4 or D5 with a maximum displacement of 21% observed at the D4 receptor. At 30 μ M, teduglutide inhibited [³H]-radioligand binding at the α -adrenergic and muscarinic receptors (M1 and M2) by 35% and 45% respectively. Teduglutide minimally increased cAMP accumulation in HEK293 cells transiently expressing the human GLP-1R by 2% and 3% at 10 μ M and 30 μ M, respectively. Teduglutide concentrations ranging from 1 pM to 1 μ M did not increase cAMP production in CHO cells stably expressing GLP IR.

Effect of Teduglutide in a Rat Growth and Body Composition Model (PH03-008)

The objective of this study was to evaluate growth promoting effects of teduglutide in rats. In this study, rats were administered vehicle (10 mM ammonium bicarbonate, pH 8), teduglutide or porcine GLP-2 (p-glycine-GLP-2) by once daily SC injection at a dose of 0.2 mg/kg/day for 14 days. The following parameters were studied: body weight, food consumption and DEXA (Dual X-Ray Absorptiometry) analysis to determine body composition.

No statistically significant differences were observed in body weight, food intake or feed efficiency between the teduglutide treated and vehicle-treated groups. Percentage lean body mass was not affected by teduglutide treatment as measured by DEXA. There was a trend towards a higher percentage fat mass in the teduglutide treated group (8%).

4.3 Safety Pharmacology

Cardiovascular and Respiratory Effects in the Anaesthetized Dog Following Intravenous Administration

The review of this study (1621/009-D6146) is incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

1. **Cardiovascular and Respiratory Effects in Anesthetized Dogs:** In propofol anaesthetized dogs, ALX-0600 at an intravenous dose of 0.1 mg/kg produced increase in heart rate, the force ventricle contraction dp/dtmax (from 2589 to 3303 mmHg/s) and femoral blood flow (from 101 to 142 ml/min) which reflected a decrease in femoral resistance. T wave height decreased by 26% and, PR and QT intervals were affected after 20 min of the administration of the compound. These findings indicated that the compound could affect heart conductivity. However, the effects were not seen at higher doses of 1 and 10 mg/kg, i.v. ALX-0600. The test was repeated with isoflurane anesthesia in 3 dogs, a low dose of 0.1 mg/kg ALX-0600 did not affect cardiovascular effects and an increase of 48.3% in minute volume, respiratory rate and peak respiratory flow were seen. The intermediate dose produced only small changes in these parameters.

Effects of Teduglutide on Cloned hERG Channels Expressed in Mammalian Cells (031203.OQQ)

The current study examined the *in vitro* effects of teduglutide on the hERG channel current expressed in HEK293 cell line. It is to be noted here that this study was intended to examine the effects of ALX1-11, rhPTH (1-84) on hERG channel current. However, after completion of the hERG portion of the study, it was determined that the compound sent from the Sponsor was not ALX1-11 as intended, but rather teduglutide. Dose solution analysis was not completed. The data presented herein reflected transformations made to the raw data to adjust for the testing of ALX-0600 instead of ALX1-11. It should be noted that an additional GLP study, 070320.OQQ, was conducted to further evaluate the effect of teduglutide on hERG current. In this study, teduglutide was tested at 0.05, 0.5 and 50 ng/mL concentrations. Positive control was terfenadine (60 nM) in DMSO.

Teduglutide inhibited hERG current by 2.1% at 0.05 ng/mL, 4.4% at 0.5 ng/mL, 2.8% at 5 ng/mL and 4.1% at 50 ng/mL versus 1.3 % in the vehicle control (HEPES buffered saline). The IC₅₀ was not determined but was estimated to be >50 ng/mL. Terfenadine (62.25 nM) inhibited hERG current by 80.8%. The following table (from page 15 of the report) shows the summary data for teduglutide.

Table 2: Summary statistics for ALX-0600 inhibition of hERG current

Mean percent inhibition at each ALX-0600 concentration (Mean), standard deviation (SD), standard error of the mean (SEM), and number of cells (N).

Concentration (ng/mL)	Mean	SD	SEM	N
0	1.3%	0.3%	0.2%	3
0.05	2.1%	1.0%	0.6%	3
0.5	4.4%*	1.7%	1.0%	3
5	2.8%	0.9%	0.5%	3
50	4.1%*	0.7%	0.4%	3

* Values were statistically different from vehicle alone.

The sponsor stated that errors associated with the handling of test article in this study would have been anticipated to result in exposure of cells to unusually low concentrations of the test article. Although there was no indication that the test article significantly inhibited hERG current, the concentrations expected to have been achieved would have provided fractional multiples of anticipated therapeutic plasma levels in animals and humans, and therefore the data generated from this study were considered of minimal value. This study was subsequently repeated by the above study (070320.0QQ) using more relevant concentrations of test article.

Effects of Teduglutide on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (070320.0QQ)

The objective of this study was to examine the *in vitro* effects of teduglutide (30 and 300 µg/mL, n = 3) on the hERG (human ether-à-go-go-related gene) channel current (IKr, delayed rectifier potassium current). In this assay, hERG potassium channels were expressed in a human embryonic kidney (HEK293) 293 cell line that lacks endogenous IKr. The vehicle used was HEPES-buffered physiological saline. The positive control was terfenadine (60 nM) in dimethylsulfoxide (DMSO).

Teduglutide inhibited hERG current by 0.4% at 30 µg/mL and 0.6 % at 300 µg/mL versus 0.6% in the control. However, this inhibition was not statistically significant. The IC₅₀ for the inhibitory effect of teduglutide on hERG potassium current could not be calculated. Under identical conditions, the positive control (60 nM terfenadine) inhibited hERG current by 83%. The following table (from page 19 of the report) shows the

summary data for teduglutide. Overall, teduglutide did not have any effect on hERG current under the conditions of the experiment.

Table 2: ALX-0600 Summary Statistics

Mean percent inhibition at each ALX-0600 concentration (Mean), standard deviation (SD), standard error of the mean (SEM) and number of cells (N).

Concentration ($\mu\text{g/mL}$)	Mean	SD	SEM	N
Vehicle	0.6%	0.1%	0.1%	3
30	0.4%	0.5%	0.3%	3
300	0.6%	0.5%	0.3%	3

Effect of Teduglutide on Cardiac Action Potential in Isolated Canine Purkinje Fibers (1150-002)

In this study, teduglutide was tested at 0.3, 1.0, 3.0, and 10 $\mu\text{g/mL}$ concentrations. Modified Tyrode's Solution (MTS) with 0.1% phosphate buffer and sotalol (30 μM) were used as the vehicle and positive control, respectively. Purkinje fibers were exposed to sequential, ascending applications of vehicle or teduglutide for a period of approximately 15 minutes at each concentration. After exposure at each concentration, action potentials from the Purkinje fiber cells were recorded at each stimulation rate of 2.0, 1.0, and 0.5 Hz.

Teduglutide had no effect on action potential duration (APD_{50} and APD_{90}), rate of depolarization (V_{max}), overshoot (OS), and resting membrane potential (EM). The positive control, sotalol, produced the anticipated effects (increase in APD, and decrease in V_{max} and OS and slightly less negative resting membrane potential as compared to the vehicle control).

Effects of Teduglutide on Action Potentials in Isolated Canine Cardiac Purkinje Fibers (031202.OQQ)

This study was intended to examine the effects of ALX1-11, rhPTH (1-84) on action potentials in isolated canine Purkinje fibers. However, after completion of the action potential duration (APD) portion of the study, it was determined that the compound sent from the sponsor was not ALX1-11 as intended, but rather teduglutide (ALX-0600). The reported, nominal concentrations for teduglutide formulations were calculated based on the chemical information provided in the Certificate of Analysis and the raw data for solution preparation. Dose solution analysis was not completed, so the concentration that the fibers were exposed to was not verified. The sponsor stated that errors

associated with the handling of test article in this study would have been anticipated to result in exposure of Purkinje fibers to unusually low concentrations of the test article. Although it was concluded that the test article did not prolong APD, the concentrations expected to have been achieved would have provided fractional multiples of anticipated therapeutic plasma levels in animals and humans, and therefore the data generated were considered of minimal value.

In this study, three concentrations of teduglutide (0.05, 0.5 and 5 ng/mL) were added sequentially to each of four fiber preparations at three stimulus intervals (basic cycle lengths of 2, 1 and 0.5 s) and the effects of teduglutide on action potential parameters were compared to time-matched vehicle control sequences.

APD₉₀: At a 2 s basic cycle length (BCL) that causes bradycardia, the average prolongation in APD₉₀ was 0.8, 3.8 and 5.5% at 0.05, 0.5 and 5 ng/mL concentrations, respectively. At 1 and 0.5 s BCLs that cause normocardia and tachycardia, respectively, the average change in APD₉₀ was 1.4, 3.6 and 5.4% and 1.3, 3.3 and 5.8% at 0.05, 0.5 and 5.0 ng/mL, respectively. Teduglutide increased APD₉₀ when compared to time-matched vehicle controls at above three concentrations.

APD₆₀: At a 2s BCL, the average prolongation in APD₆₀ was 2.0, 5.6 and 8.1% at 0.05, 0.5 and 5 ng/mL concentrations, respectively. At 1 and 0.5 s BCL, the average prolongations in APD₆₀ were 3.0, 5.4 and 8.3%, and 2.8, 4.9 and 8.4% at 0.05, 0.5 and 5 ng/mL, respectively.

Teduglutide did not affect resting membrane potential, action potential amplitude and V_{max} at any of the tested concentrations and at three stimulus intervals. In contrast, 100 μ M sotalol, the positive control, produced statistically significant prolongation of the APD₆₀ (51.9, 44.7 and 26.5% at stimulus intervals of 2, 1 and 0.5 s BCL, respectively) and APD₉₀ (52, 40.7 and 29.2% at stimulus intervals of 2, 1 and 0.5 s BCL, respectively) at all stimulus intervals. The following table (from page 17 of the report) shows the summary results for teduglutide.

Table 1. Summary of the Effects of ALX-0600

2 s BCL					
Concentration (ng/mL)	APD₆₀ (Δ%)	APD₉₀ (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
0.05	2.0 ± 1.6	0.8 ± 1.4	-1.3 ± 0.5	0.8 ± 0.5	-5.5 ± 2.7
0.5	5.6 ± 3.0	3.8 ± 2.4	-2.2 ± 1.3	0.2 ± 0.7	-7.5 ± 4.5
5	8.1 ± 2.1	5.5 ± 1.8	-1.8 ± 0.8	-1.3 ± 1.3	-14.1 ± 4.8

1 s BCL					
Concentration (ng/mL)	APD₆₀ (Δ%)	APD₉₀ (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
0.05	3.0 ± 1.9	1.4 ± 1.5	-1.8 ± 0.5	1.5 ± 0.8	-5.5 ± 3.9
0.5	5.4 ± 3.3	3.6 ± 2.5	-2.4 ± 1.4	0.8 ± 1.0	-8.6 ± 4.6
5	8.3 ± 2.5	5.4 ± 2.0	-1.9 ± 0.9	-0.3 ± 1.2	-14.0 ± 5.8

0.5 s BCL					
Concentration (ng/mL)	APD₆₀ (Δ%)	APD₉₀ (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
0.05	2.8 ± 1.6	1.3 ± 1.4	-1.8 ± 0.8	1.5 ± 0.9	-4.3 ± 2.9
0.5	4.9 ± 2.6	3.3 ± 2.1	-2.2 ± 1.6	0.8 ± 1.2	-7.6 ± 3.4
5	8.4 ± 2.1	5.8 ± 1.8	-2.0 ± 1.1	0.0 ± 1.2	-13.4 ± 4.9

BCL, Basic Cycle Length; APD₆₀ and APD₉₀, action potential duration measured at 60% and 90% repolarization; Δ%, Percent change from baseline values (Mean ± SEM, n = 4 fibers); ΔmV, absolute change from baseline in millivolts; RMP, resting membrane potential; APA, action potential amplitude; Vmax, maximum rate of depolarization.

The vehicle control data is shown in the table below (from page 18 of the report).

Table 2. Summary of the Effects of Vehicle Control

2 s BCL					
Sequence Number	APD ₆₀ (Δ%)	APD ₉₀ (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
1	0.1 ± 1.5	-0.4 ± 1.3	-0.7 ± 0.5	0.1 ± 0.6	-0.1 ± 0.9
2	4.9 ± 1.3	3.3 ± 1.6	-1.4 ± 1.1	2.3 ± 1.2	5.3 ± 4.3
3	4.9 ± 1.6	3.0 ± 1.4	-1.5 ± 1.4	0.7 ± 1.4	-3.5 ± 7.2

1 s BCL					
Sequence Number	APD ₆₀ (Δ%)	APD ₉₀ (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
1	0.4 ± 2.0	0.3 ± 1.7	-0.1 ± 0.7	-0.1 ± 0.1	-1.5 ± 0.5
2	4.2 ± 0.8	3.3 ± 0.9	-1.3 ± 0.9	1.9 ± 1.1	1.8 ± 4.1
3	4.4 ± 1.8	3.3 ± 1.7	-1.1 ± 1.1	0.3 ± 1.7	-2.1 ± 8.7

0.5 s BCL					
Sequence Number	APD ₆₀ (Δ%)	APD ₉₀ (Δ%)	RMP (ΔmV)	APA (ΔmV)	Vmax (Δ%)
1	0.1 ± 1.7	0.0 ± 1.7	-0.2 ± 1.0	0.2 ± 0.3	-1.4 ± 0.8
2	3.7 ± 0.9	2.7 ± 1.1	-1.0 ± 1.0	1.2 ± 1.6	4.2 ± 4.7
3	4.6 ± 1.7	3.2 ± 1.7	-1.1 ± 1.0	0.1 ± 2.0	-2.9 ± 8.7

BCL, Basic Cycle Length; APD₆₀ and APD₉₀, action potential duration measured at 60% and 90% repolarization; Δ%, Percent change from baseline values (Mean ± SEM, n = 4 fibers); ΔmV, absolute change from baseline in millivolts; RMP, resting membrane potential; APA, action potential amplitude; Vmax, maximum rate of depolarization.

In conclusion, teduglutide slightly prolonged APD₆₀ (2.0 to 8.4% increase) and APD₉₀ (0.8 to 5.8% increase) at all tested doses when compared to the time-matched vehicle controls. However, teduglutide did not cause significant changes in resting membrane potential, action potential amplitude and the maximum rate of depolarization (V_{max}) at any of the tested concentrations and at three stimulus intervals.

Neuropharmacological Profile of Teduglutide in Rats (0200RN12.001)

In this study, three groups of male Sprague Dawley (SD) rats (n = 10/dose) were administered teduglutide at SC doses of 1, 5 or 25 mg/kg (dose volume of 2.5 mL/kg). An additional group of ten male rats was administered the vehicle (phosphate buffered solution) at a dose volume of 2.5 mL/kg, SC. The rats were observed at 15, 30 and 45 minutes, 1, 2, 3, 4 and 24 hours post-dose. Body temperatures were measured at 60 minutes post-dose.

Teduglutide did not produce any significant effect on any neuropharmacological parameters (abnormal posture, ataxia, awareness reaction, tremors, corneal reflex, grip strength, irritability, loss of righting, motor activity, pain response, startle response, and

seizure/convulsion, etc.) at any of the tested doses. Overall, teduglutide did not appear to have any adverse effect on the CNS in rats in this study.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption:

The reviews are incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION:**Bioavailability Following Administration of Intravenous and Subcutaneous Doses in Rabbits (Study # 7203-105)**

Methods: Eight groups of rabbits (3/sex/group) were administered either subcutaneously or intravenously with ALX-0600 at 1 and 25 mg/kg (single dose, 1.25 ml/kg). Blood samples were collected at predose, 0.083, 0.167, 0.333, 0.5, 1.4, 2, 8 and 24 hr after the treatment. The plasma concentration of compound was determined by a validated enzyme-linked immunosorbent assay (ELISA) method.

Results: After i.v. administration, ALX-0600 plasma level declined rapidly and reached background level within 8 to 24 hours. The mean C_{max} at 1 and 25 mg/kg were 6,502 ng/ml in males and 5,862 ng/ml in females, and 16,418 in males and 17,4412 ng/ml in females, respectively. The mean AUC_{0-t} values following i.v. administration at 1 and 25 mg/kg were 1,950 ng.hr/ml in males and 1,918 ng.hr/ml in females and 53,491 ng.hr/ml in males and 57,266 ng.hr/ml in females, respectively. The increase in exposure was dose proportional.

After subcutaneous administration, ALX-0600 was rapidly taken up into the systemic circulation with T_{max} values ranged from 0.5 to 1 hour. The mean C_{max} at 1 and 25 mg/kg were 1,169 ng/ml in males and 738 ng/ml in females, and 9,602 in males and 8,571 ng/ml in females, respectively. The mean AUC_{0-t} values following s.c. administration at 1 and 25 mg/kg were 1,822 ng.hr/ml in males and 1,532 ng.hr/ml in females and 44,536 ng.hr/ml in males and 56,889 ng.hr/ml in females, respectively. The increase in C_{max} appeared to be less than dose proportional. The $T_{1/2}$ ranged from 0.870 to 1.71 hours.

Overall, there were no apparent gender differences in C_{max} and AUC values for both dose levels and dose routes. The absolute bioavailability (F) after s.c. administration ranged from 80-99%.

The mean pharmacokinetic (PK) parameters are shown in the following table (data extracted from Amendment 26, page 13 of sponsor's submission):

Route	Group	Dose (mg/kg)	Sex	C _{max} (ng/ml)	T _{max} (Hr)	AUC _{0-t} (ng.hr/ml)	T _{1/2} (Hr)	F
I.V.	1	1	M	6502	0.083	1950	NA	ND
			F	5862	0.083	1918	NA	ND
	2	25	M	16418	0.083	53491	NA	ND
			F	174412	0.083	57266	NA	ND
S.C.	3	1	M	1169	0.50	1822	NA	ND
			F	738	0.50	1532	0.87	0.80
	4	25	M	9062	1.0	44536	1.59	0.83
			F	8571	1.0	56889	1.71	0.99

ND: Not determined

NA: Not available

Bioavailability Following Administration of Intravenous and Subcutaneous Doses in Mice (Study # 7203-107)

Methods: In this study, two groups of CD-1 mice (50/sex/group) were subcutaneously administered single bolus doses of ALX-0600 at 1 and 25 mg/kg (5 ml/kg). Two other groups of animals (50/sex/group) were administered ALX-0600 at i.v. bolus doses of 1 and 25 mg/kg (5 ml/kg). Blood samples were collected from 5 animals/sex/time point at predose, 0.083, 0.167, 0.333, 0.5, 1, 2, 4, 8 and 24 hr post-dose. The plasma concentration of ALX-0600 was determined by a validated ELISA method.

Results: After i.v. dose of 1 mg/kg, exposures (AUC_{0-4 hr}) to ALX-0600 were 3,517 and 2,270 ng.hr/ml in males and females, respectively. At 25 mg/kg, AUC_{0-4hr} values were 89,457 and 63,716 ng.hr/ml in males and females, respectively. Increase in exposure appeared to be dose-proportional. The t_{1/2} values ranged from 0.388 to 1.11 hours.

After s.c. dose of 1 mg/kg, AUC_{0-4 hr} values were 2,911 and 1,913 ng.hr/ml in males and females, respectively. The AUC_{0-4 hr} values at 25 mg/kg were 69,948 and 49,213 ng.hr/ml in males and females, respectively. The t_{1/2} ranged from 0.368 to 0.580 hours.

The absolute bioavailability after s.c. administration was 77.2 to 84.4%, with a mean of 80.7%. The following table (from Amendment 30, page 14 of sponsor's submission) shows the summary of pharmacokinetic parameters for ALX-0600 in mouse plasma.

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Summary of Pharmacokinetic Parameters for ALX-0600 in Mouse Plasma

Dose Route	Dose Group	Dose Level (mg/kg)	Sex	C _{max} (ng/mL)	T _{max} (Hours)	AUC _{0-t} (ng·hr/mL)	AUC _{0-∞} (ng·hr/mL)	K _e (Hours ⁻¹)	t _{1/2} (Hours)	F	CL (mL/hr/kg)	V _d (mL/kg)	V _{ss} (mL/kg)	MRT (Hours)
Intravenous	1	1	M	10967	0.0830	3517	3518	1.79	0.388	NA	284	159	67.8	0.239
			F	7599	0.0830	2270	2270	1.68	0.413	NA	441	262	85.5	0.194
	2	25	M	271353	0.0830	89457	89464	0.754	0.919	NA	279	371	62.9	0.225
			F	207232	0.0830	63716	63722	0.625	1.11	NA	392	628	67.5	0.172
Subcutaneous	3	1	M	2825	0.333	2911	2913	1.88	0.368	0.828	NA	NA	NA	NA
			F	2164	0.333	1913	1916	1.65	0.420	0.844	NA	NA	NA	NA
	4	25	M	46337	0.333	69948	69953	1.21	0.571	0.782	NA	NA	NA	NA
			F	31368	0.333	49213	49217	1.20	0.580	0.772	NA	NA	NA	NA

NA Not applicable.

(b)
(4)

Bioavailability Following Administration of Intravenous and Subcutaneous Doses in Cynomolgus Monkeys (Study # 7203-106)

Methods: The aim of the study was to determine absolute bioavailability of ALX-0600 following s.c. administration in monkeys. Two groups of monkeys (2/sex/group) were administered a single bolus dose of ALX-0600 at either s.c. or i.v. at 0.5 or 12.5 mg/kg (0.625 mL/kg), respectively. The study design is shown below (from Amendment 30, Vol. 1, page 82 f sponsor’s submission). Blood samples (2/sex/group) were collected at predose, 0.083, 0.167, 0.333, 0.5, 1, 2, 3, 4, 8 and 24 hours post-dose. Plasma samples were analyzed by a validated ELISA method.

Results: After i.v. administration, plasma concentrations declined rapidly (in a bi-exponential manner), with t_{1/2} values ranging from 1.05 to 3.13 hours. The AUC_{0-t} values were 3,175 and 64,089 ng·hr/ml in males and 3,159 and 62,748 ng·hr/ml in females at 0.5

Group /Phase	Number of Animals ^a		Target Dose Level (mg/kg)	Target Dose Concentration (mg/mL)	Dose Route	Target Dose Volume (mL/kg)	Sample Collection
	Male	Female					
1/1	2	2	0.5	0.8	IV	0.625	Blood
1/1	2	2	0.5	0.8	SC	0.625	Blood
2/1	2	2	12.5	20	IV	0.625	Blood
2/1	2	2	12.5	20	SC	0.625	Blood
1/2	2	2	0.5	0.8	SC	0.625	Blood
1/2	2	2	0.5	0.8	IV	0.625	Blood
2/2	2	2	12.5	20	SC	0.625	Blood
2/2	2	2	12.5	20	IV	0.625	Blood

IV Intravenous.
SC Subcutaneous.

^a Control blood samples were collected from 2 animals/sex/time point at three points over the course of a day (approximately 6 hours apart) between Phases 1 and 2.

Note: The animals that received the IV dose in Phase 1 received the SC dose in Phase 2, and the animals that received the SC dose in Phase 1 received the IV dose in Phase 2. Phase 2 initiated 3 days after Phase 1 dose administration.

and 12.5 mg/kg, respectively. The exposure appeared to increase nearly dose proportionately. There were no apparent gender differences in the exposure.

After s.c. administration, ALX-0600 was rapidly taken up into the systemic circulation with T_{max} ranged from 0.833 to 2.67 hours. Mean AUC_{0-t} values at low and high dose were 2,452 and 75,284 ng.hr/ml in males and 2,650 and 50,381 ng.hr/ml in females, respectively. There were no marked gender differences in the exposure. The exposure appeared to increased dose proportionately. The $t_{1/2}$ ranged from 1.44 to 1.94 hours.

The PK parameters are shown below (data extracted from Amendment 30, page 93 of sponsor's submission):

Route	Group	Dose (mg/kg)	Sex	Cmax (ng/ml)	Tmax (Hr)	AUC _{0-t} (ng.hr/ml)	T _{1/2} (Hr)	F
I.V.	1	0.5	M	7111	0.083	3175	1.05	NA
			F	6357	0.083	3159	1.14	NA
	2	12.5	M	157172	0.083	64089	3.13	NA
			F	138922	0.083	62748	2.79	NA
S.C.	1	0.5	M	754	0.833	2452	1.44	0.745
			F	814	1.25	2650	1.25	0.839
	2	12.5	M	12872	2.67	75284	1.86	1.12
			F	9261	2.67	50381	1.94	0.833

NA = Not applicable

F= Absolute bioavailability

The females appeared to have greater bioavailability than males. The absolute bioavailability after s.c. administration in males and female monkeys was 74.5 and 83.9%, respectively. The overall bioavailability was about 88%.

Pharmacokinetic Study in Juvenile Minipigs (Study No. 51170)

Methods: The objective of this study was to evaluate the pharmacokinetics of ALX-0600 administered subcutaneously (SC) and intravenously (IV) to juvenile minipigs. This study was conducted in 24 weaned piglets (n =12/sex) allocated into 4 groups. The drug was administered twice daily (8-hours interval between doses) on Days 1-6 and 15-20, and a single morning dose on Days 7 and 21. On Days 8-14 the animals were not treated (wash-out period). The animals were treated in a cross-over fashion. The dose volume was 1.25 ml/kg (0.625 ml/kg x 2). The vehicle contains dibasic and monobasic sodium phosphate, L-histidine, D-mannitol in sterile Water for Injection, USP (pH = 7.2-7.6). The animals in Groups 1 and 3 were treated subcutaneously with 0.5 and 12.5 mg/kg bid of ALX-0600, respectively from Days 1-7. From Days 15-21 the same animals in Groups 1 and 3 were treated intravenously with 0.5 and 12.5 mg/kg bid of ALX-0600, respectively. The animals in Groups 2 and 4 were treated intravenously with 0.5 and 12.5 mg bid of ALX-0600, respectively from Days 1-7. From Days 15-21 the same animals in Groups 2 and 4 were treated subcutaneously with 0.5 and 12.5 mg/kg bid of ALX-0600, respectively. Clinical signs were recorded daily and the body weights were recorded twice weekly. Blood samples were collected at 0 (predose), 15, 30, 45, 60, 120, 240 and 480 minutes after the first dose on Days 1, 7, 15 and 21. The following table shows the study design (from page 17 of the study report).

Treatment

The animals were divided into 4 groups each of 3 males and 3 females. The animals were dosed in a cross-over fashion as detailed in the tables below:

Animal No			Study Day								
			1	2	3	4	5	6	7	8-14	
			Dosing*	x	x	x	x	x	x	x	
		Sampling**	x							x	
Males	Females		Treatments***								
1 - 3	4 - 6	Group 1	A	A	A	A	A	A	A	A	WO
7 - 9	10 - 12	Group 2	B	B	B	B	B	B	B	B	WO
13 - 15	16 - 18	Group 3	C	C	C	C	C	C	C	C	WO
19 - 21	22 - 24	Group 4	D	D	D	D	D	D	D	D	WO

Animal No			Study Day							
			15	16	17	18	19	20	21	
			Dosing*	x	x	x	x	x	x	x
		Sampling**	x							x
Males	Females		Treatments***							
1 - 3	4 - 6	Group 1	B	B	B	B	B	B	B	B
7 - 9	10 - 12	Group 2	A	A	A	A	A	A	A	A
13 - 15	16 - 18	Group 3	D	D	D	D	D	D	D	D
19 - 21	22 - 24	Group 4	C	C	C	C	C	C	C	C

* Dosing was twice daily (8-hours interval between doses) on Days 1-6 and 15-20, and a single morning dose on Days 7 and 21.

** PK sampling was at 0 (predose), 15 min, 30 min, 45 min, 60 min, 120 min, 240 min and 480 minutes after the first dose on Days 1, 7, 15 and 21. (480 minute sample was taken prior to administration of second daily dose.)

*** Treatments: A= low dose s.c., B=low dose i.v., C=high dose s.c., D=high dose i.v., WO=washout

Results: There were no treatment-related clinical signs and body weights. After intravenous administration, ALX-0600 concentrations declined in a bi-exponential manner. The C_{max} and AUC_{0-t} were generally similar after single and multiple dosing, indicating no accumulation after multiple dosing. The increases in C_{max} or AUC_{0-t} were approximately dose proportional.

After subcutaneous administration, the mean T_{max} ranged from 0.667 to 1.63 hours after a single dose or after multiple dosing. The increases in AUC_{0-t} were generally dose proportional following subcutaneous dosing. No marked gender difference was observed

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in C_{max} or AUC_{0-t} values. ALX-0600 bioavailability was estimated to be approximately 85% after s.c. administration.

The results are shown in the following tables (from page 72-74 of the study report).

Table A: Summary of Mean Pharmacokinetic Parameters for ALX-0600 in Minipig Plasma After Intravenous Administration Single Dose

Dose (mg/kg/day)	Gender		C_{max} (ng/mL)	T_{max} (Hours)	C_s (ng/mL)	AUC_{0-t} (ng·hr/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	K_e (Hours ⁻¹)	$t_{1/2}$ (Hours)	CL (mL/hr/kg)	V_d (mL/kg)	MRT (Hours)
1	Male	Mean	1995	0.256	5762	1702	1860	0.8511	0.807	584	264	0.408
		SD	547	0	2128	519	585	0.0547	0.052	200	100	0.063
		N	6	6	6	6	6	6	6	6	6	6
	Female	Mean	1381	0.250	4913	1440	1445	0.7796	0.902	744	275	0.364
		SD	543	0	1495	424	477	0.1087	0.136	238	116	0.042
		N	6	6	6	6	3	3	3	3	3	3
25	Male	Mean	51628	0.250	142214	44250	44284	0.7278	0.939	604	244	0.397
		SD	12963	0	41957	11300	11305	0.0668	0.083	187	96	0.047
		N	6	6	6	6	6	6	6	6	6	6
	Female	Mean	50045	0.250	144706	42613	42641	0.7860	0.921	616	237	0.374
		SD	10477	0	47971	9665	9661	0.1823	0.208	162	96	0.071
		N	6	6	6	6	6	6	6	6	6	6

Multiple Dose

Dose (mg/kg/day)	Gender		C_{max} (ng/mL)	T_{max} (Hours)	C_s (ng/mL)	AUC_{0-t} (ng·hr/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	K_e (Hours ⁻¹)	$t_{1/2}$ (Hours)	CL (mL/hr/kg)	V_d (mL/kg)	MRT (Hours)
1	Male	Mean	1996	0.250	5249	1541	1641	0.8190	0.862	626	228	0.436
		SD	347	0	628	288	288	NA	NA	112	NA	NA
		N	6	6	6	6	6	2	2	6	2	2
	Female	Mean	1396	0.250	4082	1290	1290	0.4476	1.55	822	286	0.413
		SD	345	0	952	281	281	NA	NA	262	NA	NA
		N	6	6	6	6	5	1	1	6	1	1
25	Male	Mean	48115	0.250	114658	39859	39859	0.8013	0.890	633	257	0.405
		SD	4964	0	10463	4103	4103	0.1524	0.160	70	45	0.061
		N	6	6	6	6	6	6	6	6	6	6
	Female	Mean	48121	0.250	121445	39075	39075	0.8152	0.880	649	238	0.365
		SD	6647	0	25228	5465	5465	0.1583	0.193	84	42	0.021
		N	5	5	5	5	5	5	5	5	5	5

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Table B: Summary of Mean Pharmacokinetic Parameters for ALX-0600 in Minipig Plasma After Subcutaneous Administration

		Single Dose							
Dose (mg/kg/day)	Gender		C_{max} (ng/mL)	T_{max} (Hours)	AUC_{0-24} (ng·hr/mL)	AUC_{0-48} (ng·hr/mL)	K_{el} (Hours ⁻¹)	$t_{1/2}$ (Hours)	F
1	Male	Mean	639	0.833	1378	1380	0.8722	0.803	0.882
		SD	160	0.129	261	261	0.0967	0.091	0.355
		N	6	6	6	6	6	6	4
	Female	Mean	640	0.833	1160	1162	0.8431	0.829	0.970
		SD	168	0.204	307	307	0.0788	0.086	0.458
		N	6	6	6	6	6	6	3
25	Male	Mean	9436	0.900	33292	34727	0.4993	1.35	0.770
		SD	4651	0.720	11763	12386	0.1194	0.37	0.352
		N	5	5	5	5	5	5	5
	Female	Mean	7958	1.63	32477	35473	0.3980	1.97	0.870
		SD	1563	0.59	5130	6488	0.1489	0.75	0.247
		N	6	6	6	6	6	6	6

Table B (Continued): Summary of Mean Pharmacokinetic Parameters for ALX-0600 in Minipig Plasma After Subcutaneous Administration

		Multiple Dose							
Dose (mg/kg/day)	Gender		C_{max} (ng/mL)	T_{max} (Hours)	AUC_{0-24} (ng·hr/mL)	AUC_{0-48} (ng·hr/mL)	K_{el} (Hours ⁻¹)	$t_{1/2}$ (Hours)	F
1	Male	Mean	609	0.708	1332	1332	0.8213	0.848	0.830
		SD	240	0.292	338	338	0.0604	0.063	0.174
		N	6	6	5	5	5	5	5
	Female	Mean	573	0.667	1175	1175	0.8108	0.859	0.986
		SD	145	0.258	441	441	0.0595	0.067	0.505
		N	6	6	6	6	6	6	6
25	Male	Mean	9142	1.46	32732	33492	0.4302	1.76	0.853
		SD	2207	0.60	5555	5851	0.1406	0.58	0.149
		N	6	6	6	5	6	6	5
	Female	Mean	9674	1.04	30367	30367	0.5250	1.56	0.703
		SD	3572	0.51	8714	8714	0.2160	0.74	0.111
		N	6	6	6	6	6	6	5

Comparator Study in the Wistar and Sprague Dawley Rat Following a Single Subcutaneous Injection Dose (800759)

Methods: The objective of this study was to examine the comparative pharmacokinetics (PK) in the Wistar Han and Sprague Dawley (SD) rat following a single SC injection of teduglutide. In this study, SD rats (n = 9/sex/dose) were administered teduglutide at 15 mg/kg and Wistar rats (n = 9/sex/dose) were treated at 1.5 and 15 mg/kg by SC route. The following parameters were evaluated: clinical signs, body weight and pharmacokinetics. Blood samples were collected at 0.25 h, 0.50 h, 1 h, 2 h, 4 h and 8 h post-dose. The following table (from page 11 of the report) shows the study design.

Text Table 1 Study Design

Group No. Identification	Strain	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg/dose)	Number of Animals	
					Males	Females
1 ALX-0600	Sprague Dawley	15	12	1.25	9	9
2 ALX-0600	Wistar	15	12	1.25	9	9
3 ALX-0600	Wistar	1.5	1.2	1.25	9	9

Results: Terminal $t_{1/2}$ ranged from 0.363 to 0.672 hours. There were no apparent gender or species differences in $t_{1/2}$. In both male and female Wistar rats, $t_{1/2}$ was lower at 1.5 mg/kg when compared to the 15 mg/kg group. Peak concentrations of teduglutide were generally observed at 0.5 hour post dose.

Exposure ($AUC_{0-\alpha}$) was higher in males when compared to females in the SD rats; however, no significant gender differences were observed in the Wistar Han rats. The exposure ($AUC_{0-\alpha}$) was higher in SD rats when compared to Wistar rats in both genders at 15 mg/kg. In this study, the mean combined gender $AUC_{0-\alpha}$ values at 15 mg/kg were 10624 ng.hr/mL and 7644 ng.hr/mL for the SD and Wistar Han rats, respectively. The following tables (pages 70 and 71 of the report) show the PK parameters.

Appendix 5
Table PK-2

Pharmacokinetic Parameters of ALX-0600 (teduglutide) Following a Single Subcutaneous Administration of ALX-0600 (teduglutide) to the Sprague-Dawley and Wistar Han Rat

(b) (4) Project No. 800759

Day 1

Gender	Group No.	Dose Level (mg/kg)	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-_{last}} (ng·h/mL)	k (1/h)	t _{1/2} (h)	AUC _{0-inf.} (ng·h/mL)	% Extrapolation AUC _{0-inf.}
Males	1	15	0.5	5483	8	12777	1.09	0.636	12782	0.0359
	2	15	0.5	5377	8	7879	1.04	0.665	7881	0.0243
	3	1.5	0.5	663	4	768	1.68	0.412	769	0.154
Females	1	15	0.25	5149	8	8463	1.03	0.672	8466	0.0343
	2	15	0.5	5805	8	7405	1.14	0.610	7406	0.0119
	3	1.5	0.5	751	4	691	1.91	0.363	692	0.0757

Appendix 5
Table PK-3Plasma ALX-0600 (teduglutide) C_{max} and AUC_{0-inf.} in the Wistar Han Rat and the Proportional Change of Each Parameter Relative to the Target Low Dose Following a Single Subcutaneous Administration of ALX-0600 (teduglutide)

(b) (4) Project No. 800759

Day 1

Gender	Group No.	Dose Level (mg/kg)	C _{max} (ng/mL)	AUC _{0-inf.} (ng·h/mL)	Dose Proportionality	C _{max} Ratio	AUC _{0-inf.} Ratio
Males	2	15	5377	7881	10	8.11	10.2
	3	1.5	663	769	1	1	1
Females	2	15	5805	7406	10	7.73	10.7
	3	1.5	751	692	1	1	1

Preliminary Pharmacokinetics of ALX-0600: Estimation of Pharmacokinetic Parameters After Single Intravenous or Subcutaneous Bolus Doses of ALX-0600 to Male and Female Sprague-Dawley Rats (ALX0600-10101-R-DM01-002)

The review of the above study is incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

1. Preliminary Pharmacokinetics After Single Intravenous/Subcutaneous Bolus Dose in Male and Female SD Rats:
(ALX-0600-10101R)

Methods: ALX-0600 was administered in 4 groups of male rats (5/group) as a bolus single intravenous dose of 0.1, 1, 3 or 10 mg/kg (volume = 1 ml/kg) to determine pharmacokinetics of ALX-0600. In other 2 groups (5/sex), 1 mg/kg ALX-0600 was given intravenously or subcutaneously and the blood samples were collected at the time intervals as described earlier. The plasma concentration of compound was determined by ELISA method.

Results: A dose related plasma concentration ($AUC_{0-24 \text{ hr}}$) with the half life varying from 32 to 40 min was seen in animals of 1, 3 and 10 mg/kg treatment groups. The half life was 24 to 40 min in 0.1 to 10 mg/kg treated animals. The other PK values of the study are shown below (Sponsor Table 1, vol 1:5, pp 139):

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Table 1. Preliminary pharmacokinetic parameters estimated following an I.v. bolus dose of 0.1, 1, 3 or 10 mg/kg of ALX-0600 to male rats.

Dose I.v. (mg/kg)	n	AUC(0-∞) (min µg/mL)	Elimination $t_{1/2}$ (min)	Total Body Clearance (CL) (mL/min/kg)	Steady-state Volume of Distribution (V_{ss}) (mL/kg)	Mean Residence Time (MRT) (min)
0.1	5	11.4 ± 2.2	23.9 ± 3.1	14.8 ± 2.4	288 ± 45	18.4 ± 0.9
1	5	78.2 ± 10.9	40.0 ± 8.0*	10.7 ± 1.59*	220 ± 30*	20.6 ± 1.0*
3	5	300 ± 28	82.0 ± 3.3*	9.40 ± 0.87*	182 ± 7*	20.5 ± 1.5*
10	5	1225 ± 298	38.7 ± 4.7*	7.82 ± 1.58*	158 ± 38*	19.8 ± 1.5*

The data represent the mean ± S.D. for n rats.

*Statistically significant compared to 0.1 mg/kg dose group ($p < 0.05$)

After a SC dose of 1 mg/kg ALX-0600 in male rats, C_{max} was 657±197 ng/ml, $AUC_{(0-\infty)} = 78 \pm 18.4$, $t_{max} = 42.0$ min, Mean residence time = 88.1±6.9 min and mean absorptive time (MAT)=67.4 ± 6.9 min. The estimated bioavailability was 80.5%.

Distribution:**Lacteal Excretion and Placental Transfer of ALX-0600 Following Administration of Subcutaneous Doses to Lactating Rats and Pregnant Rabbits (Study # 7203-104)**

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

Lacteal Excretion and Placental Transfer of ALX-0600 after Subcutaneous Administration to Lactating Rats and Pregnant Rabbits (Study # 7203-104)

Methods: Four pregnant female rabbits were used in the study to assess the extent of which ALX-0600 crosses the placenta. Three pregnant rabbits were treated with 25 mg/kg, s.c. of ALX-0600 from gestation day 6 (GD6) to GD18. Maternal and fetal blood was collected 1.5 hours post-treatment (estimated T_{max}). A fourth rabbit was used as a control.

Female rats were used to assess the extent to which ALX-0600 is secreted into milk. Six female rats (12 days after giving birth) were administered a single s.c. dose of 25 mg/kg ALX-0600. Milk was collected at 1.5 hours (estimated T_{max}) and 4 hours after dosing. A seventh rat was used as a control.

Results:

Concentrations of ALX-0600 in the rabbit plasma (Maternal and Fetal): Mean concentrations of 6,845.6 and 8.6 ng/ml were noted in the maternal and fetal plasma, respectively. The ratio of fetal to maternal concentration of ALX-0600 was 0.13%. The results indicated that ALX-066 has the potential to cross placental barrier.

Concentrations of ALX-0600 in the rat plasma and milk: After 1.5 hr of the treatment with ALX-0600, mean maternal plasma concentration was 89, 77.3 ng/ml and the plasma level was reduced to 465.7 ng/ml at 4 hr observation period. The concentration of ALX-0600 in the milk was 82.1 and 13.6 ng/ml at 1.5 and 4 hr post-treatment, respectively. The milk to plasma ratio was 0.92% and 2.91% at 1.5 and 4 hours post-treatment, respectively, indicating that ALX-0600 might has the potential to be excreted through breast milk.

Overall, the results of this study indicated that ALX-0600 has the potential to cross placental barrier in rabbits and is capable of being secreted through breast milk in rats. These findings are important from the perspective of labeling.

The results of this study are shown in the following table (from Amendment 24, Vol. 1, page 69 of sponsor's submission).

(b) (4) BEST AVAILABLE COPY

Table 3. Individual and Mean Concentrations of ALX-0600 in Rabbit Maternal and Fetal Plasma and Rat Plasma and Milk and Mean Fetal Plasma to Maternal Plasma and Milk to Plasma Concentration Ratios

Animal Number	Species	Collection Time (hr)	ALX-0600 Concentration (ng/mL)			Ratio (%)	
			Maternal Plasma	Fetal Plasma	Milk	Rabbit Fetal Plasma: Maternal Plasma	Rat Milk: Plasma
F09245	Rabbit	1.5	5089.4	8.1	NA	0.16	NA
F09246			4931.2	2.8	NA	0.06	NA
F09247			10516.2	15.0	NA	0.14	NA
Mean			6845.6	8.6	NA	0.12	NA
SD			3179.8	6.1	NA	NA	NA
C03681	Rat	1.5	8951.5	NA	56.7	NA	0.92
C03682			9434.0	NA	87.8	NA	0.87
C03683			8546.5	NA	101.8	NA	0.96
Mean			8977.3	NA	82.1	NA	0.92
SD			444.3	NA	23.7	NA	NA
C03684	Rat	4	321.5	NA	8.7	NA	4.22
C03685			675.7	NA	15.2	NA	2.01
C03686			400.0	NA	16.8	NA	3.39
Mean			465.7	NA	13.6	NA	3.21
SD			186.0	NA	4.3	NA	NA

NA Not applicable.
SD Standard deviation.

The sponsor submitted an amended report of this lacteal excretion study (7203-104) in this NDA. The mean rat milk:plasma ratios were calculated incorrectly in the above Table 3 of the report. The following Table 3 (from page 3 of the amended report) replaces the above table.

Table 3. Individual and Mean Concentrations of ALX-0600 in Rabbit Maternal and Fetal Plasma and Rat Plasma and Milk and Mean Fetal Plasma to Maternal Plasma and Milk to Plasma Concentration Ratios

Animal Number	Species	Collection Time (hr)	ALX-0600 Concentration (ng/mL)			Ratio (%)	
			Maternal Plasma	Fetal Plasma	Milk	Rabbit Fetal Plasma: Maternal Plasma	Rat Milk:Plasma
F09245	Rabbit	1.5	5089.4	8.1	NA	0.16	NA
F09246			4931.2	2.8	NA	0.06	NA
F09247			10516.2	15.0	NA	0.14	NA
Mean			6845.6	8.6	NA	0.12	NA
SD			3179.8	6.1	NA	NA	NA
C03681	Rat	1.5	8951.5	NA	56.7	NA	0.63
C03682			9434.0	NA	87.8	NA	0.93
C03683			8546.5	NA	101.8	NA	1.19
Mean			8977.3	NA	82.1	NA	0.92 ^a
SD			444.3	NA	23.1	NA	NA
C03684	Rat	4	321.5	NA	8.7	NA	2.71
C03685			675.7	NA	15.2	NA	2.25
C03686			400.0	NA	16.8	NA	4.20
Mean			465.7	NA	13.6	NA	2.91 ^a
SD			186.0	NA	4.3	NA	NA

NA Not applicable.

SD Standard deviation.

a: Values are obtained by the mean milk value divided by the mean plasma value.

Determination of ALX-0600 concentration in Cerebrospinal Fluid and Plasma Following Subcutaneous Bolus Injection in Rats (ALX-0600-10102-R)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

2. Determination of ALX-0600 concentration in Cerebrospinal Fluid and Plasma Following Subcutaneous Bolus Injection in Rats:
(ALX-0600-10102-R)

Methods: Twelve male anesthetized rats (SD strain) weighing between 260 to 300 g with catheters in cisterna magna were divided in to 3 groups and treated with 0, 3 or 10 mg/kg ALX-0600 (volume = 1 ml/kg). The csf samples (rate = 2 ul/min) were collected for over 6 hr period on day 1 and 29. Blood samples (0.25 ml) were collected at 3 hr of csf collection intervals. The plasma and csf concentrations of ALX-0600 and its metabolites were determined by ELISA procedure.

Results: A dose dependent plasma peak concentration of 1.37 and 2.76 ug/ml ALX-0600 was seen in males treated with 3 and 10 mg/kg with t_{max} 135 and 170 min. About 5.3 and 1.8 % of the plasma concentrations of the compound were seen in CSF after 135 to 345 and 135 to 285 min in animals belonging to 3 and 10 mg/kg ALX-0600 treatment groups. PK data suggested that ALX-0600 crossed blood CSF barrier in rats as seen in the table (Sponsor Table in vol 1:5, pp 149):

Table 1. Maximum plasma and csf concentrations (C_{max}) and corresponding t_{max} values following s.c. doses of 1, 3 and 10 mg/kg ALX-0600 to male rats.

Dose s.c.	n	Plasma		Csf	
		t_{max}	C_{max}	t_{max}	C_{max}
(mg/kg)		(min)	($\mu\text{g/mL}$)	(min)	($\mu\text{g/mL}$)
1 ^a	5	42.0 \pm 16.4	0.657 \pm 0.197	nd ^b	nd
3	5	75.0 \pm 30.0	1.37 \pm 0.27	135 \pm 47	0.0664 \pm 0.0643
10	2	60	2.76	170	0.0117

The data represent the mean \pm S.D. for *n* rats.

^a data from Report #ALX0600-10101R.

^b nd represents not determined.

In summary, intravenously administered dose of ALX-0600 attained a non-linear plasma concentration with a half-life varied from 24 to 40 min. The total body clearance and steady state volume of distribution were decreased at the higher doses indicating saturable kinetics. By subcutaneous route, peak plasma concentration of 1.37 $\mu\text{g/mL}$ was seen after 42 min and its bioavailability (as compared with IV route) was 81%. ALX-0600 was seen to cross BBB as was detected in CSF.

5.2 Toxicokinetics

(Included in toxicity studies)

6 General Toxicology

6.1 Single-Dose Toxicity

Acute Subcutaneous Toxicity Study in Mouse (Study # 88614)

The review is incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

Acute Toxicity Studies:1. **Acute Subcutaneous Toxicity Study in Mouse:** (Study #88614)

Methods: This is a GLP study and conducted at sponsor lab from May 4, 1998 to January 20, 1999 in 10 Swiss Crl:CD^R-1(ICR)BR strain 40+1 days old male mice. These animals were randomly administered subcutaneous doses of 100 mg/kg/day ALX-0600 (batch # D05GL98b in 2 divided doses) in phosphate buffered saline (PBS) and clinical signs, mortality and body weight and blood counts and chemistry were conducted for 14 days post treatment.

Results: None of the treated animals died or showed any change in body weight or food consumption. There were no treatment related gross pathological or histopathological adverse effects. A dose of 100 mg/kg/day ALX-0600 was identified as a 'well tolerated dose' in the study.

6.2 Repeat-Dose Toxicity**Mouse****14-Day Subacute Toxicity Study in Mice (Study # 88617)****1-Month Subcutaneous Toxicity Study with 1-Month Recovery in Mice (Study # 88730)**

The reviews of the above studies are incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

Subacute Toxicity Studies:**1. 14-Day Subcutaneous Toxicity Study in Mouse:**

(Project # 88617)

Testing Laboratory:

(b)(4)

(b)(4)

Dates of Start and Completion of the study: May 4, 1998 and January 20, 1999.**GLP & QAU Requirements:** Statement of compliance to US GLP regulations was included.**Species and Strain:** Swiss Crl:CD^R-1(ICR)BR strain 40₊₁ day old male mice**Batch #:** D05GL98b

Methods: One hundred seventy four male mice were randomly divided into 3 main study and 3 satellite groups. Thirty animals were used in main study groups (10/group) and 144 animals were included in satellite groups (for TK estimation). These were administered subcutaneous doses of either 0, 10 and 20 mg/kg/day ALX-0600 (batch # D05GL98b in 2 divided doses) in phosphate buffered saline (PBS) for 14 days in a volume of 20 or 40 ml/kg/bid. The dose selection was based on the existing toxicity and solubility information of the compound. All animals included of main study group were observed once during a week prior to treatment and twice daily during the study for mortality and adverse effects. The body weights and food consumption were recorded on day 0, 1, 3, 6, 9, 12 and 14 after the treatment and ophthalmic examination was done before dosing and 2 weeks after treatment. The blood samples from the main study group animals for the estimation of hematological and blood chemistry parameters were drawn at study week 2. Blood samples for estimating toxicokinetic parameters from the 3 satellite groups animals (3 mice/time interval) were collected from orbital sinus on day 1 and 14 once prior to dosing, 5, 10, 15, 30, 45, 60 and

90 min post dosing. All animals of the main study groups were killed at the termination of the study and complete gross pathology of the animals was performed. The following tissues of control and 20 mg/kg/day treatment groups were separated, weighed and examined microscopically: adrenal, brain, kidneys, liver, spleen, uterus, prostate, testes/ovaries, skeletal muscle, pituitary glands, thymus & thyroid. The other tissues of these 2 groups of animals separated for histopathological abnormalities were: aorta, duodenum, testes + epididymides, esophagus, injection site, jejunum, mandibular lymph nodes, pancreas, stomach, mammary glands, salivary glands, sciatic nerve, seminal vesicle, skeletal muscle, spinal cord - cervical/thoracic and lumber, trachea, bone marrow, lungs + bronchi, uterus/prostate and urinary bladder of the animals included the animals in 0 and 20 mg/kg/day treatment groups. Only injection site, stomach, duodenum, jejunum, ileum, cecum, colon and rectum of the animals included in 10 mg/kg/day treatment group were dissected for histopathological examination.

Results:

- a. **Observed Effects:** None of the animals showed any change in physical signs after treatment.
- b. **Mortality:** None of the animals died.
- c. **Body Weight/Food Consumption Changes:** The mean body weight gain of 6.3 and 5.6% was observed in animals included in 10 and 20 mg/kg/day ALX-0600 treatment groups. The initial (on day 1) and final mean body weights (on day 14) of control animals were 25.26 and 28.98 g, respectively. The initial and final food consumption of control animals were 10.6 (day 1) and 10.7 g (study day 14). The food consumption was not different among 0, 10 and 20 mg/kg/day ALX-0600 treatment groups animals.
- d. **Hematology/Coagulation/Bone Marrow Changes:** An increase in total neutrophil counts (21.5% vs 14.2% in control) and a decrease in lymphocytes counts (73.9% vs 84.3% in control) was seen in animals belonging to 20 mg/kg/day treatment group.
- e. **Blood Chemistry/Urinalysis Changes:** There were dose or treatment related changes although a minor decrease in serum glucose levels (164.0 vs 223 mg/dl in control) and an increase in phosphorus concentration (6.98 vs 10.63 mg/dl in control) were seen in animals belonging to 20 mg/kg/day treatment group.

These changes were not of clinical importance.

f. **Physical Examination and Ophthalmic Test Changes:** No adverse effects were seen on visual examinations.

g. **Toxicokinetics Analysis:** A dose proportional increase in the plasma concentrations was seen in 10 and 20 mg/kg/day treatment groups animals within 30 to 45 minutes of administration. The plasma concentrations were about 18 to 21% greater on day 14 than on day 1 in the 2 groups of animals. There was no sign of accumulation as at time 0, just before the administration of the compound, it was not detected. Cmax and AUC_(0-∞) data of the animals on study day 1 and 14 had been shown in the following table:

Table 5.11. Toxicokinetic parameters of ALX-0600 following single (Day 1) and repeated (Day 14) subcutaneous injections of ALX-0600 in the male mouse

Dose (mg/kg)	Tmax (min)	Cmax (ng/mL)	AUC(0-last) (ng.h/mL)
Day 1			
10	30	4797	255798
20	45	7641	470762
Day 14			
10	45	5703	205349
20	30	8123	355837

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h. **Organ Weight Changes:** Slight treatment related increase in absolute weight of liver and spleen among males and not females. A dose related increase in absolute weight and length of cecum, colon, ileum and duodenum was seen in increased number in animals of 10 and 20 mg/kg/day treatment groups.

i. **Gross Pathology Findings:** No pathological changes of clinical importance were reported.

j. **Histopathological Changes:** The mucosal hyperplasia of cecum, colon, ileum and duodenum was seen in a dose related manner among animals belonging to 10 and 20 mg/kg/day treatment groups. One animal of 20 mg/kg/day treatment group showed jejunal congestion (bleeding). Dilation and hyperplasia of renal tubular cells were seen in 2 out of 10 animals included in 20 mg/kg/day treatment group. Renal cyst in 1 and 2 animals of control and 20 mg/kg/day treatment groups and, lymphoid atrophy of thymus in 2 out of 10 animals included in 20 mg/kg/day treatment group were seen.

Fibrosis and inflammation were seen in higher incidences among the animals included in 10 and 20 mg/kg/day treatment groups. The fibrosis was present in 1, 10 and 10 out of 10 animals in all groups (control and 2 treatment groups) and the incidences of inflammation were 1 and 2 among 10 and 20 mg/kg/day ALX-0600 treatment groups.

In summary, ALX-0600 up to 20 mg/kg/day, s.c. (in 2 daily divided doses) attained dose proportional plasma concentrations and produced an increase in small and large intestines mass, inflammation and fibrosis at the site of injection, lymphoid atrophy of thymus and renal nephropathy. The target organs of toxicity were kidneys, thymus and site of injection. A subcutaneous dose of 10 mg/kg/day was considered as the 'highest tolerable dose' in the study.

2. 1-Month Subcutaneous Toxicity Study with One Month Recovery in Mice: (Project # 88730)

Testing Laboratory: [REDACTED] (b)(4)

Dates of Start and Completion of the study: August 21, 1998 and April 13, 1999.

GLP & QAU Requirement: Statement of compliance with US GLP regulations was included.

Species and Strain: Swiss Cr1:CD^R-1(ICR)BR strain 31+1 days old mice.

Batch #: 8106801.

Methods: Five hundred and four animals (252/sex) were randomly divided into 4 groups (21/sex/group-15 in main and 6/sex in recovery groups) and administered subcutaneous doses of either 0, 0.2, 0.6 and 2 mg/kg/day ALX-0600 (in 2 divided doses with 8 hr interval) in phosphate buffered saline (PBS) for 28 days. The dose selection was based on the existing toxicity information and 14-day subcutaneous toxicity study in mouse (Project #88617). A dose of 10 mg/kg/day was identified as a 'highest tolerated dose'. All animals were observed once for 1 week prior to treatment and, twice daily during the study for mortality and adverse effects. The body weights and food consumption were recorded on day 0, weekly during the study and before termination (on day 28).

Ophthalmoscopic examination was done once before, during week 4 and during recovery week 4 on study animals. The blood samples from the overnight fasting 7/sex/group of main study and, 3/sex/group of recovery groups were drawn for hematological and blood chemistry parameters. The blood samples for estimating the toxicokinetic data were collected from satellite groups of 3 animals/sex/time period from orbital sinus on day 1 and 28 at 15, 30, 45, 60, 90, 120 and 150 min post dosing. All animals were killed at the termination of the study and complete gross pathology of animals was performed. Adrenals, brain, heart, gastrointestinal tract, kidneys, liver, pituitary, prostate, spleen, testes, thymus and thyroid of each treatment group animals were weighed. The histopathological examination of above enumerated tissues and aorta, bone marrow, cecum, colon, duodenum, epididymides, esophagus, eye, gall bladder, ileum, injection site, jejunum, lymph nodes, mammary glands, optic nerve, pancreas, rectum, salivary glands, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord, stomach, tongue, trachea and urinary bladder of the animals included in 0 and 2 mg/kg/day treatment groups was done. The tissues showing treatment related findings and gross lesions of animals included in 0.2 and 0.6 mg/kg/day treatment groups were examined microscopically.

Results:

- a. **Observed Effects:** The animals included in ALX-0600 treatment groups did not show dose or treatment related changes in the clinical signs.
- b. **Mortality:** None of the animals died during the study.
- c. **Body Weight/Food Consumption/Water consumption:** During the treatment period, the mean body weight gains in males and females included in ALX-0600 treatment groups were significantly ($p < 0.01$) greater than the control group animals. The body weights of control animals at the initiation, after treatment period and recovery period were 28.13, 33.2 and 35.57 g for male and, 22.64, 26.3 and 28.1g, respectively for females. The food consumption of study male and female animals included 2 mg/kg/day ALX-0600 treatment group was more than control animals. The daily food consumption of control males was 5.3, 6.0 and 6.0 g and, control females was 4.7, 4.9 and 5.3 g/day/animal on study day -1, day 28 and day 56.

d. **Hematology/Coagulation/Bone Marrow Changes:** There was no treatment related changes of clinical or statistical importance in animals included in 0, 0.2, 0.6 or 2.0 mg/kg/day ALX-0600 treatment groups.

e. **Blood Chemistry/Urinalysis Changes:** No dose related significant changes in blood chemistry were seen in animals included in 0, 0.2, 0.6 and 2.0 mg/kg/day ALX-0600 treatment groups.

f. **Toxicokinetic Parameters:** ALX-0600 attained a peak plasma concentration in approximately 35 min after the administration of the compound and AUC values in male and female animals were similar after a single dose or repeat treatment of 28 days. The half life of the compound was 0.3 and 0.6 hr in males and females, respectively.

Toxicokinetic Parameters of ALX-0600 Following Repeat Subcutaneous Injection of ALX-0600 in Male and female Mice (day 1)

Gender	Group No.	t _{max} h	C _{max} ng/mL	AUC _{0-tlast} ng·h/mL	AUC _{15-tlast} ng·h/mL	AUC _{0-inf} ng·h/mL	AUC _{15-inf} ng·h/mL	% Extrapolation AUC _{0-inf}	t _{1/2el} (h)	k _{el} (h ⁻¹)
Males	2	0.75	110	122	110	125	113	2.5	0.47	1.47967
	3	0.50	390	375	344	379	349	1.1	0.28	2.48658
	4	0.75	1112	1476	1362	1568	1455	5.9	0.56	1.23750
Females	2	0.50	87.7	90.7	85.5	92.7	87.5	2.2	0.30	2.31148
	3	0.75	248	289	261	301	273	4.0	0.47	1.47057
	4	0.50	965	991	893	1104	1006	10.2	0.69	1.00916

* Toxicokinetic parameters are calculated from a composite plasma concentration-time profile consisting of one sample from each mouse (N=3 mice/time point)

Toxicokinetic Parameters of ALX-0600 Following Repeat Subcutaneous Injection of ALX-0600 in Male and Female Mice (Day 28)

Gender	Group No.	t _{max} h	C _{max} ng/mL	AUC _{15-tlast} ng·h/mL	AUC _{15-inf} ng·h/mL	% Extrapolation AUC _{15-inf}	t _{1/2el} (h)	k _{el} (h ⁻¹)
Males	2	0.50	134	113	121	6.8	0.62	1.11776
	3	0.50	445	471	474	0.8	0.25	2.76295
	4	0.50	1878	1878	1922	2.3	0.33	2.12112
Females	2	0.50	87.0	82.9	84.5	1.9	0.33	2.12353
	3	0.50	406	403	409	1.6	0.32	2.19451
	4	0.75	1563	1474	1510	2.4	0.35	2.00447

* Toxicokinetic parameters are calculated from a composite plasma concentration-time profile consisting of one sample from each mouse (N=3 mice/time point)

g. **Physical Examination and Ophthalmic Test:** There were no treatment related adverse effects in ophthalmoscopic and visual examinations in animals included in the study.

h. **Organ Weight Changes**: A significant treatment related increase in weight of small and large intestines was reported. The mean increase in weight of small intestines were 84.7, 80.9 and 116.1 % in males and, 75.7, 96.3 and 124.6 % among females included in 3 treatment study groups. Mean length of small intestines was increased by 33.8, 37.0 and 56.1 % among males and, 9.4, 13.2 and 15.1 % among females of 3 treatment groups. The mean length of large intestines was increased by 9.7, 12.5 and 15.6% in males and, 6.3, 6.0 and 7.0 % among females of the study groups. After the recovery period of 2 weeks, the weight and length of small and large intestines were similar to the control group animals.

i. **Gross Pathology Findings**: A dose related red to brown coloration at the injection site was reported in animals of the study.

j. **Histopathological Changes**: Mucosal hyperplasia of slight to mild nature in cecum, small intestine and rectum was reported in males and females included in 0.2, 0.6 and 2 mg/kg/day treatment groups (see table below). The significant ($p < 0.01$ to 0.05) weight increase of small intestines, large intestines and duodenum was because of drug induced hyperplasia in males and females animals (see table below). Subacute to chronic subcutaneous inflammation at the site of injection indicating the irritation at the site of injection, was observed in 3, 4, 12 and 14 males and 6, 13, 13 and 15 females belonging to 0, 0.2, 0.6 and 2 mg/kg/day treatment groups. Epithelial hypertrophy with reduced cytoplasmic vacuolation of gall bladder was seen (see table below). Renal tubular vacuolation was seen in 0, 2, 0 and 4 females belonging to 0, 0.2, 0.6 and 2 mg/kg/day treatment groups. The average villus length (micrometer) of duodenum, jejunum and ileum of the animals belonging to treatment groups was significantly increased ($p < 0.01$). The average mucosal height of cecum, colon and rectum of males and females belonging to 0.2, 0.6 and 2 mg/kg/day treatment groups was increased. After the recovery period, these histopathological changes were resolved in animals.

TABLE
Histopathological Changes During 1-Month Toxicity Study
in Mice (15 animals/sex/group)

Histopathological Findings		ALX-0600 Dose Used (mg/kg/day)			
		0	0.2	0.6	2.0
% Average Increase in Length (mm)*					
Small intestine	Males	469.8	7.1	7.9	12.8
	Females	451.1	9.4	13.2	15.1
Large Intestines	Males	111.3	9.7	12.5	15.6
	Females	114.3	6.2	6.0	7.1
# Mucosal Hyperplasia*					
a. Cecum	Males	0	11	13	14
	Females	0	11	15	12
b. Colon	Males	0	1	0	5
	Females	0	0	0	2
c. Duodenum	Males	0	12	9	15
	Females	0	11	13	15
d. Jejunum	Males	0	13	13	15
	Females	0	12	12	14
e. Ileum	Males	0	13	14	13
	Females	0	12	13	15
f. Rectum	Males	0	6	4	10
	Females	0	3	8	11
% Average Mucosal Height Increase (um)*					
a. Cecum	Males	162	45.72	19.3	24.4
	Females	154	49.3	48.7	59.7
b. Colon	Males	217	17.3	19.3	24.4
	Females	219	16.9	20.1	32.9
C Rectum	Males	182	21.4	20.3	31.3
	Females	178	25.8	27.8	36.5
% Average villus length Increase (um)*					
a. Duodenum	Males	557	32.2	26.2	32.9
	Females	543	30.6	31.9	37.6
b. Jejunum	Males	321	32.2	61.0	74.1
	Females	327	56.7	54.1	76.8
c. Ileum	Males	227	37.4	38.3	52.4
	Females	230	43.9	47.4	57.4
%Average Crypt Depth Increase (um)*					
a. Duodenum	Males	129	22.5	20.1	26.3
	Females	133	17.3	27.9	19.8
b. Jejunum	Males	118	16.1	10.2	22.3
	Females	121	12.4	16.5	19.8
c. Ileum	Males	108	17.6	13.9	21.2
	Females	114	11.4	14.0	19.2
Gall Bladder Epithelial Hypertrophy (#)	Males	0	10	10	10
	Females	0	6	9	12
#Injection Site Inflammation	Males	3	4	12	14
	Females	6	13	13	15
# Renal Tubular Vacuolation	Males	0	0	0	0
	Females	0	2	0	4

* = Under control (0) group, the absolute values of organ height, length and depth of control animals given

In summary, ALX-0600 from a dose of 0.2 to 2 mg/kg/day (in 2 daily divided doses) produced a dose proportional plasma concentration in male and female mouse. No sex related differences in the plasma concentration of the compound, were seen in the study. A dose of 0.6 mg/kg/day produced mild but desired pharmacological effects of hyperplasia of gastrointestinal tract. The site of injection and gall bladder were the target organs of toxicity. A 'no effect dose' was not established and a dose of 0.6 mg/kg/day was 'maximum tolerable dose' in the study.

90-Day Subcutaneous Toxicity and Toxicokinetic Study in Mice with 1-Month Recovery Period (Study # 0470MN12.001)

26-Week Subcutaneous Toxicity in Mice with 8-Week Recovery Period (Study # 7203-112)

The reviews of the above studies are incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

90-Day Subcutaneous Toxicity and Toxicokinetic Study with ALX-0600 in Mice with 1-Month Recovery Period

Study No.: 0470MN12.001

Volume and Page: Amendment No. 028, Vol. 1, page 1

Testing Laboratory: (b) (4)

Dates of Initiation and Completion of Study: February 1, 2001 and February 13, 2003

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted. The QAU statement was also included.

Species and Strain: CD-1 mice (approximately 6 to 7 weeks old) with mean body weight of 23 to 35 g in males and 17 to 26 g in females were used in this study.

Batch and Purity: 0850001, 98.9%

Vehicle: Phosphate buffer with L-histidine and mannitol

Methods: One hundred sixty mice were randomly divided into 4 main study groups (20/sex/group) and were administered s.c. with ALX-0600 at 2, 10 or 50 mg/kg/day (1.0, 5.0 and 25.0 mg/kg bid, 8 hours apart, 5 ml/kg). In addition, 5 animals/sex/group were used for the recovery period of 30 days. Additionally, four groups of mice (30/sex in the control and 84/sex/group for ALX-0600) were designated as toxicokinetic (TK) animals. Dose selections were based on the results of the previous toxicology studies (details were not provided) and expected clinical doses. The study design is shown below (from Amendment 028, Vol. 1, page 26).

6. METHODS AND EXPERIMENTAL DESIGN

Toxicology Groups (Groups 1-4)

Group Treatment	Dose Level (mg/kg/dose)	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals*	
					Male	Female
1. Control (vehicle)	0	0	0	5.0	20	20
2. Low-dose	1.0	2.0	0.2	5.0	20	20
3. Mid-dose	5.0	10.0	1.0	5.0	20	20
4. High-dose	25.0	50.0	5.0	5.0	20	20

* On Day 91, 15 animals/sex/group were euthanized. The remaining animals remained on test, untreated, until they were euthanized on Day 120.

Pharmacokinetic Groups (Groups 5-8)

Group Treatment	Dose Level (mg/kg/dose)	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals*	
					Male	Female
5. Control	0	0	0	5.0	30	30
6. Low-dose	1.0	2.0	0.2	5.0	84	84
7. Mid-dose	5.0	10.0	1.0	5.0	84	84
8. High-dose	25.0	50.0	5.0	5.0	84	84

Animals were observed twice daily (prior to dosing and 1 hr after the dose) for mortality, moribundity and clinical signs. Body weights and food consumption were recorded on a weekly basis. The recovery group animals were weighed on Day 92, 99, 106, 113 and 119. Ophthalmic examinations were conducted once before treatment and prior to termination of the treatment period. Hematology, serum chemistry and urinalysis were conducted at necropsy (Day 91) and at the end of the recovery period (Day 120). Blood samples were collected from TK animals (Groups 5-8) on Days 1, 30, 90 and 120. Following organs were weighed: adrenals, kidneys, ovaries, liver, brain, spleen, heart, testes and thyroid/parathyroid. Following tissues were examined histopathologically: adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, exorbital lacrimal glands, eyes, femur, gallbladder, heart, ileum, injection site, jejunum, kidneys, liver with gall bladder, lungs, mammary glands, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skin, spinal cord, spleen, sternum, stomach, thigh musculature, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus, vagina, mesenteric lymph node and ovaries. In addition, lengths of large and small intestines were also measured.

Results:

Observed Effects: Some animals had scab formations at the site of injection. These observations were noted across all groups (Group 1-M, 2 animals; Group 3-M, 3 animals; Group 3-F, 2 animals; Group 4-M, 3 animals) and appeared to be related to the repeated injections and not related to test article.

Mortality: Three animals (2 males and 1 female) of the main study group died. One female at 2 mg/kg/day treatment group was found dead on Day 39, 1 male in the control (moribund sacrifice on Day 89) group and one male at 50 mg/kg/day was found dead on Day 51. The animal at the high dose treatment group showed decreased activity, loss of righting reflex and cold to touch on Day 50. Only the Group 2 female had treatment-related hyperplasia/hypertrophy in the intestine and in the gall bladder. A total of eight toxicokinetic animals died during the study. No cause of death was determined for these animals and these animals were not examined. Mortalities are shown in the following table (from Vol. 1, page 15 of sponsor's submission).

Toxicology Animals that died during the study:

Animal No.	Group-Gender	Treatment	Study Day	Fate
4101	2-F	ALX-0600 2 mg/kg/day	39	found dead
4077	4-M	ALX-0600 50 mg/kg/day	51	found dead
4013	1-M	Control (vehicle)	89	moribund sacrifice

Pharmacokinetic Animals that died during the study:

Animal No.	Group-Gender	Treatment	Study Day	Fate
4238	6-M	ALX-0600 2 mg/kg/day	13	found dead
4231	6-M	ALX-0600 2 mg/kg/day	15	found dead
4548	6-F	ALX-0600 2 mg/kg/day	20	found dead
4691	8-F	ALX-0600 50 mg/kg/day	28	found dead
4188	5-M	Control (vehicle)	58	found dead
4261	6-M	ALX-0600 2 mg/kg/day	77	found dead
4431	8-M	ALX-0600 50 mg/kg/day	85	accidentally killed
4272	6-M	ALX-0600 2 mg/kg/day	103	found dead

Body Weight: The mean initial and final body weights of control males were 27 and 35 g, respectively. The mean initial and final body weight of control females were 23 and 30 g, respectively. Generally, following 90 days of treatment, mean body weight and mean body weight gains were increased when compared to control at all dose levels. However, mean body weights and body weight gains were comparable to control during the majority of the recovery period.

Food Consumption: The mean initial and final food consumption in control males were 6.71 and 5.86 g/animal/day, respectively. The mean initial and final food consumption in control females were 5.0 and 7.1 g/animal/day, respectively. There were no treatment-related differences in mean food consumption values between Groups 1, 2, 3, and 4 during the treatment phase and recovery phase periods.

Hematology: There were no treatment-related changes in the hematology parameters during the treatment or the recovery period.

Serum Chemistry: On day 90, treatment-related decreases in serum triglycerides were seen in males and females at 2 and 50 mg/kg/day (29.7, 10.2 and 20.3% decrease in males and 24.6, 29.2 and 16.9% decrease in females at 2, 10 and 50 mg/kg/day, respectively). However, serum triglyceride levels in the recovery period were similar to the control group animals.

Toxicokinetics: Exposure to ALX-0600 increased approximately dose proportionately. The C_{max} also increased approximately dose proportionately between 2 and 10 mg/kg/day. However, this increase was not dose proportional between 10 and 50 mg/kg/day. The $t_{1/2}$ ranged from 0.312 to 0.787 hours. There appeared to be an apparent gender difference in AUC_{0-8h} and C_{max} values. Generally, C_{max} and AUC_{0-8h} values were higher in males than females. There appears to be no accumulation of drug after multiple dosing. Toxicokinetic parameters are shown in the following table.

Group	Dose (mg/kg/day)	Tmax (hr)	Cmax (ng/ml)	T _{1/2} (Hr)	AUC _(0-8h) (ng.hr/ml)
Males:					
Day 1					
6	2	0.25	2960	0.312	2648
7	10	0.25	12052	0.541	11408
8	50	0.25	53684	0.588	56948
Day 30					
6	2	0.5	3104	0.374	2800
7	10	0.5	11482	0.598	13640
8	50	0.5	47239	0.574	65485
Day 90					
6	2	0.5	2391	0.787	3802
7	10	0.5	15173	0.628	18487
8	50	0.5	34524	0.620	77142
Female:					
Day 1					
6	2	0.25	2279	0.330	1720
7	10	0.25	13231	0.527	10819
8	50	0.25	43819	0.574	46030
Day 30					
6	2	0.25	3102	0.338	1840
7	10	0.5	8636	0.615	10427
8	50	0.25	30784	0.619	42684
Day 90					
6	2	0.25	2118	NC	3259
7	10	0.5	8045	0.634	11767
8	50	0.25	32153	0.664	41902

Detection of Antibodies in the Plasma: The samples of the present study were negative to ALX-0600 antibody test.

Ophthalmoscopy: On Day 90, ophthalmic examination revealed blepharitis in one high dose male and focal keratitis in one female in the control group. These were considered isolated cases and were not considered as treatment-related.

Organ Weight: Mean absolute thymus weight was decreased by 52.7, 31.7 and 53.5% in males and 16.8, 12, 4 and 32.75% in females at 2, 10 and 50 mg/kg/day, respectively, compared to control (6.05 and 4.75 g in males and females, respectively). Absolute weight of stomach (maximum increase up to 111%), small intestines (213-301% of control), large intestines (167-183% of control) and liver (12-221% of control) were increased at all dose levels. Small and large intestine lengths were also significantly increased at all dose levels compared to control. Small intestine length in Groups 2 and 4 females and large intestine weight in Groups 2 and 3 males remained significantly increased following the 30-day recovery period in all animals treated with ALX- 0600.

Gross Pathology: Gall bladder distension was seen in 2, 0, 4 and 3 males and, 0, 1, 1 and 2 females at 0, 2, 10 and 50 mg/kg/day, respectively. The scab formation and thickening at the site of injection was also seen in 1 and 0 males and 2 and 4 females at 0 and 50 mg/kg/day, respectively.

Histopathology: Treatment-related histopathological changes were observed in the duodenum, jejunum, ileum, cecum and gallbladder. Histopathological changes were mostly characterized by hypertrophy/hyperplasia. The incidence and severity of hypertrophy/hyperplasia of the gallbladder epithelium and of the villi of the duodenum, jejunum, or ileum and the mucosa of the cecum were increased at all dose levels when compared to control. At the end of the recovery period, only the jejunum and gallbladder had an increased incidence of hypertrophy/hyperplasia in males and females in all treated groups. The following table shows incidences and the percent increase (in parentheses) in incidences of hypertrophy/hyperplasia in different organs/tissues.

Organs	Sex	ALX-0600 Dose (mg/kg/day)			
		0	2	10	50
Stomach	Male	0	-	-	0
	Female	1	-	-	1
Duodenum	Male	2(13.3)	14(93.3)	15 (100)	15 (100)*
	Female	2(13.3)	14 (87.5)	13 (86.7)	14(93.3)
Jejunum	Male	3(18.7)	14 (93.3)	15(100)	15 (93.3)
	Female	3(20.0)	16(100)	15 (100)	15(100)
Ileum	Male	3(20.0)	15(73.3)	13(86.7)	12(75.0)
	Female	2(13.3)	16(100)	14(100)	15(100)
Cecum	Male	5(31.2)	14(93.3)	14(100)	14(97.5)
	Female	2(15.4)	16(100)	14(100)	14(100)
Pancreas (lymphocytic infiltrates)	Male	2(12.5)	-	-	1(6.7)
	Female	1(6.7)	0	-	0
Gallbladder	Male	0	12 (80.0)	12 (80.0)	12 (75.0)
	Female	0	0	25 (0.5)	75 (1.5)

*: The values in the parentheses are percentages.

In a 90-day s.c. toxicity study in CD-1 mice, animals were treated at 2, 10 and 50 mg/kg/kg (1, 5 and 25 mg/kg/day, bid, 8 hours apart). The target organ of toxicity appeared to be the duodenum, jejunum, ileum, cecum and gallbladder. The incidence and severity of hypertrophy/hyperplasia of the gallbladder epithelium and of the villi of the duodenum, jejunum, or ileum and the mucosa of the cecum were increased were seen at all dose levels. These effects appeared to be related to the pharmacological effects of ALX-0600. At the end of the recovery period, only the jejunum and gallbladder had an increased incidence of hypertrophy/hyperplasia in males and females in all treated groups. The NOAEL could not be established as treatment-related effects were seen at all tested doses.

26-Week Subcutaneous Toxicity Study in Mice with 8-Week Recovery Period

The draft report of the study was submitted under Amendment #56 dated February 27, 2004 and the final report was submitted under Amendment #64 dated June 24, 2004. Overall, the results and interpretations of the draft report do not seem to differ from that of the final report.

Study No.: 7203-112

Volume and Page: Amendment No. 56, Vol. 1, page 1; Amendment 64, Vol. 1, page 1

Testing Laboratory: (b) (4)

Dates of Initiation and Completion of Study: December 20, 2002 and February 26, 2004

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted. The QAU statement was also included.

Species and Strain: CD-1 mice (approximately 41 to 48 days old) with mean body weight of 26.1 to 39.7 g in males and 20.0 to 30.2 g females were used in this study.

Batch (Purity): 0850202 (98.2%) & 0850203 (97.4%)

Vehicle: Phosphate buffer with L-histidine and mannitol

Methods: Two hundred and eight CD-1 mice (104/sex) were randomly divided into 4 main study groups (Groups 1-4) and were treated with ALX-0600 at 2, 10 and 25/50 mg/kg/day (1, 5 and 12.5/25 mg/kg bid, 8 hr apart, 1.25 ml/kg) for 26 weeks. The high dose (Group 4 and 7) was increased to 50 mg/kg/day on Day 14/15 (F/M). Additionally 3 groups of 39/sex were included for toxicokinetic analyses (Groups 5, 6 and 7). The sponsor did not provide the basis of dose selection. The following table shows the study design (from Amendment 56, Vol. 1, page 18 of sponsor's submission).

Group	No. of Animals ^{a,b}		Dose Level ^{c,d}		Dose Concentration
	Male	Female	(mg/kg/day)	(mg/kg/dose)	(mg/mL/dose)
Toxicity Animals					
1 (Control) ^e	31	31	0	0	0
2 (Low)	21	21	2	1	0.8
3 (Mid)	21	21	10	5	4
4 (High)	31	31	25 /50	25	20
Toxicokinetic Animals					
5 (Low)	39	39	2	1	0.8
6 (Mid)	39	39	10	5	4
7 (High)	39	39	25 /50	25	20

a Toxicity animals designated for recovery sacrifice (10 animals/sex in Groups 1 and 4) will undergo 4 weeks of recovery following dose administration.

b The first three remaining toxicity animals/sex/group will undergo blood collection for antibody testing during Week 13 and at the scheduled terminal sacrifice.

c Animals will be dosed twice daily; daily doses will be given approximately 8 hours apart.

d Doses will be administered at a volume of 1.25 mL/kg/dose.

e Animals in control group will receive control article only.

Animals were observed twice daily for the clinical signs and mortality. Body weights were recorded prior to treatment, weekly for Weeks 1-14, once every 4 week thereafter and, at Weeks 27, 31 and 35. Food consumption was recorded weekly for Weeks 1-13 and once every 4 weeks thereafter. Ophthalmic examination was conducted once before treatment and during Week 26 and 34. Hematological and serum chemistry parameters were determined at scheduled terminal and recovery sacrifices. Blood samples were collected for TK analyses from 3/sex/group animals during prestudy on Day 1 and during Week 26 at 15, 30, 60 min and, 2, 4 and 8 hr post-treatment. In addition, blood samples (3/sex/group) were also collected from all treated animals during Week 13, and at termination for antibody analysis. Fifteen/sex/group animals were killed during Week 27 of treatment and the remaining animals in Group 1 and 4 were killed at the end of the recovery period, i.e., Week 35. Gross pathology was conducted at necropsy. Following organs were weighed: adrenals, brain, heart, kidneys, liver, gall bladder, lungs, ovary, pituitary, prostate, salivary glands, spleen, seminal vesicles, small intestines, large intestines, testes with epididymides, thymus, thyroid with parathyroid and uterus. The length of small and large intestines was also measured from the main study group animals. Histopathological examinations were conducted on the following tissues: adrenal, brain, cecum, colon, injection site, pancreas, pituitary gland, ovary, prostate, rectum, duodenum, epididymides, esophagus, eye, femur with bone marrow, gallbladder,

heart, Harderian gland, ileum, jejunum, kidneys, lesions, liver, lung with bronchi, lymph node, (mesenteric), mammary gland, optic nerve, salivary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum, stomach, testis, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

Results:

Observed Effects: The following clinical signs were observed: rough hair coat on ears of 1, 2, 2 and 3 males and 0, 0, 1 and 0 females and, sore/scab in 0, 0, 1 and 2 males and 0, 0, 0, and 1 females at 0, 2, 10 and 50 mg/kg/day, respectively.

Mortality: One control female and three TK animals (1 each in 10 on Day 155 and two animals at 50 mg/kg/day on Day 107 and 96) animals were found dead.

Body Weight: The mean initial body weights of control males and females were 32.2 and 24.6 g, respectively. The mean final body weights of males and females of control group were 45.5 and 37.7 g, respectively. There was a dose-related increase in the body weights at all doses (117.6, 122.6 and 115.0% of control in males and 144.2, 142.1 and 129.5% of control in females at 2, 10 and 50 mg/kg/day, respectively). At recovery period (Week 35 of the study), the body weight of the high dose animals were slightly higher than the control group (47.8 and 49.5 g in males and 36.2 and 37.1 g in females at 0 and 50 mg/kg/day, respectively).

Food Consumption: The mean initial and final food consumption in control males were 6.04 and 6.55 g/animal/day, respectively. The mean initial and final food consumption in control females were 4.61 and 5.68 g/animal/day, respectively. There were no significant treatment-related changes on food consumption in either sex.

Hematology: Treatment-related hematology changes included higher white blood cell (WBC) count, absolute lymphocyte count and absolute eosinophil count in both sexes at all dose levels. On Week 26, the absolute neutrophils counts were increased in females (165%, 160%, and 187% of control, control = $0.57 \times 10^3/\text{ul}$); however, this decrease was not evident in males (114%, 97% and 93.2% of control, control = $90 \times 10^3/\text{ul}$) at 2, 10 and 50 mg/kg/day, respectively.

Blood Chemistry: There were no significant treatment-related changes.

Toxicokinetics: Increases in mean C_{max} values were less than dose proportional and mean $\text{AUC}_{0-8\text{h}}$ values were approximately dose proportional on Day 1, but less than dose proportional on Week 26. On Day 1, mean values for C_{max} increased 1:3.1:6.7-fold for males and 1:4.1:6.9-fold for females for a 1:5:13-fold increases in the dose level. Mean values for $\text{AUC}_{0-8\text{h}}$ increased 1:4.7:13-fold for males and 1:6.5:16-fold in females for a 1:5:13-fold increase in the dose level. During Week 26, mean values for C_{max} increased 1:3.1:9.7-fold for males and 1:2.5:12-fold in females for a 1:5:25-fold increases in the

dose level. Mean values for AUC_{0-8h} increased 1:4.4:17-fold for males and 1:2.8:11-fold in females for a 1:5:25-fold increase in the dose level. Mean T_{max} values ranged from 0.25 to 0.50 hours on Day 1 and 0.25 to 1.00 hours on Week 26. Mean $t_{1/2}$ values ranged from 0.276 to 0.565 hours on Day 1 and 0.873 to 2.36 hours on Week 26. Except at 10 mg/kg/day on Week 26, no marked (>2-fold) gender differences were observed in mean C_{max} or AUC_{0-8h} values, but generally males tended to have higher exposure. The TK parameters are shown in the following table (from Vol. 1 and page 28 of sponsor's submission).

Toxicokinetic Parameters for ALX-0600 in Mouse Plasma

Group	Dose Level (mg/kg/day)	Gender	C_{max} (ng/mL)	T_{max} (Hours)	AUC_{0-1} (ng·hr/mL)	AUC_{0-8} (ng·hr/mL)	$t_{1/2}$ (Hours)
Day 1							
5	2	M	3529	0.25	2575	2576	0.276
		F	2513	0.25	1391	1392	0.304
6	10	M	10944	0.25	12205	12205	0.547
		F	10348	0.25	9114	9114	0.528
7	25	M	23588	0.50	34182	34182	0.563
		F	17317	0.25	22186	22186	0.565
Week 26							
5	2	M	4336	0.50	5130	5130	NA
		F	2144	1.00	5209	5209	2.36
6	10	M	13549	0.50	22392	22392	0.876
		F	5429	0.25	12601	14564	1.22
7	50	M	41961	0.50	84495	84495	0.928
		F	24576	1.00	58449	58449	0.873

Antibody Analysis in the Plasma: No change in the antibody status was detected from baseline to Weeks 13 or 27.

Ophthalmoscopy: No treatment-related changes were observed.

Organ Weight: Treatment-related organ weight changes included: kidney (105-113% of control, control = 0.5-0.8 g), spleen (120-168% of control, control = 0.114-0.132 g), large intestine (128-147% of control, control = 0.5 g), and small intestine (170-224% of control, control = 2.5-3.0 g). In addition, treatment-related increases in the length of small (117-131% of control) and large intestines (104-121% of control) of both male and females were also observed at all dose levels.

Gross Pathology Findings: Injection site reactions were observed at all dose levels including control, which included lytic necrosis of the subcutis, intralesional eosinophilic material, subacute inflammation, chronic inflammation, macrophage infiltrate and fibroplasia/fibrosis.

Histopathology: Treatment-related microscopic changes were observed in the following tissues: small and large intestine (epithelial and villus hypertrophy/hyperplasia), liver (hepatocellular hypertrophy), gallbladder (increased lymphohistiocytic infiltrates, increased cytoplasmic secretory product, and epithelial hypertrophy/hyperplasia accompanied by subacute inflammation, luminal suppurative exudates, and edema. Additionally, hypertrophy/hyperplasia, subacute inflammation, and/or increased secretory product were noted rarely in the bile duct), sternal bone marrow (myeloid hyperplasia), spleen (extramedullary hematopoiesis and lymphocytic hyperplasia), skin and injection sites (lytic necrosis of the subcutis, intralesional eosinophilic material, subacute and/or chronic inflammation, macrophage infiltrates, and fibroplasia/fibrosis; many of these observations were also seen at the injection sites of control animals, although at lower mean severity scores) in both sexes at all dose levels. The following table shows the histopathological changes.

Sex	Male				Female			
Dose (mg/kg/day)	0	2	10	50	0	2	10	50
Number Examined (N)	15	15	15	15	15	15	15	15
Liver								
Heptocellular hypertrophy	1	3	5	3	0	2	6	2
Chronic biliary inflammation	2	3	0	0	0	0	1	2
Gall Bladder (hypertrophy/hyperplasia)								
Incidences	0	5	9	12	0	15	15	14
Mean severity grade	0.0	0.6	1.1	1.6	0.0	1.7	1.9	1.3
Pancreas								
Periductal inflammation	0	0	3	0	0	1	0	1
Duodenum (hypertrophy/hyperplasia)								
Incidences	1	7	11	9	0	11	12	15
Mean severity grade	0.1	0.5	1.0	1.2	0.0	0.8	1.5	1.7
Ileum (hypertrophy/hyperplasia)								
Incidences	0	2	5	6	0	3	3	2
Mean severity grade	0	0.2	0.3	0.8	0.0	0.2	0.2	0.3
Cecum (hypertrophy/hyperplasia)								
Incidences	0	1	7	8	0	6	3	8
Mean severity grade	0.0	0.1	0.5	0.5	0.0	0.5	0.2	0.6
Colon (hypertrophy/hyperplasia)								
Incidences	0	1	1	8	0	1	0	2
Mean severity grade	0.0	0.1	0.1	0.7	0.0	0.1	0.0	0.1
Urinary Bladder								
Lymphohistiocytic inflammation	1	-	-	4	1	-	-	2
Seminal Vesicle								
Lymphocytic infiltration	2	-	-	5	-	-	-	-

In a 26-week s.c. toxicity study in CD-1 mice, animals were treated at 2, 10 or 50 mg/kg/day (1, 5 and 25 mg/kg bid, 8 hours apart). The target organ of toxicity appeared to be the small and large intestine (epithelial and villus hypertrophy/hyperplasia), liver

(hepatocellular hypertrophy), gallbladder (increased lymphohistiocytic infiltrates, increased cytoplasmic secretory product, and epithelial hypertrophy/hyperplasia accompanied by subacute inflammation, luminal suppurative exudates, and edema. Additionally, hypertrophy/hyperplasia, subacute inflammation, and/ or increased secretory product were noted rarely in the bile duct), sternal bone marrow (myeloid hyperplasia), spleen (extramedullary hematopoiesis and lymphocytic hyperplasia), skin and injection sites (lytic necrosis of the subcutis, intralesional eosinophilic material, subacute and/or chronic inflammation, macrophage infiltrates, and fibroplasia/fibrosis; many of these observations were also seen at the injection sites of control animals, although at lower mean severity scores). All of the microscopic effects were reversible following the 8- week recovery period, with the exception of findings in the liver, spleen, and some injection sites. The NOAEL could not be determined as treatment-related effects were observed at all dose levels.

Rat:

14-Day Subcutaneous Toxicity Study in CD-Rats (Study # 02-2776)

The review of this study is incorporated below from the pharmacology review of IND 58,213 dated December 5, 2003.

2. 14-Day Subcutaneous Toxicity Study in CD-Rats:
(Study #02-2776)

The submitted study is a Draft Final Report.

Testing Laboratory:

(b) (4)

(b) (4)

Dates of Start and Completion of Study: September 16, 2002 and February 4, 2003

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted.

Species and Strain: Sprague-Dawley CD rats approximately 7 weeks old with mean body weight of 211 to 289 g (males) and 160 to 210 g (females)

Batch #: 0850201 (98.5% pure)

Methods: Eighty rats (40/sex) were randomly divided into 4 main study (10 sex/group) and 3 toxicokinetic groups (0/sex in control and 3 treatment groups (9/sex/treatment group). ALX-0600 was subcutaneously administered at the doses of 0, 10, 25 or 50 mg/kg/day in two divided (8 hr apart in phosphate buffer solution) in a volume of 1.25 ml/kg. The low dose was selected on the basis of pharmacology and toxicokinetic data in the rats as well as toxicology data from mice and monkeys toxicity studies. The mid and high doses were multiples of the proposed clinical doses. The randomization and dose schedule of the study are given in the following table:

Group	Dose* (mg/kg/day)	Main Study Gr (# Animals/gr)		Satellite Gr* (#Animals/gr)	
		Male	Female	Male	Female
1	0 (Vehicle)	10	10	0	0
2	Low (10)	10	10	9	9
3	Mid (25)	10	10	9	9
4	High (50)	10	10	9	9

^a = Administered subcutaneously in a volume of 2.5 ml/kg (in 2 divided doses). Vehicle - aqueous in sterile water. Control group animals given sterile phosphate buffer solution containing histidine and mannitol.

*Satellite group animals will be used exclusively for TK evaluation.

The study animals were observed twice daily for the mortality

and general condition changes after each dose during treatment period. The body weights and food consumption were recorded prior to treatment and weekly during the study. The hematological (including blood coagulation parameters) and blood chemistry parameters and were determined on the blood samples collected from the overnight fasted animals of the main study groups. The urinalysis was done on the samples obtained from fasted animals. The plasma toxicokinetics parameters of the compound were estimated on the blood samples of 3/sex/group (1 ml) collected from overnight fasted animals of treatment groups at 0.25, 0.5, 1, 2, 4 and 8 hr (immediately before second dose) on day 1 and in week 4 and 13. Blood samples for the future estimation of the antibodies of ALX-0600 and E. coli were collected on day 0 and 14. All animals found dead or killed at the termination of the study were necropsied. The gross pathology on each of the animals was performed and the organs cleaned and weighed were adrenals, brain, heart, kidneys, small/large intestines, liver, spleen, testes, thymus, thyroid + parathyroid. The histopathological examination of all of the tissues of the animals included in control and 50 mg/kg/day treatment groups was performed. The tissues included were adrenals, aorta, bone and marrow (sternum, femur), bone (femoral head), brain (3 levels), epididymides, esophagus, eyes, heart, injection site, kidneys, lacrimal glands, larynx, liver (2 samples), lungs, lymph nodes (mediastinal, mesenteric), mammary glands in females, nasal cavities, optic nerves, ovaries, pancreas, pituitary glands, preputial/clitoral glands, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscles, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid lobes and thyroids, tongue, trachea, urinary bladder, uterus, vagina, vertebra (lumbar). The euthanized animals fixed in Zenker fluid. The effects of the compound on all treatment group animals were reported. The lesions produced in the animals, gastrointestinal tract tissues and cecum of control, low, mid and high dose treatment groups were also examined microscopically.

Results:

- a. **Observed Effects:** No treatment related effects were reported in the study animals.
- b. **Mortality:** None of the animals died during the study.
- c. **Body Weight & Food Consumption Changes:** The body weights of the study group animals and control group animals were similar. The initial and final body weights of the control group males were 186 g and 324 g, and of females were 152 and 220 g,

respectively. The food consumption changes were not treatment/dose related during the study.

d. **Hematological Changes**: A slight decrease ($p < 0.05$) of total WBC counts was reported in 50 mg/kg/day treatment group. A slight increase in MCH and MCHC was reported in males included in 25 and 50 mg/kg/day treatment groups. The changes were clinically insignificant and not seen among females. Among females, a statistically significant ($p = 0.01 - 0.05$) decrease in platelets was reported (1208, 872, 1281 and 912 $\times 1000/\mu\text{l}$). PT and APTT were decreased (PT = 12.9, 12.0, 11.9 and 11.7 sec in males and not affected in females). APTT in males of 50 mg/kg/day treatment group was decreased ($p < 0.01$) but was increased in females of this group ($p < 0.05$).

e. **Blood Chemistry Changes**: A trend of an increase in the ALT enzyme was seen in animals of 50 mg/kg/day treatment group (56 vs 74 U/l in control and high dose treatment group males; 33 vs 64 U/l among females), alkaline phosphatase was decreased among males and not affected among females. Total BUN was slightly increased in 50 mg/kg/day treatment group (13.4 vs 17.2 mg/dl in males and, 14.4 vs 17.1 mg/dl among females of 50 mg/kg/day treatment group).

f. **Toxicokinetic Parameters**: Quality assurance certificate will be included in the final report. The subcutaneously administered ALX-0600 was rapidly absorbed and the plasma peaks were seen in 1 hr in day 1 of the study. On day 1, the AUC values were 5534, 15572 and 29686 ng.hr/ml in males and, 4619, 13416 and 28913 ng.hr/ml in females included in 10, 25 and 50 mg/kg/day treatment groups. On day 13, the AUC values were slightly higher than on day 1 and were 8217, 23782 and 53386 ng.hr/ml in males and, 6019, 20171 and 43858 ng.hr/ml in females included in study treatment groups. The time to peak concentration on day 13 varied from 1 to 2 hr in males and, 0.5 to 2 hr in females included in 10, 25 and 50 mg/kg/day treatment groups. The half-life of the compound was 0.837, 0.979 and 1.15 hr in males and, 0.705, 0.648 and 0.779 in females included in 10, 25 and 50 mg/kg/day treatment groups.

g. **Organ Weight Changes**: The absolute weight of jejunum, ileum, cecum, rectum and colon were increased ($p < 0.05$) in males and females of 10, 25 and 50 mg/kg/day treatment group. These were attributed to the hypertrophy of the tissues.

g. **Pathological Changes**: A distended duodenum, ileum, and jejunum was reported in 1 of 10 males in 50 mg/kg/day treatment group and, 1 and 2 females of 25 and 50 mg/kg/day treatment groups.

h. **Histopathological Changes**: Mucosal epithelial hyperplasia/hypertrophy of minimal to slight nature was seen in cecum, colon and duodenum of the animals of both sexes included in 10, 25 and 50 mg/kg/day treatment groups. The incidences, intensity and distribution of hyperplasia in the study animals are shown in the following table. Subcutaneous tissue hemorrhage was seen in male and females of 50 mg/kg/day treatment group.

Table
Incidence and Distribution of Histopathological Changes in 14-Day Toxicity study in Rats

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
# Examined	10	10	10	10	10	10	10	10
Stomach Hyperpla.								
# Incidences	8	-	-	10	10	-	-	10
							-	0
Duodenum Hyperpla.								
# Incidences	0	10	10	10	0	10	10	10
Jejunum Hyperpla.								
# incidences	0	10	10	10	0	10	10	10
Ileum Hyperplasia								
# Incidences	0	10	10	10	0	10	9	10
Cecum Hyperplasia								
# Incidences	0	4	5	4	0	3	4	2
Colon Hyperplasia								
# incidences	0	0	0	2	0	0	0	1
Liver								
Hepato. cell. Necrosis #	0	-	-	0	0	0	0	1
Acute Inflammation	1	0	0	0	0	0	0	1

In summary, subcutaneously administered ALX-0600 from the doses of 10 to 50 mg/kg/day produced treatment related mucosal hyperplasia/hypertrophy of duodenum, ileum, rectum, jejunum and large intestines in male and female rats. The target organs of toxicity were gastrointestinal tract and site of injection. A dose of 50 mg/kg/day was identified as the 'highest tolerable dose' and a dose of 25 mg/kg/day was the 'no effect dose'.

14-Day Comparator Subcutaneous Toxicity and Toxicokinetic Study in Sprague-Dawley (CD), Wistar Han and Fischer-344 Rats Under Fully Fed Condition (Study # 800869)

The review of this study report is incorporated below from the pharmacology review of IND 58,213 dated June 16, 2005.

Study Title: 14-Day Comparator Subcutaneous Toxicity and Toxicokinetic Study in Sprague-Dawley (CD), Wistar Han and Fischer-344 Rats Under Fully Fed Condition

Key Study Findings: In a 2-week comparator study in Wistar Han, CD and Fischer-344 rats, animals were treated subcutaneously at 10, 25 and 50 mg/kg/day (5, 12.5 and 25 mg/kg bid, 8 hours apart). The CD rats were only treated at 50 mg/kg/day. The target organs of toxicity appeared to be the gastrointestinal tract (hyperplasia and lengthening of duodenum, ileum and jejunum and large intestines), lymph node (hyperplasia), and eye (mineralization of cornea in Fischer-344 rats). The mineralization of cornea in Fisher-344 rats during the study appeared to be the only notable difference between this and CD or Wistar rats. The exposure to ALX-0600 in different strains of rats appeared to be comparable. Overall, CD rats and Wistar Han rats showed comparable toxicity and toxicokinetic profile following s.c. administration of ALX-0600 under fully fed condition.

Study No.: 800869

Volume and Page No.: Vol. 2, page 1

Conducting Laboratory and Location: (b) (4)

Date of Initiation: April 19, 2004

Date of Completion: November 1, 2004

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Test Article, Batch # and Purity: ALX-0600, Lot No. 8503031, 98.7%

Species and Strain:

- a. Sprague-Dawley [CrI:CD®(SD)IGBR] rats with mean body weight of 140.3 g (male) and 118.7 g (female)
- b. Fischer (F-344)/CrI:CrBR rats with body weight range of 95.7 to 97.2 g in males and 84.5 to 86.2 g in females
- c. Wistar Han IGS [CrI:WI(Gix/BR/Han) IGSBR] rats with body weight range of 126.5 to 129.5 g in males and 106.6 to 111.2 g in females

Methods: The study was conducted in 3 different strains of rats (Sprague-Dawley, Fishers-344 rats and Wistar Han) to compare the toxicity and toxicokinetic of the compound across three

different strains of rats following subcutaneous administration of ALX-0600. The study included a single group of Sprague-Dawley rats (28/sex) treated at 50 mg/kg/day only, 4 groups of Fishers-344 rats (28/sex/group) and 4 groups (28/sex/group) of Wistar Han rats treated at 10, 25 and 50 mg/kg/day (5, 12.5 and 25 mg/kg/day bid, 8 hours apart, 1.25 ml/kg). The vehicle was phosphate buffer containing mannitol and L-histidine. There were 10 rats/sex/group in the main study and an additional 18/sex/group was included in the TK part of the study. The study design is shown in the table (from Vol. 2, page 16 of sponsor's submission).

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PROJECT NO. 800869

Group No. Identification	Strain	Daily Dose mg/kg/day	Dose Concentration mg/mL	Dose * Level mg/kg/dose	Dose Volume mL/kg/dose	Main Study Animal Numbers	
						Males	Females
1 ALX-0600	Sprague Dawley	50	20.0	25.0	1.25	1001 -1010	1501 -1510
2 Control	Wistar Han	0	0	0	1.25	2001 -2010	2501 -2510
3 ALX-0600	Wistar Han	10	4.0	5.0	1.25	3001 -3010	3501 -3510
4 ALX-0600	Wistar Han	25	10.0	12.5	1.25	4001 -4010	4501 -4510
5 ALX-0600	Wistar Han	50	20.0	25.0	1.25	5001 -5010	5501 -5510
6 Control	Fischer	0	0	0	1.25	6001 -6010	6501 -6506 6508- 6511
7 ALX-0600	Fischer	10	4.0	5.0	1.25	7001 -7010	7501 -7510
8 ALX-0600	Fischer	25	10.0	12.5	1.25	8001 -8010	8502 -8510 8530
9 ALX-0600	Fischer	50	20.0	25.0	1.25	9001 -9010	9501 -9510

Group No. Identification	Strain	Daily Dose mg/kg/day	Dose Concentration mg/mL	Dose* Level mg/kg/dose	Dose Volume mL/kg/dose	Toxicokinetic Study Animal Numbers	
						Males	Females
1 ALX-0600	Sprague Dawley	50	20.0	25.0	1.25	1011 -1028	1511 -1528
2 Control	Wistar Han	0	0	0	1.25	-	-
3 ALX-0600	Wistar Han	10	4.0	5.0	1.25	3011 -3028	3511 -3528
4 ALX-0600	Wistar Han	25	10.0	12.5	1.25	4011 -4028	4511 -4528
5 ALX-0600	Wistar Han	50	20.0	25.0	1.25	5011 -5028	5511 -5528
6 Control	Fischer	0	0	0	1.25	-	-
7 ALX-0600	Fischer	10	4.0	5.0	1.25	7011 -7028	7511 -7528
8 ALX-0600	Fischer	25	10.0	12.5	1.25	8011 -8028	8511 -8528
9 ALX-0600	Fischer	50	20.0	25.0	1.25	9011 -9028	9511 -9528

*Twice daily dosing 8 hr±15 minutes apart.

Animals 6507, 8501 and 8529 were replaced by animals 6511, 8529 and 8530 respectively on Days -4, -2 and -1 respectively. These animals were replaced due to clinical signs, such as malocclusion, missing eyeball and swollen limbs.

The dose selection was based on the anticipation that the high dose would produce specific effects and the low dose would be the no adverse effect level (NOAEL). It is to be mentioned here that in females, a dose of 25 mg/kg/day twice a day was also recommended as 'the maximum tolerated dose' by ExecCAC. The main study group animals were observed for adverse effects and weighed daily but only weekly changes calculated. The hematology, clinical chemistry parameters and urinalysis tests were done on all main study animals. On day 1 and 14, blood samples for TK analysis were collected. On day 14, the animals were anesthetized, necropsied and the adrenal gland, brain, heart, kidneys, small intestine (duodenum, jejunum, and ileum), liver, lungs, ovaries/testes, pituitary, prostate, large intestines, spleen, thymus, thyroid lobes/parathyroid glands and uterus were separated and weighed. The length to body weight ratio were determined. The tissues mentioned above and, other tissues like cecum, colon, stomach, small intestine (duodenum, jejunum, and ileum), esophagus, eye, harderian gland, ileum, injection site, jejunum, lacrimal glands, larynx, liver, lungs, lymph nodes, mammary gland, pancreas, prostate, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroids, vagina and zymbal gland were dissected out and processed for the histopathological examination.

Results:

Observed Effects: There were no significant differences in clinical signs across three strains of rats e.g., CD, Fishers-344 and Wistar Han. In Fishers-344 rats, the thinning of forepaw fur was seen in 0 of 10, 2 of 10, 2 of 10 and 1 of 10 males and, 1 of 10, 1 of 10, 2 of 10 and 2 of 10 females. In CD rats, this was also observed in 3 of 10 males and 4 of 10 females.

Mortality: None of the animals died during the study.

Body Weight: Body weight gains of Fishers and Wistar strain rats were similar during the study. The body weight increase in 3 strains of rats at 50 mg/kg/day was similar, i.e., 185.3% in SD rats, 173.0% in Wistar rats and 173.2% in Fischer rats. On day 14, the mean final body weights of male and female SD rats at 50 mg/kg/day were 260.0 g and 189.3 g, respectively. The initial and final body weights of Wistar Han male rats were 126.5, 129.5, 127.3 and 127.0 g and, 214.2, 221.9, 220.9 and 219.7 g, respectively. The initial and final body weights of Wistar Han female rats were 109.9, 106.6, 110.2 and 111.2 g and, 150.0, 152.8, 151.7 and 156.9 g, respectively. The initial and final body weights of male Fischer rats were 96.9, 95.4, 95.7 and 97.2 g and, 163.9, 165.6, 164.2 and 168.4 g, respectively. The initial and final body weights of female Fischer rats were 85.5, 85.2, 84.5 and 86.2 g and, 119.5, 1123.9, 122.6 and 125.2 g, respectively.

Food Consumption: The initial and final food consumption of the male and female rats of 3 strains was not significantly different. The mean initial and final food consumption in CD males was 20 and 27 g/animal/day and 17 and 21 g/animal/day in females, respectively. The mean initial and final food consumption in male Wistar rats was 21 and 23 g/animal/day and 15 and 17 g/animal/day in females, respectively. The mean initial and final food consumption in male

Fischer-344 rats was 13 and 17 g/animal/day and 12 and 13 g/animal/day in females, respectively. There were no significant treatment-related changes in any strain.

Hematology: There were no significant treatment or dose related changes of clinical importance in these parameters in any of the treatment groups of the 3 strains of rats.

Serum Chemistry: There were no significant changes of clinical importance in any strain.

Urinalysis: There were no significant treatment-related changes.

Toxicokinetics: Generally, the peak plasma (C_{max}) concentrations of the compound attained in 30 to 60 min posttreatment and there were no significant differences in the plasma concentrations in any of these strains of rats (as shown below in the sponsor's tables). The plasma concentrations appeared to be greater in males compared to females of all of the treatment groups. The half-life of the compound was determined to be about 8 hr in all strains. Overall, the TK parameters appeared to be comparable across different strains. The TK parameters in Wistar Han, Fischer-344 and CD rats are shown in the following table (combined from different tables of sponsor's submission).

Toxicokinetic Parameters of ALX-0600 Following Subcutaneous Injection

Day 14 (Females)

Group No.	Daily Dose (mg/kg/day)	t _{max} (h)	C _{max} (ng/mL)	t _{1/2} (h)	AUC _{0-8h} (ng·h/mL)	AUC _{daily} (ng·h/mL)	
Wistar Han Rat	3	10	0.5	2308	8	4819	9637
	4	25	1	6666	8	14462	28924
		50		8581	8	30537	61073
Group No.	Daily Dose (mg/kg/day)	t _{max} (h)	C _{max} (ng/mL)	t _{1/2} (h)	AUC _{0-8h} (ng·h/mL)	AUC _{daily} (ng·h/mL)	
Fischer-344 Rat		10	0.5	3330	8	5332	10664
	6	25		7186	8	14693	29385
	9	50		12462	8	35355	70709
Group No.	Daily Dose (mg/kg/day)	t _{max} (h)	C _{max} (ng/mL)	t _{1/2} (h)	AUC _{0-8h} (ng·h/mL)	AUC _{daily} (ng·h/mL)	
Sprague-Dawley CD #	1	50	0.5	11720	8	33627	67255

Toxicokinetic Parameters of ALX-0600 Following Subcutaneous Injection
Day 14 (Males)

Group No	Daily Dose (mg/kg/day)	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-8h} (ng·h/mL)	AUC _{daily} (ng·h/mL)
Wistar Han Rat 4	10		3463	8	6915	13829
	25	1	6768	8	20130	40260
	50		13640	8	52257	104515
Group No	Daily Dose (mg/kg/day)	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-8h} (ng·h/mL)	AUC _{daily} (ng·h/mL)
Fischer-344 Rat 8	10	0.25	4082	8	8820	17639
	25	0.5	9487	8	23609	47219
	50	1	15578	8	49790	99580
Group No	Daily Dose (mg/kg/day)	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-8h} (ng·h/mL)	AUC _{daily} (ng·h/mL)
Sprague-Dawley CD @ 1	50	0.5	12868	8	53677	107354

Organ Weight: Treatment-related increases were observed for large intestines, spleen and liver. Overall, organ weight changes appeared to be comparable in Wistar (Small intestine: Male: 194-222%; Female: 197-231%; Large intestine: Male: 122-129%; Female: 109-131%) and CD (Small intestine: Male: 212%; Female: 227%; Large intestine: Male: 129%; Female: 122%) rats. However, the changes in the tissue weights of Fisher rats appeared to be different (Small intestine: Male: 178-189%; Female: 182-195%; Large intestine: Male: 122-124%; Female: 121-134%) when compared to Wistar Han and CD rats. The increase in the length of small intestines at 50 mg/kg/day across CD and Wistar Han rats were also appeared to be comparable (1361 and 1245.4 mm in CD male and female rats, respectively, and 1184.9 and 1092 mm in male and female of Wistar rats, respectively). In Fisher-344 rats, this was slightly low (1045 and 965.2 mm in males and females, respectively, at 50 mg/kg/day).

Gross Pathology: The following changes were observed in CD males at 50 mg/kg/day (# affected/# of total animals): thickening of the duodenum (10/10), jejunum (7/10), ileum (5/5) and dark thickened area in stomach (2/10). In Wistar rats, the thickening of the duodenum was noted in 0, 9, 10 and 10 out of 10 males and, 0, 7, 9 and 8 females out of 10 females at 0, 10, 25 and 50

mg/kg/day, respectively. The thickening of ileum was seen in 0, 1, 5 and 2 males out of 10 males and, 0, 0, 1 and 1 female out of 10 females. The thickening of jejunum was observed in 0, 7, 7 and 8 males and, 0, 4, 5 and 5 females out of 10 animals. The dark thickened foci in the stomach were seen in 2, 5, 6 and 7 males and, 0, 2, 1 and 5 females out of 10/sex animals. The necropsy findings in Wistar rats and CD rats appeared to be similar.

Histopathology: In CD rats, treatment related mucosal hyperplasia of cecum and colon was seen in 9 males and 10 females out of 10/sex/group. There were subcutaneous inflammatory reactions in CD rats and similar reactions were also observed at the site of injections in Wistar Han and Fisher-344 rats. Ileum hyperplasia (villous/mucosal) was seen in both male and female animals at 10, 25 and 50 mg/kg/day treatment groups. None of the control group animals showed these changes in intestines. Increased number of incidences of inflammation at subcutaneous sites was seen in treated rats. Rectal hyperplasia was seen in 3 males and 1 female rat of CD strain. The incidences, intensity and distribution of hyperplasia of ileum, jejunum and lymph nodes are shown in the following table (data extracted from sponsor's table in Vol. 3, pp 120-167).

Sex	Male					Female				
Strain Used	CD	Wistar Han				CD	Wistar Han			
Doses Used	50	0	10	25	50	50	0	10	25	50
# Examined	10	10	10	10	10	10	10	10	10	10
<u>Cecum: Hyperplasia</u>										
# Incidences	10	0	10	10	10	10	0	7	9	10
<u>Colon: Hyperplasia</u>										
# Incidences	10	0	9	10	10	10	0	8	4	10
<u>Duodenum: Hyperplasia</u>										
# Incidences	10	0	10	10	10	10	0	10	10	10
<u>Ileum: Hyperplasia</u>										
# Incidences	10	0	10	10	10	10	0	8	10	10
<u>Jejunum: Hyperplasia</u>										
# Incidences	10	0	10	10	10	10	0	9	10	10
<u>Inflammation: Subcutaneous</u>										
# Incidences	4	0	1	1	5	2	0	0	0	2
<u>Liver: Extramedullary Hematopoiesis</u>										
# Incidences	6	1	4	2	3	5	0	1	0	0
<u>Lymph Node: Hyperplasia</u>										
# Incidences	7	0	0	0	4	3	1	0	0	1
<u>Spleen: Extramedullary haematopoiesis</u>										
# Incidences	8	9	0	0	10	5	1	0	0	4
<u>Stomach: Inflammation/congestion</u>										
# Incidences	1	0	4	4	4	0	0	1	1	3

In a 2-week comparator study in Wistar Han, CD and Fischer-344 rats, animals were treated subcutaneously at 10, 25 and 50 mg/kg/day (5, 12.5 and 25 mg/kg bid, 8 hours apart) except CD rats were treated at 50 mg/kg/day. The target organs of toxicity appeared to be the gastrointestinal tract (hyperplasia and lengthening of duodenum, ileum and jejunum and large intestines), lymph node (hyperplasia), and eye (mineralization of cornea in Fischer-344 rats).

The mineralization of cornea in Fisher-344 rats during the study appeared to be the only notable difference between this and ED or Wistar rats. The exposure to ALX-0600 in three different strains appeared to be comparable. Overall, CD rats and Wistar Han rats showed comparable toxicity and toxicokinetic profile following s.c. administration of ALX-0600 under fully fed condition.

**13-Week Subcutaneous Injection Study in CD-Rat with Dietary Optimization:
(Study #800069)**

The review of this study is incorporated below from the pharmacology review of IND 58,213 dated December 5, 2003.

1. 13-Week Subcutaneous Injection Study in CD-Rat with Dietary Optimization: (Study #800069)

Testing Laboratory:

(b) (4)

(b) (4)

Dates of Start and Completion of Study: January 9, 2003 and September 12, 2003

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted.

Species and Strain: Sprague-Dawley CD rats (*Rattus norvegicus*) approximately 6 weeks old with mean body weight of 181 to 185 g (males) and 142 to 146 g (females)

Batch #: 0850203/0850204 (97.4/98.5% pure)

Methods: One hundred and eighty rats were randomly divided into 4 main (15/sex/group) and 4 toxicokinetic groups (3/sex in control and 9/sex/treatment group). The study was conducted under restricted food conditions, i.e., 5 and 4 pellets/day (22 g/day and 17 g/day) were offered to male and female animals, respectively. ALX-0600 was administered subcutaneously at the doses of 0, 10, 25 or 50 mg/kg/day in two equally divided doses (8 hr apart in phosphate buffer solution) in a volume of 1.25 ml/kg. The dose selection of the study was based on the estimated human exposure and existing toxicity of the compound in the rats and 14-day subcutaneous toxicity study in rats (study #02-2776). The study was conducted at the doses of 0, 10, 25 and 50 mg/kg/day. The highest dose of 50 mg/kg/day was estimated as the 'highest tolerable dose'. The randomization, doses and other particulars of the study are shown in the following table:

Group	Dose* (mg/kg/day)	Main Study Gr (# Animals/gr)		Satellite Gr* (#Animals/gr)	
		Male	Female	Male	Female
1	0 (Vehicle)	15	15	3	3
2	Low (10)	15	15	9	9
3	Mid (25)	15	15	9	9
4	High (50)	15	15	9	9

^a = Administered subcutaneously in a volume of 2.5 ml/kg (in 2 divided doses). Vehicle - sodium phosphate buffer. *Satellite group animals will be used exclusively for TK evaluation.

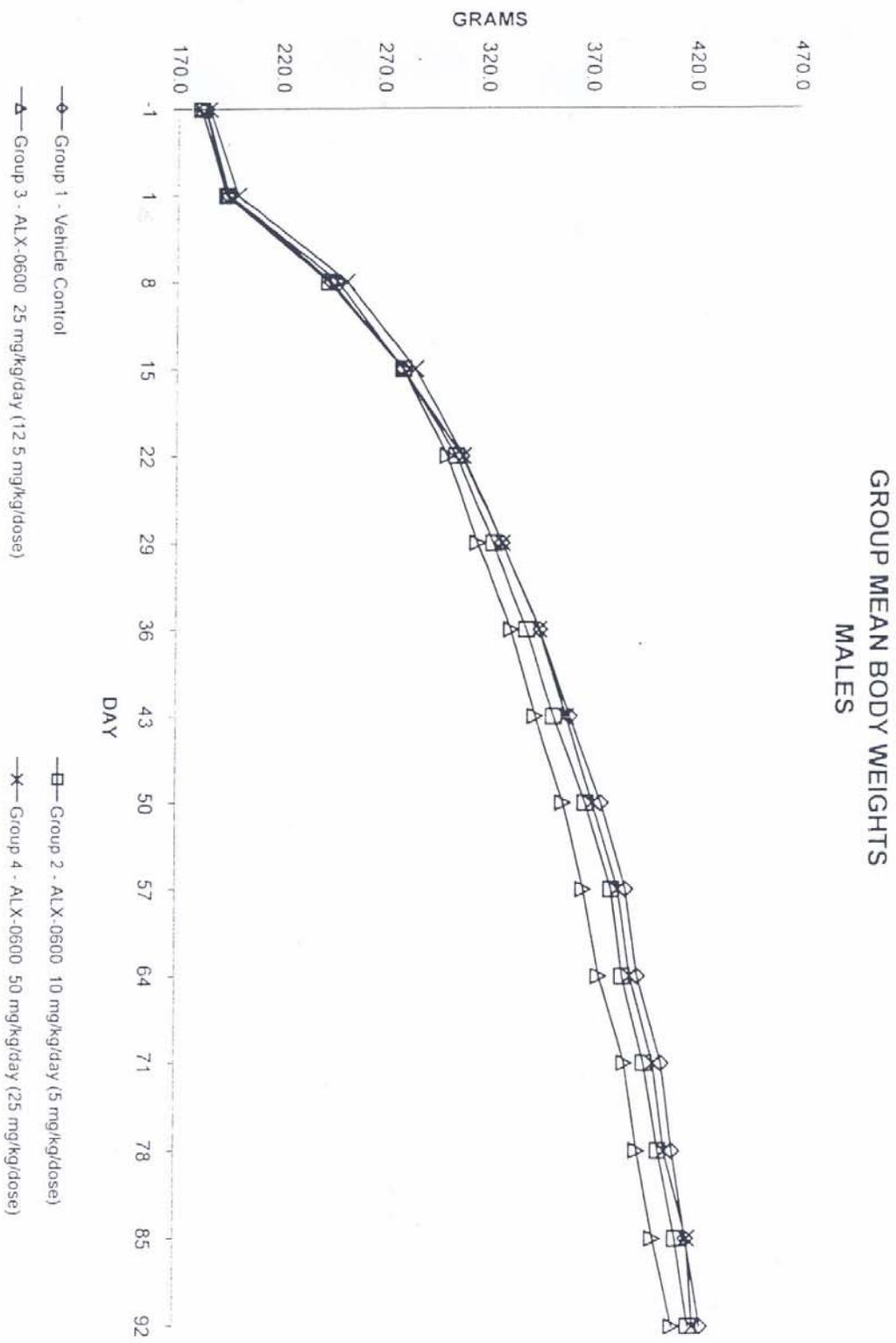
The study animals were observed for the toxic signs, mortality and symptoms during treatment period once during pretreatment period and weekly thereafter during the study. The body weights and food consumption were recorded prior to treatment and weekly during the study. The hematological and blood chemistry parameters and, urinalysis were determined on the blood and urine samples collected at the termination of the study from overnight fasted animals of the main study groups animals. The plasma toxicokinetics parameters of the compound were estimated on the blood samples of 3/sex/group (1 ml) collected from overnight fasted animals of treatment groups at 0.25, 0.5, 1, 2, 4 and 8 hr (immediately before second dose) on day 1 and in week 4 and 13. Blood samples for the estimation of the ALX-0600 and E. coli antibodies were collected in week 1, 4 and 13. All animals found dead or killed during or at the termination of the study were necropsied. The gross pathology on each of the animals was performed. The organs of each of the animals separated, cleaned and weighed were adrenals, brain, heart, kidneys, liver, lungs, pituitary, spleen, seminal vesicles, testes, thymus, thyroid + parathyroid and uterus. The histopathological examination of all of the tissues of the animals included in 0 and 50 mg/kg/day treatment groups was done. These included the gross abnormalities, adrenals, aorta, bone and marrow, (sternum), bone (femoral head), brain (3 levels), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, harderian glands, heart, ileum, jejunum, kidneys, lacrimal glands, larynx, liver (2 samples), lungs, lymph nodes (cervical, mandibular, mesenteric), mammary glands (inguinal) females, nasal cavities, optic nerves, ovaries, pancreas, pituitary glands, preputial/clitoral glands, prostate, *rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscles, skin, spinal cord (cervical, thoracic, lumber), spleen, stomach, testes, thymus, thyroid lobes and thyroids, tongue, trachea, urinary bladder, uterus, vagina, vertebra (lumber). The gross lesions, gastrointestinal tract tissues and cecum of low and mid dose treatment groups were also examined microscopically. The euthanized animals were fixed in Zenker fluid. The data were analyzed by standard statistical on-way analysis of variance, Dunnett's test for t'test, etc.

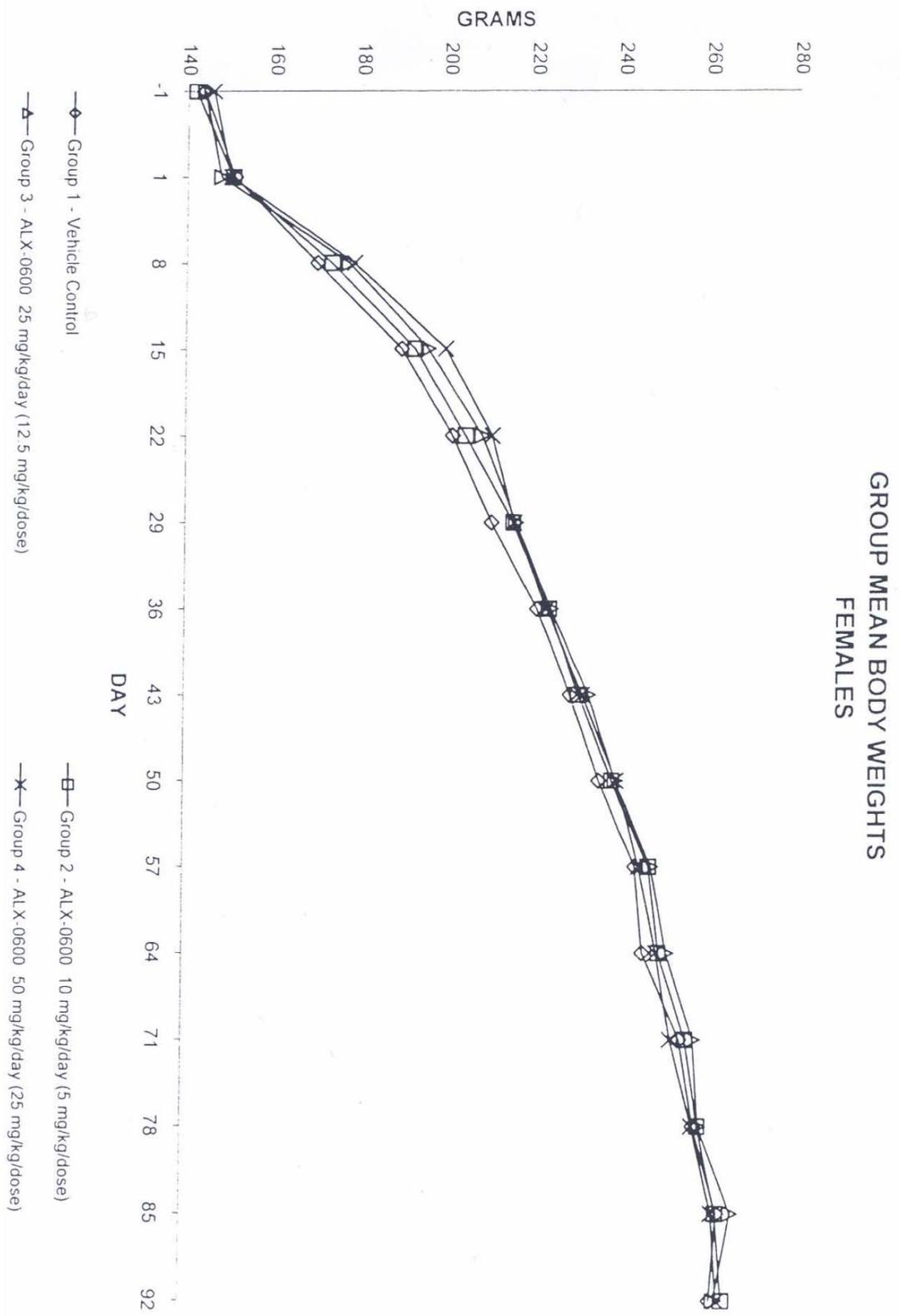
Results:

a. **Observed Effects**: Dry skin/tail was seen in 1, 1, 3 and 3 males out of 15 males and, oily fur in 2, 2, 7 and 8 females of 0, 10, 25 and 50 mg/kg/day treatment groups.

b. **Mortality**: One female (#4505) of the 50 mg/kg/day treatment group was in moribund condition and died on study day 37. Multifocal necrosis and thromboembolism were seen in the lungs of the animal.

c. **Body Weight/Food Consumption/Water Consumption Changes**: The body weight gain of the animals belonging to treatment groups and control group animals was similar. The mean initial body weights of control males and females were 183.0 and 143.7 g, respectively. The mean final body weights of males and females of control group on day 92 were 424.4 and 261.4 g, respectively. The study was conducted under restricted food intake, i.e., 5 and 4 pellets/day (22 g/day and 17 g/day) were given to male and female animals, respectively. The body weight changes are shown below in the following figures (taken from sponsor's submission).





d. **Hematology/Coagulation/Bone Marrow Changes**: There was a slight increase ($p < 0.001-0.05$) in the number of platelets in males on week 14 of the study, i.e., 1151.7, 1341.3, 1270 and 1297 thousand (Th)/ul in 0, 10, 25 and 50 mg/kg/day treatment groups. The mean platelet volume (MPV) was increased slightly but only in a statistical significant manner in males of the study.

e. **Blood Chemistry/Urinalysis Changes**: On day 90, a statistical significant ($p < 0.05-0.001$) increase in serum ALT was seen in animals included in 10, 25 and 50 mg/kg/day treatment group males (ALT = 31.3, 45.2, 46.8 and 51.3 U/l in males and 25.9, 42.9, 40.3 and 41.6 u/l in females in control and 3 treatment groups). No changes of clinical importance were seen in the urinalysis of animals included in ALX-0600 treatment groups.

f. **Toxicokinetics**: On study week 1, a dose related but non-dose proportional peak plasma concentrations were seen. T_{max} was about 1 hr after first subcutaneous dose of the compound in both male and female animals. On study week 4, the peak plasma concentrations were seen after 1 hr and 0.25 to 4 hr in males and females, respectively. The plasma peak concentrations on week 13 were 0.25, 1 and 4 hr in males and, 0.25, 0.25 and 1 hr in females of 3 treatment groups. At week 4, the AUC values were dose proportional, i.e., 7263, 22428 and 42510 in males and, 6107, 18578 and 30353 in females. These were 3.1 and 5.8 times greater in males and 3 and 5 times greater in females than in rats included 10 mg/kg/day treatment group (see scanned table below). On week 13 the concentrations of the compound in males of 50 mg/kg/day were only slightly increased. The half-lives of the compound were 0.689, 0.806 and 2.16 hr in males and, 0.73, 0.61 and 0.775 hr in females of the 3 study treatment group animals. The plasma exposures were about 6, 20 and 48 times higher in males and, 4, 26 and 30 times higher in females of the study than the plasma concentrations achieved at maximum human dose of 0.2 mg/kg. The half-lives of the compound on study week 1, 4 and 13 were similar in male and female animals. Thus the compound was not cumulated on its prolonged administration during the study. The toxicokinetic parameters of the compound on week 1, 4 and 13 are shown in the following tables (sponsor's tables in vol. 42:1 pp 41 and 42)

APPENDIX NO. 17 Toxicokinetic Parameters of ALX-0600 Following Subcutaneous Administration of ALX-0600 to the CD Rat
TABLE NO. TK-4 PROJECT NO. 800069

Week 1

Gender	Group No.	Dose Level mg/kg/day	Dose Level mg/kg/dose	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-1ast} (ng·h/mL)	k (1/h)	t _{1/2} (h)	AUC _{0-inf} (ng·h/mL)	% Extrapolation AUC _{0-inf}
Males	2	10	5	1	2455	8	5816	1.11	0.624	5817	0.0227
	3	25	12.5	1	7506	8	15272	1.09	0.636	15277	0.0315
	4	50	25	1	8864	8	19984	0.866	0.800	20011	0.134
Females	2	10	5	0.5	2820	8	4493	1.00	0.692	4494	0.0244
	3	25	12.5	1	7723	8	13746	1.08	0.641	13749	0.0229
	4	50	25	1	10782	8	21612	1.09	0.634	21618	0.0261

Week 4

Gender	Group No.	Dose Level mg/kg/day	Dose Level mg/kg/dose	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-1ast} (ng·h/mL)	k (1/h)	t _{1/2} (h)	AUC _{0-inf} (ng·h/mL)	% Extrapolation AUC _{0-inf}
Males	2	10	5	1	4280	8	7260	0.996	0.696	7263	0.0346
	3	25	12.5	1	9985	8	22412	0.981	0.707	22428	0.0706
	4	50	25	1	11773	8	42455	0.900	0.770	42510	0.131
Females	2	10	5	0.25	4728	8	6105	0.999	0.694	6107	0.0300
	3	25	12.5	0.25	7348	8	18572	1.13	0.615	18578	0.0280
	4	50	25	0.25	12783	8	30307	0.879	0.789	30353	0.153

Week 13

Gender	Group No.	Dose Level mg/kg/day	Dose Level mg/kg/dose	t _{max} (h)	C _{max} (ng/mL)	t _{last} (h)	AUC _{0-1ast} (ng·h/mL)	k (1/h)	t _{1/2} (h)	AUC _{0-inf} (ng·h/mL)	% Extrapolation AUC _{0-inf}
Males	2	10	5	0.25	3132	8	7934	1.01	0.689	7939	0.0635
	3	25	12.5	1	7478	8	24378	0.860	0.805	24415	0.150
	4	50	25	4	9578	8	54169	0.321	2.16	58159	6.8602
Females	2	10	5	0.25	3388	8	5435	0.953	0.728	5437	0.0438
	3	25	12.5	0.25	9632	8	32201	1.14	0.607	32211	0.0307
	4	50	25	1	10005	8	36703	0.894	0.775	36758	0.151

g. Detection of Antibodies in Plasma: Sponsor enclosed a summary table for the detection of antibodies to ALX-0600 Amendment # 42). The blood samples collected on day 1 and week 13 of the animals included in 12.5 and 50 mg/kg/day treatment

groups did not show the positive results for the presence of the antibody titres.

h. Organ Weight Changes: An insignificant increase in mean absolute weight of thymus was seen in males and females of the study. The absolute weight of thymus in control was 0.249 and 0.232 g in male and female animals. The absolute weight of spleen was increased in males and females included in 10, 25 and 50 mg/kg/day treatment groups as shown in the following table. As expected, a dose related marked and maximum increase in the absolute weight of small and large intestines was seen in males and females of animals included in 10, 25 and 50 mg/kg/day treatment groups as shown in the following table.

Organ Weights and Percent Organ weight Changes in animals of 13-Week Toxicity Study in CD-Rats (15 Animals/sex/Group)

Organs		ALX-0600 Dose Used (mg/kg/day)			
		Organ Wt (g)	Treated Grs.Organ Wt in g & (%) Changes		
		0 (Control)	10	25	50
Small Intestine	Male	7.6933	15.811(205.5)	15.286(198.7)	15.944(207.2)
	Female	6.4737	13.5033(208.6)	13.851(213.9)	14.073(297.1)
Large Intestines	Male	3.9008	5.114(131.1)	4.995(128.0)	5.761(147.7)
	Female	2.9837	3.9744(133.2)	4.183(140.2)	3.999(134.0)
Liver	Male	9.7323	11.008(113.1)	10.465(107.5)	10.572(108.0)
	Female	6.5025	7.6235(117.2)	7.963(122.5)	7.9079(121.6)
Spleen	Male	0.6697	0.7485(111.8)	0.7295(108.9)	0.7795(116.4)
	Female	0.5115	0.539(105.5)	0.5925(115.8)	0.6009(117.5)
Adrenal	Male	0.5009	0.0552(110.3)	0.05075(101.3)	0.05003(100.0)
	Female	0.5446	0.06217(114.1)	0.06505(119.4)	0.05858(119.6)

j. Gross Pathology Findings: Treatment related thickening of duodenum, ileum, stomach, rectum and colon was seen in male and females of treatment groups. The dark areas at the site of injection, i.e., dorsal cervical area in 1, 1, 1 and 3 males and, 1, 2, 1 and 1 female included in control and 3 treatment groups (10, 25 and 50 mg/kg/day treatment groups). A treatment related increase in the length of small and large intestines was reported ($p < 0.05$). The increase in the length of small intestine (in comparison to control group) was 18.2, 16.7 and 15.8% in males and, 20.0, 20.7 and 20.7% in females of rats included in treatment groups. The increase in the length of large intestine was 7.5, 4.5 and 17.0% in males and, 13.6 15.9 and 12.5% in females of treatment groups.

k. Histopathological Changes: A treatment related mucosal hyperplasia/hypertrophy of glandular stomach, duodenum, jejunum, ileum, cecum, colon, jejunum and rectum (grade 2 to 3)

was seen in both male and female animals included in 10, 25 and 50 mg/kg/day treatment groups. The incidences and distribution of hyperplasia in the study animals are shown in the following table (data extracted from sponsor table vol 038, vol 2:3 pp. 81-109). The changes seen in treated animals were almost completely reversed during recovery period. Ovarian pigment deposit due to the ovarian hemorrhage in 1 of 15 animals of 50 mg/kg/day treatment group was reported. Increased number of incidences of hemorrhage and subacute/granulomatous inflammation of the tissue (both left and right side injection sites) were seen in animals of 50 mg/kg/day treatment group. Mononuclear cell infiltration and acinar cell atrophy were seen in 1 and 3 males of 0 and 50 mg/kg/day treatment groups. Only edema was seen 1 female of high dose treatment group. Among females, bile duct hyperplasia was seen in 1 of the 15 females and, centrilobular necrosis was seen in 1 other female of the 50 mg/kg/day treatment group. These were not seen in control, 10 and 25 mg/kg/day treatment groups females.

Table
Incidence and Distribution of Hypertrophy/Hyperplasia in the Stomach, Bile Duct, Small and Large Intestines, Spleen, Preputial Gland and Prostate

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
# Examined	15	15	15	15	15	15	15	15
Stomach								
# Incidences	0	0	0	2	0	0	0	3
Duodenum								
# Incidences	0	15	15	15	1	15	15	14
Jejunum								
# incidences	0	15	15	15	0	15	15	14
Ileum								
# Incidences	0	11	13	11	0	12	13	13
Cecum								
# Incidences	0	15	15	15	0	15	15	14
Colon								
# incidences	0	15	15	15	0	15	14	14
Liver								
Necrosis # incidences	0	-	-	0	0	-	-	1 [#]
Bile Duct Hyperplasia	0	-	-	0	0	0	0	1 ^{#1}
Rectum								
# Incidences	0	15	15	15	0	15	14	15
Preputial Gland								
Inflammation/Infilt. Mixed cells	0	-	-	2	-	-	-	-
Prostate								
Mononuc. cell Infilt.	1	-	-	3	-	-	-	-

[#] = grade 4 (animal # 4505); ^{#1} = grade 1 (animal # 4501)

In summary, subcutaneously administered ALX-0600 from the doses of 10 to 50 mg/kg/day was shown to produce treatment related mucosal hyperplasia/hypertrophy of duodenum, ileum, rectum, jejunum and large intestines in male and female rats. A dose related increased incidences of inflammation associated with hemorrhage edema, necrosis and fibrosis at the site of injection and bile duct hyperplasia suggested the target organs of toxicity as bile duct and site of injection. The dose of 25 mg/kg/day was the highest tolerable dose.

Minipig

14-Day Subcutaneous Toxicity Study in Juvenile Minipigs (Study # 51153)

The review of this study report is incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

Study Title: 14-Day Subcutaneous Toxicity Study in Minipigs

Study No.: 51153

Volume and Page: Amendment No. 094, Vol. 2, page 1

Testing Laboratory: [REDACTED] (b) (4)

Dates of Initiation and Completion of Study: August 7, 2003 and November 25, 2004

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted. The QAU statement was also included.

Species and Strain: Juvenile minipigs

Batch and Purity: Lot No. 0850205. Purity data not provide.

Vehicle: Phosphate buffer with L-histidine and mannitol

Methods: The objective of this study was to evaluate the effect of ALX-0600 administered twice daily (8-hour interval between doses) by subcutaneous injection in juvenile minipigs in order to select doses for subsequent studies. In this study, minipigs were randomized and assigned to 6 sows to give each 8 piglets (4 males and 4 females) on lactation day 1 (LD 1). The 6 sows each with 8 piglets were distributed in 3 groups. On first day of the study (LD 7) two piglets (1 male and 1 female) were discarded from each sow. The animals in Groups 2 and 3 were treated with total daily doses of 5 and 25 mg/kg/day (1.25 ml/kg). The animals in Group 1 (control) were treated with the vehicle (phosphate buffer containing histidine and mannitol). Treatment started from LD1 to LD20 for a period of 14 days. The following table (from page 17 of the study report) shows the study design.

Treatment

The juvenile minipigs were treated according to the following table:

Group	Animal Nos		Target dose level (mg/kg b.wt/day)	Target dose level (mg/kg b.wt/dose)	Target dose concentration (mg/mL/day)	Target dose volume (mL/kg/day)	Target dose volume (mL/kg/dose)	Colour code
	Males	Females						
1	11-16	21-26	0	0	0	1.25	0.625	White
2	31-36	41-46	5	2.5	4	1.25	0.625	Blue
3	51-56	61-66	25	12.5	20	1.25	0.625	Green

Clinical signs were recorded daily (pre-dose in the morning and 1 and 2 hours after the first daily dose). The offspring were weighed on LD 1 and twice weekly thereafter. Blood samples were collected for hematology and clinical chemistry at pre-dose on LD 6 and on Day 14 (LD 20) prior to dosing. Additionally, on Days 1 (LD 7) and 14 (LD 20) blood samples were collected for determination of ALX-0600 in the plasma. At the end of the in-life phase, the offspring were sacrificed and subjected to a macroscopic necropsy. Selected organs/tissues (as shown in the following table from page 25 of the study report) were weighed, sampled and subjected to a microscopic examination. In addition, the length of small and large intestines of each piglet was measured at necropsy.

Organs and tissues	W e i g h	F i x	M i c r o	Organs and tissues	W e i g h	F i x	M i c r o
Abnormalities (gross lesions)		x		Pancreas	x	x	x
Adrenals	x	x		Parathyroids (when possible)		x	
Aorta (thoracic)		x		Pituitary	x	x	
Bile duct	x	x	x	Prostate	x	x	
Brain	x	x		Salivary gland (submandibular, parotid, sublingual)		x	
Epididymides		x		Sciatic nerve		x	
Eyes and with lens/optic nerve		x		Seminal vesicle		x	
Gall bladder	x	x	x	Skeletal muscle		x	
Femur (medial condyl of right femur)		x		Skin		x	
Heart with aortic arch	x	x	x	Spinal cord (thoracic, lumbar)		x	
Injection site		x	x	Spleen	x	x	x
Intestine small (duodenum, jejunum, ileum) Length measurement*	x	x	x	Sternum (for bone marrow)		x	
Intestine large (caecum, colon, rectum) Length measurement*	x	x	x	Stomach (glandular, non glandular)	x	x	x
Kidneys	x	x	x	Testes	x	x	
Knee joint		x		Thymus	x	x	
Larynx		x		Thyroid	x	x	x
Liver (all main lobes)	x	x	x	Tongue		x	
Lungs (cranial and caudal lobes, both sides)		x		Trachea		x	
Lymph nodes (right mandibular and mesenteric)		x		Urinary bladder and ureters		x	
Mammary gland		x		Uterus (horn, cervix)	x	x	
Oesophagus		x		Vagina		x	
Ovaries	x	x		Vertebrum (thoracic, lumbar)		x	

* The length of small and large intestine of each piglet was measured at necropsy.

Results:

Clinical Signs: There were no treatment-related clinical signs.

Mortality: There were no treatment-related mortalities.

Body Weight: The mean initial and final body weights of control males were 537 and 1202 g, respectively. The mean initial and final body weight of control females were 478 and 1144 g, respectively. The mean final body weights of males in Groups 2 and 3 were 86 and 108% of control, respectively. In females, the final body weights in Groups 2 and 3 were 109 and 116% of control, respectively.

Hematology: There were no significant treatment-related changes in the hematology parameters.

Serum Chemistry: No significant treatment-related changes were observed.

Organ Weights: Treatment-related increases in weights (absolute and/or relative) of the following organs were observed when compared to control: gall bladder (Groups 2 and 3), small intestines (Groups 2 and 3), large intestines (Groups 2 and 3), liver (Groups 2 and 3), spleen (Group 3) and thyroid (Groups 2 and 3). For the thyroid the same tendency was also seen in Group 3 females.

A statistically significant dose-related increase in the length of the small intestines was observed for Groups 2 and 3 males. Similar tendency was also seen for Groups 2 and 3 females, however these were not statistically significant. A tendency towards a dose-related increase in the length of the large intestines was seen in both sexes in Groups 2 and 3. The following table (from page 28 of the study report) shows the organ weight changes.

	Percent difference from the control					
	Male			Female		
	Control value	5 mg/kg b.wt.	25 mg/kg b.wt.	Control value	5 mg/kg b.wt.	25 mg/kg b.wt.
Small intestine weight	62.57	+75	-145	66.63	+104	+151
Large intestine weight	22.95	+44	-51	25.92	+37	+33
Small intestine length	343	+43	-60	490.5	+3	+11
Large intestine length	70.8	+10	-23	77	+4	+24

+ indicates the percent increase above control for the intestineal weight and length.

Gross Pathology: There were no significant treatment-related changes.

Histopathology: Treatment-related findings were observed in the intestinal tract (hyperplasia), gall bladder (mucosal hyperplasia associated with increased bile accumulation), bile duct (hyperplasia), the spleen (extramedullary hematopoiesis), liver (extramedullary hematopoiesis) and at the injection site (inflammation and necrosis). The following table shows the histopathological changes.

Organs	Sex (n = 6/sex)	ALX-0600 Dose (mg/kg/day)			
		0	5	25	
Stomach, nonglandular -hyperplasia, mucosa	Male	-	2	1	
	Female	-	2	2	
Stomach, nonglandular -ulceration, mucosa	Male	-	-	1	
	Female	1	1	-	
Stomach, nonglandular -erosion, mucosa	Male	-	1	-	
	Female	1	1	2	
Duodenum -hyperplasia	Male	0	3	6	
	Female	-	3	6	
Jejunum -hyperplasia	Male	-	2	6	
	Female	-	-	6	
Ileum -hyperplasia	Male	-	1	6	
	Female	-	1	6	
Cecum -hyperplasia	Male	-	2	2	
	Female	-	-	-	
Bile duct -hyperplasia, mucosal	Male	-	3	4	
	Female	-	2	5	
Gallbladder -hyperplasia, mucosal	Male	-	6	6	
	Female	-	6	6	
Colon -hyperplasia	Male	-	1	6	
	Female	-	-	3	
Rectum -hyperplasia	Male	-	-	4	
	Female	-	-	3	
Spleen -Hematopoiesis, extramedullary	Male	3	5	6	
	Female	4	3	6	
Liver -Hematopoiesis, Extramedullary	Male	2	5	5	
	Female	3	3	4	

Toxicokinetics: Exposure to ALX-0600 increased as the dose level increased from 5 to 25 mg/kg/day. The T max value was 1.00 hour on Day 1 and ranged from 0.500 to 2.00 hours on day 14. The $t_{1/2}$ values ranged from 0.662 to 1.51 hours on Day 1 and from 0.679 to 1.23 hours on Day 14. No apparent gender differences in $t_{1/2}$ were observed. No marked (>2-fold) gender differences were observed in Cmax or AUC_{0.25-8h} values. The Cmax and AUC values on Day 14 were not markedly different from those on Day 1, indicating no accumulation of ALX-0600 after multiple dosing.

The Cmax and AUC_{0.25-8h} values increased as the dose level increased from 5 to 25 mg/kg/day in both sexes. Increases in Cmax and AUC were approximately dose proportional. In males on Day 1, Cmax and AUC values increased 1:2.5 fold and 1:4.6 fold, respectively, with a 1:5 fold increase in the dose level. In females on Day 1, Cmax and AUC values increased 1:3.6 fold and 1:5.9 fold with a 1:5 fold increase in the dose level. In males on Day 14, Cmax and AUC values both increased 1:3.5 fold with a 1:5 fold increase in the dose level. In females on Day 14, Cmax and AUC values increased

1:3.8 fold and 1:5.6 fold with a 1:5 fold increase in the dose level. The following table (from page 179 of the study report) shows the toxicokinetic parameters.

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Toxicokinetic parameters for ALX-0600 in juvenile minipig plasma

Group	Dose Level (mg/kg/day) ^a	Gender	C _{max} (ng/mL)	T _{max} (Hours)	AUC _{0-24h} (ng·hr/mL)	Daily AUC (ng·hr/mL)	t _{1/2} (Hours)
<u>Day 1</u>							
2	5	M	2974	1.00	6727	13453	0.673
		F	2094	1.00	5373	10746	0.662
3	25	M	7300	1.00	30751	61502	1.26
		F	7493	1.00	31947	63881	1.51
<u>Day 14</u>							
2	5	M	2438	0.500	8015	16030	0.679
		F	2043	1.00	5960	11939	0.934
3	25	M	8467	1.00	28226	56452	1.08
		F	7757	2.00	33225	66450	1.23

^a Dosed twice daily (approximately 8 hours apart).

In a 14-day s.c. toxicity study in minipigs, animals were treated at total daily doses of 0, 5 and 25 mg/kg/day (0, 2.5 and 12.5 mg/kg, bid, 8 hours apart). The target organs appeared to be the intestinal tract (hyperplasia), gall bladder (mucosal hyperplasia associated with increased bile accumulation), bile duct (hyperplasia), the spleen (extramedullary hematopoiesis), liver (extramedullary hematopoiesis) and injection site (inflammation and necrosis).

Study title: 90-Day Subcutaneous Toxicity Study in Juvenile Minipigs with 28-Day Recovery Period

Study no.: 66585
Study report location: EDR 4.2.3.5.4.1
Conducting laboratory and location: (b) (4)
Date of study initiation: December 13, 2007
Date of study completion: July 8, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Teduglutide, Lot No. 08505061 and >95%

Key Study Findings:

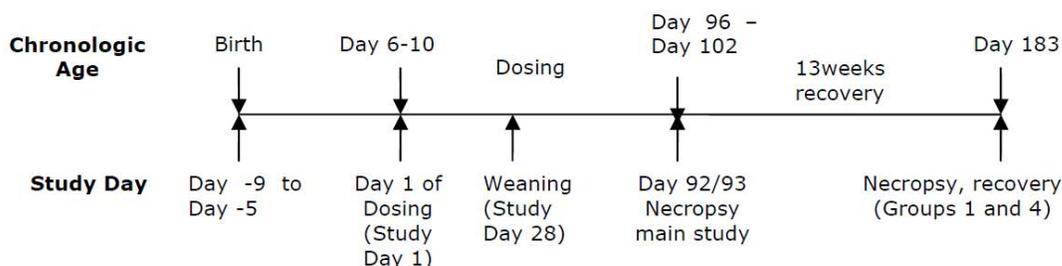
- Juvenile minipigs (7 days old) were treated at 1, 5, and 25 mg/kg/day, SC (BID dosing) for 13 weeks.
- A total of 36 piglets died (10 piglets in Group 1, 8 piglets in Group 2, 8 piglets in Group 3 and 10 piglets in Group 4) and were replaced with extra piglets. The cause of death in these piglets could not be ascertained; however, the deaths were not considered related to treatment with teduglutide as mortalities were also observed in the control group.
- Clinical signs in the piglets included constipation, diarrhea, vomiting/yellowish froth or salivation, subdued behavior, respiratory distress, convulsions, and cyanosis.
- There were no significant treatment-related effects on body weight, food consumption, ophthalmoscopy.
- In males at Week 13, teduglutide increased the P wave, PR, QT (mid and high dose) and RR intervals at all doses compared to respective controls. The ECG changes were predominantly seen in males (although females had higher plasma exposure to teduglutide than males at all doses at Week 13) only at one time point (Week 13), magnitude of these changes were small and the changes were also not dose-related. Moreover, there were no significant treatment-related effects on QTc (Fridericia) in either sex. The QTc values were comparable across all groups. Overall, these ECG changes are not meaningful and not toxicologically significant.
- Treatment-related histopathology changes were observed in the gall bladder (cystic mucous hyperplasia), extrahepatic bile duct (cystic mucous hyperplasia), small intestines (minimal/slight villous hypertrophy) and injection sites (inflammatory changes including myofibre degeneration/inflammation and granulomatous inflammation in all groups of animals including controls with increased severity for treated animals).
- The NOAEL could not be identified as treatment related effects were seen in all dose groups.

Methods:

Doses: 1, 5 and 25 mg/kg/day
 Frequency of dosing: BID (8 hours apart)
 Route of administration: Subcutaneous injection
 Dose volume: 0.625 mL/kg
 Formulation/Vehicle: Phosphate buffer with L-histidine and mannitol
 Species/Strain: Juvenile minipigs (Ellegaard Göttingen SPF minipigs)
 Number/Sex/Group: Group assignment is presented in the following table (from page 12 of the report)
 Age: 7 days old
 Weight: 412 to 1351 g
 Satellite groups: Recovery (control and high dose)
 Unique study design: The study design is shown in the figure below (from page 13 of the report).
 Deviation from study protocol: Protocol deviations were considered not to have affected the outcome of the study.

Text Table 1 Number of Animals Assigned to Main and Recovery Groups

Group	Dose mg/kg/day	Main Study		Recovery	
		Male	Female	Male	Female
1	0	8	8	3	4
2	1	8	8	-	-
3	5	8	8	-	-
4	25	6	7	5	3

**Observations and Results:**

Mortality: There was no mortality in sows. A total of 36 piglets died prior to start or during the first 23 days of the study (10 piglets in Group 1, 8 piglets in Group 2, 8 piglets in Group 3 and 10 piglets in Group 4) and were replaced with extra piglets. The cause

of death in these piglets could not be ascertained; however, the deaths were not considered related to treatment with teduglutide as mortalities were also observed in the control group. A spectrum of findings was reported in these decedent animals and the changes considered to be factors contributory to death are shown in the following table (from page 76 of the report) below:

Text Table 47 Factors Contributory to Death

FCTD GROUP	MALE				FEMALE			
	1	2	3	4	1	2	3	4
ALX-0600 (teduglutide) mg/kg/day	0	1	5	25	0	1	5	25
Unknown	1	1	0	0	1	1	1	0
Peritonitis	3	0	2	4	3	2	3**	4
Intracellular basophilic particles epithelium small intestines	2	1	0	0	1*	1	0	0
Oesophageal ulcer	0	0	0	1	0	0	0	0
Intrabronchial debris	0	1	0	0	0	0	0	0
No of minipigs ex	4	3	2	5	5	3	4	4

* This animal also has a moderate ulcer in the non glandular stomach.

** Includes one animal with moderate erosion in the non glandular stomach.

Clinical Signs: Clinical signs were recorded 1 and 2 hours after the first daily dose and 0.5 hour after the second daily dose. Clinical signs in sows included lethargy. There was a decrease in milk production post-farrowing (giving birth). Additional clinical signs in sows included increased body temperature, vomiting, reduced food consumption, diarrhea, depression, agalactia (complete stoppage of milk production, one sow) and discharge from the vulva (one sow). The following table (from page 357 of the report) shows the overview of clinical signs in sows.

Table 15 Overview of clinical signs on Sows – Individual values

Sow number	Symptoms (From Day -7 to Day 12)							Treated
	Increased body temperature	Vomitus	Reduced food consumption	Diarrhea	Depression	Vulval discharge	Agalactia ²⁾	
1	X	X	X		X			X
2 ¹⁾								
3	X		X	X	X			X
4	X	X	X	X	X			X
5	X		X		X			X
6	X	X	X		X			X
7	X	X	X	X	X	X	X	X
8	X		X	X	X			X
9		X	X		X			
10		X	X					
11	X		X					X
12								
13	X		X		X			X
14	X							

¹⁾Treated with antibiotics 2 days prior to farrowing (depressed, reduced food consumption).

²⁾ Agalactia = complete stop in milk production

Clinical signs in the piglets included constipation, diarrhea, vomiting/yellowish froth or salivation, subdued behavior, respiratory distress, convulsions, and cyanosis and death. These clinical signs were not considered treatment-related and were attributed to excessive handling of the piglets and agalactia in the sows prior to the treatment and during the initiation of the study (Days -7 through 23).

Body Weights: Body weights were measured twice weekly. The mean initial (at birth) and final (Day 92) body weights of the male piglets were 0.445 and 8.75 kg, respectively. The mean initial (at birth) and final (Day 92) body weights of the female piglets were 0.461 and 8.72 kg, respectively. In males, the final weights of the treated animals were 88%, 88%, 88.2% of control at low, mid and high doses, respectively. In females, the final weights of the treated animals were 95%, 99%, 104% of control at low, mid and high doses, respectively.

Food Consumption: Food consumption was recorded after weaning. Details of the timing were not provided. There were no significant treatment-related effects.

Ophthalmoscopy: Ophthalmoscopy was performed on Day 41 and during Week 13. There were no significant treatment-related effects.

Electrocardiography (ECG): Electrocardiography was performed prior to treatment, on Day 33 of the treatment period, during Week 13 and during the last week of recovery. During the dosing period, the electrocardiography was performed approximately 1 hour after the first daily dose. The following parameters were evaluated: heart rate (BPM), P wave duration (msec), PR (msec), QRS (msec), QT (msec) and RR- intervals (msec).

P wave duration: In males at Week 13, although not dose-related, P wave duration was increased at all doses (103%, 134% and 119% of control at 1, 5 and 25 mg/kg/day, respectively) compared to control; however, this increase in P wave was statistically significant only in Group 3 males (5 mg/kg/day) with one animal (Animal No 255) reaching a P wave duration of 60 msec. In females at Week 13, P wave duration was increased at all doses (116%, 105% and 113% of control at 1, 5 and 25 mg/kg/day, respectively; not statistically significant) compared to control.

PR Interval: In males at Week 13, PR interval was increased at all doses (110%, 109% and 117% of control at 1, 5 and 25 mg/kg/day, respectively) compared to control; however, the changes were not dose-related. In females at Week 13, PR interval was also increased at all doses (120%, 112% and 106% of control at 1, 5 and 25 mg/kg/day, respectively) compared to control (not dose-related).

QT Interval: In males at Week 13, the QT interval increased slightly at mid and high dose (101%, 108% and 107% of control at 1, 5 and 25 mg/kg/day, respectively) compared to control. The sponsor did not provide the QTc data. QTc values for Week 13 were calculated using the Fridericia method. In males at Week 13, the QTc (Fridericia) values were 0.294, 0.289, 0.285 and 0.282 at 0, 1, 5, and 25 mg/kg/day, respectively. There were no significant treatment-related effects on QT/QTc in females.

The QTc values were comparable across all dose groups. Overall, there were no significant treatment-related effects on QTc in either sex.

RR Interval: In males, at Week 13, a dose-related increase (102%, 131% and 135% of control at 1, 5 and 25 mg/kg/day, respectively) in the RR interval was observed. In females at Week 13, RR interval was also increased (116%, 112% and 110% of control at 1, 5 and 25 mg/kg/day, respectively) at all doses; however, this was not dose-related.

The following table (from page 50 of the study report) shows the ECG data in males at Week 13.

Text Table 11 ECG Data for Male Minipigs During Week 13

Göttingen minipig	Dose (mg/kg/day)	ECG Value					
		HR bpm	P msec (S.D.)	PR msec (S.D.)	QRS msec (S.D.)	QT msec (S.D.)	RR msec (S.D.)
Study	Control 0	167.1 (48.6)	38.6 (6.9)	78.6 (14.6)	48.6 (6.9)	208.6 (31.3)	386.1 (120.2)
	1	156.7 (15.3)	40.0 (10.0)	86.7 (5.8)	43.3 (5.8)	210. (20.0)	392.3 (39.7)
	5	120.0 (12.2)	52.0 ²⁾ (4.5)	86.0 (5.5)	50.0 (7.1)	226.0 (8.9)	507.2 (45.3)
	25	120.0 (12.2)	46.0 (8.9)	92.0 (11.0)	46.0 (8.9)	224.0 (18.2)	522.0 ²⁾ (52.9)
(b)(4) ω historical data ¹⁾ (upper limit)	N.A.	181.01	56.89	108.43	52.28	308.96	743.69

- 1) (b)(4) historical data, 4-7 month old minipigs
2) Statistical significance

The following tables (from page 130 of the study report) show the ECG data for females at Week 13.

Group mean values - Week 13

Females

GROUP	HR (bpm)				P (msec)				PR (msec)				QRS (msec)			
	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p	MEAN	S.D.	N	p
1	161.7	19.4	6		38.3	4.1	6		78.3	4.1	6		45.0	5.5	6	
2	140.0	7.1	5		44.0	5.5	5		94.0	5.5	5		48.0	8.4	5	
3	143.3	20.8	3		40.0	0.0	3		86.7	11.5	3		43.3	5.8	3	
4	153.3	24.2	6		43.3	5.2	6		83.3	16.3	6		46.7	8.2	6	

Group mean values - Week 13

Females

GROUP	QT (msec)				RR (msec)			
	MEAN	S.D.	N	p	MEAN	S.D.	N	p
1	211.7	14.7	6		379.8	54.2	6	
2	220.0	33.9	5		442.0	22.5	5	
3	206.7	15.3	3		423.7	70.8	3	
4	211.7	19.4	6		417.0	71.2	6	

$p > 0.05$, versus control group; S.D. = Standard deviation; N = No. of animals

All values observed in this study were within the historical control values reported for 4-7 months old Göttingen minipigs (shown in the table below from 805 of the report).

(b) (4) HISTORICAL DATA

Minipig toxicity studies

Electrocardiography - 4 to 7 months old animals

SEX	PARAMETER	Number of studies	Number of animals	MEAN	MIN	MAX	95% CONFIDENCE INTERVALS	
							LOWER LIMIT	UPPER LIMIT
male	HR bpm	68	329	122.10	93.33	160.00	63.19	181.01
female	HR bpm	73	327	117.94	90.00	164.44	68.69	167.20
male	P m sec	68	329	44.81	35.00	56.67	32.72	56.89
female	P m sec	73	327	44.30	33.33	53.33	32.08	56.51
male	PR m sec	68	329	87.55	72.00	113.33	66.66	108.43
female	PR m sec	73	327	86.95	76.00	105.00	66.33	107.56
male	QRS m sec	68	329	41.06	26.67	62.00	29.84	52.28
female	QRS m sec	73	327	40.07	26.67	56.67	29.96	50.19
male	QT m sec	68	329	251.57	213.33	306.67	194.17	308.96
female	QT m sec	73	327	252.63	196.67	306.67	197.50	307.76
male	RR m sec	15	53	558.61	444.50	662.00	373.53	743.69
female	RR m sec	21	71	575.37	485.00	696.00	377.46	773.27

Overall, teduglutide increased the P wave, PR, QT (mid and high dose) and RR intervals at all doses in males at Week 13 compared to respective controls. The ECG changes were predominantly seen in males; however, the plasma exposure (AUC) of teduglutide was higher in females than that in males at Week 13 at all doses. In addition, these changes were seen at only one time point (Week 13), in one species (juvenile minipigs) and the magnitude of these changes were small and these changes were also not dose-related. Moreover, there were no significant treatment-related effects on QTc (Fridericia) in either sex. The QTc values were comparable across all groups. Overall, these ECG changes are not meaningful and not toxicologically significant.

Hematology: Blood samples were collected for clinical pathology prior to treatment and on Days 14 and 28, during Week 13, and again during the last week of recovery. A statistically significant increase over control values was observed in the platelet count in Groups 3 and 4 in Week 13 in both sexes. In addition, statistically significant increases over control values were noted for activated partial thromboplastin time (APTT) in Group 4 males on Day 28 and in Week 13. A statistically significant increase over the control value was also noted in thrombin time (TT) for Group 3 females in Week 13. At Week 13, statistically significant decreases in red blood cells (Groups 2 and 4 females), hemoglobin (Hb) (Group 4 males and Groups 2, 3, and 4 females), hematocrit (HT) (Group 4 males and females), and mean hemoglobin concentration (MCH) (Group 3 and 4 males). The changes in clotting factors and the RBC parameters were considered to be treatment-related. Group mean values for the above hematology changes are shown in the following tables.

Males:

GROUP	Platelet (10 ⁹ /L)	APTT (Sec)	TT (Sec)	RBC (10 ¹² /L)	Hb (mmol/L)	HT (mL/100 mL)	MCH (fmol)
1	472	29.9	23.37	8.74	8.61	42.9	1.00
2	549	31.1	20.93	8.38	7.73	39.0	0.93
3	687	35.3	27.90	7.95	7.82	40.0	0.98
4	914	51.2	20.32	7.39	7.10	36.2	0.98

Females:

GROUP	Platelet (10 ⁹ /L)	APTT (Sec)	TT (Sec)	RBC (10 ¹² /L)	Hb (mmol/L)	HT (mL/100 mL)	MCH (fmol)
1	442	34.7	17.00	9.08	9.03	44.8	1.00
2	627	31.3	22.62	7.65	7.76	39.6	1.04
3	692	34.1	29.00	8.09	7.87	40.3	1.00
4	740	36.2	21.92	7.65	7.43	38.0	1.00

Clinical Chemistry: Blood samples were collected for serum chemistry prior to treatment and on Days 14 and 28, during Week 13, and again during the last week of recovery. Statistically significant decreases in alkaline phosphatase (ALKPH) values compared to controls were observed on Days 14 (Group 2 females and Group 3 males) and on Day 28 (Groups 2 and 4 females), and in Week 13 (Group 4 females). In addition, statistically significant increased globulin values were observed on Day 14 (Groups 3 and 4 males) and in Week 13 (Group 4 females). Statistically significant decreases in albumin values were observed on Week 13 (Group 4 females); and albumin/globulin ratio on Days 14 (Groups 3 and 4 males) and in Week 13 (Group 4 females). Furthermore, after 13 weeks of treatment, statistically significant decreases in creatinine values were observed in Groups 3 and 4 males, compared to control values. Overall, the clinical chemistry changes appeared to be scattered, not clearly dose-related and did not appear to be meaningful or treatment-related. The following table shows the clinical chemistry changes in males and females.

Day 14:

Group	Male					Female					
	Parameter	ALKPH (μ kat/L)	Globulin (g/L)	ALB/G Ratio	ALB (G/L)	CREAT (μ M/L)	ALKPH (μ kat/L)	Globulin (g/L)	ALB/G Ratio	ALB (G/L)	CREAT (μ M/L)
1		8.76	15.5	2.49	37.7	47.6	8.32	18.8	2.08	38.0	41.0
2		6.71	19.2	1.86	35.3	39.7	5.01	18.1	2.22	33.5	52.2
3		4.61	24.0	1.48	34.0	43.5	5.96	22.1	1.71	35.7	47.3

4	5.59	23.8	1.49	34.3	47.1	5.80	22.2	1.81	35.9	68.0
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Day 28:

Group	Male					Female				
	Parameter	ALKPH (µkat/L)	Globulin (g/L)	ALB/G Ratio	ALB (G/L)	CREAT (µM/L)	ALKPH (µkat/L)	Globulin (g/L)	ALB/G Ratio	ALB (G/L)
1	6.93	11.2	4.06	44.9	48.7	7.58	11.9	3.86	43.7	44.8
2	5.86	10.2	4.09	41.0	56.3	5.34	12.8	3.93	45.8	50.2
3	6.19	14.7	3.06	41.5	50.5	5.77	13.1	3.84	45.3	46.0
4	6.60	13.5	2.88	36.8	48.2	5.10	13.0	3.34	41.7	48.8

Week 13:

Group	Male					Female				
	Parameter	ALKPH (µkat/L)	Globulin (g/L)	ALB/G Ratio	ALB (G/L)	CREAT (µM/L)	ALKPH (µkat/L)	Globulin (g/L)	ALB/G Ratio	ALB (G/L)
1	4.19	13.8	3.16	42.6	69.9	4.19	11.0	4.24	44.8	60.2
2	3.97	16.1	2.53	40.3	62.7	3.38	13.0	3.33	42.4	63.6
3	3.49	16.3	2.53	40.2	54.8	3.38	13.8	3.28	44.0	58.3
4	3.39	17.4	2.40	40.4	50.4	2.57	19.9	2.04	38.3	57.5

Urinalysis: Urine was collected during Week 13 and during the last week of recovery. At Week 13, decreased values of potassium (Males: 76%, 57% and 55% of control at low, mid and high dose, respectively; control = 223.0 mmol/L) and chloride (Males: 84%, 66%, and 60% of control at low, mid and high dose, respectively; control = 170.9 mmol/L) were observed.

Gross Pathology: Gross pathology was conducted at necropsy. Red discoloration at the injection sites was observed in a dose-related manner. Gross observations were noted in the gall bladder of one male animal (animal No 253, Group 3) at 5 mg/kg/day and two male animals at 25 mg/kg/day (animal No. 272 and 474, Group 4). Gall bladder wall was thickened in one animal at 5 mg/kg/day (animal No 253) and one of the animals at 25 mg/kg/day (animal No 474). Several grey/white foci up to 4 mm in diameter were observed on the gall bladder in another animal at 25 mg/kg/day (animal No 272). No remarkable findings were noted in 13-week recovery animals.

Organ Weights: The following table (from page 41 of the report) shows the organs and tissues collected for weights and histopathology.

Text Table 9 Organ and Tissue Collection

Organs and tissues	W e i g h	F i x	M i c r o	Organs and tissues	W e i g h	F i x	M i c r o
Abnormalities (gross lesions)		x	x	Pancreas		x	x
Adrenals	x	x	x	Parathyroids (when possible)		x	x
Aorta (thoracic)		x	x	Pituitary	x	x	x
Bile duct		x	x	Prostate	x	x	x
Brain	x	x	x	Salivary glands (right parotid, sublingual and submandibular)		x	x
Bone marrow smear		x		Sciatic nerve		x	x
Bones (medial condyl of right femur)		x	x	Seminal vesicles		x	x
Epididymides		x	x	Skeletal muscle		x	x
Eyes with lens/optic nerve		x	x	Skin, non-treated area		x	x
Gall bladder		x	x	Spinal cord (thoracic, lumbar)		x	x
Heart with aortic arch	x	x	x	Spleen	x	x	x
Injection site		x	x	Sternum (for bone marrow)		x	x
Intestine small (duodenum, jejunum, ileum) Length measurement*	x	x	x	Stomach (glandular, non glandular)		x	x
Intestine large (caecum, colon, rectum) Length measurement*	x	x	x	Testes	x	x	x
Joint (knee)		x	x	Thymus	x	x	x
Kidneys	x	x	x	Thyroid	x	x	x
Larynx		x	x	Tongue		x	x
Liver (all main lobes)	x	x	x	Trachea		x	x
Lungs (cranial and caudal lobes, both sides)		x	x	Ureters		x	x
Lymph nodes (right mandibular and mesenteric)		x	x	Urinary bladder		x	x
Mammary gland		x	x	Uterus (horn, cervix and oviducts)	x	x	x
Oesophagus		x	x	Vagina		x	x
Ovaries	x	x	x	Vertebrum (thoracic, lumbar)		x	

Treatment-related effects were observed in the intestines (weights and lengths), liver (increased weight), adrenals (increased weight) and kidneys (increased weight). The weight and length of the intestine increased due to treatment. The effect was more pronounced in the small intestine than the large intestine. Mean small intestine weight in Groups 2, 3 and 4 as a percent of mean Group 1 small intestine weight is summarized in the table (from page 56 of the report) below.

Text Table 15 Small Intestinal Weight as a Percent of Control: Main Study

Group/Sex	Absolute weight	Weight relative to body weight	Weight relative to brain weight
2/Male	214.23*	232.87*	227.58*
3/Male	244.71*	272.69*	268.86*
4/Male	251.58*	333.80	274.70*
2/Female	297.37*	310.38*	283.96*
3/Female	308.23*	316.39*	294.64*
4/Female	293.98*	284.70*	287.82*

* = statistically significant

Mean large intestinal weight in Groups 2, 3 and 4 as a percent of mean Group 1 large intestinal weight is summarized in the table (from page 60 of the report) below.

Text Table 23 Large Intestinal Weight as a Percent of Control: Main Study

Group/Sex	Absolute weight	Weight relative to body weight	Weight relative to brain weight
2/Male	126.06	138.38	134.38
3/Male	143.97	161.62	159.38*
4/Male	116.03	150.81	126.71
2/Female	121.11	123.98	122.73
3/Female	121.29	121.95	123.12
4/Female	113.23	107.72	116.85

* = statistically significant

The small intestinal length was also increased by the treatment. The data are shown in the following table (from page 65 of the report).

Text Table 31 Small Intestinal Length as a Percent of Control: Main Study

Group/Sex	Absolute length	Length relative to body weight	Length relative to brain weight
2/Male	136.98	151.40	146.20
3/Male	139.45*	161.20	155.46*
4/Male	148.41*	196.40*	162.54*
2/Female	130.39*	134.06*	130.96*
3/Female	142.40*	144.02*	143.17*
4/Female	130.89*	124.70*	133.91*

* = statistically significant

The data for the large intestinal length are shown below (from page 69 of the report).

Text Table 39 Large Intestinal Length as a Percent of Control: Main Study

Group/Sex	Absolute length	Length relative to body weight	Length relative to brain weight
2/Male	98.80	111.54	105.57
3/Male	106.70	121.43	118.42
4/Male	100.06	132.42	109.42
2/Female	99.05	100.00	98.93
3/Female	106.32	107.25	106.99
4/Female	95.58	90.34	97.75

At Week 13, liver weights were generally increased for all treated animals when compared to controls. Statistically significant differences from control values were noted in Group 3 males (liver weight relative to brain weight) and Groups 2 and 3 females (both absolute and relative values). After a 13-week recovery period, male No 278 (Group 4) and female No 289 (Group 4) had higher liver weight in absolute and both relative values (relative to both body weight and brain weight) compared to control values.

Adrenal weights were also increased by the treatment in both sexes. In Group 4 males, a statistically significant increase in adrenal weight (relative to body weight) and a tendency for increased weight in absolute values and relative to brain weight was observed. In Groups 3 and 4 females, a statistically significant increase in adrenal weight (relative to brain weight) was observed, and also a tendency for increased weight in absolute values and relative to body weight was observed. After a 13-week recovery period, male No 278 (Group 4) had higher adrenal weight in absolute and both relative (relative to both body weight and brain weight) values compared to control values. However, the females had comparable values.

In Group 4 males, a statistically significant increased kidney weight (relative to body weight) was observed. After the 13-week recovery period, the kidney weight was still slightly increased in female animal No 289 compared to control values.

Overall, there were treatment-related increases in the liver, adrenal, and kidney weight. Absolute and/or relative liver weights were higher in teduglutide treated animals compared to controls. Absolute and/or relative adrenal weights were increased by teduglutide treatment when compared to control. In addition, at Week 13, relative kidney weight for Group 4 males was increased by teduglutide treatment compared to control. These changes in organ weights appeared to be treatment-related. However, there were no corresponding histopathological findings in the liver, adrenals or kidneys.

Histopathology: The list of tissues for histopathological examination was shown in the above table.

Adequate Battery: Yes

Peer Review: Yes

Histological Findings: Treatment-related changes were observed in the gall bladder (cystic mucous hyperplasia), extrahepatic bile duct (cystic mucous hyperplasia), small intestines (minimal/slight villous hypertrophy) and injection sites (inflammatory changes including myofibre degeneration/inflammation and granulomatous inflammation in all groups of animals including controls with increased severity for treated animals). The incidence of cystic mucous hyperplasia is shown in the table (from page 77 of the report) below.

Text Table 48 Cystic Mucous Hyperplasia: Gall Bladder and Extrahepatic Bile Duct

Gall bladder/ extrahepatic bile duct	MALE				FEMALE			
	1	2	3	4	1	2	3	4
ALX-0600 (teduglutide) mg/kg/day	0	1	5	25	0	1	5	25
Gall bladder Cystic mucous hyperplasia								
Total	0	3	5	3	0	2	3	4
Minimal	0	1	0	0	0	0	1	1
Slight	0	2	3	0	0	2	1	0
Moderate	0	0	2	2	0	0	1	3
Marked	0	0	0	1	0	0	0	0
Extrahepatic bile duct Cystic mucous hyperplasia								
Total	0	0	3	3	0	0	0	3
Minimal	0	0	2	0	0	0	0	1
Slight	0	0	1	2	0	0	0	2
Marked	0	0	0	1	0	0	0	0
No minipigs ex	5	3	5	3	3	5	3	4

The incidence of the small intestinal findings is shown in the following table (from page 78 of the report).

Text Table 49 Villous Hypertrophy: Small Intestines

Small intestine	MALE				FEMALE			
	1	2	3	4	1	2	3	4
ALX-0600 (teduglutide) mg/kg/day	0	1	5	25	0	1	5	25
Small intestine Villous hypertrophy								
Total	0	2	4	3	0	2	3	3
Minimal	0	1	4	3	0	2	3	3
Slight	0	1	0	0	0	0	0	0
No of minipigs ex	5	3	5	3	3	5	3	4

Toxicokinetics: Blood samples were collected on Day 1, Day 44 and Day 91 at 15 min, 0.5 h, 1 h, 2 h, 4 h, and 8 h post-treatment. Exposure to teduglutide generally increased with the increase in dose level from 1 to 25 mg/kg/day. The increases in C_{max} and AUC_{0-8} were generally less than dose proportional. In general, no marked (> 2-fold) sex differences were observed in C_{max} and AUC_{0-8h} values, except for the 5 mg/kg/day group

on Day 91 in which females demonstrated 2.4- and 1.4-fold greater exposure than males in terms of C_{max} and AUC_{0-8} , respectively. No apparent accumulation of teduglutide was observed after multiple dosing. The following table (from page 11 of the toxicokinetic report) shows the TK data.

Table 1. Toxicokinetic Parameters for ALX-0600 in Minipig Plasma

Day	Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/day)]	T_{max} (hr)	AUC_{0-4} (ng-hr/mL)	AUC_{0-8} (ng-hr/mL)	DN AUC_{0-8} [(ng-hr/mL)/(mg/kg/day)]	$AUC_{0-\infty}$ (ng-hr/mL)	$t_{1/2}$ (hr)	
1	2	1	M	727	727	0.250	1022	1050	1050	1034	0.581	
			F	951	951	0.250	1476	1501	1501	1485	0.499	
	3	5	M	2860	572	1.00	7540	7540	1508	7542	0.618	
			F	2520	504	2.00	7369	7369	1474	7369	0.486	
	4	25	M	6247	250	1.00	22986	22986	919	26899	2.96	
			F	7247	290	0.500	29799	29799	1192	31066	1.65	
44	2	1	M	674	674	0.500	1175	1303	1303	NA	1.14	
			F	412	412	1.00	1130	1130	1130	NA	0.636	
	3	5	M	1260	252	1.00	4390	4390	878	NA	0.840	
			F	989	198	2.00	3161	3161	632	NA	0.939	
	4	25	M	4945	198	0.250	15388	15388	616	NA	2.36	
			F	5060	202	0.250	15073	15073	603	NA	1.77	
	91	2	1	M	389	389	0.500	1476	1476	1476	NA	0.711
				F	574	574	0.500	1589	1589	1589	NA	0.724
		3	5	M	1140	228	1.00	2647	2647	529	NA	1.63
				F	2710	542	0.500	3760	3760	752	NA	1.25
		4	25	M	6315	253	0.250	15120	15120	605	NA	1.13
				F	11500	460	0.500	28419	28419	1137	NA	1.36

Dosing Formulation Analysis: Triplicate samples were collected on Day 1, Weeks 6 and 13 from each dose formulation. All formulations were in the range 92.25% to 106.03% of nominal value and were within the acceptance criteria (acceptance criteria 90-110% of nominal).

Summary: In a 90-day toxicology study in juvenile minipigs (7 days old), animals were treated at 1, 5, and 25 mg/kg/day, SC (BID dosing) for 13 weeks. A total of 36 piglets died in this study. The cause of deaths could not be determined; however, the deaths were not considered related to treatment as mortalities were also observed in the control group. Clinical signs in the piglets included constipation, diarrhea, vomiting/yellowish froth or salivation, subdued behavior, respiratory distress, convulsions, and cyanosis. There were no significant treatment-related effects on body weight, food consumption, and ophthalmoscopy. Teduglutide increased the P wave, PR, QT (mid and high dose) and RR intervals at all doses in males at Week 13 compared to respective controls. The ECG changes were predominantly seen in males; however, the plasma exposure (AUC) of teduglutide was higher in females than that in males at Week 13 at all doses. In addition, these changes were seen at only one time point (Week 13), in one species (juvenile minipigs) and the magnitude of these changes were small and these changes were also not dose-related. Moreover, there were no significant treatment-related effects on QTc (Fridericia) in either sex. The QTc values

were comparable across all groups. Overall, these ECG changes are not meaningful and not toxicologically significant. Treatment-related histopathology changes were observed in the gall bladder (cystic mucous hyperplasia), extrahepatic bile duct (cystic mucous hyperplasia), small intestines (minimal/slight villous hypertrophy) and injection sites (inflammatory changes including myofibre degeneration/inflammation and granulomatous inflammation in all groups of animals including controls with increased severity for treated animals). The NOAEL could not be identified as treatment related adverse effects were seen in all dose groups.

Monkey:

3-Day Preliminary Subcutaneous Injection Study in Cynomolgus Monkeys (88616)

14-Day Subcutaneous Injection Study in Cynomolgus Monkeys (88619)

1-Month Subcutaneous Injection Study in Cynomolgus Monkeys with 1-Month Recovery (88729)

The reviews of the above study reports are incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

3. 14-Day Subcutaneous Toxicity Study in Cynomolgus Monkeys:
(Project # 88619)

Testing Laboratory: [REDACTED] (b) (4)

Dates of Start and Completion of the study: June 9, 1998 and
January 20, 1999.

QAU GLP & Requirements: This study was not conducted according
to GLP regulations.

Species and Strain: Ten male adult Cynomolgus monkeys (Macaca
fascicularis) mean body weight of 2.3 to 2.7 kg were used in the
study.

Batch #: D05GL98b and D05GL98c.

Methods: Nine male monkeys were randomly divided into 3 groups
(3/group) and administered 0, 10 or 20 mg/kg/day, s.c. ALX-0600
(in two divided doses 8 hr apart) in phosphate buffer solution
in a volume of 2, 1 and 2 ml/kg, respectively for 14 days to
assess the toxicity of the compound. The basis of dose
selection was 3-day preliminary s.c. toxicity study in 1/sex
monkeys (Study # 88616) at the dose of 50 mg/kg (25 mg/kg/day
bid) for 3 days. No toxicity was observed and this was regarded
as the 'highest tolerable dose'. In the present study, the
animals were observed twice daily for the toxic signs and
symptoms. The body weights and food consumption were recorded
at least once before treatment, on study day 1, 3, 6, 9, 12 and
14/15 (prior to necropsy). Ophthalmic examination by
fundoscopy/slit lamp was done once before, and during week 2 of

the study. The blood samples were collected prior to treatment and on treatment day 14 for determining hematological and blood chemistry parameters. Urine samples (overnight) were collected before treatment and on study day 14. The plasma concentration of the compound was determined on day 1 and 14 after 5, 10, 15, 30, 45, 60 and 90 min post AM dosing. All animals were killed at the termination of the study and gross pathology on each of the animals was performed. The organs of each of the animals were separated, cleaned and, adrenals, brain, gastrointestinal tract (duodenum, ileum, jejunum, cecum, colon and rectum), heart, kidneys, liver, lungs, pituitary, spleen, testes, thymus and thyroid were weighed. The esophagus and stomach of each of the animals were also weighed. The histopathological examination of the above tissues and all other tissues and organs of the animals included in 0 and 20 mg/kg/day treatment groups was conducted. The affected tissues and gross lesions of animals included in 10 mg/kg/day treatment group were examined microscopically.

Results:

- a. **Observed Effects:** Sponsor stated that there were no significant clinical signs on day 14 of the study. No table or data were submitted to substantiate their claims.
- b. **Mortality:** None of the animals died during the study.
- c. **Body Weight/Food Consumption/Water Consumption:** The body weight gain was similar to that of the control group animals. The final body weights of animals on study day 12 were: 2.53, 2.63 and 2.5 kg in control, 10 and 20 mg/kg/day treatment groups, respectively. The food consumption of these animals was also similar, i.e., 5.3, 5.7 and 5.3 cookies/day in male animals of control and 2 ALX-0600 treatment groups.
- d. **Hematology/Coagulation/Bone Marrow Changes:** No changes in hematological parameters were produced by ALX-0600 treatment. It did not affect coagulation parameters in animals.
- e. **Blood Chemistry/Urinalysis Changes:** No dose/treatment related changes in blood chemistry and urinalysis were seen in animals included in ALX-0600 treatment groups.
- f. **Toxicokinetic Parameters:** The peak plasma concentration was observed in 48 min of the administration of the single 5 and 10

mg/kg/BID dose of the compound. The second dose of the compound showed a peak plasma concentration after 20 to 30 min.

Table
Toxicokinetic Parameters of ALX-0600 in Cynomolgus Monkeys

Group #	ALX-0600 (mg/kg/BID)	Tmax (hr)	Cmax (ng/ml)	AUC (ng.hr/ml)
Day 14: 2	5	70 ± 35	4106.7 ± 1302	228436.7±89348.7
3	10	75± 26	11083.0 ± 3043.2	629481.8±170789.8
Day 1: 2	5	50 ± 9	3603 ± 848.8	189501.2±57920.7
3	10	45 ± N.A.	7482.5 ± N.A.	4396975 ± N.A.

The peak plasma concentrations in animals on day 1 and 14 of these 2 treatment groups were similar and dose proportional following subcutaneous dose of the compound.

g. **Physical Examination and Ophthalmic Test:** No adverse effects on ophthalmoscopic and visual examinations were reported in animals of the 3 treatment and control groups of the study.

h. **Organ Weight Changes:** The mean weight of stomach, small intestines and large intestines was increased in treated animals. The weight increases (as compared with day 0) in 10 and 20 mg/kg/day treatment groups were 16.0 and 55.3% in stomach, 100 and 124% in small intestines and 31.9 and 53.2% in large intestines, respectively. The length of small intestines in animals included in 10 and 20 mg/kg/day treatment groups was increased by 1.45 and 1.25 times and, of large intestines was increased by 1.2 and 1.3 times (Vs control), respectively.

i. **Gross Pathology Findings:** The thickening of tissues at the injection site was in 2 and 3 animals out of 3 animals in each of 10 and 20 mg/kg/day treatment groups. Musculo-skeletal clot was seen in 1 animal in each of 10 and 20 mg/kg/day treatment groups.

j. **Histopathological Changes:** Fibrosis and cellulitis at the site of injection were seen in 3 animals in each of 10 and 20 mg/kg/day treatment groups. One animal of control group had subcutaneous fibrosis. Lymphadenitis and lymphoid hyperplasia were seen in 1 monkey of 20 mg/kg/day treatment group. Pancreatic duct hyperplasia in one and biliary duct hyperplasia in another animal of 20 mg/kg/day treatment group was observed.

In summary, ALX-0600 at 20 mg/kg/day, s.c. dose produced increase in the weight and length small and large intestines and produced biliary hyperplasia and inflammatory reactions at the

site of injection with cellulitis. A dose of 10 mg/kg/day was a 'highest tolerable dose' in this study and identified target organs of toxicity were liver and site of injection.

4. 1-Month Subcutaneous Toxicity Study with 1-Month Recovery Period study in Cynomolgus Monkeys: (Project # 88729)

Testing Laboratory: (b) (4)

Dates of Start and Completion of Study: August 21, 1998 and April 13, 1999.

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted.

Species and Strain: Ten male adult monkeys (*Macaca fascicularis*) with mean body weight of 2.57 kg (males) and 2.35 kg (females) were used in the study.

Batch #: 8106801.

Methods: Forty monkeys (20/sex) were randomly divided into main and toxicokinetic groups. In the main study groups, 4 groups (5/sex/group) were administered 0, 0.2, 0.6 or 2.0 mg/kg/day, s.c. ALX-0600 (in two divided doses 8 hr apart) in phosphate buffer solution in a volume of 1 ml/kg, bid, respectively for 28 days to assess the toxicity of the compound. Two animals/sex/group of the main study were used for the recovery period of 1 month. The dose selection of the study was based on 14-day s.c. toxicity study in monkeys in which a dose of 10 mg/kg/day was a 'highest tolerable dose' (Study # 88619). The animals of the present study were observed twice daily for the toxic signs and symptoms during treatment and recovery period and just before termination of the study. The body weights and food consumption were recorded weekly during pretest period, treatment and recovery prior to necropsy. Ophthalmic examination was done once before, and during week 4 of the study and recovery period by fundoscopy. The blood samples for determining hematological and blood chemistry parameters were collected 2 weeks prior to treatment, weekly during the study and during recovery week 4 from overnight fasted animals. Urine samples (overnight) for its analysis from all animals were collected before treatment and on study week 4. The plasma concentration and toxicokinetics of the compound was determined

on day 1 and 28 after 0, 30, 45, 60, 90, 120, 180 and 240 min after the administration of the AM dose of the compound. D-Xylose absorption test was conducted on all animals of the study prior to the treatment, weekly during treatment and recovery periods of the study. All animals were killed at the termination of the study and gross pathology on each of the animals was performed. The organs of each of the animals were separated and cleaned and, adrenals, brain, gastrointestinal tract, heart, kidneys, liver, lungs, pituitary, spleen, testes, thymus and thyroid were weighed. The esophagus and stomach of each of the animals were also weighed. The histopathological examination of the above enumerated tissues and all other tissues and organs of the animals included in 0 and 2.0 mg/kg/day treatment groups was conducted. The affected tissues, gross lesions, site of injection, pancreas, liver, stomach, small and large intestines of animals included in 0.2 and 0.6 mg/kg/day treatment groups were examined microscopically.

Results:

- a. **Observed Effects:** There were no changes in the clinical signs of the animals included in 3 treatment groups during the study.
- b. **Mortality:** None of the animals died during the study.
- c. **Body Weight/Food Consumption/Water Consumption Changes:** During the study, the body weight gain in male monkeys was similar to that of the control group animals. The initial and final body weights of control animals on study day 1, 28 and 56 were: 2.46, 2.84 and 2.6 kg among males and, 2.3, 2.5 and 2.75 kg among females, respectively. The food consumption data of the animals was not provided in the submission.
- d. **Hematology/Coagulation/Bone Marrow Changes:** There were no changes in the hematological parameters of animals included in ALX-0600 treatment groups excepting that significant ($p < 0.05$) increase in the segmented neutrophils in only females included in 0.6 mg/kg/day treatment group. Since no change was seen in high dose female and in male animals, this was considered of no importance. The treatment did not affect any change in coagulation parameters of animals.

e. **Blood Chemistry/Urinalysis Changes:** No dose or treatment related changes in blood chemistry and urinalysis of clinical significance, were seen in animals included in ALX-0600 treatment groups.

f. **Toxicokinetics:** Dose proportional peak plasma concentrations were observed in about 1 hr after the administration of a single dose of the compound in both male and female animals (t_{max} = 0.94 and 1.07 hr in male and female). The peak of plasma concentrations after repeated dosing of the compound were dose proportional with similar t_{max} in male and females animals (see table below). The elimination half lives of the compound on day 1 were: 0.77 to 1.04 hr in male and, 0.81 to 0.83 hr in females of the 3 treatment groups of the study. The half-lives on day 28 were also similar, i.e., 0.75 to 1.02 hr in male and, 0.59 to 1.11 hr in female animals. The compound did not tend to accumulate on its prolonged administration for 28 days. The toxicokinetic of the compound had been shown in the following table (table prepared from sponsor's tables in vol 9:12 at pp 495 and 496).

TABLE
Toxicokinetic Parameters of ALX-0600 in Cynomolgus Monkeys

Group #	Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{(0-\infty)}$ (ng.hr/ml)
Males:				
Day 1: 2	0.2	0.91	114 \pm 20	236.2 \pm 25.3
3	0.6	0.75	296 \pm 109	816 \pm 130.5
4	2.0	1.30	717 \pm 69	1899.08 \pm 114.75
Day 28: 2	0.2	0.95 \pm 0.4	142 \pm 12	308.9 \pm N.A.
3	0.6	1.2 \pm 0.3	317 \pm 153	829 \pm 211.9
4	2.0	1.25 \pm 0.5	745 \pm 339	3193 \pm 252.8
Females:				
Day 1: 2	0.2	0.5 \pm 0.0	134 \pm 26	236 \pm 35.06
3	0.6	1.05 \pm 0.45	291 \pm 69	802 \pm 84.42
4	2.0	1.1 \pm 0.42	1203 \pm 300	2828.3 \pm 474.7
Day 28: 2	0.2	0.95 \pm 0.11	171 \pm 41	303.64 \pm 48.3
3	0.6	1.1 \pm 0.22	417 \pm 91	851.32 \pm 119.8
4	2.0	0.95 \pm 0.33	1067 \pm 280	2744.1 \pm 272.3

The peak plasma concentrations following subcutaneous dose of the compound on study day 1 and 14 in 2 treatment groups animals were similar and dose proportional.

- g. **Physical Examination and Ophthalmic Test:** No treatment related adverse effects were reported in fundoscopic and slit lamp visual examinations in study animals.
- h. **Organ Weight Changes:** An increase in the mean relative weight of liver to body weight was seen in males and females of 2.0 mg/kg/day treatment group ($p < 0.05$). The increase was reversed in recovery period animals. The absolute weight of stomach ($p < 0.5$), small intestines ($p < 0.01$) and large intestines (only a trend in males and $p < 0.05$ in females) were increased in animals included in 0.2, 0.6 and 2.0 mg/kg/day treatment group animals. The relative weights of gastrointestinal tract were not affected. The organ weight of recovery animals was similar to the control group animals (recovery group).
- i. **Gross Pathology Findings:** Dark areas were seen at the site of injection in study animals and 1 out of 3 females in 2 mg/kg/day treatment group had bilateral single ovarian cyst. There were no other treatment related gross pathological changes in animals during the study.
- j. **Histopathological Changes:** A dose related mucosal hyperplasia of duodenum, ileum and jejunum was reported in both males and females of the study included in 0.2, 0.6 and 2.0 mg/kg/day treatment groups. There were no incidences of hyperplasia in control group animals. Average lengths of duodenal villi, jejunum and ileum in both males and females included in 0.2, 0.6 and 2 mg/kg/day treatment groups were increased significantly (p values < 0.01 or 0.05). Average mucosal height of rectum was increased ($p < 0.01$) in animals treated from 0.2 to 2 mg/kg/day for 28 days and the increase in height was not dose related. The mucosal heights of cecum and colon in male and female animals were not significantly affected. The villus:crypt ratio of intestine and rectum was not changed. These changes were reversible as either not seen or insignificant in animals of recovery group. An increased number of incidences of cellulitis and mixed cell infiltration were reported at the site of injection in animals included in ALX-0600 treatment groups. Mixed cell infiltration at the site of injection were seen in 1, 1, 3, 3, males and, 0, 1, 1 and 3

females out of 5/sex animals belonging to 0, 0.2, 0.6 and 2.0 mg/kg/day treatment groups, respectively. Slight to mild intraductal biliary hyperplasia was seen in 0, 3, 1 and 2 males and, 0, 3, 3 and 2 females monkeys out of 5/sex in 0, 0.2, 0.6 and 2.0 mg/kg/day treatment groups. Pancreatic duct hyperplasia was also reported (shown in the table below):

TABLE

Percent Increase of Gastrointestinal Tract Organ Weights and Histopathological incidences in 1-Month Toxicity Study in Cynomolgus Monkeys (3 Animals/Group)

Histopathological Findings		ALX-0600 Dose Used (mg/kg/day)			
		0	0.2	0.6	2.0
%Organ Wt. Increase					
a. Stomach	Male	0	23.5	45.0	45.0
	Female	0	27.6	55.7	58.1
b. Small Intestestine	Male	0	89.1	94.3	174.1
	Female	0	87.0	133.0	166.0
c. Large Intestine	Male	0	0	19.7	22.2
	Female	0	21.8	40.3	42.9
Mucosal Hyperplasia (%)					
Duodenum	Male	0	100	66.6	100
	Female	0	100	66.6	100
Jejunum	Male	0	100	100	100
	Female	0	100	100	100
Ileum	Male	0	66.6	100	100
	Female	0	100	100	100
Pancreas	Male	0	66.6	33.3	100
	Female	0	66.6	66.6	66.6
Average Increase in Length (%cm)					
Small Intestine	Male	0	4.7	3.3	22.2
	Female	0	12.5	19.9	25.5
Large Intestine	Male	--	10.9	2.0	12.1
	Female	--	21.2	12.1	24.5
Cellulitis (%)	Male	0	0	66.6	100
	Female	0	0	100	100
Intraductal Biliary Hyperplasia %	Male	0	100	33.3	66.6
	Female	0	100	100	66.6
Pancreatic Ductal Hyperplasia	Male	0	66.6	33.3	100
	Female	0	66.6	66.6	100

In summary, ALX-0600 at subcutaneous dose from 0.2 to 6 mg/kg/day for 28 days produced treatment related desired weight increase and hyperplasia of stomach, small and large intestines. Treatment related intraductal biliary and pancreatic ductal hyperplasia were seen in animals of treated groups. Inflammatory reactions at the site of injection were seen in animals of 2 mg/kg/day treatment group. A dose of 0.6 mg/kg/day was considered as a 'maximal tolerable dose' and site of injection, liver and pancreas were identified as the target organs of

toxicity in this study. This study provided sufficient safety margin for the proposed dose.

13-Week Subcutaneous Toxicity and Toxicokinetic Study with 1-Month Recovery Period in Cynomolgus Monkeys (Study # 7203-100)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated April 1, 2004.

1. **13-Week Subcutaneous Toxicity and Toxicokinetic Study with 1-Month Recovery Period in Cynomolgus Monkeys:**
(Study # 7203-100)

Testing Laboratory: [REDACTED]

(b) (4)

Dates of Start and Completion of Study: February 13, 2001 and April 8, 2002.

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted.

Species and Strain: Cynomolgus monkeys (*Macaca fascicularis*) approximately 2 to 3 years old with mean body weight of 2.2 to 2.3 kg (males) and 2.3 to 2.4 kg (females)

Batch #: 0850001

Methods: Forty-eight monkeys (24/sex) were randomly divided into 4 groups and were administered 0, 1, 5 or 25 mg/kg/day, S.C. ALX-0600 (in two divided doses 8 hr apart) in phosphate buffer solution in a volume of 0.625 ml/kg, bid, respectively. Two animals/sex/group were used for the recovery period of 30 days. The sponsor did not provide the basis of the dose selection of the study. The study animals were observed daily for the toxic signs and symptoms during treatment and recovery period and just before termination of the study. The body weights and food consumption were recorded prior to treatment and on the 1st day treatment then weekly during the study. Ophthalmic examination was done once before treatment and during week 13 of the study. The blood pressure and EKG tracings were recorded after 30 to 90 min of the first daily dose in conscious males on study day 0, 2, 27 and 85 and, on day 0, 3, 28 and 86 in conscious females. The hematological and blood chemistry parameters and, urinalysis were determined on the blood and urine samples collected from overnight fasted animals during week 0 (prior to treatment), 6, 13 and 17, weekly during the study and during recovery week 4 from overnight fasted animals. The plasma concentrations and toxicokinetics parameters of the compound were estimated on the blood samples collected from overnight fasted animals on day 1, 30 and 90. The blood samples (0.5 ml) on day 1 were collected after 0, 0.25, 0.5, 1, 2, 4 and 8 hr of the first dose (immediately before second dose), 8.25, 8.5, 9, 10, 12 and 16 hr after the administration of first dose of the compound. The blood samples on day 30 and 90 were collected for 8 hr (i.e., 0, 0.25, 0.5, 1, 2, 4 and 8 hr of the first dose). Four/sex/group animals were killed after 13 weeks of treatment and the remaining 2/sex/group were killed at the end of the recovery period.

The gross pathology on each of the animals was performed. The following organs of each of the animals were separated, cleaned and weighed: adrenals, brain, heart, kidneys, liver, lungs, pituitary, spleen, seminal vesicles, testes with epididymides, thymus, thyroid + parathyroid and uterus. The histopathological examination of the above enumerated tissues (used for organ weight determination) and other tissues and organs of the animals included in 1, 5 and 25 mg/kg/day treatment groups was done. The affected tissues, gross lesions, site of injection, gastrointestinal tract tissues and all other organs and tissues were preserved. The gastrointestinal tract, pancreas, pancreatic duct, spleen, gall bladder and cystic duct, liver and hepatic ducts, common bile duct and injection sites from all groups of animals were examined microscopically.

Results:

a. **Observed Effects:** Emesis was seen in 1, 2 and 3 males out of 6 in each of 1, 5 and 25 mg/kg/day treatment groups and in 1 out of 6 females of 25 mg/kg/day treatment group. The swellings at the dose sites were reported in 5 and 6 males out of 6 males of 5 and 25 mg/kg/day treatment groups. Five and 6 out of 6 females of each of 5 and 25 mg/kg/day treatment groups also showed swellings at the dose sites. Masses at the injection site were seen in 4 out of 6 males and 3 of 6 females of 25 mg/kg/day treatment group. These masses were resolved partially during the recovery period and the swelling was seen in 1 and 2 males and, 0 and 2 females in 5 and 25 mg/kg/day treatment groups.

b. **Mortality:** None of the animals died during the study.

c. **Body Weight/Food Consumption/Water Consumption Changes:** The body weight gain in monkeys belonging to treatment groups and control group animals was similar. The mean initial body weights of control males and females were 1.85 and 1.9 kg, respectively. The mean final body weights of males and females of control group on week 13 were 2.35 and 2.45 kg. Sponsor in the submission did not provide the food consumption data of the animals.

d. **Hematology/Coagulation/Bone Marrow Changes:** There was a slight but significant ($P < 0.5$) increase in the number of platelets in females on week 13 of the study, i.e., 344,

400, 450 and 447 thousand (th)/ul in 0, 1, 5 and 25 mg/kg/day treatment groups. A treatment related slight increase from 11.7 to 17.2 th/ul of total mean WBCs count was noted in only females included in 25 mg/kg/day treatment group. Absolute WBC counts were 407 and 344 th/ul in males and females of study controls. The males of this group were not affected. The absolute neutrophils counts were increased slightly from mean of 4.3 th/ul in control to 5.0, 6.2 and 8.2 th/ul in females and from 3.0 in control males to 3.8, 3.3 and 5.5 th/ul in males included in 1, 5 and 25 mg/kg/day treatment groups, respectively. On study week 6, the eosinophils counts were increased ($p < 0.05$) from 0.1 th/ul in control to 0.2 and 0.3 th/ul in males of 5 and 25 mg/kg/day treatment groups, respectively. No effect on eosinophils was seen among females at this time. On study week 13, the eosinophils counts were increased further to 0.4 and 0.5 th/ul in males. At this time, an increase from 0.1 th/ul (control group value) to 1.3 and 1.0 th/ul was seen in females included in 5 and 25 mg/kg/day treatment group. The coagulation parameters of the animals were not affected during the study.

e. **Blood Chemistry/Urinalysis Changes:** On week 13, a statistical significant ($p < 0.05$) increase in serum aspartate aminotransferase (AST) was seen in animals included in 25 mg/kg/day treatment group (60, 89, 88, 85 u/l in males and 35, 69, 44 and 52 u/l in females in control and 3 treatment groups). Serum triglycerides increase by 2, 1.95 and 2 times in males and, 1.8, 1.7 and 2.04 times in females of 3 treatment group animals. No changes of clinical importance were seen in the urinalysis of animals included in ALX-0600 treatment groups.

f. **Toxicokinetics:** On day 1, a dose proportional peak plasma concentration was seen in about 2 to 3 hr after first or second daily dose of subcutaneously administered compound in both male and female animals. On study days 30 and 90, the peak plasma concentrations were dose related but non-dose proportional. The peak plasma concentrations were seen after 1 to 2 hr of the administration of the compound as shown in the following table. The half-lives of the compound was increased with the increasing dose as on day 1 the half lives were 0.83, 0.87 and 1.7 hr in males and, 0.82, 0.93 and 3.29 hr in females of the 3 treatment groups of the study. The half-lives of the compound on study day 30 and 90 were similar, i.e., 0.82 to 1.48 hr in

male and, 0.825 to 2.31 hr in female animals. Thus the compound did not accumulate after its 90 days prolonged administration. The AUC values of the compound in 3 treatment groups animals were greater on day 1 than either on day 30 and 90. The AUC values on day 30 were smaller than on day 1 and were 0.45, 0.5 and 0.39 times of day 1 concentration in males and, by 0.42, 0.43 and 0.25 times lesser than on day 1 in females included in 3 treatment groups than on day 30. The AUC values were decreased further and on day 90, these were 0.43, 0.28 and 0.19 times of the day 1 concentrations in males and, 0.4, 0.32 and 0.24 times of the day 1 concentrations in females. The toxicokinetic parameters of the compound on study days are shown in the following tables (sponsor's tables in vol. 10 of 3, pp. 184 and 185).

Group	Dose Level (mg/kg/day)	Gender		C _{max} (ng/mL)	T _{max} (Hours)	AUC ₀₋₈ (ng·hr/mL)	AUC ₀₋₁ (ng·hr/mL)	AUC _{0-∞} (ng·hr/mL)	t _{1/2} (Hours)
<u>Day 1</u>									
2	1	M	Mean	1055	2.33	2239	4462	4466	0.831
			SD	198	3.27	395	879	881	0.055
			N	6	6	6	6	6	6
		F	Mean	1019	3.50	2389	4683	4687	0.825
			SD	98	4.07	345	768	768	0.076
			N	6	6	6	6	6	6
3	5	M	Mean	3845	3.83	11785	22904	22945	0.873
			SD	402	4.40	1079	966	985	0.112
			N	6	6	6	6	6	6
		F	Mean	3406	2.67	11377	21590	21640	0.929
			SD	1055	3.14	2714	4330	4337	0.117
			N	6	6	6	6	6	6
4	25	M	Mean	13533	5.50	55648	105057	109077	1.71
			SD	1130	4.59	5073	11618	15369	0.91
			N	6	6	6	6	6	6
		F	Mean	10973	2.17	53630	99424	112937	3.29
			SD	2234	0.98	7794	14174	18157	1.12
			N	6	6	6	6	6	6

Group	Dose Level (mg/kg/day)	Gender		C_{max} (ng/mL)	T_{max} (Hours)	AUC_{0-4} (ng·hr/mL)	$t_{1/2}$ (Hours)
<u>Day 30</u>							
2	1	M	Mean	777	0.833	2045	0.819
			SD	165	0.258	343	0.060
			N	6	6	6	6
		F	Mean	724	1.00	1980	0.825
			SD	132	0	388	0.070
			N	6	6	6	6
3	5	M	Mean	2886	1.83	11191	1.21
			SD	870	0.41	2907	0.43
			N	6	6	6	6
		F	Mean	2385	1.83	9350	1.16
			SD	692	0.41	1986	0.27
			N	6	6	6	6
4	25	M	Mean	10198	2.00	42006	1.48
			SD	2006	0	10854	0.53
			N	6	6	6	6
		F	Mean	5876	1.83	28018	2.31
			SD	1874	0.41	8534	1.21
			N	6	6	6	6
<u>Day 90</u>							
2	1	M	Mean	759	0.667	1951	0.890
			SD	169	0.258	381	0.205
			N	6	6	6	6
		F	Mean	707	0.667	1877	0.852
			SD	169	0.258	457	0.060
			N	6	6	6	6
3	5	M	Mean	1469	2.00	6297	1.98
			SD	658	0	2132	0.76
			N	4	4	4	4
		F	Mean	2323	1.25	7016	1.11
			SD	862	0.61	2142	0.38
			N	6	6	6	6
4	25	M	Mean	6228	0.917	20680	1.40
			SD	1100	0.204	4762	0.37
			N	6	6	6	6
		F	Mean	5877	1.33	26900	2.85
			SD	1695	0.52	6769	2.35
			N	6	6	6	6

Note AUC_{0-4} is equivalent to AUC_{0-1} .

g. **Detection of Antibodies in Plasma:** On study day 30, antibodies to ALX-0600 were detected in 1/sex out of 6/sex animals of 25 mg/kg/day treatment group. On month 3, the incidences were increased to 5/sex among 25 mg/kg/day treatment group animals and the antibodies were also seen 1 of 6 males of 5 mg/kg/day treatment group. In recovery period groups of animals, 2 out of 2 males of 25 mg/kg/day treatment group were positive for antibodies. The presence of antibodies during the study indicated that the responses of the compound would be variable during its use.

h. **Physical Examination and Ophthalmic Test:** Only a slight decrease (8.2%) of systolic and mean blood pressure (6.2%) was observed in males on study day 86 but not in females. During the study, EKG tracings of the animals were obtained but no table/data was submitted. Sponsor stated that no changes in the EKG pattern of the animals were observed. Sponsor claims could not be confirmed.

i. **Organ Weight Changes:** The mean absolute weight of thymus was decreased in males and females in a treatment/dose related manner. The absolute weight of thymus was 6.05 and 4.75 g in male and female animals. The organ weight was reduced in treated animals and was 48, 68 and 47% of the thymus weight in control in males and, 88, 83 and 67% of the thymus weight in control females included in 1, 5 and 25 mg/kg/day treatment groups. The organ to brain weight ratio was decreased from 0.086 in control to 0.04 in males and, from 0.074 to 0.052 in females included in 25 mg/kg/day treatment group. The absolute weight liver and gall bladder were increased in animals included in 1, 5 and 25 mg/kg/day treatment group animals as shown in the following table. A treatment related marked and maximum increase in the absolute weight of jejunum followed by increase in duodenum, ileum and colon weight was seen in males and females of animals included in 1, 5 and 25 mg/kg/day treatment groups. A dose related decrease 38.8, 41.2 and 43.5% in absolute weight of seminal vesicle was observed in animals of 1, 5 and 25 mg/kg/day treatment groups. The weight of testes was also decreased by 37.5, 65.5 and 65.5% in these males. The organ weight of seminal vesicle and testes in control males were 0.85 and 3.2 g, respectively. The organ weight changes in other tissues like liver and cecum were small as shown in the table below.

Organ Weights and Percent Organ weight Changes in animals of 3-Month Toxicity Study in
Cynomolgus Monkeys (6 Animals/sex/Group)

Organs		ALX-0600 Dose Used (mg/kg/day)			
		Organ Wt 0 (Control)	Treated Grs. Percent Organ Wt Changes		
			1	5	25
Stomach	Male	15.2	38.5	91.1	21.0
	Female	16.0	50.9	41.1	59.0
Jejunum	Male	33.16	271	361	303
	Female	37.43	242	261	337
Duodenum	Male	5.75	116	153.9	83.5
	Female	5.46	120.7	126.4	187.2
Ileum	Male	3.8	98.7	126.6	92.7
	Female	4.99	62.1	44.0	75.9
Colon	Male	30.5	42.6	68.5	35.1
	Female	34.6	23.8	24.0	43.3
Cecum	Male	5.5	22.5	40.2	23.2
	Female	5.0	46.5	38.0	23.3
Gall Bladder /Liver	Male	51	63	66	62
	Female	56	59	65	68

(-) = %Decrease in weight

j. **Gross Pathology Findings:** Treatment related thickening at the site of injection was seen 1, 1 and 4 males and, 0, 2 and 4 females included in 0, 1, 5 and 25 mg/kg/day treatment groups. Raised areas in the colon was seen in 1 and 2 females of control and 25 mg/kg/day treatment groups and it was attributed to parasitic granulomatous.

k. **Histopathological Changes:** The treatment related mucosal hyperplasia/hypertrophy of glandular stomach, duodenum, ileum and jejunum were seen in animals included in both male and female animals included in 1, 5 and 25 mg/kg/day treatment groups. The incidences, intensity and distribution of hyperplasia in the study animals are shown in the following table (data extracted from sponsor table vol 10.3, pp. 173). Average lengths of jejunum in both males and females included in 1, 5 and 25 mg/kg/day treatment groups were increased significantly ($p < 0.5$). None of the control group animals showed these changes in intestines. The changes seen in treated animals were almost completely reversed during recovery period. Increased number of incidences of granulomatous inflammation associated with hemorrhage edema, necrosis, degeneration and necrosis were reported at the site of injection in 2 and 4 animals/sex included in 5 and 25 mg/kg/day ALX-0600 treatment groups. Fibrinoid necrosis at

injection site was reported in 1 and 4 males and 0 and 4 females included in 5 and 25 mg/kg/day treatment group. Biliary duct hyperplasia/hypertrophy was observed in 2, 3 and 3 males and, 3, 2 and 4 females monkeys out of 6/sex in 1, 5 and 25 mg/kg/day treatment groups. This was associated with enlarged and some degree of chronic inflammation, enlarged bile ducts (containing basophilic, hypertrophic/hyperplastic epithelium of bile ducts. None of the control animals showed any change during the study and the lesion was completely resolved during the recovery period. Incidences of chronic inflammation of liver was seen in 2 and 1 males and, 2 and 1 females out of 6/sex animals in 5 and 25 mg/kg/day treatment groups. Minimal to moderately severe gastric mucosal hypertrophy/hyperplasia in cardiac and pyloric region was reported in animals included in ALX-0600 treatment groups. The lesions were more severe in females than in males. In few of the animals, the lesion was seen in fundic region. Minimal to slight hypertrophy/hyperplasia in the gall bladder mucosa associated with chronic inflammation was observed in animals belonging to treatment groups and none of the control group animals had the lesion. During recovery period, hypertrophy/hyperplasia of small and large intestines and, gall bladder were resolved completely. Pancreatic ductal epithelial hyperplasia with some degree of inflammation of pancreas was present in 3, 3 and 4 males and, 4, 4, and 4 females of 1, 5 and 25 mg/kg/day treatment groups, respectively. This was characterized by sponsor as an increase in both number and size of mucosal epithelial cells. Chronic inflammation of some degree was reported in pancreatic duct of animals of treatment groups. During recovery period, the hyperplasia/hypertrophy were resolved completely, as there were no incidences of hypertrophy/hyperplasia or other lesions in study treatment groups animals. There were no incidences of hyperplasia or hypertrophy in control group males or females.

Table
Incidence, Severity, and Distribution of Hypertrophy/Hyperplasia in the Bile Duct, Gallbladder, Stomach, Pancreatic Duct, and Small and Large Intestines

Sex	Male				Female			
	1	2	3	4	1	2	3	4
Group	1	2	3	4	1	2	3	4
# Examined	4	4	4	4	4	4	4	4
Bile Duct								
# Incidences	0	2	3	3	0	3	2	4
Mean Severity Grade	0.0	0.8	1.0	1.0	0.0	0.8	0.8	2.5
Gall Bladder								
# Incidences	0	1	1	1	0	0	1	3

Mean Severity Grade	0.0	0.5	0.3	0.5	0.0	0.0	0.5	1.5
Stomach								
# Incidences	0	1	4	4	0	4	3	4
Mean Severity Grade	0.0	1.5	2.8	1.5	0.0	2.3	2.0	3.0
Pancreas								
# Incidences	0	3	3	4	0	4	4	4
Mean Severity Grade	0.0	1.0	1.3	2.3	0.0	2.3	2.0	1.5
Duodenum m								
# Incidences	0	4	4	4	0	4	4	4
Mean Severity Grade	0.0	2.8	3.0	2.5	0.0	2.8	2.8	3.0
Jejunum								
# Incidences	0	4	4	4	0	4	4	4
Mean Severity Grade	0.0	2.3	2.5	2.5	0.0	2.3	2.5	3.3
Ileum								
# Incidences	0	4	4	4	0	4	4	4
Mean Severity Grade	0.0	1.8	1.5	2.0	0.0	1.8	2.0	2.3
Cecum								
# Incidences	0	4	4	4	0	4	4	4
Mean Severity Grade	0.0	2.0	2.0	1.5	0.0	1.0	1.3	2.0
Colon								
# incidences	0	4	4	4	0	3	4	4
Mean Severity Grade	0.0	2.3	2.3	2.0	0.0	1.0	1.5	2.3
Rectum								
# Incidences	0	4	3	4	0	1	4	4
Mean Severity Grade	0.0	1.5	1.8	1.8	0.0	0.5	1.0	1.3

In summary, subcutaneously administered ALX-0600 from the doses of 1 to 25 mg/kg/day produced treatment related desired mucosal hyperplasia/hypertrophy of duodenum, ileum and jejunum and large intestines in male and female monkeys. Average maximum increase was seen in length of jejunum in both males and females which was followed by duodenum and ileum and other tissues. The treatment produced an increase in the numerous villi with increased villus and crypt heights which were completely reversed during recovery period. A dose related increase incidences of granulomatous inflammation associated with hemorrhage edema, necrosis, fibrosis, degeneration and necrosis at the site of injection were seen. Biliary duct hyperplasia/hypertrophy, gastric and pancreatic ductal hyperplasia was observed in males and females monkeys of the treated group. The biliary ductal hyperplasia had enlarged ducts and some degree of chronic inflammation (containing basophilic and hypertrophic hyperplastic epithelium of bile ducts). During recovery period, hypertrophy/hyperplasia of small and large intestines and, gall bladder were resolved completely. The pancreatic mucosal hyperplasia was characterized by sponsor by both increased in number and size of epithelial cells. Chronic inflammation of some degree was reported in

pancreatic duct of animals of treatment groups. During recovery period, the hyperplasia/hypertrophy and other lesions were resolved completely. The antibodies to ALX-0600 were detected in animals included in the treatment and recovery groups. A dose of 1 mg/kg/day was considered as a 'maximal tolerable dose' and liver, biliary duct, pancreatic duct, gallbladder were identified as the target organs of toxicity in this study.

The following review of the amendment to toxicokinetic report of the above 13-week study (7203-100) in Cynomolgus monkeys is incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

Amendment to Toxicokinetic Report of a 13-Week Subcutaneous Toxicity and Toxicokinetic Study (7203-100) in Cynomolgus Monkeys with 1-Month Recovery Period

The sponsor submitted an amendment to the TK report of the above study under Amendment 024 dated February 4, 2003 following recalculation of the TK parameters using plasma concentration data obtained after each of two daily doses on Day 1. In this amendment the sponsor submitted pages 468, 468a to 515 for incorporation into the final report of the above study submitted under Amendment 018 dated April 9, 2002.

The parameters were recalculated by non-compartmental analysis by the method of Gibaldi and Perrier (1982) to estimate TK data. Measurable amounts of the compound were seen several plasma samples at Day 1 (up to 16 hours), Day 30 (up to 8 hours) and Day 90 (up to 8 hours) as shown in the following tables (from Amendment 024, page 22, 24, 26 and 28 of sponsor's submission).

(b) (4) 7203-100
AMENDED

(b) (4) 7203-100
Amended

Table 1-1
Plasma Concentrations (ng/mL) of ALX-0600 in Monkeys: Day 1

13-Week Subcutaneous Toxicity and Toxicokinetic Study with ALX-0600 in Cynomolgus Monkeys Followed by a 30-Day Recovery Period

Group	Dose Level (mg/kg/day)	Gender	Animal Number	Time After First Dose of the Day (Hours)								
				0	0.25	0.5	1	2	4	8		
1	0	M	152132	(b) (4)								
			152133	(b) (4)								
			152134	(b) (4)								
			152135	(b) (4)								
			152136	(b) (4)								
			152137	(b) (4)								
		F	152156	(b) (4)								
			152157	(b) (4)								
			152158	(b) (4)								
			152159	(b) (4)								
			152160	(b) (4)								
			152161	(b) (4)								
2	1	M	152138	(b) (4)								
			152139	(b) (4)								
			152140	(b) (4)								
			152141	(b) (4)								
			152142	(b) (4)								
			152143	(b) (4)								
			Mean	0.200	545	900	1024	488	86.6	2.73		
			SD	0.310	100	174	220	103	44.9	1.47		
			RSD (%)	155	18	19	21	21	52	54		
1	0	F	152162	(b) (4)								
			152163	(b) (4)								
			152164	(b) (4)								
			152165	(b) (4)								
			152166	(b) (4)								
			152167	(b) (4)								
					Mean	0.133	527	895	963	541	127	3.38
					SD	0.327	103	80	156	106	52	0.71
					RSD (%)	245	19	9	16	20	41	21
		3	5	M	152144	(b) (4)						
					152145	(b) (4)						
					152146	(b) (4)						
152147	(b) (4)											
152148	(b) (4)											
152149	(b) (4)											
					Mean	0.0833	1154	2028	3251	2756	1362	38.9
					SD	0.2041	286	560	647	114	272	30.9
					RSD (%)	245	25	28	20	4	20	79
F	152168			(b) (4)								
	152169			(b) (4)								
	152170			(b) (4)								
	152171	(b) (4)										
	152172	(b) (4)										
	152173	(b) (4)										
			Mean	0.250	1181	2122	3030	2959	1168	35.3		
			SD	0.418	662	1015	1372	957	187	16.6		
			RSD (%)	167	56	48	45	32	16	47		

Note: Values below the limit of quantitation are reported as zero.

(b) (4) 7203-100
AMENDED

(b) (4) 203-100
Amended

Table 1-1 (Continued)
Plasma Concentrations (ng/mL) of ALX-0600 in Monkeys: Day 1
13-Week Subcutaneous Toxicity and Toxicokinetic Study with ALX-0600 in
Cynomolgus Monkeys Followed by a 30-Day Recovery Period

Group	Dose Level (mg/kg/day)	Gender	Animal Number	Time After First Dose of the Day (Hours)						
				8 ^a	8.25	8.5	9	10	12	16
1	0	M	I52132	(b) (4)						
			I52133	(b) (4)						
			I52134	(b) (4)						
			I52135	(b) (4)						
			I52136	(b) (4)						
			I52137	(b) (4)						
		F	I52156	(b) (4)						
			I52157	(b) (4)						
			I52158	(b) (4)						
			I52159	(b) (4)						
			I52160	(b) (4)						
2	1	M	I52138	(b) (4)						
			I52139	(b) (4)						
			I52140	(b) (4)						
			I52141	(b) (4)						
			I52142	(b) (4)						
			I52143	(b) (4)						
		F	I52162	(b) (4)						
			I52163	(b) (4)						
			I52164	(b) (4)						
			I52165	(b) (4)						
			I52166	(b) (4)						
3	5	M	I52144	(b) (4)						
			I52145	(b) (4)						
			I52146	(b) (4)						
			I52147	(b) (4)						
			I52148	(b) (4)						
			I52149	(b) (4)						
		F	I52168	(b) (4)						
			I52169	(b) (4)						
			I52170	(b) (4)						
			I52171	(b) (4)						
			I52172	(b) (4)						
			Mean	38.9	835	2074	2958	3223	1004	30.9
			SD	30.9	260	586	866	516	307	17.5
			RSD (%)	79	31	28	29	16	31	57
			Mean	35.3	872	2179	2648	2705	1020	35.4
			SD	16.6	371	991	482	586	250	18.4
			RSD (%)	47	43	45	18	22	24	52

Notes: Test article was administered twice daily and the second dose of the day was given approximately 8 hours after the first dose. Values below the limit of quantitation are reported as zero.
a 8 hour sample was collected before the administration of the second dose.

(b) (4) 7203-100
AMENDED

(b) (4) 7203-100
Amended

Table 1-2
Plasma Concentrations (ng/mL) of ALX-0600 in Monkeys: Day 30
13-Week Subcutaneous Toxicity and Toxicokinetic Study with ALX-0600 in
Cynomolgus Monkeys Followed by a 30-Day Recovery Period

Group	Dose Level (mg/kg/day)	Gender	Animal Number	Time After First Dose of the Day (Hours)															
				0	0.25	0.5	1	2	4	8									
1	0	M	I52132	(b) (4)															
			I52133																
			I52134																
			I52135																
			I52136																
			I52137																
			I52156																
		F	I52157																
			I52158																
			I52159																
			I52160																
			I52161																
			Mean									0.850	379	650	769	514	121	3.43	
			SD									0.339	120	224	159	79	43	1.48	
RSD (%)	40	32	34	21	15	36	43												
2	1	M	I52138	(b) (4)															
			I52139																
			I52140																
			I52141																
			I52142																
			I52143																
			Mean									0.733	384	641	724	490	119	3.30	
		SD	0.186									95	131	132	104	41	1.00		
		RSD (%)	25									25	20	18	21	34	30		
		F	I52162									(b) (4)							
			I52163																
			I52164																
			I52165																
			I52166																
I52167																			
Mean	3.45		957	1960	2189	2825	1377	102											
SD	0.88	251	741	1173	821	371	95												
RSD (%)	25	26	38	54	29	27	92												
3	5	M	I52144	(b) (4)															
			I52145																
			I52146																
			I52147																
			I52148																
			I52149																
			Mean									3.20	919	1506	2032	2303	1146	70.8	
		SD	0.98									421	745	808	547	271	38.1		
		RSD (%)	31									46	49	40	24	24	54		
		F	I52168									(b) (4)							
			I52169																
			I52170																
			I52171																
			I52172																
I52173																			
Mean	3.20		919	1506	2032	2303	1146	70.8											
SD	0.98	421	745	808	547	271	38.1												
RSD (%)	31	46	49	40	24	24	54												

Note
a Values below the limit of quantitation are reported as zero.
Values treated as anomalous and excluded from mean calculations.

(b) (4) 203-100
AMENDED

(b) (4) 203-100
Amended

Table 1-3
Plasma Concentrations (ng/mL) of ALX-0600 in Monkeys: Day 90
13-Week Subcutaneous Toxicity and Toxicokinetic Study with ALX-0600 in
Cynomolgus Monkeys Followed by a 30-Day Recovery Period

Group	Dose Level (mg/kg/day)	Gender	Animal Number	Time After First Dose of the Day (Hours)						
				0	0.25	0.5	1	2	4	8
1	0	M	I52132	(b) (4)						
			I52133							
			I52134							
			I52135							
			I52136							
			I52137							
1	0	F	I52156	(b) (4)						
			I52157							
			I52158							
			I52159							
			I52160							
			I52161							
2	1	M	I52138	(b) (4)						
			I52139							
			I52140							
			I52141							
			I52142							
			I52143							
			Mean	0.650	324	727	689	455	130	4.60
			SD	0.351	116	148	195	109	33	3.48
			RSD (%)	54	36	20	28	24	26	76
2	1	F	I52162	(b) (4)						
			I52163							
			I52164							
			I52165							
			I52166							
			I52167							
			Mean	0.767	377	663	637	446	127	3.83
			SD	0.622	99	186	136	115	60	2.02
			RSD (%)	81	26	28	21	26	47	53
3	5	M	I52144*	(b) (4)						
			I52145*							
			I52146							
			I52147							
			I52148							
			I52149							
			Mean	13.6	461	1018	1064	1465	821	166
			SD	4.6	332	834	429	658	331	127
			RSD (%)	34	72	82	40	45	40	76
3	5	F	I52168	(b) (4)						
			I52169							
			I52170							
			I52171							
			I52172							
			I52173							
			Mean	7.88	980	1732	2222	1768	568	48.4
			SD	4.45	436	708	944	809	325	62.6
			RSD (%)	56	44	41	42	46	57	129

Note: Values below the limit of quantitation are reported as zero.
a All values excluded from the mean calculation since sample identity was in doubt for 2 time points.
b 2 tubes (instead of 1) were received. Both were labeled as 1 hour samples. Because the sample identity is in doubt no data was reported for this time point.

These values were relatively low in comparison to levels of the drug in the treated samples and were comparable to some treated samples at 0 hour time point. The sponsor contended that these values could represent endogenous levels of the native hormone. The results indicated that the sponsor's method may not specific enough to detect the

drug and the potential contamination with the test article cannot be ruled out also. The sponsor should conduct further investigation regarding this issue. Concentrations of the drug in several control plasma samples appear to be small when compared to the concentration of the drug in treated samples. Low amounts of the drug found in the control samples do not appear to affect the outcome of the study. However, the sponsor should further investigate this matter to find out the source of contamination or might consider repeating the study. The re-calculated TK data for $t_{1/2}$ of ALX-0600 does not appear to affect the existing data in a significant way (the $t_{1/2}$ of 0.819 to 3.29 hr was re-calculated to be 0.795 to 3.29 hr). It is to be mentioned here that in a 1-year s.c. toxicity study (1368-1001, 6-month interim report) in cynomolgus monkeys at 1, 5 and 25 mg/kg/day, ALX-0600 was also detected in control animals on Day 1 (0.6 to 1.6 ng/ml) and week 13 (0.8 to 2.0 ng/ml) as discussed previously.

1-Year Subcutaneous Toxicity Study in Cynomolgus Monkeys with a 13-Week Recovery Period: 6-Month Interim Report (1368-100) and the Final Report (1368-100)

The reviews of the above interim and final study reports are incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006. This to be noted here that the study number (1368-1001) in the following reviews was incorrect and is replaced by study number 1368-100.

1-Year Subcutaneous Toxicity Study in Cynomolgus Monkeys with a 13-Week Recovery Period: 6-Month Interim Report

The sponsor previously submitted an interim draft report of this study under Amendment 033 dated September 3, 2003. The sponsor submitted additional TK data of this study under Amendment 036 dated September 19, 2003. The following review covers these submissions.

Study No.: 1368-1001

Volume and Page No.: Amendment 052, Vol. 1, page 1

Testing Laboratory: (b)(4)

Dates of Study Initiation: July 26, 2002

Date of Study Completion: January 15, 2004

GLP & QAU Requirements: A statement of compliance with GLP regulations and QAU statement were included.

Species and Strain: Cynomolgus monkeys (*Macaca fascicularis*) approximately 3.2 to 5.4 years (males) and 2.5 to 4.8 (female) with mean body weight of 2.5 to 5.1 kg (males) and 2.2 to 3.7 kg (females) were used in this study.

Batch (Purity): 0850201 (98.5%) & 0850202 (98.2%)

Methods: Sixty four monkeys (32/sex) were randomly divided into 4 main study (6/sex/group) groups as shown in the following table and were treated with either vehicle (phosphate buffer with L-histidine and mannitol) or ALX-0600 subcutaneously (0.625 ml/kg) at 1.0, 5.0 and 25.0 mg/kg/day (0.5, 2.5 and 12.5 mg/kg/day bid, 8 hours apart).

Group	Number of males/females	Dose (mg/kg/day)	Dose (mg/kg)	Dose Volume (ml/kg)	Conc. Of Dose Solution
1	10/10	0.0	0.0	0.625	0.0
2	6/6	1.0	0.5	0.625	0.8
3	6/6	5.0	2.5	0.625	4.0
4	10/10	25.0	12.5	0.625	20.0

Twenty-three animals (4/sex in group 1, 2/sex in groups 2 and 3 and, 3 males and 4 females in group 4) were euthanized on Week 27 (after 6 months of treatment). Thirty two animals (4/sex/group) were scheduled to be sacrificed during Week 53 (i.e., after 1 year of treatment). An additional 8 animals (2/sex each in control and high dose treatment groups) will continue on study without further treatment for a recovery period of 13 weeks and then will be euthanized. Animals were observed twice daily for clinical signs and mortality during treatment. Body weights were recorded prior to treatment and weekly thereafter. Food consumption was recorded on a daily basis.

Electrocardiography (ECG) was obtained in Week 0 (pre-study day), 13, 26 and 52 and at the time of termination in recovery group animals. Ophthalmic examination was conducted once before treatment (pre-study Day 0), and at Week 13, 26 and 52 and at the time of termination in recovery group animals. Hematological and serum chemistry parameters and urinalysis were determined on Day 1 (prior to treatment), Week 13, 26, 39 and 52 and antibody analyses were performed at pre-test, and in Weeks 13, 26, 39, and for the recovery animals during the week prior to necropsy. Blood samples were/will be collected for TK analysis at 0.25, 0.5, 1, 2, 4, and 8 hr post-treatment on Day 1 and on Week 13, 26, 39 and 52. Following organs of each of the animals were/will be weighed: adrenals, brain, heart, kidneys, liver, lungs, ovary, pituitary, prostate, spleen, cecum, colon, small intestines, testes with epididymides, thymus, and thyroid with parathyroid. The length of small and large intestine were/will be measured from the main study group animals. Following tissues will be examined for histopathological examinations from all animals: aorta, heart, salivary gland (mandibular), tongue, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum), pancreas, liver, gall bladder, trachea, lung, bone marrow, thymus, spleen, lymph nodes, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicle, ovaries, uterus, cervix, vagina, adrenals, pituitary, thyroid with parathyroid, skin/mammary gland, bone, skeletal muscle, eyes with optic nerve, sciatic nerve, brain, spinal cord, gross lesions and injection site.

Results:

Observed Effects: Test article-related clinical signs included injection site changes (thickening, ulcers, and/or masses), distended abdomen, and/or swollen prepuce/scrotum. Distended abdomen was seen in 0, 3, 5 and 10 males and in 1, 4, 6 and 8 females at 0, 1, 5 and 25 mg/kg/day, respectively and was considered to be due to the increased size and weight of stomach. Swollen scrotum and/or prepuce were noted in 1 of 10, 5 of 5, 6 of 6 and 10 of 10 males at control, low, mid and high dose, respectively. Thickening, ulcer and masses were noted at the site of injection of 10 of 10 males and 6 of 10 females. One control and 3 females of mid-dose treatment groups exhibited red mucus and/or watery

diarrhea on Day 157 to 165 and this was not considered treatment-related effect and was considered to be due to bacterial (campylobacter) infection.

Mortality: One female of high dose treatment group was sacrificed in moribund condition on Day 150 and this animal had preputial swelling.

Body Weight/Food Consumption: There was a dose related increase in the body weights of animals at all dose levels. Final body weights of males in Week 12 were 4.0, 4.0, 4.2 and 4.3 kg and 3.0, 2.9, 3.0 and 3.0 kg in females at 0, 1, 5 and 25 mg/kg/day, respectively. On Week 26, the body weights were 4.3, 4.4, 4.5 and 4.6 kg in males and, 3.2, 3.1, 3.1 and 3.1 kg in females at 0, 1, 5 and 25 mg/kg/day, respectively. Food consumption data was not provided by the sponsor.

Hematology: On week 27, there was a consistent increase in the number of absolute eosinophils in males (113, 121, 100 and 284% of control at 0, 1, 5 and 25 mg/kg/day, respectively). There were no changes in the mean fibrinogen concentration, mean prothrombin time and mean activated partial thromboplastin time.

Blood Chemistry/Urinalysis Changes: On Week 13 and 26, a decrease in albumin was noted (12 and 16% reduction, respectively, compared to pre-study mean) in the high dose animals. The total protein levels were increased significantly in Group 4 males. Mean globulins were increased in Group 4 males and females on Week 26. No changes of clinical importance were seen in the urinalysis of animals included in ALX-0600 treatment groups.

Toxicokinetics: Generally, peak plasma concentrations (C_{max}) were achieved within 0.8 to 2.2 hr post-treatment. In males, mean AUC_{0-8h} values were 2,143, 11,946 and 53,822 ng.hr/ml at 1, 5 and 25 mg/kg/day, respectively. In females, AUC_{0-8h} values were 2,639, 11,562, and 55,324 ng.hr/ml at 1, 5 and 25 mg/kg/day, respectively. The $t_{1/2}$ varied from 0.912 to 3.16 hr. There was no apparent gender difference in the C_{max} and AUC_{0-8h} values. Overall, increases in C_{max} were not dose-proportional and increases in AUC_{0-8h} were approximately dose-proportional. There seems to be no accumulation of the drug over time. Mean TK parameters are shown in the following table (from Vol. 3.3, page 186 of sponsor's submission).

Mean Toxicokinetic Parameters for ALX-0600 in Monkey Plasma

Group	Dose Level (mg/kg/day)	Gender		C _{max} (ng/mL)	t _{max} (Hours)	AUC _{0-t} (ng·hr/mL)	t _{1/2} (Hours)
<u>Day 1</u>							
2	1	M	Mean	696	1.33	2143	0.912
			SD	143	0.52	359	0.110
			N	6	6	6	6
		F	Mean	919	0.833	2639	1.01
			SD	282	0.258	708	0.13
			N	6	6	6	6
3	5	M	Mean	2921	1.83	11946	1.53
			SD	651	0.41	2211	0.36
			N	6	6	6	6
		F	Mean	2870	1.83	11562	1.16
			SD	918	1.17	1722	0.39
			N	6	6	6	5
4	25	M	Mean	11190	2.20	53822	2.87
			SD	4109	1.03	12532	1.36
			N	10	10	10	8
		F	Mean	11024	2.20	55324	3.16
			SD	1859	0.63	4171	1.02
			N	10	10	10	9

Contamination of Control Samples: On Day 1 and Week 13, some plasma samples of control group animals were positive for ALX-0600. ALX-0600 was detected (0.6 to 1.6 ng/ml) in control group animals, on Day 1 as shown in the table below (from Vol. 3.3, page 199 of sponsor's submission). The presence of similar amounts of the compound was also reported in Week 13 samples (0.8 to 2.0 ng/ml) as shown in sponsor's Table 2 (Vol. 3.3, page 201). Test article was detected in control samples in toxicity studies in Cynomolgus monkeys. The sponsor contended that these levels could be due to endogenous GLP-2 or a related peptide. Sponsor's explanation may be appropriate; however, the following issues may also be considered 1) this was not found in any other toxicity study in mice. It is possible that monkey GLP-2 may be more analogous to test article compared to that of mice, 2) the assay method (ELISA) may not be specific to detect the test article and 3) the potential contamination with the test article may not be ruled out. The sponsor may be asked to further address this issue. The sponsor may also be asked to submit sequence homology of this peptide to monkey GLP-2 and mice GLP-2. This information may be useful to explain this issue.

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Table 1
Plasma concentrations (ng/mL) of ALX-0600 in monkeys: Day 1

Group	Dose Level (mg/kg/day)	Gender	Animal Number	Time After Dosing (Hours)											
				0	0.25	0.5	1	2	4	8					
1	0	M	F18932M	(b) (4)											
			F18933M												
			F18940M												
			F18941M												
			F18946M												
			F19405M												
			F19924M												
			F20204M												
			F20919M												
			F20958M												
		F	F18180F												
			F189118F												
			F18965F												
			F18983F												
			F18987F												
			F18997F												
			F202104F												
			F202117F												
			F20280F												
			F20598F												
2	1	M	F18939M	(b) (4)											
			F19939M												
			F20211M												
			F20222M												
			F20233M												
		F20912M													
		Mean	0.683							384	529	677	562	162	6.67
		SD	0.714							143	130	146	103	89	3.85
		RSD (%)	104							37	25	22	18	55	58
		F	F18162F							(b) (4)					
F18666F															
F18993F															
F18995F															
F20279F															
F20568F															
Mean	1.53	396	696							853	682	198	11.7		
SD	1.15	102	65							358	190	75	6.7		
RSD (%)	75	26	9							42	28	38	57		

Note: Results below the limit of quantitation (<0.5 ng/mL) are reported as zero.

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Table 2
Plasma concentrations (ng/mL) of ALX-0600 in monkeys: Week 13

Group	Dose Level (mg/kg/day)	Gender	Animal Number	Time After Dosing (Hours)																	
				0	0.25	0.5	1	2	4	8											
1	0	M	F18932M	(b) (4)																	
			F18933M																		
			F18940M																		
			F18941M																		
			F18946M																		
			F19405M																		
			F19924M																		
			F20204M																		
			F20919M																		
			F20958M																		
		F	F18180F																		
			F189118F																		
			F18965F																		
			F18983F																		
			F18987F																		
			F18997F																		
			F202104F																		
			F202117F																		
			F20280F																		
			F20598F																		
2	1	M	F18939M	(b) (4)																	
			F19939M																		
			F20211M																		
			F20222M																		
			F20233M																		
			F20912M																		
		Mean	2.67								262	475	595	476	185	24.4					
		SD	1.75								129	213	211	104	70	13.6					
		RSD (%)	66								49	45	35	22	38	56					
		F	F18162F								(b) (4)										
F18666F																					
F18993F																					
F18995F																					
F20279F																					
F20568F																					
Mean	2.87	337	553															640	448	169	14.1
SD	1.58	126	247															157	60	44	9.3
RSD (%)	55	37	45															25	13	26	66

Note: Results below the limit of quantitation (<0.5 ng/mL) are reported as zero.

Plasma Antibody Analysis: Sponsor did not provide report on the presence of ALX-0600 antibody analysis and stated the report will be submitted on the completion of the study (final report).

Ophthalmoscopy: There were no treatment-related changes.

Organ Weight: Organ weight changes were observed for the following organs: thymus (male: 103, 77, and 44% of control, control = 3.49 g; female: 84, 92 and 61% of control, control = 2.698 g, at 0, 1, 5 and 25 mg/kg/day, respectively), spleen (males: 119-235% of control, control = 4.510 g), kidneys (108-131% of control, control = 13-17g), lungs (male: 11-114% of control, control = 22.27 g), and liver (male: 110-150%, control = 76 g). In addition, a treatment-related increase in the length of small and large intestines of the both males and females was also observed.

Gross Pathology: Treatment-related gross pathological changes included increased thickness of large and small intestine, stomach, axillary lymph node, injection site.

Histopathology: Treatment-related histopathological changes were observed in the gastrointestinal tract (mucosal hyperplasia, increased mucosal surface area and thickness at all doses in both sexes), pancreas (minimal to moderate hypertrophy/hyperplasia of pancreatic duct at all doses), liver and gall bladder (hypertrophy/hyperplasia of the biliary epithelium in medium to large bile ducts at all tested doses with an increase in severity at high dose; minimal to mild hypertrophy/hyperplasia of gallbladder mucosa and mononuclear cell infiltrate into the lamina propria of the gallbladder mucosa leading to formation of lymphoid follicles with an apparent increase in severity at high dose), injection site (inflammatory changes characterized by the presence of vasocentric, mixed cells of blood vessels and inflammation of subcutis, eosinophilic/granulomatous), lymphoid tissue (plasmacytosis with granulomatous infiltrate at high dose and lymphoid hyperplasia at all doses), adrenal gland (minimal to moderate hypertrophy of the cortical epithelium of the adrenal gland in Group 3 and 4 animals), and scrotal and perineal skin (mild scrotal or perineal edema in Group 3 animals). The following table summarizes the histopathological changes in different tissues (data extracted from Vol. 2, page 104-116 of sponsor's submission).

Tissue Finding	Grade	Males				Females			
		0	1	5	25	0	1	5	25
Dose (mg/kg/day)		0	1	5	25	0	1	5	25
Number of Animals (N)		4	2	2	3	4	2	2	4
Liver -Hypertrophy/hyperplasia, epithelium, bile ducts	1	0	2	2	2	0	1	1	1
	2	0	0	0	1	0	1	1	3
	3	0	0	0	1	-	-	-	-
	4	-	-	-	-	-	-	-	-
Stomach -Hyperplasia, mucosa, pylorus	1	0	0	0	1	-	-	-	-
	2	0	1	2	1	0	1	1	2
	3	0	1	0	2	0	0	0	1
	4	-	-	-	-	-	-	-	-
Duodenum	1	-	-	-	-	0	1	0	0

-Hyperplasia, mucosa	2	0	0	0	1	0	0	1	1
	3	0	2	1	3	0	1	1	3
	4	-	-	-	-	-	-	-	-
Ileum - Hyperplasia, mucosa	1	0	0	0	2	0	1	0	0
	2	0	1	2	2	0	1	2	2
	3	0	1	0	0	0	0	0	2
	4	-	-	-	-	-	-	-	-
Pancreas - Hypertrophy/hyperplasia, epithelium, bile ducts	1	0	0	0	1	0	0	0	1
	2	0	1	0	2	0	2	2	2
	3	0	0	2	1	-	-	-	-
	4	-	-	-	-	-	-	-	-
Gallbladder -Hyperplasia, mucosa	1	0	0	1	1	0	0	0	2
	2	0	1	0	1	0	0	1	2
	3	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-
Gallbladder -Infiltrate, mononuclear cell	1	1	0	1	2	2	2	0	0
	2	0	0	1	0	1	0	0	1
	3	0	0	0	1	0	0	0	3
	4	0	1	0	0	0	0	1	0
Lymphoid Node, Axillary -Hyperplasia, Lymphoid	1	1	1	0	1	0	0	1	1
	2	1	1	0	1	1	0	1	3
	3	0	0	2	0	-	-	-	-
	4	-	-	-	-	-	-	-	-
Lymphoid Node, Axillary -Plasmacytosis	1	-	-	-	-	1	0	0	1
	2	0	0	0	1	-	-	-	-
	3	0	0	0	1	0	0	0	2
	4	0	0	0	1	-	-	-	-
Adrenal Gland -Hypertrophy, cortical epithelium	1	0	0	0	2	0	0	1	1
	2	0	0	1	1	-	-	-	-
	3	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-
Skin, scrotal -Edema	1	0	0	1	3	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-
Injection Site -Inflammation, Vasocentric, Mixed Cell	1	1	1	0	0	2	0	0	0
	2	0	0	2	2	0	1	1	0
	3	0	0	0	1	0	0	1	2
	4	-	-	-	-	0	0	0	2
Injection Site -Inflammation, Subcutis, Eosinophilic/Granulomato us	1	0	1	0	0	0	1	0	0
	2	0	0	2	1	0	1	0	0
	3	0	0	0	1	0	0	2	1
	4	0	0	0	2	0	0	0	3
Injection Site -Hemorrhage	1	0	1	0	1	1	1	0	0
	2	4	1	1	0	2	0	2	2
	3	0	0	0	1	-	-	-	-
	4	-	-	-	-	-	-	-	-
Injection Site -Fibrosis	1	1	1	0	0	1	0	0	0
	2	0	1	0	1	0	0	1	0
	3	0	0	1	2	0	0	1	2
	4	0	0	0	1	0	0	0	1
Injection Site	1	-	-	-	-	-	-	-	-

-Edema	2	-	-	-	-	0	0	0	1
	3	0	0	1	0	0	1	1	1
	4	-	-	-	-	-	-	-	-
Injection Site -Ulceration, epidermis	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	0	0	0	1	-	-	-	-
	4	-	-	-	-	-	-	-	-

1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked; - : Not observed

In a 6-month (interim report of a 1-year study) s.c. toxicity study in Cynomolgus monkeys, animals were treated at 1, 5 and 25 mg/kg/day (0.5, 2.5 and 12.5 mg/kg bid, 8 hours apart). The target organ of toxicity appeared to be the gastrointestinal tract (mucosal hyperplasia, increased mucosal surface area and thickness), pancreas (minimal to moderate hypertrophy/hyperplasia of pancreatic duct), liver and gall bladder (hypertrophy/hyperplasia of the biliary epithelium in medium to large bile ducts; minimal to mild hypertrophy/hyperplasia of gallbladder mucosa and mononuclear cell infiltrate into the lamina propria of the gallbladder mucosa leading to formation of lymphoid follicles), injection site (inflammatory changes characterized by the presence of vasocentric, mixed cells of blood vessels and inflammation of subcutis, eosinophilic/granulomatous), lymphoid tissue (plasmacytosis with granulomatous infiltrate and lymphoid hyperplasia), adrenal gland (minimal to moderate hypertrophy of the cortical epithelium of the adrenal gland), and scrotal and perineal skin (mild scrotal or perineal edema). The NOAEL could not be determined as treatment-related effects were seen at all tested doses. It is to be mentioned here that on Day 1 and Week 13, some plasma samples of control group animals were positive for ALX-0600. Potential contamination of the test solution with the test article can not be ruled out in this case. This contamination issue should be further investigated.

1-Year Subcutaneous Chronic Toxicity Study in Cynomolgus Monkeys with a 13-Week Recovery Period

Study No.: 1368-1001.

Volume and Page: Amendment 069, page 1

Testing Laboratory: (b) (4)

Dates of Start and Completion of Study: July 26, 2002 and September 2, 2004.

GLP & QAU Requirements: A statement of compliance with GLP regulations and a QAU statement were included.

Species and Strain: Cynomolgus monkeys (*Macaca fascicularis*) approximately 3.2 to 5.4 years of age in males and 2.5 to 4.8 years of age in females with mean body weight of 2.5 to 5.1 kg in males and 2.2 to 3.7 kg in females were used in this study.

Batch (Purity): 0850001 (98.9%), 0850201 (98.5%), 0850202 (98.2%), 0850203 (97.4%), 0850204 (98.5%) and 0850205 (98.6%)

Methods: In this study, sixty four monkeys (32/sex) were randomly divided into 4 main study (10/sex for Group 1, 6/sex for Groups 2 and 3 and 10/sex for Group 4) groups. Twenty three animals (4/sex in group 1, 2/sex in groups 2 and 3 and, 4 males and 4 females in group 4) were killed on Week 27 (after 6 months of treatment). Thirty two animals (4/sex/group) were sacrificed during study Week 53 (i.e., after 1 year of treatment). An additional 8 animals (2/sex/group) were continued on study without further treatment for 13 weeks recovery period and then sacrificed. Animals were treated subcutaneously with ALX-0600 at (scapular region) 0 (Group 1), 1 (0.5 mg/kg bid, Group 2), 5 (2.5 mg/kg, bid, Group 3) and 25 (12.5 mg/kg, bid, Group 4) mg/kg/day (vehicle-phosphate buffer with mannitol and L-histidine; dose volume = 1.25 ml/kg) in 2 divided doses 8 hr apart for 56 weeks. Study animals were observed twice daily for the

toxic signs and symptoms and mortality during treatment. Body weights were recorded prior to treatment (Day-1) and weekly thereafter. Food consumption was recorded once daily. Physical examinations, electrocardiographic (ECG) evaluations, and ophthalmic examinations were conducted at prestudy, and during Weeks 13, 26 and 52. Hematology, serum chemistry parameters and urinalysis were determined on Day 1 (prior to treatment), Week 13, 26, 39 and 52 and at the time of termination in the recovery group animals at 0.25, 0.5, 1, 2, 4, and 8 hr postdose. Plasma concentrations and toxicokinetic parameters of the compound were estimated on these blood samples. Blood samples for antibody estimation were collected in Week 13, 26, 39 and 52 and for recovery animals during the week prior to necropsy. Following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovary, pituitary, spleen, cecum, colon, small intestines, testes with epididymides, thymus, thyroid and parathyroid. In addition, lengths of small and large intestines were also measured from the main study group animals. Histopathological examinations were conducted with the following tissues from all animals: aorta, heart, salivary gland (mandibular), tongue, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum), pancreas, liver, gallbladder, trachea, lung, bone marrow, thymus, spleen, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicle, ovaries, uterus, cervix, vagina, adrenals, pituitary, thyroid and parathyroid, skin/mammary gland, bone (femoral head), bone (7th rib), skeletal muscle (thigh), eyes with optic nerve, sciatic nerve, brain and spinal cord.

Results:

Observed Effects: Treatment-related clinical signs included injection site changes (thickening, raised, swelling, ulcers, and/or masses), distended abdomen, and swollen prepuce/scrotum. The injection site reactions were dose-related. Two animals had mass under the left or both armpits, which correspond to enlarged axillary lymph nodes resulting from the inflammation at the site of injection. The distended abdomen was considered to be due to increased size of the gastrointestinal tract. Scrotal/preputial swelling was attributed to scrotal or subcutaneous perineal edema.

Mortality: One male at 25 mg/kg/day was sacrificed in moribund condition on Day 150 and this animal had swollen scrotum, distended abdomen and hunched appearance.

Body Weight: The mean initial and final food body weight in control males were 3.7 and 5.3 kg, respectively. The mean initial and final body weight of control females were 2.7 and 3.9 kg, respectively. There were no significant treatment-related effects.

Food Consumption: The sponsor did not provide food consumption data.

Hematology: Treatment-related hematology changes at Week 52 included: increase in the number of eosinophils (males: 1.87%, 1.28%, 3.25% and 8.00% at 0, 1, 5 and 25 mg/kg/day, respectively; females: 1.93%, 4.53%, 4.90% and 4.88% of baseline at 0, 1, 5 and 25 mg/kg/day, respectively), neutrophil counts (males: 19.93, 22.38, 30.05 and 25.57% at 0, 1, 5 and 25 mg/kg/day, respectively; females: 16.07, 24.50, 32.28 and 38.98% at 0, 1, 5 and 25 mg/kg/day, respectively), white blood cell count (males: 10.8,

11.7, 16.5 and 14.7 x 10³/µl at 0, 1, 5 and 25 mg/kg/day, respectively; females: 10.3, 12.8, 16.5 and 16.5 x 10³/µl at 0, 1, 5 and 25 mg/kg/day, respectively). Additionally, in Group 3 and 4, a slight decrease of hematocrit and hemoglobin and in red blood cell indices, mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were also seen. No changes were observed in the mean fibrinogen concentration, mean prothrombin time and mean activated partial thromboplastin time.

Serum Chemistry: Treatment-related serum chemistry changes included decreases in cholesterol (at high dose), and increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and mean triglyceride and globulin concentration (at high dose). A decrease in serum albumin of 12, 15.2 and 13.1% in males at high dose was noted in Week 13, 26, and 52, respectively, compared to control. Alkaline phosphatase (ALP) levels on Week 52 were 343, 382, 315 and 270 U/L in males and 193, 227, 283 and 356 U/L in females at 0, 1, 5 and 25 mg/kg/day, respectively. ALT values were 37, 57, 99 and 69 U/L in males and 70, 111, 86 and 63 U/L at 0, 1, 5 and 25 mg/kg/day, respectively. On Week 52, albumin to globulin ratio was decreased (3.1 and 1.5 in males and 2.5 and 1.2 in females at 0 and 25 mg/kg/day, respectively). Serum triglycerides showed a non-dose-dependent increase of 188, 163.7, 132% in males and, 154.4, 140.9 and 143.9% in females at 1, 5 and 25 mg/kg/day, respectively. On Week 26, a treatment-related increase in lipase level was also observed (27, 55, 57 IU/L in males and 31, 44, 41 and 68 IU/L in females at 0, 1, 5 and 25 mg/kg/day, respectively). This increase on Week 52 was similar but during the recovery period, these changes were recovered.

Urinalysis: There were no significant treatment-related changes.

Toxicokinetics: Exposure to ALX-0600 increased with increasing dose. ALX-0600 was absorbed rapidly and the plasma level declined rapidly with mean terminal T_{1/2} ranged from 0.912 to 4.50 hours. There were no marked gender differences in the C_{max} and AUC_{0-8h} values. Increases in C_{max} and AUC_{0-8h} were roughly dose-proportional to the increase in dose. There was no apparent accumulation of the drug following multiple exposure. As mentioned before, ALX-0600 was detected (0.6 to 16 ng/ml) in control group animals on Day 1, Week 13, Week 39 and Week 52. This issue has been discussed before. The potential contamination of the test solution with the compound can not be ruled out. The toxicokinetic parameters are shown in the following table.

Parameter	Dose (mg/kg/day)	Males			Females		
		Day 1	Week 26	Week 52	Day 1	Week 26	Week 52
C _{max} (ng/ml)	1	696	644	431	919	645	507
C _{max} (ng/ml)	5	919	2482	2322	2870	2519	3289
C _{max} (ng/ml)	25	11190	11469	12168	11024	14838	15371
AUC _{0-8h} (ng.hr/ml)	1	2143	1893	1498	2639	2176	1784
AUC _{0-8h} (ng.hr/ml)	5	11946	8954	9130	11562	11035	16346

AUC _{0-24h} (ng.hr/ml)	25	53822	51282	64645	55324	68916	65898
T _{1/2} (h)	1	0.912	1.38	1.89	1.01	1.40	1.49
T _{1/2} (h)	5	1.53	2.32	2.31	1.16	2.67	2.05
T _{1/2} (h)	25	2.87	2.83	4.30	3.16	2.21	3.51

Detection of Antibodies in Plasma: Seven of 12 animals at 25 mg/kg/day showed appreciable increase in titre for antibody to ALX-0600 in Week 52 when compared to Day 1. No antibody was detected at Day 449. It is to be mentioned here that in a previous 13-week study (7203-100) in Cynomolgus monkeys, antibodies were also detected at 25 mg/kg/day in both sexes at Day 30 and 90. The relative titer values at Week 26, 36 and 52 are shown in the following table (from Vol. 7, page 152 of sponsor's submission):

Analysis of Cynomolgus Monkey Plasma Samples for the Detection of Antibodies to ALX-0600 using ELISA to Support (b) (4) Study # 1368-100

Group	Sample Set #	Sex	Animal Number	Relative Titer (compared to Day 1)				
				Day 1	Week 13	Week 26	Week 39	Week 52
3 5 mg/kg/day	B	M	F19915M	-	N/T	N/T	N/T	No change
		M	F19449M	-	N/T	N/T	N/T	No change
		F	F202115F	-	N/T	N/T	N/T	No change
		F	F202112F	-	N/T	N/T	N/T	No change
	C	M	F20956M	-	N/T	N/T	N/T	No change
		M	F18953M	-	N/T	N/T	N/T	No change
		F	F199100F	-	N/T	N/T	N/T	No change
		F	F189101F	-	N/T	N/T	N/T	No change

Group	Sample Set #	Sex	Animal Number	Relative Titer (compared to Day 1)					
				Day 1	Week 13	Week 26	Week 39	Week 52	Day 449
4	A	M	F18955M	-	366	241	344	1025	NA
		M	F19919M	-	No change	No change	No change	633	No change
		M	F18942M	-	267	No change	No change	391	No change
		F	F202116F	-	312	No change	243	923	No change
		F	F18973F	-	N/T	N/T	N/T	No change	NA
	B	M	F20217M	-	No change	No change	No change	605	NA
		F	F202109F	-	N/T	N/T	N/T	No change	N/T
		F	F20260F	-	466	1213	754	844	NA
	C	M	F20219M	-	N/T	N/T	N/T	No change	NA
		M	F20234M	-	253	No change	No change	1144	NA
		F	F194109F	-	N/T	N/T	N/T	No change	NA
		F	F20262F	-	N/T	N/T	N/T	No change	NA

N/A - sample was not available

N/T - sample was not tested

No Change - titer did not change when compared to Day 1

- = No value available as Day 1 is a baseline

Ophthalmoscopy: There were no treatment-related effects.

Electrocardiography: No significant treatment-related changes were observed.

Organ Weight: Test article-related effects included: increased intestinal weight (male: 252, 310 and 227% of control at 1, 5 and 25 mg/kg/day, respectively; female: 250, 201 and 280% of control at 1, 5 and 25 mg/kg/day, respectively), small intestine length (male: 158, 138 and 146% of control at 1, 5 and 25 mg/kg/day, respectively; female: 135, 157 and 153% of control at 1, 5 and 25 mg/kg/day, respectively), increased liver weight (male: 131, 124 and 118% of control at 1, 5, and 25 mg/kg/day, respectively; female: 110, 108 and 133% of control at 1, 5 and 25 mg/kg./day, respectively), decreased thymus weight (78%±1.35, 63.6%±1.1 and 98.2% of control in males and 3.2, 55.3%, 46.2% and 26.5% of control in females at 0, 1, 5, and 25 mg/kg/day, respectively) and increased spleen weight (male: 141, 109 and 136% of control at 1, 5 and 25 mg/kg/day, respectively; female: 122, 185 and 136% of control at 1, 5 and 25 mg/kg/day, respectively).

Gross Pathology: Treatment-related findings included increased thickness of the stomach, duodenum, jejunum, colon and a white nodule in the liver (one Group 4 male, F20201, at Week 27), increased thickness of the injection site, increased size of the axillary and other lymph nodes and edema around the scrotum or perineum.

Histopathology: Treatment-related histopathological changes were observed in the stomach, small intestine, large intestine, pancreatic ducts, bile ducts in the liver and gallbladder at all time points and generally consisted of hyperplasia and/or hypertrophy of the luminal and/or ductular epithelium. Other test article-related changes were

inflammation at the injection sites and lymphoid follicle formation in the gallbladder. The following table shows the histopathological changes.

Sex	Male				Female			
Dose (mg/kg/day)	0	1	5	25	0	1	5	25
Number Examined	4	2	2	4	4	2	2	4
Liver								
Heptocellular hypertrophy	1	3	5	3	0	2	6	2
Chronic biliary Inflammation.	2	3	0	0	0	0	1	2
Gall Bladder								
Hypertrophy/hyperplasia of the epithelium of the bile duct in the gall bladder	0	5	9	12	0	15	15	14
Pancreas								
Periductal inflammation	0	0	3	0	0	1	0	1
Duodenum								
Mucosal hyperplasia	1	7	11	9	0	11	12	15
Ileum								
Mucosal hyperplasia	0	2	5	6	0	3	3	2
Cecum								
Mucosal hyperplasia	0	1	7	8	0	6	3	8
Colon								
Mucosal hyperplasia	0	1	1	8	0	1	0	2
Urinary Bladder								
Lymphohistiocytic inflammation	1	-	-	4	1	-	-	2
Seminal Vesicle								
Lymphocytic Infiltration.	2	-	-	5	-	-	-	-

Leiomyosarcoma: One Group 2 (1 mg/kg/day) female monkey (F20279F) sacrificed in Week 53 had a leiomyosarcoma in the muscularis of her rectum. The leiomyosarcoma consisted of numerous interweaving spindle-shaped cells with abundant cytoplasm, and plump, cigar shaped, open-faced nuclei with numerous mitotic figures. The tumor was not encapsulated and infiltrated surrounding smooth muscle. This tumor was positive for smooth muscle actin, and negative for desmin, S100, GFAP (glial fibrillary acidic protein), CD117 and CD34. This tumor was found in the distal end of the colon. This animal had minimal mucosal hyperplasia affecting mucosal epithelium. The sponsor stated that incidental leiomyosarcomas, leiomyomas or gastrointestinal stromal tumors have been reported in other species of macaques in rare occasions. However, a test article-relationship could not be ruled out. Another high-dose male monkey (F20232) also had minimal mucosal epithelial hyperplasia of the rectum, which was observed at Week 27.

In a 1-year subcutaneous toxicity study in cynomolgus monkeys, animals were treated at 1, 5 and 25 mg/kg/day (0.5, 2.5 and 12.5 mg/kg bid, 8 hours apart). The target organ of toxicity appeared to be the small intestine, pancreas, bile ducts, liver, gallbladder (hyperplasia and/or hypertrophy of the luminal and/or ductular epithelium) and injection site (inflammation characterized by vasocentric and/or granulomatous with the presence of eosinophils and mononuclear cell infiltrate). One female monkey at 1 mg/kg/day had possible treatment-related leiomyosarcoma in the muscularis of the rectum. Appreciable amount of antibody was detected at 25 mg/kg/day, which was also observed in a previous 13-week study in Cynomolgus monkeys.

7 Genetic Toxicology

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

Induction of Reverse Mutations in Microbial Mutagenicity Test (Ames Test): (Study # 88665)

The review of the above study is incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

1. Induction of Reverse Mutations in Microbial Mutagenicity Test (Ames Test): (Study #G98AU51.502; Proj. # 88665)

Conducting Laboratory: [REDACTED]

(b)(4)

(b)(4)

Dates of Initiation and Completion: May 11, 1998 and July 13, 1998.

GLP Statement: A statement that the study complied with GLP requirements was enclosed.

Materials and Methods:

Strains Used: a. Salmonella - TA98, TA100, TA1537 and TA 1535 and; b. E. Coli - WP2uvrA.

Chemical: ALX-0600 (batch #D06GL98a); Positive controls (lot #, concentrations and the strain name): Methyl-methanesulfonate (MMS), 2-nitrofluorene (2NF), 9-aminoacridine (9AmAc), 2-aminoanthracene (2AA) and sodium azide. These positive controls and ALX-0600 were dissolved in dimethylsulfoxide (DMSO).

Methods: The compound was tested for mutagenic potential according to the Ames method in the absence and presence of S-9 metabolic system. In preliminary toxicity test, ALX-0600 was tested with salmonella strains TA98, TA100, TA1537 and TA1538 and, E. coli strain WP2 uvrA (one plate/dose with no overnight cultures) at the concentration of 6.7, 10, 33.7, 67, 100, 333, 667, 1000, 3333 and 5000 ug/plate. Based on the results of the preliminary test, ALX-0600 was tested in triplicate at 0, 100, 333, 1000, 3333 and 5000 ug/plate concentrations in mutagenicity assay in the absence and presence of S-9 mix. In this assay, 0.5 ml S-9 or sham mix was mixed with 100 ul of bacterial tester strain and 50 ul vehicle/test article. For activated assay, the tester strain was combined with 2 ml top agar and S-9 mix. Each of the culture solution was prepared in triplicate and all tubes incubated at 37°C for 48 to 72 hr. Revertant colonies were counted after the incubation. The methodology was adequately

described and the criteria for assessing the positive effect were if the number of revertants in the treated cultures exceeded the control by at least twofold (TA98, TA100, and WP2uvrA') or at least 3 folds (strain TA1535 and TA1537) for 2 successive concentrations of the article.

Results: In toxicity part of the test, a reduction in the number of mean values of revertant colonies for salmonella strains TA1535, TA1537, TA98 and TA100 and, in E. coli WP2uvrA' strain was reported at a concentration of greater than 500 ug/plate ALX-0600. The number of revertant colonies in a plate containing 5000 ug or less of the compound was not affected. In mutagenicity test, the mean number revertant colonies in plates containing the compound were similar to the negative control as shown in the following table (sponsor's table vol 10:12, pp 194):

TABLE
Salmonella/E.coli Mutagenicity Assay
Summary of Results

Test Article	• ALX-0600				
Study Number	• G98AU51.502		Experiment No : BI		
Average Revertants Per Plate \pm Standard Deviation					
A. Liver Microsomes: None					
Dose (ug)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	11 \pm 2	113 \pm 12	9 \pm 1	7 \pm 2	16 \pm 2
100	13 \pm 2	116 \pm 15	7 \pm 3	9 \pm 3	18 \pm 4
333	14 \pm 1	117 \pm 4	9 \pm 3	7 \pm 1	16 \pm 1
1000	12 \pm 1	119 \pm 9	9 \pm 3	7 \pm 2	17 \pm 4
3333	13 \pm 2	116 \pm 11	10 \pm 2	6 \pm 2	22 \pm 1
5000	11 \pm 3	120 \pm 6	10 \pm 2	6 \pm 1	17 \pm 4
Pos	246 \pm 10	597 \pm 56	308 \pm 13	262 \pm 16	168 \pm 12
B. Liver Microsomes' Rat liver S9					
B. Liver Microsomes' Rat liver S9					
Dose (ug)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	21 \pm 2	133 \pm 21	13 \pm 2	9 \pm 1	17 \pm 2
100	18 \pm 2	141 \pm 12	9 \pm 1	7 \pm 0	13 \pm 1
333	16 \pm 3	126 \pm 9	9 \pm 2	6 \pm 3	17 \pm 4
1000	19 \pm 3	140 \pm 8	9 \pm 1	6 \pm 2	13 \pm 2
3333	14 \pm 4	142 \pm 3	11 \pm 0	6 \pm 1	18 \pm 5
5000	15 \pm 4	122 \pm 5	12 \pm 1	6 \pm 2	18 \pm 3
Pos	642 \pm 68	713 \pm 81	82 \pm 16	79 \pm 13	387 \pm 24

0.0 - Vehicle plating aliquot of 50 μ l

Pos = Positive Control concentrations as specified in Materials and Methods section

The sensitivity of the assay was confirmed by the response with positive control 2AA (as shown in the table above). ALX-0600 was non-mutagenic in this test.

7.2 *In Vitro* Assays in Mammalian Cells

In-Vitro Assessment of Clastogenic Activity in Chinese Hamster Ovarian Cells: (Study # 88666)

The review of the above study is incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

2. In-Vitro Assessment of Clastogenic Activity in Chinese Hamster Ovarian Cells: (Study #G98AU51.331; Project #88666)

Conducting Lab: [REDACTED] (b)(4)

Dates of Initiation and Completion of Study: May 12, 1998 and October 21, 1998.

GLP and QAU Statement: A statement was included.

Materials and Methods

Drugs and Chemicals: ALX-0600 (Batch #D06GL98a, D06GL98c, D08GL98a, D07GL98a). Positive controls - Cyclophosphamide (CP), Mitomycin C (MMC). The test was done in the presence and absence of metabolic activator, rat liver S-9 fraction.

Methods: Chinese hamster ovarian (CHO-K₁) cells (CCL61 with chromosomes 20) obtained from [REDACTED] (b)(4) were seeded at approximately 5X10⁵ cells/cm² per flask and incubated for 16 to 24 hr. The culture was maintained in McCoy's 5A medium for non-activated medium or with S-9 mix (4 ml serum free medium + 1 ml S-9) for activated medium. To this culture medium, 50 ul of the solvent/compound to be tested was added. These cells (subclone of WBL) were treated for 4 hr, the media removed and cells washed and returned for incubation for a total of 20 hr from the initiation of treatment. The cells were harvested by trypsinization and counted by Coulter counter. Cell viability was determined by trypan blue dye exclusion. In chromosomal aberration test, Colemid (0.1 ug/ml) was added in duplicate flasks 2 hr prior to schedule cell harvest to arrest cell division in metaphase and returned to incubator till cell collection. The cells were counted in 3 test compound concentrations. The metaphases collected after centrifuging were resuspended in hypotonic 0.075 M KCl solution, recentrifuged and cell pellet resuspended in a small amount of fixative and dropped on a slide, stained with Giesma for determining the aberrations. The chromosomal aberrations including breaks, and exchanges counted. In the toxicity study without S-9 activation, 1500, 2000 and 3000 ug/ml ALX-0600 concentrations were tested without S-9 mix. The concentrations

in the presence of S-9 activation were 250, 500, 1000 and 2000 ug/ml ALX-0600. The test was considered as positive if it showed a significant ($p < 0.05$) increase in the chromosomal aberrations in a dose related manner.

Results: ALX-0600 up to 3000 ug/ml concentration did not show toxicity after 4 hr of incubation and the mitotic index was not changed. Based on this, the concentrations selected for the main test for estimating the chromosomal aberration were 1500, 2000 and 3000 ug/ml ALX-0600 in the cultures incubated for 4 hr. MMC and CP were used as positive control in the absence and presence of S-9 activation, respectively. An additional set of 1500, 2000 and 3000 ug/ml ALX-0600 cultures in triplicate, was used for 20 hr incubation. No toxicity was observed up to 3000 ug/ml ALX-0600 concentrations and the mitotic indices up to 2000 ug/ml were not different from those of control cultures. The mitotic indices of cultures containing ALX-0600 in the presence and absence of S-9 mix were similar (as shown in the table). The chromosomal aberrations in positive controls containing 0.08 ug/ml MMC and 10 ug/ml CP were significantly ($p < 0.01$) more than the controls (11.0 and 20.5% vs 2.5% in DMSO culture as shown in the table (sponsor's table 9, vol 1:10, pp 230). ALX-0600 was not clastogenic in this test.

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TABLE 9

SUMMARY

Treatment	S9 Activation	Treatment Time (Hours)	Mitotic Index	Cells Scored	Aberrations Per Cell (Mean ± SD)	Cells With Aberrations (%)	
						Numerical	Structural
DMSO	-	4	5.8	200	0.030 ± 0.198	2.0	2.5
ALX-0600							
1500 µg/mL	-	4	6.0	200	0.030 ± 0.171	2.0	3.0
2000 µg/mL	-	4	6.7	200	0.045 ± 0.252	1.5	3.5
3000 µg/mL	-	4	7.2	200	0.030 ± 0.171	1.5	3.0
MMC, 0.08 µg/mL	-	4	7.0	200	0.120 ± 0.355	1.0	11.0**
DMSO	+	4	8.7	200	0.025 ± 0.157	2.0	2.5
ALX-0600							
1500 µg/mL	+	4	7.7	200	0.035 ± 0.184	2.0	3.5
2000 µg/mL	+	4	6.9	200	0.035 ± 0.184	3.5	3.5
3000 µg/mL	+	4	7.8	200	0.045 ± 0.208	4.0	4.5
CP, 10 µg/mL	+	4	5.5	200	0.280 ± 0.627	3.5	20.5**
DMSO	-	20	8.2	200	0.015 ± 0.122	1.5	1.5
ALX-0600							
1500 µg/mL	-	20	6.8	200	0.030 ± 0.171	1.0	3.0
2000 µg/mL	-	20	7.8	200	0.035 ± 0.184	1.0	3.5
3000 µg/mL	-	20	8.4	200	0.030 ± 0.171	0.5	3.0
MMC, 0.08 µg/mL	-	20	4.8	200	0.160 ± 0.430	2.5	14.0**

¹ Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

² Severely damaged cells were counted as 10 aberrations.

³ *, p<0.05; **, p<0.01; Fisher's exact test.

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

In Vivo Mouse Micronucleus Assay Following Subcutaneous Administration of Teduglutide (Study No. AA65WK.123.BTL)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

Study Title: *In Vivo* Mouse Micronucleus Assay Following Subcutaneous Administration of ALX-0600

Key Findings: Negative

Study No.: AA65WK.123.BTL

Study Type: *In vivo* bone marrow micronucleus assay

Volume #, and Page #: Amendment 026 (page 52) and Amendment 072 (page 6)

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: September 25, 2002

GLP Compliance: A statement of compliance was included

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: ALX-0600, Lot No. 0850201, 98.5%

Formulation/Vehicle: Solution, 0.9% sodium chloride

Methods:

Strains/Species/Cell Line: Male mice/CD-1 (ICR)

Dose Selection Criteria:

Basis of dose selection: The doses were selected based on physico-chemical properties and limit of subcutaneous dose of 20 ml/kg.

Test Agent Stability: The stability of the test solutions were determined by the sponsor.

Controls:

Negative Control: Phosphate buffer (b) (4) with L-histidine (b) (4) and (b) (4) mannitol

Positive controls: Cyclophosphamide (50 mg/kg, i.p.)

Exposure Conditions:

Sampling Times: Blood samples were collected at 24 (from five groups) and 48 hours (from two groups) after treatment

Doses: ALX-0600 was administered twice by two subcutaneous injections 8 hours apart at a dose volume of 20 ml/kg. The study consisted of seven groups, each containing 5 males and 5 females. Animals in five of these groups were treated either with the controls (negative and positive) or with ALX-0600 at a total dose of 177 (100 + 77), 400 (200 + 200) and 800 (400 + 400) mg/kg and were euthanized at 24 hours after treatment. Animals in the other two groups were treated either with the negative control or ALX-0600 at 800 mg/kg and were euthanized at 48 hours post-treatment. For the toxicokinetic portion of the study, mice were assigned to one group of three male and one group of three female mice each.

Study Design: The study design is shown in the table below (from page 9 of sponsor's report).

Treatment (20 mL/kg/administration; 2 administrations, 8 hrs apart)	Treatment/ Administration (mg/kg)	Number of Mice/Sex Used For		
		Bone Marrow Collection		Toxicokinetics
		After Last Dose Administration		
		24 hr	48 hr	2 hr
Negative Control: PB w/Mannitol and L-Histidine	0.0/0.0	5	5	0
Test Article: ALX-0600 (total dose)				
Low dose (177 mg/kg/day)**	100/77	5	0	0
Mid dose (400 mg/kg/day)	200/200	5	0	0
High dose (800 mg/kg/day)	400/400	5	5	3
Positive Control: CP*	50*	5	0	0

*Positive control was administered only once, at the time of second test article dose administration

**Total dose was planned to be 200 mg/kg, however the results of the concentration analysis indicated that only a dose of 177 mg/kg was administered

Analysis:

Counting Method: Manual. Micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes per animal were scored for the presence of micronuclei.

Criteria for Positive Results: The following criterion was established for a positive response: a statistically significant dose-related increase in the number of micronucleated reticulocytes and one or more doses were statistically elevated relative to negative control at any sampling time.

Summary:

Study Validity: All criteria for a valid study were met.

Study Outcome: Negative. The following table (from Vol. 12 page 16 of sponsor's submission) summarizes the results of the *in vivo* mice bone marrow micronucleus assay.

Table 2: Summary of Bone Marrow Micronucleus Analysis After Subcutaneous Administration of ALX-0600 in CD-1 (ICR) Mice

Treatment (2 x20 mL/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
						Number per 1000 PCEs (Mean +/- SD)	Number per PCEs Scored ¹
PB w/Mannitol and L-Histidine							
	M	24	5	0.484 ± 0.03	---	0.5 ± 0.50	5 / 10000
	F	24	5	0.441 ± 0.04	---	0.4 ± 0.22	4 / 10000
ALX-0600 (total dose)							
177 mg/kg	M	24	5	0.471 ± 0.06	-3	0.5 ± 0.00	5 / 10000
	F	24	5	0.444 ± 0.02	1	0.5 ± 0.35	5 / 10000
400 mg/kg	M	24	5	0.417 ± 0.04	-14	0.7 ± 0.27	7 / 10000
	F	24	5	0.479 ± 0.04	9	0.4 ± 0.22	4 / 10000
800 mg/kg	M	24	5	0.434 ± 0.02	-10	0.5 ± 0.00	5 / 10000
	F	24	5	0.430 ± 0.04	-2	0.5 ± 0.35	5 / 10000
CP**							
50 mg/kg	M	24	5	0.325 ± 0.04	-33	24.3 ± 4.44	*243 / 10000
	F	24	5	0.353 ± 0.09	-20	24.4 ± 4.02	*244 / 10000
PB w/Mannitol and L-Histidine							
	M	48	5	0.487 ± 0.03	---	0.3 ± 0.27	3 / 10000
	F	48	5	0.512 ± 0.06	---	0.6 ± 0.22	6 / 10000
ALX-0600 (total dose)							
800 mg/kg	M	48	5	0.480 ± 0.05	-1	0.7 ± 0.27	7 / 10000
	F	48	5	0.487 ± 0.06	-5	0.7 ± 0.27	7 / 10000

¹*Statistically significant, p≤0.05 (Kastenbaum-Bowman Tables)

** Positive control was administered only once, at the time of second test article dose administration

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

104-Week Subcutaneous Carcinogenicity Study in Wistar Han IGS Rats (Study No. 800070)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated October 14, 2008.

**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET
Review of Carcinogenicity Study Results**

P/T REVIEWER(s): Tamal K. Chakraborti, Ph.D.

DATE: October 8, 2008

IND: 58,213

DRUG CODE#: ALX-0600

CAS#: Not available

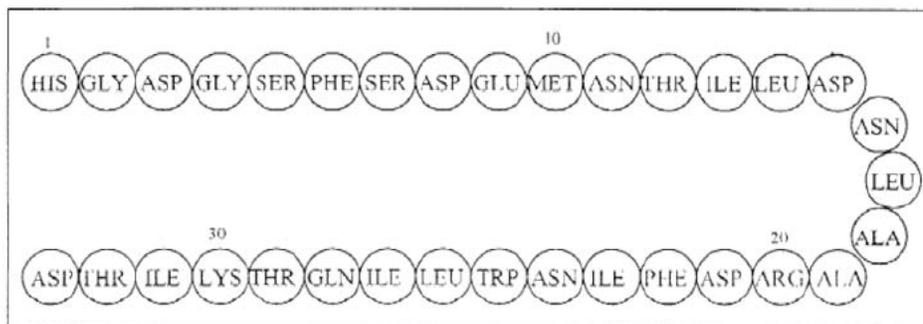
DIVISION: Division of Gastroenterology Products (DGP)

DRUG NAME: ALX-0600

CHEMICAL STRUCTURE: ALX-0600, a 33-amino acid peptide, is a recombinant analog of human regulatory gut glucagon-like peptide-2 (GLP-2). It differs from natural GLP-2 in the substitution of glycine for alanine at position 2. ALX-0600 was synthesized from *Escherichia coli* (Strain No. PAL2000) carrying (b) (4)

(b) (4) The structure of ALX-0600 is shown below from the sponsor's submission.

Figure A1. Primary structure of ALX-0600



SPONSOR: NPS Pharmaceuticals, Inc.

LABORATORY: (b) (4)

CARCINOGENICITY STUDY REPORT DATE: December 21, 2007

THERAPEUTIC CATEGORY: Human Glucagon-like Peptide/Intestinal Peptide. The drug is being developed for the treatment of short bowel syndrome (SBS)

PHARMACOLOGICAL CLASSIFICATION: Recombinant analogue of Human Glucagon-like Peptide-2 (GLP-2)

MUTAGENIC/GENOTOXIC: Negative. ALX-0600 was negative in the Ames test, *in vitro* chromosomal aberration test in Chinese hamster ovary (CHO) cells, *in vitro* mammalian erythrocytes micronucleus test and *in vivo* mouse micronucleus assay.

RAT CARCINOGENICITY STUDY:

STUDY DURATION (weeks): 104

STUDY STARTING DATE: June 29, 2004

STUDY ENDING DATE: December 21, 2007

RAT STRAIN: Wistar Han IGS (CrI:WI(Glx/BR/Han)IGSBR)

ROUTE: Subcutaneous (SC)

DOSING COMMENTS: Initially, the sponsor submitted (November 13, 2003) a dose selection and protocol for 104-week subcutaneous carcinogenicity study in SD rats with dietary restrictions at 0, 1.5, 5.0 and 25 mg/kg, BID or 0, 3, 10 and 50 mg/kg/day (pharmacology review dated December 5, 2003). The doses were selected based on plasma exposure levels. The ExecCAC did not concur with the proposed doses as sufficient data on long-term effects of dietary restriction on plasma exposure were lacking (ExecCAC meeting minutes dated December 19, 2003, Attachment-I). The Committee preferred to use the toxicity endpoint (maximum tolerated dose/MTD) instead of AUC comparisons. Based upon increased serum alanine transaminase (ALT), liver necrosis, and bile duct hyperplasia at 50 mg/kg/day in the 13-week study in SD rats, the MTD was identified as 50 mg/kg/day in females. However, the MTD in males could not be identified in the absence of dose limiting toxicity in males. The ExecCAC recommended 10, 25 and 50 mg/kg/day doses for female rats based on the identified MTD from the 13-week study (800069) in SD rats with restricted diet. However, the Committee could not recommend the doses for the male, as a MTD could not be established for males.

NPS communicated (February 17, 2004) to the Agency that the recommended high dose of 50 mg/kg/day was not technically feasible in the light of possible severe injection site reactions, which might be incompatible with the survival of SD rats for the span of the carcinogenicity study. These injection site reactions (necrosis, microscopic inflammatory findings and acute phase reactions manifested by changes in clinical pathology parameters) were consistently observed in all species (mouse, rat and monkey) tested and progressed in incidence and severity when dosed twice daily longer than 90 days and were considered part of the dose limiting toxicity. As an alternative, based on a technical feasibility standpoint, NPS proposed to switch to the Wistar (Han) rats (instead of SD rats) using *ad libitum* feeding and proposed to conduct the carcinogenicity study at 3, 10 and 30 mg/kg/day based on 25-fold ratio of exposure (AUC) at the maximum recommended human dose (MRHD, 0.2 mg/kg/day). Historical data in the Wistar (Han) strain at (b) (4) the laboratory contracted to perform the carcinogenicity study, showed a survival rate ranging from 50 to 86% at Week 104. In contrast, *ad libitum* fed SD rats had a survival rate ranging from 22 to 52%. The Division responded (Division letter dated February 26, 2004) with input from Dr. Kenneth L. Hastings, Associate Director, ODE II and III that the switch to the Wistar (Han) strain would be allowed provided NPS conducts a comparative two-week subcutaneous toxicity and toxicokinetic (TK) study in Wistar (Han) and SD rats under fully fed conditions to demonstrate equivalency of the strains in terms of toxicity and toxicokinetics. The Agency recommended that if equivalency was established, TK data from this study could be used to support the dose selection for the proposed 104-week study in

Wistar (Han) rats. NPS conducted a 14-day comparator SC study (800869) using three strains of rats (Wistar Han, SD and Fischer-344) under fully fed conditions as recommended by the Division. The exposure levels were comparable in three strains of rats. Overall, SD rats and Wistar Han rats were considered to be equivalent in terms of toxicity and toxicokinetic profile following SC administration of ALX-0600 under fully fed condition. This fulfilled the Division criteria for using Wistar Han rats instead of SD rats with *ad libitum* feeding for the proposed carcinogenicity study (pharmacology review dated June 16, 2005).

Subsequently, the sponsor submitted (April 20, 2005) a second dose selection and protocol for carcinogenicity study (pharmacology review dated June 16, 2005) in Wistar Han rats at 3, 10 and 35 mg/kg/day. However, the sponsor had already initiated the carcinogenicity study in Wistar Han rats on June 29, 2004 prior to the submission of second dose selection protocol on April 20, 2005. The doses (3, 10, and 35 mg/kg/day) were selected based on the pharmacokinetic (PK) endpoint. The ExecCAC did not concur with the proposed dose selection for the following reasons (ExecCAC meeting minutes dated June 28, 2005, Attachment-II).

“

1. *The Committee did not consider it appropriate to comment on the dose selection at this stage, as the study has already been initiated. The Committee will evaluate the appropriateness and merits of the carcinogenicity study after the submission of the study report.*
2. *A dose producing an exposure (plasma AUC) of 25-fold multiple of human exposure would be generally acceptable as high dose provided it does not produce excessive mortality.*
3. *In the future, if the sponsor needs feedback on the protocol and dose selection, the information should be submitted to the Agency for input prior to initiation of the study.”*

NUMBER OF RATS:

- Control-1 (C1): 50/sex
- Low Dose (LD): 50/sex
- Middle Dose (MD): 50/sex
- High Dose-1 (HD1): 50/sex

RAT DOSE LEVELS:

- Low Dose: 3 mg/kg/day
- Middle Dose: 10 mg/kg/day
- High Dose-1: 35 mg/kg/day

BASIS FOR DOSES SELECTED: AUC ratio (Pharmacokinetic endpoint)

PRIOR FDA DOSE CONCURRENCE: No (ExecCAC meeting minutes dated June 28, 2005, Attachment-II).

RAT CARCINOGENICITY: Males: Positive for benign adenoma in the bile duct and jejunum;
Females: Negative

RAT TUMOR FINDINGS: In females, the trend test was negative ($p > 0.05$). However, in males, the trend test was significant for benign pheochromocytoma ($p = 0.0206$) in the adrenal gland, adenoma in the bile duct ($p = 0.0037$), adenoma in the jejunum ($p = 0.0031$), and c-cell carcinoma in the thyroid ($p = 0.0145$). However, benign pheochromocytoma in the adrenal and C-cell carcinoma in the thyroid were considered as common tumors as the incidences in the control were higher than 1% and were not considered as significant ($p > 0.005$). The following Table (from page 854 of the study report) shows the p-values for the trend test in males.

ORGAN NAME	TUMOR NAME	P-VALUE
Adrenal	Benign Pheochromocytoma	0.0206
Bile Duct	Adenoma	0.0037
Jejunum	Adenoma	0.0031
Thyroid	Carcinoma c-cell	0.0145

In addition to the overall trend test, pair-wise comparisons were also made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the one in the control group 1. In females, pair-wise comparisons were negative ($p > 0.05$). However, in males, the incidence rates of bile duct adenoma ($p = 0.0465$) and jejunum adenoma ($p = 0.0332$) were statistically significantly higher in group 4 compared to respective controls. These p-values for pair-wise comparisons for the high-dose males are shown in the following table (from page 854 of the study report):

Organ Name	Tumor Name	Comparison	P-Value
Bile Duct	Adenoma	1 vs 4	0.0465
Jejunum	Adenoma	1 vs 4	0.0332

Overall, ALX-0600 caused statistically significant increases in the incidences of adenomas in the bile duct ($p = 0.0037$, trend test, 0 of 50, 0 of 50, 1 of 50 and 4 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.050$, pairwise at high dose) and jejunum ($p = 0.0031$, trend test, 0 of 50, 1 of 50, 0 of 50 and 5 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.024$, pairwise at high dose) of male rats. There were no drug-related tumor findings in females.

RAT STUDY COMMENTS: The dose selection based on the pharmacokinetic endpoint (AUC ratio) appears to be appropriate and acceptable, as the high dose produced about a 25-fold human

exposure (total daily AUC of about $30 \mu\text{g}\cdot\text{hr}/\text{ml}$ in rats, as compared to $1.2 \mu\text{g}\cdot\text{hr}/\text{ml}$ at the expected maximum human dose of $0.2 \text{ mg}/\text{kg}/\text{day}$). Also, the mortality incidence at the high dose did not interfere with the interpretation of the study results. The strain selection was also acceptable to the Division as discussed above. Overall, the study was conducted in a valid manner. The study conduct appears to be acceptable.

CARCINOGENICITY:**Study title:** 104-Week Subcutaneous Carcinogenicity Study in Wistar Han Rats

Key study findings: In a 104-week subcutaneous carcinogenicity study in Wistar Han rats, animals (n = 50/sex/dose) were treated at 0 (solution/phosphate buffer with mannitol and L-histidine), 3, 10, and 35 mg/kg/day. The dose selection was based on a pharmacokinetic endpoint (AUC ratio). The dose selection appears to be acceptable, as the high dose produced about a 25-fold human exposure (total daily AUC of about 30 µg•hr/ml in rats, as compared to 1.2 µg•hr/ml at the expected maximum human dose of 0.2 mg/kg/day). Furthermore, the high dose did not produce excessive mortality. In males, survival appears to be comparable at the low- and mid-dose when compared to the control. However, the survival rate of the high-dose males (62%) was lower than control (76%). In females, survival rates appeared to be comparable among three treatment groups. However, the survival rates at all doses were lower than the control. There was no significant meaningful effect of treatment on bodyweight or food consumption. A high incidence of corneal opacities (superficial punctate keratopathy) was seen in all groups. An increased incidence of diffuse retinal degeneration was observed in males and females of group 4 (week 104). These findings did not correlate with any treatment-related histopathological findings. Treatment-related increases were seen in the weights of the small and large intestine, liver and pancreas. Treatment-related gross pathology changes (thickening, dilatation, and enlargement) were observed in injection sites, small and large intestines, kidneys, mesenteric lymph nodes, abdominal cavity and extra-hepatic bile ducts. Treatment-related non-neoplastic lesions were observed in the bile ducts (dilatation, mural fibrosis or thickening, epithelial hyperplasia and proliferation of mural ductules), liver (hyperplasia and proliferation of portal bile ducts, often with an associated periductular fibrosis, as well as a small number of billiary cysts), intestinal tract (villous/mucosal hyperplasia of the intestinal mucosa characterized by a pronounced elongation of villi in the small intestine), injection sites (localized inflammation/fibrosis/degeneration and/or degeneration of the panniculus muscle) and mesenteric lymph nodes (enlarged or dark mesenteric lymph nodes associated with one or more of lymphoid hyperplasia with or without histiocytosis, dilatation of lymph node sinuses and/or erythrophagocytosis). Overall, ALX-0600 caused statistically significant increases in the incidences of adenomas in the bile duct (p = 0.0037, trend test, 0 of 50, 0 of 50, 1 of 50 and 4 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; p = 0.050, pairwise at high dose) and jejunum (p = 0.0031, trend test, 0 of 50, 1 of 50, 0 of 50 and 5 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; p = 0.024, pairwise at high dose) of male rats. There were no drug-related tumor findings in females. The study conduct was acceptable and valid.

Study number: 800070

Volume #, and page #: EDR submission dated February 25, 2008

Conducting laboratory and location:

(b) (4)

(b) (4)

Date of study initiation: June 29, 2004

GLP compliance: A statement of compliance was included.

QA report: yes (X) no ()

Drug, lot #, and % purity: ALX-0600. The lot numbers and the purity data are shown in the following table (from page 24 of the study report).

Text Table 4 Lot Numbers, Expiry Dates and Purity of ALX-0600

Lot Number	Expiry Date	Purity
0850203	July 2005	97.4
0850204	July 2005	98.5
08503021	February 2006	98.7
08503031	March 2006	98.7
08504011	03 November 2006	98.6
08504021	31 November 2006	98.6
08504031	25 May 2007	98.0
08505011	13 July 2007	97.5
08505021	20 July 2007	97.9
08505031	03 August 2007	98.0
08505041	10 August 2007	98.0
08505051	28 October 2007	98.1

CAC concurrence: No (ExecCAC meeting minutes dated December 19, 2003 and June 28, 2005)

Study Type: 2-year bioassay

Species/strain: Rats/Wistar Han

Number/sex/group; age at start of study: 50/sex/group; 6 weeks old

Animal housing: Animals were housed individually in stainless steel wire mesh-bottomed cages equipped with an automatic watering valve and/or water bottle. The targeted conditions for animal room environment and photoperiod were as follows: Temperature: $22 \pm 3^\circ\text{C}$, Humidity: $50 \pm 20\%$, Light cycle: 12 hours light and 12 hours dark. The overall average temperature and relative humidity during the study were 22.84°C and 47.65% , respectively.

Formulation/vehicle: Solution/Phosphate buffer with mannitol and L-histidine

Drug stability/homogeneity: Dose formulations were determined to be stable for 48 hours at room temperature. Triplicate samples were retained from the first dose formulation on the day of preparation on Days 1 and 28 and on Weeks 13, 26, 39, 52, 65, 78, 91 and 104 for concentration verification. The dose formulation analyses were within 10% of nominal concentration on all occasions with the exception of samples from Week 52 at 1.2 mg/mL where measured concentrations of ALX-0600 were within 17% of the nominal concentration. This variation was considered to have no adverse impact on the outcome of the study results due the total length of

study and the magnitude of dose concentrations involved. ALX-0600 was not detected in control samples.

Methods:

Doses: 3, 10, and 35 mg/kg/day (1.5, 5.0 and 17.5 mg/kg BID, 8 hours apart)

Basis of dose selection: AUC ratio

Restriction paradigm for dietary restriction studies: Not applicable

Route of administration: Subcutaneous.

Frequency of drug administration: Twice daily (8 hours apart)

Dual controls employed: No

Interim sacrifices: None

Study Design: The study design is shown in the table below (from page 23 of the study report).

Text Table 2 Original Study Design (Weeks 1 to 30)

Group Number Identification	Dose Level (mg/kg/day)	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)	Number of Animals			
				Main Study ^a		Toxicokinetic ^b	
				Males	Females	Males	Females
1/ Vehicle Control	0	0	0	50	50	-	-
2/ ALX-0600	3	1.5	1.2	50	50	12	12
3/ ALX-0600	10	5	4	50	50	12	12
4/ ALX-0600	35	17.5	14	50	50	12	12
5/ Antibody Baseline	-	-	-	12	12	-	-

a Euthanized in Week 105/106.

b Euthanized in Week 52/53.

Based on clinical findings, the dose volume for Groups 1 and 4 was increased from 1.25 to 1.75 mL/kg/dose beginning at Week 30 in order to decrease the dose concentration from 14 to 10 mg/mL at injection sites in Group 4.

Text Table 3 Study Design (Weeks 30 to 106)

Group Number Identification	Dose Level (mg/kg/day)	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)	Number of Animals			
				Main Study ^a		Toxicokinetic ^b	
				Males	Females	Males	Females
1/ Vehicle Control	0	0	0	50	50	-	-
2/ ALX-0600	3	1.5	1.2	50	50	12	12
3/ ALX-0600	10	5	4	50	50	12	12
4/ ALX-0600	35	17.5	10	50	50	12	12
5/ Antibody Baseline	-	-	-	12	12	-	-

a Euthanized in Week 105/106.

b Euthanized in Week 52/53.

Satellite group for toxicokinetics: Yes (shown in the above table).

Deviations from original study protocol: There were minor protocol deviations which did not seem to have any impact on the results and interpretations.

Statistical methods:

Mortality: Mortality rates were assessed using the logrank test (homogeneity test). Whenever the homogeneity comparison revealed a significant difference among the considered groups, the significance of a dose-related trend in mortality was evaluated using the method of Tarone.

Tumor Data: For the purpose of the statistical analysis, hemangiosarcomas were combined across all the different sites in which they appear. The fatal and incidental tumors were analyzed separately by Proc Multtest as per survival adjusted trend test (Peto et al., 1980). For each site/neoplasm combination as well as for hemangiosarcoma data, the significance of a linear dose-related increase in tumor occurrence rates was evaluated using Peto's survival-adjusted trend test. Furthermore, Peto's one-tailed trend test was also used to test whether or not the tumor rate in each treated group was significantly higher than the one in the control group.

Observations and times:

Mortality: Twice daily

Clinical Signs: Twice daily

Body weights: Weekly throughout the first 16 weeks of treatment and then monthly

Food consumption: Weekly throughout the first 13 weeks of treatment, and then monthly

Ophthalmoscopy: Once prior to the start of treatment (all animals) and during Weeks 52 and 104 (excluding toxicokinetic animals)

Hematology: At necropsy.

Serum Chemistry: At necropsy

Antibody Analysis: Blood samples were collected for possible future analysis for antibodies to ALX-0600 from 12 main study animals/sex/group 5 (1 day prior to treatment start) from the abdominal aorta at necropsy. In addition, blood samples were collected from the jugular vein from 12 toxicokinetic animals/sex/group at approximately 4 hours post dose during Weeks 13 and 26.

Gross pathology: At necropsy

Organ Weights: For each main study animal at necropsy and for each toxicokinetic animal at Week 52, the following organs were weighed: small intestine (duodenum, ileum, jejunum measured together), large intestine (cecum, colon, rectum measured together), pancreas, liver and brain.

Histopathology: All tissues from all main study animals were examined. The bile duct, liver, pancreas (with pancreatic duct), and small and large intestine from the toxicokinetic animals sacrificed on Week 52 were examined. The tissues that were examined in the main study groups are shown in the following list (from page 34-36 of the study report).

abnormalities
animal identification^{****}
adrenals
aorta (thoracic)
bile duct⁺⁺⁺
bone and marrow (sternum)**
bone (femoral head)**
brain (3 levels)
cecum (2 sections)⁺⁺⁺
colon (2 sections)⁺⁺⁺
drainage lymph node of clinically observed external mass^{***}
duodenum⁺⁺⁺
epididymides*
esophagus

eyes*
 Harderian glands
 heart (including section of aorta)
 ileum (2 sections)⁺⁺⁺
 injection sites (2)
 jejunum (3 sections)⁺⁺⁺
 kidneys
 lacrimal glands
 liver (sample of 2 lobes)⁺⁺⁺
 lungs (sample of 2 lobes)⁺⁺
 lymph nodes (mandibular and mesenteric)
 mammary gland (inguinal) (females)⁺
 optic nerves⁺⁺
 ovaries
 pancreas (with pancreatic duct)⁺⁺⁺
 pituitary
 preputial/clitoral gland
 prostate
 rectum⁺⁺⁺
 salivary gland (mandibular, sublingual and parotid)
 sciatic nerve
 seminal vesicles
 skeletal muscle
 skin (inguinal)
 spinal cord (cervical, thoracic and lumbar)
 spleen
 stomach
 testes*
 thymus⁻
 thyroid lobes (and parathyroids)⁺
 tongue
 trachea
 urinary bladder

uterus (cervix, horns and body)
 vagina

Toxicokinetics: On day 28 and on weeks 13, 26 and 52, blood samples were obtained from subgroups of 3-toxicokinetic animals/sex/group/time-point at 0.25, 0.5, 1, 2, 4, 8 and 24 hours post-dose. The following table (from page 32 of the study report) shows the sample collection scheme for TK analysis.

Text Table 5 Toxicokinetic Sample Collection Scheme

Sampling Occasion	Post Dose Sampling Times and Group Assignment			
	0.25 and 4 h	0.5 and 8 h	1 and 24 h	2 h
Day 28	A	B	C	D
Week 13	B	C	D	A
Week 26	C	D	A	B
Week 52	D	A	B	C

Results:

Mortality: In males, survival rate was comparable at the low- and mid-dose compared to control. At high dose, survival rate (62%) of males was lower than control (76%). In females, survival rates were comparable among three treatment groups; however, survival rates of all three treated groups were lower than the control. The following table (from page 41 of the study report) shows the survival data at the end of the treatment period.

Text Table 6 Survival

Group Number Identification	Dose Level (mg/kg/day)	Survival			
		Males	%	Females	%
1/ Vehicle	0	38/50	76	38/50	76
2/ ALX-0600	3	37/50	74	34/50	68
3/ ALX-0600	10	35/50	70	33/50	66
4/ ALX-0600	35	31/50	62	33/50	66

The survival curves for males and females are shown in the following Figures (from page 57 and 58 of the study report).

Figure 1 Survival Curves (%) - Males

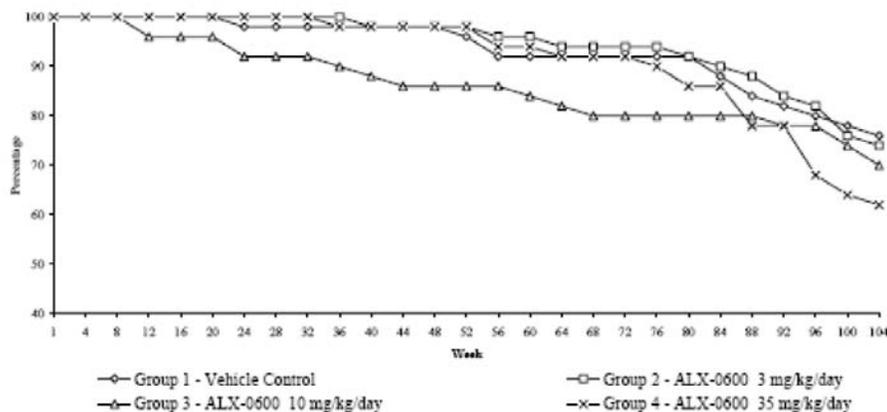
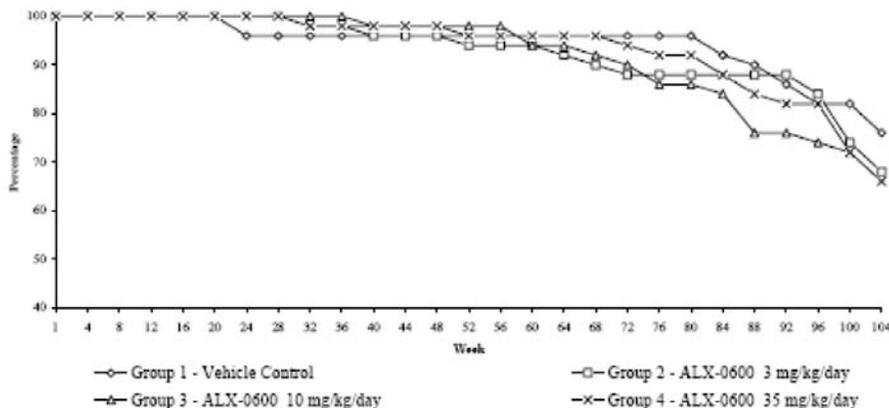


Figure 2 Survival Curves (%) - Females



Clinical signs: Clinically observed masses were noted in higher incidences in all dose groups in males and in high dose females. These masses generally correlated with consistent thickening of the subcutis at the injection sites. These signs were also generally associated with thin fur cover and/or skin papules. The following table (from page 41 of the study report) shows the clinically observed masses.

Text Table 7 **Animals with Clinically Observed Masses**

Group Number Identification	Dose Level (mg/kg/day)	Masses			
		Males	%	Females	%
1/ Vehicle	0	15/50	30	21/50	42
2/ ALX-0600	3	24/50	48	9/50	18
3/ ALX-0600	10	42/50	84	7/50	14
4/ ALX-0600	35	29/50	58	31/50	62

Text Table 8 **Animals with Clinically Observed Masses at Treatment site***

Group Number Identification	Dose Level (mg/kg/day)	Masses			
		Males	%	Females	%
1/ Vehicle	0	2/50	4	0/50	0
2/ ALX-0600	3	0/50	0	0/50	0
3/ ALX-0600	10	2/50	4	0/50	0
4/ ALX-0600	35	5/50	10	13/50	26

* Treatment site includes dorsal thoracic, lumbar, sacral regions as well as numbered treatment sites.

Clinical signs observed across the groups included prominent backbone, thin condition, oily fur, areas of fur staining, and thin fur cover remote from the injection site. Clinical signs associated with generally poor or deteriorating condition were observed in moribund rats from the control and/or treated groups. These signs included abdominal distension, abnormal respiration, decreased activity, cold to touch, dehydration, hunched posture, weak condition, generalized skin pallor, and tremors.

Bodyweights: The mean initial (Week -2) and final (Week 104) weights of control (Group 1) males were 109.6 and 572.5 g, respectively. The mean initial and final weights of control (group 1) females were 96.1 and 389.1 g, respectively. In males, final bodyweights were 97%, 100%, and 99% of control at 3, 10 and 35 mg/kg/day, respectively. In females, final body weights were 86%, 86% and 90% of control at 3, 10 and 35 mg/kg/day, respectively. There was no significant effect of treatment on bodyweight. The following table shows the absolute bodyweights (g) and bodyweight gains (g) for males and females.

Male	1*	2*	3*	4*
Wk 0	110	108	108	108
Wk 24	460	451	455	456
% of Control, Wk 24	100.00	98.04	98.91	99.13
Δ Wk24-Wk0	350	343	347	348
BW Gain, % of Initial BW	318.18	317.59	321.30	322.22
BW Gain, % Of Control	100	99.81	100.98	101.27

Male	1	2	3	4
Wk 0	110	108	108	108
Wk 52	532	517	534	528
% of Control, Wk 52	100.00	97.18	100.38	99.25
Δ Wk52-Wk0	422	409	426	420
BW Gain, % of Initial BW	383.64	378.70	394.44	388.89
BW Gain, % Of Control	100	98.71	102.82	101.37

Male	1	2	3	4
Wk 0	110	108	108	108
Wk 80	568	558	569	558
% of Control, Wk 80	100.00	98.24	100.18	98.24
Δ Wk80-Wk0	458	450	461	450
BW Gain, % of Initial BW	416.36	416.67	426.85	416.67
BW Gain, % Of Control	100	100.07	102.52	100.07

Male	1	2	3	4
Wk 0	110	108	108	108
Wk 104	572	558	576	568
% of Control, Wk 104	100.00	97.55	100.70	99.30
Δ Wk104-Wk0	462	450	468	460
BW Gain, % of Initial BW	420.00	416.67	433.33	425.93
BW Gain, % Of Control	100	99.21	103.17	101.41

Female	1	2	3	4
Wk 0	96	97	96	98
Wk 24	260	253	256	263
% of Control, Wk 24	100.00	97.31	98.46	101.15
Δ Wk24-Wk0	164	156	160	165
BW Gain, % of Initial BW	170.83	160.82	166.67	168.37
BW Gain, % Of Control	100	94.14	97.56	98.56

Female	1	2	3	4
Wk 0	96	97	96	98
Wk 52	301	279	286	294
% of Control, Wk 52	100.00	92.69	95.02	97.67
Δ Wk52-Wk0	205	182	190	196
BW Gain, % of Initial BW	213.54	187.63	197.92	200.00

BW Gain, % Of Control	100	87.87	92.68	93.66
Female	1	2	3	4
Wk 0	96	97	96	98
Wk 80	353	312	313	326
% of Control, Wk 80	100.00	88.39	88.67	92.35
Δ Wk80-Wk0	257	215	217	228
BW Gain, % of Initial BW	267.71	221.65	226.04	232.65
BW Gain, % Of Control	100	82.80	84.44	86.91
Female	1	2	3	4
Wk 0	96	97	96	98
Wk 104	389	336	335	352
% of Control, Wk 104	100.00	86.38	86.12	90.49
Δ Wk104-Wk0	293	239	239	254
BW Gain, % of Initial BW	305.21	246.39	248.96	259.18
BW Gain, % Of Control	100	80.73	81.57	84.92

*GROUP:

1. Control (Water)
2. 3 mg/kg/day
3. 10 mg/kg/day
4. 35 mg/kg/day

The following figures (from page 51 and 52 of the study report) show the growth curves in males and females.

Figure 3 Group Mean Body Weights - Males

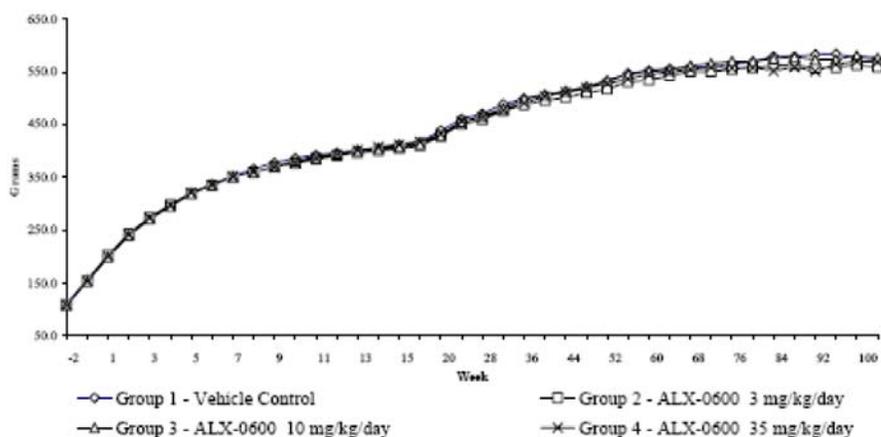
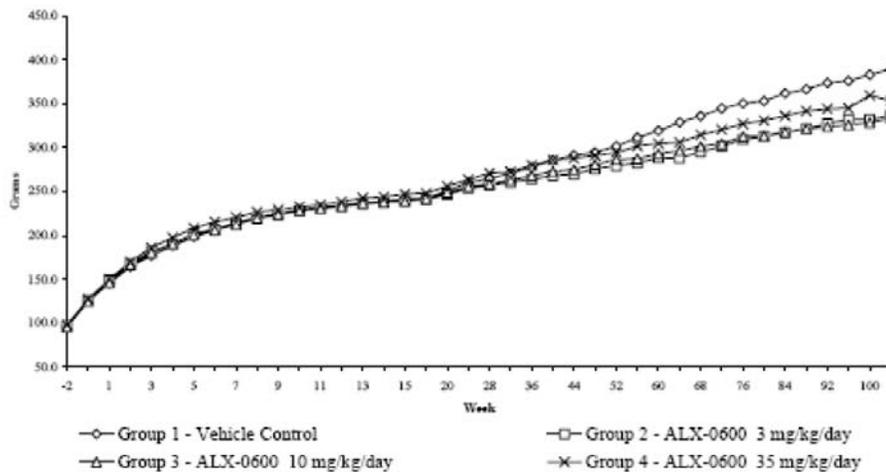


Figure 4 Group Mean Body Weights - Females



Food consumption: The mean initial (week -1) and final (week 104) food consumption in control (group 1) males was 22 and 24 g/animal/day, respectively. The mean initial (week -1) and final (week 104) food consumption in control (group 1) females was 17 and 21 g/animal/day, respectively. There was no significant effect of treatment on food consumption.

Ophthalmoscopy: Corneal opacities (superficial punctate keratopathy) were seen in all groups including control. An increased incidence of diffuse retinal degeneration was observed in males and females of group 4 at week 104. However, there were no histopathological correlates. The following tables (from pg. 128-131 of the study report) show the ophthalmological findings in males and females at week 104.

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Table 5 Incidence of Ophthalmological Findings

Clinical Sign\Site	Week 104 - Males			
	Group 1 - Vehicle Control Group 2 - ALX-0600 3 mg/kg/day	Group 3 - ALX-0600 10 mg/kg/day Group 4 - ALX-0600 35 mg/kg/day	Group	
	1	2	3	4
Number of animals per group	38	37	35	31
Anterior Cortical Cataract\Left	1	5	1	4
Anterior Cortical Cataract\Right	4	6	1	4
Anterior Suture Cataract\Left	-	1	-	-
Anterior Suture Cataract\Right	-	1	1	-
Corneal Opacity\Right	1	-	-	-
Corneal Vascularization\Left	-	1	-	-
Corneal Vascularization\Right	1	2	1	-
Diffuse Retinal Degeneration\Left	1	7	5	9
Diffuse Retinal Degeneration\Right	1	7	5	9
Focal Choriorretinal Atrophy\Left	-	2	-	2
Iritis\Left	-	-	-	1
Mature Cortical Cataract\Left	-	-	-	1
Phthisis Bulbi\Left	1	-	-	-
Large Area of Corneal Ectasia\Right	1	-	-	-
Enlarged Globe\Left	-	-	-	1
Pale Vessels\Left	-	1	-	1
Pale Vessels\Right	-	1	-	1

Table 5 Incidence of Ophthalmological Findings

Week 104 - Males

Clinical Sign/Site	Group			
	1	2	3	4
Persistent Pupillary Membrane\Left	2	.	.	.
Persistent Pupillary Membrane\Right	2	.	.	.
Posterior Cortical Cataract\Left	.	1	.	.
Posterior Cortical Cataract\Right	.	1	.	.
Reddish Discharge\Left	.	.	1	.
Superficial Punctate Keratopathy\Left	37	37	34	30
Superficial Punctate Keratopathy\Right	38	37	34	31

Table 5 Incidence of Ophthalmological Findings

Week 104 - Females

Clinical Sign/Site	Group			
	1	2	3	4
Number of animals per group	38	34	33	33
Anterior Cortical Cataract\Left	2	3	5	3
Anterior Cortical Cataract\Right	5	1	4	4
Anterior Polar Cataract\Right	.	.	1	.
Anterior Suture Cataract\Left	.	4	2	2
Anterior Suture Cataract\Right	.	4	1	2
Corneal Vascularization\Right	.	.	1	.
Diffuse Retinal Degeneration\Left	4	4	4	3
Diffuse Retinal Degeneration\Right	5	3	4	4
Focal Chorioretinal Atrophy\Right	1	1	.	.
Focal Nuclear Opacity\Left	2	.	.	.
Focal Nuclear Opacity\Right	2	.	.	.
Iritis\Right	1	.	1	.
Mature Cortical Cataract\Right	.	.	1	.
Phthisis Bulbi\Right	.	.	1	.
Pale Vessels\Left	1	.	.	.
Pale Vessels\Right	1	.	.	.
Persistent Pupillary Membrane\Left	.	1	1	.

Table 5 Incidence of Ophthalmological Findings

Week 104 - Females				
Group 1 - Vehicle Control	Group 3 - ALX-0600 10 mg/kg/day		Group 4 - ALX-0600 35 mg/kg/day	
Group 2 - ALX-0600 3 mg/kg/day				
Clinical Sign/Site	Group			
	1	2	3	4
Persistent Pupillary Membrane/Right	1	1	1	.
Posterior Cortical Cataract/Right	.	.	.	1
Reddish Discharge/Left	1	.	2	2
Reddish Discharge/Right	.	.	1	.
Superficial Punctate Keratopathy/Left	38	29	31	30
Superficial Punctate Keratopathy/Right	36	29	28	30

Hematology: During week 104, statistically significant increases in absolute and percent neutrophils, absolute monocytes and decrease in percent lymphocytes were noted in males and females at 35 mg/kg/day when compared to respective controls. Statistically significant decreases in red blood cells were noted in treated males when compared to controls. Statistically significant decreases in percent lymphocytes and increases in absolute monocytes were noted in females treated at 10 mg/kg/day when compared to controls and statistically significant decreases in red blood cell counts were noted in females treated at 3 mg/kg/day when compared to controls. At termination, statistically significant increases in absolute neutrophils were noted in females treated at 3 and 35 mg/kg/day. These changes although statistically significant were minimal in nature and did not have any meaningful significance. The following Table shows treatment related changes in mean WBC differential counts (percent and absolute) at Week 104.

WEEK 104 (MALES)						
Group	Neutrophil		Lymphocyte		Monocyte	
	%	Absolute (10 ³ /μL)	%	Absolute (10 ³ /μL)	%	Absolute (10 ³ /μL)
1	28.04	1.498	66.57	3.467	2.51	0.131
2	34.53	1.670	59.73	2.914	2.30	0.111
3	26.18	1.184	68.79	3.311	2.44	0.115
4	38.00*	2.393*	55.41*	3.346	3.22	0.202*
WEEK 104 (FEMALES)						
1	21.17	0.637	72.48	2.258	2.77	0.079
2	25.11	0.820	68.15	2.207	2.93	0.094
3	28.28	1.113	64.30	2.348	3.51	0.103
4	33.46*	1.577*	59.56*	2.802	3.07	0.145

*: P ≤ 0.05

Serum Chemistry: There were no significant treatment-related changes.

Organ Weights: Treatment-related increases were found in the weights of the small and large intestine, liver, and pancreas. Group mean differences are summarized in the following table (from page 46 of the study report) below.

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Text Table 11 Noteworthy Differences in Organ Weights Compared to Control Groups as Percentage Differences

Sex	Dose (mg/kg/day)	Male				Female			
		0	3	10	35	0	3	10	35
Body (gms)		548	519	543	537	363	303	305	327
Small Intestine									
Absolute		-	148	151	206	-	154	151	185
% body		-	160	150	213	-	204	196	216
% brain		-	151	152	213	-	163	154	192
Large Intestine									
Absolute		-	42	36	42	-	38	37	44
% body		-	48	37	45	-	65	62	61
% brain		-	44	37	46	-	43	39	48
Liver									
Absolute		-	26	20	24	-	5	5	16
% body		-	33	20	27	-	26	25	29
% brain		-	27	21	27	-	9	7	19
Pancreas									
Absolute		-	0	-1	7	-	-4	6	10
% body		-	5	-1	9	-	16	25	23
% brain		-	-	-1	10	-	-1	7	13

Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group - $p \leq 0.05$; refer to data tables for actual significance levels and tests used.

Gross pathology: Treatment-related gross pathology changes (thickening, dilatation, enlargement) were observed in injection sites, small and large intestines, kidneys, mesenteric lymph nodes, the abdominal cavity, and extra-hepatic bile ducts. The incidences of the principal treatment-related macroscopic findings are shown in the following table (from page 47 of the study report).

Text Table 12 Incidence of Treatment-related Macroscopic Findings

Tissue/Finding	Sex	Male				Female				
		Dose (mg/kg/day)	0	3	10	35	0	3	10	35
Number of animals examined		50	50	50	50	50	50	50	50	50
Bile duct										
	Dilatation	5	20	27	25	11	19	25	23	
Injection sites										
	Thickening - dorsal thoracic left	-	-	4	17	-	-	2	21	
	Thickening - dorsal thoracic right	-	-	3	19	1	-	1	21	
	Thickening - lumbar left	-	-	6	15	-	-	3	18	
	Thickening - lumbar right	-	-	7	17	-	-	6	18	
	Thickening - scapular left	-	1	-	11	-	-	1	12	
	Thickening - scapular right	-	2	-	12	1	-	2	13	
Stomach										
	Thickening	9	14	21	22	2	9	15	18	
Duodenum										
	Thickening	-	36	36	38	1	36	33	38	
	Mass	-	-	-	-	-	-	-	1	
Jejunum										
	Thickening	-	35	37	39	-	35	34	38	
	Mass	-	1	1	6	-	1	-	-	
Ileum										
	Thickening	-	22	23	34	-	23	27	29	
Cecum										
	Thickening	-	7	7	7	-	5	4	4	
Colon										
	Thickening	1	6	8	9	-	6	7	4	
Rectum										
	Thickening	-	6	8	9	1	5	6	6	
Kidney										
	Discoloration dark	3	9	8	10	14	34	36	40	
Lymph node: mesenteric										
	Enlargement	5	13	11	18	2	10	11	13	
Abdomen										
	Mass	3	1	2	5	1	3	3	5	

An increase in the incidence of abdominal masses was observed at 35 mg/kg/day. Thickening of the subcutis at injection sites was seen at 35 and 10 mg/kg/day. Thickening of the intestines, particularly the small intestine (duodenum, jejunum and ileum) was consistently observed in animals from all treatment groups. A small number of masses in the jejunum and one instance in the duodenum were also recorded, primarily at the high dose level in male animals. Increased incidences in enlarged mesenteric lymph nodes were seen in all treatment groups. Areas of dark discoloration, principally in the corticomedullary region, were reported more frequently in the kidneys of treated animals. This change was especially evident in female animals. The following table (from page 48 of the study report) shows the treatment-related increase in intestinal length.

Text Table 13 Intestinal Group Mean Lengths at Termination

Tissue/Finding	Sex	Male				Female			
		Dose (mg/kg/day)	0	3	10	35	0	3	10
Number of animals examined		38	37	35	31	38	34	33	33
Small intestine									
	Length (mm)	1212	1446	1478	1524	1088	1339	1372	1438
	% difference from control	-	19	22	26	-	23	26	32
Large intestine									
	Length (mm)	237	267	273	290	213	235	246	257
	% difference from control	-	13	15	22	-	10	15	21

Histopathology:

Non-neoplastic: Treatment-related non-neoplastic lesions were observed in the bile ducts (dilatation, mural fibrosis or thickening, epithelial hyperplasia, and proliferation of mural ductules), liver (hyperplasia and proliferation of portal bile ducts, often with an associated periductular fibrosis, as well as a small number of biliary cysts), intestinal tract (villous/mucosal hyperplasia of the intestinal mucosa characterized by a pronounced elongation of villi in the small intestine), injection sites (localized inflammation/fibrosis/degeneration and/or degeneration of the panniculus muscle) and mesenteric lymph nodes (enlarged or dark mesenteric lymph nodes associated with one or more of lymphoid hyperplasia with or without histiocytosis, dilatation of lymph node sinuses and/or erythrophagocytosis). The following tables (pages 50-52 of the study report) show non-neoplastic findings.

Text Table 16 Incidence of Non-Neoplastic Lesions of the Biliary Ducts

Tissue/Finding	Sex	Male				Female			
		Dose (mg/kg/day)	0	3	10	35	0	3	10
Number of animals examined		46	47	44	48	47	46	46	48
Extrahepatic bile duct									
	Epithelial hyperplasia	1	5	17	15	1	2	6	11
	Mural fibrosis/thickening	-	11	25	26	9	19	19	25
	Proliferation of mural ductules	6	9	16	21	-	8	11	14
	Dilatation	9	23	28	36	12	21	31	35
	Number of animals examined	50	50	50	50	50	50	50	50
Liver									
	Bile duct hyperplasia	3	13	25	32	5	11	16	20
	Biliary cysts	1	2	4	2	1	2	1	7

Text Table 17 Incidence of Non-Neoplastic Changes of the Intestine

Tissue/Finding	Sex	Male				Female			
		0	3	10	35	0	3	10	35
Dose (mg/kg/day)		0	3	10	35	0	3	10	35
Number of animals examined		50	50	50	50	50	50	50	50
Duodenum									
Hyperplasia: villous/mucosal		2	42	46	46	2	45	43	47
Jejunum									
Hyperplasia: villous/mucosal		-	42	47	47	-	44	42	46
Ileum									
Hyperplasia: villous/mucosal		-	37	44	45	-	43	37	44
Cecum									
Hyperplasia: villous/mucosal		-	11	11	25	1	12	11	14
Colon									
Hyperplasia: villous/mucosal		-	7	3	17	-	9	8	10
Rectum									
Hyperplasia: villous/mucosal		-	2	-	7	-	4	1	1

Text Table 18 Incidence of Injection Site Lesions

Tissue/Finding	Sex	Male				Female			
		0	3	10	35	0	3	10	35
Dose (mg/kg/day)		0	3	10	35	0	3	10	35
Number of animals examined		50	50	50	50	50	50	50	50
Injection sites									
Inflammation/fibrosis/degeneration		1	3	25	47	2	1	21	50
Degeneration: panniculus muscle		-	-	-	5	-	-	1	5

Text Table 19 Incidence of Mesenteric Lymph-Node Lesions

Tissue/Finding	Sex	Male				Female			
		0	3	10	35	0	3	10	35
Dose (mg/kg/day)		0	3	10	35	0	3	10	35
Number of animals examined		50	50	50	50	50	50	50	50
Mesenteric lymph nodes									
Lymphoid hyperplasia		-	-	3	7	1	-	5	3
Histiocytosis		1	5	2	4	-	9	11	9
Sinusal dilatation		-	3	8	14	1	2	3	4
Erythrophagocytosis		12	13	17	21	9	16	23	19

Neoplastic: In females, there were no significant treatment-related tumor findings. However, in males, trend test was significant for pheochromocytoma in the adrenal ($p = 0.0206$), adenoma in the bile duct ($p = 0.0037$), adenoma in the jejunum ($p = 0.0031$), and c-cell carcinoma in the thyroid ($p = 0.0145$). It is to be mentioned here that adrenal benign pheochromocytoma and thyroid C-cell carcinoma were considered as common tumors, as the incidences were higher than 1% in control and was not considered significant ($p > 0.005$).

Overall, ALX-0600 caused statistically significant increases in the incidences of adenomas in the bile duct ($p = 0.0037$, trend test, 0 of 50, 0 of 50, 1 of 50 and 4 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.050$, pairwise at high dose) and jejunum ($p = 0.0031$, trend test, 0 of 50, 1 of 50, 0 of 50 and 5 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.024$, pairwise at high dose) of male rats. There were no drug-related tumor findings in females.

The following table shows the incidences of neoplastic lesions.

Organ/Tumor	Control (n = 50)	3 mg/kg/day (n = 50)	10 mg/kg/day (n = 50)	35 mg/kg/day (n = 50)		p- Value*	
MALES							
Abdomen							
Osteosarcoma	0	0	0	1/3 (33%)			
Carcinoma: Metastasis	0	0	0	1/3 (33%)			
Adrenal							
Pheochromocytoma (Benign)	2/50 (4%)	1/50 (2%)	0/50	5/50 (10%)		0.0206*	
Pheochromocytoma (Malignant)	1/50 (2%)	0/50	0/50	0/50		1.000	
Adenoma (B, Cortical)	2/50 (4%)	0/50	0/50	1/50 (2%)		0.5281	
Bile Duct							
Adenoma	0/50	0/50	1/44(2.3%)	4/48 (8%)		0.0037**	
Brain							
Oligodendroglioma (Malignant)	0/50	0/50	1/50 (2%)	0/50		0.4740	
Astrocytoma (Malignant)	1/50 (2%)	1/50 (2%)	0/50	0/50		0.9357	
Colon							
Adenoma	0/50	0/50	0/50	1/50 (2%)		0.4286	
Epididymis							
Mesothelioma (B)	0/50	0/50	2/50 (4%)	0/50		0.4529	
Eye							
Schwannoma (B, Intraocular)	0/50	0/50	0/50	1/50 (2%)		0.2199	
Fat							
Metastasis (C)	0/2	0/2	0/6	1/6 (17%)		NA	
Injection Site							
Squamous cell , Papilloma	0/50	1/50 (2%)	0/50	0/50		0.7305	
Histiocytoma, Fibrous	0/50	0/50	1/50 (2%)	0/50		0.4681	
Jejunum							
Adenoma	0/50	1/50 (2%)	0/50	5/50 (10%)		0.0031*	
Adenocarcinoma	0/50	1/50 (2%)	0/50	0/50		0.7305	
Fibrosarcoma	0/50	0/50	1/50 (2%)	0/50		0.4681	
Liver							
Adenoma,Hepatocellular	2/50 (4%)	1/50 (2%)	0/50	1/50 (2%)		0.6208	
Hepatocellular	1/50 (2%)	0/50	0/50	0/50		1.00	

carcinoma							
Cholangioma	0/50	0/50	0/50	1/50 (2%)		0.2199	
Carcinoma: Metastasis	1/50 (2%)	0/50	0/50	1/50 (2%)		NA	
Lung							
Adenoma, Alveolar/Bronchiolar	0/49	0/50	1/50 (2%)	0/50		0.4714	
Sarcoma, metastasis	1/49 (2%)	1/50 (2%)	1/50 (2%)	0/50			
Lymph Node							
Hemangioma	0/27	2/29 (7%)	1/25 (4%)	0/27		0.6525	
Hemangiosarcoma (Mesenteric)	0/50	1/49 (2%)	0/50	0/50		0.8036	
Mammary Gland							
Fibroadenoma	0/50	0/50	1/50 (2%)	0/50		0.4681	
Pancreas							
Adenoma, Acinar cell	1/50 (2%)	1/50 (2%)	1/49 (2%)	2/49 (4%)		0.1602	
Adenoma, Islet cell	0/50	1/50 (2%)	0/50	0/50		0.7305	
Carcinoma, Islet cell	0/50	1/50 (2%)	0/49	1/49 (2%)		0.2733	
Pituitary							
Malignant Schwannoma	0/50	0/49	0/50	1/50 (2%)		0.2609	
Prostate							
Carcinosarcoma	0/50	0/50	0/50	1/50 (2%)		0.2581	
Seminal Vesicle							
Carcinoma, Metastasis	0/50	0/50	0/50	1/50 (2%)		NA	
Skin							
Carcinoma: Squamous cell	0/43	1/35(2.8%)	0/30	0/37		0.7368	
Squamous cell papilloma	0/43	0/35	2/30(6.7%)	1/37(2.7%)		0.1620	
Subcutaneous Tissue							
Fibroma	1/5 (20%)	0/3	2/9 (22%)	2/6 (33%)		0.1802	
Hemangioma	0/5	0/3	1/9 (11%)	1/6 (17%)		0.1570	
Malignant Schwannoma	0/5	2/3 (67%)	1/9 (11%)	0/6		0.7118	
Lipoma	0/5	0/5	1/9 (11%)	1/6 (17%)		0.2388	
Testes							
Adenoma, Interstitial cell	0/50	2/50 (4%)	2/50 (4%)	3/50 (6%)		0.0641	
Thyroid							
Adenoma, Follicular cell	1/50 (2%)	2/50 (4%)	1/50 (2%)	0/50		0.8465	
Adenoma: C-Cell	6/50(12%)	1/50 (2%)	3/50 (6%)	2/50 (4%)		0.8097	
Carcinoma: Follicular cell	1/50 (2%)	1/50 (2%)	0/50	0/50		0.9288	
Carcinoma: C-Cell	1/50 (2%)	0/50	0/50	4/50 (8%)		0.0145*	

Organ/Tumor	Control (n = 50)	3 mg/kg/day (n = 50)	10 mg/kg/day (n = 50)	35 mg/kg/day (n = 50)		p- Value*	
FEMALES							
Abdomen							
Adenocarcinoma	0/2	1/2 (50%)	0/1	0/4		NA	
Metastasis	1/2 (50%)	0/2	1/1 (100%)	0/4		NA	
Carcinoma	0/2	0/2	0/1	1/4 (25%)		NA	
Granular Cell Tumor	0/2	0/2	0/1	1/4 (25%)		NA	
Adrenal							
Pheochromocytoma (Benign)	1/50 (2%)	1/50 (2%)	0/50	0/50		0.9276	
Pheochromocytoma (Malignant)	0/50	0/50	1/50 (2%)	0/50		0.4783	
Adenoma: Cortical	3/50 (6%)	1/50 (2%)	0/50	0/50		0.9949	
Carcinoma: metastasis	0/50	1/50 (2%)	0/50	0/50		NA	
Bile Duct							
Adenoma	0/47	0/46	0/46	1/48 (2%)		0.2370	
Brain							
Granular cell tumor (Benign)	0/50	0/50	1/50 (2%)	0/50		0.4783	
Astrocytoma (Malignant)	0/50	1/50 (2%)	1/50 (2%)	0/50		0.6113	
Colon							
Carcinoma:metastasis	0/50	1/50 (2%)	0/50	0/50		NA	
Fat							
Carcinoma	0/6	2/6 (33%)	0/3	0/5		NA	
Heart							
Malignant Schwannoma: endocardial	0/50	1/50 (2%)	0/50	0/50		0.7372	
Hemolymphoreticular Tissue							
Malignant lymphoma	1/50 (2%)	3/50 (6%)	2/50 (4%)	1/50 (2%)		0.6691	
Ileum							
Adenoma	0/50	0/50	1/50 (2%)	0/50		0.4643	
Histiocytoma, Fibrous	0/50	0/50	1/50 (2%)	0/50		NA	
Jejunum							
Carcinoma	0/50	1/50 (2%)	0/50	0/50		NA	
Kidney							
Adenoma: Tubular cell	0/50	0/50	1/50 (2%)	0/50		0.4783	
Liver							
Adenoma, Hepatocellular	1/50 (2%)	1/50 (2%)	2/50 (4%)	1/50 (2%)		0.4641	
Carcinoma	0/50	1/50 (2%)	0/50	1/50 (2%)		NA	
Sarcoma	0/50	0/50	1/50 (2%)	0/50		NA	
Lymph Node							
Sarcoma, metastasis	0/23	0/27	0/26	1/24 (4%)		NA	
Lymph Node:Mesenteric							

Hemangioma	0/50	1/50 (2%)	2/50 (4%)	2/50 (4%)		0.1773	
Hemangiosarcoma	0/50	2/50 (4%)	2/50 (4%)	0/50		0.7200	
Mammary Gland							
Adenoma	2/50 (4%)	2/50 (4%)	0/50	0/50		0.9817	
Adenocarcinoma	0/50	1/50 (2%)	0/50	1/50 (2%)		0.2897	
Fibroadenoma	5/50 (10%)	1/50 (2%)	4/50 (8%)	6/50 (12%)		0.0924	
Muscle							
Hemangioma	0	0	1 (2%)	0			
Pancreas							
Adenoma, Acinar cell	1 (2%)	1 (2%)	1 (2%)	2 (4%)			
Adenoma, Islet cell	0	1 (2%)	0	0			
Carcinoma, Islet cell	0	1 (2%)	0	1 (2%)			
Ovary							
Cystadenoma	0/50	1/50 (2%)	1/50 (2%)	0/50		0.5970	
Adenoma: Sertoliform tubular	3/50 (6%)	1/50 (2%)	0/50	0/50		0.9949	
Granulosa cell tumor	0/50	0/50	0/50	1/50 (2%)		0.2391	
Carcinoma	0/50	1/50 (2%)	0/50	1/50 (2%)		NA	
Sarcoma: metastasis	0/50	0/50	1/50 (2%)	0/50		NA	
Pancreas							
Carcinoma: Islet Cell	0/49	0/49	1/48 (2%)	1/48 (2%)		0.2391	
Carcinoma: metastasis	0/49	1/49 (2%)	0/48	2/48 (4%)		NA	
Sarcoma: metastasis	1/49 (2%)	0/49	2/48 (4%)	0/48		NA	
Pituitary							
Adenoma: Pars distalis	32/50 (64%)	31/50 (62%)	28/50 (56%)	31/50 (62%)		0.4873	
Adenoma: Pars intermedia	0/50	0/50	1/50 (2%)	0/50		0.4783	
Rectum							
Papilloma	0/50	0/50	1/50 (2%)	0/50		0.4783	
Skin Miscellaneous							
Keratoacanthoma	0/25	0/25	0/14	1/25 (4%)		0.2391	
Stomach							
Adenocarcinoma	0/50	0/50	1/50 (2%)	0/50		0.4745	
Carcinoma	0/50	1/50 (2%)	0/50	2/50 (4%)			
Subcutaneous Tissue							
Malignant Schwannoma	0/4	0/4	0/2	1/11 (9%)		0.2547	
Carcinoma: metastasis	0/4	1/4 (25%)	0/2	0/11		NA	
Thyroid							
Adenoma, Follicular cell	0/50	0/50	2/50 (4%)	1/50 (2%)		0.1889	
Adenoma: C-Cell	4/50 (8%)	1/50 (2%)	2/50 (4%)	3/50 (6%)		0.3833	
Carcinoma: Follicular cell	1/50 (2%)	1/50 (2%)	0/50	0/50		NA	
Carcinoma: C-Cell	3/50 (6%)	0/50	1/50 (2%)	0/50		0.9321	
Urinary Bladder							
Sarcoma: metastasis	0/50	0/50	0/50	1/49 (2%)		NA	
Carcinoma:	0/50	1/50 (2%)	0/50	0/49		NA	

metastasis							
Uterus							
Adenocarcinoma: endometrial	1/50 (2%)	4/50 (8%)	1/50 (2%)	4/50 (8%)		0.1488	
Adenoma: endometrial	0/50	1/50 (2%)	1/50 (2%)	0/50		0.5970	
Leiomyoma	0/50	0/50	1/50 (2%)	0/50		0.4783	
Sarcoma: endometrial stromal	0/50	1/50 (2%)	2/50 (4%)	0/50		0.6563	
Malignant schwannoma	2/50 (4%)	0/50	1/50 (2%)	2/50 (4%)		0.2771	
Deciduoma	0/50	1/50 (2%)	0/50	0/50		NA	
Polyp: endometrial stromal	5/50(10%)	2/50 (8%)	5/50 (10%)	6/50 (12%)		0.1829	
Carcinoma: metastasis	0/50	1/50 (2%)	0/50	0/50		NA	

*: Significant in Trend test (p <0.05 for rare tumors and <0.005 for common tumors)

Toxicokinetics: T_{max} was reached between 0.28-1.0 hours post-dose. The T_{max} values showed no apparent gender differences. Both C_{max} and $AUC_{0-t_{last}}$ values increased with dose and were generally higher in males compared to females from all groups on all sampling occasions. Overall, C_{max} increased in a less than proportional manner with increasing dose in both genders, but $AUC_{0-t_{last}}$ generally increased in a proportional manner. The TK parameters are shown in the following table (from page 45 of the report).

Text Table 10 Toxicokinetic Parameters of ALX-0600 in Plasma Following Subcutaneous Injection of ALX-0600

Gender	Group Number	Dose Level (mg/kg/day)	Day 28				Week 52			
			t_{max} (h)	C_{max} (ng/mL)	t_{last} (h)	$AUC_{0-t_{last}}$ (ng•h/mL)	t_{max} (h)	C_{max} (ng/mL)	t_{last} (h)	$AUC_{0-t_{last}}$ (ng•h/mL)
Males	2	3	0.5	1634	8	2447	0.5	1558	8	2279
	3	10	0.5	4056	8	7279	1	2805	8	7494
	4	35	1	10431	8	29357	1	9241	8	36494
Females	2	3	0.283	1348	4	1500	0.5	1128	8	2243
	3	10	0.5	2756	8	4076	1	1992	8	4875
	4	35	0.5	4853	8	10827	0.5	8026	8	22875

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The study conduct appears to be appropriate and acceptable. The dose selection based on a pharmacokinetic endpoint (AUC ratio) appears to be acceptable, as the high dose produced about a 25-fold human exposure (total daily AUC of about 30 $\mu\text{g} \cdot \text{hr}/\text{ml}$ in rats, as compared to 1.2 $\mu\text{g} \cdot \text{hr}/\text{ml}$ at the

expected maximum human dose of 0.2 mg/kg/day). Furthermore, the high dose did not produce excessive mortality. The test model (Wistar Han rats) selection was in concurrence with the Agency. Overall, the study was conducted in a valid and acceptable manner.

Evaluation of tumor findings: In females, the trend test was negative ($p > 0.05$). However, in males, the trend test was significant for benign pheochromocytoma ($p = 0.0206$) in the adrenal gland, adenoma in the bile duct ($p = 0.0037$), adenoma in the jejunum ($p = 0.0031$), and c-cell carcinoma in the thyroid ($p = 0.0145$). However, benign pheochromocytoma in the adrenal and C-cell carcinoma in the thyroid were considered as common tumors as the incidences in the control were higher than 1% and were not considered as significant ($p > 0.005$). The following table (from page 854 of the study report) shows the p-values for the trend test in males.

ORGAN NAME	TUMOR NAME	P-VALUE
Adrenal	Benign Pheochromocytoma	0.0206
Bile Duct	Adenoma	0.0037
Jejunum	Adenoma	0.0031
Thyroid	Carcinoma c-cell	0.0145

In addition to the overall trend test, pair-wise comparisons were also made using Peto's one-sided trend test to determine if the tumor rate in each treated group was significantly higher than the one in the control group 1. In females, pair-wise comparisons were negative ($p > 0.05$). However, in males, the incidence rates of bile duct adenoma ($p = 0.0465$) and jejunum adenoma ($p = 0.0332$) were statistically significantly higher in group 4 compared to respective controls. These p-values for pair-wise comparisons for the high-dose males are shown in the following table (from page 854 of the study report):

Organ Name	Tumor Name	Comparison	P-Value
Bile Duct	Adenoma	1 vs 4	0.0465
Jejunum	Adenoma	1 vs 4	0.0332

Overall, ALX-0600 caused statistically significant increases in the incidences of adenomas in the bile duct ($p = 0.0037$, trend test, 0 of 50, 0 of 50, 1 of 50 and 4 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.050$, pairwise at high dose) and jejunum ($p = 0.0031$, trend test, 0 of 50, 1 of 50, 0 of 50 and 5 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.024$, pairwise at high dose) of male rats. There were no drug-related tumor findings in females.

Carcinogenicity summary: In a 104-week subcutaneous carcinogenicity study in Wistar Han rats, animals ($n = 50$ /sex/dose) were treated at 0 (solution/phosphate buffer with Mannitol and L-

histidine), 3, 10, and 35 mg/kg/day. The dose selection was based on a pharmacokinetic endpoint (AUC ratio). The dose selection appears to be acceptable, as the high dose produced about a 25-fold human exposure (total daily AUC of about 30 $\mu\text{g}\cdot\text{hr}/\text{ml}$ in rats, as compared to 1.2 $\mu\text{g}\cdot\text{hr}/\text{ml}$ at the expected maximum human dose of 0.2 mg/kg/day). Furthermore, the high dose did not produce excessive mortality. In males, survival appears to be comparable at the low- and mid-dose when compared to the control. However, at high dose, the survival rate (62%) of males was lower than control (76%). In females, survival rates appeared to be comparable among three treatment groups, however, the survival rates at all doses were lower than the control. There was no significant meaningful effect of treatment on body weight or food consumption. High incidence of corneal opacities (superficial punctate keratopathy) was seen in all groups. An increased incidence of diffuse retinal degeneration was observed in males and females of group 4 (week 104). These findings did not correlate with any treatment-related histopathological findings. Treatment-related increases were seen in the weights of the small and large intestine, liver and pancreas. Treatment-related gross pathology changes (thickening, dilatation, and enlargement) were observed in injection sites, small and large intestines, kidneys, mesenteric lymph nodes, abdominal cavity and extra-hepatic bile ducts. Treatment-related non-neoplastic lesions were observed in the bile ducts (dilatation, mural fibrosis or thickening, epithelial hyperplasia and proliferation of mural ductules), liver (hyperplasia and proliferation of portal bile ducts, often with an associated periductular fibrosis, as well as a small number of biliary cysts), intestinal tract (villous/mucosal hyperplasia of the intestinal mucosa characterized by a pronounced elongation of villi in the small intestine), injection sites (localized inflammation/fibrosis/degeneration and/or degeneration of the panniculus muscle) and mesenteric lymph nodes (enlarged or dark mesenteric lymph nodes associated with one or more of lymphoid hyperplasia with or without histiocytosis, dilatation of lymph node sinuses and/or erythrophagocytosis). Overall, ALX-0600 caused statistically significant increases in the incidences of adenomas in the bile duct ($p = 0.0037$, trend test, 0 of 50, 0 of 50, 1 of 50 and 4 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.050$, pairwise at high dose) and jejunum ($p = 0.0031$, trend test, 0 of 50, 1 of 50, 0 of 50 and 5 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.024$, pairwise at high dose) of male rats. There were no drug-related tumor findings in females. The study conduct was acceptable and valid.

Carcinogenicity conclusions: ALX-0600 caused statistically significant increases in the incidences of adenomas in the bile duct ($p = 0.0037$, trend test, 0 of 50, 0 of 50, 1 of 50 and 4 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.050$, pairwise at high dose) and jejunum ($p = 0.0031$, trend test, 0 of 50, 1 of 50, 0 of 50 and 5 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.024$, pairwise at high dose) of male rats. There were no drug-related tumor findings in females.

SUMMARY AND EVALUATION: ALX-0600 is a 33-amino acid recombinant analog of human glucagon-like peptide (GLP-2). It has been shown to increase the absorptive surface and weight of small and large intestines in rats, mice and monkeys. The sponsor is developing the drug for the treatment of short bowel syndrome (SBS). In this submission, the sponsor presented the final report of a 104-week carcinogenicity study in Wistar Han rats.

In a 104-week subcutaneous carcinogenicity study in Wistar Han rats, animals ($n = 50/\text{sex}/\text{dose}$) were treated at 0 (solution/phosphate buffer with Mannitol and L-histidine), 3, 10, and

35 mg/kg/day. The dose selection was based on a pharmacokinetic endpoint (AUC ratio). The dose selection appears to be acceptable, as the high dose produced about a 25-fold human exposure (total daily AUC of about 30 $\mu\text{g}\cdot\text{hr}/\text{ml}$ in rats, as compared to 1.2 $\mu\text{g}\cdot\text{hr}/\text{ml}$ at the expected maximum human dose of 0.2 mg/kg/day). Furthermore, the high dose did not produce excessive mortality. In males, survival appears to be comparable at the low- and mid-dose when compared to the control. However, at high dose, the survival rate (62%) of males was lower than control (76%). In females, survival rates appeared to be comparable among three treatment groups, however, the survival rates at all doses were lower than the control. There was no significant meaningful effect of treatment on bodyweight or food consumption. High incidence of corneal opacities (superficial punctate keratopathy) was seen in all groups. An increased incidence of diffuse retinal degeneration was observed in males and females of group 4 (week 104). These findings did not correlate with any treatment-related histopathological findings. Treatment-related increases were seen in the weights of the small and large intestine, liver and pancreas. Treatment-related gross pathology changes (thickening, dilatation, and enlargement) were observed in injection sites, small and large intestines, kidneys, mesenteric lymph nodes, abdominal cavity, and extra-hepatic bile ducts. Treatment-related non-neoplastic lesions were observed in the bile ducts (dilatation, mural fibrosis or thickening, epithelial hyperplasia and proliferation of mural ductules), liver (hyperplasia and proliferation of portal bile ducts, often with an associated periductular fibrosis, as well as a small number of biliary cysts), intestinal tract (villous/mucosal hyperplasia of the intestinal mucosa characterized by a pronounced elongation of villi in the small intestine), injection sites (localized inflammation/fibrosis/ degeneration and/or degeneration of the panniculus muscle) and mesenteric lymph nodes (enlarged or dark mesenteric lymph nodes associated with one or more of lymphoid hyperplasia with or without histiocytosis, dilatation of lymph node sinuses and/or erythrophagocytosis). Overall, ALX-0600 caused statistically significant increases in the incidences of adenomas in the bile duct ($p = 0.0037$, trend test, 0 of 50, 0 of 50, 1 of 50 and 4 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.050$, pairwise at high dose) and jejunum ($p = 0.0031$, trend test, 0 of 50, 1 of 50, 0 of 50 and 5 of 50 animals at 0, 3, 10 and 35 mg/kg/day, respectively; $p = 0.024$, pairwise at high dose) of male rats. There were no drug-related tumor findings in females. The study conduct was acceptable and valid.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Fertility and Reproductive Performance Study in Rats (Segment I) by Subcutaneous Route (Study No. 98357)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

Fertility and Reproductive Performance Study in Rats (Segment I) by Subcutaneous Route

Study No.: 98357

Volume and Page: Amendment 56, Vol. 6, page 1

Testing Laboratory: [REDACTED] (b) (4)

Dates of Start and Completion of Study: September 15, 2003 and February 18, 2004.

GLP & QAU Requirements: A statement of compliance with GLP regulations was submitted. A QAU statement was also included in the report.

Species and Strain: Crl:CD (SD)IGS BR rats. Male rats were approximately 12 weeks old with mean body weight of 349 to 431 g. Female rats were 9 weeks of age and weighed between 183 and 227 g.

Batch and Purity: 08503021 and 98.7%

Methods: ALX-0600 was subcutaneously administered to 4 groups (22/sex) of rats at doses of 0 (vehicle), 2, 10, or 50 mg/kg/day (in 2 divided doses, 8 hr apart) to study its effects on the reproduction and fertility of rats. Males were treated 28 days prior to mating, during and following mating till terminal sacrifice, and females were treated for a period of 2 weeks prior to mating, during mating and until GD7 (gestation day 7). The mating of 1/sex animal was done in a cage for a maximum period of 21 days and the day of positive presence of sperm in vaginal lavage was GD0. The vehicle was phosphate buffer containing mannitol and L-histidine. The dose volume was 1.25 ml/kg. Dose selection was based on the potential human exposure, existing toxicity data and any limitations imposed by the test article. Male rats were observed daily for general condition and weighed twice weekly during the treatment period. Mated female body weights were determined on GD0-3, 3-7, 7-10 and 10-13 and food consumption of mated females were determined on GD0-3, 3-7, 7-10 and 10-13. Females were euthanized on GD13. The males were sacrificed approximately 3 weeks after the end of the mating period and epididymides, small and large intestines, prostate, seminal vesicles and testes were separated and weighed. A computer assisted sperm analyzer program was used to study sperm morphology, counts and motility. All females were subjected to necropsies in which the external examination, lesions and internal organ examination and whole reproductive tract was examined. The following parameters were studied: mating index, fertility index, conception rate, the number of corpora lutea, live fetuses, resorptions, pre- and post-implantation losses. For each male in the control and high dose groups, the right testes were prepared for histopathological examinations. Testicular histopathological evaluations included the assessment of spermatogenic cycle.

Results: There were no mortalities. Tail bleeding was seen in 13, 14, 14 and 17 males and, 11, 11, 13 and 6 females out of 22/sex at 0, 2, 10 and 50 mg/kg/day, respectively. Dark areas were seen at left scapular injection site in 5, 3 and 10 males at 2, 10 and 50 mg/kg/day. The initial body weights of the males during the pre-mating period were 489.5, 480.2, 484.2 and 474.5 g at 0, 2, 10 and 50 mg/kg/day, respectively. On study Day 66 (termination day of males) body weights of males were 565.8, 542.7, 542.2 and 432.0 g, respectively at 0, 2, 10 and 50 mg/kg/day. There was a retardation of body weight gain of 4.1, 4.1 and 56.0 % in males at 2, 10 and 50 mg/kg/day, respectively, when compared to control. The body weight of females during the pre-mating period and up to GD13 was similar. The absolute weight of large and small intestines in males treated with ALX-0600 was increased in dose proportional manner. The increase of large intestine weights were 118.5, 119.1 and 126.0% of control in males and 104.3, 109.5, 113.4% of control in females (control males and female weights were 4.961 and 3.53 g, respectively) at 2, 10 and 50 mg/kg/day, respectively. Thickening of duodenum was noted in 0, 15, 20 and 21 males and 0, 8, 11 and 16 in females at 0, 2, 10, and 50

mg/kg/day, respectively. The increase of small intestine weights was 169.1, 178.5 and 184.1 % in males and, 121.1, 126.9 and 152.8% in females of the control group (control males/female weighed 11.74/7.99 g) at 2, 10 and 50 mg/kg/day, respectively.

The average number of estrus cycles were 2.4, 2.9, 2.5 and 2.7 with an average cycle length of 4.3, 3.9, 4.2 and 4.2 at 0, 2, 10 and 50 mg/kg/day, respectively. All dams survived to the scheduled sacrifice, with no gross pathologic changes observed. No treatment-related effects on mating and fertility index, uterine findings (number of corpora lutea, implantations, live and dead embryos, resorptions and pre- and post-implantation losses). The following table represents a summary of the reproductive performance parameters of male and female rats examined.

Parameters	Dose (mg/kg/day)			
	0	2	10	50
# Animals	22	22	22	22
# Matings	22	21	22	22
Mating Index (%)	100	95.5	100.0	100.0
Fertility Index (%)	100	90.9	95.5	100
Conception Rate (%)	100	95.2	95.5	100
# Corpora lutea	19.2 ± 2.5	17.7 ± 1.5	18.4 ± 2.4	18.5 ± 2.5
# Implantations	17.0 ± 1.9	16.3 ± 1.6	16.5 ± 2.1	16.8 ± 2.2
# Live embryos	16.3 ± 1.6	15.5 ± 1.93	16.0 ± 2.2	16.1 ± 2.0
# Dead embryos	0	0	0	0
Sum of early resorptions and dead embryos	0.7 ± 0.9	0.7 ± 0.98	0.6 ± 1.33	0.7 ± 1.09
# Early Resorptions	0.7 ± 0.88	0.7 ± 0.98	0.6 ± 1.33	0.7 ± 1.09
Pre-Implantation Losses (%)	10.9 ± 7.9	7.9 ± 5.4	10.1 ± 6.4	8.7 ± 7.8
Post-implantation loss (%)	4.1 ± 4.8	4.4 ± 5.9	3.3 ± 7.1	4.0 ± 6.3
Sperm Evaluation				
Cauda Epididymis Wt.	0.37 ± 0.042	0.36 ± 0.36	0.35 ± 0.03	0.38 ± 0.37
Spermatozoa/g (in 10 ⁶)	670.8 ± 108.7	648.0 ± 118.6	660.3 ± 132.6	653.6 ± 104.8
Motility (%)	68.1 ± 8.9	69.5 ± 8.7	67.7 ± 9.1	69.7 ± 5.8

In a subcutaneous Segment I study in rats, groups of males and females were treated with ALX-0600 at 2, 10 and 50 mg/kg (in 2 divided doses, 8 hours apart). Male and female reproductive performances were not affected by the treatment. The NOAEL for male and female reproductive performance was considered as 50 mg/kg/day. There was no effect on early embryonic development at ≤50 mg/kg/day, s.c.

9.2 Embryonic Fetal Development

Subcutaneous Segment II Teratology Study in Rats (Study No. 7203-117)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

Study No. 7203-117

Volume and Page: Amendment 050, Vol. 1, Page 1

Testing Laboratories:

(b) (4)

Study Started: March 20, 2003

Study Completed: December 4, 2003

GLP Requirements: A Statement of compliance with the GLP regulations and quality assurance unit was included.

Animals: Sprague Dawley pregnant female rats (CrI:CD(SD)IGSBR), 10 to 12 weeks of age, weighing 222–296 g.

Batch No. and Purity: 0850204, 98.5%

Methods: One hundred time mated pregnant female rats (25/group, four groups) were administered ALX-0600 at the subcutaneous doses of 0.0 (vehicle control), 2, 10, and 50 mg/kg/day (1, 5 and 25 mg/kg bid) in 2 divided doses (8 hr apart) from GD6 through GD17. ALX-0600 was administered in a phosphate buffer containing L-histidine and mannitol at pH to 7.26 to 7.4 in a volume of 1.25 ml/kg. The basis of dose selection was not provided by the sponsor. Clinical observations were made twice daily for mortality and moribundity throughout the study, i.e., 1 hr after the first and the second dose. The body weights and daily food intake of the main study animals were recorded on GD0, 4, 6, 8, 10, 12, 14, 16, 18 and 20. The blood samples from animals of toxicokinetic subgroup (Groups 2-4, 9/group) were collected on GD6 and GD17 at 0, 0.25, 0.5, 1, 2, 4 and 8 hr postdose. On GD20, all dams were necropsied and uteri were evaluated for the number of live and dead fetuses and resorptions and ovaries were evaluated for the number of corpora lutea. Live fetuses were sexed, weighed and examined for external, visceral and skeletal abnormalities.

Results: There were no mortalities. Body weights of treated rats were similar to those of control animals. Mean initial body weights were 224.4, 225.2, 223.7 and 225.4 g at control, 2, 10 and 50 mg/kg/day, respectively. On GD 20, final body weights were 378.3, 372.1, 371.6 and 373.7 g at 0, 2, 10 and 50 mg/kg/day, respectively. The mean initial and final food consumption in control animals were 25.3 and 28.2 g/animal/day, respectively. The mean final food consumption in treated animals was 27.1, 27.6 and 28.1 g/animal/day at 2, 10 and 50 mg/kg/day, respectively. There were no significant treatment-related effects on food consumption. There were no maternal necropsy findings, uterine weight or cesarean section parameters. Fetal viability and body weights, fetal external findings and visceral findings were also similar across groups. There was a

significant increase in the incidence of incomplete ossification of the skull and wavy/bent ribs in all treated groups. These types of fetal variations are generally considered reversible and are not considered to be incompatible to survival. There were no skeletal malformations. The cesarean findings are shown in the following table.

	Vehicle Control	ALX-0600 (mg/kg/day)		
		2	10	50
#Pregnant Animals Used	24	25	24	24
Cesarean Parameters				
Mean #Corpora Lutea + SD	16.0 ± 2.7	14.3 ± 2.4	16.1 ± 2.8	15.4 ± 2.2
Mean #Implantation Sites ± SD	14.1 ± 1.7	12.9 ± 2.3	13.5 ± 2.7	13.5 ± 2.1
Mean % Pre-Implant. Loss	10.9 ± 9.0	9.5 ± 11.9	16.0 ± 11.4	12.2 ± 10.2
Post implantation loss, %	0.8 ± 2.2	1.8 ± 4.1	4.3 ± 9.3	5.2 ± 20.5
Non Viable ¹ SD	0	0	0	0
Resorptions				
Total (%) ± SD	0.1 ± 0.3	0.2 ± 0.5	0.5 ± 1.0	0.4 ± 1.1
Early ± SD	0.1 ± 0.3	0.2 ± 0.5	0.2 ± 0.4	0.4 ± 1.1
Late ¹ ± SD	0.0	0.0	0.3 ± 1.0	0.0 ± 0.0
# Live fetuses	14 ± 1.6	12.7 ± 2.3	13.0 ± 3.1	13.2 ± 2.9
Sex Ratio (M/F)	53/47	51/49	48/52	52/48
Fetal Body Wt.				
Males(g) ± SD	3.83 ± 0.30	3.92 ± 0.26	3.8 ± 0.31	3.74 ± 0.30
Females(g) ± SD	3.61 ± 0.30	3.71 ± 0.15	3.6 ± 0.30	3.57 ± 0.32

¹: Values indicate the mean value per litter

The tabulated summary of the incidence of fetal external, visceral and skeletal variations and malformations is shown below.

Parameter	0 mg/kg/day	2 mg/kg/day	10 mg/kg/day	50 mg/kg/day
External Observations				
No. of Litters examined	24	25	24	23
No. of fetuses examined	336	317	312	316
No. of fetuses with abnormalities	0	0	0	0
Visceral Observations				
No. of dams	24	25	24	23
No. of fetuses examined	169	158	156	156
No. of visceral variations	1	3	3	1
Variations of the major vessels	0	0	1	0
Intermediate lobe of lung small/missing	0	0	1	0
Dilated ureter	0	0	1	1
Dilated cerebral ventricles	1	3	0	0

Skeletal Observations				
Litters Evaluated	24	25	24	23
Fetuses Evaluated	167	158	156	159
No. of skeletal anomalies	16	14	11	12
Unossified hyoid body	21	38	22	25
Incomplete ossification of skull	4	28	18	25
Less than four caudal vertebrae ossified	37	30	55	44
Wavy/bent ribs	0	7	9	12
5 th /6 th sternebrae incomplete ossification	48	55	62	40

Toxicokinetics: After subcutaneous administration, ALX-0600 exposure (C_{max} and AUC_{0-8h}) was approximately dose proportional. No marked gender differences in exposure were observed on Day 6 or Day 17. Values for C_{max} and AUC_{0-8h} on Day 17 appeared to be higher than those observed on Day 6, indicating possible accumulation following multiple exposures. The TK parameters are shown in the following table.

Gestation Day	Dose (mg/kg/day)	T _{max} (hr)	C _{max} (ng/ml)	AUC _(0-8h) (ng.hr/ml)	T _{1/2} (hr)
6	2	0.25	1398	1644	0.55
	10	0.5	4109	5863	0.651
	50	1.0	13602	39228	0.808
17	2	0.25	1023	1569	0.632
	10	0.50	2298	5514	0.654
	50	0.50	11162	51493	1.71

In a Segment II s.c. teratology study in rats, animals were treated with ALX-0600 at 2.0, 10 or 50 mg/kg/day (1, 5 and 25 mg/kg, bid, 8 hours apart) during the period of organogenesis (GD6-17). ALX-0600 did not cause any significant maternal or fetal toxicity. ALX-0600 does not appear to be teratogenic in rats at the tested doses. The NOAEL for developmental toxicity appeared to be ≥ 50 mg/kg/day.

Subcutaneous Teratology Study in Rabbits (Study No. (b) (4)-487001)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated December 19, 2006.

Subcutaneous Segment II Teratology Study in Rabbits

Study No.: (b) (4)-487001

Volume and Page: Amendment 056, Vol. 8, page 1

Testing Laboratories: (b) (4)

Initiation Date: August 14, 2003

Completion Date: February 24, 2004

GLP Requirements: Statement of compliance with the GLP regulations and quality assurance unit (QAU) statement were included.

Animals: Artificially inseminated Female New Zealand White Rabbits [Hra:(NZW)SPF], 6 months of age, weighing 3.22-3.26 kg.

Drug Batch No. and Purity: 08503021 and 98.7%

Methods: Pregnant New Zealand White Rabbits (22/group) were administered ALX-0600 at the subcutaneous doses of 0.0 (vehicle control), 2, 10, and 50 mg/kg/day (1.25 ml/kg) in 2 divided doses (8 hours apart) during GD7 through GD20. The highest dose was selected based on the solubility of the test article. The sponsor stated that this (50 mg/kg) is the maximum feasible dose (MFD) based on the solubility. Clinical observations were made twice daily for mortality and moribundity throughout the study, i.e., 1 hr after the first and the second dose. The body weights and daily food intake of the main study animals were recorded on GD0, 7 to 21, 24, and 29. Mean body weight of TK animals were determined on GD0 and 7 to 20. Blood samples were collected from TK animals on GD7 and 20 at 0, 15, 30 min, 1, 2, 4 and 8 hr postdose. On GD29, all dams were necropsied, with determinations of the numbers of corpora lutea, live fetuses (viable implants), resorptions (nonviable implants), and any abnormal uterine conditions were recorded. Fetuses were identified, weighed and examined externally. Live fetuses were sacrificed and then examined for external, visceral and skeletal abnormalities.

Results: One rabbit each in the vehicle control and 50 mg/kg dose groups aborted on GD29 and GD21, respectively. The mean initial and final body weight of control animals were 3.242 and 4.008 kg, respectively. There were no significant treatment-related changes in the body weight. The mean initial and final food consumption in control animals were 213 and 144 g/animal/day, respectively. There were no significant treatment-related effects on food consumption. Three, 2, 3 and 5 out of 22/group rabbits at 0, 2, 10 and 50 mg/kg/day treatment groups were non-gravid (ammonium sulfide test negative). Thickening of the tissues at the injection site were seen in 0, 3, 5 and 16 rabbits and, red areas were seen in 2, 1, 4 and 11 rabbits of 22/group animals at 0, 2, 10 and 50 mg/kg/day, respectively.

The number of late resorptions at 2 mg/kg/day (6.5% per litter) was significantly higher when compared to, which also exceeded (b) (4) developmental historical control data (6.2% per litter). However, this increase was not evident at 10 or 50 mg/kg/day and was not considered to be treatment-related. The results showed a slight but statistically significant trend for early resorptions in 50 mg/kg/day treatment group compared to control. This was not considered treatment-related. This was attributed, in part, to female no. 38396, which had 60% early resorptions. In addition, this increase was within

the ^{(b) (4)} historical control range (the 25th and 75th quartile values for early resorptions were 4.9% and 9.6% per litter, respectively). Thus, the increasing trend for early resorptions does not appear to be a biologically significant effect. The Cesarean section data are shown in the following table.

	Vehicle Control	ALX-0600, SC (mg/kg/day)		
		2	10	50
# Pregnant Rabbits	18	20	19	16
Cesarean Parameters				
No. of Implantation Sites	6.8 ± 2.84	7.1 ± 2.80	7.0 ± 7.3	7.3 ± 1.91
Mean % Living Fetuses	98.7	89.7*	98.5	92.5
No. Corpora Lutea (Mean) ± SD	9.0 ± 2.35	8.8 ± 2.55	8.6 ± 2.0	8.8 ± 1.94
No. of Dead Fetuses	0	0	0	0
Resorptions				
Total (%)	1.3 ± 3.89	10.3 ± 13.63*	1.5 ± 4.48	7.5 ± 15.79
Early ± SD	1.3 ± 3.89	3.8 ± 7.25	1.5 ± 4.48	6.8 ± 15.88
Late ± SD	0	6.5 ± 11.5*	0.0 ± 0.0	0.6 ± 2.5
Sex Ratio (M/F)	4.3/2.3	3.2/3.1	3.6/3.3	3.8/3.1
Fetal Body Wt.				
Males(g) ± SD	46.4 ± 4.59	46.1 ± 4.46	47.7 ± 5.73	47.3 ± 5.16
Females(g) ± SD	45.5 ± 5.25	45.4 ± 5.17	45.9 ± 4.59	46.0 ± 5.47

* Significantly less than vehicle control (p < 0.01)

There were no significant test article-related fetal external, visceral or skeletal malformations. Two and four fetuses at 2 and 10 mg/kg/day, respectively, had visceral malformations. Fetus nos. 38366-10 and 38398-02 at the 10 mg/kg/day exhibited hydrocephaly characterized by increased cavitation of the lateral (bilateral) and third ventricles. Lobular agnesis of the lungs (absent right accessory lobe) was noted for fetus nos. 38433-03 and 38436-05 at 2 mg/kg/day and in fetus nos. 384060-09 and 38419-09 at the 10 mg/kg/day. The incidences of external and visceral variations/malformations were not dose- or treatment-related. There were no single type of variation/malformation, which occurred in more than one litter, and none of the findings appeared related to dose or treatment-related. Thus, the observed incidence of external and visceral malformations probably represents chance occurrences and was not considered related to treatment. The incidences of fetal external, visceral and skeletal anomalies are shown in the table below.

Parameter	0 mg/kg/day	2 mg/kg/day	10 mg/kg/day	50 mg/kg/day
External Observations				
No. of Litters examined	18	20	19	16
No. of fetuses examined	120	125	131	109
No. of fetuses with abnormalities	2	2	5	1

Thoracogastroschisis	0	0	1	0
Adactyly	0	0	1	0
Malrotated paw	0	0	1	0
Hemimelia	0	0	1	0
Visceral Observations				
No. of dams	18	20	19	16
No. of fetuses examined	120	125	131	109
No. of fetuses with anomalies	0	2	4	0
Hydrocephaly	0	0	2	0
Lung-lobular agnesis	0	2	2	0
Interventricular septal defect	0	0	1	0
Heart- great vessel anomaly	0	1	0	0
Skeletal Observations				
Litters Evaluated	18	20	19	16
Fetuses Evaluated	120	125	131	109
No. of skeletal anomalies	1	0	2	1
Rib anomaly	1	0	0	0
Vertebral anomaly	0	0	1	1
Scapula absent	0	0	1	0

Toxicokinetics: The exposure (AUC_{0-8h}) to ALX-0600 increased in a dose proportional manner over a dose range of 2 to 50 mg/kg/day. Exposure to the drug increased upon repeated dosing, however, there did not appear to be any significant accumulation of the drug. The T_{max} reached at 0.5 to 2 hours postdose on GD7 and 0.25 to 1 hour postdose on GD20. The mean TK data is shown below:

Gestation Day	Dose (mg/kg/day)	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-8h} (ng.hr/ml)
7	2	0.5	699	1617
	10	1.0	2227	7140
	50	1.5	5129	28184
20	2	0.8	839	2401
	10	0.8	3051	8478
	50	1.0	11671	49306

In a Segment II s.c. teratology study in rabbits, animals were administered subcutaneously with ALX-0600 at doses of 0 (vehicle), 2, 10 or 50 mg/kg/day (0, 1, 5 and 25 mg/kg/day, bid, 8 hours apart) during the period of organogenesis (GD7-20). ALX-0600 did not produce any developmental adverse effect in fetuses during the study. The NOAEL for developmental toxicity was considered as ≥ 50 mg/kg/day. ALX-0600 does not appear to be teratogenic in rabbits at the tested doses.

9.3 Prenatal and Postnatal Development

Study title: Subcutaneous Developmental and Perinatal/Postnatal Reproduction Toxicity Study in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.: Report No. XGW00008; Study No. TX-0600-G-007
Study report location: EDR 4.2.3.5.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: November 12, 2006
Date of completion: February 8, 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Teduglutide, 08505051, 98.3%

Key Study Findings:

- In a subcutaneous pre and postnatal development study in rats, animals were treated at 10, 25 and 50 mg/kg/day (5, 12.5 and 25 mg/kg BID, 8 hours apart) from DG 7 through DL 20.
- There were no significant treatment-related effects on F0 body weight, food consumption, pregnancy, and delivery or uterine parameters.
- No significant treatment-related effects were observed in F1 development, sexual maturation, behavior, mating and fertility index and Caesarean-sectioning parameters.
- There were no significant treatment-related F2 gross external changes.
- Overall, teduglutide did not show any significant adverse effect on pre and postnatal development in rats at the tested doses.

Methods: Pregnant female rats were randomly assigned to four dose groups (Groups I through IV, n = 22/group). Teduglutide or vehicle (sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate with mannitol and L-histidine) were administered subcutaneously twice daily (approximately 8 hours apart) from day 7 of gestation (DG 7) through day 20 of lactation (DL 20) or DG 24 (rats that did not deliver a litter) at dosages of 0 (Vehicle), 5, 12.5, and 25 mg/kg BID (dose volume = 1.25 mL/kg) for a daily total dosage of 0 (Vehicle), 10, 25, and 50 mg/kg/day. Injection sites were rotated to minimize irritation. F1 generation pups were not directly administered the test article or vehicle, but have been possibly exposed to the test article or vehicle during maternal gestation (*in utero* exposure) or *via* maternal milk during the lactation period.

Doses: 5, 12.5, and 25 mg/kg BID (10, 25, and 50 mg/kg/day)*

Basis of dose selection: Dosages were selected based on the results of the 13-week subcutaneous injection study (800069) in the rat.

Frequency of dosing: Twice daily (8 hours apart)

Dose volume: 1.25 mL/kg

Route of administration: Subcutaneous

Formulation/Vehicle: Sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate with mannitol and L-histidine

Species/Strain: Sprague Dawley rats

Number/Sex/Group: 22/dose

Satellite groups: None

Study design: The study design is shown in the tables below (from page 29 of the report).

Deviation from study protocol: Protocol deviations did not adversely affect the data and the interpretations of the results.

4.5.1. F0 Generation Rats

Dosage Group	Dosage ^a (mg/kg/dose)	Dosage ^a (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned F0 Generation Rat Numbers
I	0 (Vehicle)	0 (Vehicle)	0	1.25	22	25701 – 25722
II	5	10	4	1.25	22	25723 – 25744
III	12.5	25	10	1.25	22	25745 – 25766
IV	25	50	20	1.25	22	25767 - 25788

a. The test article/substance was considered 100% active/pure for the purpose of dosage calculations.

4.5.2. F1 Generation Rats

Dosage Group	Maternal Dosage (mg/kg/day)	Number of Rats Per Sex	Assigned F1 Generation Rat Numbers	
			Male Rats	Female Rats
I	0 (Vehicle)	22	22401-22422	22501-22522
II	10	22	22423-22444	22523-22544
III	25	22	22445-22466	22545-22566
IV	50	22	22467-22488	22567-22588

Observations:**F₀ Dams:**

- Survival: Mortality was observed twice daily.
- Clinical signs: Clinical signs were examined on a twice daily basis.
- Body weight: Body weights were recorded on a weekly basis.
- Feed consumption: Food consumption values were recorded on DGs 0, 7, 10, 12, 15, 18, 20 and 25 and DLs 1, 4, 7, 10 and 14.
- Uterine content: After completion of the 28-day postpartum period, female rats were sacrificed and a gross necropsy was performed. The number and distribution of implantation sites was recorded.
- Necropsy observation: After completion of the 28-day postpartum period, female rats were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed.
- Toxicokinetics: None
- Dosing Formulation Analysis: Concentration results were considered acceptable if the difference between the actual mean value and the targeted concentration was $\leq 10\%$.

F₁ Generation:

- Survival: Each litter was evaluated for viability at least twice daily.
- Clinical signs: Clinical observations were recorded once daily.
- Body weight: Pup body weights were recorded on days 1 (birth), 4, 7, 14 and 21 postpartum and on the day of sacrifice.
- Food consumption: Food consumption was recorded on a weekly basis.
- Physical development: Female rats were evaluated for the age of vaginal patency, beginning on day 28 postpartum. Male rats were evaluated for the age of preputial separation, beginning on day 39 postpartum.
- Neurological assessment: Neurological assessments included motor activity (days 22 and 59 to 61 postpartum), passive avoidance (day 24 postpartum), water M-maze test (day 70 postpartum)
- Reproduction: At approximately 90 days of age, the F1 generation rats within each dosage group were assigned to cohabitation, one male rat per female rat. The cohabitation period consisted of a maximum of 19 days. Male rats were sacrificed after completion of the 19-day cohabitation period. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Testes and epididymides of male rats were excised and paired organ weights were recorded. Female rats were sacrificed on DG 21, Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Female rats were examined for number and distribution of corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions.

F₂ Generation:

- Survival: Yes
- Body weight: Yes
- External evaluation: Gross external evaluations
- Male/Female ratio: The sponsor did not report the male/female ratio. However, the sponsor presented the data for the number of live male fetuses.

Results:

F₀ in-life: There were no mortalities. One female rat at 50 mg/kg/day died on DL 10 prior to scheduled sacrifice. Clinical observations in this rat included red perinasal substance on the day of death. This rat lost body weight beginning on DL 8, while food consumption values appeared unaffected by the treatment with the test article. At

necropsy, the stomach was distended with a brown fluid; all other tissues appeared normal. The litter for this dam consisted of 13 male and 6 female pups, of which three pups (one female and two males) died on PND 9 and one pup (male) was missing on PND 2. All surviving pups were sacrificed following the death of the dam. Death was not considered treatment-related as it was a single event with no other signs of toxicity occurring in any of the rats in this dosage group. All other rats survived to scheduled necropsy. There were no other treatment-related clinical signs in other F0 rats.

The mean initial (Day 0) and final (Day 20) body weight of control females were 240 and 392 g, respectively. Body weights and body weight gains during the gestation and lactation periods were unaffected by treatment with teduglutide. The mean initial (Day 0-7) and final (Day 18-20) absolute food consumptions in control females were 22.4 and 25.0 g/animal/day. There were no significant treatment-related changes in final absolute food consumption.

F₀ necropsy: There were no significant treatment-related necropsy observations. Distention of the stomach with a brown fluid was the only gross lesion which was seen in a decedent female rat at 50 mg/kg/day (25778) as described above.

Natural delivery and litter observations were unaffected by the treatment. Values for the uterine parameters (numbers of dams delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index, the numbers of dams with stillborn pups and of dams with all pups dying, litter sizes, surviving pups per litter, percent male pups per number of pups sexed per litter and live litter size at weighing) were comparable among the four dosage groups. The lactation index [number of live pups on day 28 (weaning) postpartum/number of live pups on day 4 postpartum] at 50 mg/kg/day dosage group was significantly reduced in comparison to the vehicle control group value (91.3% vs. 98.8%). However, this reduction in lactation index was not considered treatment-related for the following reasons: 1) the overall number of pups delivered in this dosage group was lower compared to the vehicle control group value (274 vs. 337); and 2) a litter of pups that were sacrificed due to the unscheduled death of their dam (25778) on DL 10, and the reduction in viability index (number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum) compared to the vehicle control group values, and these reductions in lactation index was not dose-related. The following tables (from page 72 and 73 of the report) show the delivery data.

NDA 203441

Tamal K. Chakraborti, Ph.D.

PROTOCOL XGW00008: ALX-0600: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S STUDY NUMBER: TX-0600-G-007)

TABLE A11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0 (VEHICLE)	II 10	III 25	IV 50
RATS ASSIGNED TO NATURAL DELIVERY	N	22	22	22	22
PREGNANT	N	21 (95.4)	22 (100.0)	19 (86.4)	18 (81.8)
DELIVERED LITTERS	N(%)	21 (100.0)	22 (100.0)	19 (100.0)	18 (100.0)
DURATION OF GESTATION b	MEAN±S.D.	22.7 ± 0.5	22.4 ± 0.5	22.7 ± 0.5	22.7 ± 0.6
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	354 16.8 ± 2.1	366 16.6 ± 2.1	307 16.2 ± 3.3	286 15.9 ± 3.9
DAMS WITH STILLBORN PUPS	N(%)	4 (19.0)	1 (4.5)	3 (15.8)	4 (22.2)
DAMS WITH NO LIVEBORN PUPS	N	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
GESTATION INDEX c	% N/N	100.0 21/ 21	100.0 22/ 22	100.0 19/ 19	100.0 18/ 18
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0) d

- a. Two doses were to be administered on day 7 of gestation through day 20 of lactation.
b. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the time the first pup was delivered.
c. Number of rats with live offspring/number of pregnant rats.
d. Excludes value for dam 25778, which was found dead on day 10 of lactation.

PROTOCOL XGW00008: ALX-0600: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S STUDY NUMBER: TX-0600-G-007)

TABLE A12 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP DOSAGE (MG/KG/DAY) a		I 0 (VEHICLE)	II 10	III 25	IV 50
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	21	22	19	18
PUPS DELIVERED (TOTAL)	N MEAN±S.D.	337 16.0 ± 1.8	342 15.5 ± 2.0	291 15.3 ± 3.1	274 15.2 ± 3.8
LIVEBORN	MEAN±S.D. N(%)	15.8 ± 1.9 332 (98.5)	15.4 ± 2.1 340 (99.4)	15.2 ± 3.1 288 (99.0)	14.9 ± 3.7 269 (98.2)
STILLBORN	MEAN±S.D. N(%)	0.2 ± 0.5 5 (1.5)	0.1 ± 0.4 2 (0.6)	0.2 ± 0.4 3 (1.0)	0.3 ± 0.6 5 (1.8)
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	5/332 (1.5)	14/340 (3.2)**	3/288 (1.0)	2/269 (0.7)
DAYS 2- 4	N/N(%)	5/326 (1.5)	21/326 (6.4)**	7/285 (2.4)	2/267 (0.7)
DAYS 5- 7	N/N(%)	3/322 (0.9)	3/305 (1.0)	1/278 (0.4)	2/265 (0.8)
DAYS 8-14	N/N(%)	1/319 (0.3)	5/302 (1.6)	3/276 (1.1)	4/247 (1.6) b
DAYS 15-21	N/N(%)	0/318 (0.0)	2/296 (0.7)	3/272 (1.1)	2/244 (0.8)
DAYS 22-28	N/N(%)	0/318 (0.0)	0/295 (0.0)	0/271 (0.0)	0/242 (0.0)
VIABILITY INDEX c	% N/N	97.0 322/332	89.7 305/340**	96.5 278/288	98.5 265/269
LACTATION INDEX d	% N/N	98.8 318/322	96.7 295/305	97.5 271/278	91.3 242/265b**

- DAY(S) = DAY(S) POSTPARTUM
a. Two doses were to be administered on day 7 of gestation through day 20 of lactation.
b. Excludes 15 pups from litter 25778 that were sacrificed on day 10 of lactation due to death of dam.
c. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
d. Number of live pups on day 28 (weaning) postpartum/number of live pups on day 4 postpartum.
** significantly different from the vehicle control group value (p≤0.01).

F₁ physical development: There were no treatment-related deaths in the F1 generation rats. There were no significant treatment-related clinical signs. No reflex and physical development parameters (surface righting, acoustic startle, air righting and pupil constriction) of the F1 generation pups were affected by maternal treatment with teduglutide. The statistically significant reduction in the percentage of F1 pups at 25 and

50 mg/kg/day that meet criterion for air righting on day 17 postpartum was not considered treatment-related in the absence of a dose response. Body weights and body weight gains of the F1 generation male and female rats were unaffected by maternal treatment with teduglutide. Food consumption was unaffected by the treatment. Sexual maturation was unaffected by the treatment. The average day on which preputial separation was observed in male rats or vaginal patency was observed in female rats was comparable among the four maternal dosage groups.

F₁ behavioral evaluation: Motor activity, passive avoidance, water maze test parameters (learning and retention) were not significantly affected by treatment with teduglutide.

F₁ reproduction: There were no significant treatment-related effects on mating and fertility parameters in F1 male and female rats. Values for the number of days in cohabitation, the number of rats that mated, the fertility index, the number of rats with confirmed mating dates during the first, second and/or third weeks of cohabitation, and the number of pregnancies per number of rats in cohabitation were comparable among the four maternal dosage groups and did not significantly differ. The following tables (from pages 225 and 226 of the report) show the mating fertility data for F1 rats.

PROTOCOL XGW00008: ALX-0600: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S STUDY NUMBER: TX-0600-G-007)

TABLE B23 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	25	50
RATS IN COHABITATION	N	22	22	22	22
DAYS IN COHABITATION a	MEAN±S.D.	3.7 ± 4.0	2.8 ± 1.4	3.0 ± 3.0	3.4 ± 2.7 [21]
RATS THAT MATED b	N(%)	21(95.4)	22(100.0)	21(95.4)	21(95.4)
FERTILITY INDEX c,d	N/N (%)	18/21 (85.7)	21/22 (95.4)	17/21 (81.0)	19/21 (90.5)
RATS WITH CONFIRMED MATING DATES	N	21	22	21	20
MATED WITH FEMALE e					
DAYS 1-7	N(%)	19(90.5)	22(100.0)	20(95.2)	20(100.0)
DAYS 8-14	N(%)	2(9.5)	0(0.0)	1(4.8)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION d	N/N (%)	18/22 (81.8)	21/22 (95.4)	17/22 (77.3)	19/22 (86.4)

[] = NUMBER OF VALUES AVERAGED

a. Restricted to rats with a confirmed mating date and rats that did not mate.

b. Includes only one mating for each male rat.

c. Number of pregnancies/number of rats that mated.

d. Includes only one pregnancy for each rat that impregnated more than one female rat.

e. Restricted to rats with a confirmed mating date.

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Tamal K. Chakraborti, Ph.D.

TABLE B24 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 10	III 25	IV 50
RATS IN COHABITATION	N	22	22	22	22
DAYS IN COHABITATION a	MEAN±S.D.	3.8 ± 4.1	2.8 ± 1.4	3.1 ± 3.2	3.5 ± 2.9 [21]
RATS THAT MATED	N(%)	22(100.0)	22(100.0)	22(100.0)	22(100.0)
FERTILITY INDEX b	N/N (%)	19/22 (86.4)	21/22 (95.4)	17/22 (77.3)	20/22 (90.9)
RATS WITH CONFIRMED MATING DATES	N	22	22	22	21
MATED BY FIRST MALE c					
DAYS 1-7	N(%)	19(86.4)	22(100.0)	20(90.9)	20(95.2)
DAYS 8-14	N(%)	2(9.1)	0(0.0)	1(4.5)	0(0.0)
MATED BY SECOND MALE c					
DAYS 15-21	N(%)	1(4.5)	0(0.0)	1(4.5)	1(4.8)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	19/22 (86.4)	21/22 (95.4)	17/22 (77.3)	20/22 (90.9)

[] = NUMBER OF VALUES AVERAGED

a. Restricted to rats with a confirmed mating date and rats that did not mate.

b. Number of pregnancies/number of rats that mated.

c. Restricted to rats with a confirmed mating date.

No Caesarean-sectioning or litter parameters for F1 generation were affected by the treatment with teduglutide. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, the percentage of resorbed conceptuses, and the percentage of live male fetuses were comparable among the four maternal dosage groups and did not significantly differ. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. Placenta appeared normal. The following tables (from pages 227 and 228) show the Caesarean-sectioning data for F1 generation rats.

TABLE B25 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP MATERNAL DOSAGE (MG/KG/DAY)		I 0 (VEHICLE)	II 10	III 25	IV 50
RATS TESTED	N	22	22	22	22
PREGNANT	N(%)	19(86.4)	21(95.4)	17(77.3)	20(90.9)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	19	21	17	20a
CORPORA LUTEA	MEAN±S.D.	16.9 ± 2.7	17.3 ± 2.6	17.0 ± 1.5	17.2 ± 1.9
IMPLANTATIONS	MEAN±S.D.	14.9 ± 3.8	15.4 ± 2.7	16.6 ± 1.6	16.6 ± 2.8
LITTER SIZES	MEAN±S.D.	14.5 ± 4.1	14.6 ± 3.0	15.8 ± 1.8	15.8 ± 2.6
LIVE FETUSES	N	275	306	269	316
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.4 ± 0.5	0.8 ± 0.9	0.8 ± 0.7	0.8 ± 0.8
EARLY RESORPTIONS	N	7	16	14	15
LATE RESORPTIONS	N	1	1	0	1
DAMS WITH ANY RESORPTIONS	N(%)	8(42.1)	12(57.1)	11(64.7)	11(55.0)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	19(100.0)	21(100.0)	17(100.0)	20(100.0)
PLACENTAE APPEARED NORMAL	N(%)	19(100.0)	21(100.0)	17(100.0)	20(100.0)

a. Includes values for dam 22579, which did not have a confirmed mating date.

TABLE B26 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F2 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	25	50
LITTERS WITH ONE OR MORE LIVE FETUSES	N	19	21	17	20a
IMPLANTATIONS	MEAN±S.D.	14.9 ± 3.8	15.4 ± 2.7	16.6 ± 1.6	16.6 ± 2.8
LIVE FETUSES	N	275	306	269	316
	MEAN±S.D.	14.5 ± 4.1	14.6 ± 3.0	15.8 ± 1.8	15.8 ± 2.6
LIVE MALE FETUSES	N	137	147	132	151
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	49.6 ± 11.1	48.8 ± 12.9	48.7 ± 11.8	48.5 ± 13.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.06 ± 0.43	5.11 ± 0.29	4.89 ± 0.26	4.91 ± 0.39 [19]b
MALE FETUSES	MEAN±S.D.	5.23 ± 0.48	5.23 ± 0.33	5.06 ± 0.32	5.06 ± 0.40 [19]b
FEMALE FETUSES	MEAN±S.D.	4.91 ± 0.40	4.99 ± 0.29	4.75 ± 0.27	4.77 ± 0.39 [19]b
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	3.6 ± 4.7	5.5 ± 6.1	5.0 ± 4.4	4.6 ± 4.8

[] = NUMBER OF VALUES AVERAGED

a. Includes values for litter 22579; the dam did not have a confirmed mating date.

b. Excludes values for litter 22579; the dam did not have a confirmed mating date.

F₂ findings: There were no significant treatment-related F₂ gross external alterations (malformations or variations). Fetal gross external alteration data is shown in the table (from page 229 of the report) below.

TABLE B27 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY - F2 GENERATION LITTERS/FETUSES

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	25	50
LITTERS EVALUATED	N	19	21	17	20
FETUSES EVALUATED	N	275	306	269	316
LIVE	N	275	306	269	316
TAIL: SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.8)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3)a	0(0.0)	0(0.0)
BODY: TRUNK SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.8)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3)a	0(0.0)	0(0.0)

a. Fetus 22544-4 had other gross external alterations.

Summary: In a subcutaneous pre and postnatal development study in rats, animals were treated at 10, 25 and 50 mg/kg/day (5, 12.5 and 25 mg/kg BID, 8 hours apart) from DG 7 through DL 20. There were no significant treatment-related effects on F₀ body weight, food consumption, pregnancy, and delivery or uterine parameters. No significant treatment-related effects were observed in F₁ development, sexual maturation, behavior, mating and fertility index and Caesarean-sectioning parameters. There were no significant treatment-related F₂ gross external changes. Overall, teduglutide did not show any significant adverse effect on pre and postnatal development in rats at the tested doses.

10 Special Toxicology Studies

Acute Intravenous/Perivenous/Intrararterial Tolerance Study in Male Rabbits (Study # 7203-101)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated April 1, 2004.

2. Acute Intravenous/Perivenous/Intrararterial Tolerance Study in Male Rabbits (Study # 7203-101)

Methods: This study was done to evaluate the local tolerance of ALX-0600 in 18 adult male rabbits (Hra:NZW SPF strain). Eighteen animals weighing 2.252 to 2.582 kg were employed and were given a single dose of intravenous, perivenous or intra-arterial injection in phosphate buffer with mannitol and histidine. The intended route of administration of the compound in human was intravenous but the sponsor had conducted the present study with the compound by perivascular and intra-arterial routes additionally with the intention to detect the tolerance of the accidentally spill- over/administered compound on these sites. ALX-0600 was injected as per the following schedule (table taken from sponsor submission (vol 1.3 pp 10)):

Group	Route of Injection	Study Design		Dose Volume (Dose Level)	Number of Animals
		Right Ear	Left Ear		
1	Intravenous			1.25 mL/kg (25 mg/kg)	6
2	Perivenous	Vehicle Control Article	ALX-0600	0.1 mL/injection (approximately 0.84 mg/kg)	6
3	Intraarterial			1.25 mL/kg (25 mg/kg)	6†

* The first three animals assigned to each group were sacrificed on Day 4; the remaining three animals/group were sacrificed on Day 15.

† Due to difficulty in delivering the entire dose to the original animal, one animal in this group was replaced with another animal on the same day of treatment.

The injection of the compound (left ear) or control vehicle (right ear) were made directly in the marginal vein (group 1), near to marginal vein (group 2) and in the central ear artery (group 3). The animals were observed on day 1 before injecting the compound and 1 hr post dosing. The

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animals were weighed on day 1 and day 15 (on day of necropsy). On day 4 or 15 of the study, the animals were sacrificed and the site of injection were dissected, cleaned and examined macroscopically. The injection sites were preserved for microscopic examination.

Results:

a. General Observations and Body Weight Changes: None of the animals died during the study. The mean body weights of the animals were similar in the 3 groups. On day 1, the initial body weights the animals included in intravenous, perivenous and intra-arterially administered groups were 2.391, 2.379 and 2.412 kg on day 1. The body weights in 3 groups of animals sacrificed on day 4 were 2.470, 2.370 and 2.415 kg and, on day 15 terminal day, these were 2.521, 2.582 and 2.478 kg, respectively.

b. Group Observations:

Intravenous Group: In vehicle control or treated animals, no erythema, or area of irritation was reported.

Perivenous Injection Group: In vehicle control group animals, erythema was seen in 2 of the 3 rabbits at 4 hr post dosing on day 1 and from study day 2 to 4. But in left ear of animals (compound treated), area of irritation and erythema were seen in 2 of the 3 animals killed on day 4. Small hematomas were seen for 4 to 6 days in 3 of the 3 animals scheduled to be killed on day 15. Thus, ALX-0600 caused irritation after the injection, which persisted for 2 to 5 days. These reactions were resolved from day 5 of the study. The compound produced erythema and areas of irritation at the site of injection.

Intra-arterial Injection Group: In vehicle control group animals, no reactions were observed in animals scheduled to be killed on day 4. One of 3 animals of vehicle control group scheduled to be killed on day 15, showed erythema on day 2 to day 9. In 1 of the 3 animals left ear treated with ALX-0600, a large hematoma was reported from day 2 to 4. In 1 out of 3 animals to be killed on study day 15, small erythema and areas of irritation were reported on study day 2 to 3. The erythema was resolved by day 7 and area of irritation resolved from day 4 of the study.

Hemorrhage and collagen degeneration/necrosis were present in ear treated with intra-arterial route, which was resolved by day 7. Thus, a single dose of intra-arterially administered ALX-0600 caused irritation for 2 to 5 days and was resolved by study day 7.

In summary, ALX-0600 produced local irritation, hematoma and erythema after its single perivenous or intra-arterial injection.

13-Day Subcutaneous Injection Toxicity Study with Teduglutide in Minipigs (6852-175)

Methods: The purpose of this study was to evaluate the local dermal toxicity of teduglutide when administered *via* SC injection to minipigs (n = 4/group, 5 months old, body weight ranged from 11.0 to 13.7 kg) at different concentrations either daily or every fourth day (four doses) for 13 days. In this study, female Göttingen minipigs were assigned to two groups as shown in the following table (from page 8 of the report). Animals were administered dose formulations at six test sites by subcutaneous injection. The following parameters were evaluated: mortality, clinical signs, dermal irritation scoring, body weights, food consumption, and histopathology.

Group/Dose Interval	No. of Animals	Dose Concentration (mg/mL)					
		Test Site					
	Female	1a,b	2a,c	3a,d	4a,e	5a,f	6g
1 (Daily)	4	0	0	20	30	50	50
2 (Every Fourth Day - Days 1, 5, 9, and 13)	4	0	0	20	30	50	50
a	Dose volume was 1 mL/dose/test site.						
b	Vehicle A [35mM sodium phosphate (pH 7.4 ± 0.1), 50mM L-histidine, 3% mannitol in reverse osmosis water] was administered at Test Site 1.						
c	Vehicle B [60mM sodium phosphate (pH 7.8 ± 0.1), 20mM L-histidine, 3% mannitol in reverse osmosis water] was administered at Test Site 2.						
d	54-mg ALX-0600/vial lyophilized powder reconstituted with Sterile Water for Injection was administered at Test Site 3. The vehicle components were similar to Vehicle A.						
e	50-mg ALX-0600/mL solution diluted to 30 mg/mL with Vehicle B was administered at Test Site 4.						
f	50-mg ALX-0600/mL solution was administered as supplied at Test Site 5. The vehicle components were similar to Vehicle B.						
g	50-mg ALX-0600/mL solution was administered at an injection volume of 0.6 mL for a total dose of 30 mg at Test Site 6.						

Results: All animals survived to scheduled necropsy. There were no significant treatment-related effects on clinical signs, body weight and food consumption. No skin irritation was observed in animals that were treated every fourth day (Group 2). In the animals dosed daily for 13 days (Group 1), test article-related dermal observations were limited to very slight to slight edema and atonia (impairment of skin elasticity). The time of onset, frequency of observed irritation, and severity were dose and volume-related (Ranking: Test Site 5 > Test Site 6 > Test Site 4 > Test Site 3). The following tables (from page 26 and 27 of the report) show the results.

Table 2
Summary of Dermal Observations - Predose Days 1 through 13 of the Dosing Phase and on the Day of Scheduled Sacrifice

Category Sign	Sex: Group: Dose Interval: Dose Volume: Number in Group:	Females	
		1 Daily 1 mL ^a 4	2 Every 4 th Day 1 mL ^a 4
		N	N
Test Site 4			
Edema, Very Slight		3	0
Test Site 5			
Atonia, Yes		2	0
Edema, Slight		1	0
Edema, Very Slight		4	0
Test Site 6			
Edema, Slight		1	0
Edema, Very Slight		3	0

N = Number of animals with observed sign
a The dose volume was 1 mL for Test Sites 1 through 5 and 0.6 mL for Test Site 6.

Table 3
Summary of Dermal Observations - 3 to 4 Hours Postdose Days 1 through 13 of the Dosing Phase

Category Sign	Sex: Group: Dose Interval: Dose Volume: Number in Group:	Females	
		1 Daily 1 mL ^a 4	2 Every 4 th Day 1 mL ^a 4
		N	N
Test Site 3			
Edema, Very Slight		2	0
Test Site 4			
Atonia, Yes		1	0
Edema, Slight		1	0
Edema, Very Slight		3	0
Test Site 5			
Atonia, Yes		4	0
Edema, Slight		4	0
Edema, Very Slight		4	0
Test Site 6			
Atonia, Yes		1	0
Edema, Slight		2	0
Edema, Very Slight		4	0

N = Number of animals with observed sign
a The dose volume was 1 mL for Test Sites 1 through 5 and 0.6 mL for Test Site 6.

Treatment with teduglutide on a daily basis caused irritation at the injection sites characterized by edema, fibrosis, hemorrhage, marked giant cell infiltrates, lymphocyte and macrophage infiltrates, chronic-active inflammation, and minimal to marked necrosis (shown in the following table from page 21 of the report).

Text Table 3
Incidence and Mean Severity Scores of Microscopic Findings - Skin and Injection Sites, Daily Administration for 13 Days (Group 1)

Group 1	Skin	1	2	3	4	5	6
ALX-0600 (mg/mL)		0	0	20	30	50	50
Degeneration, skeletal muscle	0 (0.0) ^a	1 (0.3)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Edema	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)	2 (1.5)	3 (2.3)
Fibrosis	0 (0.0)	1 (0.3)	1 (0.5)	3 (2.5)	4 (3.3)	4 (3.5)	4 (3.3)
Hemorrhage	1 (0.5)	1 (0.3)	0 (0.0)	1 (0.3)	4 (2.3)	2 (1.3)	2 (1.3)
Infiltrate, giant cells	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	4 (3.3)	4 (3.5)	4 (3.5)
Infiltrate, lymphocytes/macrophages	0 (0.0)	3 (0.8)	3 (1.0)	1 (0.8)	2 (1.3)	1 (0.8)	0 (0.0)
Inflammation, chronic-active	1 (0.3)	0 (0.0)	0 (0.0)	3 (1.8)	2 (1.5)	3 (2.3)	4 (3.0)
Necrosis	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	2 (1.3)	4 (2.8)	4 (2.5)

Severity scores: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, and 5 = severe.

a Incidence and average severity.

No macroscopic findings were noted for the sites treated every fourth day. When the test article was administered every fourth day, microscopic findings were reduced (as shown in the table below, from page 22 of the report) when compared with Group 1. The findings limited to sites treated with the test article were minimal to slight hemorrhage, minimal to moderate chronic-active inflammation, moderate necrosis, and minimal giant cell infiltrates. There was no apparent dose response for the hemorrhage and chronic-active inflammation.

Text Table 4
Incidence and Mean Severity Scores of Microscopic Findings - Skin and Injection Sites, Administration Every Fourth Day for Four Doses (Group 2)

Group 2	Skin	1	2	3	4	5	6
ALX-0600 (mg/mL)		0	0	20	30	50	50
Degeneration, skeletal muscle	0 (0.0) ^a	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Edema	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fibrosis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.3)	1 (0.5)	1 (0.5)
Infiltrate, giant cells	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)
Infiltrate, lymphocytes/macrophages	0 (0.0)	4 (1.0)	2 (0.5)	4 (1.0)	1 (0.3)	2 (0.8)	3 (0.8)
Inflammation, chronic-active	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.8)	2 (1.3)	1 (0.3)
Necrosis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)	0 (0.0)

Severity scores: 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, and 5 = severe.

a Incidence and average severity.

Overall, no skin irritation was observed in animals that were treated every fourth day (Group 2). In the animals dosed daily for 13 days (Group 1), test article-related dermal observations included edema, fibrosis, hemorrhage, marked giant cell infiltrates, lymphocyte and macrophage infiltrates, chronic-active inflammation, and minimal to marked necrosis.

**In Vitro Hemolytic Potential and Blood Compatibility Assessment of Teduglutide:
#7203-102)**

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated April 1, 2004.

3. In Vitro Hemolytic Potential and Blood Compatibility Assessment of ALX-0600: #7203-102)

Methods: The test was performed to determine the hemolytic potential and compatibility of ALX-0600 in mouse, minipig, cynomolgus monkeys and human whole blood. The hemolysis test was done using ALX-0600 (Batch # 0850001, purity 98.9%) in vehicle phosphate buffer with mannitol and histidine. To 0.5 ml blood sample (mouse, minipig/cynomolgus monkey or human blood), 0.5 ml of solution containing 0, 0.2, 2 or 20 mg/ml ALX-0600 or 0.5 ml saponin (positive control) was mixed in the tubes. The tubes were shaken gently and incubated for 43 min at approx. 37°C. The supernatant was harvested and concentration of hemoglobin in each tube was measured by spectrophotometric method. The test was positive if the solution contained hemoglobin in greater concentration than or equal to 500 mg/l of negative control.

Blood Compatibility:

Thirty two test tubes were divided in to 8 groups (4/group) and to these was added 0.5 ml mouse serum (4 tubes) or 0.5 plasma (4 tubes), or 0.5 ml minipig serum (4 tubes) or minipig plasma (4 tubes), or 0.5 ml cynomolgus monkey serum (4 tubes) or monkey plasma (4 tubes) or 0.5 ml human serum (4 tubes) or human plasma (4 tubes). To each tube of a serum or plasma of one of the species, ALX-0600 solution containing 0, 0.2 or 2.0 or 20 mg/ml was added. The final concentration of the compound in each tube was ½ the concentration of the formulation (0.1, 1.0 or 10 mg/ml). The tubes were shaken to mix and incubated for 33 min at 23.6°C. The occurrence of precipitate or coagulation was examined by macroscopically/microscopically (for sign of incompatibility). The absence of precipitate of coagulation was an indication of no incompatibility.

Results: Hemolysis was seen in the tube containing saponin (control positive) indicating the conditions of the test were optimum. No hemolysis was seen in tubes containing 0.2, 2 or 20 mg/ml ALX-0600. Thus, ALX-0600 when mixed with equal amounts of serum/plasma samples of animals and human did not produce hemolysis or flocculation.

ALX-0600 did not produce precipitate on mixing with mouse, minipig, monkey or human sera or plasmas.

Induction of Delayed Type Hypersensitivity (DTH) Response in Mouse: (Study #47063; 0711XA27.001)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

1. **Induction of Delayed Type Hypersensitivity (DTH) Response in Mouse:** (Study #47063;0711XA27.001)

Name of the Conducting Laboratory: [REDACTED]

Methods: Sixty BALB/cByJ female mice weighing approximately 18 to 25 g were divided in to 6 groups (10/group) and given an intradermal injection of PBS (control), ALX-0600 (2 groups of animals in the presence and 2 groups without adjuvant) or OVA (1

group of animal) at the doses and schedule as shown in the following table:

Group	Treatment	Dose (mg/kg)	Adjuvant
1	Vehicle	-	+
2	Test Article	0.1	+
3	Test Article	1	+
4	OVA	5	+
5	Test Article	0.1	-
6	Test Article	1	-

The animals in each group were given a single injection per week for 3 weeks. The sensitized mice were challenged with 30 ul of 1 mg/kg ALX-0600 solution intradermally in right ear pinna, one week after the sensitization. The group of mice sensitized with OVA were injected with 30 ul of 0.5% OVA solution in right ear pinna. An intradermal injection of vehicle (PBS) was given in the left ear pinna in each of the mice and it served as control. The hypersensitivity was assessed by estimating the increased swelling at treated sites (Vs control vehicle site). All of the study animals were sacrificed at the end of the study.

Results: Three animals died during the study. These were 1 each in 0.1 mg/kg and 1 mg/kg ALX-0600 and, 1 in OVA treatment group. The deaths were attributed not to the treatment related causes. The thickness of the ear pinna treated with ALX-0600 was similar to that of vehicle treated pinna. A significant increase in the ear thickness was seen in OVA treated animals ($p < 0.001$). ALX-0600 did not produce delayed hypersensitivity in mice.

Immunogenicity of Teduglutide in New Zealand White Rabbits (Study # 47063)

The review of the above study report is incorporated below from the pharmacology review of IND 58,213 dated May 24, 2002.

2. Immunogenicity of ALX-0600 in NewZealand White Rabbits:
(Study # 47063)

Name of the Conducting Laboratory: [REDACTED]

Methods: Twelve (6/sex) adult New Zealand white rabbits about 6 months old weighing approximately 3.1 to 3.5 kg (males) and 3.0 to 3.3 kg (females) were used to assess the immunogenicity of subcutaneously or intravenously administered ALX-0600. The animals were divided in to 2 groups (3/sex/group) and administered 1 ml of 0.25 mg/ml solution of ovalbumin or ALX-0600. For priming immunization, the freshly mixed solution of ALX-0600 with complete Freund's solution (IFA, in 1:1 ratio) was administered subcutaneously in to the scapular region on day 1. A control group (3/sex) received freshly mixed solution of ovalbumin with complete Freund's solution. Subsequent booster immunization (primary and secondary) was done at the end of week 4 and 6. All animals were observed twice daily for mortality and other changes. During the pretreatment period and at the end of week 4 and 6, 5 ml of the blood was collected from each animal in to clot tubes and serum from each of the sample was separated and preserved at -80°C. The presence of anti-sera immunogenicity (specific IgM and IgG antibodies for ALX-0600) was determined by using double immunodiffusion assay. The slides were stained with Coomassie Brilliant blue for making the assay sensitive to 25 ug/ml. All animals were killed after the study and after the blood collection and discarded.

Results: None of the animals died during the study. Serum samples from 1 male and 1 female were diluted serially, 1:10, 1:100, 1:1000 and 1: 10000 and ALX-0600. Ovalbumin was used at the concentration of 1, 0.5, 0.25, 0.125, and 0.625 mg/ml in the present test. The serum dilution for the control was undiluted and 1:10. The precipitin lines were seen in serum sample with undiluted anti-rabbit IgM and IgG antibodies. These lines were also seen in male and female rabbit samples containing 0.125 and 0.25 mg/ml ovalbumin and at 1:10 and 1:100 dilutions of ovalbumin. No precipitin lines were seen in serum samples of rabbits (male/female) containing 0.125 or 0.25 mg/ml ALX-0600. ALX-0600 did not produce immunogenic response in rabbits.

Exploratory Mechanistic Toxicity Study in Cynomolgus Monkeys on the Potential Increase of C-Reactive Protein (Study # 519157)

Methods: This was an exploratory mechanistic study in the Cynomolgus monkey to assess the effects of SC administration of teduglutide on C-reactive protein (CRP) using a crossover design. Each administration was separated by a 7-day wash-out period. Three batches of teduglutide with varying (b) (4) content were used to examine if (b) (4) in the drug product would cause temporary increases in CRP.

In this study, six female Cynomolgus monkeys were administered a SC injection of teduglutide at a dose level of 10 mg/kg on Days 1, 8, 15 and 22. Three different batches of teduglutide were used on the study with varying (b) (4) content. One additional batch was identified as a teduglutide placebo. The study design is shown in the table (from page 2 of the study report) below.

Animal No./Sex	Dose 1 Day 1	Dose 2 Day 8	Dose 3 Day 15	Dose 4 Day 22
1F	Placebo lot CL9-316A	teduglutide lot CL9-123 (b) (4)	teduglutide lot CL9-124 (b) (4)	teduglutide lot NPS-016-071910 (b) (4)
2F	teduglutide lot CL9-123 (b) (4)	teduglutide lot CL9-124 (b) (4)	teduglutide lot NPS-016-071910 (b) (4)	Placebo lot CL9-316A
3F	teduglutide lot CL9-124 (b) (4)	teduglutide lot NPS-016-071910 (b) (4)	Placebo lot CL9-316A	teduglutide lot CL9-123 (b) (4)
4F	teduglutide lot NPS-016-071910 (b) (4)	Placebo lot CL9-316A	teduglutide lot CL9-123 (b) (4)	teduglutide lot CL9-124 (b) (4)
5F	Placebo lot CL9-316A	teduglutide lot CL9-123 (b) (4)	teduglutide lot CL9-124 (b) (4)	teduglutide lot NPS-016-071910 (b) (4)
6F	teduglutide lot CL9-123 (b) (4)	teduglutide lot CL9-124 (b) (4)	teduglutide lot NPS-016-071910 (b) (4)	Placebo lot CL9-316A

Blood samples were collected for the determination of CRP concentration during pretest and at the following time points on Days 1, 8, 15 and 22: predose, 6, 12, 24 and 48 hours postdose. The study was terminated after the final blood sampling on Day 24.

Results: There were no treatment-related clinical signs. Body weight was not affected by the treatment. At 12 hour post-dose, CRP concentration increased and generally peaked at 24 hour post-dose in all animals. CRP concentrations returned to pre-dose levels at 48 hour post-dose in all animals. Higher concentrations of CRP were observed in animals treated with teduglutide batches with standard and high (b) (4) content when compared with those receiving placebo or teduglutide containing no (b) (4). The initial administration of teduglutide containing (b) (4) resulted in higher CRP levels than the second administration regardless of (b) (4) level.

Overall, the results of the study indicated that there was a teduglutide (b) (4) content-related increase in CRP concentrations with a peak at 24 hour post-dose in all animals. However, the initial administration of teduglutide containing (b) (4) resulted in higher CRP levels than the second administration regardless of (b) (4) level.

11 Integrated Summary and Safety Evaluation

The applicant has conducted adequate nonclinical studies with teduglutide which included pharmacology, safety pharmacology, pharmacokinetics, acute toxicology studies mice, repeated dose toxicology studies in mice (14 days to 26 weeks duration), rats (14 day to 13 weeks duration), Cynomolgus monkeys (14 to 1 year duration), toxicology studies in juvenile minipigs (14 days to 90 days duration), genotoxicity studies (Ames test, chromosome aberration test in Chinese hamster ovary cells, *in vivo* micronucleus test in mice), reproductive toxicology studies (fertility and early embryonic development in rats, embryofetal development in rats and rabbits, and pre and postnatal development in rats), and special toxicology studies (antigenicity and local tolerance studies). Toxicology studies were conducted using the subcutaneous (SC) route, the intended clinical route of administration.

Pharmacology studies examined the intestinotrophic activity of teduglutide in several species including mice, rats, ferrets, dogs, minipigs and monkeys. In CD1 mice, teduglutide increased weight and length of the small intestine in a dose-related manner with an ED₅₀ of 0.98 µg/day (0.05 mg/kg/day). Teduglutide enhanced epithelial barrier function as it reduced both paracellular and transcellular permeability in mice. Teduglutide also increased the absorptive function of the intestinal mucosa as evidenced by enhanced D-xylose absorption in rats and monkeys. In dogs, teduglutide showed excitatory and inhibitory actions on the motor activity of the gastrointestinal tract. Teduglutide decreased spontaneous contractions in isolated segments from rat colon in a dose-related manner. Teduglutide prevented TPN-induced hypoplasia in rats. In a proof of concept study in a rat model of SBS, teduglutide increased the rate or magnitude of the intestinal adaptive response to intestinal resection (75% resection) when administered at a dose of 0.2 mg/kg/day for 21 days. In neonatal piglets with an 80% jejunioileal resection, teduglutide administration resulted in significant improvements in crypt-villus architecture in the small intestine as well as duodenal, jejunal and ileal glucose transport and jejunal glutamine transport. Teduglutide improved disease related histopathology in rodent models of intestinal damage such as indomethacin-induced enteritis, dextran sulfate-induced colitis, chemotherapy-induced mucositis, and irradiation-induced intestinal damage. Overall, pharmacology studies appeared to support the proposed use of teduglutide in SBS.

In vivo pharmacokinetic studies showed that, following SC administration, teduglutide was rapidly absorbed into the systemic circulation in all species with C_{max} occurring between 20 and 60 min after dosing. Subsequently plasma teduglutide concentrations declined rapidly with a mean terminal phase half-life (t_{1/2}) of less than 2 hr. Teduglutide

did not appear to accumulate in the plasma upon multiple dosing. Generally, the pharmacokinetics of teduglutide in all species was linear and the AUC values were typically dose proportional. In pharmacokinetic studies in mice and monkeys, generally there were no significant gender differences in C_{max} and AUC values. The absolute bioavailability after SC administration in monkeys ranged from 80 to 99%. In mice, the absolute bioavailability after SC administration ranged from 77.2 to 84.4%, with a mean of 80.7%. The t_{1/2} ranged from 0.870 to 1.71 hours in monkeys following SC administration. In mice, t_{1/2} ranged from 0.368 to 0.580 hours following SC administration. In rats, there was limited (2 to 5% of the administered dose) penetration of teduglutide through the blood-brain barrier. In addition, there was minimal placental transfer of 0.1% teduglutide into the fetus of pregnant rabbits and the concentration in breast milk in lactating rats ranged from 0.9 to 2.9% of the plasma concentrations following a single subcutaneous dose of 25 mg/kg. Teduglutide was considered non-immunogenic in mice, rats and rabbits, while it induced a weak humoral immune response in monkeys. Antibodies in monkeys occurred after 4 weeks of twice daily SC administration. However, occurrence of anti-teduglutide antibodies in monkeys did not appear to be associated with a reduction in its pharmacological activity or decline in the systemic exposure.

In pivotal repeated dose toxicology studies, major treatment-related effects were related to the pharmacological activity of teduglutide which were seen in all species. In the 26-week study in mice at 2, 10 and 50 mg/kg/day, major treatment-related histopathological changes were seen at all doses in the small and large intestine (epithelial and villus hypertrophy and hyperplasia), gall bladder (epithelial hypertrophy and hyperplasia accompanied by subacute inflammation), sternal bone marrow (myeloid hyperplasia) and injection site (inflammation and necrosis). In the 13-week study in rats at 10, 25 and 50 mg/kg/day, major treatment-related histopathological changes were seen at all doses in the small and large intestine (mucosal hypertrophy and hyperplasia) and injection site (inflammation and necrosis). In the 1-year study in Cynomolgus monkeys at 1, 5 and 25 mg/kg/day, major treatment-related histopathological changes were seen at all doses in the small and large intestine (mucosal hyperplasia), stomach (mucosal hyperplasia), pancreas (hypertrophy/hyperplasia of the pancreatic duct epithelium), liver and gall bladder (epithelial hypertrophy and hyperplasia of the bile duct in the liver and mucosal hypertrophy/hyperplasia of the gall bladder) and injection site (inflammation and necrosis).

Teduglutide was also tested in juvenile minipigs in 14-day and 90-day toxicology studies up to 25 mg/kg/day (about 500 times the recommended daily human dose of 0.05 mg/kg). In the 14-day study at 5 and 25 mg/kg/day, major treatment-related histopathological changes were seen at all doses in the nonglandular stomach (mucosal hyperplasia associated with ulceration/erosion), small and large intestinal tract (hyperplasia), gall bladder (mucosal hyperplasia), bile duct (mucosal hyperplasia) and injection site (inflammation and necrosis). In the 90-day study at 1, 5 and 25 mg/kg/day, major treatment-related histopathological changes were observed at all doses in the small intestines (minimal/slight villous hypertrophy), gall bladder (cystic mucosal hyperplasia at all doses), extrahepatic bile duct (cystic mucosal hyperplasia), and

injection site (inflammation and necrosis). In the 90-day study, teduglutide increased the P wave, PR, QT (mid and high dose) and RR intervals at all doses in males at Week 13 compared to respective controls. The ECG changes were predominantly seen in males; however, the plasma exposure (AUC) to teduglutide was higher in females than that in males at Week 13 at all doses. In addition, these changes were seen at only one time point (Week 13), in one species and the magnitude of these changes were small and these changes were also not dose-related. Moreover, there were no significant treatment-related effects on QTc (Fridericia) in either sex. The QTc values were comparable across all groups. Overall, these ECG changes are not meaningful and not toxicologically significant.

Teduglutide was negative in the Ames test, *in vitro* chromosomal aberration test in Chinese hamster ovary (CHO) cells, and *in vivo* mouse micronucleus test. In a 2-year carcinogenicity study by subcutaneous route in Wistar Han rats at 3, 10 and 35 mg/kg/day (about 60, 200 and 700 times the recommended daily human dose of 0.05 mg/kg, respectively), teduglutide caused statistically significant increases in the incidences of adenomas in the bile duct and jejunum of male rats. There were no drug related tumor findings in females. A 2-year mouse carcinogenicity study is ongoing. By virtue of its mechanism of action (intestintrophic activity or growth promoting pharmacological effect) and the findings of the carcinogenicity study in rats, teduglutide has the potential to cause hyperplastic changes including carcinogenicity in humans.

In the subcutaneous fertility and early embryonic development study in rats at 2, 10 and 50 mg/kg/day, teduglutide did not show any adverse effects on early embryonic development or fertility parameters up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg). In the subcutaneous embryofetal development study in rats at 2, 10 and 50 mg/kg/day, teduglutide was not teratogenic up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg). In the subcutaneous embryofetal development study in rabbits at 2, 10 and 50 mg/kg/day, teduglutide was not teratogenic up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg). In the subcutaneous pre and postnatal development study in rats at 10, 25 and 50 mg/kg/day, teduglutide did not show any significant adverse effect on pre and postnatal development up to 50 mg/kg/day (about 1000 times the recommended daily human dose of 0.05 mg/kg).

The following table (from page 32 of Section 2.4 of the submission) shows the summary of exposure margins from various toxicology studies over the human exposure at the proposed clinical dose of 0.05 mg/kg/day.

Table 1 Summary of NOEL/NOAEL Doses and Exposure Comparisons

Species	Dosing Duration	NOAEL (mg/kg/day)	AUC ₀₋₈ (µg•hr/mL) ^a	MOE ^b
Mouse	13 Weeks	50	41.9	178.3
Mouse	26 Weeks	50	58.5	248.9
Rat (Sprague-Dawley)	14 Days	50	43.9	186.8
Rat (Wistar)	14 Days	50	30.5	129.8
Rat (Sprague-Dawley)	13 Weeks	50	36.7	156.2
Monkey	28 Days	2	2.7	11.5
Monkey	13 Weeks	5	6.3	26.8
Monkey	52 Weeks	5	9.1	38.7
Rat (Wistar)	104 Weeks	3 ^c	2.3	9.8
Rat (Sprague-Dawley)	GD 6 to 17	50 (Maternal & Developmental)	51.5	219.2
Rabbit	GD 7 to 20	50 (Maternal & Developmental)	49.3	209.8
Juvenile Minipigs	13 Weeks	<1	<1.5	<6.4

NOAEL=no observed adverse effect level; NOEL=no observed effect level; GD = gestation day; MOE = margin of exposure.

^a = Lowest AUC value reported (either male or female) except for 104-week rat carcinogenicity study where the value for males is reported.

^b = Human AUC_{0-∞} at the clinical dose of 0.05 mg/kg/day is 0.235 µg•hr/mL (Study NPS-RAS-004).

^c = NOEL assigned in 104-week rat carcinogenicity study.

Source: Section 2.6.6.10, Table 32

Overall, nonclinical safety of teduglutide has been adequately tested in several toxicology studies. Nonclinical studies conducted with teduglutide provide adequate assurance of safety and support its proposed use at the intended therapeutic dosage and in accordance with the proposed product labeling. However, by virtue of its mechanism of action (intestintrophic activity or growth promoting pharmacological effect) and the findings of the carcinogenicity study in rats, teduglutide has the potential to cause hyperplastic changes including carcinogenicity in humans.

12 Appendix/Attachments

None

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/s/

TAMAL K CHAKRABORTI
08/03/2012

SUSHANTA K CHAKDER
08/03/2012

Comments on N203441 teduglutide

From: A. Jacobs

Date: July 25, 2012

1. I concur that there are no nonclinical approval issues and that the pregnancy category should be B

2. I suggest that the (b) (4) section of labeling be removed. This section is NOT supposed to be a general summary of toxicity, but rather for those few instances where unexpected, unmonitorable toxicity is seen at exposures relevant to humana, and might affect clinical use.

I have discussed other comments with the reviewer and they will be addressed as appropriate.

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

/s/

ABIGAIL C JACOBS
08/09/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 203441

**Applicant: NPS Pharmaceuticals, Stamp Date: 11/30/11
Inc.**

**Drug Name: Teduglutide
(Gattex®)**

NDA Type: New NDA

Submit Date: 11/30/11

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?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
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)?	√		
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).	√		
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 <u>submitted</u> a rationale to justify the alternative route?	√		
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?	√		

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
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		
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?	√		The proposed labeling sections relevant to nonclinical studies may need to be revised during the labeling review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		N/A
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _____ YES.

If the NDA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant. _____N/A

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

Tamal K. Chakrabortit, Ph.D. December 30, 2011

 Reviewing Pharmacologist Date

Sushanta K. Chakder, Ph.D. December 30, 2011

 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/

TAMAL K CHAKRABORTI
12/30/2011

SUSHANTA K CHAKDER
12/30/2011